THE BIOCHEMICAL EFFECTS OF *CUSSONIA ARBOREA* ROOT BARK EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

BY

ABA, PATRICK EMEKA
PG/PhD/11/58702

DEPARTMENT OF VETERINARY PHYSIOLOGY AND PHARMACOLOGY,
UNIVERSITY OF NIGERIA, NSUKKA

NOVEMBER, 2015

TITLE PAGE

THE BIOCHEMICAL EFFECTS OF *CUSSONIA ARBOREA* ROOT BARK EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

BY

ABA, PATRICK EMEKA
DVM, MSc (UNN)
PG/PhD/11/58702

THESIS SUBMITTED TO THE DEPARTMENT OF VETERINARY PHYSIOLOGY AND PHARMACOLOGY, FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF NIGERIA, NSUKKA FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY

NOVEMBER, 2015

CERTIFICATION

ABA, PATRICK EMEKA, a postgraduate student in the Department of Veterinary Physiology and Pharmacology, with registration number PG/PhD/11/58702 has satisfactorily completed the requirements for the degree of Doctor of Philosophy in Veterinary Biochemistry.

The work embodied in this thesis is original and has not been submitted in part or full for any diploma or degree of this or any other University.

PROF. I.U ASUZU	**PROF. S.C UDEM**
(SUPERVISOR)	**(HEAD OF DEPARTMENT)**
PROF. B.M AGAIE	**PROF. C.O NWOSU**
(EXTERNAL EXAMINER)	**(DEAN, FACULTY OF VETERINARY MEDICINE)**

DEDICATION

TO GOD ALMIGHTY BE ALL THE GLORY

ACKNOWLEDGEMENTS

My first line of appreciation goes to God Almighty, the author and finisher of my life. His loving kindness in my life is unending. I bless His name.

I equally wish to announce my unreserved appreciation to my family members- my lovely wife, beautiful daughter, caring mother, wonderful sisters and great in-laws for the encouragement I received from them during this study. I sincerely appreciate them.

One of my most important unadulterated, effusive and profound gratitudes goes to my supervisor, Professor I.U ASUZU, the Vice Chancellor of Federal University, Oye-Ekiti, Ekiti state, who equally doubles as my role model and mentor. I thank him not only as my supervisor whose emulative qualities are unparalleled but as a father, spiritual motivator, morale booster, friend and above all, for being highly instrumental for whatever I have become today in my life. I owe all to his instrumentality. Despite his tight schedules as the Vice Chancellor, he still has time to meticulously and timely attend to my needs. Many a time, I feel words are not enough to say THANK YOU. However, gold and silver I have not; I just wish to say THANK YOU and may God Almighty continue to shower you with His blessings.

This acknowledgement would be grossly inadequate if I fail to appreciate the sincere aid rendered to me in the course of this onerous task by the following members of staff- Prof. S.C Udem (Head of Department), Prof. J.I Ihedioha (Director of Veterinary Hospital), Prof. P.A Onyeyili, Prof. S.M Anika, Prof. C.N Uchendu, Prof. A.O Anaga, Prof. I.R Obidike, Prof. I.I Madubunyi, Prof. R.C Ezeokonkwo, Dr. L.O Aka. Also worthy of mention are my research fellows- Dr. V.U Omoja, Dr.(Mrs) R.I Odo, Dr. I.G Eke, Dr. I.K Idika, Dr. C.O Okorie-Kanu, Dr. J. Bosha, Dr. E.C Mbegbu, Dr.(Mrs) T.E Ihedioha, Dr.(Mrs), C.O Nwaigwe, Mrs V. Madu,

Mrs. M.C Onu, Miss Odinaka and other members of staff of the Department of Veterinary Physiology and Pharmacology (VPP) to mention only but a few.

Lastly but definitely not the least, I wish to heartily recognize the professional and technical assistance of Dr. J. Igoli of the University of Strathchyde, Glasgow , Scotland, UK, Prof. P.O Ukoha, of Chemistry Department and Dean Faculty of Physical sciences, Mr. E Mbaoji of the Department of Chemistry and Mr A. O Ozioko formally of Plant Science Department. Thank you all.

DR. PATRICK EMEKA ABA

TABLE OF CONTENTS

CHAPTER THREE

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

APPENDIX

ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. It is a growing public health concern worldwide affecting humans and animals. Synthetic drugs available for the treatment of the ailment have serious side effects, complicated mode of intake and are costly. Literature search revealed that *Cussonia arborea* is used folklorically in the management of Diabetes mellitus. The aim of this study is to isolate, characterize and elucidate the active principle responsible for its hypoglycaemic activity using alloxan-induced diabetic rats. The root bark of *C. arborea* (2 kg) was extracted with 80% methanol by cold maceration method. Acute toxicity study was done in 35 rats assigned into 7 groups of 5 rats per group. Groups 1, 2,3,4,5 and 6 rats were orally administered with graded doses (500, 1000, 2000, 3000, 4000, 5000 mg/kg bw) of the extract respectively. The rats in group 7 received 10 ml/kg distilled water (DW) to serve as negative control group. They were observed closely for 48 hours for signs of toxicity. Assessment of hypoglycemic activities of the extract was done using oral glucose tolerance test (OGTT), acute and chronic antidiabetic studies. In OGTT, 30 rats were randomly assigned into 5 groups of six rats per group. Groups 1, 2 and 3 rats received 250, 500 and 1000 mg/kg bw of the extract respectively while groups 4 and 5 rats received 2 mg/kg bw glibenclamide and 10 ml/kg DW respectively after 18 h fasting and prior to 2000 mg/kg of glucose load. The fasting blood glucose (FBG) levels of the rats were determined after 30, 60, 120 and 180 min post glucose challenge. Diabetes was induced by single intraperitoneal administration of alloxan monohydrate at the dose of 160 mg/kg bw. Rats with FBG levels above 126 mg/dl (7 mMol/L) were considered diabetic. Thirty male albino Wistar rats assigned into 6 groups of 5 rats per group were used for acute antidiabetic studies. Groups 1, 2, 3, 4 and 5 were diabetic rats treated with 250, 500 1000 mg/kg bw of the extract, 2 mg/kg bw glibenclamide and 10 ml/kg DW respectively while group 6 rats were non diabetic but administered with 10 ml/kg DW. The FBG levels of the rats were determined 1 h, 3 h, 6 h and 24 h post treatment. Seventy two (72) male albino wistar rats weighing between 100 and 105 g were assigned into six groups of 12 rats per group for chronic antidiabetic studies. Groups 1, 2, 3, 4 and 5 rats were made diabetic as described earlier and treated with 62.5, 125, 250 mg/kg bw of the extracts, 2 mg/kg bw glibenclamide and 10 ml/kg DW respectively while the non diabetic group 6 rats received 10 ml/kg DW and served as normal control rats. The treatment was daily through the oral route for

84 days. The biochemical (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglyceride, high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total protein, albumin, globulin, blood urea nitrogen, creatinine, total bilirubin, conjugated bilirubin, unconjugated bilirubin, malondiadehyde (MDA), superoxide dismutase (SOD), catalase, and reduced glutathione) and haematological (red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), differential leucocytes (lymphocytes, neutrophils, basophils, eosinophils and monocytes), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)) parameters were assayed on days 28, 56 and 84 post treatment while the FBG values and weight changes were determined every two weeks. The glycosylated haemoglobin values were measured on days 42, 56 and 84 post treatment. Three rats per group were humanly sacrificed on days 28, 56 and 84 for the assessment of the histomorphology of various organs (pancreas, kidney, liver and heart). *In vitro* antioxidant assay was carried out using ferric reducing antioxidant power (FRAP) and diphenyl picryl hydrazyl (DPPH) photometric assay models. Chromatographic separation of the plant extract was done using column and thin layer chromatographic techniques. The ^{1}H proton and ^{13}carbon nuclear magnetic resonance (NMR) were used for structural elucidation of the active hypoglycaemic principle. No sign of toxicity was observed after acute toxicity test. The phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenes. The mean FBG level of the rats treated with 250 mg/kg bw of the extract in acute anti-diabetic study, at 3 h post extract administration was significantly ($p<0.05$) lower than the FBG before administration of the extract. In the chronic anti-diabetic study, the mean FBG level of rats treated with 125 mg/kg bw of the extract reduced significantly ($p<0.05$), the post induction FBG from 315.33± 10.08 mg/dl to 93.00 ±8.50 mg/dl, 14 days post administration and ameliorated the glycosylation of haemoglobin compared to the negative control group. Administration of 250 mg/kg bw of the extract in glucose challenged rats reduced the post challenge glucose level from 147.66 ±1.85 mg/dl to 83.00 ±3.5 mg/dl, 180 min post treatment. Assessment of the biochemical and haematological parameters in chronically-treated diabetic rats showed that the administration of 125 mg/kg bw of the extract, significantly ($p<0.05$) reduced the activities of AST, ALT and the levels of total cholesterol, triglyceride, VLDL, LDL BUN, creatinine, total bilirubin, malondialdehyde but significantly ($p<0.05$) increased the activities of SOD, catalase and the

levels of HDL, total protein, conjugated bilirubin, reduced glutathione , RBC, Hb and PCV when compared to the diabetic untreated group (negative control). The extract yielded seven fractions. The FBG of the rats treated with 12.5 mg/kg bw of fraction 2 was significantly ($p<0.05$) lower compared to the negative control group. Subfraction 1 (2 mg/kg) of fraction 2 significantly ($p<0.05$) reduced the FBG levels from 310.00±5.77 mg/dl to 74.00±0.57 mg/dl. Rats treated with 125 mg/kg bw of the extract showed milder histopathologic lesions in various tissues compared with negative control. The DPPH *in vitro* antioxidant assay showed a concentration dependent activities with 400 µg/ml of the extract and ascorbic acid showing 74% and 92% activities respectively. The FRAP values of the extract and ascorbic acid at 400µg/ml were 1.60 and 2µm respectively. The NMR spectroscopy revealed a pentacyclic triterpenoid, {(3ϐ)-3-hydroxyolean-12-en-28-oic acid} as the active compound. The study showed that the root bark extract of *C. arborea* did not only possess antihyperglycaemic, hypolipidemic, antioxidant and antianemic properties but also reduced haemoglobin glycosylation and ameliorated the severe degenerative lesions in the pancreas, kidney, liver and cardiac myocytes occasioned by *Diabetes mellitus*.

`CHAPTER ONE

INTRODUCTION

Diabetes is a complex and a multifarious group of metabolic disorders that disturbs the metabolism of carbohydrates, fats and protein (Kahn and Shechter, 1991**;** Bliss, 2000). It is characterized by increased fasting and postprandial blood glucose levels. *Diabetes mellitus* is a group of metabolic disorder resulting from defects in insulin secretion or reduced sensitivity of the tissues to insulin action or both (Lanza *et al.,* 2001). It is a disease characterized by inability to regulate blood glucose as a result of relative or absolute deficiency in insulin. This results to hyperglycemia often accompanied by glycosuria, polydipsia and polyuria (Celik *et al.,*2002) Besides hyperglycaemia, several other factors like hyperlipidaemia and enhanced oxidative stress play a major role in diabetes pathogenesis. The disease is progressive and is associated with high risk of complication (Dewanjee *et al.,* 2008). It is one of the most common endocrine diseases and has a prevalence rate varying from 1- 50% (King and Rewers, 1993**).**

The term ñ*Diabetes Mellitus*ò is derived from the Greek words dia (through), bainein (to go) and diabetes literally means pass through. The disease causes loss of weight as if the body mass is passed through the urine. Although it was known for centuries that the urine of patients with diabetes was sweet, it was not until 1674 that the physician named Willis coined the term *Diabetes mellitus* (from the Greek word for honey) (Vasudevan and Kumari, 2005).

Although many types of *Diabetes mellitus* exist (Tierney *et al*, 2002), it has been broadly classified into two by World Health Organization (WHO, 1980). Type I diabetes mellitus ï insulin dependent *Diabetes mellitus* and type 2 *Diabetes mellitus*-Non insulin dependent *Diabetes mellitus*. Type 1 *Diabetes mellitus* is characterized by loss of the insulin-producing beta cells of the islet of langerhans in the pancreas, leading to insulin deficiency. This type can be

further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated auto immune attack (Rother, 2007) Type 2 *Diabetes mellitus* is characterized by insulin resistance which may be combined with relatively reduced insulin secretion (Shoback, 2011). Type 2 diabetes is the most common type. Other forms of diabetes that have been described include Gestational diabetes mellitus, type 3 diabetes mellitus (Alzeibers disease) and non specific types (Shoback, 2011). Gestational *Diabetes mellitus* (GDM) is a third major category of diabetes. It occurs in at least 5 to 14 percent of pregnancies. It may improve or disappear after delivery (Cousens, 2008). Alzheimerôs disease has a strong connection with diabetes, obesity and heart disease. It is such strong connection that Alzheimerôs is being referred to by some scientists as type 3 diabetes (Rivera *et al*., 2005). Alzeheimerôs disease is a chronic neuro-degenerative disease that usually starts slowly and gets worse over time (Burns and Lliffe, 2009). The most common early symptom is difficulty in remembering recent events (Short term memory loss) (Burns and LLiffe, 2009). Mental and physical exercise and avoiding obesity may decrease the risk of Alzheimers disease (Ballard *et al*., 2011).

Other specific types of *Diabetes mellitus* include Latent autoimmune diabetes of adults (LADA) which is a condition in which type 1 *Diabetes mellitus* develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM based on age rather than etiology. Genetic mutations (autosomal or mitochrondrial) can lead to defects in beta cell function. Any disease that can cause extensive damage to the pancreas may lead to diabetes (for example chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone

excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells (WHO, 1999).

The incidence of *Diabetes mellitus* in dogs and cats has been noted. Middle to older dogs and cats are mainly affected. Females are affected twice as often as males and the incidence appears to be increased in certain small breeds such as chow chow, Alashan Malamute, poorly (Kahn, 2005). *Diabetes mellitus* affects 1 in 400 (0.25%) cats through recent studies. Mc Cann *et al.,* (2007) noted that it is becoming more common lately in cats. The prevalence of diabetes mellitus in world population is increasing in epidemic proportion. In 1995, it was estimated that around 135 million people were affected by this condition and it was expected to affect 200 million by the year 2015 (King *et al.,* 1998). The World Health Organization warns that deaths due to diabetes will increase globally by as much as 80 percent in some regions over the next 10 years (WHO, 2005).

Common clinical signs of diabetes in dogs include polydipsia, polyuria, polyphagia with weight loss, bilateral cataract and weakness (Kahn, 2005). In cats the back legs may become weak and the gait may become stilted or wobbly consequent upon peripheral neuropathy. Untreated, the condition leads to increasingly weak legs in cats and eventually mal-nutrition, ketoacidosis and or dehydration and death but prompt effective treatment can lead to diabetic remission (Rand and Marshal, 2005). There are two forms of complications-acute and chronic complications. Diabetic Ketoacidosis (DKA) is one of the acute complications. Elevated levels of ketone bodies in the blood decrease the blood pH leading to DKA with classical signs of abdominal pains, lethargy, coma, hypotension, shock and death. Urine analysis will reveal significant levels of Ketone bodies (which appear in the urine after exceeding its renal threshold) (Aiello, 1998). Another acute complication is hyperglycemic hypoosmolar state in which water

will osmotically be withdrawn from the cells of a patient with very high blood sugar level, into the blood causing dehydration and increase in blood osmolarlity (Meyes, 2000).

Respiratory infection is another acute complication. The immune response is impaired in individuals with diabetes mellitus. Cellular studies have shown that hyperglycemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function which leads to an increase in susceptibility to respiratory infections such as pneumonia and influenza among individuals with diabetes. Several studies show that diabetes is associated with slower recovery from respiratory infection (Ahmed *et al.,* 2008).

All forms of diabetes increase the risk of long term complications. The major long term complications are relative to blood vessel damage (Boussageon *et al.,* 2011). The damage to small blood vessels leads to microangiopathy which has been incriminated in other chronic complications such as diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and diabetic cardiomyopathy, while damage to the large vessels (Macrovascular disease) is sequel to cardiovascular disease such as coronary artery disease, and diabetic myonecrosis (Muscle wasting) (Aristides *et al.,* 2007). Another chronic complication is diabetic encephalopathy in which there is increased cognitive decline and the risk of dementia observed in diabetics. Various mechanisms are proposed including alterations to vascular supply of the brain and the interaction of insulin with the brain itself (Gispen and Biessels, 2000).

The diagnosis of *Diabetes mellitus* is based on persistent fasting hyperglycemia and glycosurias. Assay of glycosylated haemoglobin and fructoseamine may also help to differentiate between *Diabetes mellitus* and stress-induced hyperglycemia (Goldstein *et al.,* 1984). Other aids to diagnosis include clinical signs such as polyruria and polydipsia accompanied by weight loss, vision changes and unexplained fatigue or by observation of diabetes associated disease such as

poor wound healing, heart attack, stroke neuropathy, foot ulcers, certain fungal infections or delivery of baby with macrosomia (Dewanjee *et al.,* 2008; Twari and Rao, 2002). The normal fasting blood sugar value in dogs and cats is between 7-20 mMol/L (Kahn, 2005). In humans, diagnosis of diabetes is by demonstration of fasting plasma glucose level at or above 126 mg/dl (7 mMol/L) or plasma glucose at or above 200 mg/dl (11./Mmol/l) 2 hours after oral glucose load as in glucose tolerance test (WHO, 1999). Glucose tolerance test is a confirmatory method for the diagnosis of diabetes (WHO 1999**)** and it is determined by evaluating the concentration of the blood glucose at specific intervals before and after oral or intravenous administration of a given quantity of glucose. Both oral and intravenous routes may be used in the dog, however, in ruminants; the intravenous method must be used since there is no blood glucose response to oral administration of carbohydrate (Nelson, 2000). Glucose is irreversibly bound to hemoglobin (termed glycoslylated hemoglobin or HbA_{1C}). An elevated glucose level of 6% or higher is considered abnormal by most laboratories (Weykamp and panders, 1994). Glycosylated hemoglobin is used as a treatment-tracking test reflecting average blood glucose levels over the preceeding 90 days (approximately) which is the average life span of the red blood cells which contain hemoglobin in most patients (Goldstein *et al.,* 1984).

Insulin sensitivity test and insulin assay using enzyme-linked immunosorbent assay (ELISA) method can be used to aid diagnosis of diabetes mellitus. In insulin sensitivity, about 0.2 units of insulin is administered to the patient followed by monitoring of the blood glucose level over an interval of some hours (2-3 hrs). The blood glucose level will be lowered in patients that have absolute deficiency or reduced insulin secretion or absolute lack of insulin secretion. There will be no significant decrease in patients that have insulin dysfunction or resistance (Guyton, 1996). Insulin assay is used to differentiate between type 1 and type2

diabetes. In type 1 diabetic patients, no insulin will be detected after the assay, but in a type 2 patient, insulin level may be normal, reduced or high (WHO, 2006).

Treatment and management involve a combination of weight reduction diet, insulin and possibly oral hypoglycemic compounds. In cats, the use of high protein, low carbohydrate diets and in dogs, diets that are high in fiber and complex carbohydrate are preferred (Kahn, 2005) Dietary control is the cornerstone in the treatment of diabetes, irrespective of the severity of the symptom (Nelson, 2000). The diet should be targeted towards reduction of body weight, blood glucose levels and maintenance of good health. The oral hypoglycemic agents lower blood glucose level by different mechanisms. Sulfonylureas such as glyburide, meglitinides (repaglinide) and D- phenylalanine derivatives increase insulin production from the pancreas. Biguanides such as metformin decrease glucose release from the liver while alpha-glucosidase inhibitors such as glucobay (acarbose) slow down absorption of sugar from the gut. Thiazolidinediones (TZD or glitazone) such as pioglitazone, increase glucose uptake by fat and muscle cells. Exanatide and DPP-4 inhibitors such as sitagliptin reduce appetite, increase insulin production from the pancreas and decrease glucose release from the liver at once (Blumer, 2010). The first generation sulfonylureas include tolbutamide, acetohexamide, tolazamide and chlorpropamide while the second generation includes glibenclamide (glyburide) glipizide and gliclizide. The second generation agents are more potent than the first. Some pancreatic insulin secretary capacity must exist for sulfonylurea to be effective in improving glycaemic control (Nelson 2000). Sulfonylurea is ineffective as the sole form of treatment for insulin-dependent *Diabetes mellitus* (IDDM) and has not been effective in diabetic dogs presumably because of the high incidence of IDDM in this species. Sulfonylurea has the side effect of hypoglyemia (commonly), vomiting, nausea and cholestatic jaundice (Rang *et al.,* 2003). The biguanides like

metformin are used in patients with type 2 *Diabetes mellitus*. The commonest unwanted side effects are dose-related gastro-intestinal disturbances such as diarrhoea, nausea and anorexia (Grandhipuran *et al.,* 2006). Lactic acidosis is rare but potentially fatally toxic and metformin should not be given to a patient with renal or hepatic disease (Figmognari-pastorelli and Incalzi, 2006). Acarbose, oral alpha glucosidase inhibitors have been used in cats at the dosage of 125-250 mg. The most common side effects of acarbose include increased flatulence, diarrhoea abdominal bloating and kidney tumors in long term use. In humans, combination of diets, exercise and weight loss with oral diabetic drugs and use of insulin have proved to be effective. Exercise plays important role in the management of hyperglycaemic conditions by helping to promote weight loss as well as elimination of the insulin resistance induced by obesity (Edward *et al.,* 1987). Exercise also exerts a glucose lowering effect by increasing the absorption of insulin from injection site, increasing blood flow to exercising muscles and stimulating translocation of glucose effect (Nelson, 2000). Life style modification such as smoking less, wearing diabetic shoes or taking drugs that will minimize blood pressure will help to reduce the development of cardiovascular disease (Adler *et al.,* 2000). Insulin replacement therapy is another aid in the management of diabetes mellitus. Insulin lowers the concentration of glucose in the blood by inhibiting hepatic glucose production and by stimulating uptake and metabolism of glucose by muscle and adipose tissue (Rang *et al.,* 2003). Insulin is the main stake in the treatment of virtually all IDDM patients in who there is absolute lack of insulin. It is also used in the NIDDM patient that does not respond well to diet, physical exercise and hypoglycaemic agent treatment (Anaga and Asuzu, 2010). Diabetic dogs are mainly treated by exogenous insulin administration due to high incidence of IDDM in them (Nelson, 2000). There is no practical cure at this time for type 1 diabetes. The only time type 1 diabetes mellitus is said to have been

cured is in a condition of kidney-pancreas transplant (Vinik *et al.,* 2004). This can be done when diabetic nephropathy has developed. A simultenous pancreas-kidney transplant is a promising solution showing similar or improved survival rates over a kidney transplant alone (Strata and Alloway, 1998). Still, they remain on long term immunosuppressive drugs and there is a possibility that the immune system will mount a host versus graft response against the transplanted organ (Vinik *et al*., 2004). Transplant of exogenous beta cells have been performed experimentally in both mice and humans, but this measure is not yet practical in regular clinical practice partly due to the limited number of beta cell donors. Like any other transplant, it has provoked an immune reaction and long term immunosuppressants have been needed to protect the transplanted tissue (Shapiro *et al.,* 2006). Stem cell research has also been suggested as a potential avenue for cure since it may permit the re-growth of islet cells which are genetically part of the treated individual, thus, perhaps eliminating the need for immunosuppresants (Vinik *et al.,* 2004). In a trial, researchers implanted diabetes type 1 patient with their own stem cells raised from their own bone marrow. The stem cell transplant led to an appreciable repopulation of functioning insulin-producing beta cells in the pancreas, so the patients became insulin free. Most of these patients became insulin independents for a mean period of 18 months (Couri *et al.,* 2008). At present time, autologous non-myeloblastic Hematopoietic Stem-Cell Transplantation (HSCT) remains the only treatment capable of reversing type 1 *Diabetes mellitus* in humans.

In type 2 *Diabetes mellitus* in human, a gastric by-pass surgery can normalize blood glucose levels in 80-100% of severely obese patients. This approach may be standard for people with type 2 *Diabetes mellitus* in the near future (Rubino and Gagner, 2002).

The use of plants in the treatment of ailments dates back to the prehistoric times. Herbalism is a traditional medicine or a folk medicine-practice based on the use of plant and

plant extracts (Akah *et al.,* 1998). Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. Africa is endowed with many plants that can be used for medicinal purposes. In fact, out of the approximated 64000 plant species used in tropical Africa, more than 4000 are used as medicine plants (Akah *et al.,* 1998). A recent review article presents the profile plants with hypoglycaemic properties reported in literature from 1990 to 2000 and states that medicinal plants play an important role in the management of diabetes mellitus especially in the developing countries where resources are meager (Bnouham, 2006). The first use of anti-diabetic drugs was as herbal extracts used by Indians in the Amazon Basin for the treatment of type 2 diabetes and today promoted as vegetable insulin although not formally an insulin analog (Soumyanath, 2005).

Some of the traditional plant treatment for diabetes include but are not limited to *Agrimonia eupararia* (Agrimony), *Juniperus communis* (Junipe) *Allium sativum* (garlic) (Swant son *et al.,* 1990). Others include *Strophantus hispidus, Gongronema latifolia, Morinda lucida, Vernonia colorata, Viscum album, Garcinia cola, Pterocarpus marsupium* also known as *Indian Kino, Malaban kino* and *Pitasara nenga* etc. (Kavishankar *et al*., 2011)

Cussonia arborea (Hochst) is a tree that originated from Africa and has its center of distribution in South Africa and Madagascar (Tennant, 2010). The tree can grow up to 11m tall with bole of up to 0.75 diameter; bark deeply fissured and corky. Leaves are digitately compound; petiole up to 87cm long and 9 mm wide but usually much smaller, mortly glabrous or some what hairy in places. Leaflets: 5-9 sessile, chartaceous to coriaceous to oblanceolate, ovate and obviate up top 23cm long by 10.5cm wide. Flowering spikes up to about 26 together but mostly less than 12, up to 46 cm long sometimes galled.

The different local names of the plant are Mufenje by Yoruba, Hannun kuturuu (Lepers Hand), takandar giiwaa (elephant sugarcane) by Hausa, Ityovor by TIV people and Kijagaajaga by Bukoba rural district in Tanzania. No specific Igbo name has been associated with the plant.

1.2 Background of the study

The search and development of a novel chemical molecule from medicinal plants that can be used for treatment of diabetes with little or no side effects is the driving force for this study. Most of the drugs in use are known to cause serious adverse side effects with various disadvantages.

The sulfonylureas for instance have increased risk of hypoglycaemia. They have high risk of death compared to melformin. The biguanides such as metformin are associated with increased risk of gastrointestinal problems. They are contraindicated for people with moderate or severe kidney disease or heart failure because of risk of lactic acidosis due to alcoholism. Metformin has increased risk of causing Vitamin B12 deficiency (Agabegi and Agabegi, 2008). It is also less convenient in dosing due to its metallic taste. The Alpha glucosidase inhibitors such as acarbose are less effective than most other diabetes pills in decreasing glycated hemoglobin. They also have increased risk of gastrointestinal problems than other diabetes pills except metformin. They are expensive and inconvenient in dosing with increased risk of edema and anaemias with increases in low density lipoprotein while the thiazolidinediones such as Pioglitazone have slow onset of action and requires monitoring for hepatotoxicity. They are expensive and have increased risk of limb fractures. They have also been associated with high risk of bladder cancer (Cambon-Thomsen *et al.,* 2007; Bennet *et al.,* 2011).

1.3 Statement of the problem

1. In the last two decades, research into antidiabetic medicinal plants has not been rewarded with marketable novel drugs (Dewanjee *et al.,* 2008).
2. The available synthetic hypoglycaemic agents used in clinical practice have serious adverse effects; therefore the search for more effective and safer antidiabetic agents has become an area of active research.
3. The synthetic drugs used presently are given for a long duration and have complicated mode of intake. There is no single agent that yields optimal glucose lowering effects in all treated patients.
4. Most ethnomedical practices lack scientific procedures and their effects sometimes are over exaggerated or based on trial and error.

1.4 Objectives of the study

The main objectives of this study include;

1. To establish the pharmacological basis for the use of the root bark of *C. arborea* in the treatment of symptoms of diabetes mellitus with a view to determining the efficacy and possibly biomedical mechanism(s) of action of the plant.
2. To isolate, characterize and carry out structural elucidation of the active compound(s) responsible for the anti-diabetic activity of the plant.
3. To evaluate the antioxidant potential of the plant.
4. To assess the toxicity of the extract.
5. To determine the biochemical effects associated with the plant in the treatment of *Diabetes mellitus*.

CHAPTER TWO
LITERATURE REVIEW

2.1 History

The term *Diabetes mellitus* is a group of metabolic diseases in which a person has high blood sugar either because the body does not produce enough insulin or because cells do not respond to the normally produced insulin (Shoback, 2011). Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE. Type 1 was associated with youth and type 2 with obesity (Leonid, 2009). The term mellitus or from honey was added by Thomas willis in the late 1600s to separate the condition from *Diabetes insipidus* which is also associated with frequent urination.

The first complete clinical description of diabetes was given by the ancient Greek physician Aretacus of Cappadocia (1st century CE), who also noted the excessive amount of urine which passed through the kidneys (Dallas, 2011). Although diabetes has been recognized since antiquity, and treatments of various efficacy have been known in various regions since the middle ages, and in legend for much longer, pathogenesis of diabetes has only been understood experimentally since about 1900 (Patlak, 2002). An effective treatment was only developed after the Canadians Frederick Banting and Charles Best first used insulin in 1921 and 1922 (Leonid, 2009).

The role of the pancreas in diabetes is generally ascribed to Joseph Von Mering and Oskar mikowski, who in 1889 found that dogs whose pancreases were removed, developed all the signs and symptoms of diabetes and died shortly afterwards (Von Mehring and Minkowski, 1890). In 1910, Sir Edward Albert Sharpey-Schafer suggested that people with diabetes were deficient in a single chemical that was normally produced by the pancreas. He proposed calling this substance insulin, from the Latin insulin meaning Island, in reference to the insulin-

producing Islets of langerhans in the pancreas. The islet of langerhans was discovered in 1869 by an anatomist named Paul Langerhans. He identified the keys cell in the pancreas which produces the main substance that controls glucose levels in the body (Bryan, 2004). Other landmark discoveries include development of the long acting insulin NPH in the 1940ôs Novo-Nordisk (Leonid, 2009), identification of the first sulfonylurea in 1942, reintroduction of the use of biguanides for type 2 diabetes in the late 1950s. Further more amino acid sequence of insulin was determined by Sir Frederic Sanger for which he received a Nobel prize. Insulin was the first protein that the amino acid structure was determined. There was also discovery of the three dimensional structure of insulin. The radioimmunoassay for insulin was discovered by Rosalyn Yalow and Solomon Berson (Yallow and Benson, 1960**)**. Thiazolidinediones were identified as effective insulin sensitizer during the 1990s (Polonsky, 2012).

2.2 Prevalence and incidence of DM

Middle aged to older dogs and cats are mainly affected by diabetes. In dogs, females are affected twice as often as males and the incidence appears to be increased in certain small breeds such as chow chow, Alaskan malamute, poodle etc. (Kahn, 2005). *Diabetes mellitus* affects 1:400 (0.25%) cats, though recent veterinary studies (McCann *et. al.,* 2007) noted that it is becoming more common lately in cats. In 2000, according to the WHO, at least 320 million people world wide suffered from diabetes (Wild *et. al.,* 2004). The prevalence of diabetes mellitus increases with age, and the numbers of older persons with diabetes are expected to grow as the elderly population increases in number. (Harris *et. al.,* **1998**).

2.3 Classification of Diabetes mellitus

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (WHO, 1980) and in modified form in 1985 (WHO 1985). The 1980 Expert committee proposed two major classes of *Diabetes mellitus* and named them insulin-dependent *Diabetes*

mellitus or type 1 and Non-insulin-dependent *Diabetes mellitus* or type 2. Gestational *Diabetes mellitus* (GDM) was regarded as the third. The recent classification as suggested by Kuzuya and Matsuda (1997) incude both the clinical stages and etiological types of *Diabetes mellitus*. The etiological classification reflects the fact that the defect or process which may lead to diabetes may be identifiable at any stage in the development of diabetes-even at the stage of nomoglycaemias. Therefore, the presence of islet cell antibodies in a normoglycaemic individual makes it likely that the person has the type 1 auto-immune process. The etiological types designate defects, disorders or processes which often result in *Diabetes mellitus*.

1. Type 1-Beta-cell destruction usually leading to absolute insulin deficiency.

a. Auto immune

b. Idiopathic

2. Type 2 (which may range from prominently insulin resistance with relative insulin deficiency to a predominantly secreatory defect with or without insulin resistance).

3. Gestational diabetes (which occur during pregnancy and may resolve after delivery or result to type 2).

4. Other specific types

a. Genetic defects of beta-cell function

i. Chromosome 20, HNF4$\propto$ (MODY 1)

ii. Chromosome 7, glucokinase (MODY2)

iii. Chromosome 12, HNFI$\propto$ (MODY 3)

iv. Chromosome 13, IPF-1 (MODY 4)

v. Mitochondrial DNA 3243 mutation

vi. Others

b. Genetic defects in insulin action

i. Type A insulin resistance

ii. Leprechaunism

iii. Rabson-Mendenhall syndrome

iv. Lipoatrophic diabetes

v. Others.

c. Disease of the exocrine pancreas

i. Fibrocalculous pancreatopathy

ii. Pancreatitis

iii. Trauma/pancreatectomy

iv. Neoplasia

v. Cystic fibrosis

vi. Haemochromatosis

vii. Others.

d. Endocrinopathies

i. Cushingôs syndrome

ii. Acromegaly

iii. Phaeochromocytoma

iv. Glucagonoma

v. Hyperthyroidism

vi. Somatostatinoma

vii. Others.

e. Drug or chemical induced

i. Nicotinic acid

ii. Glucocorticoids

iii. Thyroid homones

iv. Alpha-adrenergic agonists

v. Beta-adrenergeic agonists

vi. Thiazides

vii. Dilantin

viii. Pentamidine

ix. Vacor

x. Interferon-alpha theraphy

xi. Others.

f. Infections

i. Congenital rubella.

ii. Cytomegalovirus

iii. Others.

e. Uncommon forms of immune-mediated diabetes

i. Insulin autoimmune syndrome (antibodies to insulin).

ii. Anti-insulin receptor antibodies

iii. Staff man syndrome

iv. Others

g. Other genetic syndromes sometimes associated with diabetes

i. Downôs syndrome

ii. Friedreichôs ataxis

iii. Huntingtonôs chores

iv. Klinefelterôs syndrome

v. Lawrence-moon-Biedel syndrome

vi. Myofonic dystrophy

vii. Porphyrines

viii. Prader-Willi syndrome

ix. Turnerôs syndrome

x. Wolframôs syndrome

xi. Others

5. Type 3 diabetes mellitus (Alzheimerôs diseases)

It is also noteworthy that some researchers are currently considering Alzheimerôs disease as Type 3 diabetes mellitus.

2.3.1 Type 1 *Diabetes mellitus* (T_1DM)

Type 1 *Diabetes mellitus* results from the bodyôs failure to produce insulin and presently requires the patient to inject insulin for survival, thus insulin dependent *Diabetes mellitus* (IDDM). Type 1 *Diabetes mellitus* is characterized by loss of the insulin-producing beta cells of the islets of langerhans in the pancreas leading to insulin deficiency. The loss of beta cells could be immune-mediated or idiopathic. The majority of type 1 diabetes is of auto-immune-mediated nature in which beta cell loss is a T- cell-mediated autoimmune attack (Rother, 2007). There is no known preventive measure against type 1 diabetes which causes approximately 10% of *Diabetes mellitus* cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed juvenile diabetes because a majority of these diabetes cases were in children (Humphray *et al.,* 1998).

2.3.1.1 Autoimmune type 1 *Diabetes mellitus*

It results from auto-immune mediated destruction of the beta cells of the pancreas. The rate of destruction varies being rapid in some individuals and slow in other (Zimmet *et al.,* 1994). The rapidly progressive form is commonly observed in children but may also occur in adults (Humphray *et al.,* 1998). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Individuals with this form of type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis (Willis *et al.,* 1996). At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C- peptide (Hother-Nielsen *et al.*, 1988). The peak incidence of this form of type 1 diabetes occurs in childhood and

adolescence but the onset may occur at any age, ranging from childhood to the ninth decade of life (Molbak *et al.,* 1994). There is genetic predisposition to autoimmune destruction of beta cells, and it is also related to environmental factors that are still poorly defined. Although patients are usually not obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients may also have other auto-immune disorders such as Gravesô disease, Hashimotoôs thyroiditis and Addisonôs disease (Betterle *et al.,* 1983).

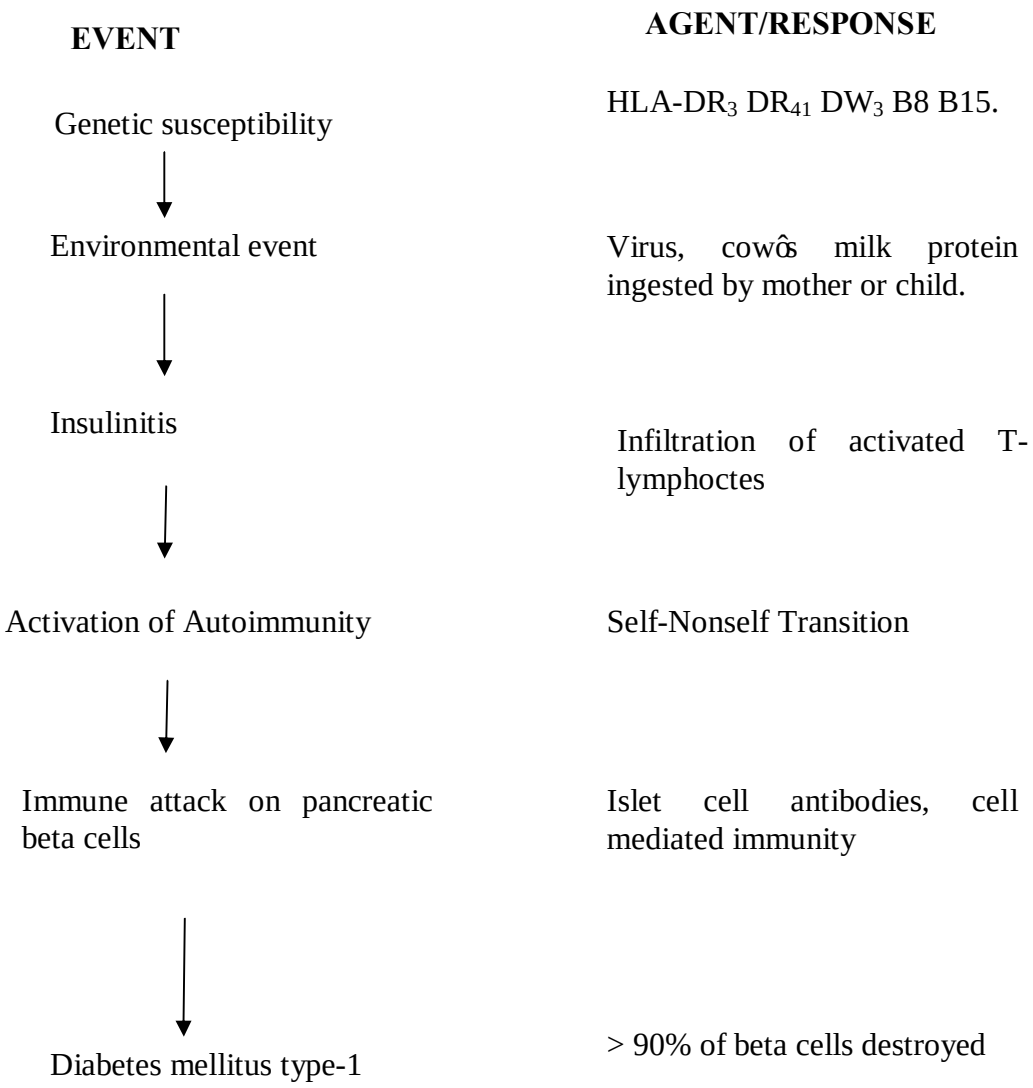
2.3.1.2 Idiopatic type 1 *Diabetes mellitus*

This is a form of type 1 diabetes mellitus which has no known aetiology. Some of these patients have permanent insulinopenia and are susceptible to keto-acidosis but have no evidence of autoimmunity (Mclarty *et al.,* 1990). This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans, an absolute requirement for insulin replacement therapy in affected patients may come and go and patients periodically develop ketoacidosis (Ahren and Corrigan, 1984).

2.3.1.3 Pathogenesis of type1 DM

Its transmission is believed to be an autosomal dominant, recessive or mixed chromosome, although no mechanism is proven. Researchers believe that onset of type 1 seems to be linked with an environmental insult, an allergen such as cowôs milk or a virus that initiate this process in genetically susceptible individual. There are certain viruses that seem to destroy the pancreatic beta cells directly rather than through an autoimmune reaction (Mijac *et al.,* 1997). Researchers have also found a significant correlation between antibodies to cowôs milk protein especially to bovine serum albulmin in the onset of IDDM (Karjalainen *et al.,* 1992). Cowôs milk seems to be strongly linked to the onset of type 1.

Figure 1: Diagrammatic representation of the pathogenesis of type 1 DM



Source: Harrison's principle of internal medicine

2.3.2 Type 2 *Diabletes mellitus*

Type 2 *Diabetes mellitus* is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Shoback, 2011**)** due to defection in insulin receptor. *Diabetes mellitus* of this type previously included non-insulin dependent diabetes or adult onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. Individuals who have this type of diabetes are frequently resistant to the action of insulin (Lillioja *et al.,* 1993). The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (Bogardus *et al.,* 1985). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah *et al.,* 1982). Ketoacidosis is infrequent in this type of diabetes, when seen it usually arises in association with the stress of another illness such as infection (Banerji *et al.,* 1994**)** whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels in these diabetes patients would be expected to result in even higher insulin values had their beta-cell function been normal (Polonsky *et al.,* 1996). Thus, insulin secretion is defective and insufficient to compensate for the insulin resistance. On the other hand some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and or pharmacological treatment of hyperglycemia but is not restored to normal (Wing *et al.,* 1994).

2.3.2.1 Pathogenesis of type 2 Dm

The main cause of insulin resistance is a lack of communication between the insulin, a chemical messenger and the receivers of the signal, a significant amount of which are called GLUT-4 transporters. Glut-4 transporters are proteins within the cell that rise to the cellôs

membrane, take hold of the glucose and bring it inside the cell. Insulin resistance implies that cells do not receive the insulin signaling message clearly and do not respond appropriately. Consequently, blood sugar levels remain high, causing pancreatic beta cells to pump out more insulin to ñknockò louder on the cell walls. At the onset of the disease process, the glucose is let in, even with insulin resistance. This is referred to as compensated insulin resistance as the pancreas has put out more insulin and glucose level stabilizes for a while. Eventually, over time, the beta cells of the pancreas are either inflamed or worn out. About 30 percent of the people with type 2 diabetes do inject insulin daily because their insulin-producing cells have become destroyed (Cousen, 2008). These type 2 (NIDDM) diabetics become insulin-dependent diabetes (IDDM).

2.3.3 Gestational *Diabetes mellitus* (GDM)

Gestational *Diabetes mellitus* (GDM) is third major category of diabetes. Gestational diabetes is carbohydrate intolerance resulting in hyperglycemias of variable severity with onset or first recognition during pregnancy. Gestational *Diabetes mellitus* resembles type 2 diabetes in several respects involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2-5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20-50% of affected women develop type 2 diabetes later in life. In fact, the rate of diabetes in expectant mothers has more than doubled in the past six years (Lawrence *et al.,* 2008). This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential for the children of diabetic mothers to become diabetic in the future.

2.3.3.1 Pathogenesis of GDM

This is as a result of the metabolic changes that occur during a normal pregnancy to conserve sugar for the foetus. The motherôs placenta produces hormones that naturally increase insulin resistance, thus redirecting some of the sugar to her fetus that before pregnancy would have gone to her cells. Early in pregnancy maternal estrogen and progesterone increase and help in pancreatic beta cell type hyperplasias and increased insulin production (Kuhl and Holst, 1976). This rise in insulin enhances peripheral glucose utilization and glycogen storage and lower glucose levels, but this shifts as the pregnancy progresses. Cortisol which has the highest diabetic creating potency peaks at 26 weeks. Progesterone with anti-insulin qualities peaks at 32 weeks. These two periods 26 and 32 weeks, encompasses an important time during which the pancreas releases 1.5 to 2.5 times more insulin to respond to the resistance (Freinkel, 1980).

2.3.3.2 Table 1: The diabetogenic potency of hormones in pregnancy

Hormone	1 peak elevation	Diabetigenic potency
Prolaction	10	Weak
Estradiol	26	Very weak
HCG	16	Moderate
Cortisol	26	Very strong
Progesterone	32	Strong

(Cousens, 2008)

2.3.4 Other specific types

2.3.4.1 Genetic defects of beta-cell function

These defects are usually autosomal in inheritance. Maturity onset diabetes of the young (MODY) is one form that is usually characterized by impaired insulin secretion with minimal or no defect in insulin action (Clement *et al.,* 1996).

The most common form is associated with mutations on chromosome 12 in a hepatic nuclear transcription factor referred to as HNF_1 (Yamagata, *et al.,* 1996). A second form is associated with mutations of the glucokinase gene on chromosome 71 (Froguel *et al.,* 1992). Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which in turn stimulates insulin secretion by the beta cell. This glucokinase serves as the ñglucose sensorô for the beta cell. Because of the defect in glucokinase gene, increased levels of glucose are necessary to elicit normal level of insulin secretion. A third form is associated with a mutation in the $HNF_{4,}$ (a transcription factor which is involved in the regulation of the expression of HNF_1 gene) on chromosome 20_q (Yamagata *et al.,* 1996). Fourth variant has recently been ascribed to mutation in another transcription factor gene, IPF-1 which in its homozygous form leads to total pancreatic agenesis (Stoffers *et al*., 1997). Specific genetic defects in other individuals who have a similar clinical presentation are currently being defined. Point mutations in mitochrondria DNA have been found to be associated with diabetes mellitus and deafness (Walker and Turnbull, 1997). The most common mutation occurs at position 3243 in the tRNA leucine gene leading to an A to G substitution. An identical lesion occurs in the MELA syndrome (Mitochondria myopathy, encephalopathy, lactic acidosis and stroke-like syndrome), however, diabetes is not part of this syndrome, suggesting that there are other reasons different from phenotypic expression of the genetic lesion (Johns, 1995).

Genetic abnormalities that result in the inability to convert pro insulin to insulin have been identified in a few families. Such traits are usually inherited in an autosomal pattern (Robbins *et al.,* 1984) and the resultant carbohydrate intolerance is mild. Similarly mutant insulin molecules with impaired receptor binding have been identified in a few families. These are also associated with autosomal inheritance and either normal or only mildly impaired carbohydrate metabolism (Haneda *et al.*, 1984).

2.3.4.2 Genetic defects in insulin action

There are some unusual causes of diabetes which result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinaemia and modest hyperglycaemia to symptomatic diabetes (Taylor, 1992). Some individuals with these mutations have Acanthosis nigricans. Women may have virilization and have enlarged cystic ovaries. In the past, this syndrome was termed type A insulin resistance (Kahn *et al.,* 1976) Leprechaunism and Rabsom-mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance (Taylor, 1992).

2.3.4.3 Disease of the exocrine pancreas

Any process that generally injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatic carcinoma and pancreatectomy (Larsens *et al.,* 1987). With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in beta cell mass

(Permert *et al.,* 1994). If extensive enough, cystic fibrosis and haemochromatosis will also damage beta cells and impair insulin secretion (Moran *et al*., 1994**).**

2.3.4.4 Endocrinopathies

Some hormones (e.g growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases such as Acromegaly, Cushingôs syndrome, Glucagonoma and Phaeochromoly associated with excess secretion of these hormones can cause diabetes (McFarlane, 1997). These forms of hyperglycecmia typically resolve when the hormone excess is removed.

Somatostatinoma and aldosteronoma-induced hypokalemia can cause diabetes at least in part by inhibiting insulin secretion (Krejs *et al.,* **1**979).

2.3.4.5 Drug or chemical-induced DM

The drugs that can impair insulin secretion may not by themselves cause diabetes but they may precipitate diabetes in individuals with insulin resistance (Pandit *et al.,* 1993). In such cases, the classification is ambiguous as the primacy of beta cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poision) and pentamidine can permanently destroy pancreatic beta cells (Esposti *et al.,* 1996). There are also many drugs and hormones which can impair insulin action. Examples include nicotinic acid and glucocorticoids (Yajnik *et al.,* 1992).

2.3.4.6 Infection

Some viruses have been associated with beta cell destruction. Diabetes occurs in some patients with congenital rubella (Forrest *et al.,* 1971). In addition, coxasackie B, Cytomegalovirus and other viruses (eg adeno virus and mumps) have been implicated in inducing the disease (Pak *et al.,* 1988)**.**

Uncommon but specific forms of immune-mediated *Diabetes mellitus*

Diabetes may be associated with several immunological diseases with aetiology different from that which leads to the type 1 diabetes process. Postprandial hyperglycaemia of a severity sufficient to fulfill the criteria for diabetes has been reported in rare individuals who spontenously develop insulin auto antibodies (Bodansky *et al.*, 1986). However these individuals generally present with symptoms of hypoglycaemia rather than hyperglycaemia. The ñstiff manò syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms (Solimena and Decamilli, 1991). Affected people usually have high titres of the GAD auto antibodies and approximately one half will develop diabetes. Patients receiving interferon alpha have been reported to develop diabetes associated with islet cell auto antibodies and in certain instances, severe insulin deficiency (Fabris *et al.*, 1992).

Anti-insulin receptor antibodies can precipitate diabetes by binding to the insulin receptor, thereby reducing the binding of insulin to target tissues (Flier, 1992). However, these antibodies also can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycaemia (Kahn *et al.,* 1977). Anti-insulin receptor antibodies are occasionally found in patients with systemic hispus erythematous and other autoimmune diseases (Tsokos *et al.,* 1985).

2.3.4.7 Other genetic syndromes sometimes associated with diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Downôs syndrome, Klinefelters syndrome and Turnerôs syndrome. Wolfranôs syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of beta cells at autopsy (Barret *et al.,* 1995). Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy and neural deafness.

2.3.5 Type-3 *Diabetes mellitus* (Alzheimer's disease)

Alzheimerôs disease is a neurological disorder characterized by profound memory loss and progressive cognitive and behavioural decline due to selective loss or dysfunction of neurons in specific brain regions/neural circuits, including the neocortex, hippocampus, and basal forebrain (Mattson, 2004). Alzheimerôs disease is currently being associated with diabetes, obesity and heart disease. Some researches refer to the disease as type 3 DM (Rivera *et al.,* 2005). A variety of studies have shown that individuals with type-2 diabetes have greater chances of developing Alzheimerôs. The current theories implicate poor brain circulation caused by diabetes as a primary factor as well as insulin resistance in the areas of the brain responsible for memory and recognition, such as the limbic system, hippocampal, cortical and precortical areas. New research linking diabetes and Alzheimerôs suggest that the high blood sugar of diabetes can lead to the formation of advanced glycation end products (AGEs) (Takeuchi *et al.*, 2004).

2.4 Clinical signs of DM

The classic signs and symptoms of *Diabetes mellitus* are the three polysô---------polyuria (excessive urination), polydipsia (excessive thirst) and polyphagia (excessive hunger).

The kidneys cannot absorb the ever-increasing glucose, so the excess is excreted in the urine. The brain is prompted by this loss of fluid signals, thirst and hunger. If this process continues, stored fats are metabolized which leads to lower pH levels and acidosis. The drop in PH levels and loss of ketones in urine signals the onset of ketoacidosis. Intracellular potassium ions are exchanged for hydrogen along with sodium, magnesium and phosphorus. Blood volume drops increasing haematocrit, haemoglobin and white blood cell counts. Respiratory

compensation results in laboured, deep respiration (Kussmaul respiration) in an attempt to lower the partial pressure of carbon dioxide (PCo_2) values. This process is acute but may extend over several days. Keto acidosis is a likely presenting symptom in an initial type 1 diagnosis (ADA, 1997).

In addition to the classic signs of diabetes listed above, type 1 diabetes presents the following signs and symptoms.

- Weakness and fatigue
- Drowsiness.
- Irritability
- Blurred vision or any change in sight
- Nausea and vomiting
- Sudden unexplained weight loss (Cook and Plotnic, 2008)

In type 2 *Diabetes mellitus*, the symptoms are usually insidious (develops slowly) and also include the following:

- Polyuria, polydipsia, polyphagia, weakness and fatigue, drowsiness, irritability, blurred vision, tingling or numbness in legs, feet or finger, slow healing of cuts (especially on feet), frequent skin or vaginal infections or itchy skin (Cook and Plotnick, 2008).

Signs and symptoms of diabetic ketoacidosis found in type-1 include: hyperglycaemia, Glycosuria, weight loss, nausea and vomiting, deep labored respiration, fatigue and abdominal pain (Bruno *et al*., 1990).

In dogs (with usually type 1 DM), the common signs include polydipsia, polyuria, polyphagia with weight loss, bilateral cataracts and weakness. The renal threshold for glucose is 180 mg/dl in dogs and 240 mg/dl in cats. Cataract formation in dogs is related to the unique

sorbitol pathway in which glucose is metabolized in the lens which leads to edema of lens and disruption of normal light transmission (Aiello *et al.,* 1998).

In cats, (with usually type 2 DM), the first obvious symptoms are sudden weight loss or gain accompanied by excessive drinking and urination; for example, cats can appear to develop an obsession with water and lurk around faulets or water bowls. Appetite is either ravenous or absent. In cats, the back legs may become stilted wobbly because of peripheral neuropathy. Lethargy and limpness are acute symptoms indicating likely ketoacidosis and or dehydration (Rand and Marshal, 2005).

Generally speaking, diabetic animals have decreased resistance to bacterial and fungal recurrent infections such as cystitis, prostitis bronchopneumonia and dermatitis (Rand and Marshal, 2005).

2.5 Complications of *Diabetes mellitus*

2.5.1 Acute complication

Acute complications of diabetes mellitus include diabetic ketoacidosis (DKA), hyperglycemic hyperosmolar state, respiratory infections, hypoglycemia or hyperglycemia.

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. Low insulin levels cause the liver to turn to fat for fuel leading to production of ketone bodies (ketosis). Elevated levels of ketone bodies in the blood decrease the blood pH leading to DKA. Common signs of DKA include abdominal pains, lethargy, coma, hypotension, shock and death. Urine analysis will reveal significant levels of ketone bodies (which appear in the urine after exceeding its renal threshold) (Aiello *et al.*, 1998). Therapy include correcting dehydration, by administration of IV fluids such as 0.09% NaCl or lactated Ringers solution; reducing hyperglycemia and ketosis by administration of crystalline Zinc (regular) insulin,

maintaining serum electrolyte levels especially potassium; insulin regimen such as regular insulin at 0.2 mg/kg IM in the initial dose, followed by hourly administration of 0.1mg/kg (Kahn, 2005).

DKA may be the presenting symptom in individuals previously undiagnosed with type-1 DM and it is likely seen in type 1 diabetics if they have not received enough insulin during times of illness. Type-2 diabetes may experience DKA if they are very ill and not able to eat sufficient calories. Other factors include overeating and physical inactivity. DKA usually occurs when blood glucose levels are over 240 mg/dl. Mortality rates range from less than 5% to 14% (Cherry-pepper *et al*., 1992).

Hyperglycemic hyperosmolar syndrome (HHS) accounts for 5-15% of hospital admission for diabetes coma (Patterson, 2005). Mortality rates are reported as high as 50% (Cherry-pepper, *et al.*, 1992). This disorder resembles DKA (dehydration, altered mental status) although blood glucose levels are much higher (up to 2000 mg/dl), Kussmaul respiration is rare and there is absence of ketosis. There is a reduction in the rate of glomerular filteration and glucose excretion (Patterson, 2005). Hyperglycemic hyperosmolar syndrome occurs mostly in diagnosed and undiagnosed type-2 diabetics over the age of 60. Conditions or events that precipitate HHS include: History of type 2 diabetes, chronic illness, acute illness (eg stroke, myocardial infarction), mild renal insufficiency, lack of normal thirst drive or access to water, drugs (diuretic, glucocorticoids) surgery, dialysis, (Cherry-pepper *et al*., 1992). Individuals may exhibit central nervous system distress (e.g hallucinations, focal or grand mal seizures). Treatment is similar to that of DKA and consists of intravenous administration of fluids, electrolytes and insulin. The result of the treatment may make the patient sensitive to further insulin. Glucose control can be attained through a combination of diet, exercise and oral hypoglycemic agents or

insulin. Patient education should include an understanding of the warning signs of onset of HHS and glucose monitoring techniques (Rees, 1994).

Hyperglycemia is another diabetic emergency. If left unchecked, chronic hyperglycemia can result in diabetic ketoacidosis or hyperglycemic hyperosmolar nonketotic coma. These conditions develop over days but will develop more quickly if the individual is concurrently suffering an illness or infection (Cherry-Pepper, *et al.,* 1992).

The most common acute diabetic emergency in the dental office is hypoglycemia (FDA, 2010). Most diabetics begin to have symptoms when the blood glucose level falls below 70 mg/dl. Persons with long standing type 1 diabetes may have decreased ability to sense impending hypoglycemia (Oliver and Tellervo, 1993). Symptoms of hypoglycemia may include: headache, hunger, moist skin, pallor, weakness, dizziness, anxiety and confusion. If left untreated, these symptoms may progress to severe hypoglycemia, loss of consciousness leading to seizures and possible death (Cherry-Pepper- *et al.,* 1992). In the condition of hypoglycemia, individuals should consume 10-15 g of rapidly absorbable carbohydrate such as 3 glucose tablets, fruit juice, 5-7 hard candies, milk, 1 tablespoon of sugar or sugar cube, regular soft drink (not diet), cake icing.

A respiratory infection is another acute complication. The immune response is impaired in individuals with *Diabetes mellitus*. Cellular studies have shown that hyperglycemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function, which leads to an increase in susceptibility for respiratory infections such as pneumonia and influenza among individuals with diabetes. Several studies also show diabetes associated with a worse disease course and slower recovery from respiratory infections (Ahmed *et al.,* 2008).

2.5.2 Chronic complication

The underlying cause of chronic complications in both type-1 and type-2 diabetes are consequent upon two main metabolic problems: the glycosylated proteins/lipids and the intracellular accumulation of sorbitol.

For example glycosylated low-density lipoproteins (LDL) do not bind to LDL receptors and are therefore not capable of blocking off the endogenous cholesterol synthesis. The result is increased tendency of cholesterol accumulation. Sorbitol accumulation inside the cells is another major serious metabolic problem. In a normal physioiology, once the sorbitol is produced, it is metabolized in polyol pathway by polyol dehydrogenase to fructose. This conversion to fructose allows it to be excreted from the cell. In the diabetics with excessive blood sugars, the sorbitol accumulates and plays a major role in the development of secondary complications.

Diabetic nephropathy

Diabetic nephropathy is a chronic complication of both type-1 DM and type-2 DM. There are five stages in the development of diabetic nephropathy.

Diabetic nephropathy is a common cause of high rates of dialysis and death. Once people begin kidney dialysis, the diabetic process affects the kidneys in a few ways: glomerulosclerosis, and arteriosclerosis of the entering and leaving renal arteries; arteriosclerosis of the renal artery and its internal branches; and deposits of glycogen, fat and glycopolysaccharides around the tubules. Early on, nephropathy has no symptoms, but as it advances we see edema (swelling, particularly around the eye), nausea, fatigue, headache, and generalized itching (Cousens, 2013).

Diabetic cardiomyopathy

Diabetic cardiomyopathy refers to a disease process which affects the myocardium in diabetic patients causing a wide range of structural abnormalities eventually leading to left

ventricular hypertrophy and diastolic and systolic dysfunction or a combination of these. The concept of diabetic cardiomyopathy is based upon the idea that diabetes is that factor which leads to changes of the cellular levels leading to structural abnormalities (Factor *et al*., 1981).

Molecular basis for diabetic cardiomyopathy can be explained by the interaction among hyperglycemic, hyperlipidemic and increased ROS (reactive oxygen species) which induce alterations in down-stream transcription factors that result in changes in gene expression, myocardial substrate utilization, myocyte growth, endothelial function and myocardial compliance (Zhou *et al.*, 2001).

Diabetic encephalopathy

The brain makes use of glucose as the main fuel to generate energy, mainly by oxidative metabolism. However, a prolonged increase in blood glucose levels, even in the absence of *Diabetes mellitus* symptoms will eventually lead to brain damage (Rosseti *et al.*, 1990). Thus hyperglycaemia-induced neurotoxicity has been implicated as one of the main causes of diabetic encephalopathy (McCall, 2004). The advanced glycation end products (AGEs) AGE receptor (RAGE) axis and the polyol pathways may represent molecular mechanisms of glucose neurotoxicity (Browlee, 2000).

Diabetic neuropathy

Diabetic neuropathies are family of nerve disorders caused by diabetes. People with diabetes can over time, develop nerve damage throughout the body such as pain, tingling or numbness in hands, arms, feet and legs, wasting of the muscles, indigestion, nausea or vomiting, diarrhoea, constipation, dizziness due to a drop in blood pressure after standing or sitting up, problems with urination, erectile dysfunction in men or vaginal dryness in woman, weakness etc.

Diabetic neuropathies could be peripherial neuropathy, autonomic neuropathy, proximal neuropathy or focal neuropathy.

Diabetic neuropathy is associated with decreased sensory and nerve conduction velocities. Diabetic neuropathy also seems to be associated with sorbitol accumulation. Sorbitol accumulation leads to myoinosotol loss. Inositol helps create healthy nerve conduction. Typically, this peripheral neuropathy is associated with parenthesis, hyperesthesias and pain. On the neurological exam, almost every diabetic has some level of poor vibratoy sense, altered pain and temperature sense and poor deep tendon reflexes (Wyngaarden *et al*., 1992**)**

Diabetic foot complications are the most common cause of non trauma-based lower extremity amputations in the industrialized world. Neuropathy, a major etiologic component of most diabetic ulcerations, is present in more than 82 percent of diabetic patients with foot wounds (Pecoraro *et al*.,1990).

Diabetic retinopathy

Diabetic retinopathy is the leading cause of blindness in diabetics. In diabetic retinopathy, the retinal vessels in the eye weaken and develop micro aneurisms that leak blood plasma out of the capillaries. This results in scarring in the eye, which leads to gradual blindness. It is related to glycosylated hemoglobin and seems to increase when the glycosylated hemoglobin goes above 6%.

In diabetic retinopathy, the pericytes of the retinal capillaries are injured, which is associated with defective capillary function (Saphieha *et al.,* 2010). Such capillary deficiency is associated with defects in proper oxygen delivery and nutrient supply resulting in vascular endothelial growth factor (VEGF) over production in the retina (Kermorvant-Duchemin *et. al.*, 2010). This VEGF over production is also associated with abnormal angiogenesis and enhanced

retinal capillary permeability, resulting in retinal dysfunction associated with the loss of visual acuity in these patients (Saphieha *et al.*, 2010).

Dental disease

Periodontal (gum) disease is more common among people with diabetes. Among young adults, those with diabetes have about twice the risk of those without diabetes. Almost one-third of people with diabetes have severe periodontal disease with loss of attachment of gums to the teeth measuring 5 millimeters or more. Improving your dental health can also help with glycemic control (Ross-Flanigan, 1999).

Diabetic cataract

During hyperglycemia, extracellular glucose diffuse into the lens, this can lead to the post-translational modification. Cataract progresses from the production and accumulation of excessive sorbitol in the lens fibre and consequent osmotic stress. Sorbitol which is synthesized from aldose reductase utilizing the NADPH does not cross the cell membranes; it can accumulate in the cells and can cause cell damage due to disturbing osmotic homeostasis (Gupta *et al*., 2009). Reduction in concentration of glutathione, antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase with increase in age were the main factor involving the generation of cataract (Graw, 2009).

Diabetic angiopathy

Vascular diseases such as damage of blood vessels (angiopathy) are consequent upon chronic elevation of blood glucose levels. The endothelial cells lining the blood vessels take in more glucose than normal, since they do not depend on insulin. This makes them to form more surface glycoprotein than normal and cause the basement membrane to grow thicker and weaker. Non-diabetic off-spring of type 2 diabetics has been found to have increased arterial stiffness and

neuropathy despite normal blood glucose levels (associated with diabetic renal disease) (Ban and Twigg, 2008).

The damage to small blood vessels (microangropathy) has been incriminated in the pathogenesis of diabetic neuropathy, nephropathy, encephalopathy, retinopathy and cardiomyopathies as discussed earlier.

2.6 Diagnosis of DM

Diabetes mellitus can be diagnosed using the following:

1. Clinical signs

2. Fasting blood sugar

3. Oral glucose tolerance test

4. Glycated haemoglobin HbA_{1c}

5. Insulin sensitivity test

6. Insulin assay

7. Urinary sugar

For individuals with symptoms of diabetes such as excessive thirst and urination or unexplained weight loss, only elevated fasting plasma glucose (Ó140 mg/dl) or random plasma glucose Ó200 mg/dl is required to confirm the diagnosis.

In 1997, the first expert committee on the diagnosis and classification of *Diabetes mellitus* revised the diagnostic criteria between Fasting Plasma Glucose (FPG) levels and presence of retinopathy as the key factor with which to identify threshold glucose levels. The committee examined data from three cross-sectional epidemiological studies that assessed retinopathy with fundus photography or direct ophthalmoscope and glycemia as FPG, 2-h plasma

glucose (PG), and glycosylated haemoglobin (HbA_{1c}). These studies demonstrated glycemic levels below which there was little prevalent retinopathy and above which the prevalence of retinopathy increased in an apparently linear fashion. The deciles of the three measures at which retinopathy began to increase were the same for each measure within each population. Moreover, the glycemic values above which retinopathy increased were similar among the populations. These analyses helped to inform a new diagnosis cut point Ó126 mg/dl (7.0 mmol/l) for FPG and confirmed the long-standing diagnostic 2-h plasma glucose (PG) value of Ó200mg/dl (11.1mmol/l) (IEC, 2009).

Glycosylated haemoglobin (HbA_{1c}) is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a two to three month period of time. The test plays a critical role in the management of the patient with diabetes since it correlates well with both microvascular and to a lesser extent, macrovascular complication and it is widely used as standard biomarker for the adequacy of glycemic management (IEC, 2009). An international expert committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the HbA_{1c} test to diagnose diabetes with a threshold of ≥ 6.5% and American diabetes Association affirms this. The diagnostic HbA_{1c} cut point of 6.5% is associated with an inflection point for retinopathy prevalence, as well as the diagnostic thresholds for FPG and 2-h plasma glucose (IEC, 2009).

2.6.1 Formation of glycosylated haemoglobin (HbA_{1c})

Glycosylated haemoglobin is the result of simple chemical reaction between haemoglobin and sugars after synthesis of hemoglobin is complete that is post-translational modifications (Bunn *et al.*, 1976). The reaction proceeds in 2 stages.

1. Glucose combines with alpha amino group of the valine residual N-terminus of beta (β) globin chains to form aldimine compound (Schiff base). This reaction is reversible and dissociation to native hemoglobin and glucose occurs readily.
2. Internal rearrangement of the aldimine intermediate by the Amadori reaction yields a stable ketoamine derivative.

Early workers confirmed that glycosylation begins during erythropoeiesis and continues slowly through out the life of hemoglobin in the circulation; concentrations reached in the red cell of diabetic subjects are consistent with their known life span of about 120 days (Bunn *et al.,* 1976).

2.6.2 Criteria for diagnosis of diabetes in non pregnant adult

Any one of the following as shown on the table below is considered diagnostic of diabetes.

Table 2: Diagnosing diabetes

Test	Result	Interpretation
HbA1c	6.5% or higher	Diabetes
	5.7-6.4%	Impaired glucose tolerance
	Lower than 5.7%	Normal
Random plasma	200 mg/dl or higher	Diabetes
	140-199mg/dl	Impaired glucose tolerance
	Lower than 140 mg/L	Normal
Fasting plasma	126 mg/dl or higher	Diabetes
Glucose	100-125 mg/dl	Impaired glucose tolerance
	Lower than 100mg/dl	Normal

(Amantel *et al*., 2013)

Table 3: Values for diagnosis of *Diabetes mellitus* and other categories of the hyperglycemic glucose concentrations (mMol/L (mg/dl))

Diabetes mellitus	Whole venous	Blood capillary	Plasma venous
Fasting or 2-hour post glucose load	Ó6.1 (Ó110) Ó10 (Ó180)	Ó6.1 (Ó110) Ó11.1 (Ó200)	Ó7.0 (Ó126) Ó11.1 (Ó200)
Impairs glucose tolerance: fasting (4 measured) and 2-hour post glucose load	>6.1 (>110) Ó6.7 (Ó120) and >100 (>180)	> 6.1 (> 110) Ó7.8 (Ó140) and >11.1 (<200)	>7.0 (>126) Ó7.8 (Ó140) and <11.1 (<200)
Impaired fasting glycaemia fasting and (if measured 2-hour doit glucose load	Ó5.6 (Ó110) and 6.1 (<110 < 6.7 (<120)	Ó5.6 (Ó100)and < 6.1 (<110) < 7.8 (<140)	Ó6.1 (Ó110) and <7.0 (126) <7.8 (<140)

(Amantel *et al*., 2013)

In 1997 and 2003, the expert committee on diagnosis and classification of *Diabetes mellitus* (Genuth *et al.,* 2003) recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal. These people were defined as having impaired fasting glucose (IFG) FPG level of 100 mg/dl (5.6mmol/L) to 199mg/dl 11.0 mmol/L).

Individuals with IFG and or IGT have been referred to as having pre-diabetes, indicating the relatively high risk for the future development of diabetes. IFG and IGT can be observed as intermediate stages in some disease process. They are associated with obesity (especially abdominal or visceral obesity), dyslipidemia with high triglycerides and or low HDL-cholesterol and hypertension. Structure life style intervention, aimed at increasing physical activity and producing 5-7 % loss of body weight and certain pharmacological agents have been demonstrated to present or delay the development of diabetes in people with IGT, the potential impact of such interventions to reduce mortality or the incidence of cardiovascular disease has not been demonstrated to date.

2.6.3 The oral glucose tolerance test (OGTT)

In symptomatic individuals with random plasma glucose value >200 mg/dl, the OGTT is not required for a diagnosis of diabetes. However, in asymptomatic individual and to establish a diagnosis of IGT, the OGTT is necessary (WHO, 1980; WHO, 1985). The test should be performed in the morning on subjects who have had at least 3 days of unrestricted diet. The subject should have fasted overnight for 10-16 hours and remain seated and not smoke throughout the test. A fasting blood sample should be collected, after which the subjects should drink 75 g of glucose in a concentration no greater than 25 g per 100 ml. The OGTT is the

internationally recognized standard for diagnosing a symptomatic NIDDM (WHO, 1980**;** WHO 1985)**.**

Fasting plasma glucose (FPG)

In the U.S population, there is a broad distribution of FPG among adults with undiagnosed NIDDM and only approximately 26 percent of people age 20-74 years with undiagnosed NIDDM have fasting hyperglycemia (Ó140 mg/dl) (Harris *et al.,* 1985). Other studies have also found that as many as 80% of diabetes cases discovered in population screening by OGTT have EPG < 140 mg/dl (Clement *et al.*, 1993).

Sensitivity is somewhat lower in women compared with men, but there is little effect of age or ethnicity. Body mass index (BMI) < 23 is associated with considerably lower sensitivity with no difference between the two higher BMI categories (23-26.9 and Ó 27). However individuals with BMI < 23 constitute only about 10 % of all NIDDM cases. Hypertension, treated or untreated, has no consistent effect on sensitivity. In summary variations in sensitivity by age, sex, ethnic group, BMI or blood pressure status appear to be too small to have practice implications regarding the effectiveness of screening by FPG (Modan and Harris, 1994).

Screening by other methods

Other methods of screening for undiagnosed NIDDM has been evaluated and found to be inadequate (Harris and Modan, 1994**).** Glycosylated haemoglobin has the same advantage as FPG requiring only one blood sample and minimal patient cooperation, and in addition is not affected by time of day or recent food intake. Measurement of casual or random blood glucose or urine glucose are not acceptable screening methods because these cannot be standardized with regards to risk of having diabetes or developing its complications, due to considerable

fluctuations of blood and urine glucose level according to the interval since the preceding meal, the unstandardized content of the meal, and the often unknown renal threshold for glycosuria.

2.7 Management and treatment of *Diabetes mellitus*

2.7.1 Pharmacologic interventions

2.7.1.1 Use of oral hypoglycemic/ antidiabetic drugs

Diabetes mellitus is a chronic disease that requires life-long pharmacological and non-pharmacological management to prevent complications such as cardiovascular disease, retinopathy, nephropathy and neuropathy (Qaseem, 2012**).** In the United States of America, eleven classes of medications are approved for management of *Diabetes mellitus*, these include eight oral agents such as-biguanides, sulfonylureaôs, meglitinides, thiazolidinediones (glitazones), alpha glucosidase inhibitors, DPP-4 inhibitors, bile acid sequestrants, dopamine-2 agonist and 3 injectable agents such as-GLP-1 receptor agonists (Incretins), amylin analogues and insulin (Alexander, 2008**).** The 18th WHO expert committee on the selection and use of essential medicines in 2011 requested a review of the current oral hypoglycemic medicines for use in the adult to determine if updates to the EML are needed (WHO, 2011). Currently the EML contains two oral hypoglycemics, glibenclamide (sulfonylurea) and metformin.

2.7.1.1.1 Sulfonylurea

The hypoglycemic effect of sulfonylureas was first discovered in France during World War II as a chance finding in the course of investigations concerning the antibiotic properties of modified sulfonamides.

Since the early 1950s sulfonylureas, mainly tolbulamide and chlorpropamide have been used extensively in the treatment of diabetes. The incidence of adverse reactions is low, with a total rate for all side effects estimated at 3.2 percent for tolbutamide and 6 percent for

chlorpropamide (Shen and Bressler, **1977**). The most frequently described significant side effects are haematologic (agranulocytosis, bone marrow aplasia, red cell aplasia) and gastrointestinal (nausea, vomiting, heart burn, abnormal liver function tests, Jaudice). Other described effects include cutaneous reactions (rashes, pruritis), vasomotor effects (disulfiram-like reaction to alcohol-most commonly seen with chlorpropamide) possible hypothroidism, and dilutional hyponatremia with water intoxication (due to the anti-diuretic action of chlorpropamide and perhaps tolbutamide) (Pannekoek, 1975).

Mechanism of actions of sulfonylurea drugs

During the late 1950s and early 1960s most studies seemed to indicate that the primary action was an increase in insulin release. More recently, however, a large body of clinical investigative data has caused serious doubts on the significance of the role that insulin-secretary effects of sulfonylureas play in their chronic anti-diabetic action.

Sulfonylureas and glucagon secretion

A decrease in glucagon secretion by the sulfonylureas could explain their anti-diabetic action. Early studies in which sulfonylureas failed to lower the blood glucose in alloxan-diabetic animals were used as evidence to rule out an effect of these agents on glucagon secretion. More recent studies, both *in vivo* and *in vitro,* have evaluated the effects of sulfonylureas on glucagon secretion by measurement of glucagon with radioimmunology assay techniques. While studies with duck pancreas have shown sulfonylurea-induced suppression of glucagon secretion (Salmols *et al.,* 1969), those with rat pancreas show either stimulation or inhibition, depending on the experimental conditions (Grodsky *et al.*, 1977). There is no good evidence that acute or chronic sulfonylurea therapy alters glucagon secretion in normal subjects or patients with

diabetes mellitus (Peks *et al.,* 1972). Thus, it is unlikely that the anti-diabetic action of sulfonylureas is related to an alteration in glucagon secretion.

New sulfonylurea (third generation)

Glimeperide, a third generation sufonylurea binds to a 65kd protein of the putative sulfonylurea receptor different from the 140 kd protein targeted by other sulfonylurea (Kramer *et al.*, 1994). It has a three fold faster rate of association and nine fold faster rate of dissociation than glibenclamide (Kramer *et al.*, 1994) and thus has rapid onset and prolonged duration of action, permitting once daily administration (Draeger, 1995). Though, its initial action is stimulation of insulin secretion, it has also an insulin-mimetic effect in peripheral tissues possibly mediated by GLUT-4 recruitment (Muller and Wied, 1993). The extra pancreatic effects may explain lesser degree of stimulated hyperinsulinaemia. Unlike glibenclamide, this drug prevents post-exercise insulin release, thereby decreasing the risk of hypoglycaemia (Draeger, 1995). It is absorbed completely in either fasting or fed state. It does not accumulate in the body with reducing renal function (Up to GFR 10ml/min) and its hydroxyl metabolite has negligible effects on blood glucose and is excreted equally by the liver and kidney. Hence, it is safe in renal failure and in the elderly (Draeger, 1995). It does not exhibit any drug interaction and because of its poor binding to extrapancreatic, myocardial and vascular system ATP dependent K^+ channels, the risk of coronary vasoconstriction and adverse cardiovascular events are reduced in comparison to other sulfonylureas (Smits and Thieri, 1995). On a weight for weight basis, glimeperide is the most potent sulfonylurea with suggested dose between 1-6 mg once daily but dose up to 8mg may give additional glucose lowering effect (Smits and Thieri, **1995**).

The following Table summarizes the names of first, second and third generation sulfonylurea their structures, dosage range and tablet.

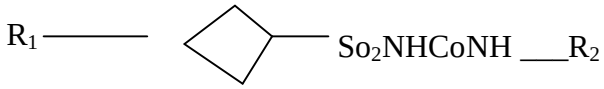
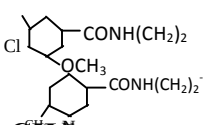
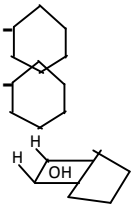


Table 4: First, second and third generation sulfonylureas

First generation

	NAME	R_1	R_2	Dosage Range(mg)	Tablet Size (mg)
1.	Tolbutamide	CH_3-	-$(CH_2)_3CH_3$	500-3000	500
2.	Chlorpropamide	CC-	-$(CH_2)_2CH_3$	100-500	100, 200
3.	Tolazamide	CH_3-	- 	100-750	100, 200
4.	Acetohexamide	CH_3CO-	- 	500,- 1,500 200, 500	

Second generation

5.	**Glibenclemide**			**2.5-20**	**5**
6.	**Glipizide**			**2.5-45**	**5**
7.	**Glibornride**	**CH_3**		**12.5-100**	**12.5**

Third generation

8.	**Glimeperide**			**1-6**	

(Adapted from The Academy of Dental Learning and OSHA Training. www.ada.org/cerp)

2.7.1.1.2 Biguanides

Biguanides were banned in the United States during the 1970ôs because they were linked to lactic acidosis. A safer biguanide, metformin has been released (brand name Glucophage) and the risk of lactic acidosis appears to be minimal. These drugs work by decreasing the amount of glucose release from the liver

Metformin is the first-line drug treatment for type 2 diabetes. According to national and international guidelines, metformin is the recommended first line oral therapy for the treatment of type 2 diabetes (Inzucchi *et al.*, 2012). This is due to several factors including the impressive safety record of the drug, having been in clinical use for over 50 years and the fact that metformin treatment is weight neutral. In addition, there are likely to be other beneficial effects including reduction in cardiovascular disease and mortality compared with non-intensive treatment and a possible reduction in cancer incidence which has been seen in some (Noto *et al.*, 2012) but not in all patients (Van Staa *et al.*, 2012). Chemically, biguanides such as metformin are composed of two guanides groups joined together with the loss of ammonia. Antihyperglycaemic effects have been observed in response to many but not all, guanidine-containing compounds. For metformin, these effects are uniquely dissociated from toxicity.

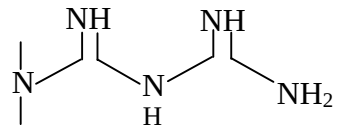
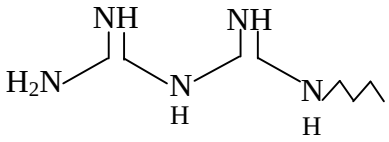
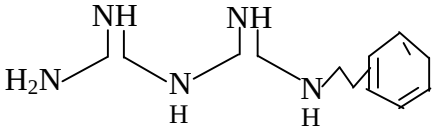
The liver is the main site of action of metformin

Metformin is not metabolized and is excreted in the urine and bile in an unmodified form. The pharmacokinetics of metformin is largely determined by its active transport by key organic cation transporters. Members of this transporter family (Koepsell *et al.*, 2007) are involved in action transport across the gut epithelum and hence determine rates of absorption of plasma membrane monoamine transporter (PMAT) and organic cation transporter (OCT3); they transport metformin into hepatocytes (OCTI) and from hepatocytes into the bile (multidrug and

toxic compound extrusion (MATE) and finally into the renal tubular epithehal cells (OCT2) and into the renal tuble (MATE2) (Rena *et al.*, 2012).

Although metformin exerts its major effects through inhibition of hepatic glucose production, enhanced glucose disposal has also been described. Some early studies suggested that metformin exerts its insulin-sensitizing effect (Bailey and Puah, 1986) and or promotes glucose transport independently of the insulin receptor-mediated proximal signaling pathway in skeletal muscle (Turban *et al.,* 2012). Other biguanides are phenformin and buformin which have been withdrawn from market for their toxic effects.

Table 5: Structures of biguanides

S/No	Name	Chemical name	Chemical structure
1	Metformin	Dimethyl biguanidine	
2	Buformin	A butyl derivative of biguanide	
3	Phenformin	A phenethylated biguanidine	

Source: Tonascia and Meinert, (1986**)**

The most common side effect of biguanide is diarrhoea and dyspepsia, occurring in up to 30% of patients. The most important and serious side effect is lactic acidosis. Metformin is therefore contraindicated in renal insufficiency. Renal functions should be assessed before starting metformin treatment. Phenformin and buformin are more prone to cause acidosis than metfomin, therefore they have been practically replaced by it. However, when metformin is

combined with other drugs (combination therapy) hypoglycemia and other side effects are possible.

2.7.1.1.3 Thiazolidinedione (TZD)

They are also known as glitazones and are a class of medication or drugs used in the treatment of diabetes mellitus type 2. They were introduced in the late 1990s.

Thiazolidinediones are used clinically to reduce blood glucose, triglyceride and free fatty acid levels in animal models of insulin resistance type 2 diabetes and in humans with these conditions (Diamart and Heine, 2003).

Mechanism of action

Thiazolidinediones act by activating PPARs (peroxisome proliferator-activated receptors), a group of nuclear receptors, with greatest specificity for PPARɔ (gamma). The endogenous ligands for these receptors are free fatty acids (FFAs) and eicosanoids. When activated, the receptor binds to DNA in complex with the retionoid X receptor (P XR), another nuclear receptor, increasing transcription of a number of specific genes and decreasing transcription of others (Mangelsdorf *et al.,* 1995). The PPARɔ can be activated by naturally occurring compounds such as the long chain fatty acid derivative 15-deoxy-æ (12, 14)-prostaglandin (Forman *et al*., 1995; Kliewer *et al.,* 1995). In addition to TZDs, the N- 9-fluorenylmethyloxy carbonyl leucine derivative, FMOC- L- Lucine (F-L- Len), is another chemically distinct synthetic ligand of PPARɔ that also shows insulin-sensitizing action *in vivo*. Although some effect of TZDs are PPARɔ, independent, this nuclear receptor is the major functional receptor that mediates the pharmacological actions of these drugs, because their clinical potency correlates closely with their respective capacity to bind to this receptor (Willson *et al*., 1996**).**

Fat redistribution

One result of PPARɔ activation is enhanced differentiation and proliferation of preadipocytes into mature fat cells, particularly in non-visceral (peripheral/subcutenous) fat depots. There is an upregulation of enzymes/transporters in adipocytes to facilitate their uptake of fatty acids (e.g increase in lipoprotein lipase, fatty-acid transporter 1 and glycerol kinase) (Lee *et al.,* 2003). It is notable and probably important, that most of these consequences of PPARɔ stimulation are not seen in visceral adipocytes, even though; these cells have abundant PPARɔ receptors. Visceral adipocytes are also metabolically quite different to peripheral adopocytes in other ways. For example, they are less responsible to insulin and more responsive to catecholamines. Increased fatty acid storage in subcutaneous adipocytes results in a ñLipid stealò phenomenon, leading to lower circulating fatty acids and reduced concentrations of triglycerides in musle and liver (Gurnell *et al.,* 2003). Studies in animal and humans have shown that TZDs only improve insulin action (and glycemic control in diabetes) in the presence of insulin resistance (Oakes *et al.,* 1994). This may be explained by the fact that the effects of these drugs on lipid redistribution are only beneficial if there is excess tissue lipid availability. The ñLipid stealò effect of TZDs may therefore be a major contributor to improved insulin action in muscle (enhanced glucose utilization) and liver (reduced hepatic glucose output), as the direct effects of PPARY stimulation in muscle and liver are unclear. The potential role of the TZDs in reducing hepatic lipid content in non-alcoholic steartohepatitis is still under investigation. The TZDs do not increase insulin. On the contrary, they reduce insulin level acutely, which may be a consequence of improved insulin sensitivity and or reduced circulating fatty acid (as fatty acids stimulate insulin secretion). In the longer term, TZDs arrest the decline in ɓ-cell function that occurs in type2 diabetes perhaps by protecting ɓ-cell from lipotoxicty (Parulkar *et al.,* 2001)

Other biological effects

The effects of the thiazolidinediones on lipid concentrations tend to increase while triglyceride concentrations decrease. Although, LDL cholesterol concentrations may increase initially, this effect lessens over time and particles are now larger and more buoyant (Gurnell *et al.*, 2003). The outcomes of ongoing large clinical trials may clarify the effect of TZDs on cardiovascular risk. Proglitazone has some PPARŬ activity, which may account for the data suggesting a more favourable effect on triglyceride and LDL cholesterol levels (Parulkar *et al.,* 2001). Another reported effect, which may not be mediated by PPARↃ, is a degree of anti-inflammatory activity and reduction in macrophage function (Gurnell *et al*., 2003). Limited evidence suggests also that the thiazolidinediones may improve insulin resistance and ovulatory function in women with polycystic ovary syndrome (Parulkar *et al.,* 2001).

Pharmacokinetics and drug interactions

The thiazolidinediones are rapidly absorbed and reach peak concentrations within a few hours (Mudaliar and Henry, 2001). Steady state is usually reached within one week, but perhaps because of the importance of fat redistribution, the full benefit may take 4-12 weeks to become evident. Rosiglitazone and proglitazone are strongly protein bound in the circulation, predominately to albumin (Mudaliar and Henry, 2001). No significant drug interactions have been reported with the thiazolidinediones, but it should be noted that in combination with the sulfonylureas, hypoglycaemia may occur due to the combination of enhanced insulin sensitivity (Thiazolidinediones) and enhanced insulin secretion (Sulfonylureus). Thiazolidinediones are metabolized by cytochrome p450 2 CB (and by CYP3A4 for pioglitazone) but at conventional doses apparently do not affect the activity of those enzymes. Caution should still be exercised when using TZDs in combination with drugs metabolized by these enzymes.

Members of thiazolidinedione

The members are derivatives of the parent compound, thiazolidinedione and they include:

1. **Rosiglitazone (Avandia):** It was put under selling restrictions in US and withdrawn from the market in Europe due to some studies suggesting an increased risk of cardiovascular events. Upon re-evaluation of new data in 2013, the FDA lifted the restriction
2. **Proglitazone (Actos):** France and Germany may have suspended its sale after a study suggested the drug could raise the risk of bladder cancer.
3. **Lobeglitazone (Duvie):** Approved for use in Korea
4. **Troglitazone (Resulin):** It was withdrawn from the market due to an increased incidence of drug-induced hepatitis

Other members of this class include

5. Netoglitazone
6. Rivoglitazone
7. Ciglitazone

Uses

The only approved use of the thiazolidinediones is in *Diabetes mellitus* type 2. It is being investigated experimentally in polycystic ovary syndrome (PCOS), non-alcoholic steatohepatitis (Belfort *et al*., 2006), Psoriasis (Krentz & Friedmann, 2006) autism (Borris, 2007), ovarian hyper stimulation syndrome (by VEGF inhibition in granulose culb (Shah *et al.,* 2010), lichen planopilaris and other conditions. There are some indications that thiazolidinediones provide some degree of protection against the initial stages of breast carcinoma development.

Side effects and contraindications

The main side effect of all thiazolidinediones is water retention, leading to edema. It occurs in less than 5% of individuals, but it is a big problem. Significant water retention, will lead to a decompensation of potentially previously unrecognized heart failure. Therefore, thiazolidinediones should be prescribed with both caution and patient warnings about the potential for water retention/weight gain especially in patients with decreased ventricular function

Though older studies suggested there may be an increased risk of coronary heart disease and heart attacks with rosiglitazome, proglitazone treatment, in contrast, has shown significant protection from both micro and macro-vascular cardiovascular events and plague progression that aided extensive media coverage led to a substantial decrease in rosiglitazone use (Nissen *et al.,* 2008**).** In November 2013, the FDA announced it would remove the usage restrictions for rosiglitazone in patients with coronary artery disease. Preliminary data from a 10- year epidemiological study from Takeda pharmaceutical company indicated a possible link between proglitazone (Actos) and bladder cancer. The findings promoted the FDA to order safety reviews for the drug in September, 2010.

2.7.1.1.4 Meglitinide

The meglitinide analogues are insulinotropic agents introduced in 1995 and approved for clinical use in adults with type 2 diabetes mellitus in 2000. They are secretagogue molecules with more rapid anti-hyperglycemic action and a shorter duration than sulfonylureas, thus providing better control of postprandial hyperglycemia and reducing the risk of late hypoglycaemias (Dornhorst, 2001; Landgraf, 2000**).**

Repaglinide

Repaglinide was the first meglinide analogue approved for clinical use in adults with Type 2 DM. Repaglinide in the S(+) enantiomer of 2- ethoxy-4-(2-((3-methyl-1-(2-(1-piperidinyl)-butyl) amino)-2-oxoethyl) benzoic acid with a molecular weight of 452.6 Da.

The mechanism of action is similar to sulfonylureas, but repaglinides exhibits distinct pharmacological properties in structure, binding profile, duration of action and mechanism of excretion (Culy and Jarvis, 2001). Like the sulforylureas, the insulinotropic action of repaglinide is mediated via adenosine triphosphate (ATP)- dependent potassium channels. Repaglinide stimulates insulin secretion by blocking ATP-dependent potassium channels (KATP) of the pancreatic beta cell, where inhibition of KATP channels results in membrane depolarization and calcium influx through voltage-gated calcium channels. These events lead to an increase in intracellular calcium and subsequent exocytosis of insulin- containing granules.

Repaglinide binds to the sulfonylurea receptor SURI and it seems to have also a separate distinct binding site on ɓ- cells co-incubated with PPP (3- (3 hydnetyphenyl)- N- (1-propy 1) piperidine), a pharmacological tool to differentiate between the two different binding sites (Fuhlendorff, 1998). Moreover, molecular studies have shown that the binding site of repaglinide is different from that of glibenclamide and nateglinide (Gromada, *et al.,* 1995**).**

Nateglinide

Nateglinide is a (N- [c trans-4-isopropyl-cyclonexyl)- carbonyl]-D- Phenylalanine A-4166) phenylalanine derivative. Like repeglinide, nateglinide also binds competitively to SUR, inhibiting KATP channels and stimulating insulin secretion, but the pharmacodynamic properties of this molecule are unique in several aspects (HU, 2002). Comparative preclinical studies *in vitro* indicate that nateglinide inhibits KATP channels more rapidly, and with a shorter duration

of action than glibenclamide, glimeperide and repaglinide, and shows a greater degree of specificity for sulfonylurea receptor (SuR), over SuRz, as compared with glibenclamide and repaglinide. Also the half life of nateglinide on the receptor is approximately 2X, much shorter when compared to that of repaglinide, which is 23min. In addition, the dissociation from the receptor is estimated to be 90X faster than that of repaglinide, indicating a very short on-off effect of nateglinide on insulin release (Hu, 2002).

Foley *et al* have demonstrated a sort of ñglucose-sensitizing propertyò of nateglinide in *in vitro* experiments on rat ɓ- cells. In fact, unlike glibenclamide and repaglinide, the potency of nateglinide increases in the presence of glucose. The inhibition of KATP current is enhanced 16 fold when the glucose concentration is raised from 3 mmol/L to 16 mmol/L. Interestingly, the glibenclamide potency is much reduced under the conditions where as the potency of repaglinide is enhanced 4- fold, which could explain the low incidence of hypoglycaemia (Hu, 2002)
Moreover, phamacodynamic studies in patients with Type 2 DM have demonstrated that the administration of nateglinide (prior to meals) induces early phase insulin secretion and significantly reduces post-prandial hyperglycaemia in a dose-dependent manner. Interestingly, insulin secretion was significantly greater when nateglinide was taken before a meal compared to neteglinide given in the fasted state or in response to just the meal (Keelson *et al.,* 2000).

Pharmacokinetic properties

Repaglinide is rapidly absorbed after oral administration. The peak plasma concentration is reached 30-60 min after administration, plasma levels decrease rapidly and the drug is eliminated within 4-6 hrs. Its absorption is not affected by food, bioavailability is 63% and the half-life is ~ 1 hour (Hatorp et *al.,* 1999)

Repaglinide has a small volume of distribution and is highly bound (more than 98%) to plasma albumin. Repaglinide is metabolized by the liver cytochrome P456 (CYP_3A_4) and eliminated rapidly through the bilary tract, without apparent accumulation in the plasma after a multiple dose (Hatorp, 2002).

Nateglinide

Nateglinide is rapidly absorbed after oral administration from the gastro intestinal tract in a dose-dependent manner and the bioavailability of the drug is approximately 72% (Weaver *et. al.,* 2001). The optimal time of oral administration of nateglinide is before the meal; infact absorption is more rapid when the drug in administered 0-30 min before meals (Luzio *et. al.,* 2001). Peak plasma concentrations are achieved within 1hr and the half-life is 1.8 h because it is rapidly eliminated from plasma. This short elimination half-life ensures no drug accumulation at any dose level. Nateglinide is metabolized mainly via the hepatic CYP2C9 and CYP3A4 isoenzymes of cytochrome p450 and eliminated primarily by the kidney. Twenty percent of a nateglinide dose is eliminated unmodified in the bile and 10% in the urine (Weaver *et al.*, 2001). Neteglinide is also extensively bound to plasma proteins (98%) and has a relatively small volume of distribution (Weaver *et al.,* 2001).

Indications and usage

Neglitinide analogues, acting on the pancreatic ϐ-cells, mimic some how the early rise of insulin secretion after meal ingestion and reduce postprandial hyperglyceamia (Landgraf, 2000). Clinical trials of nateglinide and repaglinide have shown efficacy and safety as monotherapy and in combination therapy in patients with T2DM. Both nateglinide and repaglinide have good safety profile. Moreover, repaglinide is eliminated mainly via-non renal routes and can therefore

be administered to patients with mild to moderate renal insufficiency and or in whom one of the other second line anti-hyperglycemic drugs is contraindicated (Marbury *et al*., 2000)

2.7.1.1.5 Incretin mimetics

The incretin effect describes the phenomenon that oral glucose leads to a greater insulin response than an isoglycaemic intravenous glucose load (Nauck *et al.,* 1986). There are two major incretins:

1. Glucose depedent insulinotropic polypeptide (GIP)
2. Glucagon- like peptide 1 (GLP-1)

Glucagon- like peptides are preserved in patients with type-2 DM. GLP-1 is a product of the glucagon gene, which is expressed in pancreatic Ŭ- cells and in L- cells, located mostly in the lower small intestine and colon. GLP-1 concentrations increase as early as 5 to 10 minutes following ingestion of carbohydrates and lipids, well before the nutrients pass into the lower gut where most L- cells are located (Eissele *et al.,* 1992). Once released from L-cells, GLP-1 is rapidly metabolized by a widely distributed serine protease, DDP-4, resulting in a half-life of 1 to 2 mins in the circulation. DPP-4 which is located at endothelial cells as well as in soluble form in plasma, cleaves the two N-terminal amino-acids from GLP-1, causing a substantial loss of insulinotropic activity (Vahl *et al.*, 2003). GLP-1 stimulates insulin secretion from the beta cells and inhibits glucagon secretion from alpha cells. Both actions occur in a glucose-dependent manner and lead to a normalization of postprandia and fasting hyperglyceamia (Drucker, 2006). In the gasterointestinal tract, GLP-1 has a direct effect on motility and slows gastric emptying. This effect contributes to normalization of postprandial hyperglycaemia and explains why long-term treatment with GLP-1 receptor agonists leads to weight loss (Drucker, 2006). Under

hypoglycaemic conditions, the counter-regulation by glucagon is not affected and insulin secretion is not stimulated and, therefore GLP-1 does not elicit hypoglycemia (Drucker, 2006).

Type 2 DM and incretin- based therapy

The incretin-based therapies after a good alternative choice to the established antidiabetic compounds due to their satisfying antihyperglycemic efficacy, their lack of risk of hyperglycaemia and their positive effects on body weight. In order to utilize GLP-1 action for T2DM, two options are presently available

1) GLP-1- receptor agonist (or GLP-1 mimetics) as injectable compounds
2) DPP-4 inhibitors as orally active substances

GLP-1 receptor agonists

a)Exanatide: Exenatide is the synthetic form of exendin- 4, a peptide first discovered in the saliva of the gila monster (*Heloderms suspectum*) in 1992. It has a 53% amino acid sequence homology to human GP-1 and is a GLP-1 receptor agonist (Eng, 1992). It is administered subcutanously once daily. It has a prolonged half life in comparison to native GLP-1 of approximately 3.5 hours. After subcutenous injection, sufficient plasma concentrations are reached 4-6 h (Kolterman *et al.,* 2003). In clinical studies, exenatide lowered the HbA_{1c} by 0.8-1.1% (Defronzo *et al*., 2005). Exenatide in combination with metformin (Kendall *et al.,* 2005**),** sulfonylurea (Defronzo *et al*., 2005) or both (Buse, *et al.,* 2004) resulted in significant mean HbA_{1c} reductions from baseline ranging from 0.77% to ï 0.86%. Patients also had statistically significant reductions in mean body weight from baseline (-1.6kg to- 2.8kg). Comparative

studies with insulin showed that effects of exenatide on glycaemic parameters are comparable to the improvement seen with insulin therapy (Heine *et al.,* 2005; Gallwitz, 2006).

Severe hypoglycemic events were only observed in exanatide- treated patients who had received combination therapy with sulfonylurea. For this reason, a reduction in the dosage of sulfonylurea should be considered when initiating exanatide therapy. In the comparative studies, comparing exanatide with insulin treatment, the incidence of nocturnal hypoglycaemic events was lower in the exenatide- treated patients (Barnett, 2007).

b) Liraglutide

Liraglutide is the first human GLP-1 analogue. It has two modifications in the amino acid sequence of native GLP-1 and an attachment of a fatty acid side chain to the peptide. It is injected subcutaneously once daily (Agerso *et al*., 2002). Liraglutide lowers blood glucose, body weight and food intake in animal model (Sturis *et al*.,2003). In clinical studies in approximately 4,200 Type 2 DM patients, liraglutide was efficacious and safe (Marre *et al.,* 2009; Zinman *et al*., 2009).

DPP-4 inhibitors

a) Sitagliptin

Sitagliptin was the first DPP-4 inhibitor approved for the T2DM treatment. The recommended dose of once-daily oral sitagliptin is 100 mg. At this dose, sitagliptin can inhibit ~80% of endogenous DPP-4 activity over a 24- hour period (Herman *et al.,* 2005).

b) Vildagliptin

Vildagliptin also acts by inhibiting circulating DPP-4 activity. It is available as a 50mg twice daily in combination with metformin, sulfonylurea or pioglitazone. Vildagliptin has been

studied as monotheraphy (Dejager *et al.,* 2007) and in combination with other oral anti-diabetic agents (Ahren, 2008).

2.7.1.1.6 Alpha- glucosidase inhibitor

They are oral anti-diabetic drug used for *Diabetes mellitus* type2 that work by preventing the digestion of carbohydrates. They are a class of drugs known as ñstarch blockersò. They slow absorption of certain carbohydrates in the gastrointestinal tract.

Drugs that belong to this class include

1) Acarbose (precose)
2) Miglitol (glyset)
3) Voglibose (volix)
4) Emiglitate

The guidelines issued by the American Diabetes Association suggest that the expected decrease in HbA_{1c} would be between 0.5% and 0.8% when this drug is used alone (ADA, 1997).

2.7.1.2 Insulin therapy

Normally produced by the ɓ-cells of the pancreas, insulin assists in the diffusion of glucose into the cell. When there is no insulin (Type 1 diabetes), glucose cannot enter the cell and be converted into energy. For individuals who do not produce insulin, insulin injections are necessary to balance the amount of glucose in the blood. Insulin cannot be taken orally because stomach acid will destroy it before it is effective (Dorman *et al.,* 1995).

Table 6: Common subcutaneous insulin pharmacodynamics

S/n	Action	Type	Onset	Peak	Duration
1	Rapid	Aspart (Novolog) Lispro (Humalog) Glulisine (Apridra)	0.24hours	0.5- 1.5hrs	Duration 3-6 hors
2	Short	Regular	30mins	2.5- 5 hrs	6-8 hrs
3	Intermediate	NDH	1hr	3-6 hrs	11-16 hrs
		Lente	2hr	4-8 hrs	12-18 hrs
4	Long acting	Ultralente	4-6 hrs	12-16h	Up to 3hrs
		Glargine (lantus) 70/30	1-2 hrs	peakless	24hrs
5	Mixed insulin intermediate short acting	(a) 70% NPH/ 30% regular	30 mins	1-4 hrs	4-30 hrs
		(b) 50/50: 50% NPH/50% Regular	30 mins		4-15hrs
6	Intermediate + Rapid acting	Huamalog mix 75/25 75% NPL/ 25% Lispro	0.25hrs	1-4hrs 05-6.5 hrs Dual peaks	24hrs
7	Intermediate + rapid acting	Novolog mix 70/30: 70% NPA 130% Aspart	0.25hrs	1-4 hrs dual peaks	24hrs

(Adapted from The Academy of Dental Learning and OSHA Training. www.ada.org/cerp)

2.7.1.3 Injectable amylin analogue

Amylin agonist analogues slow gastric emptying and suppress glucagon. They have all the incretins actions except stimulation of insulin secretion. As of 2007, pramlintide were the only clinically available amylin analogue. Like insulin, it is administered by subcatenous injection. The most frequent and severe adverse effect of pramlintide is nausea, which occurs mostly at the beginning of treatment and gradually reduces. Typical reductions in HbA_{1c} values are 0.5-1.0% (Buse *et al*., 2004)

Amylin is a peptide hormone that is co-secreted with insulin from the pancreatic ɓ-cell and is thus deficient in diabetic people. It inhibits glucagon secretion, delays gastric emptying, and acts as a satiety agent.

2.7.1.4 Glycourics

Sodium glucose co-transport SGLT-2 inhibitors block the re-uptake of glucose in the renal tubules, promoting loss of glucose in urine. This causes both mild weight loss, and a mild reduction in blood sugar levels with little risk of hypoglycaemia (Dietrich *et al*., 2013). Urinary tract infection is a common side effect. Example of SGLT-2 inhibitors include

1. Canagliflozin
2. Dapagliflozin
3. Empagliflozin

2.7.1.5 Medicinal plants with antidiabetic activity

The following plants are known for their hypoglycemic properties;

1.*Abelmoschus moschatus* Medik (Malvaceae),

2.*Acacia arabica* (Lam) Wild. (Mimosaceae),

3.*Achyranthes aspera* L (Amaranthaceae)

4.Achyrocline satureioides (Less) DC (Asteraceae)

5. *Acosmium panamense* Schott. (Leguminosae)

6. *Aegle marmelose* (L) Corr. (Rutaceae)

7. *Agrimony eupatoria* L. (agrimony) (Rosaceae)

8. *Ajuga iva L. Schreberr* (Medit) (Lamiaceae)

9. *Allium cepa L*. (onion): (Liliaceae)

10. *Allium sativum* L. (garlic): (Liliaceae)

11. *Aloe vera* (L) Burm.(Asphodelaceae)

12. *Andrographis paniculata* Burm. (Acanthaceae)

13. *Annona squamosa* L (Annonaceae)

14. *Artemisia herba-alba* Asso (Med).(Asteraceae)
15. *Artemisia dracunculus* L. (Asteraceae)
16. *Astragalus membranaceus Bunge* (Fisch.): (Leguminosae)
17. *Averrhoa bilimbi* L (Oxalidaceae)
18. *Azadirachta-indica A*. Juss. (Meliaceae)
19. *Bauhinia candicans* Benth (Leguminosae)
20. *Bauhinia forficate* Link. (Caesalpinaceae)
21. *Bidens pilosa* L (Asteraceae)
22. *Biophytum sensitivum* (L) DC. (Oxalidaceae)
23. *Bixa orellana* L. (Bixaceae)
24. *Brassica nigra* (L) Koch (Brassicaceae)
25. *Bryonia alba* L.(Cucurbitaceae)
26. *Bumelia sartorum* Mart. (Sapotaceae)
27. *Caesalpinia bonducella* (L) Roxb. (Caesalpinaceae)
28. *Cajanus cajan* (L) Millsp. (Papilionaceae)
29. *Casearia esculenta* Roxb. (Flacourtiaceae)
30. *Cassia auriculata* L.(Caesalpinaceae)
31. *Catharanthus roseus* (L)G.Don.(Apocynaceae)
32. Chamaemelum nobile (L) All. (Asteraceae)
33. *Cichorium intybus* L.(Asteraceae)
34. *Clausena anisata* (Willd) Benth. (Rutaceae)
35. *Coriandrum sativum* L (Apiaceae)
36. *Cuminum cyminum* L (Apiaceae)

37. *Cuminum nigrum* L (Apiaceae)

38. *Cyamopsis tetragonoloba* (L) Taubert.(Papilionaceae)

39. *Dioscorea dumetorum* (Kunth) Pax.(Dioscoreaceae)

40. *Eclipta alba* (L) Hassk. (Asteraceae)

41. *Emblica officinalis Gaertn*. (Euphorbiaceae)

42 *Enicostema littorale blume* (Gentianaceae)

43. *Ficus bengalensis* L. (Moraceae)

44. *Garcinia kola Heckel* (W & C Afr)((Clusiaceae)

45. *Gongronema latifolium Endl*. (Asclepiadaceae)

46. *Helicteres isora* L., As.(Sterculiaceae)

47. *Hypoxis hemerocallidea* conn Corm (Africanpotato) (Hypoxidaceae)

48. *Inula racemosa* Hook.f.(Asteraceae)

49. *Lagerstroemia speciosa* (L) Pers.(Lythraceae)

50. *Lepidium sativum* L. (Brassicaceae)

51. *Mangifera indica* L.(Anacardiaceae)

52. *Momordica charantia* L. (Cucurbitaceae)

53. *Morinda lucida* Benth.(Rubiaceae)

54. *Myrcia uniflora* Barb., Rods.(Myricaceae)

55. *Nigella sativa* L (Ranunculaceae)

56. *Ocimum sanctum* L. (Lamiaceae)

57. *Origanum vulgare* L. (Lamiaceae)

58. *Otholobium pubescens* L. (Papilionaceae)

59. *Paeonia lactiflora* Pall.(Paeoniaceae)

60. *Panax ginseng* C. Meyer. (Araliaceae)

61. *Phyllanthus amarus* Schum & Thonn. (Euphorbiaceae)

62. *Psidium guajava* L. (Myrtaceae)

63. *Pterocarpus marsupium* Roxb.(Papilionaceae)

64. *Retama raetam* (RR) (Forssk) Webb. (Papilionaceae)

65. *Salacia reticulate* W. (Celastraceae)

66. *Spergularia purpurea* (SP) (Pers) G, Donf.(Caryophyllaceae)

67. *Suaeda fruticosa* (SF) Euras (Chenopodiaceae)

68. *Syzigium cumini* (L) Skeels.(Myrtaceae)

69. *Tamarindus indica* L. (Caesalpinaceae)

70. *Telfaria occidentalis* Hook. (Cucurbitaceae)

(Kavishankar *et al.*, 2011)

2.7.2 Non-pharmacologic intervention

2.7.2.1 Excercise

For decades, exercise has been considered a cornerstone of diabetes management along with diet and medication. Exercise is a subset of physical activity planned, structured and repetitive bodily movement performed to improve or maintain one or more components of physical fitness while physical activity in bodily movement produced by the contraction of skeletal muscle that requires energy expenditure in excess of resting energy expenditure (Wasserman and Cherrington 1996).

Endogenous glucose production

Endogenous glucose production (EGP) is closely coupled to the increase in muscle glucose uptake during moderate exercise. Studies conducted in animal models and human subjects have

defined the importance of insulin and glucagon in the stimulation of EGP during light and moderate exercise (Wasserman and Cherrington, 1996). Glucagon also stimulates hepatic amino acid metabolism (Krishna *et al.,* 2000) providing precursors for gluconeogenesis and energy to fuel it. The decrease in insulin during exercise is necessary for the full glycogenolytic response (Wasserman and Cherrington 1996).

In contrast, in very intense aerobic exercise (> 80% of V_{O2}max), the catecholamines likely play important role. In this situation, nor-epinephrine and epinephrine levels rise as much as 15 fold from baseline, and glucose production in young fit subjects rises about 7-fold during exercise (Marliss *et al.,* 1995). In normal subjects, plasma insulin doubles soon after the end of a very intense exercise session, restoring glycemia to baseline within an hour (Marliss *et al.,* 1995**).** In contrast in type I diabetics in which endogenous insulin cannot increase, hyperglycemia after a very intense exercise lasts at least several hours (Sigal *et al.,* 1996; Purdon *et al.,* 1993). Type 2 diabetic patients with a mild to moderate elevation in glucose levels may experience a fall in glucose during exercise due to impaired endogenous glucose output. This population when maintained on diet therapy alone or diet and sulfonylurea therapy with post absorptive plasma glucose in excess of 200 mg/dl and normal basal insulin, shows a fall in glycemia of 50 mg/dl during a 45-min exercise bout (Minuk *et al.,* 1981). High-intensity intermittent exercise performed in postprandial type 2 diabetic subjects has the same plasma glucose and insulin lowering effect as moderate-intensity exercise of equivalent caloric requirement (Larsen *et al.,* 1997).

Fat metabolism

Moderate exercise is associated with an approximately 10 fold increase in fat metabolism. This is due to increased energy expenditure coupled with greater fatty acid

availability. The increase in fatty acid availability is due both to an increase in lipolysis and decreased re-esterification of Non-essential Fatty Acid (NEFA) to triglycerides (Wolfe *et al.,* 1990). Acute NEFA release from adipose tissue is regulated primarily by the actions of insulin and the catecholamines when the exercise-induced fall in insulin is prevented; the increase in NEFA levels is prevented (Wasserman and Cherrington, 1996). Metabolism of fats during exercise is quantitatively different in obese type 2 diabetic subjects in relation to healthy subjects. In this population, utilization of plasma free fatty acid is reduced, while intramuscular triglyceride untilization is increased (Blaak *et al.,* 2000). Interestingly, lean type 2 diabetic patients do not have this adaptation to exercise (Borghouts *et al.,* 2002).

Muscle glycogenolysis

Glycogen breakdown is regulated by glycogen phosphorylase. It is interesting that although muscle glycogenolysis increase with increasing work rate, phosphorylase transformation to its active phosphorylated form is not (Howlett *et al*., 1998). This suggests that allosteric regulators may be important activators of glycogen phosphorylase during exercise (Rush and Spriet, 2001). $\beta-$adrenergic receptor stimulation by catecholamines play a major role in the mobilization of muscle glycogen during exercise (Wasserman and Cherrington, 1996).

Exercise-induced muscle glucose uptake

Muscle glucose uptake requires glucose delivery from blood to muscle, membrane glucose transport and muscle-glucose phosphorylation. Exercise-induced increase in glucose delivery is so efficient at maintaining interstitial glucose that an increase in muscle fractional glucose extraction is not required for increase in muscle glucose uptake (Hargreaves *et al*., 1991). Exercise also increases glucose transport by stimulating GLUT4 translocation to the muscle cell surface (Lund *et al.,* 1995).

2.7.2.2 Diet

Essentially, eating plan for type 2 diabetic should be tailored at foods that are low in refined grains and sugar, low in saturated and trans fats and high in fiber (Cousens, 2013).

Glycemic index (G1)

Glycemic index is a scale (0-100) ranking how quickly a carbohydrate containing food will digest into glucose in our blood. High G1 foods break down quickly whereas low G1 foods breack down slowly. With low G1 foods, one feels full longer and ones bodyôs insulin has more time to perform its work of removing the glucose from the blood.

Table 7: Different food stuffs and their glycemic indices

	Low G1 foods 55 or less	Medium G1 foods 56-69	High G1 foods 70 & above
1	Whole grain bread	Couscous	White bread
2	Pumpernicle bread	Rye bread	Instant mashed potatoe
3	Oat meal	Instant oat meal	Corn flakes, Rice Krispies
4	All-bran cereal	Shredded wheat	Refined, sweetened cereals
5	Converted rice	Cream of wheat	Instant rice
6	Brown and basmati rice	Whole grain crackers	Bagels
7	Bulgur, Barley, Quinoa	Pita bread	Waffles/pancakes made
8	Firm cooked pasta	Long grain while pice	With white flour
9	Beans, peas, lenfits	Apricot, Bannana	Soda crackers
10	Apples, peaches, peers	Cantaloupe	French fries
11	Grape fruit, oranges	Pinapple, raisins	Dried dates figs
12	Berries, cherries, grapes	Canned fruit in juice	Sweetened fruit jice
13	Kiwi, mango, plum	Cranberry juice	Parsnips, pumpkin
14	Avocado	New potatoes	Rutabaga, turnip
15	Sweet potatoe	Beets	Broad beans
16	Carrots, broccoli	Sweetened conderzes milk	Refried beans
17	Cauliflower corn		Ice cream
18	Leafly vegetables		Soft drink.
19	Low fat milk, soyamilk		Glucose
	Yogurt, & cottage cheese		

Adapted from ñthe GI dietò Rick Gallopò.

www.gidiet.com or www.diabetes.calfiles/glycemicidex-08.pdf. eat more of fibres and drink less alcohol.

2.8 Experimental diabetes

Substances with diabetogenic effect in experimental animals

These include Alloxan, Chlorothiazide, Chlorizotocin, Cyclosporine, Cyproheptadine, Diazoxide, Dithizone, Furosemide Hydrochlorothiazide, L-asparaginase, Methylnitrosourea, Oxime, Streptozotocin, Styrylquinoline, Trichlormethiazides, Vacor, Xylazine (Glenn and Susan, 1981).

Alloxan

Alloxan (2, 4, 5, 6-tetra oxy pyrimidine), is a compound gotten by amalgamation of the word allantein ñand ñoxalsaò. Oxalic acidò, with molecular formular, $C_4 H_2 N_2 O_4$ is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (beta cells) when administrated to rodents and many other animals. This causes an insulin-dependent *Diabetes mellitus* (called ñalloxan diabetesò) in these animals. (Szukudelski, 2001**).**

Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT 2 glucose transporter. Alloxan in the presence of intracellular thiols generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialluric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction (Szkudelskl, 2001).

Alloxan establishes a redox with formation of superoxide to hydrogen peroxide with a simultaneous massive increase in the cytosolic calcium ion concrentration which causes rapid destruction of pancreatic beta cell (Szkudelski, 2001). The range of diabetogenic dose of alloxan is narrow that even slight over dosing may result in the death of animals (Frode and Medeiros, 2008). The most frequently used dose of alloxan is 150 mg/kg given intraperitonealy. The intravenous dose of alloxan is 65 mg/kg (Frode and Medeiros, 2008).

Moreover, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status (Federiuk *et al.,* 2004). Furthermore, alloxan has been demonstrated to be non-toxic to the human beta cells, even in a very high dose. The reason of which may be attributed to the differing glucose uptake mechanism in humans and rodents (Tyrbery *et al*., 2001).

Streptozotocin (STZ)

Streptozotocin is a nitrosurea compound derived from *Streptomyces anchromogenes*, which has also been used as an antibiotic and a cancer treatment. Streptozotocin enters the β cell via a glucose transporter (GLUT2) and causes alkylation of DNA. The DNA damage induces activation of poly ADP-ribosylation, a process that is more important for diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidize resulting in the formation of superoxide radicals consequently, hydrogen peroxide and hydroxyl radicals are also generated. Further more streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, β cells undergo the destruction by necrosis (Szkuldeski, 2001).

In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes, but higher dose are also used. It is also efficacious after intraperitoneal administration of a similar or higher dose but single doses below 40 mg/kg may be effective (Frode and Medeiros, 2008). In mice multiple low doses (40 mg/kg) are the most effective at maintaining mouse viability and inducing pancreatic dysfunction in part through immune destruction (Katherine and Laura, 2009).

Induction of diabetes with streptozocin leads to a spontenous reduction of hyperglycemia by the developments of functioning insulinoma. There is also a high incidence of kidney and liver tumors (Etuk, 2010).

2.9 Chromatography

Chromatography is the general name applied to a series of separation methods that employ a system with two phases of matter, a mobile phase and a stationary phase. Analytes in a mixture to be separated interact with the stationary phase with different affinities while moving through the system, carried along by the mobile phase. The analytes with a low affinity for the stationary phase will tend to move along rapidly while those with high affinity will tend to lag behind. Thus the separation of analytes in chromatographic system is based on the differential affinity of the analyte for the stationary verses mobile phases.

Column chromatography (CC)

Column chromatography consists of a common of particulate material such as silica or alumina that has a solvent passed through it at atmospheric, medium or low pressure. The columns are usually glass with sinter frits to hold the packing. Most systems utilize gravity to push the solvent through but medium pressure pumps are commonly used in flash column chromatography. The sample is dissolved in solvent and applied to the front of the column (wet packing) or alternatively adsorbed on a coarse silica gel (dry packing). The solvent elutes the sample through the column, allowing the component to separate.

The solvent is usually changed stepwise, and fractions are collected according to the separation required, with the eluting products usually monitored by Thin Layer Chromatography. The technique is not efficient, with relatively large volumes of solvent being used and particle size is constrained by the need to have a flow of several ml/min. The advantage is that no

expensive equipment is required and the technique can be scaled up to handle sample sizes approaching gram amounts (Guiochon 2001).

Other forms of chromatography incude: high pressure liquid chromatography, thin layer chromatography, gel permeation chromatography and gas chromatography (GC).

2.10 Spectroscopic techniques

Nuclear magnetic resonance spectroscopy (NMR)

Spectroscopy is the study of the interaction between electromagnetic radiation (EMR) and matter. Nuclear magnetic resonance spectroscopy is the study of interaction of radio frequency (RF) of the EMR with unpaired nuclear spins in an external magnetic field to extract structural information about a given sample. The chemical structures of simple and complex compounds are determined by one dimensional techniques (1D-NMR) and two dimensional techniques (2D-NMR) resspectively (Abraham *et al.,* 1988).

One dimensional NMR ID-Proton NMR (^{1}H-NMR)

Proton NMR is a plot of signals arising from absorption of RF during an NMR experiment by the different protons in a compound under study as a function of frequency (chemical shift). The area under the plots gives information about the number of protons present in the molecule, the position of the signals (the chemical shift) reveals information regarding the chemical and electronic environment of the protons, and the splitting pattern provides information about the number of neighboring (Vicinal or germinal) protons (Sanders and Hunter, 1993).

1D-Carbon NMR (13^{C} NMR)

Carbon NMR is a plot of signals arising from the different carbons as a function of chemical shift. The signals in 13c-NMR experiments usually appear as singlets because of the decoupling of the attached protons. Different techniques of recording of the 1D-Carbon NMR have been

developed so that it is possible to differentiate between the various types of carbons such as the primary, secondary, tertiary and quaternary from the 1D ^{13}C-NMR plot. The range of the chemical shift values differs between the ^{1}H (normally 0-10) and 13C- NMR (Normally 0-230) that arise from the two nucleic having different numbers of electorns around their corresponding nucleic as well as different electronic configurations (Abraham *et al.,* 1988).

Other special forms of spectroscopy include: 2d Nuclear Overhauser Enhancement Spectroscopy (NOESY), Heteronuclear Multiple Bond Correlation (HMBC), Gas Chromatography/Mass Spectrometry (GC/MS), Mass Spectrometry (MS), Liquid Chromatography/ Mass Spectrometry (LC/MS).

2.11 *Cussonia arborea*

2.11.1 Scientific classification of *Cussonia arborea*

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Order: Apiales

Family: Araliaceae

Subfamily: Aralioideae

Genus: Cussonia

Species: arborea

Other species of Cussonia

1. *Cussonia angolensis*

2. *Cussonia arenicola*
3. *Cussonia bancoensis*
4. *Cussonia brieyi*
5. *Cussonia corbisieri*
6. *Cussonia gamtoosensis*
7. *Cussonia holsti*
8. *Cussonia jatrophoides*
9. *Cussonia natalensis*
10. *Cussonia nicholsoni*
11. *Cussonia ostinii*
12. *Cussonia paniculata*
13. *Cussonia sessilis*
14. *Cussonia sphaerocephala*
15. *Cussonia spicata*
16. *Cussonia thyrsiflora*
17. *Cussonia transvaalensis*
18. *Cussonia zimmermannir*
19. *Cussonia zuluensis*

(Adapted from wikipedia **2015)**

Identification of Cussonia arborea

Fig.2 *Cussonia arborea* plant in its natural habitat

Fig.3: The stem of *Cussonia arborea* plant in its natural habitat

2.11.2 **Different local names of the plant**

The different local names of the plant are as follows: Mufenje by Yoruba,
Hannun kuturuu (Lepers Hand), Takandar giiwaa (elephant sugarcane) by Hausa Ityovor by TIV people, Kijagaajaga by Bukoba rural district in Tanzania; no specific local name has been associated with the plant in Igbo land.

2.11.3 Geographical distribution: It is widely distributed across Tropical Africa.

Cussonia arborea (Hochst) is a tree that originated from Africa and has its centre of distribution in South Africa and Madagascar (Tennant, 2010). The tree can grow up to 11m tall with a bole of up to 0.75 diameters; bark deeply fissured and corky. The leaves are digitately compound ; petiole up to 87cm long and 9 mm wide but usually much smaller, mostly glabrous or somewhat hairy in places ; leaflets 5-9, sessile, chartaceous to coriaceous to oblanceolate, ovate and obviate up to 23 cm long by 10.5 cm wide. Flowering spikes up to about 26 together, but mostly less than 12, up to 46 cm long, sometimes galled (Tennant, 2010**).** In the dry season, the tree becomes completely defoliated, and the thick stumpy branches resembling amputated and deformed limbs sticking up into the sky invoke the Bambara name in Mali ñstump of an amputated linbò and in upper Volta ñcut handò and the Hausa name in Northern Nigeria ñLeperôs handò. The wood is dirty white, soft and brittle (Tennant, 2010**).**

Habitat: In Brachystegia woodland, often in rocky terrain

Altitude: 850-1750 M

Flowering time: September to November

2.11.4 Folkloric uses of *Cussonia arborea*

1. Antifungal and for treatment of chronic diarrhea (Kinsangau *et al.*, 2007**)**
2. Antimicrobial and antimalarial effects (De villiers *et al.,* 2010)
3. For the treatment of pain, inflammation and sexually transmitted diseases (De villiers *et al.*, 2010).
4. Hypoglycemic potential (Amadou *et al.*,2008**)**

Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al.,* 2008**).** The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Ayurveda and other traditional medicinal systems for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance (Welihinda *et al.,* 1982). Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from the intestine and glucose production from liver (Hongxiang *et al.,* 2009). Insulin and oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management but there is quest for the development of more effective anti-diabetic agents.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials:

3.1.1 Chemicals and reagents

Chloroform, Methanol, Ethylacetate and Hexane were obtained from Sigma Aldrich, U.K., Test kits (Randox, UK), Glycosylated hemoglobin Kit (Spectrum, Egypt)

Ferric Reducing Antioxidant Power (FRAP) reagents

1, 1 diphenyl-2-picrynyl phenyl hydrazyl (DPPH) reagents

Glucometer strip, RBC, WBC diluting fluids, Drapkins reagent, MDA, SOD, GSH and Catalase reagents

Silica gel (Laboratory Tech. Chemicals)

3.1.2 I**nstruments and glassware**

1. Glucometer (Accu-Chek Advantage II)
2. Gastric gavage
3. Electronic weighing balance (Mettler, England)
4. Beakers, test tubes, graduated cylinder
5. Oven (Gallenkamp, England)
6. Votex machine
7. Spatular, funnel, filter paper, scissors
8. Spectrophotometer (Spectrum Lab. 752s, England)
9. Column glassware (Pyrex)
10. UV lamp (YLN, China)
11. Refrigerator (Haier Thermocool)

12. Water bath (Techmel and Techmel, Texas, USA)
13. Stirrer (glass rod)
14. Thin layer chromatography plate and container (De Saga)
15. Cotton wool
16. Crocodile clip, syringes
17. Mortar and pestle
18. Capillary tubes
19. Rotary evaporator (Buchii, Switzerland)
20. Analytical weighing balance (Scientific and Instrument Company, England)
21. Microhaematocrit Centrifuge
22. Centrifuge
23. Microscope
24. Microscope slides
25. NMR machine
26. 20cm x20cm chromatographic plates, chromatographic tank

Drugs

1. Glibenclamide
2. Alloxan monohydrate

3.1.3 Animals

Male albino Wistar rats weighing between 100 g and 105 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The rats were acclimatized for two weeks. The environmental temperature where the animals were housed varied between 28 and 32^{0C}. The animals were kept in stainless wire mesh cages and provided

with good clean water *ad libitum*. They were fed with standard commercial feed (Guinea^{R} growers, Benin).

3.1.4 Plant material

The root bark of the plant material (*Cussonia arborea*) used in this study was collected from Orukpa Local Government Area of Benue state and identified by a plant taxonomist, Mr. Ozioko at the International Centre for Ethnomedicine and Drug Development, Echara, Aku Road, Nsukka.

3.2 Methods

3.2.1 Preparation of the plant extract

Cold maceration method of extraction was employed. The root bark of *C. arborea* was air dried at a very low intensity of sunlight to avoid denaturation of the active ingredient. It was pulverized and stored in an air tight container pending its usage. About 2 kg of the powdered stem bark was soaked in 10 liters of 80% methanol with intermittent shaking every 2 h for 48 h. The mixture was filtered using Whatmann No 1 filter paper. The filtrate was concentrated using rotary evaporator and the extract was stored in a refrigerator at 4^{OC}.

Calculation of the percentage yield

$$\textbf{Percentage yield} = \frac{\text{Mass of the extract} \times 100}{\text{Mass of the starting material}}$$

Weight of beaker=Y g

Weight of extract=(X-Y) g

Weight of beaker+ extract=X g

$$\% \text{ of extract yield} = \frac{(X-Y)}{2000g} \times 100$$

3.2.2 Acute toxicity test

Acute toxicity test according to the method of Aba *et al.,* (2014) was used. Rats were divided into seven groups consisting of five rats per group. Groups 1, 2, 3, 4, 5 and 6 were given (orally) graded doses (500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg) of the extract respectively. The rats in group seven received distilled water (10 ml/kg) to represent the negative control. The rats were watched closely for 48 h for signs of toxicity such as sedation, dullness, anxiety, wretching, anaesthesia and death.

3.2.3 Induction of experimental *Diabetes mellitus*

Diabetes was induced in rats using the method described by Venugopal *et al.,* (1998). The rats were injected with alloxan monohydrate dissolved in distilled water at a dose of 150 mg/kg body weight intraperitoneally, after overnight fasting (18 hours). Meanwhile before the injection with alloxan monohydrate, the blood glucose levels of the rats were taken using Accu-Check glucometer. This was done by tail snip of the rats and allowing blood to drop on the Glucometer strip. The value was digitally read off on the screen of the glucometer. After induction, the rats were kept in clean stainless steel cages and fed with commercial feed and were also given clean water for about 2 days. The rats were fasted overnight before the assessment of their blood glucose status on the 2nd day. The fasting blood glucose values above 7 mMol/L (126 mg/dl) were considered diabetic.

3.2.4: Effect of *Cussonia arborea* extract (CAE) on blood glucose level of alloxan-induced hyperglycaemia/dose response study

Diabetes was induced by the method described by Venugopal *et al*., (1998). However, the fasting blood sugar (FBS) levels of the rats were measured before diabetes induction (pre-induction

fasting blood glucose) (FBG).The diabetic rats were randomly assigned into six groups consisting of six rats per group, their fasting blood glucose measured and regarded as Fasting Blood Glucose (FBG) 0 h and treated as follow:

Group 1: 250 mg/kg CAE (Low dose)

Group 2: 500 mg/kg CAE (Medium dose)

Group 3: 1000 mg/kg CAE (High dose)

Group 4: 2 mg/kg Glibenclamide (Standard control)

Group 5: 10 ml/kg distilled water (negative control)

Group 6: non-diabetic + 10 ml/kg distilled water (Normal control)

The FBG was measured at 1h, 3 h, 6 h and 24 h post drug and extract administration.

3.2.5 Antioxidant test

Ferric Reducing Antioxidant Power (FRAP) and 1, 1 Diphenyl- 2- picryl hydrazyl (DPPH) models for assaying *in vitro* antioxidant properties were adopted.

The method of Benzie and Strain, (**1999)** was used for FRAP test. The procedure was as follows:

A. **FRAP reagent preparation**

1. Exactly 3.1 g of Na-acetate trihydrate was weighed out and added to 16 ml of glacial acetic acid. The volume was made up to a litre with distilled water.
2. Ten millimol of TPTZ (Tripyridyltriazine) in 40 mMol HCL was prepared.
3. Twenty millimol of $FeCl_3.6H_20$ was prepared.

1, 2 and 3 above were mixed in the ratio of 10:1:1 to form a working FRAP reagent.

Sample preparation

Serial dilutions of the samples were made in triplicates. The concentrations of 10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml of the extracts were made. Ascorbic acid was used as standard. Three milliliter (3 ml) FRAP reagent was used to blank the spectrophotometer while mixture of FRAP reagent and Ascorbic acid served as the standard.

Experimental procedure

One hundred microliter (0.1 ml) of the sample was mixed with 3000 µl (3 ml) of the working FRAP reagent and the mixture was shaken properly. The absorbance was read in a spectrophotometer at 593 nm after mixing. The procedure was done for all concentrations. Thereafter, samples were placed at 37^{o}C in water bath and their absorbencies measured after 4 mins. Ascorbic acid standard was processed in the same way. The antioxidant values of the different concentrations of the sample were calculated using the formular below:

$$\text{FRAP value of sample (µm)} = \frac{\text{Change in the absorbance of sample (0-4 min)}}{\text{Change in absorbance of the standard from (0-4 min)}} \times 100$$

NB: the FRAP value of the standard (Ascorbic acid) = 2 µm

B. **DPPH model of *in vitro* analysis of antioxidant property**

Extract preparation

The method of Mensor *et al.,* (2001) was used. Six serial dilutions of the extract were prepared. The concentrations were: 10, 50, 100, 200 and 400 µg/ml. Extracts and DPPH reagent were mixed in the first triplicates of the different concentrations to serve as the test sample, while the remaining triplicates which served as blank contained the extract and methanol. One milliliter (1ml) of DPPH reagent and 2 ml of methanol served as negative control.

Test procedure

Two milliliter (2 ml) of the extract at different concentrations was mixed with 1 ml of 0.5 mMol DPPH (in methanol) in a curvet. The absorbance at 517nm was read at 0 min and 30th minute following incubation in the dark at room temperature. One ml (1 ml) of methanol plus 2 ml of the extract was used as blank while 0.2 mMol DPPH solution plus 2 ml of methanol was used as the negative control. All the experiments were carried out in triplicate.

Ascorbic acid (Vitamin C) was processed in the same way as the extract, as described earlier. Ascorbic acid was used as reference standard. The antioxidant activities of both the extract and the reference standard (Ascorbic acid) were calculated using the formular below:

$$\% \textbf{ Antioxidant Activity} = 100 - \frac{\{(abs_{Sample} - abs_{Blank}) x 100\}}{\text{Absorbance of the control}}$$

NB: Abs=absorbance

3.2.6 Table 8: Effect of CAE on oral glucose tolerance test (OGTT)

Thirty rats were randomly assigned into 5 groups, their FBG levels were measured and then the rats were treated as follows after 18 h fasting

Group	Treatments	30 mins post treatment
1	250 mg/kg CAE	Glucose (2000 mg/kg)
2	500 mg/kg CAE	Glucose (2000 mg/kg)
3	1000 mg/kg CAE	Glucose (2000 mg/kg)
4	2 mg/kg Glibenclamide	Glucose (2000 mg/kg)
5	10 ml/kg distilled water	Glucose (2000 mg/kg)

FBS checked after 30 min, 60 min, 120 min and 180 min post glucose challenge

3.2.7 Phytochemical test

The method of Trease and Evans (1989) was used to determine the phytochemical constituents of the extract.

A. Test for the presence of tannins

A volume of 3 ml of the extract was placed into 3 test tubes. A few drops of lead subacetate were added into the first test tube while 5 ml of dilute sulphuric acid was added to the second tube. Into the third tube was added a few drops of ferric chloride. Distilled water in another test tube was used as control. Brownish colour in lead acetate and yellow colour appearance in dilute sulphuric acid indicates the presence of tannins. The control showed no colour change. Bluish-black precipitate with ferric chloride shows the presence of tannins while the negative control was yellow in colour

B. Test for the presence of alkaloid

To 1 ml of the extract in three test tubes was added a few drops of Wagner`s reagent in test tube 1, Meyer`s reagent in test tube 2 and Draggendorffôs reagent in test tube 3 and distilled water in another test tube served as the control. Intense yellow colour in Wagners reagent test when the control is light yellow indicates positivity. Slight yellow in Meyer`s reagent test as the control remains colourless also indicates presence of alkaloid. Dirty yellow precipitate in Draggendorff`s reagent test shows positivity as the control has brick-red precipitate.

C. Test for the presence of flavonoids

One milliliter (1 ml) of the extract was diluted to 5 ml with distilled water in a test tube. One milliliter (1 ml) of 20% sodium hydroxide was added and water was used as control. Intense yellow colour would indicate the presence of flavonoids.

D. Test for the presence of glycosides

To 5 ml of the extract in a test tube was added 2 ml of dilute sulphuric acid, heated, cooled and neutralized with equal volume of sodium hydroxide solution. This was boiled. About 5 ml of fehling`s`solutions I and II mixture was added and heated for 5 to 10 mins and cooled. Water

was used as negative control. Brick-red precipitate at the bottom of the test tube shows a positive test for glycoside if there is no such precipitate in the control tube (tube containing H_2SO_4, NaOH, Fehling`s I and II).

E. **Test for the presence of saponins**

I. **Frothing Test**: Three milliliter (3 ml) of the extract was diluted to 10 ml with distilled water. The solution was shaken vigorously for 30 seconds and allowed to stand. If there is much foaming, saponin is present.

II. Emulsifying Test: One drop of olive oil was added to the solution in I above and shaken vigorously. Saponin was used as a positive control while distilled water served as negative control. If emulsification is observed, saponin is present.

Test for Sterols/Terpenes

Ten (10) ml of extract was evaporated to dryness in a beaker. The residue was dissolved in 1 ml acetic acid anhydride and 1 ml chloroform. This was transferred to a dry test tube into which 2 ml of concentrated sulphuric acid was added. Formation of a brownish or violet ring at the zone of contact with supernatant indicated the presence of sterols/terpenes.

3.2.8 Chronic antidiabetic study

Seventy two (72) male albino rats weighing between 100 and 105 g were assigned into six groups of 12 rats per group. Diabetes as described by Venugopal *et al.,* (1998**),** was induced in 60 rats while the remaining 12 rats served as normal control. The rats were treated as follows:

Group 1: Diabetic rats treated with 62.5 mg/kg CAE

Group 2: Diabetic rats treated with 125 mg/kg CAE

Group 3: Diabetic rats treated with 250 mg/kg CAE

Group 4: Diabetic rats treated with 2 mg/kg Glibenclamide

Group 5: Diabetic rats treated with 10 ml/kg Distilled water

Group 6: nondiabetic rats treated with 10 ml/kg Distilled water

The rats were treated daily for eighty four (84) days. The biochemical [(Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), Total Protein, Albumin, Globulin, Blood Urea Nitrogen, Creatinine, Total Bilirubin, Conjugated Bilirubin, Unconjugated Bilirubin, Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase, and Reduced Glutathione) and haematologic (Red Blood Cell (RBC), Haemoglobin (HB), Packed Cell Volume (PCV), White Blood Cell (WBC), differential leucocytes (Lymphocytes, Neutrophils, Basophils, Eosinophils and Monocytes), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC))] parameters were assayed on days 28, 56 and 84 while the Glycosylated Haemoglobin (HbA_{1c}) was measured on days 42, 56 and 84. Three rats per group, upon collection of blood samples, were also sacrificed on days 28, 56 and 84 for histomorphologic assessment. The Fasting Blood Glucose (FBG) levels and the weights of the rats were taken after every 2 weeks till the duration of the experiment. Blood samples for haematology were obtained through the medial canthus of the ratsô eyes into an EDTA bottle while the blood for serology was allowed to clot, centrifuged and serum decanted into a clean bijou bottle.

3.2.9 Glycosylated haemoglobin

This was done by ion-exchange resin method (English *et al*., 2014) using Glycosylated hemoglobin Kit (Spectrum, Egypt)

Assay principle

A hemolysed preparation of whole blood was mixed continuously for 5 mins with a weakly binding cation- exchange resin. The labile fraction was eliminated during the hemolysate preparation and during the binding. During this mixing, HBA_0 binds to the ion-exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator was used to remove the resin from the supernatant. The percent GHb was determined by measuring the ratio of the absorbances of the glycosylated haemoglobin (GHb) and the total haemoglobin fraction (THb). The ratio of the absorbances of GHb and THb of the control and test was used to calculate the percent GHb of the sample.

Procedure

A. Hemolysate preparation

Into a test tube was dispensed 0.5 ml of lysing reagent and labeled as test and control. Thereafter, 0.1 ml of the reconstituted control or well mixed sample was added into the appropriately labeled tubes and mixed until complete lysis was evident.

B. Glycosylated haemoglobin separation

The cap from the ion exchange resin was removed and labeled as control and test and 0.1ml of hemolysate from step A above was added into appropriately labeled ion exchange resin tubes. Thereafter, a resin separator was inserted into each tube so that the rubber sleeve was approximately 1cm above the liquid level of the resin suspension and the tube was vortexed continuously for 5 mins. The resin was allowed to settle and the resin separator was pushed until the resin was firmly packed and the supernatant decanted directly into a curvette and absorbance measured at 415 nm against distilled water.

C. Total haemoglobin fraction

In to a test tube labeled as test and control was dispensed 5 ml of distilled water and 0.02 ml of hemolysate from step A above added to appropriately labeled tube. The mixture was mixed well and the absorbance read against distilled water.

Calculations

Ratio of Control (R_c)= Absorbance control GHb / Absorbance control THb

Ratio of test (R_t)= Absorbance test GHb / Absorbance test THb

GHb (%) = Ratio of Test (R_t) / Ratio of control (R_c) X 10

10 is the value of the control.

3.2.10 Determinations of serum biochemistry parameters

3.2.10.1 Assay of aspartate aminotransferase activity

The method of Reitman and Frankel (1957) using Randox Commercial Enzyme kit was used.

This method is based on the principle that oxaloacetate is formed from the reaction below:

αï Oxoglutarate + L ï aspartate ⟶ L-glutamate + oxaloacetate

Glutamic-oxaloacetic acid transaminase (aspartate aminotransferase) activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine.

Reagents

	Contents	Initial concentration of reagents
1.	Buffer	
	Phosphate buffer	100 mmol/l, pH 7.4
	Lï Aspartate	100 mmol/l
	αï Oxoglutarate	2 mmol/l
2.	2, 4ï dinitrophenyl hydrazine	2 mmol/l
	Sodium hydroxide solution	0.4mol/l

1. Measurement against reagent blank

The AST substrate phosphate buffer (0.5ml each) was pipetted into both the reagent blank (B) and sample (T) test tubes respectively. The serum sample (0.6ml) was added to the sample (T) test tubes only and mixed thoroughly. Then 0.1ml of distilled water was added to the reagent blank (B). The entire reaction medium was well mixed and incubated for 30 min in a water bath at 37^{o}C.

Immediately after incubation, 2, 4-dinitrophenyl-hydrazine (0.5ml) was added to the reagent blank (B) and the sample (T) test tubes, mixed thoroughly and allowed to stand for exactly 20 min at 25^{o}C. Finally, 5.0 ml of sodium hydroxide (0.4 mol/l) solution was added to both the blank and the reagent test tubes respectively and mixed thoroughly.

The absorbance of sample was read at a wavelength of 550 nm against the reagent blank after 5 min.

2. Measurement against sample blank

The AST substrate phosphate buffer (0.5ml each) was pipetted into the sample blank (B) and sample (T) test tubes respectively. The serum sample (0.1ml) was added to the sample test (T) only and mixed immediately then incubated in a water bath for exactly 30 min at 37^{o}C.

2, 4 ï Dinitrophenylhyrazine was added to both sample blank (B) and sample (T) test tubes immediately after incubation. Also, 0.1ml of the sample was added to the sample blank (B) only. Each medium was mixed and allowed to stand for exactly 20 min at 25^{o}C. Finally, 5.0ml of sodium hydroxide (NaOH) solution 0.4 mol/l was added to both the sample blank (B) and sample (T) test tubes and mixed thoroughly. Absorbance of the sample was read with spectrophotometer at a wavelength of 550 nm against the sample blank after 5min.

3.2.10.2 Assay of alanine aminotransferase activity

The method of Reitman and Frankel (1957) using Randox Commercial Enzyme kit was used.

Alanine aminotransferase assay is based on the principle that pyruvate is formed from:

αï Oxoglutarate + L-alanine ⟶ L-glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4ï dinitrophenyl hydrazine.

Reagents

Contents	**Initial concentrations of solutions**
Buffer	
Phosphate buffer	100 mmol/l pH 7.4
Lï alanine	200 mmol/l
αï oxoglutarate	2.0 mmol/l
2, 4ï dinitrophenyl hydrazine	2.0 mmol/l
Sodium hydroxide solution	0.4 mol/l

Procedure

Measurement against sample blank

The ALT substrate phosphate buffer (0.5ml each) was pipetted into two sets of test tubes labeled B (sample blank) and T (sample test respectively). The serum (0.1ml) sample was added to the sample test (T) only and mixed properly, then incubated for exactly 30 min in a water bath at a temperature of 37^{o}C.

2, 4ï dinitrophenyl hydrazine (0.5 ml) was added to both test tubes labeled T (sample test) and B (sample blank) immediately after the incubation. Also, 0.1ml of serum sample was added to the sample blank (B) only. The entire medium was mixed thoroughly and allowed to stand for exactly 20 min at 25^{o}C. After which, 5.0 ml of sodium hydroxide (NaOH) solution (0.4 mol/l) was added to both test tubes and also mixed thoroughly. Absorbance of the sample was read at a wavelength of 546 nm after 5min.

Measurement against reagent blank

The ALT substrate phosphate buffer (0.5ml each) was pipetted into both the reagent blank (B) and sample (T) test tubes respectively. The serum sample 0.1ml was added to the sample (T) test tube only and mixed thoroughly. Then 0.1ml of distilled water was added to the reagent blank (B). The entire medium was mixed and incubated for exactly 30 min in a water bath at 37^{o}C. Immediately after incubation, 2,4 ï dinitrophenylï hydrazine (0.5ml) was added to both reagent blank and sample (T) test tubes. The contents of the tubes were mixed thoroughly and allowed to stand for exactly 20 min at 25^{o}C. Finally, 5.0 ml of sodium hydroxide solution (0.4 mol/l) was added to both the blank and reagent test tubes respectively. Each was mixed thoroughly and absorbance of sample was read at a wavelength of 546 nm against the reagent blank after 5min using spectrophotometer.

AST: ALT ratio

This was determined by dividing the AST value with corresponding ALT value (Reitman and Frankel, 1957).

3.2.10.3 Determination of total proteins

Total proteins were determined by the direct Biuret method (Tietz, 1995), for the in-vitro determination of total proteins in serum or plasma using the total proteins test kit (Randox, UK). The serum sample (0.02 ml) was mixed and reacted with Cu^{2+} contained in 1 ml of diluted Biuret reagent in a clean test tube, to form a stable coloured complex. The mixture was allowed to stand for 30 mins at room temperature. A standard was prepared by adding 1ml of the Biuret reagent and 0.02 ml of the standard into a clean test tube. This was mixed properly and allowed to stand for 30 mins. After this, the absorbances of both the sample and standard were read at 546 nm against the working reagent blank using a spectrophotometer.

The total proteins concentration was obtained with the formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5.95 = \text{Total protein (g/dl)}$$

3.2.10.4 Determination of serum albumin

The serum albumin was determined by the bromocresol green method (Doumas *et al*. 1971**),** for the *in vitro* determination of albumin in serum or plasma using albumin test kit (Randox, UK). The serum sample (0.01 ml) was mixed properly in 3.0 ml of diluted bromocresol green reagent for 5 minutes in a clean test tube to produce a coloured complex. The standard was also prepared by adding 3.0 ml of bromocresol reagent and 0.01 ml of standard. The absorbances of both the sample and standard were read immediately after reaction for 5 minutes at 630 nm against the

working reagent blank, using a Spectrophotometer. The concentration of albumin was obtained using the formula below:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 4.66 = \text{Albumin (g/dl)}$$

3.2.10.5 Determination of serum globulin

The serum globulin was obtained by the subtracting the serum albumin value from the total protein value (Colville, 2002). The concentration of serum globulin was obtained using the formular below:

Serum total protein (g/dl) ï Serum albumin (g/dl) = Serum globulin (g/dl)

3.2.10.6 Determination of serum cholesterol

The serum cholesterol was determined by cholesterol oxidase-perioxidase method (Allain *et al*., 1974**),** for the *in-vitro* determination of cholesterol in serum or plasma using cholesterol test kit (Randox, UK). The serum sample (0.01 ml) was reacted with 1.0 ml of cholesterol working reagent containing cholesterol esterase, oxidase and peroxidase to form a colored quinolic derivative. It was mixed properly and allowed to stand at room temperature for 10 mins. The standard was also prepared by adding 1.0 ml of cholesterol working reagent and 0.01 ml of standard. It was mixed properly and allowed to stand at room temperature for 10 minutes. The absorbance of both the sample and standard were read against the working reagent blank at 500 nm within 60 mins with a digital spectrophotometer. The cholesterol concentration in each sample was obtained with the formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 206 = \text{Cholesterol concentration (mg/dl)}$$

3.2.10.7 HDL-Cholesterol

Principle

Low density lipoproteins (LDL) very low density lipoproteins (VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the prescence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant was determined.

Procedure

Precipitation

Five hundred microlitre (500µL) of diluted R1 (phosphotungstic acid and magnesium chloride) was mixed with 200 µL of serum sample or standard and allowed to sit for 10 min at room temperature. Thereafter, the clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOP-PAP method (Allain *et al*., 1974).

Determination of cholesterol using the supernatant

One hundred micro liter (100 µL) of supernatant (or standard or distilled water for blank as the case may be) was mixed with 1000 µL of reagent, mixed and incubated for 10 min at room temperature.

Absorbances of the sample (Asample) and standard (Astandard) were measured against the reagent blank within 60 mins.

Calculation

$$\textbf{HDL -Cholesterol (mg/dl)} = \frac{\text{change Asample}}{\text{Change Astandard}} \times \text{concentration of standard}$$

LDL-Cholesterol (mg/dl) = Total cholesterol-(Triglyceride/5)-HDL-Cholesterol (Friedwald, 1972**)**

VLDL-Cholesterol (mg/dl) = Triglyceride/5

3.2.10.8 Triglyceride

This was done according to Allain *et al*., (1974**).**

Triglyceride measurement are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders such as diabetes mellitus.

R1a= buffer

R1b=Enzyme Reagent

R1b was reconstituted with 15 ml of R1a

Procedure

The serum sample or standard (10 µL) was mixed with 1000 µL of R1, and incubated for 10 mins at room temperature.

The absorbance of the sample (Asample) and standard (Astandard) were read against the blank (1000 µL of R1) at wavelength 500 nm

$$\text{Triglyceride conc. (mg/dl)} = \frac{\text{Asample}}{\text{Astandard}} \times \text{standard concentration}$$

Concentration of standard =191 mg/dl

3.2.10.9 Bilirubin

Both total and conjugated bilirubin were assayed according to Doumas *et al*. (1971**);** direct bilirubin (conjugated) reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the prescence of caffeine, which releases albumin bound to bilirubin by the reaction with diazotized sulphanilic acid.

Procedure

Sample blank: The serum sample (0.2 ml) was added to 0.2 ml of R1 (sulphanilic acid) and 1ml of R3 (caffeine), mixed and incubated for 10 min at room temperature. Thereafter, R4 (Tartrate) was added and incubated for further 5-30 min.

Sample: To 0.2 ml of serum sample was added 0.2ml of R1, 1 drop (50 µL) of R2 (Nitrite) and 1000 µL of R3 , mixed and incubated for 10 min at room temperature. Thereafter, 1000uL of R4 was added to the mixture and incubated for further 5-30 min.

The absorbance of sample A_{TB} was read against sample blank at wavelength 578 nm.

Calculation

Total Bilirubin (mg/dl) = 10.8x A_{TB}

Direct bilirubin assay

Sample blank: To 0.2 ml of serum sample was added 0.2 ml of R1, 2 ml of 0.9 percent of NaCl and incubated for 10 min at room temperature.

Sample: To 0.2 ml of serum sample was added 0.2 ml of R1, 2 ml of 0.9 % of NaCl , 1 drop (50 µL) of R2 and incubated for 10 min at room temperature.

The absorbance of sample A_{DB} was read against sample blank at wavelength 546 nm.

Calculation

Direct bilirubin (mg/dl)=14.4x A_{DB}

Unconjugated bilirubin = Total bilirubin - conjugated bilirubin (TB - CB)

3.2.10.10 Creatinine

The assay was according to Blass *et al*., (1974). Creatinine is derived from creatinine phosphate in muscle tissues and may be defined as nitrogenous waste product. It is produced and excreted at a constant rate which is proportional to the muscle mass. Creatinine is measured primarily to assess the kidney function and has certain advantage over the measurement of urea. The plasma

level of creatinine is relatively independent of protein ingestion, water intake, rate of urine excretion and exercise thus it has advantage over urea measurement in the assessment of kidney function. It is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease. Elevation of plasma creatinine is indicative of under-excretion, suggesting kidney impairment. Reduced levels of plasma creatinine are rare and not clinically significant.

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. This complex is directly proportional to the amount of creatinine.

Procedure

Working reagent was prepared by mixing equal volume of R1a (Picric acid) and R1b (sodium hydroxide).

The serum sample (0.1 ml) or standard solution (0.1ml) was mixed with 1 ml of working reagent and after 30 sec the absorbance A1 of the standard or sample was obtained. Exactly 2 mins, the absorbance A2 of the standard or sample was also read against air at 492 nm

Calculation

A2-A1 = change in Asample or change in Astandard

$$\text{Conc. Creatinine} = \frac{\text{change in Asample}}{\text{Change in Astandard}} \times \text{conc. of standard}$$

Concentration of standard= 2.08 mg/dl

3.2.10.11 Urea

Urease-berthelot Method (Fawcett and Scott, 1960) was used in determining serum urea concentration.

Principle

Urea in serum is hydrolysed to ammonia in the prescence of urease.

Preparation of reagent

R1a=Urease

R1b=sodium nitropruside

R2=phenol concentrate

R3=hypochlorite concentrate

The contents of R1a was transferred into bottle R1b and mixed gently to form R1. The R2 was diluted with 660 ml of distilled water while R3 was diluted with 750 ml of distilled water.

Procedure

The serum sample or standard or distilled water (10 µL) was mixed with 100 µL of R1, and incubated at 37^0C for 10 mins. Thereafter, 2.5 ml of R2 was added to either the tube containing the sample or standard or blank (distilled water). To the mixture above was also added 2.5 ml of R3, mixed immediately and incubated for 15 mins at 37^0C. The absorbance of the sample (Asample) and standard (Astandard) were read against the blank at the wavelength 546 nm.

Calculation

$$\text{Urea concentration (mg/dl)} = \frac{\text{Asample}}{\text{Astandard}} \times \text{standard concentration}$$

Concentration of the standard= 79.33 mg/dl

3.2.11 Determination of haematological parameters

3.2.11.1 Determination of packed cell volume

The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002). Micro-capillary tubes were almost filled with the anti-coagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 rpm for 5 min

using a microhaematocrit centrifuge. The PCV was read as a percentage on the microhaematocrit reader (Thrall and Weiser, 2002**).**

3.2.11.2 Determination of haemoglobin concentration

The haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method (Higgins *et al*. 2008). The blood sample (0.02 ml) was added to 5 ml of Drabkins reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 min to react. The absorbances of both sample and standard were read against a working reagent blank at the wavelength of 540 nm using a spectrophotometer (Lab-tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the factor derived from the absorbance and concentration of the standard.

3.2.11.3 Erythrocyte count

The erythrocyte count was determined by the haemocytometer method (Thrall and Weiser, **2002**). Blood sample (0.02 ml) was added to 4 ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was discharged onto the Neubaeur counting chamber and allowed to settle for 2-3 min. The light dry objective (x 40) of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per microlitre of blood (Thrall and Weiser, 2002).

3.2.11.4 Total leukocyte count

The total leukocyte count was determined by the haemocytometer method (Thrall and Weiser, **2002**). Blood sample (0.02 ml) of blood was added to 0.38 ml of white blood cell diluting fluid

(glacial acetic acid tinged with gentian violet) in a clean test tube, to make a 1:20 dilution. A drop of the diluted blood was charged onto the Neubaeur chamber and allowed to settle for 2 min. The x10 objective lens of the light microscope was used in making a total count of white blood cells on the four corner squares. The number of cells counted for each blood sample was multiplied by 50 to obtain the total leukocyte count per microlitre of blood (Thrall and Weiser, 2002).

3.2.11.5 Differential leukocyte count

Smears for differential leukocyte counts were prepared on clean slides and stained by the Leishman technique (Thrall and Weiser, 2002). The differential leukocyte count was enumerated by the battlement counting method (Coles, 1986**)**. The x100 (oil immersion) objective lens of the light microscope was used in making a differential leukocyte count and the different cells of the leukocytic series were identified and scored using the differential cell counter (Thrall and Weiser, 2002**).**

3.2.11.6 Determination of mean corpuscular values

The mean corpuscular values ï Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using standard formulae (Coles, 1986).

3.2.12 *In vivo* antioxidant assay

3.2.12.1 Estimation of Superoxide dismutase

Superoxide dismutase activity was assayed by the method of Kakkar *et al*. (1984). The assay is based on the inhibition of the formation of NADH- phenazine methosulphate-nitroblue tetrazolium formazan. The reaction is initiated by the addition of NADH. After incubation for 90

s, the reaction is stopped by adding glacial acetic acid. The colour developed at the end of the reaction is extracted into n-butanol layer and measured at 520 nm in a spectrophotometer.

The chemicals used were as follows:

1. Sodium pyrophosphate buffer, 0.052 M, pH 8.3
2. Absolute ethanol
3. Chloroform
4. n-Butanol
5. Phenazine methosulphate (PMS), 186 μmol
6. Nitroblue tetrazolium (NBT), 300 μmol
7. Reduced NADH, 780 μmol

Procedure

Plasma (0.5 ml) was diluted to 1.0 ml with ice cold water, followed by 2.5 ml ethanol and 1.5 ml chloroform (chilled reagent). The mixture was shaken for 60 seconds at $4^{o}C$ and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS and 0.3 ml of NBT and approximately diluted enzyme preparation in a total volume of 3 ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at $30^{o}C$ for 90 S, the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was separated. The colour intensity of the chromogen in the butanol layer was measured in a spectrophotometer at 520 nm. A system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme concentration, which gives 50% inhibition of

NBT reduction in one minute under assay conditions. SOD activity was expressed as U/ml of plasma.

3.2.12.2 Estimation of Catalase

The activity of catalase was assayed by the method of Sinha (1972). Dichromate in acetic acid was reduced to chromic acetate, when heated in the presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate spectrophotometrically after heating the reaction mixture. The chemicals used were as follows:

1. Phosphate buffer, 0.01 M, pH 7
2. Hydrogen peroxide, 0.2 M
3. Potassium dichromate, 5%
4. Dichromate acetic acid reagent: Potassium dichromate and glacial acetic acid were mixed in the ratio 1:3. From this 1 ml was diluted again with 4 ml of acetic acid.
5. Standard hydrogen peroxide, 0.2 mM

Procedure

To 0.9 ml of phosphate, 0.1 ml of plasma and 0.4 ml of H_2O_2 were added. The reaction was after 15, 30, 45 and 60 seconds by adding 2 ml of dichromate acetic acid mixture. The tubes were kept in a boiling water bath for 10 min and cooled. The colour developed was read at 530 nm. Standards in the concentration range of 20-100 µmoles was processed for the test. The activity of catalase was expressed as U/ml for plasma (U- µmoles of H_2O_2 Utilised / second).

$$\text{Catalase activity} = \frac{\log A/B \times 023}{0.00693}$$

3.2.12.3 Reduced glutathione

The reduced glutathione level was determined by the method of Beutler *et al.,* (1963).This method was based on the development of yellow colour when 5,5ô- dithio-bis-2-nitrobenzoic (DTNB) is added to a compound containing sulphydryl groups. The colour developed was read at 412 nm in a spectrophotometer.

The chemicals used were as follows:

1. 0.3 M Disodium hydrogen phosphate

2. 0.1% disodium salt of EDTA

3. Precipitating reagent: 1.67 g of metaphosphoric acid, 0.2 gm of EDTA disodium salt, 30 gm sodium chloride in 1 litre of distilled water.

4. 5,5ô- dithio-bis-2-nitrobenzoic (DTNB) reagent: 40 mg of DTNB in 100 ml of 1% sodium citrate.

5. Standard solution: 10 mg of reduced glutathione in 100 ml distilled water.

Procedure

0.2 ml of sample was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent was added, mixed thoroughly and kept for 5 minutes before centrifugation. To 2 ml of the filterate, 4 ml of 0.3 M disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added and the colour developed was read at 412 nm in a spectrophotometer. A set of standard solutions containing 20-100 ε g of reduced glutathione was treated similarly. The values were expressed as mg/dl for plasma. The enzyme activities were gotten by dividing the absorbances with the slope of a standard curve (0.009).

3.2.12.4 Estimation of lipid peroxidation (Malondialdehyde)

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.* (1993).

Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with three or more double bonds), ethane and pentane (from the n-terminal carbons of 3 and 6 fatty acids, respectively). MDA reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

A volume, 0.1 ml of the serum was mixed with 0.9 ml of H_2O in a test tube. A volume, 0.5 ml of 25% TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 min in a water-bath and then cooled in cold water. Then 0.1ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at 532 nm and 600 nm against a blank.

$$\% \text{ TBARS} = \frac{A_{532} - A_{600} \times 100}{0.5271 \times 0.1} \quad \text{(mg/dl)}$$

3.2.13 Histopathology examination

The histological examination of the tissues of the pancreas of Wistar male albino rats was done using the method of Drury *et al.* (1967).

A. Fixation and washing

Formalin (10%) was used as the fixative and for the purpose of preservation. A thin section of the tissue (about 1 to 2 cm in diameter) was trimmed with a sharp razor blade. The small pieces of the tissue were placed in the 10% formalin, the container was shaken gently several times to make sure that the fluid had reached all surfaces and that pieces were not sticking to the bottom. This was then incubated at 25^0C for 24 h, to allow proper fixing. The fixed tissue pieces were washed with running water for 24 h to free them from excess fixatives.

B. Dehydration

Water was removed from the tissue before embedding the tissue in paraffin. The dehydration was achieved by immersing the thin sections of the tissue in automatic tissue processor containing 12 jars.The first three (3) jars contained 70, 90 and 95% absolute alcohol respectively. This was done to remove the water content in the tissues. The absolute alcohol reduced the shrinking that occurred in the tissue. The time for each step was 30 min. A second change of absolute alcohol was included to ensure complete removal of water. This was achieved in the second three (3) jars of the automatic tissue processor.

C. Clearing

Solutions of xylene were used for clearing the tissue sections. This step was achieved in the third three (3) jars of the automatic tissue processor. Because the alcohol (ethanol) used for dehydration would not dissolve or mix with molten paraffin, the tissue was immersed in xylene solution which was miscible with both alcohol and paraffin before infiltration could take place.

Clearing was done to remove opacity from dehydrated tissue. A period of 15 min was allowed to elapse before the tissue was removed from the solution for infiltration with paraffin.

D. Infiltration with paraffin

Paraffin wax at 50 to 52°C was used to infiltrate the tissue. The tissue was transferred directly from the clearer to a bath containing melted paraffin. After 30 - 60 min incubation in the first bath, the tissue was then removed to a fresh dish of paraffin contained in the fourth three jars of the automatic tissue processor for a similar length of time.

E. Embedding (Blocking) with Paraffin

As soon as the tissue was thoroughly infiltrated with paraffin, it (paraffin) was allowed to solidify around and within the tissue.

F. Paraffin Sectioning

The embedded blocks were trimmed into squares and fixed in the microtome for sectioning after which the sections were floated on a water bath.

G. Mounting

Glass slides were thoroughly cleaned and a thin smear of albumen fixative was made on the slides. The albumenized slide was used to collect the required section from the rest of the ribbon in the water. The section on the glass slide was kept moist before staining.

H. Staining with haematoxylin

The slides were passed through a series of jars containing alcohols of decreasing strength and various staining solutions.

I. Microscopic observation of slide

The slides prepared were mounted on a photomicroscope, and viewed at different magnifications. The photograph of each of the slides was taken.

3.2.14 Bioassay-guided fractionation/isolation of the active compound (s)

3.2.14.1 Column chromatography

Column chromatography was used to separate the different phytochemical components of the plant extracts. The method of Ergon (1967) was used. The method is as follows.

Packing of the (silica gel) column

1. A piece of wire was used to add a plug of glass wool to the bottom of a glass column. Adequate wool was added but not so much as to prevent the dripping of the eluent.
2. The column was clamped to a retort stand. The clamp was cushioned with soft material to avoid breaking the column.

3. A pinch clamp was placed on the rubber tubing to avoid the escape of the initial eluent (Hexane) as the column was one-third filled with the initial eluent.
4. A slurry of the silica gel in initial eluent was made by pouring silica gel into a beaker of eluent in the ratio of 1:2
5. The slurry was constantly being stirred and quickly but carefully poured into the column to maximize the amount of silica that goes into the column.
6. The pinch clamp was removed to allow the eluent drip into the beaker used in making the slurry in order to rinse it and pour back into the column.
7. Rubber stand was used to tap the side of the column to ensure tight packing of the silica gel. Pasteur pipette was used to rinse the side of the column.
8. The eluent was then drained until none remains above the silica gel before the extract (mixed with powdered silica gel in the ratio of 1:8) was poured on the already packed silica gel.
9. Five hundred milliliter of the initial eluent (Hexane) was then carefully poured on the sample by running it on the side of the column to avoid distorting the sample and consequent formation of air bubble.
10. The pinch camp was removed to allow the eluent to drip as it separates the components.
11. Step 9 was repeated continuously but with different proportion of the eluents mixed in various ratios to make up 500 ml. The eluents used as the column progressed include chloroform, ethylacetate, and methanol (Table 2). Two eluents were mixed to make 500 ml in different proportions at a time.

Table 9: The mixture proportions of the chemicals used for column chromatography

The mixture proportions of the chemicals are summarized in the table below:

	Hexane	Chloroform	Ethylacetate	Methanol
1	100	-	-	**-**
2	80	20	-	**-**
3	60	40	-	**-**
4	40	60	-	**-**
5	20	80	-	**-**
6	-	100	-	**-**
7	-	80	20	**-**
8	-	60	40	**-**
9	-	40	60	**-**
10	-	20	80	**-**
11	-	-	100	**-**
12	-	-	80	20
13	-	-	60	40
14	-	-	40	60
15	-	-	20	80
16	**-**	**-**	**-**	100

12. The fractions were collected as different colours as the column progressed.

These fractions were then analyzed by thin layer chromatography to ascertain if the fractions collected in a container were the same.

3.2.14.2 Thin layer chromatography

This was done to analyze the purity of the compounds and fractions following column chromatography. The method of Ergon (1967) was used. .Compounds that have the same chromatographic band pattern were pooled together, the process is as follows:

1. The fractions from column chromatography were allowed to concentrate to a reasonable extent before spotting on a TLC plate.

2. The eluents/solvents (moving phase) were prepared by mixing chloroform, ethylacetate and methanol in the ratio of 3:2:1 and about 20 ml of the mixture was poured into an air-tight container.
3. A whatman No 1 filter paper was cut and put into the solvent in order to saturate the system before placement of TLC plate.
4. TLC plate was cut and pencil was used to draw a line on which the fractions were spotted using capillary tube.
5. The capillary tip was dipped into the fractions and carefully, a spot was made on the line drawn on the TLC plate. Spotting was done repeatedly to ensure success.
6. Upon saturation of the system, the filter paper was removed and replaced with TLC paper. The container was then tightly closed to allow the solvent system elute the compounds.
7. After about 10 mins, the TLC plate was removed and allowed to dry before viewing under UV light at the frequency of 254 nm and 365 nm.
8. The fractions that show the same band pattern under UV light were pooled together and concentrated in the oven at 37^{o}C.

3.2.14.3 Bioassay-guided screening of the fractions

The fractions (7) gotten after chromatography were subjected to screening to ascertain the particular fraction that has the antidiabetic properties.

Diabetes was induced in groups 1-9 rats as described before by the method of Venugopal *et al.*, (1998). The pre-extract administration blood glucose level was determined. The rats were assigned to 10 groups consisting of 3 rats each. Groups 1-7 received the same dose (12.5 mg/kg) of different fractions of CAE, group 8 rats received 2 mg/kg glibenclamie (standard control)

while rats in groups 9 and 10 received the vehicle (10 ml/kg distilled water) to serve as negative and normal controls respectively.

Following the administration of the fractions, blood was collected from the rats by tip tail cut after 1 hour, 3 hours and 6 hours post extract administration for blood glucose analysis. The fraction which was able to reduce significantly, the blood-glucose level was taken as the fraction that has the anti-hyperglycemic property.

3.2.14.4 Preparative thin layer chromatography (bioassay-guided purification of active fraction)

The active fraction obtained from section 3.2.14.4 was subjected to preparative thin layer chromatography for further purification as follows:

Preparation of preparative TLC plate

This was done using the method of Ergon (1969). Thirty five (35 g) gramme of silica gel (60G) was mixed with 85 ml of distilled water in a ceramic motar to form a slurry. The slurry was poured into a trough of movable spreader adjusted to 0.5 mm thickness. The slurry was spread on 5 glass plates measuring 20x20 cm placed in a rack. Thereafter, the plates were air dried.

Upon drying, the plates were activated in an oven for 1h at 110 degrees just before use. The active fraction was dissolved in appropriate solvent system (Methanol) and streaked on the plate to form a band. The plate was lowered into a preparative thin layer chromatographic tank that was previously loaded with predetermined solvent (Mobile phase) system and saturated with whatman filter paper. Different bands (which represent different pure compounds) were scrapped into different test tubes. Thereafter, the different bands were dissolved in appropriate solvent system (methanol), centrifuged, filtered, concentrated to dryness and stored in a refrigerator until

time of use. Bioassay testing of the different compounds eluted was again carried out by repeating the experiment in section 3.2.14.3.

3.2.14.5 Bioassay-guided preparative thin layer chromatography on the active sub-fraction (purification of fraction 2)

Diabetes again was induced in 12 rats (groups 1-4) by the method of venugopal *et al*., (1998). Fifteen rats were assigned into 5 groups of 3 rats per group. The rats in groups 1 and 2 were treated with 2 mg/kg of the sub-fraction 2 and the rats in groups 3 and 4 were treated with 2 mg/kg glibenclamide and 10 ml/kg distilled water respectively while rats in group 5 were not induced but treated with the vehicle (10 ml/kg distilled water). Thereafter, blood samples were taken 1h, 3 h and 6 h posttreatment for FBG analysis.

3.2.14.6 Characterization and structural elucidation of the active compound(s)

This was done using Nuclear Magnetic Resonance (NMR) spectroscopy (carbon 13 and proton NMR).

Nuclear Magnetic Resonance (NMR) **spectroscopy**

Spectroscopy is the study of the interaction of electromagnetic radiation (EMR) with matter. Nuclear magnetic resonance spectroscopy determines the physical and chemical properties of atoms or molecules and can provide detailed information about the structure, dynamics, reaction states and chemical environment of molecules (Shah *et al.,* 2006). It is the study of interaction of radio frequency (RF) of the EMR with unpaired nuclear spins in an external magnetic field to extract structural information about a given sample. It is routinely used by chemists to study chemical structure of simple molecules using simple one dimensional technique (1D-NMR). Two dimensional techniques (2D-NMR) are used to determine the structure of more complicated molecules (Abraham *et al.,* 1988).

3.2.14.7 Statistical analyses

Statistical package for social sciences version 20 was employed. One-Way Analysis of Variance (ANOVA) was used to compare the means of some of the parameters and their difference separated using Duncans Multiple Range test while Data on fasting blood sugar levels and glycosylated haemoglobin were correlated using pearson parametric correlation. P values <0.05 were considered significant.

CHAPTER FOUR
RESULTS

4.1 Percentage yield of the extract

The percentage yield of the extract was 12.5 %.

4.2 Acute toxicity

The result of acute toxicity test of the extract is presented in table 10. Treatment of rats orally with varying doses (500, 1000, 2000 3,000, 4000 and 5000 mg/kg) of the methanol root bark extract of *Cussonia arborea* did not result in toxicity signs nor deaths of the treated animals (Table 10). The LD_{50} therefore was not obtained.

4.3 Phytochemical test result

Table 11 shows the result of phytochemical screening of the extract of *C. arborea*. The result of the phytochemical studies of the methanol root bark extract of *Cussonia arborea* revealed the presence of saponins and terpenes in very high quantity, glycosides, alkaloids, flavonoids, in relatively high amount, tannins, were found in moderate concentrations.

4.4 The percentage antioxidant activities of the methanol root bark extract of *Cussonia arborea* using FRAP assay model

The result of the FRAP assay indicates that the antioxidant activity of the methanol root bark extract of *Cussonia arborea* increased with increase in the concentration of the extract with optimum activity of 75 % at the concentration of 400 µg/ml. This was presented in figure 4.

Table 10: Result of acute toxicity test using varying doses of the methanol root bark extract of *Cussonia arborea* on rats.

GROUP	DOSE (mg/kg)	Signs of Toxicity/Death
1	500	**None**
2	1000	**None**
3	2000	**None**
4	3000	**None**
5	4000	**None**
6	5000	**None**
7	**Distilled water**	**None**

Table 11: Phytochemistry of the methanol root bark extract of *Cussonia arborea*

Phytochemical constituents	**Tests**	**Concentration**
Saponins	Froathing	+++
	Emulsifying	+++
Glycosides	Sulphuric acid +Fehlings I and II	++
Tannins	Lead acetate	+
	Ferric chloride	+
	Sulphuric acid	+
Alkaloids	Wagner	+
	Meyer	++
	Dragendorff`s	++
Flavonoids	Sodium hydroxide	++
Terpenes	**Acetic acid anhydride+ chloroform + sulphuric acid**	+++
	+ **moderate**	
	++ **high**	
	+++ **very high**	

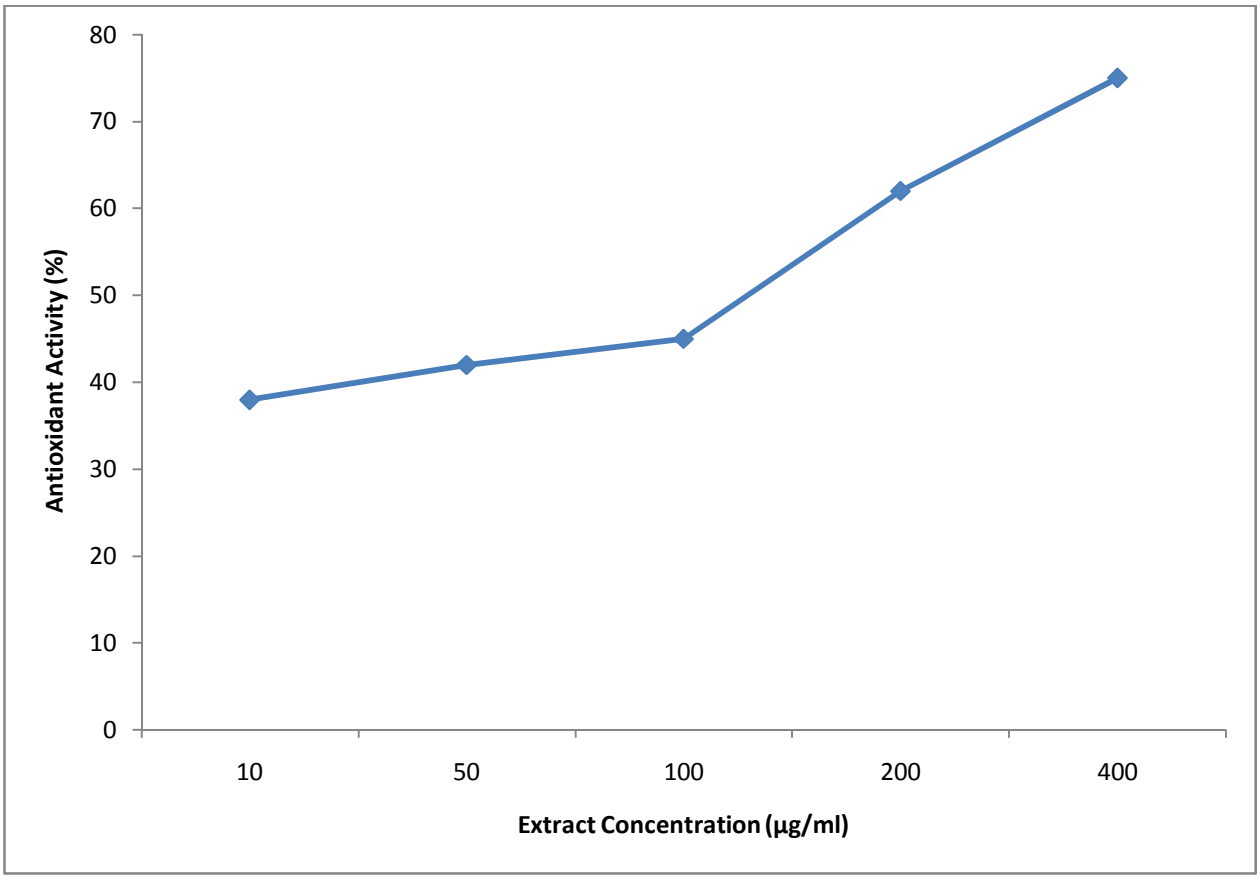


Figure 4: The percentage antioxidant activities of the methanol root bark extract of *Cussonia arborea* using FRAP assay model

4.5 The antioxidant activity of the methanol root bark extract of *Cussonia arborea* at different Concentrations using DPPH photometric assay method

The antioxidant test using DPPH assay (**Figure 5**) indicated that both the extract and the ascorbic acid/vitamin C (positive control) exhibited concentration-dependent effect on antioxidant activity with optimum activities of 74% and 92% respectively at the concentration of 400 µg/ml.

4.6 Effects of acute administration of *Cussonia arborea* root bark extract on Fasting blood glucose levels of alloxan-induced diabetic rats

The result of the acute anti diabetic study (**Table 12**) shows that the fasting blood glucose levels (FBG) of the rats in group 6 were significantly ($p< 0.05$) lower than those in other groups following induction. Six hours post treatment, the FBS levels of group one rats were significantly ($p<0.05$) lower than that of the negative control rats (Group 5) but statistically comparable to that of the normal control rats and group 4 rats till the end of the experiment (24 h).

4.7 Effect of *Cussonia arborea* extract on FBG of normoglycaemic rat (Oral glucose tolerance test)

The result indicates that 30 mins post glucose load/challenge, the sugar levels of the rats increased significantly ($p<0.05$) (**Table 13**). The sugar levels of the group 2 rats (treated with 250 mg/kg of the extract) was significantly ($p<0.05$) reduced compared to that treated with distilled water but was statistically comparable to the group treated with glibenclamide, 180 mins post glucose challenge.

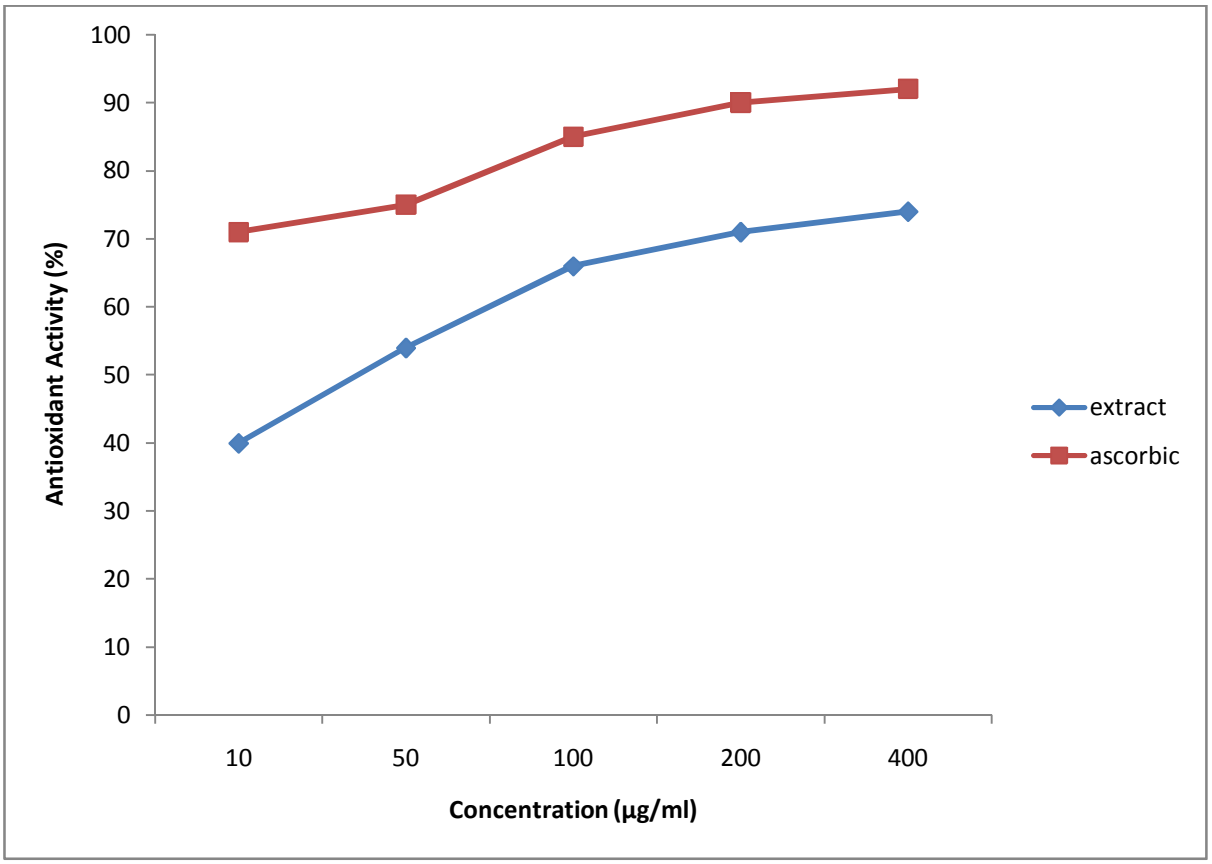


Figure 5: The antioxidant activity of the methanol root bark extract of *Cussonia arborea* at different Concentrations using DPPH photometric assay method

Table 12: Effects of acute administration of *Cussonia arborea* root bark extract on Fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

Group/dose	Preinduction FBS	FBS 0 h	FBS 1 h	FBS 3 h	FBS 6 h	FBS 24 h
1 250 mg/kg CAE	73.33 ± 0.33$^{(a)}$	326.33 ± 2.33$^{(b)}$	265.66 ± 2.96$^{(bc)}$	160.33 ± 1.45$^{(b)}$	96.66 ± 1.76$^{(a)}$	91.00 ± 4.93$^{(ab)}$
2 500 mg/kg CAE	73.33 ± 1.45$^{(a)}$	333.33 ± 29.06$^{(b)}$	285.66 ± 7.17$^{(c)}$	183.66 ± 7.05$^{(b)}$	164.33 ± 3.84$^{(b)}$	142.66 ± 6.48$^{(d)}$
3 1000 mg/kg CAE	74.00 ± 0.57$^{(a)}$	316.00 ± 18.55$^{(b)}$	301.00 ± 4.35$^{(cd)}$	278.66 ± 8.19$^{(c)}$	231.00 ± 5.85$^{(c)}$	193.00 ± 6.50$^{(d)}$
4 2 mg/kg Glibenclamide	73.33 ± 0.33$^{(a)}$	328.00 ± 14.73$^{(b)}$	240.00 ± 26.45$^{(b)}$	160.00 ± 5.77$^{(b)}$	98.66 ± 0.66$^{(a)}$	98.00 ± 0.57$^{(b)}$
5 Diabetic + 10 ml/kg DW	75.33 ± 0.88$^{(a)}$)	343.33 ± 23.33$^{(b)}$	332.00 ± 18.90$^{(d)}$	315.66 ± 20.61$^{(d)}$	345.33 ± 19.19$^{(d)}$	320.33 ± 11.83$^{(e)}$)
6 Non-diabetic +10ml/kg DW	75.33 ± 0.33$^{(a)}$	75.00 ± 0.57$^{(a)}$	74.33 ± 0.33$^{(a)}$	75.66 ± 0.33$^{(a)}$	73.00 ± 0.56$^{(a)}$	75.00 ± 0.57$^{(a)}$

Different superscripts along the same column indicate significant difference at $p<0.05$

Table 13: Effect of *Cussonia arborea* extract on FBG (mg/dl) of normoglycaemic rat (Oral glucose tolerance test)

Group	FBS (0hr)	30mins post	60mins	120min	180min
1	74.00 ± 0.57^{a}	151.00 ± 2.08^{bc}	153.66 ± 7.31^{bc}	107.33 ± 0.88^{c}	99.00 ± 0.57^{c}
2	75.00 ± 0.57^{a}	147.66 ± 1.85^{b}	142.66 ± 1.45^{b}	100.00 ± 0.57^{b}	83.00 ± 3.51^{a}
3	76.33 ± 1.20^{a}	152.33 ± 3.71^{bc}	154.00 ± 3.05^{bc}	105.33 ± 0.88^{c}	103.00 ± 1.15^{c}
4	75.00 ± 0.57^{a}	120.66 ± 1.57^{a}	118.66 ± 1.85^{a}	89.00 ± 1.52^{a}	79.33 ± 1.45^{a}
5	73.66 ± 0.33^{a}	158.00 ± 1.154^{c}	161.66 ± 2.84^{c}	111.00 ± 0.57^{d}	90.33 ± 0.88^{b}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Normoglycaemic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* + glucose (2000 mg/kg)

Group 2: Normoglycaemic rats treated with 500 mg/kg of methanol root bark extract of *Cussonia arborea* + glucose (2000 mg/kg)

Group 3: Normoglycaemic rats treated with 1000 mg/kg of methanol root bark extract of *Cussonia arborea* + glucose (2000 mg/kg)

Group 4: Normoglycaemic rats treated with 2mg/kg glibenclamide + glucose (2000 mg/kg)

Group 5: Normoglycaemic rats treated with 10 ml/kg of distilled water + glucose (2000 mg/kg)

4.8: Effects of chronic administration of *Cussonia arborea* root bark extract on Fasting blood glucose levels of alloxan-induced diabetic rats

The FBS of all the rats in groups 1, 2, 3, 4 and 5 were significantly ($p< 0.05$) higher than that of the group 6 rats (Normal control) post induction. On day 14 post treatment, the FBS levels of groups 2 and 4 rats were significantly ($p<0.05$) lower than those of the group 5 rats but statistically comparable to that of the group 6 rats (Normal control). These two groups 2 and 4 consistently compared very well with the normal control rats throughout the 84 day duration of the study (**Table 14**).

4.9: Effects of different fractions of *Cussonia arborea* on the fasting blood glucose levels of alloxan induced diabetic rats

The FBS levels of the rats treated with fraction 2 were significantly ($p< 0.05$) lower than the FBS of the diabetic untreated rats (Group 5) 3 h and 6 h post treatment but was statistically comparable to that of the normal control rats and to that of the rats treated with glibenclamide (Group 4 rats) (**Table 15**).

4.10: Effect of subfraction 2 of *Cussonia arborea* root bark extract on the fasting blood glucose levels of alloxan-induced diabetic rats

The FBG levels of the rats treated with subfraction 1 of the fraction 2 ($Subf2_1$) showed a significant ($p< 0.05$) reduction when compared with the diabetic untreated (group 4) rats 3 h and 6 h post treatment but was statistically comparable to those of the rats treated with standard drug (Glibenclamide) and normal control rats (**Table 16**).

Table 14: Effects of chronic administration of *Cussonia arborea* root bark extract on Fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

	FASTING BLOOD GLUCOSE LEVELS (mg/dl)							
GROUP	PREINDUCTION	0 h POST TREATMENT (PT)	14 DAYS PT	28 DAYS PT	42 DAYS PT	56 DAYS PT	70 DAYS PT	84 DAYS PT
1	78.00 ± 1.15 [a]	310.00 ± 15.27[b]	118.33 ± 4.40[b]	127.00 ± 3.21[c]	113.66± 3.17[b]	111.66 ± 6.00[a]	115.66 ± 2.90b	108.66 ± 4.37[b]
2	79.33 ± 1.45 [a]	315.33 ± 10.08[b]	93.00 ± 8.50[a]	94.66 ± 4.33[b]	80.00 ± 3.78[b]	79.00 ± 2.51[a]	74.00 . ± 1..57[a]	82.33 ± 3.88[a]
3	78.33 ± 1.76[a]	318.33 ± 16.42[b]	117.66 ± 6.48[b]	127.33 ± 1.76[c]	118.00± 9.01[b]	117.00 ± 5.68[b]	119.66 ± 1.85[b]	110.66 ± 4.84[b]
4	77.66 ± 2.33[a]	307.33 ± 7.33[b]	87.00 ± 1.52[a]	87.66 ± 2.72[b]	80.00 ± 5.77[a]	76.33 ± 2.40[a]	73.66 ± 1.20[a]	79.33 ± 1.20[a]
5	77.66 ± 5.23[a]	311.33 ± 9.61[b]	288.66 ± 6.96[c]	292.66 ± 3.92[d]	303.66± 3.17[c]	295.66 ± 2.84[c]	292.33 ± 7.17[c]	289.66 ± 4.91[c]
6	76.33 ± 6.02[a]	78.33 ± 4.05[a]	81.66 ± 4.09[a]	77.66 ± 2.10$_a$	82.66 ± 1.45[a]	76.66 ± 2.02[a]	73.33 ± 1.20[a]	73.00 ± . 57[a]

Different superscripts along the same column indicate significant difference at $p<0.05$

Table 15: Effect of different fractions of *Cussonia arborea* on the fasting blood glucose (mg/dl) levels of alloxan induced diabetic rats

Group	FBG **pre induction**	FBG **0 h**	FBG **1 h**	FBG **3 h**	FBG **6 h**
Diabetic rat + 12.5 mg/kg F1	79.00 ± 1.52[a]	293.00 ±1 .73[bc]	286.33 ± 2.02[c]	282.66 ± 4.80[c]	273.00 ± 11.67[c]
Diabetic rat + 12.5 mg/kg F2	77.66 ± 2.84[a]	307.00 ± 18.77[c]	211.33 ± 5.54[b]	94.67 ± 5.84[ab]	77.33 ± 3.17[a]
Diabetic rat + 12.5 mg/kg F3	75.33 ± 2.18[a]	282.00 ± 8.02b[c]	237.00 ± 9.07[c]	119.00 ± 7.23[b]	99.33 ± 6.06[b]
Diabetic rat + 12.5 mg/kg F4	75.33 ± 1.33[a]	295.33 ± 5.17[bc]	278.00 ± 4.04[c]	247.33 ± 23.66[c]	244.33 ± 21.69[bc]
Diabetic rat + 12.5 mg/kg F5	81.33 ± 2.33[a]	290.33 ± 2.60[bc]	279.66 ± 2.02[c]	277.33 ± 1.45[c]	271.66 ± 4.70[c]
Diabetic rat + 12.5 mg/kg F6	78.00 ± 5.03[a]	294.33 ± 6.17[bc]	287.00 ± 8.50[c]	279.33 ± 8.41[c]	267.00 ± 6.11[c]
Diabetic rat + 12.5 mg/kg F7	78.33 ± 4.17[a]	284.00 ± 9.07[bc]	274.00 ± 13.01[c]	269.66 ± 12.91[c]	252.33 ± 26.20[bc]
Diabetic rat + 2mg/kg Glibenclamide	75.00 ± 0.57[a]	287.33 ± 14.52[bc]	208.66 ± 3.52[b]	85.66 ± 2.60[ab]	80.33 ± 3.48[a]
Diabetic rat + 10 ml/kg Distilled water	73.00 ± 2.51[a]	273.33 ± 14.52[b]	272.00 ± 10.11[c]	257.33 ± 16.17[c]	223.00 ± 2.51[b]
Non-diabetic rat + 10 ml/kg Distilled water	74.00 ± 0.57[a]	76.00 ± 0.57[a]	74.33 ± 0.88[a]	75.33 ± 3.17[a]	76.00 ± 1.52[a]

Different superscripts along the same column indicate significant difference at $p<0.05$

Table 16: Effect of subfraction 2 of *Cussonia arborea* root bark extract on the fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

	Group	FBG pre induction	FBG 0 h	FBG 1 h	FBG 3 h	FBG 6 h
1	Diabetic rat + 1.25 mg/kg subF2$_1$	75.00±0.57^a	310.00±5.77^b	196.33±3.17^b	81.33±3.17^a	80.00±0.57a
2	Diabetic rat + 1.25 mg/kg subf2$_2$	76.33±0.88^a	313.33±12.01^b	310.33±9.66^c	297.00±3.51^b	298.66±6.35b
3	Diabetic rat + 1.25 mg/kg subf2$_3$	75.42±0.71^a	299.97±10.55^b	305.24±78^c	289.92±7.23^b	291.58±5.45^b
4	Diabetic rat + 2 mg/kg Glibenclamide	76.00±1.52^a	313.33±17.63^b	197.003.51^b	78.33±1.45^a	77.00±0.57^a
5	Diabetic rat + 10 ml/kg Distilled water	76.33±0.88^a	300.00±5.77^b	295.66±6.69^c	296.33±3.17^b	294.00±4.00^b
6	Non-diabetic rat + 10 ml/kg Distilled water	76.00±1.15^a	74.66±1.45^a	74.67±0.88^a	77.33±3.17^a	75.00±1.52^a

Different superscripts along the same column indicate significant difference at $p<0.05$

4.11: Glycosylated haemoglobin {HbA_{1c}} values of alloxan-induced diabetic rats treated with methanol root bark extract of *Cussonia arborea*

The glycosylated haemoglobin values of group 2 rats (125 mg/kg of extract) were significantly ($p<0.05$) lower than that of the group 5 rats (negative control) but was statistically comparable ($p> 0.05$) to those of group 6 rats (Normal control) and to those treated with standard drug (Glibenclemide) 42 days post treatment. On days 56 and 84, all the treatment groups showed significantly ($p< 0.05$) lower values of glycosylated hemoglobin when compared with the negative control groups.

The correlation between the glycosylated hemoglobin and fasting glucose levels indicate that they significantly ($p<0.01$) correlate with each other with r^2 value of 0.914 on day 84 post-treatment. (Mean HbA1c in % (Mean +SEM): 7.328± 1.97; Mean FBS in mg/dl (Mean +SEM: 123.94± 77.83; Pearsons correlation value (0.910) significant at 0.01 level (2 ï tailed); r^2= 0.914) (**Figure 6**).

4.12: Effects of chronic administration of *Cussonia arborea* root bark extract on the activity of aspartate aminotransferase of alloxan-induced diabetic rats

The result indicates that AST activities of the rats in groups 2 and 4 were comparable to those of the group 6 (Normal control) but were found to be significantly ($p< 0.05$) lower than those of the rats in groups 1, 3 and 5 on day 28 post treatment. On day 56, the AST activity of the rats in group 1 compared well with that in group 2 but was still higher than that of the normal control while on day 84, the AST activities of the rats in groups 1, 2 and 4 were comparable to that of the normal control. However, those of the groups 3 and 5 remained significantly ($p< 0.05$) higher than the normal control.

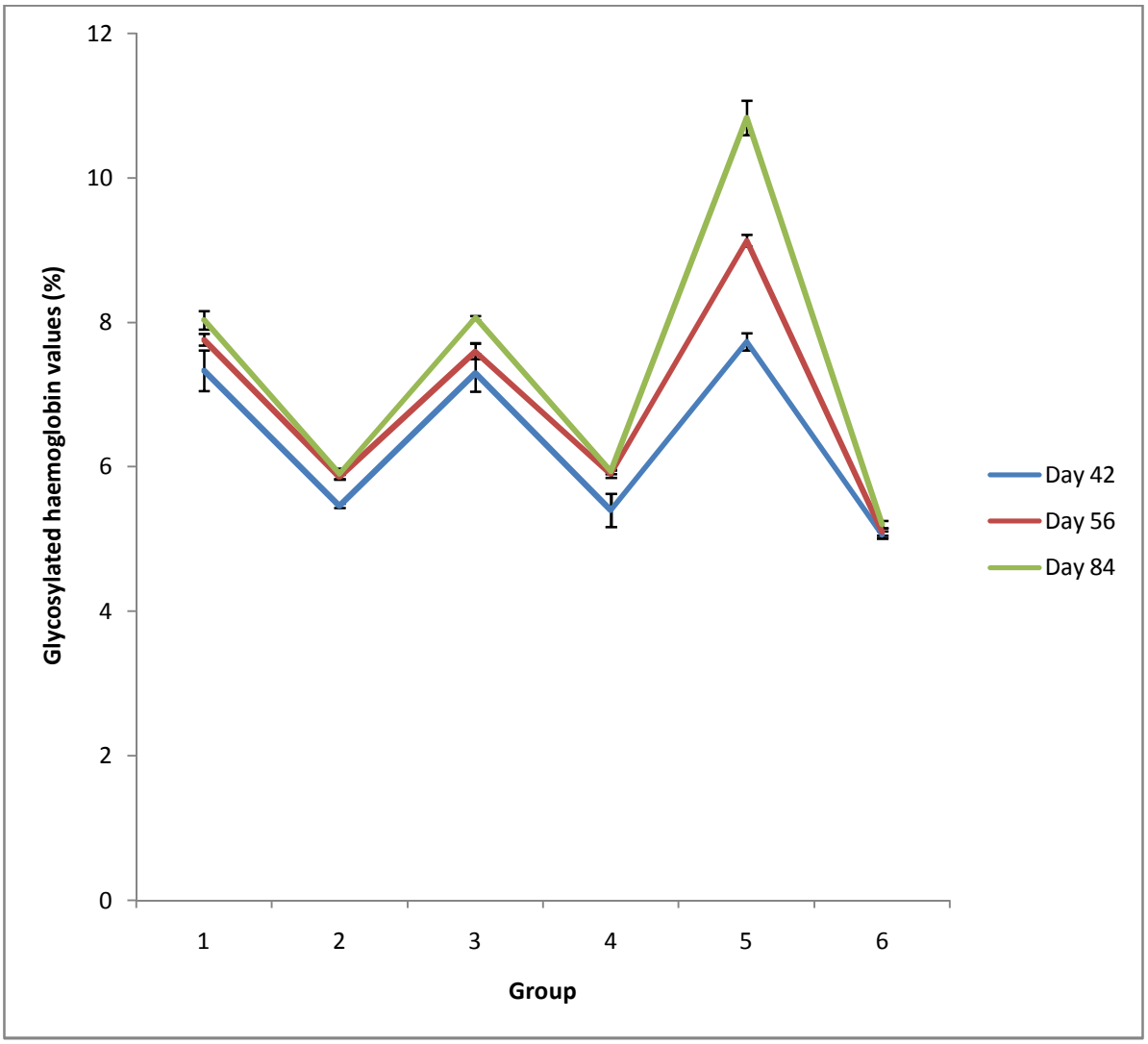


Figure 6: Glycosylated haemoglobin {HbA1c (%)} values of alloxan-induced diabetic rats treated with methanol root bark extract of *Cussonia arborea*

Table 17: Effects of chronic administration of *Cussonia arborea* root bark extract on the activity of aspartate aminotransferase (U/L) of alloxan-induced diabetic rats

GROUP	DAY 28	DAY 56	DAY 84
1	91.94 ± 1.52^{b}	88.93 ± 2.49^{b}	78.28 ± 1.84^{ab}
2	71.45 ± 1.693^{a}	78.42 ± 1.45^{ab}	76.82 ± 0.93^{ab}
3	111.67 ± 6.70^{c}	126.33 ± 5.57^{c}	82.02 ± 1.07^{bc}
4	66.16 ± 3.14^{a}	70.40 ± 2.01^{a}	77.49 ± 2.70^{ab}
5	116.85 ± 5.3^{c}	137.23 ± 4.31^{c}	85.64 ± 2.88^{c}
6	67.60 ± 3.80^{a}	68.62 ± 3.83^{a}	72.29 ± 1.55^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.13: Effects of chronic administration of *Cussonia arborea* root bark extract on the activity of alanine aminotransferase of alloxan-induced diabetic rats

On day 28 post treatment, the ALT activities of the rats in groups, 1 and 3 were statistically comparable (P>0.05) to those of the normal control (Group 6 rats) but was significantly (P < 0.05) lower than the ALT activities of the rats in group 5 (negative control). On day 56, the ALT activities of the rats in groups 2 and 4 compared very well to those in group 6 while those of the rats in group 5 remained significantly higher than those of all the groups till the end of the experiment. On day 84, ALT activities of the rats in all the groups were statistically comparable (p> 0.05) but lower than those of the group 5 (**Table 18**).

4.14: Effects of chronic administration of *Cussonia arborea* root bark extract on total serum Cholesterol of alloxan-induced diabetic rats

Total cholesterol levels of the rats in group 5 were significantly (p<0.05) higher than the cholesterol level of the rats in group 6 (Normal control) and those of the other groups across the treatment periods. On days 56 and 84 post treatment, the total cholesterol levels of groups 2 and 4 rats were statistically comparable (p> 0.05) to the normal control rats but significantly (p<0.05) lower than those of the groups 1 and 3 rats (**Table 19**).

Table 18: Effects of chronic administration of *Cussonia arborea* root bark extract on the activity of alanine aminotransferase (U/L) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	35.82± 0.31^{ab}	37.52± 0.61^{b}	35.23± 0.35^{a}
2	36.85± 1.48^{b}	32.95± 0.97^{a}	32.32± 0.54^{a}
3	35.73± 1.06^{ab}	40.25± 0.77^{c}	38.99± 0.61^{a}
4	31.86± 0.57^{a}	32.09± 0.88^{a}	32.95± 0.36^{a}
5	43.68± 2.73^{c}	50.99± 0.73^{d}	51.30± 6.54^{b}
6	31.44± 0.67^{a}	31.92± 1.07^{a}	32.60± 0.46^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 19: Effects of chronic administration of *Cussonia arborea* root bark extract on total serum Cholesterol (mg/dl) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	149.48±1.04^{c}	148.83±1.47^{b}	142.92± 1.27^{b}
2	141.20± 0.92^{b}	135.33± 1.74^{a}	133.04± 1.49^{a}
3	149.70± 1.39^{c}	162.79± 3.84^{c}	153.49± 1.55^{c}
4	135.22± 3.28^{ab}	134.68±1.73^{a}	134.08± 1.41^{a}
5	167.86± 0.37^{d}	180.11 ± 1.65^{d}	168.47± 0.52^{d}
6	130.37± 4.03^{d}	130.41 ± 2.95^{a}	131.00± 1.20^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.15: Effects of chronic administration of *Cussonia arborea* root bark extract on serum triglycerides (mg/dl) of alloxan-induced diabetic rats

The table indicates that the triglyceride levels of the rats in group 6 (Normal rats) were statistically lower than those in other groups on day 28 post treatment. However, on day 56 and 84, it was comparable ($p > 0.05$) to those in groups 2 and 4. The triglyceride levels of the rats in group 5 were significantly higher than those of the other groups throughout the experiment period (**Table 20**).

4.16: Effects of chronic administration of *Cussonia arborea* root bark extract on high density lipoprotein of alloxan-induced diabetic rats

The results of the high density lipoprotein (HDL) indicate that the HDL levels of rats in group 2 were significantly ($p < 0.05$) higher than those of the rats in groups 1,3 and 5 but were comparable to the triglyceride levels of rats in group 4 across the treatment periods (**Figure 7**).

4.17: Effects of chronic administration of *Cussonia arborea* root bark extract on very low density lipoprotein of alloxan-induced diabetic rats

Table 21 shows that the VLDL levels of rats in group 2 were significantly ($p < 0.05$) lower than those of the rats in groups 1, 3 and 5 on days 28, 56 and 84 but comparable ($p > 0.05$) to those in group 6 (Normal control rats). The VLDL levels of rats in group 5 were significantly ($p < 0.05$) higher than those of the other groups throughout the period of the experiment (**Table 21**).

Table 20: Effects of chronic administration of *Cussonia arborea* root bark extract on serum triglycerides (mg/dl) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	173.33± 2.84^{c}	171.33± 1.85^{b}	161.33± 1.85^{c}
2	165.00± 2.08^{b}	151.66± 4.17^{a}	148.00± 0.57^{a}
3	184.66±2.96^{d}	197.66± 1.45^{c}	187.66± 2.02^{d}
4	164.66± 1.20^{b}	154.00± 2.08^{a}	153.00± 1.52^{b}
5	206.00± 1.15^{e}	212.66± 1.76^{d}	200.00± 0.57^{e}
6	151.33± 0.33^{a}	151.33± 0.66^{a}	152.00± 0.57^{ab}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

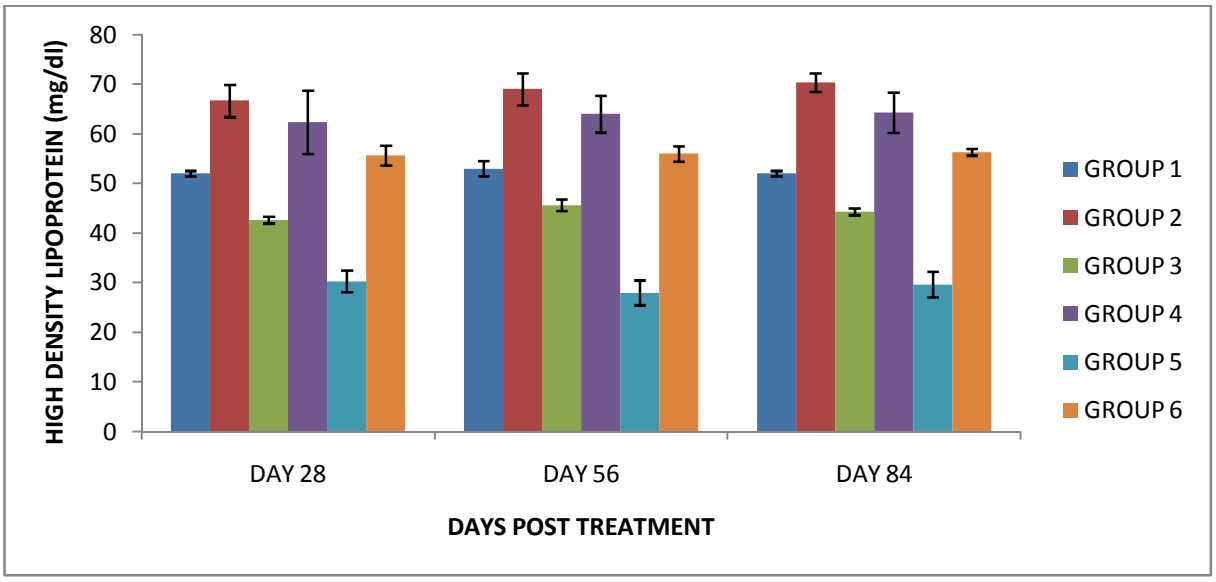


Fig. 7: Effects of chronic administration of *Cussonia arborea* root bark extract on High density lipoprotein (mg/dl) of alloxan-induced diabetic rats

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 21: Effects of chronic administration of *Cussonia arborea* root bark extract on very low density lipoprotein (mg/dl) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	34.66 ± 0.56^{c}	34.26 ± 0.37^{b}	32.26 ± 0.37^{c}
2	30.33 ± 0.29^{b}	30.33 ± 0.83^{a}	29.60 ± 0.11^{a}
3	36.93 ± 0.59^{d}	39.53 ± 0.29^{c}	37.53 ± 0.41^{d}
4	28.93 ± 0.24^{a}	30.80 ± 0.41^{a}	30.60 ± 0.30^{b}
5	41.20 ± 0.23^{e}	42.53 ± 0.35^{d}	40.00 ± 0.11^{e}
6	31.26 ± 0.52^{b}	30.26 ± 0.13^{a}	30.40 ± 0.12^{ab}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.18: Effects of chronic administration of *Cussonia arborea* root bark extract on low density lipoprotein of alloxan-induced diabetic rats

Low density liproprotein levels of the rats in groups 2 and 4 were statistically comparable to those of the normal control (Group 6) rats and significantly ($p< 0.05$) lower than those in groups 1 and 3 throughout the experimental period of 84 days (**Table 22**).

4.19: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total protein of alloxan-induced diabetic rats

The total protein level of group 5 rat shows a significant ($p< 0.05$) reduction from those of the normal control rats and other group on days 56 and 84 while the total protein levels of the rats in group 2 were statistically comparable to those of the normal control rats on these days (**Table 23**).

4.20: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total albumin of alloxan-induced diabetic rats

On day 28, the albumin levels of the rats in all the groups were statistically comparable. Albumin levels of rats in groups 1, 2, 4 and 6 also compared very well on day 56 but were significantly higher than those of group 5 rats. However on day 84, the albumin levels of the rats in group 1, 3, 4 and 5 were comparable but significantly ($p< 0.05$) lower than those of groups 2 and 6 (**Table 24**).

Table 22: Effects of chronic administration of *Cussonia arborea* root bark extract on low density lipoprotein (mg/dl) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	62.83± 1.86^{b}	61.06± 3.01^{b}	56.66± 0.65^{c}
2	44.20± 4.00^{a}	40.56 ± 1.18^{a}	33.11± 0.84^{a}
3	70.10± 1.21^{b}	77.13± 3.09^{c}	71.62± 1.94^{d}
4	43.93± 7.68^{a}	39.66± 5.78^{a}	39.15± 5.01^{ab}
5	96.33± 2.14^{c}	108.66± 0.88^{d}	98.80± 3.08^{e}
6	43.43± 6.15^{a}	44.00±4.56^{a}	46.27± 1.59^{b}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 23: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total protein (g/dL) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	5.27 ± 0.41^{b}	5.13 ± 0.12^{bc}	3.96 ± 0.03^{b}
2	5.57 ± 0.13^{c}	5.59 ± 0.10^{d}	5.43 ± 0.26^{cd}
3	4.79 ± 0.15^{ab}	4.81 ± 0.09^{b}	3.95 ± 0.03^{b}
4	516 ± 0.09^{bc}	5.25 ± 0.22^{cd}	5.01 ± 0.01^{c}
5	4.34 ± 0.17^{a}	4.0 ± 0.01^{a}	3.30 ± 0.15^{a}
6	5.41 ± 0.14^{c}	5.61 ± 0.12^{d}	5.96 ± 0.13^{d}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 24: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total albumin (g/dL) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	2.60 ± 0.25^{a}	2.89 ± 0.06^{bc}	2.18 ± 0.15^{b}
2	2.70 ± 0.15^{a}	3.00 ± 0.00^{bc}	3.04 ± 0.03^{c}
3	2.86 ± 0.13^{a}	2.58 ± 0.29^{ab}	1.69 ± 0.16^{a}
4	2.59 ± 0.21^{a}	3.04 ± 0.03^{bc}	3.04 ± 0.03^{c}
5	2.59 ± 0.29^{a}	2.33 ± 0.16^{a}	1.87 ± 0.18^{ab}
6	2.88 ± 0.32^{a}	3.34 ± 0.14^{c}	3.31 ± 0.13^{c}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.21: Effects of chronic administration of *Cussonia arborea* root bark extract on serum globulin of alloxan-induced diabetic rats

Groups 1, 2, 4 and 5 rats have comparable globulin levels on day 28. This was significantly ($p< 0.05$) higher than those of the normal control rats. The globulin levels of the normal control rats (Rats in group 6) were significantly ($p< 0.05$) higher than those of the negative control rats (group 5 rats) but where statistically comparable to those of the other groups (**Table 25**).

4.22: Effects of chronic administration of *Cussonia arborea* root bark extract on serum albumin: globulin ratio of alloxan-induced diabetic rats

There was no significant differences except on the day 28 when the rats in group 1 appear to have a significantly ($p< 0.05$) lower albumin: globulin ratio compared to normal control rats (**Table 26**).

4.23: Effects of chronic administration of *Cussonia arborea* root bark extract on blood urea nitrogen (BUN) (mg/dl) of alloxan-induced diabetic rats

The elevated BUN levels of group 5 rats were significantly ($p< 0.05$) higher than those of the rats in other groups including the normal control rats throughout the treatment period. The BUN of rats in groups 1, 2 and 4 were comparable to that of the normal control rats throughout the duration of the experiment (**Figure 8**).

Table 25: Effects of chronic administration of *Cussonia arborea* root bark extract on serum globulin (g/dL) of alloxan-induced diabetic rats

Group	DAY 28	DAY56	DAY 83
1	2.66 ± 0.37^{c}	2.24 ± 0.66^{ab}	1.78 ± 0.12^{ab}
2	2.87 ± 0.01^{c}	2.59 ± 6.10^{b}	2.39 ± 0.23^{c}
3	1.92 ± 0.03^{ab}	2.23 ± 0.28^{ab}	2.25 ± 0.14^{bc}
4	2.56 ± 0.24^{bc}	2.21 ± 0.23^{ab}	1.96 ± 6.03^{abc}
5	2.52 ± 0.22^{bc}	1.66 ± 0.16^{a}	1.43 ± 0.29^{a}
6	1.75 ± 0.11^{a}	2.27 ± 0.09^{b}	2.38 ± 0.05^{c}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 26: Effects of chronic administration of *Cussonia arborea* root bark extract on serum albumin: globulin ratio of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	0.93 ± 0.05^{a}	1.16 ± 0.03^{a}	1.29 ± 0.12^{a}
2	1.02 ± 0.21^{ab}	1.30 ± 0.00^{a}	1.24 ± 0.18^{a}
3	1.48 ± 0.06^{ab}	1.20 ± 0.26^{a}	0.76 ± 0.12^{a}
4	1.04 ± 0.17^{ab}	1.40 ± 0.15^{a}	1.55 ± 0.04^{a}
5	1.51 ± 0.24^{b}	1.46 ± 0.23^{a}	1.49 ± 0.46^{a}
6	1.25 ± 0.13^{ab}	1.50 ± 0.11^{a}	1.51 ± 0.13^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

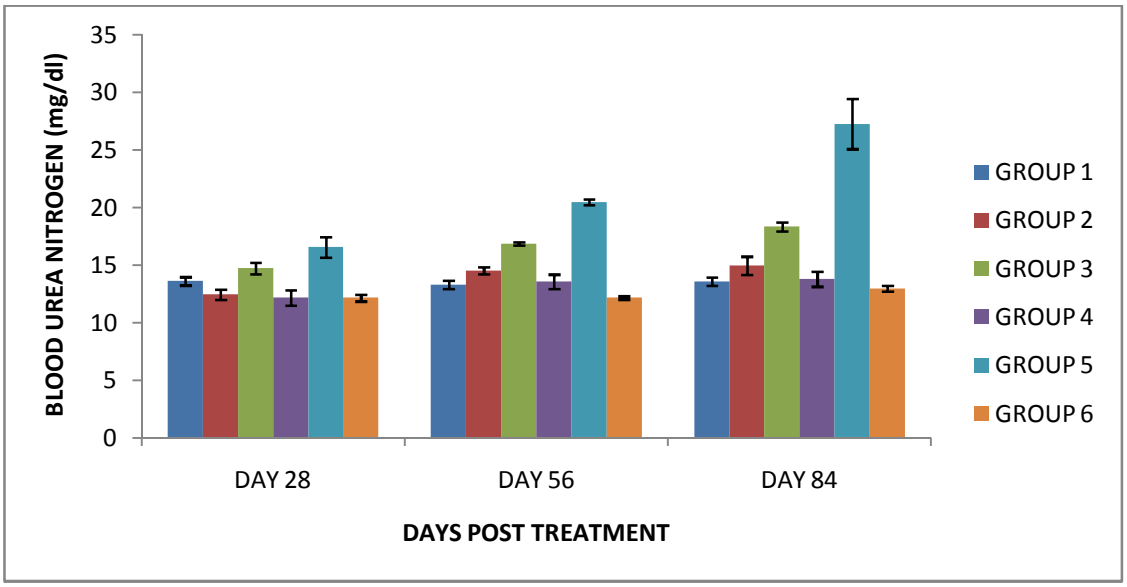


Fig. 8: Effects of chronic administration of *Cussonia arborea* root bark extract on blood urea nitrogen (BUN) (mg/dl) of alloxan-induced diabetic rats

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.24: Effects of chronic administration of *Cussonia arborea* root bark extract on serum creatinine of alloxan-induced diabetic rats

The creatinine levels of the rats in group 5 were significantly ($p<0.05$) elevated compared to that of the other groups throughout the period of the experiment. On day 28, groups 1, 2, 3 and 4 ratsô creatinine compared very well among one another but were significantly ($p<0.05$) higher than that of the normal control rats. The creatinine levels of groups 2, and 4 rats were statistically comparable to that of the group 6 rats on days 56 and 84 (**Table 27**).

4.25: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total bilirubin of alloxan-induced diabetic rats

The total bilirubin levels of the rats in group 5 were significantly ($p< 0.05$) elevated compared to the normal control rats and other groups throughout the experimental period. Groups 2, and 4 rats have comparable ($p> 0.05$) total bilirubin values as seen on day 56. The total bilirubin levels of groups 1, 2 and 4 rats compare very well among one another on day 84 but were significantly ($p< 0.05$) higher than that of the normal control (**Table 28**).

4.26: Effects of chronic administration of *Cussonia arborea* root bark extract on serum conjugated bilirubin of alloxan-induced diabetic rats

The conjugated bilirubin levels of rats in group 5 were significantly ($p<0.05$) different from those of the other groups on day 28 but compared very well with 1, 3, 4 and 5 on days 56 and 84 post treatment. On day 56, the conjugated bilirubin levels of group 2 rats were significantly ($p<0.05$) higher than that of the groups 1, 3 and 5 but statistically comparable to those of the normal control rats (**Figure 9**).

Table 27: Effects of chronic administration of *Cussonia arborea* root bark extract on serum creatinine (mg/dl) of alloxan-induced diabetic rats

Group	DAY28	DAY 56	DAY84
1	0.70 ± 0.00^{b}	0.80 ± 0.00^{bc}	0.82 ± 0.01^{b}
2	0.72 ± 0.01^{b}	0.73 ± 0.00^{ab}	0.69 ± 0.00^{a}
3	0.77 ± 0.02^{bc}	0.83 ± 0.02^{c}	0.80 ± 0.00^{b}
4	0.72 ± 0.03^{b}	0.71 ± 0.01^{a}	0.71 ± 0.01^{a}
5	0.82 ± 0.02^{d}	0.98 ± 0.04^{d}	1.12 ± 0.04^{c}
6	0.62 ± 0.02^{a}	0.66 ± 0.01^{a}	0.67 ± 0.000^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 28: Effects of chronic administration of *Cussonia arborea* root bark extract on serum total bilirubin (mg/dL) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	0.67 ± 0.09^{ab}	0.99 ± 0.01^{c}	0.91 ± 0.02^{b}
2	0.78 ± 0.01^{bc}	0.87 ± 0.01^{bc}	0.81 ± 0.1^{b}
3	0.97 ± 0.03^{c}	1.15 ± 0.08^{d}	1.063 ± 0.07^{c}
4	0.63 ± 0.09^{ab}	0.80 ± 0.01^{b}	0.82 ± 0.02^{b}
5	1.60 ± 0.11^{d}	1.71 ± 0.09^{e}	1.92 ± 0.04^{d}
6	0.50 ± 0.04^{a}	0.54 ± 0.02^{a}	0.56 ± 0.00^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

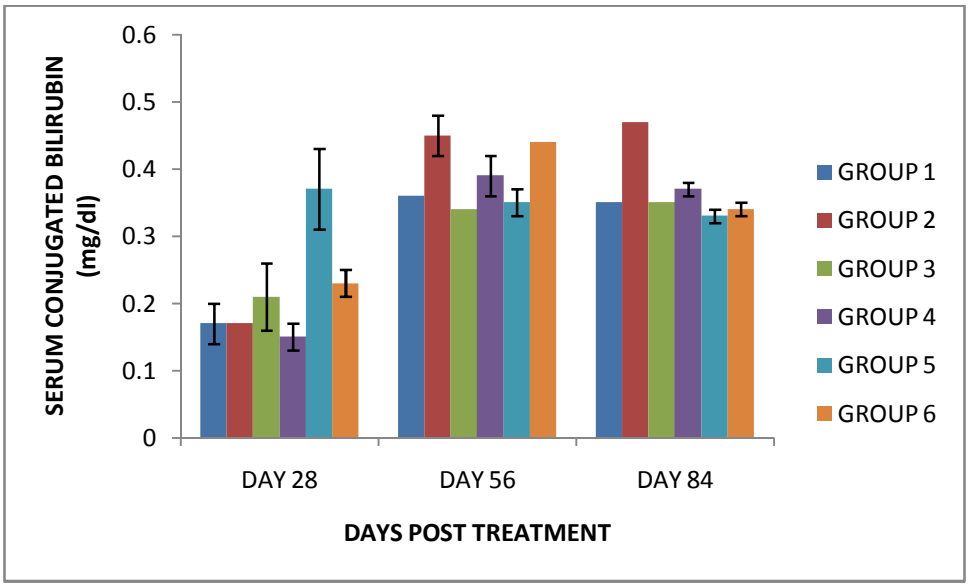


Fig. 9: Effects of chronic administration of *Cussonia arborea* root bark extract on serum conjugated bilirubin (mg/dL) of alloxan-induced diabetic rats

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.27: Effects of chronic administration of *Cussonia arborea* root bark extract on serum unconjugated bilirubin of alloxan-induced diabetic rats

Unconjugated bilirubin levels of the normal control rats compared very well with those of the rats in groups 2 and 4 on day 28 but differed significantly ($p< 0.05$) from those of the other groups. On days 56 and 84, the unconjugated bilirubin levels of the normal control rats were significantly ($p< 0.05$) lower than those of other groups (**Table 29**).

4.28: Effects of chronic administration of *Cussonia arborea* root bark extract on the ratios of serum AST: ALT activities of alloxan-induced diabetic rats

The AST: ALT ratio of the rats in groups 2 and 4 compared with those of the rats in group 6 (Normal control) throughout the experiment but were significantly ($p< 0.05$) higher than those of the negative control group on day 28 (**Table 30**).

4.29: Effects of chronic administration of *Cussonia arborea* root bark extract on BUN: Creatinine ratio of alloxan-induced diabetic rats

The Blood urea nitrogen: Creatinine ratio of the rats in group 5 (Negative control) were statistically comparable to that of the normal control rats and to other rats in other groups but was significantly ($p<0.05$) higher than those of the group 4 rats on day 28 post treatment. Groups 1, 2 and 4 ratsô BUN: creatinine ratio were statistically comparable to the normal control rats on day 84 but were significantly ($p< 0.05$) lower than those of the other groups (**Table 31**).

Table 29: Effects of chronic administration of *Cussonia arborea* root bark extract on serum unconjugated bilirubin (mg/dL) of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	0.61 ± 0.00^{bc}	0.41 ± 0.01^{b}	0.34 ± 0.02^{b}
2	0.50 ± 0.05^{abc}	0.63 ± 0.00^{c}	0.56 ± 0.01^{c}
3	0.76 ± 0.08^{c}	0.81 ± 0.07^{d}	0.71 ± 0.06^{d}
4	0.48 ± 0.08^{ab}	0.41 ± 0.05^{b}	0.44 ± 0.01^{b}
5	1.23 ± 0.14^{d}	1.38 ± 0.07^{e}	1.59 ± 0.03^{e}
6	0.27 ± 0.02^{a}	0.09 ± 0.02^{a}	0.22 ± 0.01^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 30: Effects of chronic administration of *Cussonia arborea* root bark extract on the ratios of serum AST: ALT activities of alloxan-induced diabetic rats

Group	DAY 28	DAY 56	DAY 84
1	1.94 ± 0.08^{a}	2.38 ± 0.11^{a}	2.37 ± 0.06^{b}
2	2.56 ± 0.06^{b}	2.36 ± 0.02^{a}	2.21 ± 0.06^{bc}
3	3.12 ± 0.16^{c}	3.14 ± 0.17^{c}	2.10 ± 0.01^{b}
4	2.07 ± 0.07^{a}	2.19 ± 0.02^{a}	2.35 ± 0.08^{b}
5	1.94 ± 0.13^{a}	2.69 ± 0.08^{b}	1.89 ± 0.08^{a}
6	2.91 ± 1.88^{bc}	2.14 ± 0.04^{a}	2.21 ± 0.07^{bc}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 31: Effects of chronic administration of *Cussonia arborea* root bark extract on BUN: creatinine ratio of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	18.89±0.46^{ab}	18.13±0.56^{a}	19.58±0.68^{a}
2	17.68±0.62^{ab}	18.13±0.23^{a}	18.27±1.21^{a}
3	19.01±0.21^{ab}	20.16±0.67^{ab}	22.91±0.31^{b}
4	16.83±1.51^{a}	18.94±0.98^{ab}	19.41±1.00^{a}
5	20.16±0.60^{b}	20.86±0.68^{b}	24.20±1.18^{b}
6	19.55±1.06^{ab}	18.23±6.31^{a}	19.42±0.29^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.30: Effects of chronic administration of *Cussonia arborea* root bark extract on red blood cell count of alloxan-induced diabetic rats

The red blood cell counts of the normal control rats (Group 6) were significantly ($p< 0.05$) higher than those of the other groups on days 28 and 56 post treatment. On day 84 however, the RBC counts of rats in groups 2 and 4 were statistically comparable ($p> 0.05$) to that of the normal control rats (**Table 32**).

4.31: Effects of chronic administration of *Cussonia arborea* root bark extract on total haemoglobin concentration (g/dL) of alloxan-induced diabetic rats

The total haemoglobin of diabetic untreated rats (Group 5) was significantly ($p< 0.05$) lower than the total haemoglobin of other rats in other group throughout the experiment. The rats in groups 2 and 4 have their total haemoglobin values statistically comparable ($p> 0.05$) to those of the normal control rats (Group 6) on days 56 and 84 post treatment (**Table 33**).

4.32: Effects of chronic administration of *Cussonia arborea* root bark extract on the packed cell volume of alloxan-induced diabetic rats

The packed cell volume of the rats in group 6 was significantly ($p< 0.05$) higher than the negative control (Group 5) and other groups throughout the period of the experiment except on day 84 when the values were statistically comparable ($p> 0.05$) to the packed cell volume value of the rats in group 2 (**Figure 10**).

Table 32: Effects of chronic administration of *Cussonia arborea* root bark extract on red blood cell count X 10^6 (cells/mm^3) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	5.81 ± O.03^b	5.18 ± 0.52^b	6.93 ± 0.03^c
2	6.61 ± 0.15 ^{c}	6.96 ± 0.03c	7.53 ± 0.03 ^{d}
3	4.92 ± 0.03^a	5.31 ± 0.28^b	6.41 ± 0.26^b
4	6.72 ± 0.03 ^{c}	6.96 ± 0.03^c	7.46 ± 0.03^d
5	4.83 ± 0.08^a	3.86 ± 0.06^a	3.60 ± 0.10^a
6	7.45 ± -.-3^d	7.76 ± 0.08^d	7.63 ± 0.03^d

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 33: Effects of chronic administration of *Cussonia arborea* root bark extract on total haemoglobin concentration (g/dL) of alloxan-induced diabetic rats

Group	Day 28	**Day 56**	**Day 84**
1	11.62 ± 0.10 ^{b}	11.80 ± 0.17 ^{c}	13.46 ± 0.15^{bc}
2	13.03 ± 0.26^{c}	13.93 ± 0.03^{d}	14.10 ± 0.15b^{c}
3	10.99 ± 0.34 ^{ab}	10.99 ± 0.40^{b}	13.26 ± 0.31^{b}
4	12.59 ± 0.21^{c}	13.49 ± 0.08^{d}	14.13 ± 0.18^{bc}
5	10.60 ± 0.34^{a}	10.06 ± 0.29^{a}	8.83 ± 0.44^{a}
6	14.13 ± 0.18^{d}	14.16 ± 0.16^{d}	14.33 ± 0.16^{c}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

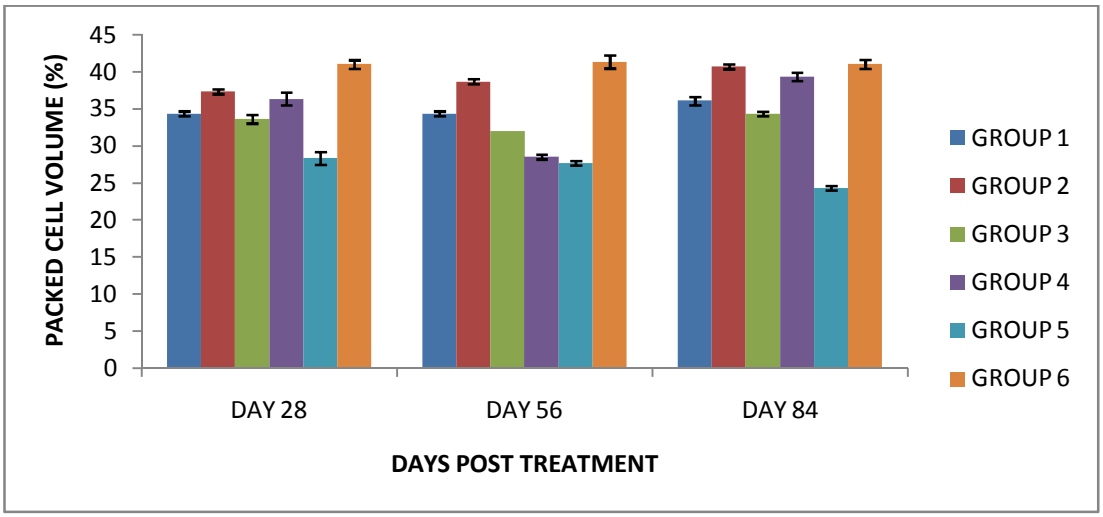


Fig. 10: Effects of chronic administration of *Cussonia arborea* root bark extract on the packed cell volume (%) of alloxan-induced diabetic rats

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.33: Effects of chronic administration of *Cussonia arborea* root bark extract on mean corpuscular volume of alloxan-induced diabetic rats

The mean corpuscular volume of groups 2 and 4 rats were statistically comparable ($p > 0.05$) to those of the negative and normal control groups on day 28. On days 56 and 84, the MCV values of groups 2, 3, 4 and 6 were statistically comparable to one another but were significantly lower than that of the negative control group (**Table 34**).

4.34: Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin concentration of alloxan-induced diabetic rats

No significant differences were observed among all the groups throughout the duration of the experiment (**Table 35**).

4.35: Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin of alloxan-induced diabetic rats

The mean corpuscular haemoglobin of all the rats in group 1,2 ,3, and 4 were significantly ($p < 0.05$) lower than that of the group 5 (Negative control rats) but statistically comparable ($p > 0.05$) to that of group 6 rats on days 56 and 84 post treatment (**Table 36**).

4.36: Effects of chronic administration of *Cussonia arborea* root bark extract on total White blood cell count of alloxan-induced diabetic rats

The total white blood cell counts of rats in groups 1 and 2 were significantly ($p < 0.05$) higher than that of the negative control group but were statistically comparable ($p > 0.05$) to that of the normal control rats on days 56 and 84 post treatment (**Table 37**).

Table 34: Effects of chronic administration of *Cussonia arborea* root bark extract on mean corpuscular volume (fl) of alloxan-induced diabetic rats

Group	Day 28	**Day 56**	**Day 84**
1	58.67 ± 0.88^{b}	67.33 ± 6.96^{b}	51.93 ± 6.67^{a}
2	56.00 ± 1.00^{ab}	55.33 ± 0.67^{a}	54.53 ± 0.57^{ab}
3	67.00 ± 1.73^{c}	61.33 ± 3.28^{ab}	53.46 ± 1.46^{ab}
4	54.00 ± 1.15^{a}	55.00 ± 0.58^{a}	52.56 ± 0.29^{ab}
5	58.33 ± 1.85^{ab}	71.33 ± 0.88^{b}	67.40 ± 0.65^{c}
6	54.66 ± 0.8^{ab}	53.33 ± 0.67^{a}	54.90 ± 1.02^{b}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 35: Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin concentration (g/dL) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	33.86 ± 0.12[a]	34.36 ± 0.49[a]	37.00 ± 2.00[a]
2	34.90 ± 0.41[a]	36.03 ± 0.29[a]	34.33 ± 0.66[a]
3	33.46 ± 1.53[a]	34.03 ± 1.54[a]	38.00 ± 1.00[a]
4	34.66 ± 0.29[a]	35.20 ± 0.20[a]	35.33 ± 0.33[a]
5	34.26 ± 3.69[a]	36.40 ± 1.44[a]	36.00 ± 1.52[a]
6	34.50 ± 0.94[a]	34.43 ± 0.93[a]	35.00 ± 0.57[a]

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 36: Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin (pg) of alloxan-induced diabetic rats

Group	Day 28	**Day 56**	**Day 84**
1	19.96 ± 0.31[a]	23.46 ± 2.68[ab]	19.36 ± 072[a]
2	19.73 ± 0.49[a]	20.03 ± 0.13[ab]	18.70 ± 0.26[a]
3	22.30 ± 0.26[b]	20.73 ± 1.08[ab]	20.73 ± 0.97[a]
4	18.73 ± 0.26[a]	19.36 ± 0.14[a]	18.90 ± 0.21[a]
5	21.93 ± 0.89[b]	25.83 ± 0.60[c]	24.00 ± 1.00[b]
6	18.63 ± 0.37[a]	18.20 ± 0.25[a]	18.76 ± 0.33[a]

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 37: Effects of chronic administration of *Cussonia arborea* root bark extract on total White blood cell count X 10^3 (cells/mm^3) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	7.13 ± 0.03^{b}	7.16 ± 0.03^{bc}	7.20 ± 0.06^{bc}
2	7.10 ± 0.05^{b}	7.13 ± 0.03^{bc}	7.27 ± 0.08^{c}
3	7.03 ± 0.03^{ab}	7.13 ± 0.03^{bc}	9.97 ± 0.12^{ab}
4	6.93 ± 0.63^{a}	7.10 ± 0.05^{b}	6.96 ± 0.06^{ab}
5	7.00 ± 0.06^{ab}	6.90 ± 0.05^{a}	6.90 ± 0.05^{a}
6	7.10 ± 0.05^{b}	7.26 ± 0.06^{c}	7.27 ± 0.09^{c}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.37: Effects of chronic administration of *Cussonia arborea* root bark extract on lymphocyte count of alloxan-induced diabetic rats

On day 28, the lymphocytes of groups 1, 2, 3 and 4 rats were significantly ($p<0.05$) higher than that of group 5 rats. On days 56 and 84 however, they were statistically comparable (**Table 38**).

4.38: Effects of chronic administration of *Cussonia arborea* root bark extract on neutrophil count of alloxan-induced diabetic rats

No significant changes were observed in the neutrophil counts of all the rats in the treatment groups throughout the duration of the experiment. The neutrophil count of the rats in group 5 was significantly ($p< 0.05$) higher than that of the normal control rats except on day 84 (**Table 39**).

4.39: Effects of chronic administration of *Cussonia arborea* root bark extract on Basophil count of alloxan-induced diabetic rats

No significant changes were observed throughout the duration of the experiment (**Table 40**).

4.40: Effects of chronic administration of *Cussonia arborea* root bark extract on Eosinophil count of alloxan-induced diabetic rats

No significant changes were observed except on the day 84, where the eosinophil counts of group 6 rats were significantly ($p< 0.05$) higher than that of the negative control rats (Group 5) **(Table 41).**

4.41: Effects of chronic administration of *Cussonia arborea* root bark extract on Monocyte (%) count of alloxan-induced diabetic rats

No significant changes were observed in the monocyte counts of the rats in all the groups across the treatment period except for rats in group 3 which had a significantly ($p< 0.05$) higher monocyte count on day 28 when compared with the negative control group (**Table 42**).

Table 38: Effects of chronic administration of *Cussonia arborea* root bark extract on lymphocyte count (%) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	57.66 ± 0.66^{b}	59.00 ± 0.57^{ab}	58.33 ± 0.67^{ab}
2	58.33 ± 0.33^{b}	59.33 ± 0.66^{ab}	59.33 ± 0.57^{b}
3	59.00 ± 0.57^{b}	60.00 ± 0.56^{ab}	57.33 ± 0.33^{a}
4	58.33 ± 0.32^{b}	59.00 ± 0.58^{ab}	57.66 ± 0.33^{ab}
5	56.00 ± 0.58^{a}	61.33 ± 1.20^{b}	57.06 ± 0.56^{a}
6	58.00 ± 0.56^{b}	58.00 ± 0.58^{a}	58.33 ± 0.33^{ab}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 39: Effects of chronic administration of *Cussonia arborea* root bark extract on Neutrophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	40.66 ± 0.33^{a}	39.33 ± 0.33^{ab}	40.00 ± 0.56^{a}
2	41.33 ± 0.67^{a}	39.66 ± 0.33^{ab}	40.66 ± 0.66^{a}
3	40.60 ± 0.58^{a}	39.00 ± 0.57^{ab}	41.33 ± 0.23^{a}
4	40.66 ± 0.58^{b}	39.66 ± 0.33^{ab}	41.00 ± 0.58^{a}
5	43.00 ± 0.58^{b}	37.33 ± 1.45^{a}	41.67 ± 0.33^{a}
6	41.00 ± 0.058^{a}	40.66 ± 0.66^{b}	40.33 ± 0.33^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 40: Effects of chronic administration of *Cussonia arborea* root bark extract on Basophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.03 ± 0.03^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2	0.07 ± 0.03^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}
3	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}
4	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}
5	0.07 ± 0.06^{a}	0.06 ± 0.03^{a}	0.03 ± 0.03^{a}
6	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 41: Effects of chronic administration of *Cussonia arborea* root bark extract on eosinophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.73 ± 0.03^{a}	0.70 ± 0.05^{a}	0.73 ± 0.03^{abc}
2	0.73 ± 0.06^{a}	0.97 ± 0.22^{a}	0.67 ± 0.06^{abc}
3	0.70 ± 0.06^{a}	0.70 ± 0.06^{a}	0.60 ± 0.05^{ab}
4	0.73 ± 0.03^{a}	0.90 ± 0.15^{a}	1.03 ± 0.21^{c}
5	0.80 ± 0.05^{a}	0.87 ± 0.06^{a}	0.56 ± 0.03^{a}
6	0.77 ± 0.03^{a}	1.00 ± 0.25^{a}	1.00 ± 0.20^{bc}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 42: Effects of chronic administration of *Cussonia arborea* root bark extract on Monocyte (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.23 ± 0.03^{ab}	0.30 ± 0.05^{a}	0.27 ± 0.03^{a}
2	0.20 ± 0.05^{ab}	0.33 ± 0.13^{a}	0.30 ± 0.06^{a}
3	0.27 ± 0.03^{b}	0.27 ± 0.03^{a}	0.37 ± 0.03^{ab}
4	0.23 ± 0.03^{ab}	0.40 ± 0.20^{a}	0.60 ± 0.10^{b}
5	0.13 ± 0.03^{a}	0.40 ± 0.25^{a}	0.36 ± 0.3^{ab}
6	0.23 ± 0.03^{ab}	0.33 ± 0.08^{a}	0.37 ± 0.12^{ab}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.42: Effects of chronic administration of *Cussonia arborea* root bark extract on plasma malondialdehyde of alloxan-induced diabetic rats

The malondialdehyde level of the rats in group 5 (Negative control) was significantly ($p< 0.05$) higher than that of the normal control rats and other groups throughout the experimental period. The rats in group 2 have a statistically comparable ($p> 0.05$) MDA value with the normal control rats on days 56 and 84 post treatment (**Table 43**).

4.43: Effects of chronic administration of *Cussonia arborea* root bark extract on the activities of superoxide dismutase (U/L) of alloxan-induced diabetic rats

The superoxide dismutase activities of the rats in group 6 (Normal control) were significantly ($p< 0.05$) higher than the negative control group and other groups on day 28 post treatment. On days 56 and 84 post treatment however, the SOD activity of the rats in group 2 were statistically comparable to that of the normal control rats but were significantly ($p< 0.05$) higher than that of the other groups. On day 84, the SOD activity of group 4 rats were statistically comparable to both the normal control group and the group 2 rats (**Table 44**).

4.44: Effects of chronic administration of *Cussonia arborea* root bark extract on serum reduced gluthatione (mg/dL) of alloxan-induced diabetic rats

The reduced glutathione values of the normal control rats were significantly ($p<0.05$) higher than that of the negative control rats throughout the period of the experiment. On day 84 post treatment however, the reduced glutathione levels of the groups 2 and 4 rats were statistically comparable ($p> 0.05$) with that of the normal control rats (**Figure 11**).

Table 43: Effects of chronic administration of *Cussonia arborea* root bark extract on plasma malondialdehyde (mg/dL) of alloxan-induced diabetic rats

GROUP	DAY 28	DAY 56	DAY 84
1	6.60 ± 0.11^{d}	6.10 ± 0.10^{c}	6.10 ± 0.25^{b}
2	5.73 ± 0.08^{c}	4.26 ± 0.06^{a}	4.53 ± 0.06^{a}
3	7.26 ± 0.31^{e}	6.73 ± 0.12^{d}	9.27 ± 0.67^{c}
4	5.13 ± 0.09^{b}	4.80 ± 0.15^{b}	4.70 ± 0.05^{a}
5	7.33 ± 6.89^{e}	9.20 ± 0.05^{e}	10.30 ± 0.05^{d}
6	4.33 ± 0.08^{a}	4.30 ± 0.05^{a}	4.50 ± 0.05^{a}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 44: Effects of chronic administration of *Cussonia arborea* root bark extract on the activities of superoxide dismutase (U/L) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day84
1	0.29 ± 0.00^{a}	0.29 ± 0.00^{b}	0.37 ± 0.02^{c}
2	$0.38\pm0.00_{c}$	0.39 ± 0.00^{d}	0.62 ± 0.03^{d}
3	0.29 ± 0.01^{a}	0.28 ± 0.01^{b}	0.28 ± 0.01^{b}
4	0.33 ± 0.01^{b}	0.37 ± 0.02^{c}	0.59 ± 0.01^{d}
5	0.28 ± 0.00^{a}	0.20 ± 0.00^{a}	0.16 ± 0.00^{a}
6	0.57 ± 0.01^{d}	0.57 ± 0.00^{d}	0.59 ± 0.00^{d}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control

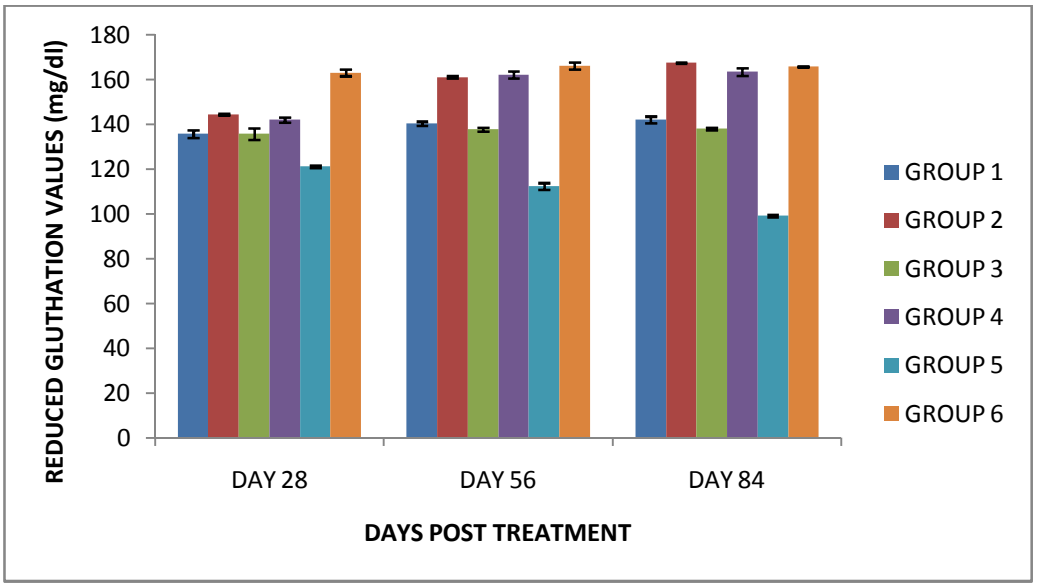


Fig 11: Effects of chronic administration of *Cussonia arborea* root bark extract on serum reduced gluthatione (mg/dL) of alloxan-induced diabetic rats

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.45: Effects of chronic administration of *Cussonia arborea* root bark extract on catalase activity of alloxan-induced diabetic rats

The catalase activity of the negative control rats were significantly ($p< 0.05$) lower than that of the other groups throughout the experimental period. On day 84 post treatment, the catalase activity of the normal control rats and that of the group 2 rats was statistically comparable (**Table 45**).

4.46: Weekly weight (g) changes associated with treatment of alloxan-induced diabetic rats with *Cussonia arborea* extract

The weights of the rats on week zero were all statistically comparable ($p> 0.05$). On week 2 post treatment, the weight of the group 5 rats were significantly ($p< 0.05$) lower than those of the other rats in other groups while the mean weight of the rats in group 6 was significantly ($p< 0.05$) higher than that of the other groups. These persisted till the end of the experiment (**Table 46**).

Table 45: Effects of chronic administration of *Cussonia arborea* root bark extract on catalase (U/L) activity of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	2.61 ± 0.06^{c}	2.71 ± 0.08^{b}	3.62 ± 0.31^{c}
2	3.80 ± 0.21^{e}	4.92 ± 0.03^{d}	5.81 ± 0.03^{e}
3	1.96 ± 6.03^{b}	2.29 ± 0.03^{d}	2.43 ± 6.29^{b}
4	3.35 ± 0.08^{d}	4.33 ± 0.33^{c}	4.59 ± 0.31^{d}
5	1.40 ± 6.00^{a}	6.69 ± 0.30^{a}	0.87 ± 0.06^{a}
6	5.35 ± 0.03^{f}	5.74 ± 0.02^{e}	5.75 ± 0.02^{e}

Different superscripts along the same column indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

Table 46: Weekly weight (g) changes associated with treatment of alloxan-induced diabetic rats with *Cussonia arborea* extract

WEEK	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
WK0	100.46±0.31^{a}	101.33±1.33^{a}	102.00±0.57^{a}	101.67±1.67^{a}	102.00±0.57^{a}	101.67±1.67^{a}
Wk 1	102.00±0.57^{ab}	101.33±0.88^{ab}	102.00±1.15^{ab}	102.33±1.85^{b}	98.00±1.52^{a}	105.33±0.88^{c}
Wk2	102.33±0.33^{b}	104.00±0.57^{b}	101.66±0.66^{b}	107.00±1.15^{b}	92.80±1.15^{a}	117.00±3.5^{c}
Wk3	104.67±0.88^{b}	110.66±1.20^{c}	104.00±0.57^{b}	112.00±1.52^{c}	90.00±0.57^{a}	128.33±2.72^{d}
Wk4	106.00±2.51^{b}	119.00±1.52^{c}	106.33±2.33^{b}	119.80±2.51^{c}	87.00±0.57^{a}	137.66±1.20^{d}
Wk5	108.33±1.20^{b}	124.33±3.38^{c}	109.00±1.15^{b}	122.33±1.85^{c}	85.66±0.88^{a}	153.33±2.40^{d}
Wk6	110.33±1.33^{6}	128.66±1.20^{c}	111.67±1.20^{b}	127.66±0.66^{c}	84.00±0.58^{a}	173.66±0.88^{d}
Wk7	116.66±1.45^{b}	133.00±0.57^{c}	118.00±1.15^{b}	132.66±1.45^{c}	80.33±0.33^{a}	182.66±1.76^{d}
Wk8	121.33±0.66^{b}	135.33±1.45^{c}	122.00±0.57^{b}	135.67±0.88^{c}	80.66±0.33^{a}	193.33±1.76^{d}
Wk9	123.66±1.85^{b}	144.00±1.15^{c}	125.33±0.88^{b}	141.33±0.88^{c}	82.00±0.57^{a}	200.00±0.57^{d}
Wk10	127.66±0.88^{b}	144.66±0.33^{c}	127.00±0.58^{b}	146.00±0.58^{c}	80.66±0.33^{a}	212.33±1.20^{d}
Wk11	129.66±0.88^{b}	153.66±1.20^{c}	128.33±0.33^{b}	152.66±1.45^{c}	78.33±0.66^{a}	244.66±2.90^{d}
Wk12	134.33±1.20^{b}	175.00±2.51^{d}	132.00±1.51^{b}	161.33±0.88^{c}	77.33±0.33^{a}	249.66±5.17^{e}

Different superscripts along the same row indicate significant difference at $p<0.05$

Group 1: Diabetic rats treated with 62.5 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 2: Diabetic rats treated with 125 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 3: Diabetic rats treated with 250 mg/kg of methanol root bark extract of *Cussonia arborea* for 84 days

Group 4: Diabetic rats treated with 2 mg/kg of glibenclamide for 84 days (Standard control)

Group 5: Diabetic rats treated with 10 ml/kg of distilled water for 84 days (Negative control)

Group 6: Non-diabetic rats treated with 10 ml/kg of distilled water for 84 days (Normal/positive control)

4.47: Pancreas of normal control rats (Group 6) showing normal cells (arrow) of the islet of langerhans

Photomicrograph of the pancreas shows a fully populated islet cell of langerhans. The islet itself is surrounded by the acinar glands of the exocrine pancreas (**Plate 1**).

4.48: Pancreas of Diabetic untreated rats (Group 5) showing depopulation of the islet cells (arrow)

The photomicrograph of pancreas shows a small sized islet of langerhans with sparse population of the islet cells as shown by the arrow (**Plate 2**).

4.49: Pancreas of group 1 rats (Diabetic + 62.5 mg/kg extract) showing moderate population of the islet cells of the langerhans (Arrow)

The photomicrograph shows islet of langerhans of pancreas with moderately populated islet cells. Acinar cells surround the islet of langerhans. It is not as populated as the normal control but it is more populated than that of the negative controlôs (**Plate 3**)

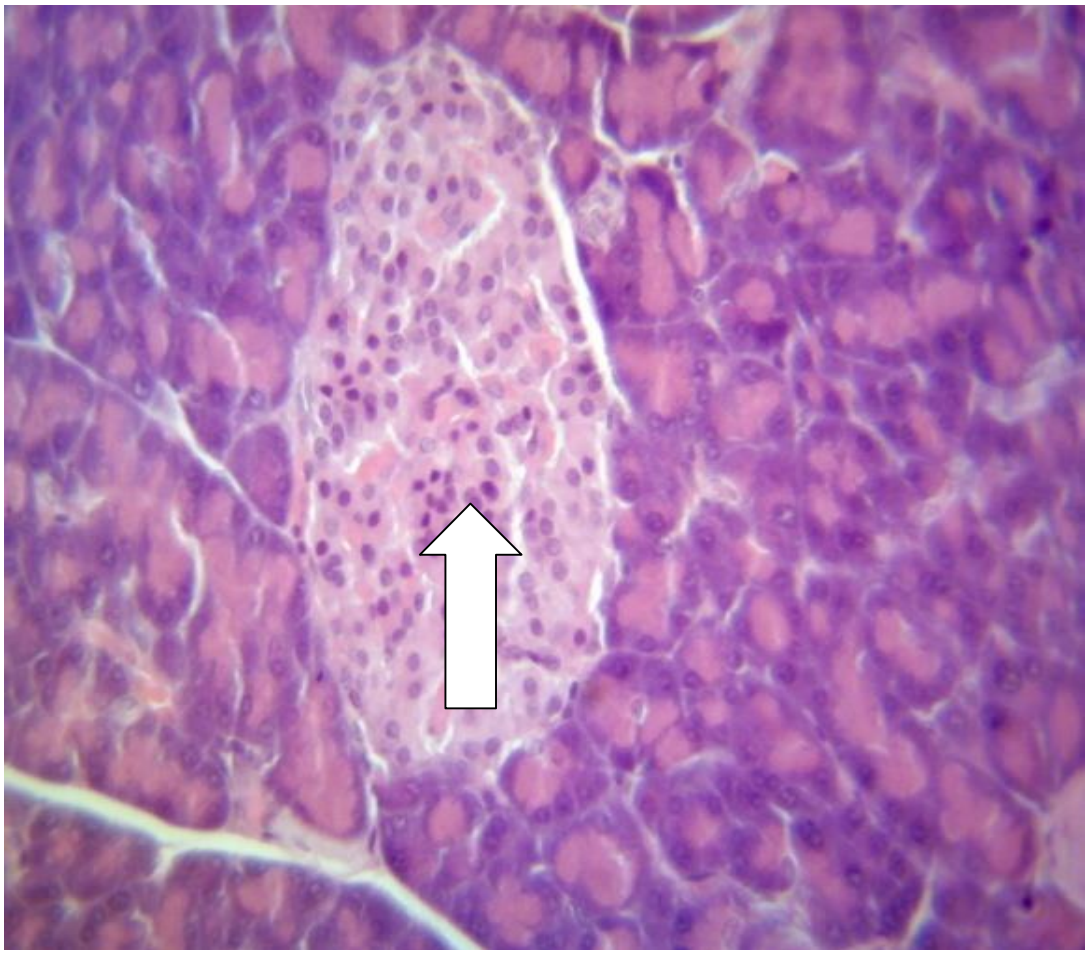


Plate 1: Pancreas of normal control rats (Group 6) showing normal cells (Arrow) of the islet of langerhans (H&E X400)

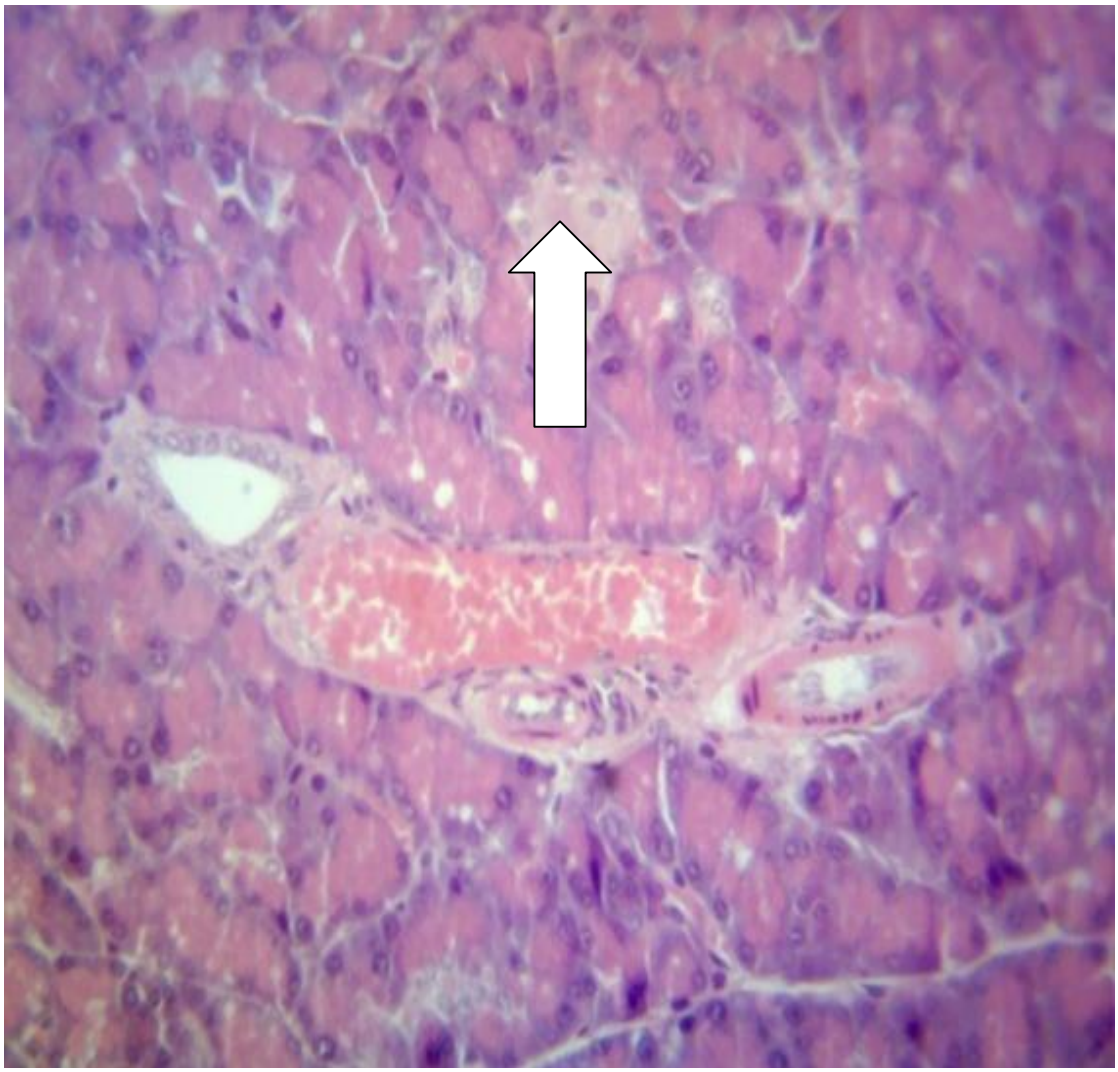


Plate 2: Pancreas of Diabetic untreated rats (Group 5) showing depopulation of the islet cells (Arrow) (H&E X400)

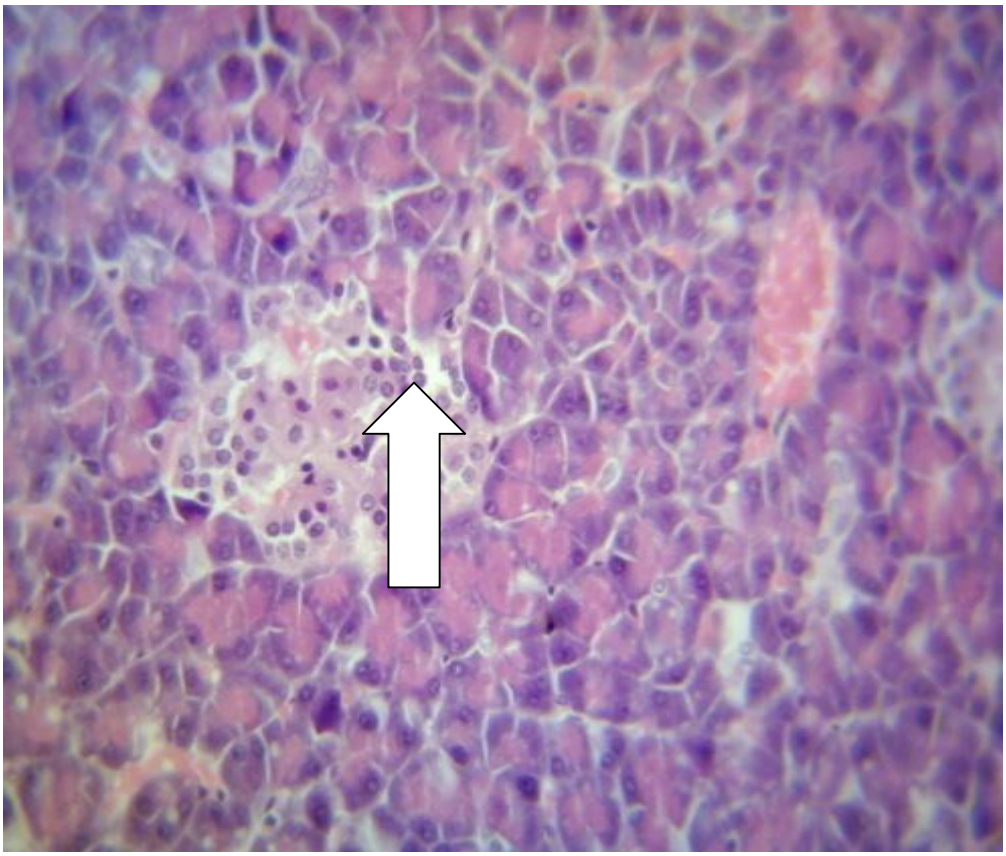


Plate 3: Pancreas of group 1 rats (Diabetic + 62.5 mg/kg extract) showing moderate population of the islet cells of the langerhans (Arrow) (H&E X400)

4.50: Pancreas of group 2 rats (Diabetic + 125 mg/kg extract) showing good population of the islet cells of the langerhans (Arrow)

The photomicrograph shows good population of the islet cells of langerhans as shown by the arrow. The islet cells appear fully populated. The acinar glands of the exocrine pancreas surround the islet (**Plate 4**).

4.51: Pancreas of group 3 rats (Diabetic + 250 mg/kg extract) showing cytoplasmic vacuolations (Arrow) and moderate population of the islet cells of the langerhans

The photomicrograph of the pancreas shows a sizeable islet of langerhans with some islet cells undergoing cytoplasmic vacuolations, a stage in cellular degeneration and necrosis (**Plate 5**).

4.52: Pancreas of group 4 rats (Diabetic + 2 mg/kg glibenclamide) showing good population of the islet cells of the langerhans (Arrows)

The Photomicrograph of pancreas shows densely populated islet cells of langerhans comparable to that seen in normal control rats (**Plate 6**).

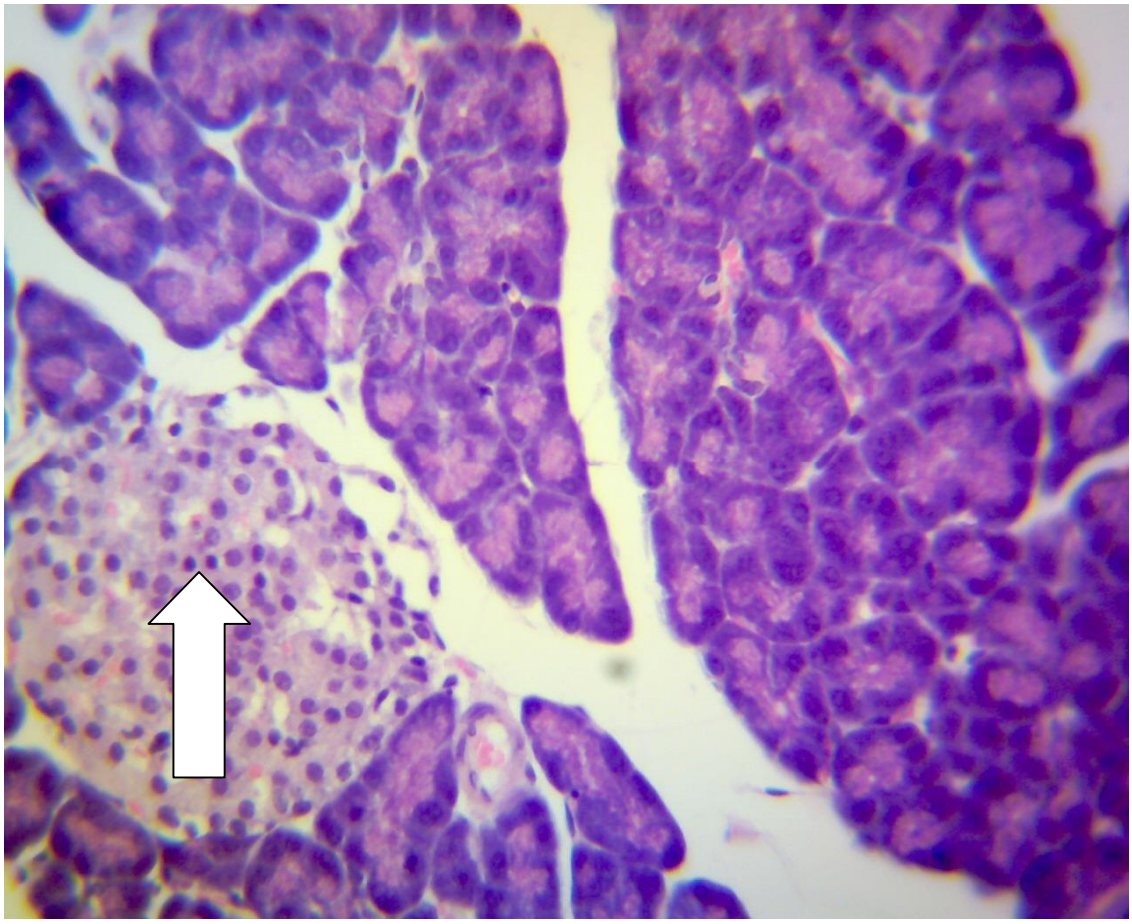


Plate 4: Pancreas of group 2 rats (Diabetic + 125 mg/kg extract) showing good population of the islet cells of the langerhans (Arrow) (H&E X400)

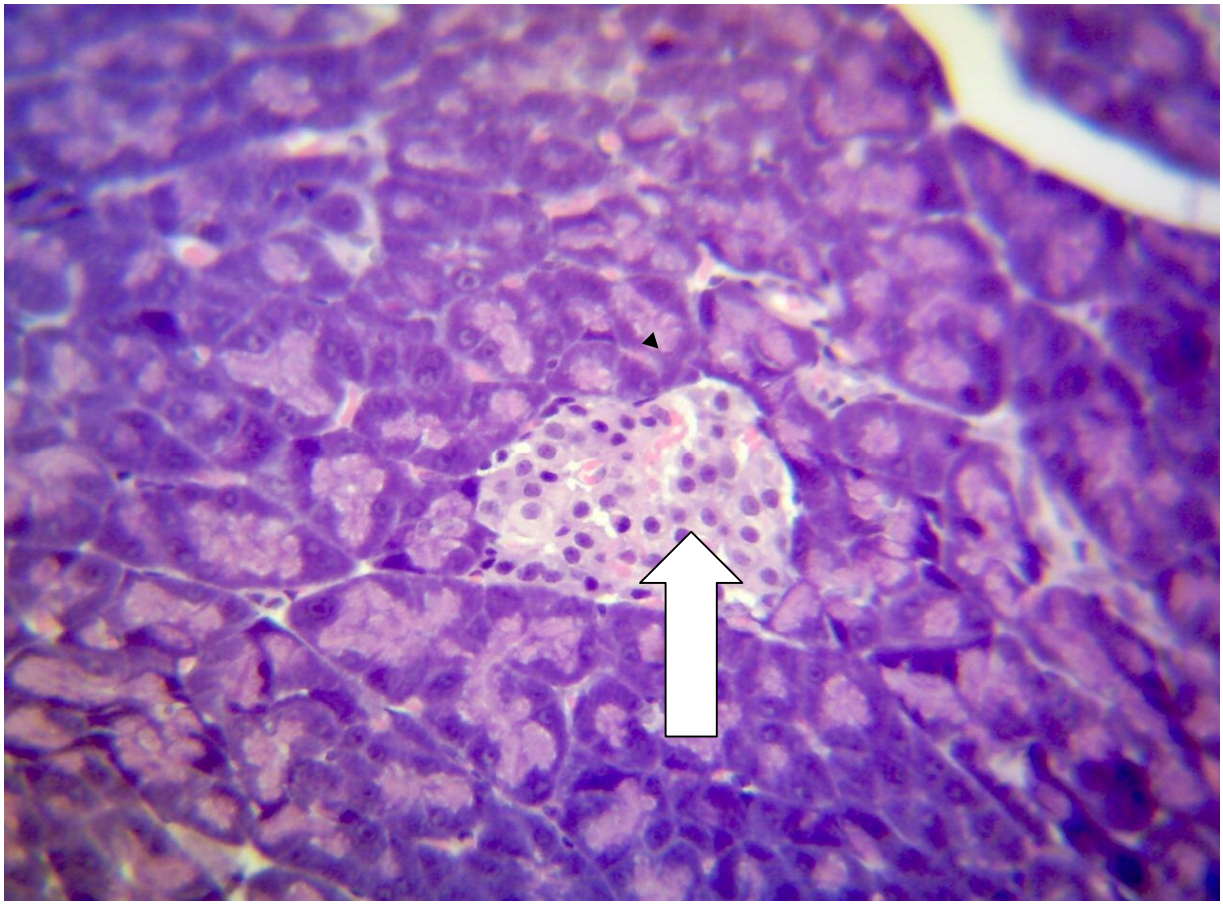


Plate 5: Pancreas of group 3 rats (Diabetic + 250 mg/kg extract) showing cytoplasmic vacuolations (Arrow) and moderate population of the islet cells of the langerhans (H&E X400)

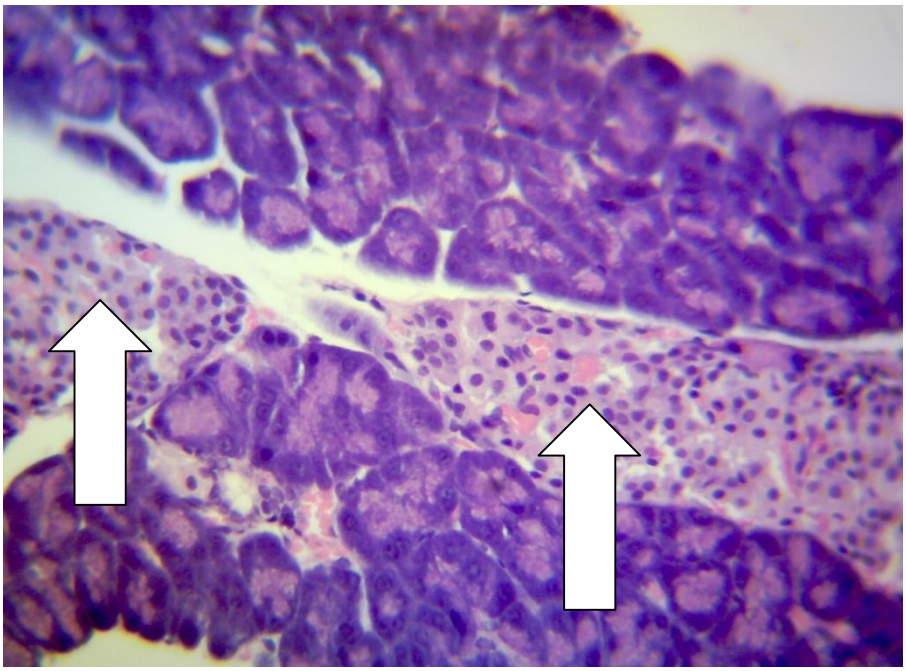


Plate 6: Pancreas of group 4 rats (Diabetic treated with 2 mg/kg glibenclamide) showing good population of the islet cells of the langerhans (Arrows) (H&E X400)

4.53: Kidney of normal control rats (Group 6) showing glomerulus (White arrow) and normal renal tubular epithelial cells (Black arrows)

The photomicrograph of kidney nephrons shows normal glomerulus and renal tubular epithelial cells lining the tubules (**Plate 7**).

4.54: Kidney of rats in group 5 showing erosion of epithelial cells of the renal tubules (White arrows) (H&E X400)

The photomicrograph shows kidney nephrons whose tubular lining epithelial cells are undergoing serious degeneration, erosion and necrosis (**Plate 8**).

4.55: Kidney of group1 rats (Diabetic rats treated with 62.5 mg/kg of extract) showing infiltration with mononuclear cells in the glomerulus (White arrow) and necrosis of the tubular epithelial cells (Black arrows)

This photomicrograph shows kidney nephrons with multifocal erosion of tubular epithelial cells into the lumen. There was also infiltration by mononuclear cells (**Plate 9**).

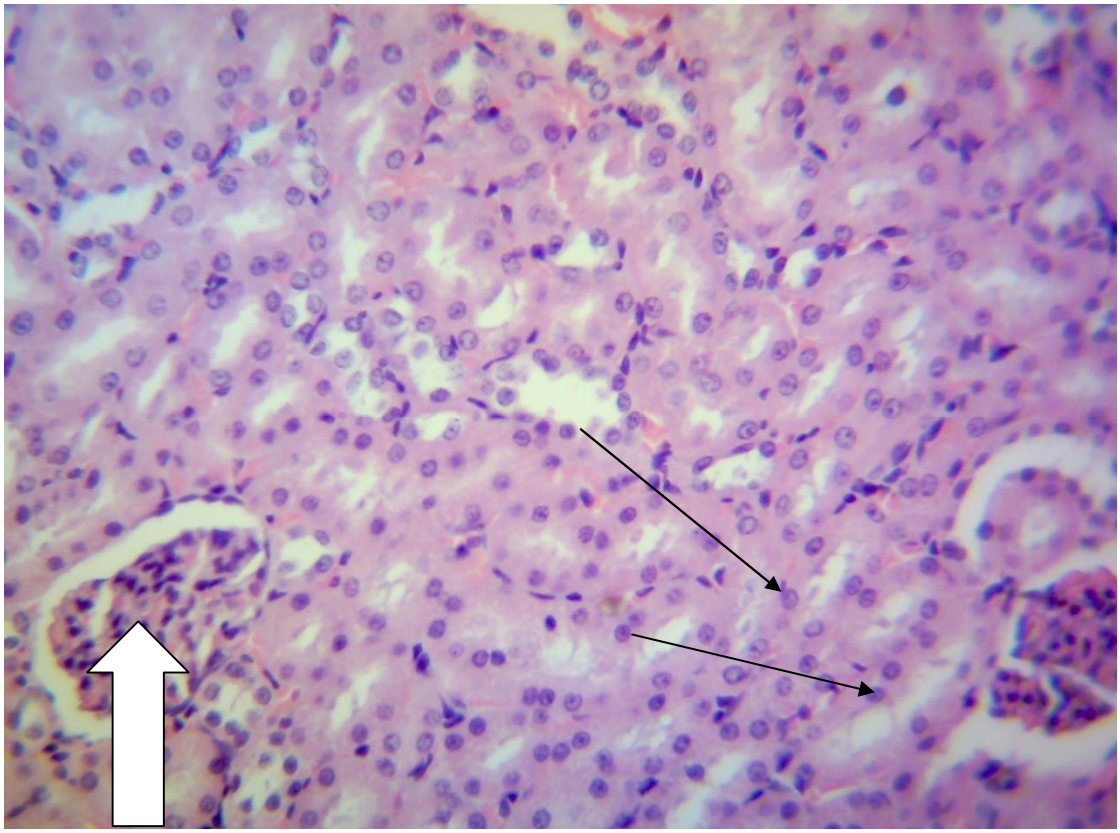


Plate 7: Kidney of normal control rats (Group 6) showing glomerulus (White arrow) and normal renal tubular epithelial cells (Black arrows) (H&E X400)

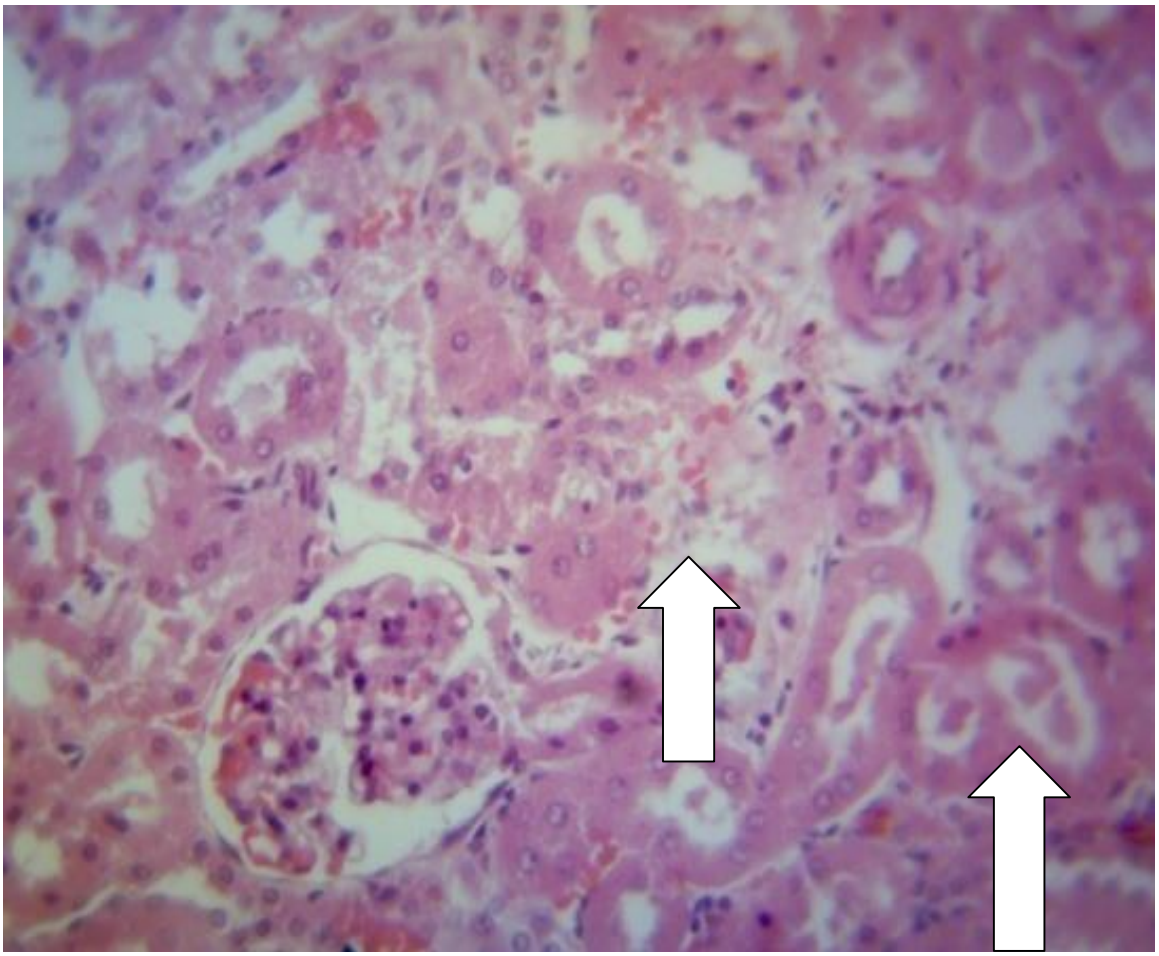


Plate 8: Kidney of rats in group 5 showing erosion of epithelial cells of the renal tubules (White arrows) (H&E X400)

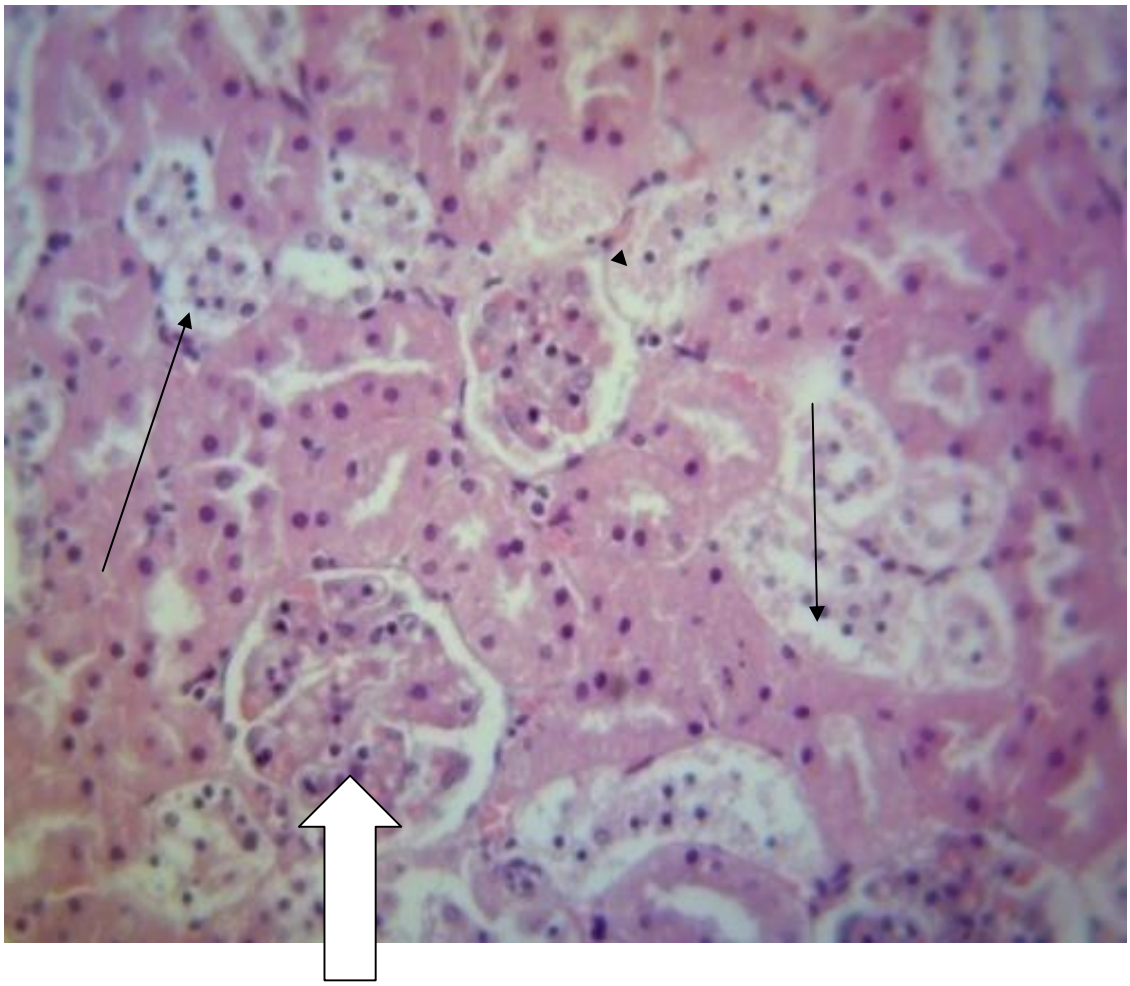


Plate 9: Kidney of group1 rats (Diabetic rats treated with 62.5 mg/kg of extract) showing infiltration with mononuclear cells in the glomerulus (White arrow) and necrosis of the tubular epithelial cells (Black arrows) (H&E X400)

4.56: Kidney of group 2 rats (Diabetic rats treated with 125 mg/kg extract) showing normal tubular epithelial cells (Arrows)

The photomicrograph of the kidney nephrons shows normal epithelial cells lining the tubules. This is comparable to what is seen in the photomicrograph of the nephrons of normal contro rats (**Plate 10**)

4.57: Kidney of group 3 rats (Diabetic rats treated with 250 mg/kg of extract) showing glomerulus (White arrow) and mild necrosis of renal tubular epithelial cells (Blackarrow)

The photomicrograph shows localized areas of tubular epithelial cell degeneration and necrosis. There is also infiltration of the glomerulus by mononuclear cells (**Plate 11**).

4.58: Kidney of diabetic rats treated with 2 mg/kg glibenclamide (Group 4) showing tubular epithelial cells (Black arrows) and glomerulus (White arrow) with no observable pathologic lesions (Arrows) (Plate 12)

This photomicrograph shows kidney nephrons with normal glomerulus and normal tubular epithelial lining cells. This was comparable to the nephron photomicrogragh of the normal control rats and rats treated with 125 mg/kg of the extract.

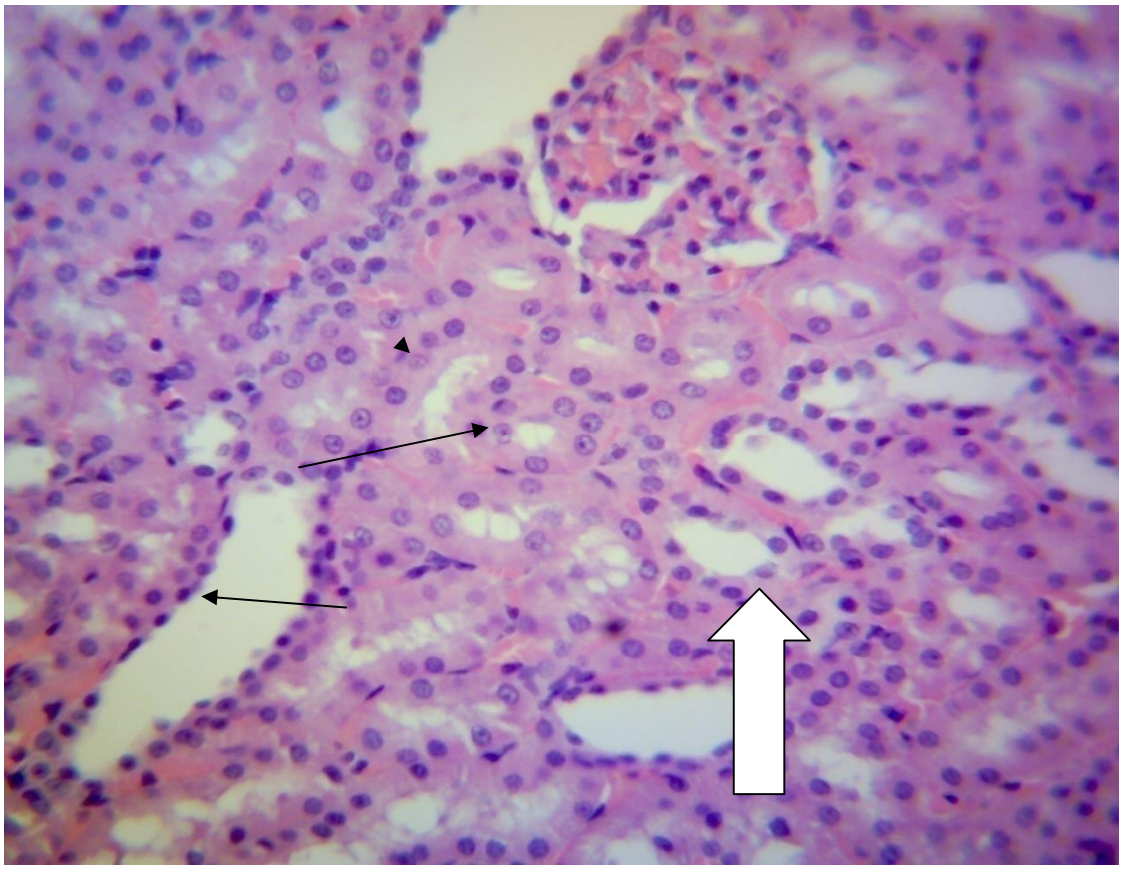


Plate 10: Kidney of group 2 rats (Diabetic rats treated with 125 mg/kg extract) showing normal tubular epithelial cells (Arrows) (H&E X400)

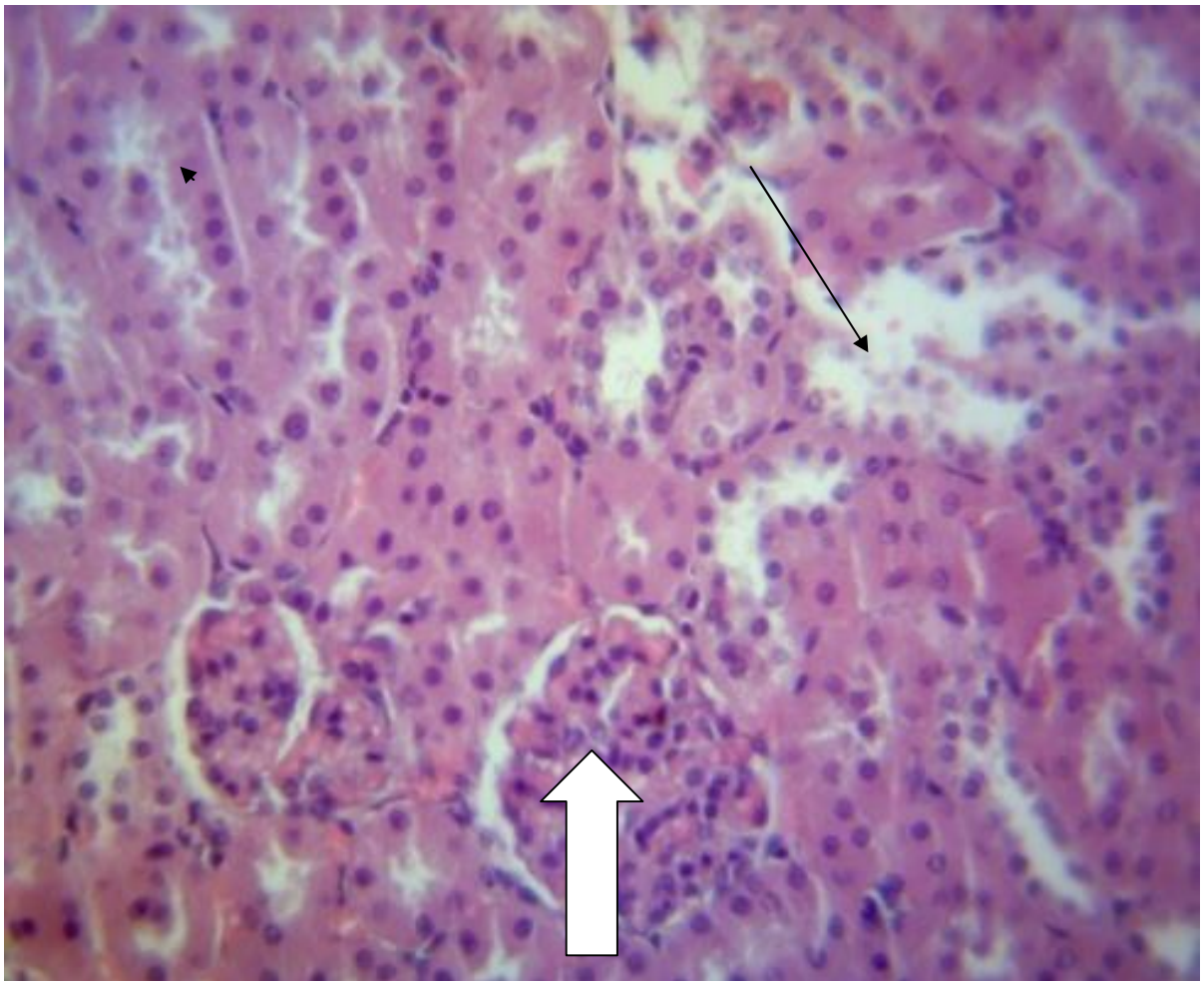


Plate 11: Kidney of group 3 rats (Diabetic rats treated with 250 mg/kg of extract) showing glomerulus (White arrow) and mild necrosis of renal tubular epithelial cells (Blackarrow) (H&E X400)

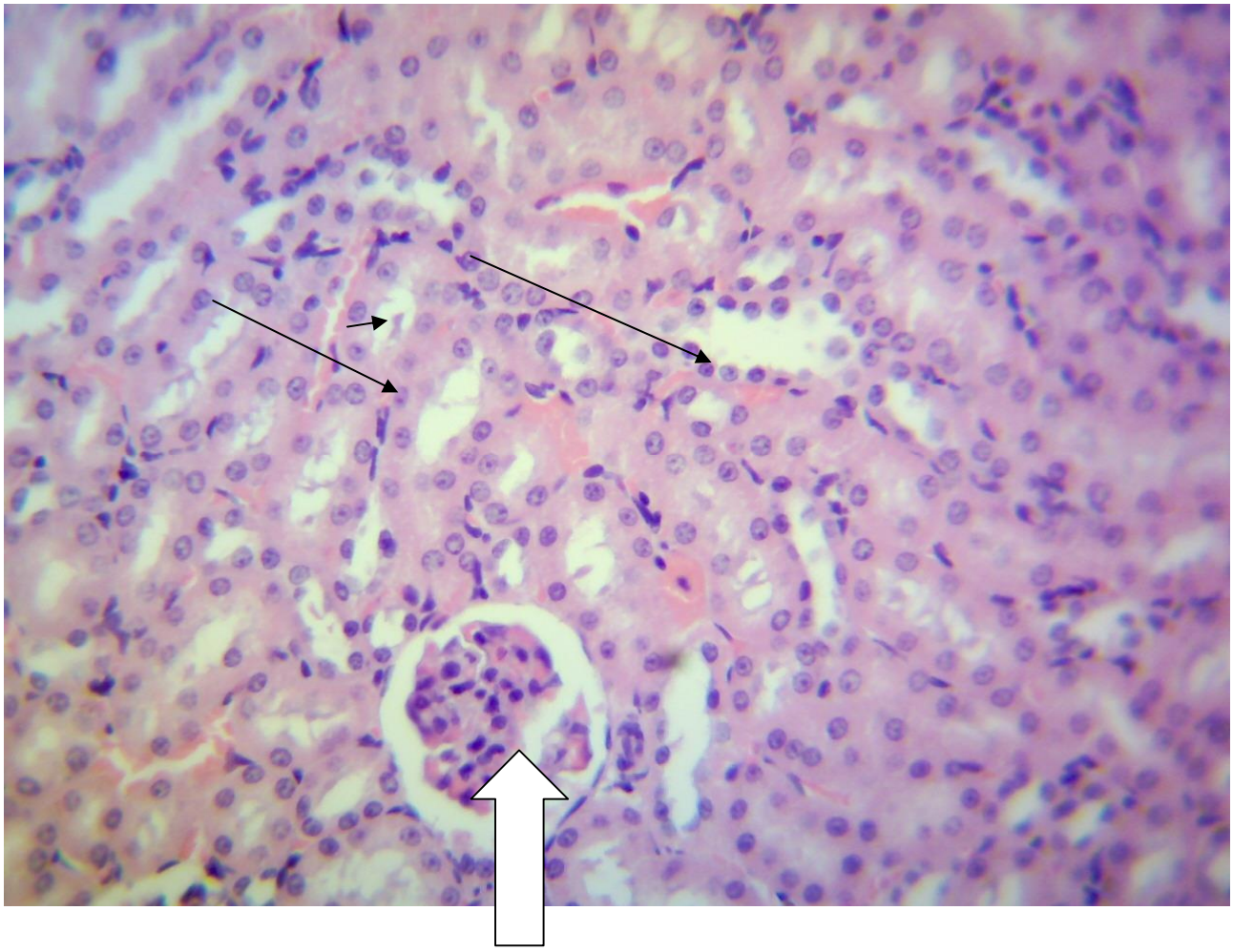


Plate 12: Kidney of diabetic rats treated with 2 mg/kg glibenclamide showing tubular epithelial cells (Black arrows) and glomerulus (White arrow) with no observable pathologic lesions (Arrows) (H&E X400)

4.59: Liver of normal control rat (Group 6) showing the central vein (White arrow) with normal arrangement of hepatocytes in cords (Black arrows)

The photomicrograph of liver shows normal liver hepatocytes arranged in cords (**Plate 13**).

4.60: Liver of diabetic untreated rats (Group 5) showing severe degeneration and necrosis of hepatocytes (White arrows) and mononuclear cells infiltration (Black arrows)

The photomicrograph of liver shows generalized degeneration and necrosisof hepatocytes with infiltration of mononuclear cells. The photomicrograph is distinctly different from that of the normal control rats (**Plate 14**).

4.61: Liver of diabetic rat treated with low dose (Group 1 rats) of extract (62.5 mg/kg) showing mild infiltration of mononuclear cells (Black arrows) and mild degeneration of hepatocytes (White arrow)

The liver photomicrograph shows hepatocytes undergoing degeneration with mild infiltration by mononuclear cells. It also shows the central vein (**Plate 15**).

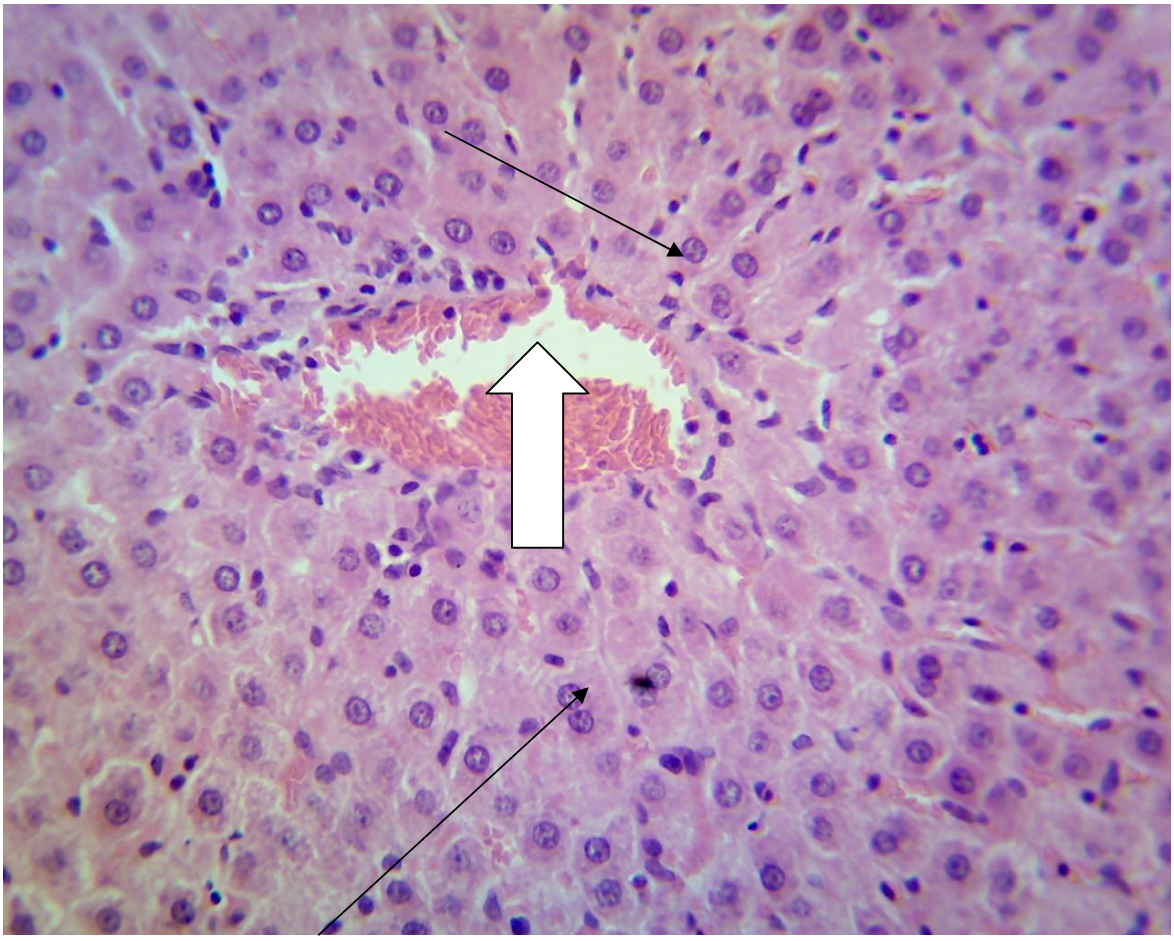


Plate 13: Liver of normal control rat showing the central vein (White arrow) with normal arrangement of hepatocytes in cords (Black arrows) (H&E X400)

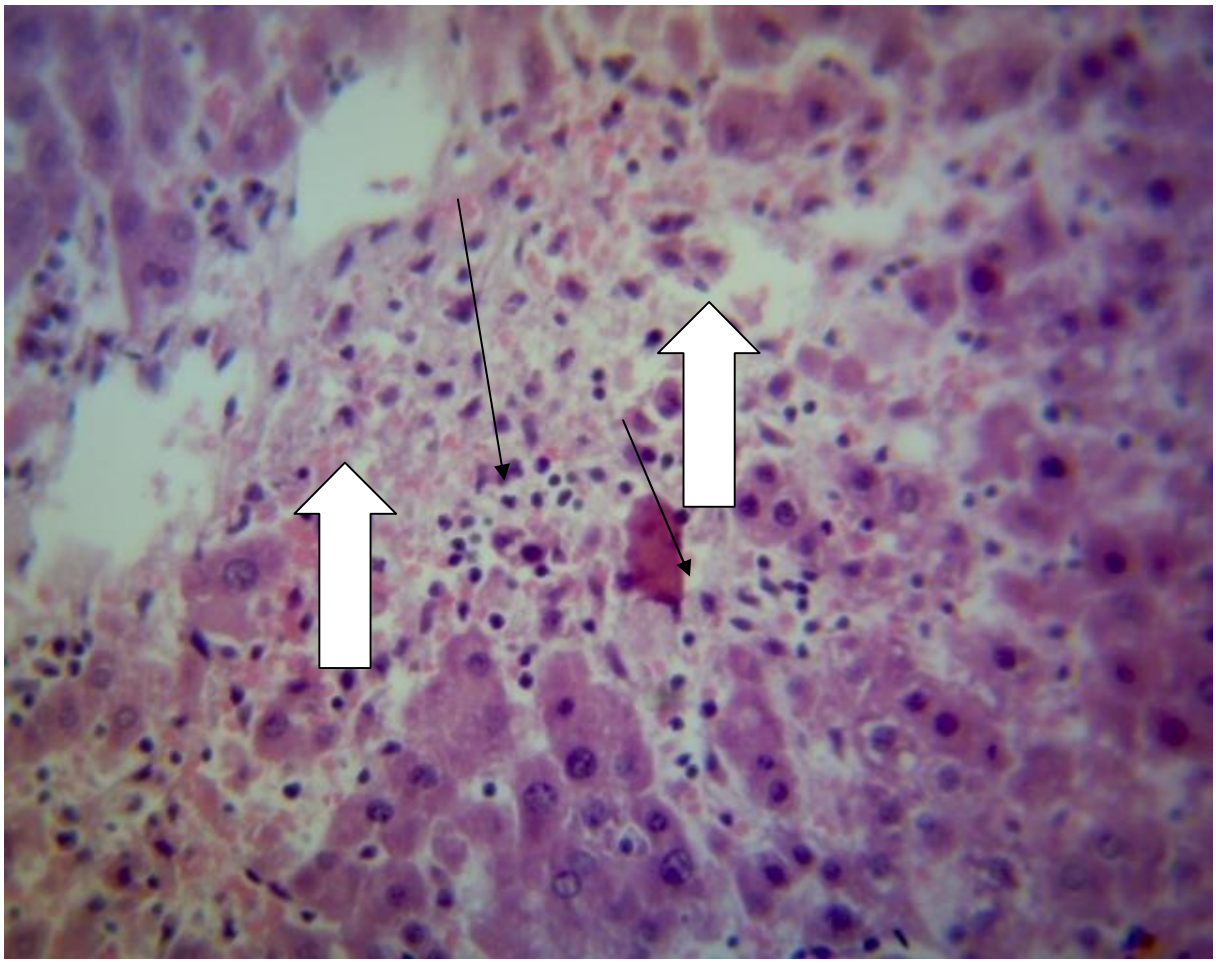


Plate 14: Liver of diabetic untreated rats (Group 5) showing severe degeneration and necrosis of hepatocytes (White arrows) and mononuclear cells infilteration (Black arrows) (H&E X400)

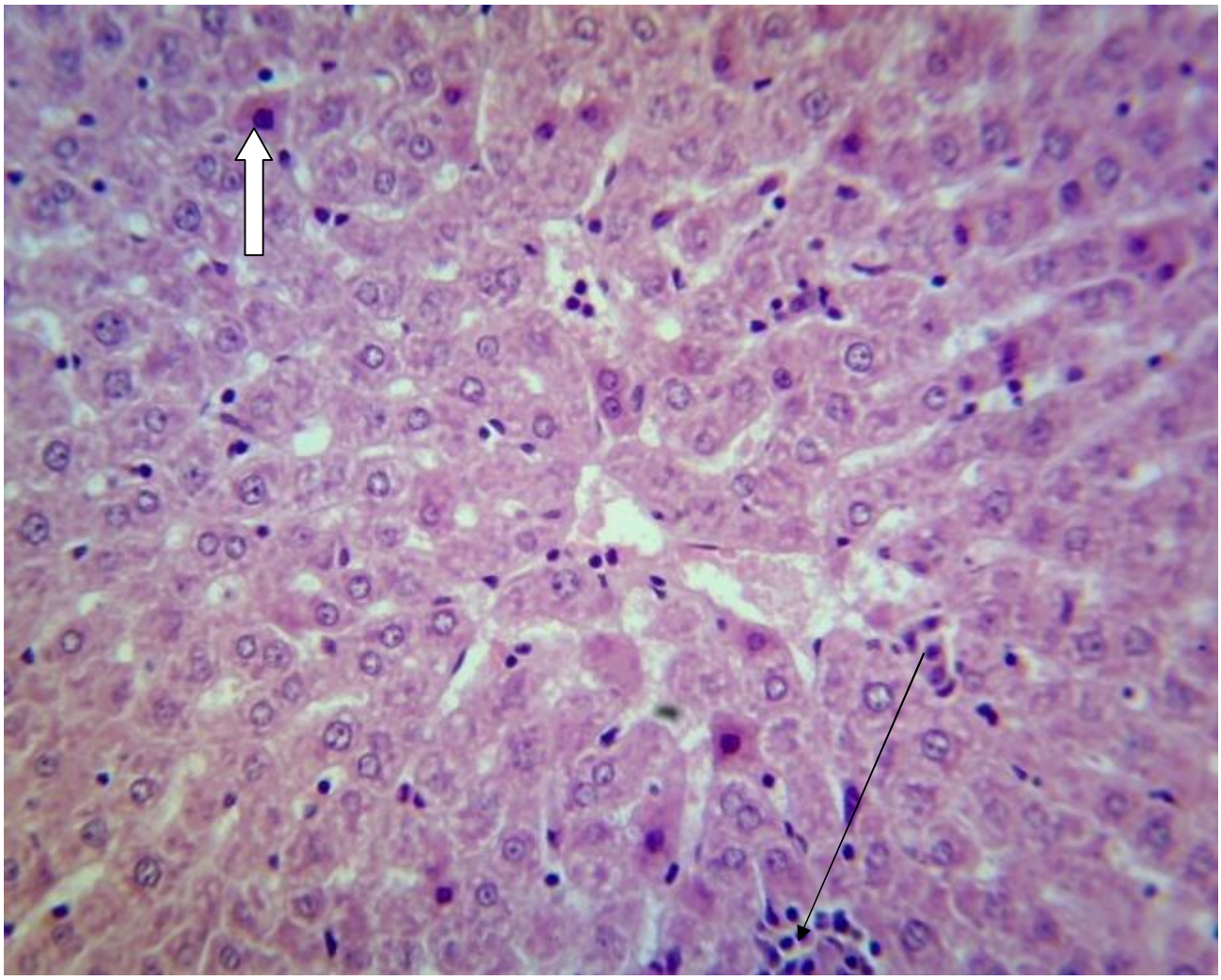


Plate 15: Liver of diabetic rat treated with low dose (Group 1 rats) of extract (62.5 mg/kg) showing mild infiltration of mononuclear cells (Black arrows) and mild degeneration of hepatocytes (White arrow) (H&E X400)

4.62: Liver of (Group 2) diabetic rat treated with middle dose (125 mg/kg of extract) showing central vein and normal hepatocyes in cords (Arrows)

This liver photomicrograph shows the central vein and normal hepatocytes arranged in cords. The photomicrograph is comparable to that seen in the normal control rats and rats treated with 2 mg/kg glibenclamide (**Plate 16**).

4.63: Liver of diabetic rat treated with high dose of extract (250 mg/kg) showing central vein (Black arrows) and hepatocytes with no obvious lesion (White arrow) This liver photomicrograph showing normal hepatocytes and central vein is comparable to that seen with the rats treated with 2 mg/kg glibenclamide, 125 mg/kg of the extract and the normal control rats **(Plate 17).**

4.64: Liver of diabetic rat treated with 2 mg/kg glibenclamide showing normal hepatocytes (Arrows).

The liver photomicragph shows normal hepatocytes and no obvious lesion. It is comparable to that of the normal control rats (**Plate 18**).

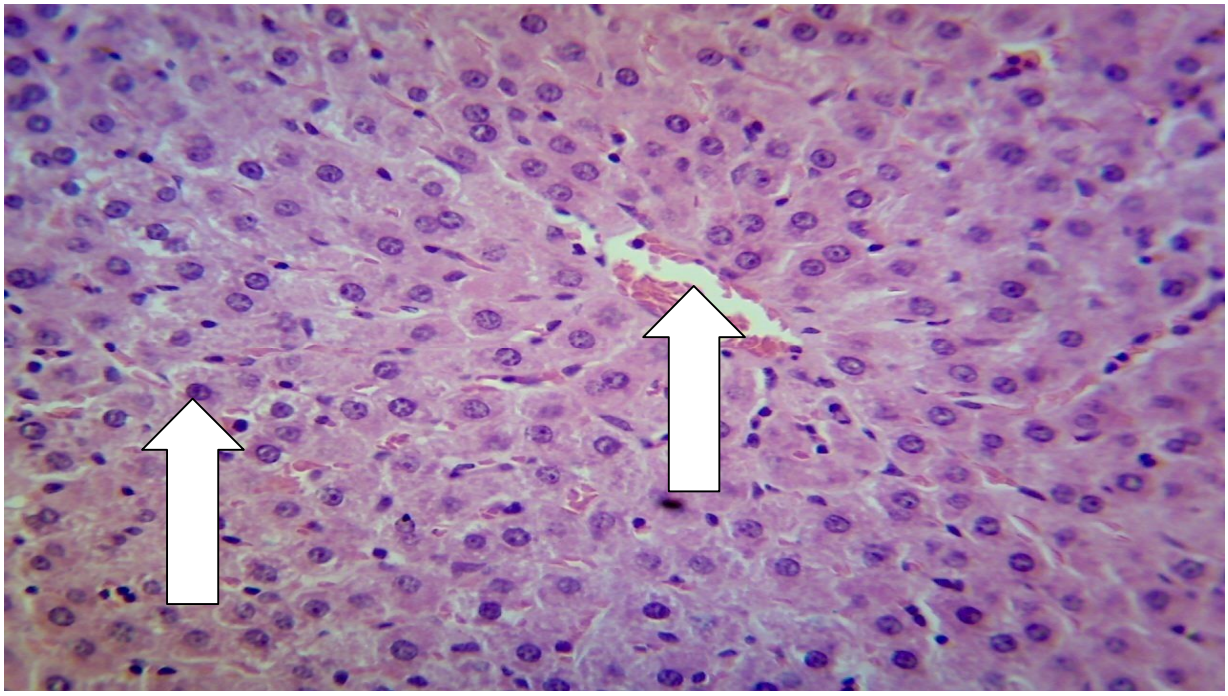


Plate 16: Liver of (Group 2) diabetic rat treated with middle dose (125 mg/kg of extract) showing central vein and normal hepatocyes in cords (Arrows) (H&E X400)

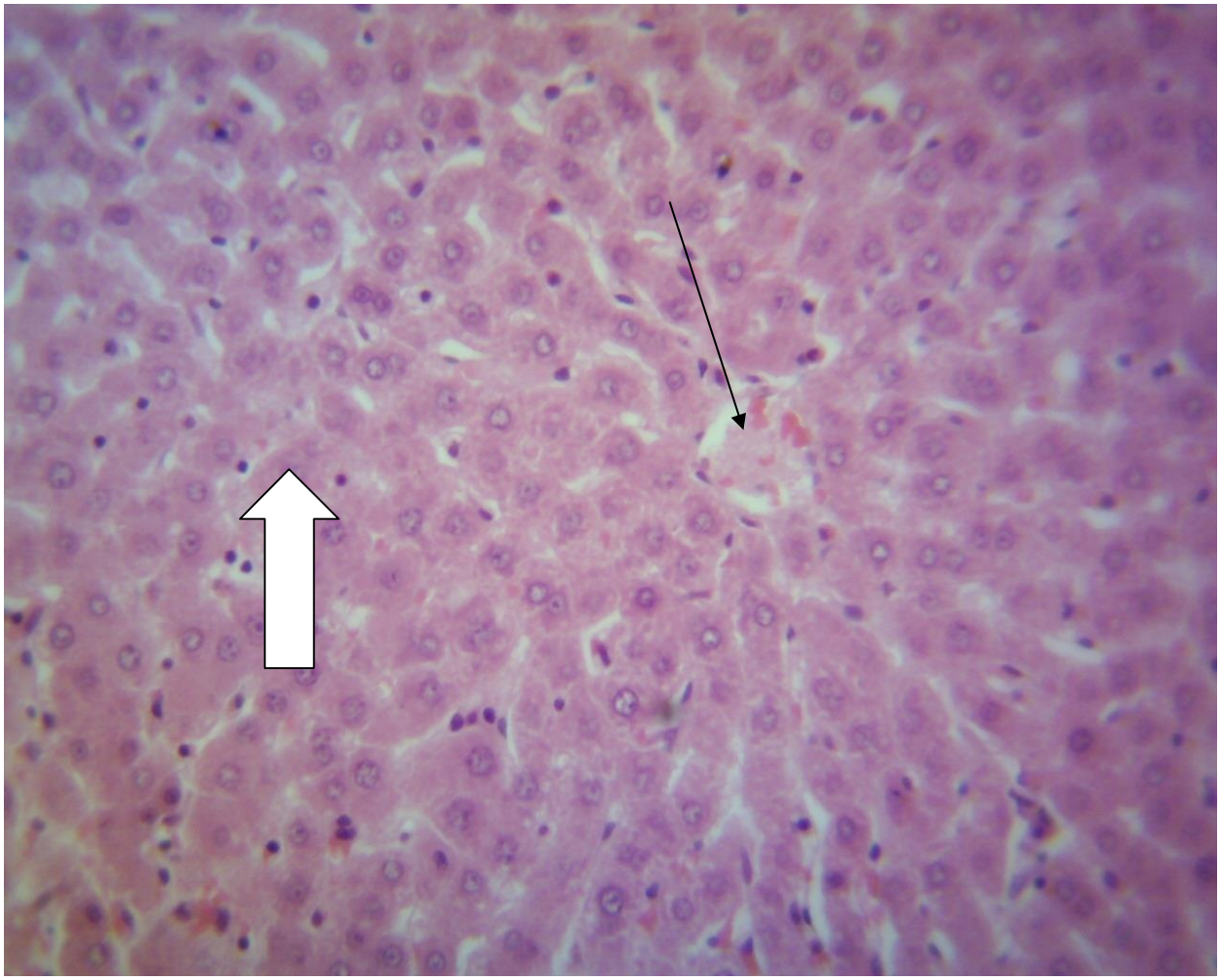


Plate 17: Liver of diabetic rat treated with high dose of extract (250 mg/kg) showing central vein (Black arrows) and hepatocytes with no obvious lesion (White arrow) (H&E X400)

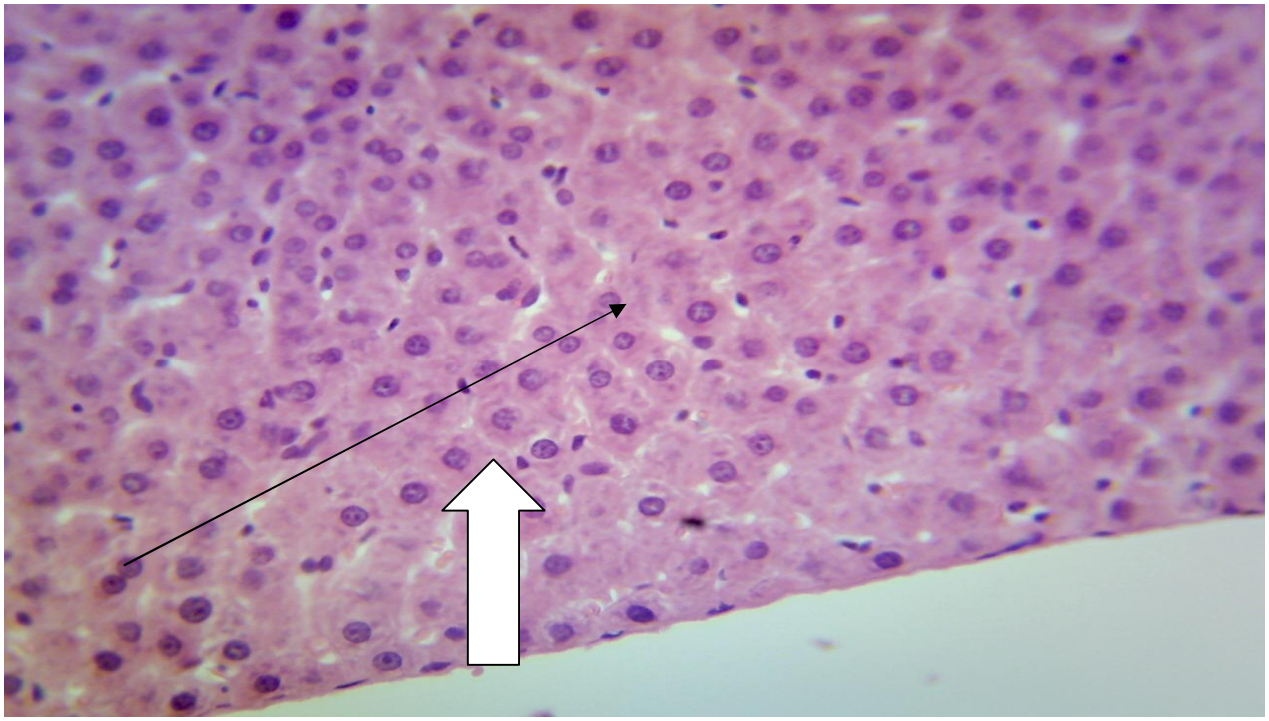


Plate 18: Liver of diabetic rat treated with 2 mg/kg glibenclamide showing normal hepatocytes (Arrows) (H&E X400)

4.65: Heart of normal control rats (Group 6) showing normal arrangement of the myocytes (arrows)

The photomicrograph shows normal spindle-shaped cardiac myocytes with no lesion seen (**Plate 19**).

4.66: Heart of diabetic untreated rats (Group 5) showing mild degeneration of myocytes (White arrows)

The photomicrograph of the heart shows necrotic myocytes and irregular arrangement of the cells. This is different from that of the normal control rats (**Plate 20**).

4.67: Heart of (Group1) diabetic rat treated with low dose (62.5 mg/kg) of the extract showing no obvious lesion

The photomicrograph of heart muscles shows cardiac myocytes in normal arrangement. This is comparable to that of the normal control rats (**Plate 21**).

4.68: Heart of (Group 2) diabetic rat treated with middle dose (125 mg/kg) of the extract with normal myocytes (Arrow) and showing no obvious lesion

The photomicrograph shows cardiac myocytes in normal arrangement with no obvious lesion. This is also comparable to that of the normal control rats (**Plate 22**).

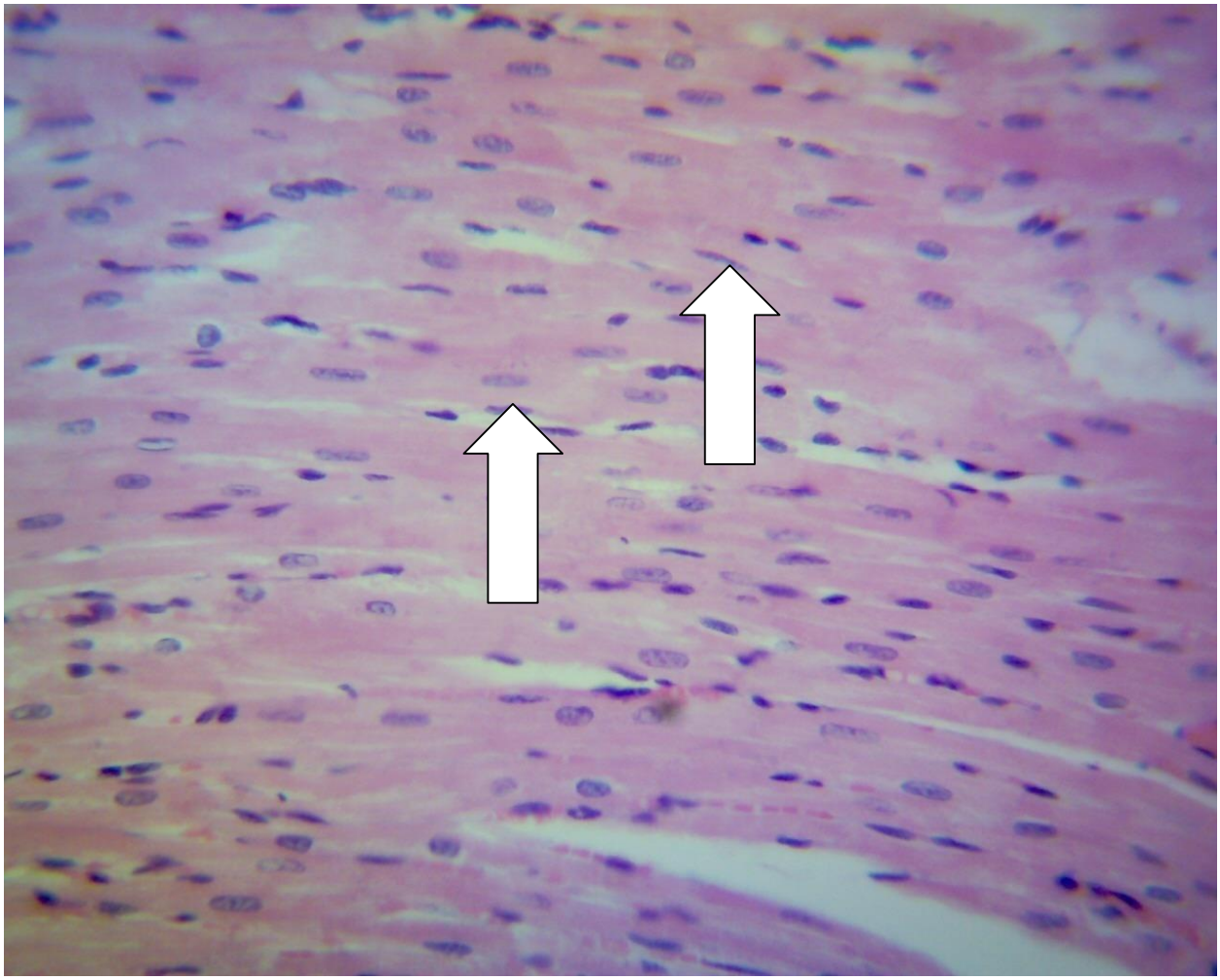


Plate 19: Heart of normal control rats (Group 6) showing normal arrangement of the myocytes (arrows) (H&E X400)

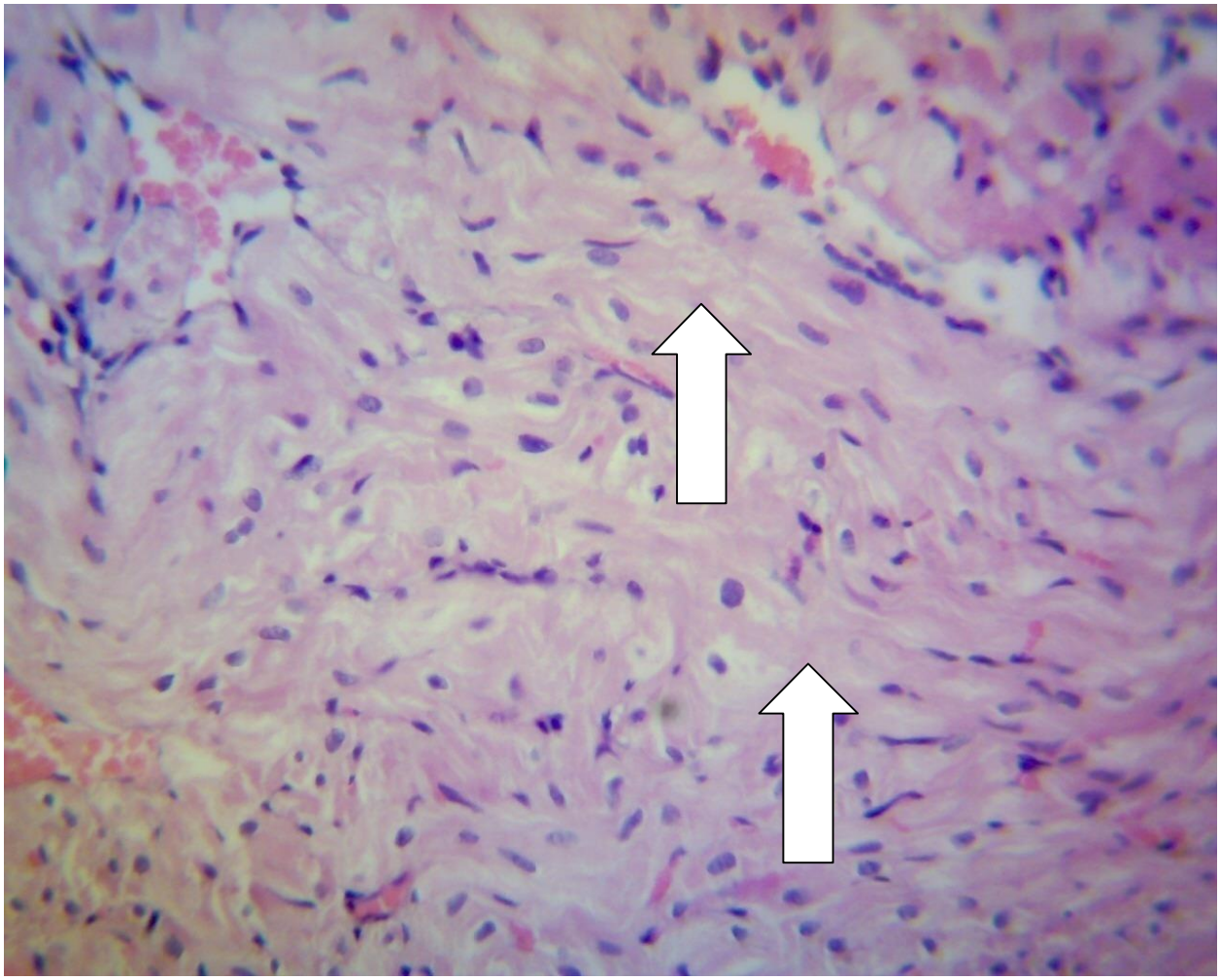


Plate 20: Heart of diabetic untreated rats (Group 5) showing mild degeneration of myocytes (White arrows) (H&E X400)

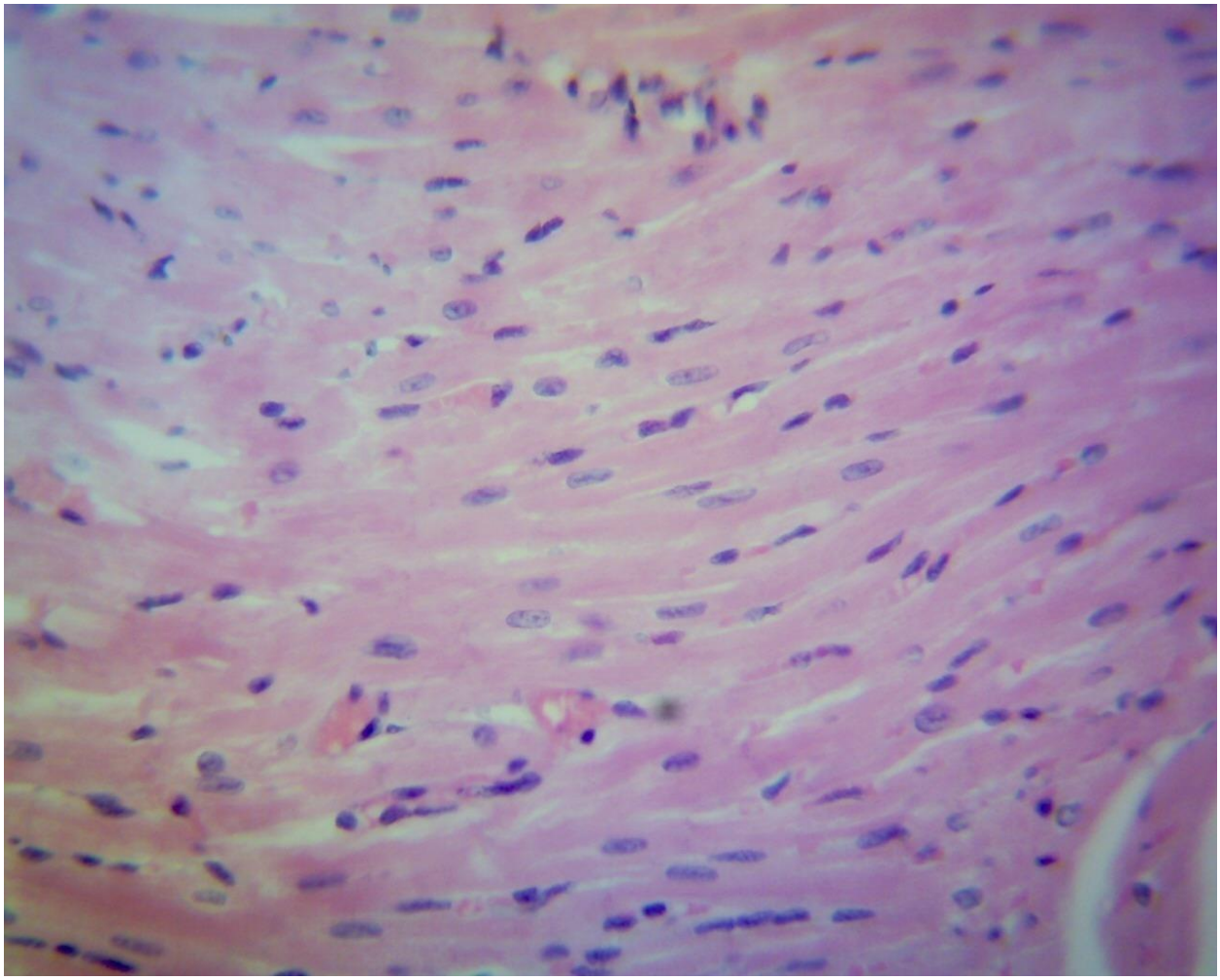


Plate 21: Heart of (Group1) diabetic rat treated with low dose (62.5 mg/kg) of the extract showing no obvious lesion (H&E X400)

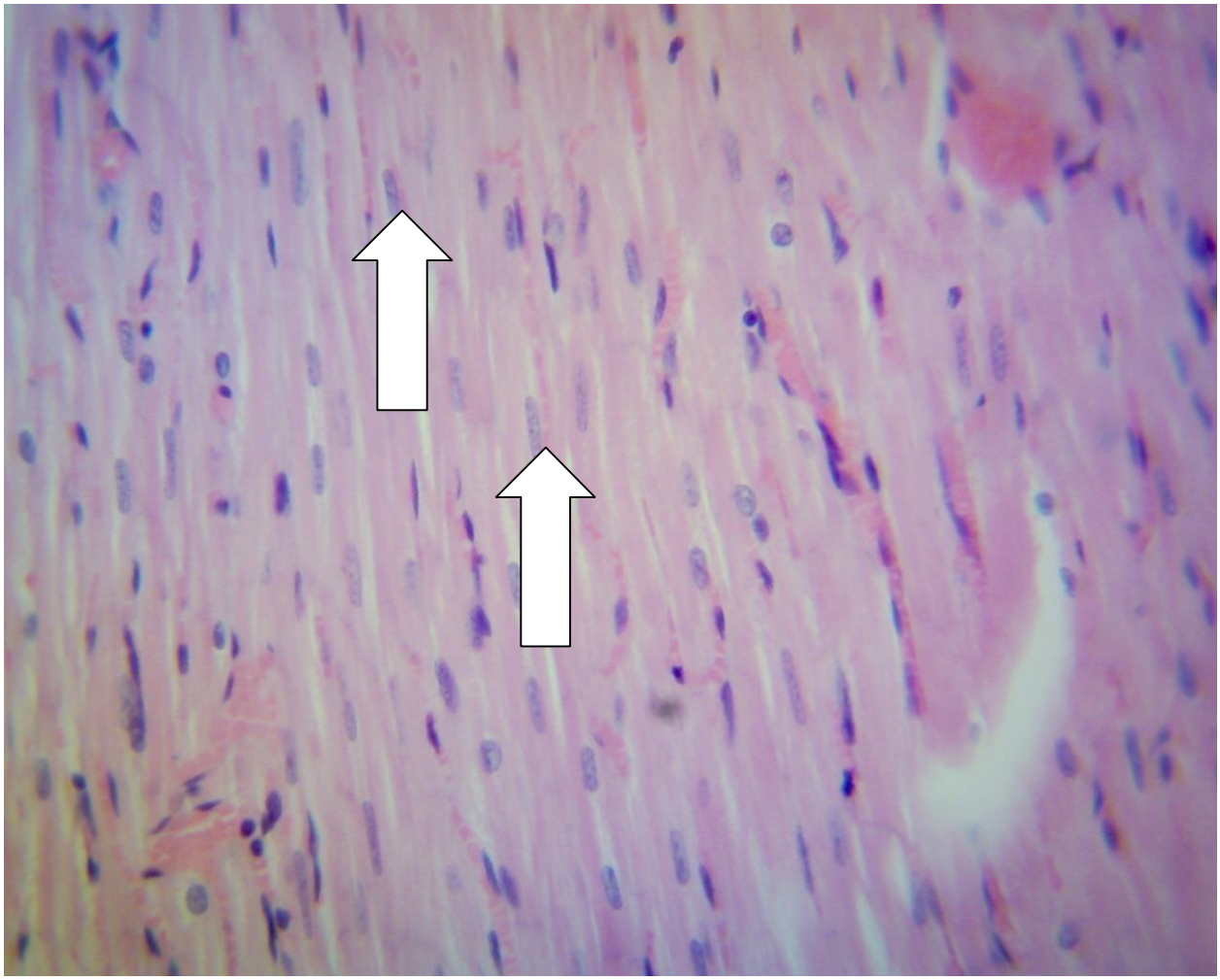


Plate 22: Heart of (Group 2) diabetic rat treated with middle dose (125 mg/kg) of the extract with normal myocytes (arrow) and showing no obvious lesion (H&E X400)

4.69: Heart of (Group 3) diabetic rat treated with high dose (250 mg/kg) of the extract showing no obvious lesion

This photomicrograph of the heart muscle cells shows normal cardiac myocytes in normal arrangement comparable to that of the normal control rats (**Plate 23**).

4.70: Heart of diabetic rat treated with 2 mg/kg of glibenclamide with normal myoctes (Arrows) and showing no obvious lesion

There is no obvious lesion seen in this photomicrograph of this cardiac photomicrograph. This is comparable to that of the normal control rats (**Plate 24**).

4.71: The purified fraction 2 (2:1) (Active compound with retention factor of 0.59)

This chromatogram of this active subfraction shows subf 2_1 with a single band, indicating purity (**Figure 12**).

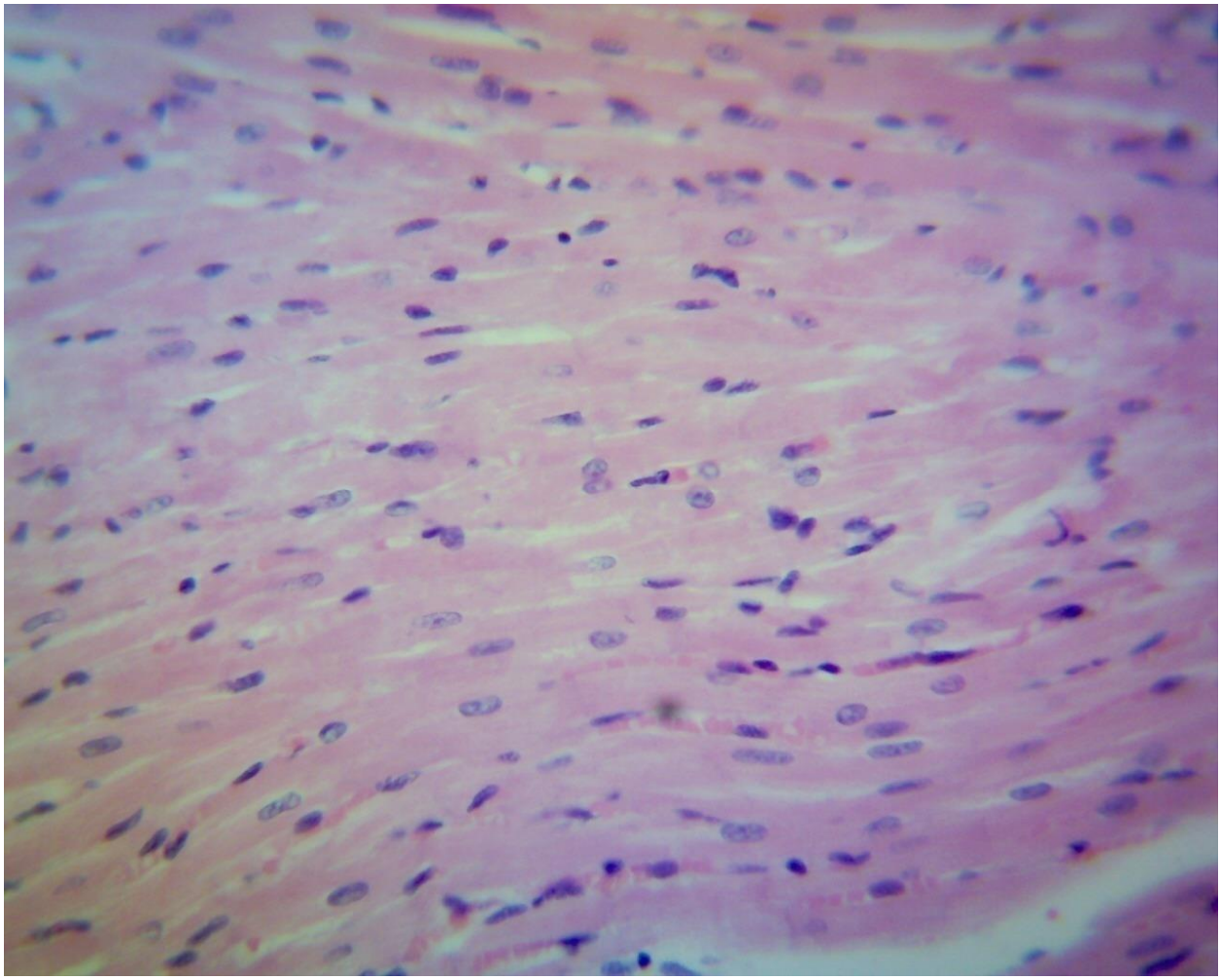


Plate 23: Heart of (Group 3) diabetic rat treated with high dose (250 mg/kg) of the extract showing no obvious lesion (H&E X400)

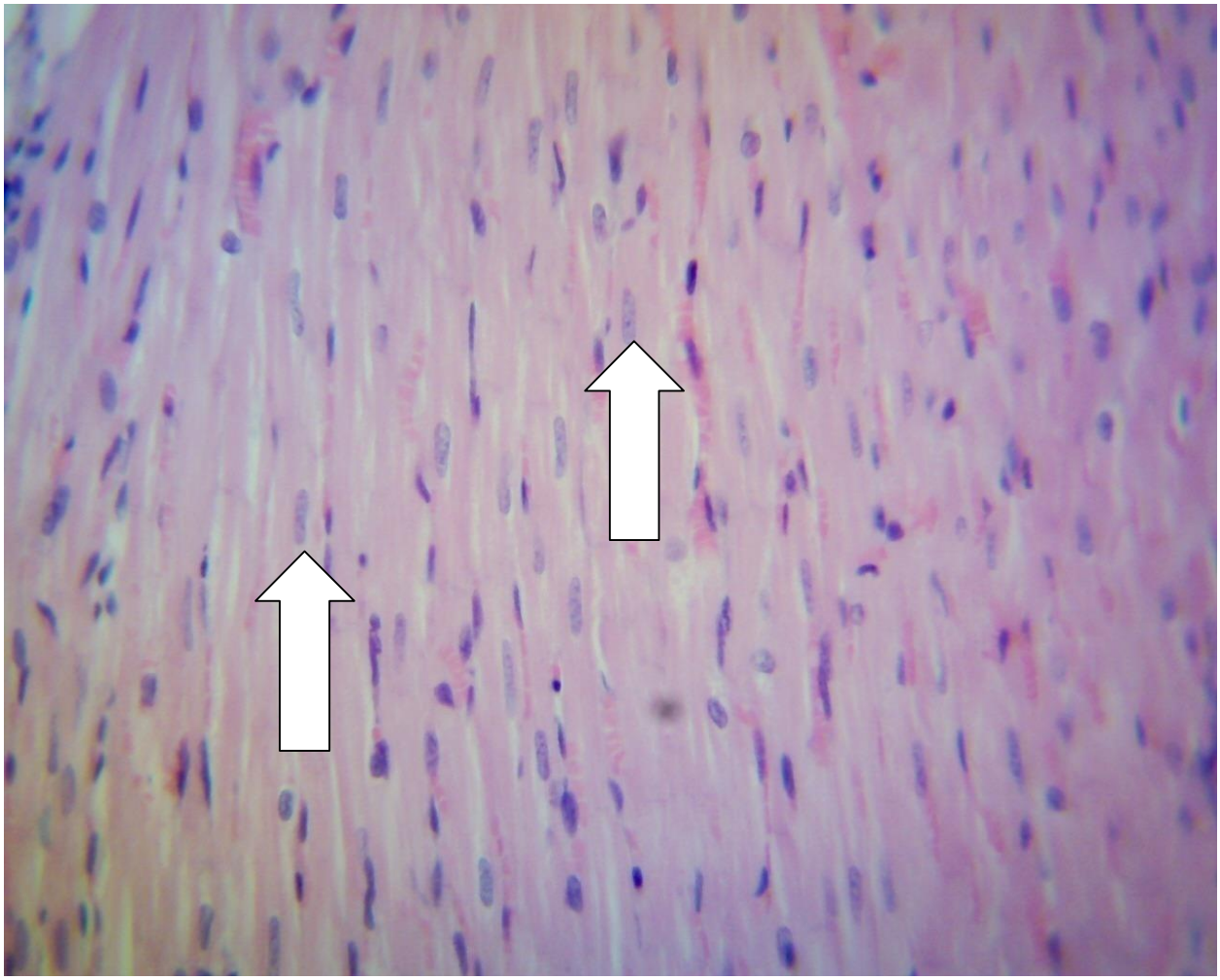
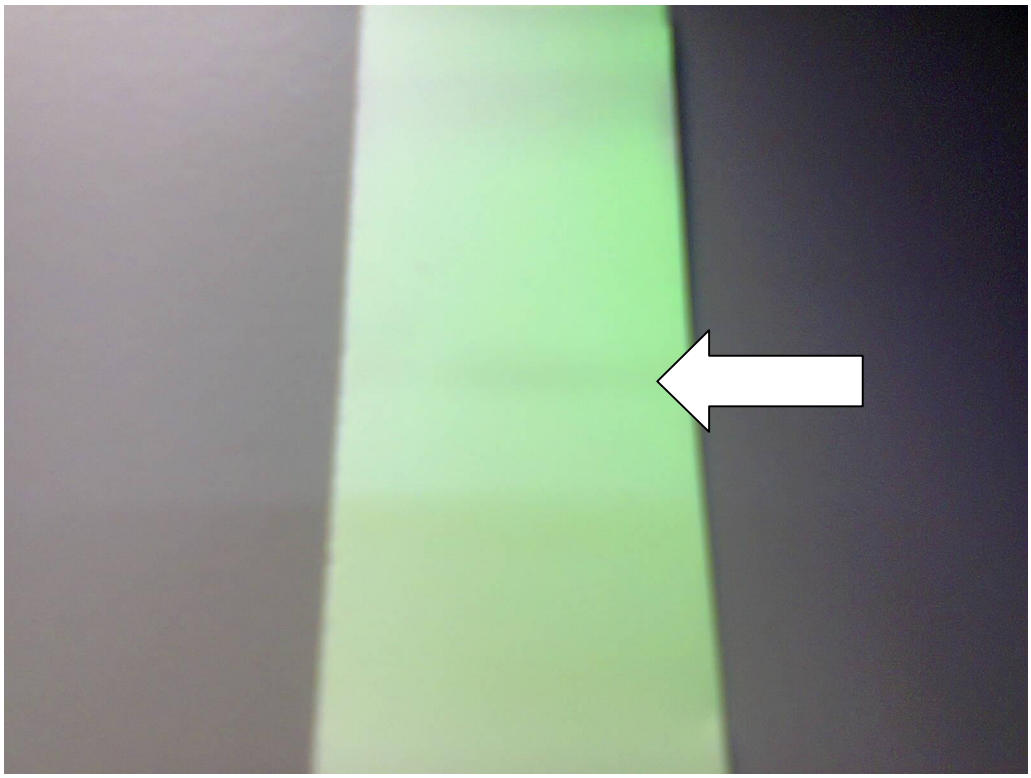


Plate 24: Heart of diabetic rat treated with 2 mg/kg of glibenclamide with normal myoctes (Arrows) and showing no obvious lesion (H&E X400)



2:1

Figure 12: The purified fraction 2 (2:1) (Active compound with retention factor of 0.59)

4.72: ^{1}H Proton Nuclear Magnetic Resonance (NMR) of the active compound

The 1H-NMR spectra of the active compound shows spikes of protons (**Figure 13**).

4.73: ^{13}C-NMR of the Active Compound

It shows different spikes indicating different carbon atoms (**Figure 14**).

4.74: Pentacylic triterpenoid, {(3β)-3-hydroxyolean-12-en-28-oic acid}

The structure of the active compound shows a pentacyclic triterpenoid with molecular formular: $C_{30}H_{48}O_3$ (**Figure 15**)

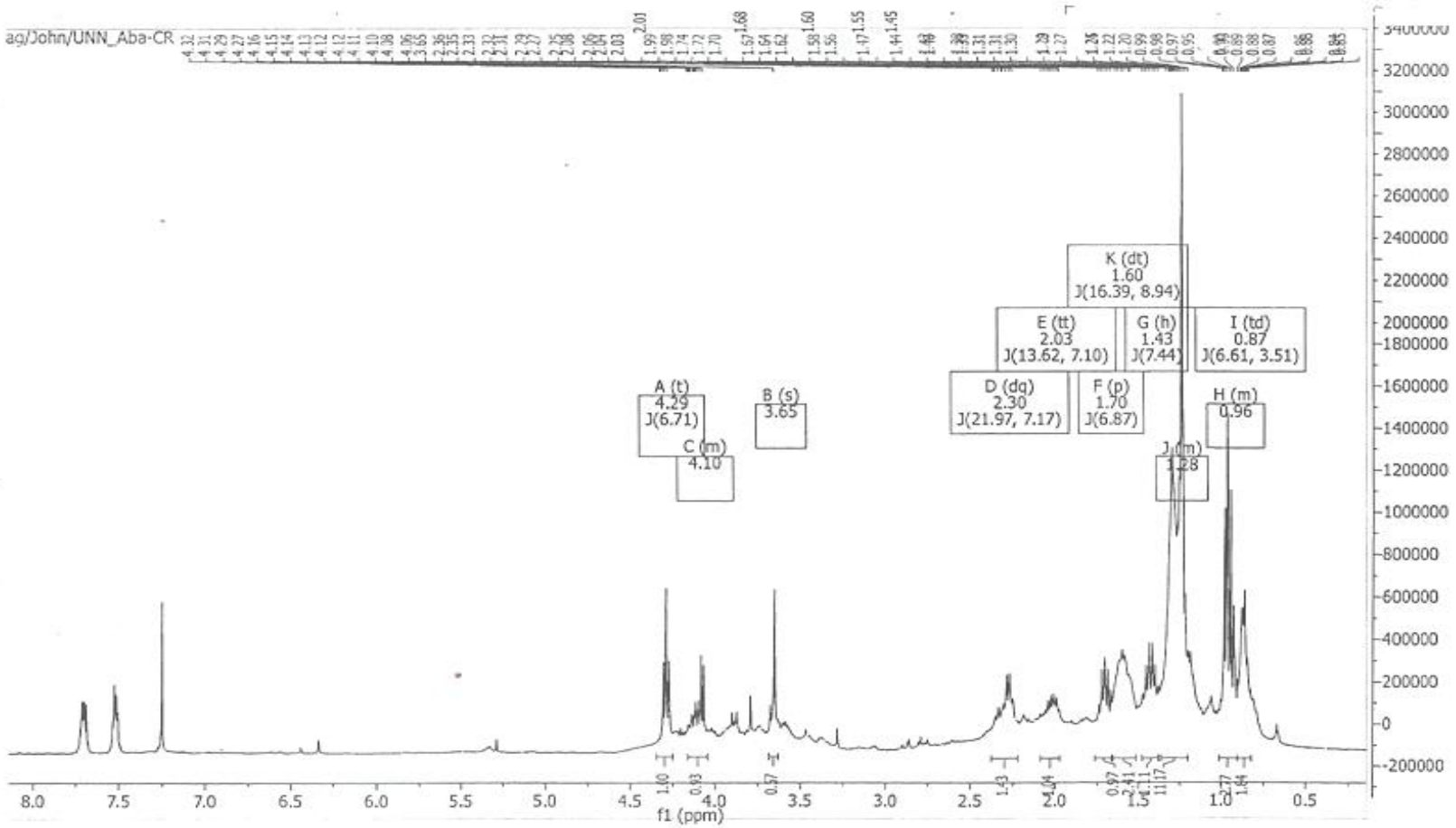


Figure 13: ^{1}H Proton Nuclear Magnetic Resonance (NMR) of the Active Compound

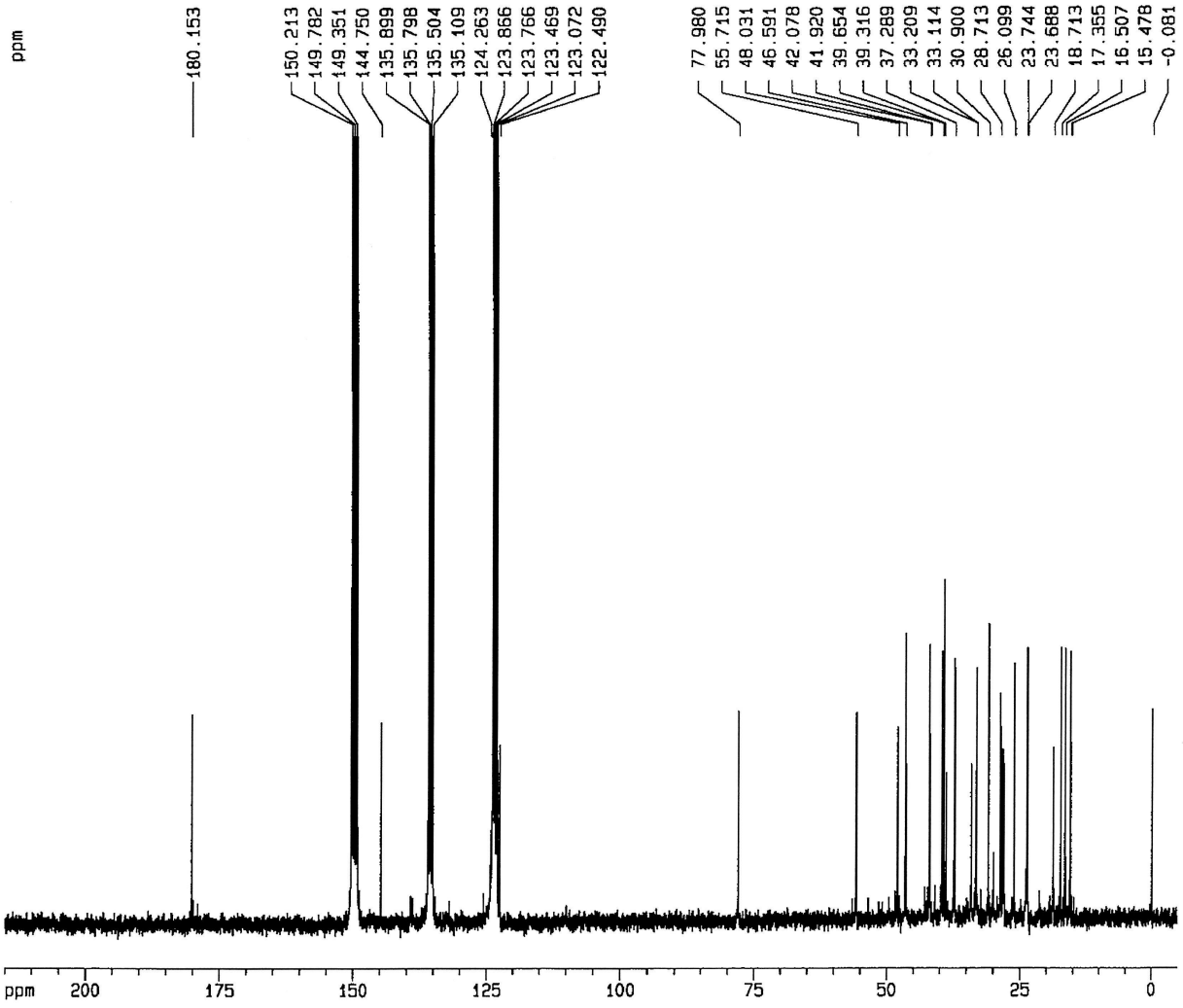


FIG 14: ^{13}C-NMR of the Active Compound

CHEMICAL STUCTURE OF THE ACTIVE COMPOUND

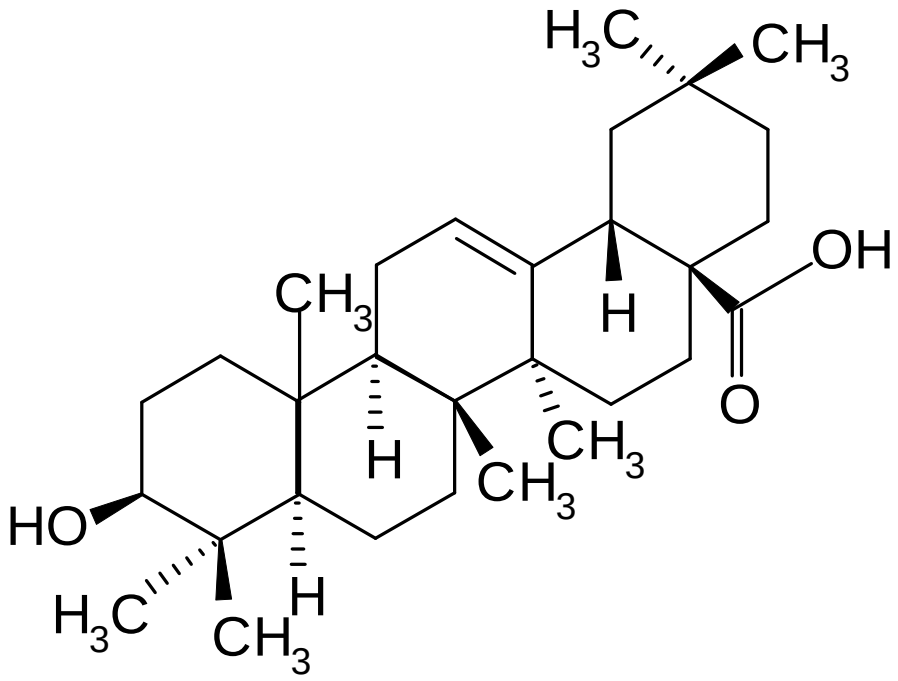


Figure 15: Pentacylic Triterpenoid, {(3β)-3-hydroxyolean-12-en-28-oic acid}.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrate, fats and protein (Kahn and Shechter 1991; Bliss, 2000) and characterized by increased fasting and postprandial blood sugar levels. It is a disorder of public health concern rapidly reaching epidemic proportion globally (Jerald *et al*., 2008). Plants have been used since time immemorial in the management of *Diabetes mellitus* (Akah *et al.,* 1998). *Cussonia arborea* is a plant used in traditional medicine for the management of *Diabetes mellitus* (Amadou *et al.,* 2008). This study intends to isolate, characterize and elucidate the active hypoglycemic principle in *C arborea* in addition to evaluating its safety, antioxidant potentials and possible haematobiochemical effects using alloxan-induced diabetic rats.

The acute toxicity study results showed that the methanol root bark extract of *C. arborea* did not produce any sign of toxicity even at the highest dose of 5000 mg/kg body weight (bw) 48 h post administration (Table 10). The low toxicity effect of medicinal plants have earlier been described by Fabricant and Farnswort (2001) who reported that medicinal plants have been documented to be safer on their long term use by humans. Phytochemical evaluation of *C. arborea* root bark extract revealed the presence of saponins, glycosides, alkaloids tannins, flavonoids and terpenes (Table 11). Researchers have reported different phytochemical compounds in medicinal plants (Tapondjou *et al.*, 2003). There is dearth of information on the phytochemical constituents of *C. arborea*. In their earlier studies Tapondjou *et al.,* (2003) and Dovgii *et al.,* (2005) reported the presence of saponins from *Cussonia bancoensis* and glycoside from *Cussonia paniculata* respectively.

The Ferric reducing antioxidant power assay was used to evaluate the antioxidant potential of *C. arborea*. Principally, FRAP assay treats the antioxidants in the sample as reductant in a redox-linked colometric reaction (Guo *et al.,* 2003). FRAP assay measures the reducing potential of an antioxidant to react on ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produce blue colour of ferrous form (Benzie and Strain, 1999) which can be detected at 593nm. The antioxidant compounds which act as reducing agents exert their effect by donating hydrogen atom to ferric complex and thus break the radical chain reaction. The higher the absorbance, the higher the antioxidant activity which is indicated by the high FRAP value.

The result of the FRAP assay showed concentration-dependent activity. Increase in concentration of the extract resulted in increase in the absorbance of the sample. The highest FRAP value (1.5µm) of *C. arborea* was obtained at the concentration of 400 µg/ml (Figure 4). This value was statistically comparable to the FRAP value of ascorbic acid which was 2 µm (Benzie and Strain, 1999).

DPPH is a commercial oxidizing radical which can be reduced by antioxidants. In this assay, the violet colour of DPPH was reduced to pale yellow colour due to the abstraction of hydrogen atom from it. The result indicated a concentration-related antioxidant activity with the optimum of 74% for the extract and 92% for ascorbic acid (standard) at concentration of 400 µg/ml (Figure 5). The more antioxidant activity in the extract the more DPPH reduction occurred (Mohammed *et al.*, 2009). High reduction of DPPH is related to the high scavenging activity performed by any particular sample (Blois, 1958). The antioxidant property of the extract could be attributed to its phytochemical constituents such as glycosides, flavonoids, terpenes and saponins. A researcher had reported the antioxidant properties of saponin, flavonoids and terpenes (Rordrigues *et al,* 2005).

The single intraperitoneal injection of alloxan monohydrate at the dose of 160 mg/kg body weight resulted in a significant ($p<0.05$) elevation of the fasting blood glucose (FBG) levels of the rats in both the acute and chronic antidiabetic studies (Tables 12 and 14). Alloxan monohydrate and its reduced product, dialuric acid establishes a redox cycle with the formation of superoxide radicals (Szukudelski, 2001). These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium ion concentration which causes rapid destruction of pancreatic beta cells of the islets of langerhans resulting in a decrease in endogenous insulin secretion and attendant elevation of the blood glucose levels (Akuodor *et al.,* 2014). During this study, rats with elevated glucose levels of Ó126 mg/dl (7 mMol/L) were considered diabetic (WHO, 1980). The results of both the acute and chronic antidiabetic studies indicated that treatment of the diabetic rats with 250 mg/kg (for acute study) and 125 mg/kg (for chronic study), significantly ($p<0.05$) reduced the elevated glucose levels 6 hours and 14 days post extract administration respectively (Tables 12 and 14). The reductions achieved by the extract at these doses were comparable to that achieved by 2 mg/kg of glibenclamide, a known anti-diabetic drug. The result is in agreement with our earlier study (Aba *et al.,* 2014) with the methanol stem bark extract of *C. arborea* which demonstrated antihyperglycaemia. Other researchers (Khazraji *et al.,* 1993**;** Lim *et al*., 2009; Liu *et al*., 2005) have also demonstrated hypoglycaemic potentials of root barks of different plants.

The results of the oral glucose tolerance test indicate that after challenging the rats with 2000 mg/kg of glucose, the blood glucose levels of the rats increased significantly (Table 13). It is a well known phenomenon that the blood glucose level increases after a meal (postprandial hyperglycemia). Pretreatment of the rats with *C. arborea* extract (250 mg/kg) showed a significant reduction of the elevated blood glucose levels compared to the distilled water

pretreated rats (Table 13). This goes a long way to confirm the antihyperglycemic properties of *C.arborea* because oral glucose tolerance measures the ability to metabolize glucose (Luzi, 1998). *C. arborea* extract caused the most significant ($p<0.05$) reduction of elevated glucose levels in the glucose-challenged rats at the dose of 250 mg/kg, 180 min post treatment. The decreases or reductions in the glucose levels of rats treated with hypoglycaemic plants in oral glucose tolerance test could be attributed to lowered intestinal glucose absorption, inhibition of renal glucose reabsorption, improved tissue glucose uptake or even gluconeogenesis inhibition which is highly activated by 18 hours fasting before glucose load (Oliveira *et al.,* 2008).

The glycosylated haemoglobin (HbA_{1c}) values of the diabetic untreated rats were significantly ($p<0.05$) higher than that of the normal control rats. However, all treatment (extract and glibenclamide) groups recorded significantly ($p<0.05$) lower HbA_{1c} value at the end of the experiments (Figure 6). Group 2, which was treated with 125 mg/kg of the extract, demonstrated the highest ameliorative effects with regards to glycosylation of haemoglobin. Glycosylated haemoglobin is a measurement that reflects both fasting and postprandial glucose concentrations over a 3-month period (Diana *et al.,* 2010). Reductions in the values of HbA_{1c} by the treated groups indicate the hypoglycaemic effect of the extracts. The result is in agreement with the findings of Anand *et al*.,(2007) and Yu *et al*., (2006) who reported the ameliorating effect of *Brassica nigra and Astragalus membranaceus* (hypoglycaemic plants) respectively on glycosylation of hemoglobin in diabetic rats. Oral hypoglycaemic agents such as glibenclamide have also been reported to lower HbA_{1c} levels of diabetic rats (Diana *et al.,* 2010).

Assesement of serum liver enzymes in alloxan-induced diabetic rats treated with graded doses of *C. arborea* root bark extract for 84 days revealed significant ($p<0.05$) decreases in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the treatment group compared to the diabetic untreated group (Tables 17 and 18). This result is in agreement with the reports of Kadarian *et al.*, (2002). They observed that *Achyrocline satureioides*-treated diabetic rats, at the dose of 300 mg/kg, inhibited the increase of liver AST and ALT. Serum liver enzymes are biochemical parameters usually evaluated to ascertain any effects on the liver (Yusuf *et al.,* 2012). Increases in the activities of AST and ALT are associated with liver injury by drugs or any other hepatotoxin (Ramaiah, 2011). However , ALT is more specific to liver and it is thus a better parameter for evaluating liver injury while serum AST may also be associated with diseases of organs such as heart and muscle (Ozer *et al*., 2008). The significant ($p<0.05$) increases in the activities of serum AST and ALT in the diabetic untreated rats (group 5) may be attributed to the effect of alloxan on the liver. Alloxan monohydrate is toxic to the liver affecting liver function (El Demerdash *et al.,* 2005). The damaged liver therefore leads to leakage and increased levels of liver enzymes into the blood (Navarro *et al.,* 1993). In the treatment groups (extract-treated and Glibenclamide-treated), the serum activities of AST and ALT were statistically comparable ($p>0.05$) to the normal control rats at the end of the experiment. The extract and the glibenclamide convincinly ameliorated the deleterious effect of alloxan monohydrate in the treated rats by antioxidation mechanism.

The lipid profile assay showed that the diabetic untreated rats had significantly ($p<0.05$) elevated serum levels of total cholesterol, triglyceride (TG), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) with significant ($p<0.05$) reductions in serum high density lipoprotein (HDL) (Tables 19-22 and Figure 7). Ozougwu *et al.,* (2013) revealed that *Diabetes*

mellitus is associated with dyslipidaemia. A cascade of pathogenic steps resulting from insulin resistance together with dysfunction of the enzyme lipoprotein lipase (LPL) could account for most of these abnormalities (dyslipidaemia). Insulin resistance in adipocytes allows exuberant lipolysis stimulated by hormone sensitive lipase resulting in excessive free fatty acid release into the blood. This results in upregulation of apolipoprotein B. Therefore the liver produces and exports an increased amount of triglyceride-rich VLDL. In diabetes, LPL activity is blunt which accounts in part for the elevated plasma VLDL-TG and the diminished HDL as well as the relatively low LDL. Secondly, when plasma VLDL-TG is elevated, cholesterol ester transfer protein (CETP) causes TG to move to HDL and LDL and conversely causes cholesterol ester to move from HDL and LDL to VLDL-TG. Hence diabetics with high plasma VLDL conconmittantly have low plasma HDL because the cholesterol ester is being transferred out of the HDL fraction, as well as a diminution in size of the LDL particle because LDL becomes TG enriched and this is converted by hepatic lipase to small dense LDL (Goldberg, 2001). Upon treatment with the extract however, the elevated total cholesterol, triglyceride, LDL, and VLDL were significantly ($p<0.05$) reduced while the levels of HDL were significantly ($p<0.05$) elevated at the end of the experiment when compared to the negative control group (Tables 19-22 and Figure. 7). This indicates that the root bark extract of *C.arborea* possesses hypolipidemic properties. These findings are in agreement with the reports of Kuma and Augusti (1989) on *Ficus bengalensis*-treated diabetic rats.

There were significant ($p<0.05$) reductions in the levels of total protein, albumin and globulin of the rats in group 5 (negative control) compared with other groups (Tables 23-25). This indicates that *Diabetes mellitus* impairs the synthetic function of the liver which may be attributed to the damage caused by alloxan monohydrate used in this case for induction of diabetes. It may also

be consequent upon loss of protein (usually albumin) in urine. Proteinuria (albuminuria) is a common finding in *Diabetes mellitus* (Olsen and Mogensen, 1996). The liver plays a vital role in protein metabolism. It helps in the synthesis of plasma proteins such as albumin, fibrinogen e.t.c (Jepson *et al.* 1986; Hasselgren *et al.,* 1984). Any damage to the liver will cause aberration in the dynamics of these products.

The increase in blood urea nitrogen (BUN) and Creatinine levels observed in group 5 rats (diabetic untreated) compared to the normal control rats may be attributed to the effect of diabetes on the kidney (diabetic nephropathy) . Diabetes has been associated with impairment of kidney function. The kidney is actively involved in the development, maintenance and resolution of hyperglycemia through gluconeogenesis and glucose excretion (Marseric, 2009). The kidney is involved in the regulation of glucose via gluconeogenesis, taking up glucose from the glomerular filterate. Glucose utilization by the kidney after overnight fast accounts approximately 10% of glucose utilized by the body (Gerich, 2010). The cellular responses of the kidney to noxious incursions vary from minor biochemical abnormalities to cell death. The effects usually reported following toxic injury to the kidney reflect decreased elimination of wastes such BUN or increased creatinine levels (Braide and Anika, 2009). The BUN and creatinine are considered important biomarkers of kidney dysfunction (Mukinda and Eagles, 2010). The BUN and creatinine levels of 125 mg/kg extract-treated rats and glibenclamide-treated rats were significantly ($p<0.05$) reduced compared to the negative control group across the duration of the experiment (Figure 8 and Table 27). This may probably be as a result of mitigation of diabetes by the agents (extract and glibenclamide) resulting to a corresponding amelioration of the complication (diabetic nephropathy). The result is in agreement with the

findings of Eidi *et al*., (2005) who reported decreases in the creatinine and BUN values of *Allium sativum*-treated diabetic rats.

The negative control rats showed significantly ($p<0.05$) elevated levels of both total and unconjugated bilirubin when compared to the normal control rats (Tables 28 and 29). This observation may be consequent upon hepatocellular injuries induced by diabetes thus impairing hepatocyte function such as bilirubin conjugation. The hepatocytes are responsible for uptaking and conjugating bilirubin (Murray, 2000). Treatment of the rats with the extract (125 mg/kg) resulted in significant ($p<0.05$) reduction of the total and unconjugated bilirubin and elevation of conjugated bilirubin compared to the negative control group (Tables 28 and 29). This indicates that the extract may have hepatocurative properties. It could also be due to the antidiabetic properties of the plant.

Haematological evaluation revealed significant ($p<0.05$) reductions in the red blood cell (RBC), total haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and packed cell volume (PCV), values of the diabetic untreated rats compared to the normal control rats (Tables 32-36 and Figure 10). These observations agree with existing literature that anaemia is a common pathophysiology associated with *Diabetes mellitus* (Akindele *et al.,* 2012). The occurrence of anaemia in *Diabetes mellitus* has been reported due to the increased non enzymatic glycosylation of RBC membrane proteins which correlates with hyperglycemia (Oyedemi *et al.,* 2011). Oxidation of these proteins and hyperglycemia in *Diabetes mellitus* causes an increase in the production of lipid peroxides that lead to haemolysis of RBCs (Arun and Ramesh, 2002). Significant ($p<0.05$) increases in the RBC, haemoglobin and PCV values of the extract-treated rats observed towards the end of the experiment (Tables 32-33 and Figure 10) indicate that the plant extract may have anti-anaemic properties. It could also be consequent

upon the antidiabetic effects of the extract which disrupted pathophysiology of anaemia. No significant ($p>0.05$) changes were observed in mean corpuscular haemoglobin concentration, basophil, eosinophil, monocyte, lymphocyte and total white blood cell counts (Tables 37-42). The reason for this observation is not very clear.

Results of oxidative stress markers showed that superoxide dismutase activities in rats treated with 125 mg/kg body weight of the extract was significantly ($p<0.05$) higher than that of the negative control but statistically comparable ($p>0.05$) to that of the normal control rats and those treated with glibenclamide (Table 43). This indicates that the extract could protect against diabetes by mopping up superoxide anions generated in the course of pathogenesis of diabetes mellitus. Superoxide dismutase has been recognized to play an important role in body defense mechanism against the deleterious effects of oxygen free radicals in biologic systems (Kakkar *et al*., 1983). It catalyses the dismutation of reaction of superoxide radical anion (O^{2-}) to hydrogen peroxide which is then catalysed to innocuous oxygen and water by glutathione peroxidase and catalase (Kakkar *et al.,* 1983). This is in agreement with the reports of Jain *et al*., (2010) that hypoglycemic plant-treated rats showed significant increases in superoxide dismutase activity when compared to the negative control group.

Malondialdehyde (MDA) values of the diabetic untreated rats (Group 5) were significantly ($p<0.05$) higher than those of the treated groups and normal control rats throughout the period of the experiment (Table 43). *Diabetes mellitus* is associated with increase in lipid peroxidation. Persistent hyperglycaemia in *Diabetes mellitus* leads to increased formation of free radicals through various mechanisms. These free radicals attack and damage lipids, proteins and nucleic acids and results in various late diabetic complications. Malondialdehyde is an end product of lipid proxidation which is an oxidative stress marker (Anit *et al.,* 2012). The rats treated with 125

mg/kg extract showed significantly lower MDA values which were comparable to that of the normal control rats. This indicates that the extract was able to ameliorate lipid peroxidation in diabetic rats. Pandar and Kar (2007) also reported such decreases in the MDA values of diabetic rats treated with hypoglycemic plants.

The reduced glutathione levels of diabetic untreated rats were significantly ($p<0.05$) lower compared to the normal control rats (Figure 11). Upon treatment with the extract, the reduced glutathione level was improved. Reduced glutathione is one of the major antioxidant constituents of the erythrocytes that play a major role in protecting against oxidative damage (Rizvi and Zaid, 2001). Its antioxidant function is related to oxidation of the thiol group of its cysteine residue with formation of disulfide (GSSG) (Rizvi and Zaid, 2001). This is in conformity with the findings of Jain *et al*., (2010), who reported a 4 times increase in the value of reduced glutathione value of *Aloe vera*-treated diabetic rats compared to the negative control.

The significantly ($p<0.05$) reduced catalase activities of the negative control (group 5) rats were significantly ($p<0.05$) increased upon treatment with the extract. The catalase activity of the extract-treated rats was statistically comparable to that of the normal control rats (Table 45). Catalase catalyses the decomposition of hydrogen peroxide to water and molecular oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) (Chelikani *et al.*, 2004). The result therefore indicates that the extract could protect cells against oxidative damage which is the case in diabetes. Researchers (Pandar and Kar, 2007; Jain *et al*., 2010) have also reported increases in the catalase activities in hypoglycemic plant-treated diabetic rats.

A significant ($p<0.05$) decrease in weight of the diabetic untreated rats compared to the normal control rats was observed. This loss in weight may probably be as a result of *Diabetes mellitus*. *Diabetes mellitus* is associated with unexplained weight loss as a cardinal sign. Treatment of the rats with the extract and Glibenclamide improved weight gain. Glibenclamide has also been noted to increase weight of diabetic patients when used for treatment (Standiford, *et al*., 2014). This also implies that the extract may have benefits in improving weight gain in diabetic patients.

The histomorphologic assessment of various organs (pancreas, kidney, liver and heart) of diabetic rats showed various degrees of lesions. The pancreas of diabetic untreated rats showed severe depopulation of the islet cells of langerhans compared to the normal control rats. This probably is attributed to the effect of alloxan monohydrate in the pancreas. Alloxan causes the destruction of beta cells causing a reduction in insulin production with attendant diabetes mellitus (Szukuldeski, 2001). Treatment of diabetic rats with the extract ameliorated to a reasonable extent, the ongoing degenerative process in the cells of the islets of langerhans caused by alloxan. Fernandez-Alvarez *et al*., (2004) reported a regeneration of beta cell mass following treatment of streptozotocin-induced diabetic rats with tungstate for one month.

In the kidney, there were also various degrees of lesions ranging from mild to severe nephritis. The severe glomerular and tubular nephritis observed in the kidney of negative control rats may be consequent upon diabetes and or the effect of alloxan monohydrate in the kidney. Diabetes is the most common cause of chronic kidney failure in the developed and developing countries (Reutens *et al.,* 2008). Reutens *et al.,* (2008) also reported intercapillary glomerulonephritis among other lesions in diabetic nephropathy. The diabetic rats treated with various doses of the extract showed milder glomerulonephritic lesions.

The liver of the diabetic rats also showed severe degenerative changes in the hepatocytes. Some of the liver diseases occurring as a consequence of *Diabetes mellitus* include glycogen deposition (Stone and Van Thiel, 1985), steatohepatitis and cirrhosis (Hano, 1968). The diabetic rats treated with various doses of the extract revealed milder lesions compared with that of the diabetic untreated rats.

The cardiac myocytes of the diabetic untreated rats were severely degenerated. Diabetes increases necrosis by four fold in cardiac myocytes, nine fold in endothelial cells and six fold in fibroblasts (Frustaci *et al.,* 2000). The authors also reported that diabetes was characterized by 85-fold, 61-fold and 26-fold increases in the apoptosis of cardiac myocytes, endothelial cells and fibroblasts respectively.

Chromatographic analysis of *C. arborea* extract yielded seven different fractions (Table 15). Upon further purification and separation by bio-assay guided technique, subfraction one of fraction two (Figures 12) demonstrated hypoglycaemic potential after reducing the fasting blood glucose levels of diabetic rats from 310.00 ±5.77 to 80.00 ±0.57 mg/dl six hours post administration (Table 16). This subfraction was eluted with Hexane: Ethlyacetate in the ratio of 3:2. The active fraction (1.25 mg/kg) achieved 74% reduction of the fasting blood glucose levels of diabetic rats compared to the crude extract (125 mg/kg) which reduced the glucose levels by 73%. Thus, at a very minimal dose, the hypoglycemic potency of the active compound compares very well with that of the crude extracts.

Nuclear Magnetic Resonance (NMR) (proton and ^{13}carbon NMR) (Figures 13 and 14) was used for structural elucidation of active fraction of the extract. It was identified as an unsaturated pentacyclic triterpene, {(3ϐ)-3-hydroxyolean-12-en-28-oic acid} (Figure 15). This is an

Oleanolic acid. Triterpenes such as Oleanolic acids have been reported to possess antidiabetic activities. Oleanolic acid is a triterpenoid natural product found in a variety of plants and has been demonstrated to have a variety of biological effects. Interestingly, Oleanolic acid has been shown to have glucose-lowering properties in animal models *in vivo* (Yoshikawa and Matsuda, 2000). The mechanism by which Oleanolic acid reduces glycemia is not established. Several possibilities have been identified including effects on reducing gastric emptying (Yoshikawa and Matsuda, 2000), enhancing acetylcholine release that in turn may augment insulin release (Hsu et al., 2006) or reducing insulin resistance (Sato *et al.,* 2007). A recent study using the glucose unresponsive pancreatic beta cell line RINm5F has also suggested that Oleanolic acid may stimulate insulin secretion (Zhang *et al*., 2004). In a study by Teodoro *et al.,* (2008), Oleanolic acid significantly enhanced insulin secretion at basal and stimulatory glucose concentrations in INS-1 832/13 cells and enhanced acute glucose-stimulated insulin secretion in isolated rat islets. In the cell line the effects of Oleanolic acid on insulin secretion were comparable to that of the sulfonylurea tolbutamide at basal glucose levels and with the incretin mimetic Exendin-4 under glucose-stimulated conditions, yet neither Ca^{2+} nor cAMP rose in response to Oleanolic acid. Chronic treatment with Oleanolic acid increased total cellular insulin protein and mRNA levels. These effects may contribute to the anti-diabetic properties of this natural product. Oleanolic acid exerts beneficial effects against diabetes and metabolic syndrome. It improves insulin response, preserves functionality and survival of beta cells and protects against diabetes complications (Castellano *et al.,* 2013). Oleanolic acid may directly modulate enzymes connected to insulin biosynthesis, secretion and signaling. However, its major contribution appear to be derived from the interaction with important transduction pathways and many of its effects are consistently related to activation of the transcription factor NrF2. Doing that, Oleanolic acid induces the

expression of antioxidant enzymes and phase II response genes, blocks NF-kB and represses the polyol pathway, AGEs production and hyperlipidaemia (Castellano *et al.,* 2013).

5.2 CONCLUSION

The acute toxicity study result showed that the extract (*C arborea*) was not toxic even at the highest dose of 5000 mg/kg body weight of the extract. Phytochemical analysis of the extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenes. Oral glucose tolerance test in normoglycaemic rats showed that the plant extract, at t he dose of 250 mg/kg bw, reduced significantly, the blood glucose levels of the treated rats 180 min post glucose load compared to the distilled water treated rats that served as control. The extract demonstrated antihyperglycemia in alloxan-induced diabetic rats. The doses of 250 mg/kg body bw and 125 mg/kg bw of the extract showed the most significant hypoglycaemic activities in acute and chronic antidiabetic studies respectively.

The results of the chronic anti-diabetic studies indicated that the extract at the dose of 125 mg/kg reduced haemoglobin glycosylation and ameliorated the severity of lesions occasioned by *Diabetes mellitus* in different organs (pancreas, kidney, liver and heart). The study also showed that the extract demonstrated hypolipidemic properties with improvements in *in vivo* antioxidant parameters. Haematological assessment revealed significant increases in the red blood cell count, packed cell volume and total haemoglobin levels in the extract-treated rats. The results of the liver and kidney function tests indicate that the extract could protect against the damages caused by diabetes in these organs. The extract demonstrated *in vitro* antioxidant potential in ferric reducing antioxidant power (FRAP) and diphenyl picryl hydrazyl (DPPH) photometric assays. Chromatographic separation of the extract yielded seven fractions. Subfraction 1 of fraction 2

(fraction 2:1) showed the most significant hypoglycaemic activity. The hypoglycaemic activity of subfraction 2_1 at the dose of 1.25 mg/kg was 74.2 % while that of the crude extract at 125 mg/kg was 73.9 %. Spectroscopic elucidation of this active compound using ^{1}H proton and ^{13}Carbon nuclear magnetic resonance revealed a pentacylic triterpenoid, {(3ϐ)-3-hydroxyolean-12-en-28-oic acid}.

In general, the study showed that the methanol root bark extract of *C. arborea* did not only possess anti-hyperglycemic, hypolipidemic, antioxidant and anti-anemic properties but also reduced haemoglobin glycosylation and ameliorated the severe degenerative lesions in the pancreas, kidney, liver and cardiac myocytes occasioned by *Diabetes mellitus*.

RECOMMENDATION

It is recommended that further research in the form of clinical trial of the isolated compound be carried out.

REFERENCES

Aba PE, Asuzu IU, Odo RI (2014) Antihyperglycemic and antioxidant potential of *Cussonia arborea* in alloxan-induced diabetic rats; *Journal of Comp. Clin. Pathol*. 23:451-458

Abraham RJ, Fisher J, Loftus P (1988) Introduction to NMR spectroscopy, John Wiley and Sons, Chichester pp.98

Adler AI, Stratton IM, Niel HA (2000) Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study *BMJ* 321: 412-419

Agabegi ED, Agabegi SS (2008) Step up to Medicine (step-up series) Hagertwon MD: Lippincott Williams and WIlins ISBN 0-7817-7153-6. Pp71

Agerso H, Jensen LB, Elbrond B, Rolan P, Zdravkovic M (2002) The pharmacokinetics, pharmacodynamics, safety and tolerability of NN 2211, a new long-acting GLP-1 derivative, in healthy men *Diabetologia* 45: 195- 202

Ahmed MS, Reid K, Khardori N (2008) Respiratory infections in diabetes: reviewing the risk and challenges. *Journal of respiratory disease* 4 (1): 82-85

Ahren B (2008) Emerging dipeptidyl peptidase- 4 inhibitors for the treatment of diabetes. Expert. Opin. Emerg. *Drugs*, 13: 593-607.

Ahren B, Corrigan CB (1984) Intermittent need for Insulin in a subgroup of diabetic patients in Tanzania. *Diabet Med.* 2: 262-264.

Aiello SE (1998) Diabetes mellitus in Aiello SE (ed) ñThe Merck Veterinary Manual, 8th ed, Merck and Co. Inc. White house station, NJ U.S.A pp 394-396.

Akah PA, Orisakwe LE, Nwafor SV, Gammaniel KS (1998) Prospects of national plant products as anit ulcer agent. *Journal of pharmaceutical research Development* 3 (2): 57-62

Akindele OA, Babatunde AI, Chinedu FM, Samuel OA, Oluwasola CA, Oluseyi AA (2012) Rat models of food induced non-obese type 2 diabetes mellitus; comparative pathophysiology and histopathology. *International Journal of Physiology, Pathophysiology and Pharmacology* 4(1): 51-58

Akuodor GC, Udia PM, Bassey A, Chilaka KC, Okozie OA (2014) Antihyperglycemic and antihyperlipidemic properties of aqueous root extract of *Icacina senegalensis* in alloxan-induced diabetic rats. *Journal of Acute Disease,* 99-103

Alexander GC (2008) National trends in the treatment of type 2 diabetes mellitus. *Arch. Intern. Med*. 168 (19): 2088- 2094

Allain, C. C, Poon, L. S., Chan, C. S., Richmond, W. and Fu, P. U (1974). Enzymatic determination of total cholesterol. *Clinical Chemistry,* 20:470-475.

Amadou MD, Anna SN, DioP MN, Guata YM, Diarral HR, Gaffary AN, Babacar F (2008). Screening of plants for antidiabetic properties; *Fundamental and clinical pharmacology*.22**:** 211-216

Amantel C, Bronec W, Cotton M, Jenkins Q (2013) Diabetes Treatment guideline. Group health Cooperative, pp 1-13

American Diabetes Association (ADA) (1997) New Recommendations to lower the diabetes diagnosis pint. *Diabetes care*. Retrieved 1997 from http://www.diabetes.org/ada/mwclass.htm

Anaga AO, Asuzu IU (2010) Antihyperglycaemic properties of the ethylacelate extract of *Dennettia tripelala* in diabetic rats. *Journal of complimentary and integrative medicine* 1:1-11

Anand P, Murali KY, Tandon V, Chandra R, Murthy PS (2007) Preliminary studies on antihyperglycemic effect of aqueous extract of *Brassica nigra* (L.) Koch in streptozotocin induced diabetic rats. *Indian J Exp Bioi,* 45: 696-701.

Anit L, Suvarna P, Neeru B, Jasbir S, Rajesh P (2012) Malondialdehyde (MDA): an oxidative stress marker in type II diabetes mellitus with and without complication. *Current trends in biotechnology and chemical Research* 2 (2):54 Ctbcr.com/index.php/biochem/article/view/54

Aristides V, Rayaz A, Malik A (2007) Diabetic Neuropathy: Clinical Management (Clinical Diabetes 2nd ed.) Humans Press New York pp 188-198

Arun GS, Ramesh KG (2002) Improvement of insulin sensitivity by perindopril in spontenously hypertensive and streptozotocin diabetic rats. *Indian J. pharmacol.* 34**:** 156-164

Bailey CJ, Puah JA (1986) Effect of metformin of glucose metabolism in mouse soleies mycle *Diabetes metab* 12: 212-218.

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimerôs diseaseò *Lancet* 377 (9770): 1019-1031

Ban CR, Twigg SM (2008) Fibrosis in diabetes complications: pathogenic mechanisms circulating and urinary markers. *Vase Health Risk Manag* 4 (3): 575-596.

Banerji MA, Chaiken RI, Huey H, Tuomi T, Norin AJ, Mackay IR (1994) GAD antibody negative NIDDM in Adult black subjects with diabetic ketoacidosis and increased frequency of human luckotyte antigen DR_3 and DR_4. Flatbush diabetes: *Diabetes.* 43:741-745

Barnett A (2007) Exenetide. Expert. *Opin. Pharmacother*. 8**:** 2593- 2608

Barrett, TG, Bundey SE, Macleod AF. (1995) Neurodegeneration and diabetes: UK nationwide study of wolfram (DIDMOAD) syndrome. *Lancet.* 346: 1458-1463

Belfort R, Harrison SA, Brown K (2006) A placebocontrolled trial of proglitazone in subjects with non-alcoholic steatohepatitis. *N. Eng. J. med*. 355 (22): 2297- 2307

Bennett WL, Maruthur NM, Segal JB, Wilson LM, Chatterjee R, Marinpoulos SS, Puhan MA, Ranasinghe P, Block L, Nicholson WK, Hutfless S, Bass EB, Bolen S (2011) ñComparative effectiveness and safety of medications for type 2 diabetes: an update including new drugs and 2-drug combinations. *Annals of internal medicine* 154 (9): 602-613.

Benzie FF, Strain JJ (1999) Ferric reducing/Antioxidant power Assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology.* 299**:** 15-23.

Betterle C, Zanette F, Pedini B, Prescotto F, Rapp LB, Monosciotti CM (1983) Clinical and subclinical Organ specific autoimmune manifestations in type 1 (Insulin dependent) diabetic patients and their first degree relatives. *Diabetologia* 26: 431-436

Beutler E, Duron O, Kelly BM (1963) Improved method for determination of blood glutathione. *J Lab. Clin. Med.* 61: 882-888

Blaak EE, Van Hggel-Leiyssen DP, Wagen-Markers AJ, Saris WH, Van Baak MA (2000) Impaired oxidation of plasma derived fatty acid in type 2 diabetic subjects during moderate- intensity exercise. *Diabetes* 49: 2102-2107.

Blass, K. G., Thiebert, R. J. and Lam, L. K. (1974). A study of the mechanism of the Jaffe reaction: *Journal of Clinical Biochemistry*, 12:336 - 343.

Bliss M. (2000) The discovery of insulin, university of Chicago press, USA pp. 321-1418

Blois MS (1958) Antioxidants determination by the use of a stable free radical. *Nature,* 181: 1199-1200.

Blumer (2010) Oral hypoglycaemics. *Journal of complementary and integrative medicine* 5 (2): 435-438

Bnouham M. (2006) Medicinal plants with potential antidiabetic activity: a review of ten years of herbal medicine research *Int. J. diabetes and Metabolism* 14:1-25.

Bodansky HJ, Grant PJ, Dean BM, McNally J, Bottazzo GF, Hamblung MH (1986) Islet cell antibodies and insulin auto-antibodies in association with common viral infections. *Lancet*. 2: 1351-1353.

Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G., (1985) Relationship between degree of obesity and *in vivo* insulin action in man. *Am. J. physiol*. 248: 286-291.

Borghouts LB, Wagen Makers AJ, Goyens PL, Keizer HA (2002) Substrate utilization in non-obsese type II diabetic patients at rest and during excerise. *Clin. Sci* (Lond) 103: 559-566.

Borris EM (2007) Effect of proglitazone treatment on behavioural symtoms in austistic children. *J. neuroinflammation* 3 (1): 4

Boussageon R, Bejan- Angoulvant T, and Saadatian Elahi M (2011) Effect of intensive glucose lowering treatment on all cause mobility cardiovascular death, and microvascular events in type2 diabetes: meta analysis of randomized controlled trials: *BMJ* 343:4169. PMC 3144314

Braide LM, Anika SM (2009) Environmental Toxicology, 1st edition SNAAP Press Ltd, Enugu**.** Pp 89-99

Brownlee M (2000). Negative consequence of glycation. *Metabolism* 49: 9-13

Bruno G, Merletti F, Pisu E, Pastore G, Marengo C, Pagano G (1990) Incidence of IDDM during 1984-1986 in population aged less than 30 yrs. Residents in Turin, Italy. *Diabetes care* 13 (10): 1051-1056

Bryan J (2004) Just the about Facts Diabetes,Chicago Illinois: Heimannan Library a division of Reed Elserier Inc. P. 7. ISBN 1-4034-4600-8.

Bunn HF, Hancy DN, Kamin S, Gabbay KH, Gallop PM (1976) The biosynthesis of human hemoglobin A.C. Slow glycosylation of hemoglobin *in vivo*. *J. Clin. Invest*. 57: 1652-1659.

Burns A, LLiffe S (2009) ñAlzheimerôs diseaseò BMH (Clinical Research ed.) 338: b158 doi: 10: 1136/bmj.b158 PMID 19196745.

Buse JB, Henry RR, Han J, KIM DD, Fineman MS, Baron AD (2004) Effects of exanatide (exendin- 4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes care* 27: 2628-2635

Cambon-Thomsen A, Rial-Sebbag E, Knoppers BM (2007) Trends in ethical and legal for use of human biobanksò. *European Respiratory Journal* 30 (3): 373-382

Castellano JM, Guinda A, Delgado T, Mirela R, Cayuela JA (2013) Biochemical basis of antidiabetic activity of oleanolic acid and related triterpenes. *America Diabetes Association* 62 (6):1791-1799

Celik IE, Yegin F, Odabasoglu (2002) Effect of experimental diabetes mellitus on plsama lactate dehdrogenase and glutamic oxaloacetic transaminase levels in rabbits, *Turkish J. Biol*. 26**:** 151-154

Chelikani P, Fita I, Loewen PC, (2004) Diversity of structures and properties among catalases. *Cell mol. Life sci*. 61**(**2):192-208

Cherry- Pepper G, Sorkin J, Andres R, Baum BJ, Ship A (1992) Salivary gland function and glucose metabolic status. *Journal of Gerontology: Medical Sciences*: 47 (4): Mizo-M134.

Clement JP, French LR, Goetz FC (1993) A re-evaluation of the effectiveness of a single fasting plasma glucose sample as a screening test for diabetes mellitus. The Wadena city health study. *Diabetes.* 42 (suppl. 1) 31A

Clement K, Pueyo ME, Vaxillare M, Rakotoambinina B, Thuillier F, Passa P (1996) Assessment of insulin sensitivity in glucoskinase- deficient subjects. *Diabetologia* 39: 82-90

Coles EH (1986) Fundamentals of Veterinary Cinical Pathology 4th edition W.B Saunders Philadelphia, USA. Pp56

Colville J (2002) Blood Chemistry In: Hendrix CM (ed) Laboratory Procedures for Veterinary Technicians. 4th edn. Mosby, St. Louis, pp 75 - 103

Cook DW, Plotmck L (2008) ñType I diabetes mellitus in Pediatricsò. *Pediatr. Rev.* 29 (11): 374-384

Couri CE, Oliveira MC, Stracieri AB (2008) C-petptide levels and insulin independence following autologous nonmyeloblative hematopetic stem cell transplantation in newly diagnosed type I diabetes mellitus. *Journal of American medical Association* 301 (15): 1573-9.

Cousen G (2008) ñThere is a cure for diabetesò. North Atlantic Books, Berkeley, California pp 1-566. ISBN 978-1-58394-544-5.

Culy CR, Jarvis B (2001) Repaglinide: a review of its therapeutic use in type 2 diabates mellitus. *Drugs* 61: 1625-1660.

Dallas J (2011) Royal College of Physicians of Edinburgh. Diabetes, Doctors and Dogs. An exhibition on Diabetes and Endocrinology by the college Library for the 43rd st. Andrewôs Day Festival Symposium

De villiers BJ, Vuuren SF, Vanzyl RL, Van BE (2010) Antimicrobial and antimalaria activity of *Cussonia arborea. Journal of Ethnopharmacology*, 7(2):323-8

Defronzo RA, Ratner RE, Han J,KIM DD, FIneman MS, Braon AD (2005) Effects of exanatide (exendin- 40 on glyceemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes care* 28: 1092-1100.

Dejager S, Razac S, Foley JE, Schweizer A (2007) Vildagliptin in drug-naïve patients with type 2 diabetes: a 24-week, double blind, randomized, placebo-controlled, multiple- doze study. *Horn metab. Res*. 39: 218- 223

Dewanjee S, Bose SK, Sahu R, Mandal SC (2008) Antidiabetic effect of matured fruits of *Diospyros peregrine* in alloxan induced diabetic rats. *International Journal of green pharmacy* pp 2:95-99

Diamart M, Heine RJ (2003) Thiazolidinediones in type 2 diabetes mellitus. *Drugs* **63**: 1373-1405.

Diana S, Kara N, Eleanor P, Jiemmy C and Hertzel C (2010) The effect of oral antidiabetic agents on A1c levels: systematic review and meta-analysis. *Diabetes care,* 33(8): 1859-1864

Dietrich E, Powell J, Taylor JR (2013) Canagliflozin: a novel treatment option for type 2 diabetes. *Drug design, development and therapy,* 22 (7): 1399-1408

Dorman JS, McCarthy BJ, Oô Leary LA, Kochler AN (1995) Risk factors for insulin dependent diabetes. In Diabetes in America (2nd ed) National Institute of health Available: http://diebetes ï in-america.s-3.com/contents/html

Dornhorst A (2001) Insulinotropic meglitinide anaologues. *Lacet,* 358-716.

Doumas BT, Perry BW, Sasse EA, Straumfjord Jr JV (1971) Standardization in bilirubin assays: evaluation of selection methods and stability of bilirubin solutions. *Clinical Chemistry,* 19:984-993

Dovgii VI, Grishkovets VV, Shashkov K (2005) Triterpene glycosides from *Cussonia paniculata*. Isolation and structure determination of glycosides A, B1, B2, C, D, G2, H1 AND H2 from leaves of *Cussonia paniculata. Springer-link Journal article.* 2:160-163

Draeger E (1995) Clinical profile of glimeperide. *Diabetes. Res. Clin pract.* 28 (suppl. 1): S 139-146

Drucker DJ (2006) Incretin-based therapies: a clinical need filled by unique metabolic effects. *Diabetes Educ.* 32 (suppl 2) 65-71

Drury RA, Wallington A, Cameroun SR (1967) "In: Carlleton's Histological Techniques". Oxford University press, New York. pp 1-420

Edward CA, Blackburn MA, Craigen L (1987) Viscosity of food gums determined *in-vitro* related to their hypoglycaemic actions. *American Journal of Clin. Nutri.* 46: 72-77

Eidi A, Eidi M, Esmaeili E (2005) Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicin*e 13: 624-629.

Eissele R, Goke R, WIllemers HP, Vermeer H, Arnold, R, Goke B (1992) Glucagon- like peptide- 1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur. J. Clin. Invent.* 22: 283-291.

El ï Dermardash FM, Yousy MV and EL-waga NIA (2005) Biochemical study on the hypoglycaemic effects of onion and garlic in alloxan-induced diabetic rats. *Food and chemical Toxicity*, 43: 57-63

Eng J, Kleinman WA, Singh L, Sing G, Ranfman JP (1992) Isolation and characterization of exendin-4, an exendin- 3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig paneceas. *J. Biol. Chem.* 267**:** 7402-7405.

English E, Milosevich ET, John WG (2014) in vitro determination of hemoglobin A1c for diabetes diagnosis and management: technology update; Received 21 March 2014; Accepted 14 April 2014; Published 16 July 2014; *Dovepress* 6: 21-31

Ergon S (1967) Thin layer chromatography (2nd edition). *Springer-verlag Berlin*. Pp 88-120

Esposti MD, Ngo A, Meyers MA (1996) Inhibition of mitochondrial complex I may account for IDDM Induced by intoxication with rodenticide, Vacor. *Diabetes* 45: 1531-1534.

Etuk EU (2010) Animal models for studying diabetes mellitus. *Asian Journal of Experimental Biological Sciences* 1: 331-336.

Fabricant DS, Farnsworths NR (2001) The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspective Supplements*. 109 (1): 66-76.

Fabris P, Betterle C, Floreani A, Greggio NA, de Lazzari F, Naccarato R (1992) Development of type -1 Diabetes mellifus during interferon alfa therapy for chronic HCV hepatitis (letter). *Lancet,* 340: 548.

Factor SM, Bhan R Minase T, Wolinsky H and Sonnenblick EH (1981) Hypertensive diabetic cardiomypathy in the rate: an experimental model of human disease. *Am. J. pathol*. 102: 219-228

Fawcett, J. K. and Scott, J. E. (1960) A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, 13:156-159.

Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK (2004) Induction of type 1 diabetes mellitus in laboratory rats by use of alloxan; route of administration, pitfalls and insulin treatment. *Comprehensive Med*. 54: 252- 257.

Fernandez-Alvarez J, Barbera A, Nadal B, Barcelo-Batkoric S, Piquer S, Claret M, guinovart JJ, Gomis R, (2004) Stable and functional regeneration of pancreatic beta-cell population in nSTZ-rats treated with tungstate beta cell neogenesis by tungstate. *Diabetologia*, 47 (3): 470

Fimognari-Pastorelli R, Incalzi RA (2006) Phenformin induced lactic acidosis in an older diabetic patient: A recurrent drama (Phenformin and lactic acidosis). *Diabetes care*, 29 (4): 956-1.

Flier JS (1992) Lecture: Syndromes of insulin resistance: from patient to gene and back again. *Diabetes,* 4**1**: 1207- 1219

Food and Drug Administration (2010) Questions and answers ï safety requirement for victoza (Liraghitude). http://www.fda.gov/Drugs/drysafety/port market drug safety information for patients and providers/ucm 198543.htm.

Forrest JA, Menser MA, Burgess JA (1971) High frequency of diabetes mellitus in young patients with congenital rubella. *Lancet,* 2: 332- 334

Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-deoxy- æ^{12} 14 prostaglandin J2 is a Ligand for the adopocyte determination factor PPARY. *Cell,* 83: 803-812.

Freinkel N (1980) Banting lecturer 1980: of pregnancy and progeny. *Diabetes,* 29: 1023-1035.

Friedwald WT, Levy RL, Fredickson DS (1972) Estimation of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18: 499-502

Frode TS, Medeiros YS (2008) Animal Models to test drugs with potential antidiabetic activity. *Journal of ethnopharmaology,* 115**:** 173-183.

Froguel P, Vaxillaire M, Sunf, Velho G, Zouali H, Butel MO (1992) Close linkage of glucokinase locus on chromosome 7p to early- onset-non-insulin-dependent diabetes. *Nature*. 356: 162-164.

Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, Nadel-Ginard B, Anversa P (2000) Myocardial cell death in human diabetes. *Circulation Research,* 87: 1123-1132

Gallwitz B (2006) Extenctide in type 2 diabetes: treatment effects in clinical studies and animal study data. *Int. J. Chin. Pract.* 60: 1654 ï 1661.

Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, knowler WC, Lebovitz H, Lernmar KA, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J and Zimmet P (2003) The expert committee on the diagnosis and classification of diabetes mellitus 2, the expert committed on the diagnosis and classification of Diabetes mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes care*. 26: 3160-3167.

Gerich JE (2010). Role of kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: Therapeutic implication. *Diabetes medicine*, 27: 136-142

Gispen, WH and Biessels GJ (2010) cogitation and synaptic plasticity in diabetes mellitus. *Trends Neurosci*. 23 (11): 542s-9

Glenn LW, Susan PL (1981) The role of chemicals in diabetes mellitus. *Toxicological pathology* 17: 357-363.

Goldberg IJ (2001) Diabetic Dyslipidemia: Statins versus Fibrates in the Treatment of Diabetic Dyslipidemia. Symposium. Program and abstracts of the 61st Scientific Sessions of the American Diabetes Association; June 22-26, Philadelphia, Pennsylvania

Goldstein D, Wield-Mayer HM, England JD, Little RR, Parker KM (1984) Recent advances in glycosylated hemoglobin measurements. *CRC crit Clin. Lab. Sci* 52: 282-291

Grandhipuran PSK, Palamissmy A, Durairay SK, Sorimuthu PS (2006) Phenformin induced lactic acidosis in an older diabetic patient: A recurrent drama (phenformin and Lactic acidosis) *Diabetes care,* 29 (4) 956-961.

Graw J (2009) Mouse model of cataract. *Journal of genetics*. 84: 469-486.

Grodsky GM, Epstein GH, Fanska R and Karan, H (1977) Pancreatic action of sulfonylures. *Fed. Proc.* 36: 2714-2719.

Gromada J, Dissings-Kofod H, Frokjaer- Jensen J (1995) Effects of the hypogycaemic drugs - Rapaglindie and glibenclamide on ATP- sensitive potassium- channel and rat pancreatic beta cells. *Diabetologia*, 38: 1025-1032

Guiochon G (2001) Basic Principles of Chromatography in: Handbook of Anal. Tech. Glinzler H and Williams A (eds). 1: 173-198 Wiley- VCH Verleg GMBH, Winheim, Germany

Guo, C; Yang J; Wei J; Li Y; Xu J. Jiang, Y; (2003). Antioxidant activities of peel, pulp, and seed fractions as determined by FRAP assay. *Nutr. Res*. 23: 1719-1726.

Gupta SK, Kalaiselvan V, Saxena R, Agrarwal CS (2009) Advance in pharmacological strategies for prevention of cataract development. *Indian Joural of ophthalmology,* 57:175-183.

Gurnell M, Savage DB, Chatterjee VK, Orahilly S, (2003) The metabolic syndrome: peroxisome proliferation activated receptor gamma and its therapeutic modulation. *J. Clin. Endocrinol. Metab*. 88: 2412- 2421

Guyton AC (1996) Textbook of medical physiology 3rd ed; W.B. Saunders Company, London pp 1093-1098.

Haneda M, Polonsky KS, Bergenstal RM, Jaspan JB, Shoelson SE, Blix PM (1984) Familial hyperinsulinaemia due to a structurally abnormal insulin. Definition of an emerging new clinical syndrome. *N. Engl. J. Med*. 3010: 1288- 1294.

Hano T (1968) Pathohistological study on the liver cirrhosis in diabetes mellitus. *Kobe J Med Sci* 14:87-106,.

Hargreaves M, Kiens B, Richter EA (1991) Effect of increased plasma fatty acid concentration or muscle metabolism in exercising men. *J. Appl. Physiol.* 70: 194-201

Harris M.I, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Bryd- Hold DD (1998) Prevalence of diabetes, Impaired fasting glucose and impaired glucose tolerance in US adults. The Third National Health and Nutrition Examination Survey. *Diabetes Care,* 21 (4): 518: 524.

Harris MI, Hadden WC, Knowler WC, Bennett PH (1985) International criteria for the diagnosi of diabetes mellitus and impaired glucox tolerance. *Diabetes care,* 8: 562-567.

Hasselgren PO, Jagenburg R, Karlstrom L, Pedersen P, Seeman T.(1984) The Changes of protein metabolism in liver and skeletal muscle following trauma complicated by sepsis. *J Trauma,* 24**:** 224-228.

Hatorp V (2002) Clinical pharmacokinetics and pharmacodynamics of repaglinide. *Clin. Pharmacokinet,* 41: 471- 483.

Hatorp V, Huang WC, Strange P, (1999) Repaglinide pharmacokinetics in healthy young adult and elderly subjects. *Clin. Ther*. 21: 702-710

Heine RJ, Van Gaal LF, Johns D, Mihm MJ, Widel MH, Brodows RG (2005). Exenatide versus insulin glargine in patients with suboptimially controlled type 2 diabetes: a randomized trial. *An intern. Med*. 143: 559-569.

Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, Desmet M, Synder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Ramaels, Gottsdiner KM, Wagner JA (2005) Pharamcollinction and pharmacodynamics of sitigliptin, an inhibitor of dipeptidylpeptidase iv, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin. Pharmacol. Ther.* 78: 675-688.

Higgins T, Beutler E, Doumas BT (2008) Measurement of Haemoglobin in Blood. In: Burtis C.A., Ashwood E. R. and Bruns D. E.(Eds.), Tietz Fundamentals of Clinical Chemistry, 6th Edition, Saunders Elsevier, Missouri. Pp 514 - 515.

Hongxiang Hui, George Tang, Vay Liang W, Go LW.(2009) Hypoglycemic herbs and their action mechanisms. *Chin Med* 4: 11-14.

Hother-Nielson O, Faber O, Sorensen NS, Beck-Nielsen H (1988) Classification of newly diagnosed diabetic patients as insulin requiring or non-insulin-requiring based on clinical and biochemical variables. *Diabetes care*. 11: 531-537.

Howlett RA, Parolin ML, Dyck DJ, Hultman E, Jones NL, Heigenhauser GF, Spriet LL (1998) Regulation of skeletal muscle glycogen phosphorylese and PDH at varying exercise power outputs. *Am. J. Physiol* 275: 418-425.

Hsu JH, Wu YC, I. Liu MJ. Cheng T. (2006) Release of acetylcholine to raise insulin secretion in Wistar rats by Oleanolic acid one of the active principles contained in *Cornus officinalis Neurosci. Lett.,* 404 :112ï 116 |

Hu S (2002) Interaction of nateglinde with k (ATP) channel in beta- cells underlies its unique insulnotropic action *Eur J. Pharmacol.* 442: 163-171

Humphray ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet P (1998) Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult-onset diabetes mellitus. *Diabetic med*. 15:113-119

International Expert Committee (IEC) (2009) International Expert Committee on the role of the HbA1c assay in the diagnosis of diabetes. *Diabetes care*, 32: 1327-1334

Inzucchi SE, Bergenstal RM, Buse JB (2012) Management of hyperglycemia in type 2 diabetes: a patient centred approach. Passion statement of the American Diabetes Association (ADA) and the European Association for the study of Diabetes (EASD). *Diabetologia*. 55**:** 1577-1596.

Jain N, Vijayaraghavan R, Pant SC, Lomash V, Ali M. (2010) Aloe vera gel alleviates cardiotoxicity in streptozocin-induced diabetes in rats. *J Pharm Pharmacol.* 62: 115-123.

Jepson MM, Pell JM, Bates PC, Millward DJ (1986) The effects of endotoxaemia protein metabolism in skeletal muscle and liver of fed and fasted rats.*Biochem J*. 235:329-336.

Jerald EE, Joshi SB and Jain DC (2008) Antidiabetic activity of flower buds of *Michehia champaca* (linn). *Indian J. Pharmacol*, 64:510-571

Johns DR. (1995) Mitochondria. DNA and disease. *N. Engl J*. Med. 33: 638- 644

Kadarian C, Broussalis AM, Miño J, Lopez P, Gorzalczany S, Ferraro G, Acevedo C (2002) Hepatoprotcctive activity of *Achyrocline satureioides* (Lam) D. C. *Pharmacol Res* 45: 57-61.

Kahn CM (2005) Diabetes Mellitus in Kahn CM (ed) ñMerk veterinary Manual, 9th edition, Merck and Co, Inc. White House Station, NJ U.S.A 439-1013

Kahn CR, Bairel KL, Flier JS, Jarrett DB (1977) Effects of autoanitbodies to the insulin receptor on Isolated adipocytes. *J. Univ. invest.* 60: 1094-1106.

Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P. Martin MM (1976) The yndromen of Insulin Resistance and acanthosis nigrican*. N. Engl. J. Med.* 294: 739-745

Kahn CR, Schechter Y (1991) Insulin oral hypoglycaemic agent and the pharmacology of the endocrine pancrease in the pharmacological basis of therapetuics. Gillman A.G Rail, Rail TW, Nies AS and Taylor. New York Pergamon Press: 1463-1495.

Kakkar SB, Viswanathan P (1983) A modified spectrophotometric assay of superoxide dismutase, Indian J. Biochem. Biophysics, 21: 130-132

Karjalainen J, Martin J, Knip M (1992) ñA Bovine albumin peptide as a possible trigger of Insulin- dependent diabetesò. *N. Eng. J. Med.* 327: 302-307

Katherine M, Laura RM (2009) Steptoxotocin, Type- 1 diabetes mellitus and Bone. *Biological procedures online* 11: 296-315.

Kavishankar GB, Lakshmidevi N, Mahadeva S, Prakash HS, Niranjana SR (2011) Diabetes and medicinal plants-A review; *Int J Pharm Biomed Sci.* 2 (3 65-80)

Keelson L, mather S, Walter YH (2000) Synergistic effects of nateglinide and meal administration on insulin secreation in patients with type 2 dibates milleitus. *J. Clin. Endocrinol metab.* 85: 1081-1086.

Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD (2005) Effects of exenatide (exendin- 4) on glycaemie control over 30 weeks in patients with type 2 diabetes, treated with metformin and a sulfonylurea. *Diabetes care,* 28**:** 1083-1011

Kermorvant-Duchemin E, Sapieha P, Sirinyan M, Beauchamp M, checchin D, Hardy P, Sennlaub F, Lachapelle P, chemtob S, (2010) Understanding ischemic retinopatheies: emerging concept from oxygen-induced retiopathy. *Doc. Ophthalmol*. 120: 51-60.

Khazraji SM, Shamaony LA, Twaij HA (1993) Hypoglycaemic effect of Artemisia herba alba.: effect of different parts andinfluence of the solvent on hypoglycemic activity. *J Ethnopharmacol*, 40**:** 163-166.

King H, Aubert R.F, Herman WH (1998). Global burden of diabetes: 1995-2025: Prevalence, numerical estimates and projection: *Diabetes care,* 21: 1414-1431

King H, Rewers M (1993) Global estimates from prevalence of diabetes Mellitus and impaired glucose tolerance in adults. WHO ad HO diabetes Reporting Group, *Diabetes Care*, 16 (1): 157-177.

Kinsangau DP, Hosea KM, Herbert VM, Cosam CJ, Zakaria HM, Pax JM, Catherine BG, Lenta NB, Krishna PD, Nobert S (2007) Plant species used in treating various HIV/AIDS-related conditions in Bukoba rural district. *Journal of Ethnobiology and Ethnomedicine* 3: 29

Kissebah AH, Vydelingum N, Murray R, Evans DF, Hartz AJ, Kalkhoff RK (1982) Relationship of body fat distribution to metabolic complications of obesity J. *Lin. Endocrinol. Metabolism* 54: 254-260.

Kliewer SA, Lenhard JM, Willson TM, Plate I, Monis DC, Lehmann JM (1995) a prostaglin J2 metabolile binds peroxisome proliferations activated receptor Y and promotes adopocyte differentiation. *Cell,* 83: 813-819

Koepsell H, Lips K, Volk C (2007) Poly specific organization transporters: structure, function, physiological roles and biopharmaceutical implications. *Pharm. Res*. 24: 1227- 1251.

Kolterman OG, Buse JB, Finemann MS, Gaires E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, baron AD (2003) synthetic exenclin ï 4 (exenatide) significantly reduces post prandiat and fasting plasma glucose in subjects with type 2 diabetes. *J. Clin. Endocrinol. Metal.,* 88: 3082-3089.

Kramer W, Muller G, Girbig F (1994) Differential interaction of glimeperide and glibenclamide with the B cell sulfonylureas receptor II. Photo affinity labeling of a 65 kda protein by (3H) glimeperide. *Bioch. Bioplys. Acta,* 119: 278-290

Krejs G, Orci L, Conlon JM Ravazzola M, Davis GR, Raskin P (1979) Somatostatinoma syndrome *N. Eng. J. med.* 301**:** 285-292

Krentz AJ, Friedmann PS (2006) Type 2 diabetes, psoriasis and thiazolidinediones *Int. J. Clin. Pract.* 60 (3): 362-363

Krishna MG, Coker RH, Lacy DB, Zinker BA Halseth AE, Wasserman DH (2000) Glucagon response to exercise is critical for accelerated hepatic glutamine metabolism and nitrogen disposed. *Am J. Physiol.* 279: 638-645

Kuhl C, Holst J.J (1976) ñPlasma glucagon and Insulin: glucagon ratio in gestational diabetes. *Diabetes.* 25:16

Kuma RV, Augusti KT (1989) Antidiabetic effect of a leucocyanidin derivative isolated from the bark of *Ficus bengalensis* Linn. *Indian J Biochem Biophys* 26: 400-404.

Kuzuya T, Matsuda A (1997) Classification of diabetes on the basis of aetiologies versus degree of insulin deficiency. *Diabetes care;* 20: 2019-220.

Landgraf R (2000) Meglitinide analogues in the treatment of type 2 diabetes mellitus. *Drugs Aging.* 17: 411-425.

Lanza RP, Eeker DM, Kuhtreigber UM, Marsh JP, Ringelin J, Chink WL (2001) Transplantation of Islets using micro encapsulation: studies in diabetic rodents and dogs. *J. mol. Med*. 77: 206-210

Larsen JJ, Dela F, Kjaer M, Galbo H (1997) The effect of moderate exercise on postprandial glucose homeoostasis in NIDDM patients. *Diabetologia* 40: 447-453

Larsens, Hilsted J. Tronier B, Worning H, (1987) Metabolic control and B cell function in patients with insulin-dependent diabetes mellitus secondary to chronic pancreatits. *Metabolism*.36: 964-967

Lawrence JM, Contreras R, Chen W, Sacks DA (2008) ñTrends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially /ethnically diverse population of pregnant women. *Diabetes care,* 31 (5): 899-904

Lee CH, Olson P, Evans RM (2003) Mini review: lipid metabolism, metabolic diseases, and peroxisome proliferator- activated receptors. *Endocrinology* 144: 2201-2207

Leonid P (2009) Principles of diabetes mellitus (2nd ed) New York: Springer p. 3 ISBN 978-0-387-09840-1

Lillioja S, Mott D.M, Spraul M, Ferraro R, Foley JE, Ravussin E (1993) Insulin resistance in insulin secretory dysfunction as precursors of non-insulin dependent diabetes prospective study of Pima Indians .*N. Eng. J. Med*. 329**:** 1988-1992

Lim S, Yoon JW, Choi SH, Cho BJ, Kim JT, Chang HS, Park HS, Park KS, Lee HK, Kim YB, Jang HC (2009) Effect of ginsam, a vinegar extract from *Panax ginseng,* on body weight and glucosehomeostasis in an obese insulin-resistant rat model. *Metabolism* 58: 8-15.

Liu TP, Liu IM, Cheng JT. (2005) Improvement of insulin resistance by *panax ginseng* in fructose-rich chow-fed rats. *Horm Metab Res.,* 37**:** 146-151.

Lund S, Holman GD Schmitz O, Pedersen O (1995) Contraction stimulates translocation transporter GLUt4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc. Natl. Acad. Sci USA* 92: 5817-5821

Luzi L (1988) Pancreas transplantation and diabetic complications; *New England of Medicine*, 339: 116-117

Luzio SD, Anderson DM, Owens DR (2001) Effects of timing of administration and meal composition on the pharmacokinetic and pharmacodynamic characteristics of the short-acting oral hypoglyceamic agent nateglinide in healthy subjects. *J. Clin Endocrinol metab.* 86: 4874-4880

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastne P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor super family: The second decade. *Cell,* 83: 835-839

Marbury TC, Ruckle JL, Hatorp V (2000) Phamacokinetics of repaglinide in subjects with renal impairment. *Clin. Pharmacol. Ther.* 67**:** 7-15

Marliss EB, Sigal RJ, Manzon A, Halter JB, Vramic M (1995) Glucoregulation in intense exercise and its implication for persons with diabetes mellitus. In proceedings of the IDF satellite symposium on Exercise, fuel homestasis and Diabetes mellitus, Nara, Japan 12-13 Nov. 1994. London Smith Gordon

Marre M, Shaw J, Brandle M, Bebakar WM, Kamaruddin RA, Strand J, Zdravkovic M, Li Thi, TD, Colagiuri S (2009) Liraglutide, a once daily human GLP-1 analogue, added to a sulfongular over 26 weeks produces greater improvement in glycaemic and weight control compared with adding rosighitazone or placebo in subjects with Type 2 diabetes (LEAD- ISu)- *Diabet. Med.* 26: 268-278.

Marseric O (2009) Glucose control by the kidney: an emerging target in diabetes. *Am. J. Kidney Disease*, 53:875-883

Mattson MP (2004) Pathways towards and away from Alzheimerôs disease. *Nature,* 430: 631-639.

McCall AL (2004) Cerebral glucose metabolism in diabetes mellitus. *Eur. J. Pharmacol*. 490: 147-158

McCann TM; Simpson K.E; Shaw D.I; Butt JA; Gunn-Moore DH (2007). Feline diabetes mellitus in the UK: The prevalence within an insured cat population and a questionnaire based putative risk factor analysis. *Journal of Feline Medicine and Surgery* 9 (4): 289-99

McFarlane IA (1997) Endocrine diseases and diabetes mellitus. In: pickup JC Williams G Eds. Textbooks of Diabetes 2nrd ed. Oxford: Blackwell. Pp 1-64

McLarty DG, Athaide I, Bottazzo G.F, Swai ABM, Alberti KGMM (1990) Islet cell antibodies are not specifically associated with insulin-dependent diabetes in rural Tanzanian Africans. *Diabetes Res. Clin. Pract*. 9: 219-224.

Mensor F, Luciana L, Fabia S, Menezes G, .Leitao AS, Reis T C, Dos-santos S, Coube S, Leitao G (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 15:127-130

Meyes P.A. (2000) Gluconeogenesis and control of blood sugar (Glucose). Harpers Medical Biochemistry (25th ed). Lange textbook p. 208

Mijac V, Arrieta J, Mendt C, (1997) ñRole of environmental factor in the development of insulin-dependent diabetes mellitus (DDM) in Insulin Dependent Venezuelan Childrenò *Invert Clin.* 36: 73-82

Minuk HL, vranic M, Hanna Ak, Albiser AM, Zinman B (1981) Glucoregulatory and metabolic response to exercise in obese non-insulin dependent diabetes. *Am. J. Physiol*. 240: 458-464.

Modan M, Harris MI (1994) Fasting Plasma glucose in screnning for NIDDM in the US and Isreal. *Diabetes Care*. 17: 436-439

Mohammed F, Ghafar K, Nagendra P, Kong KW, Amin I (2009) Flavanoid,, hesperidine, total phenolic contents and antioxidant activities from *Citrus species*. *Africa journal of Biotectrology* 9 (3): 326-330.

Molbak AG, Christau B, Marner B, Borch Johnsen K, Nerup J (1994) Incidence of insulin dependent diabetes mellitus in age groups over 30 years in Dennmark. *Diabet. Med*. 11: 650-655.

Moran A, Pyzdrowski KL, Weinreb J, Kahn BB, Smith SA, Adams KS (1994) Insulin sensitivity in cystic fibrosis. *Diabetes*. 43: 1020-1026.

Mudaliar S, Henry RR (2001) New oral therapies for type 2 diabetes mellitus: the glita zones or insulin sensitizers. *Annu Rev. med.* 52: 239- 257

Mukinda JT, Eagles Fk (2010) Acute and subchronic oral toxicity profile of aqueous extract of *Pohygala fruitcosa* in female mice and rats. *J. Ethnopharmacol.,* 128**:**236-240

Muller G, Wied S (1993) The sulfonylurea drug glimeperide stimulates glucose transport, glucose transporter translocation and dephosphoryletion in insulin resistant rat adipocytes *in vitro*. *Diabetes,* 42: 1852-1867.

Murray RK (2000) Porphyrins and Bile Pigment: In metabolism of proteins and amino acids. Harpers Biochemistry (25th edition). Mc Graw-Hill Publishers Pp 359-374

Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creautzfieldt W (1986). Incretin effects of increasing glucose loads in man calculated from vamas insulin and c-peptide responses. J. *Clin Endocrinol metab.* 63: 492-498.

Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM (1993) Free radicals scavenger and antihepatotoxic activity of Rosmarinus. *Plant Medicine,* 59**:**312-314

Nelson RW (2000) Diabetes Mellitus; the textbook of Veterinary internal Medicine; Disease of dog and cat in Ehinger S. J and Feldman E.C. (eds.) Elsevier Saunders, USA pp 1563-1591

Nissen SE, Nicholds SJ, Wolski K (2008) Comparison of proglitazone vs glimeperide on progression of coronary atheroscleoris in patients with type 2 diabetes: The PERISCOPE randomized controlled tiralû *JAMA* 299 (13): 1561-1573.

Noto H, Goto A, Tsujimoto T, Noda M (2012) Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis: *Plos one* **7:**e33411

Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ, Kraegen EQ (1994) A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucorepulation in the rat. *Diabetes,* 43: 1203- 1210

Oliveira HC, Dos Santos MP, Grigulo R, Lima LL, Martins DTP, Lima JCS, Stoppiglia LF (2008) Antidiabetic activity of *Vatairea macrocarpa* extract in rats. *J. ethnopharmacol*; 115: 515-519

Oliver RC, Tellervo T (1993) Perioodontitis and tooth loss; comparing diabetics with the general population. *Journal of America Dental Association.* 124: 71-76.

Olsen S, Mogensen CE (1996) How often is NIDDM complicated with non-diabeticrenal disease? An analysis of renal biopsies and the literature. *Diabetologia,* 39:1638-1645

Oyedemi SO, Yakubu MT, Afolayan AJ (2011) Antidiabetic activities of aqueous leaves extract of *leonotis leonurus* in streptozotocin induced diabetic rats. *J.med Plant Research,* 5: 119-125

Ozer J, Ratweb M, Shawe M, Vanhaelen-Fastre R, Declercq JP and Van Meerssche M (2008) The current state of serum biomarker of hepatotoxicity. *Toxicity* 245: 194-205

Ozougwu JC, Obimba KC, Belonwu C, Unakalama CB (2013) The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology* 4(4):46-57

Pak CY, Eun H, McArthur RG, Yoon J. (1988) Association of cytomegalo virus infection with autoimmune type 1 diabetes. *Lance*t, 2: 1-4.

Panda S, Kar A (2007) Antidiabetic and antioxidative effects of *Annona squamosa* leaves are possibly mediated throughquercetin-3-O-glucoside. B*iofactors* 31: 201-210.

Pandit MK, Burke J, Gustatson AB, Minocha A, Peiris AN (1993) Drug induced disorders of glucose tolerance *Ann intern. Med*. 118: 529- 540

Pannekoek JH (1975) Insulin, glucagon and oral hypoglycemic drugs. In: side effects of the Drugs, Vol. 8. Dukes MNG (ed). Amsterdam, Excerpta Medica: pp 904-927.

Parulkar AA, Pendergrass ML, Ganda-Ayala RL, Fronsela VA (2001) Hypoglycemic effects of thiazolidinediones *Ann. Intern med* 134**:** 61-71.

Patlack M (2002) ñNew weapons to combat an ancient diseaseò: treating diabetes *The FASEB Journal* 16 (14): 1853. PMID 12468446

Patterson, D (2005) Dental care guidelines, for the diabetic family gentle care. Retrieved 2005 from http://www.dentalgentlecare.com/dentalcarefordiabetics.html.

Pecoraro RE, Reiber GE, Burgess EM (1990) Pathways to diabetic Limb amputation: Basis for preventionò. *Diabetes care,* 13: 513-521.

Peks, F, Floyd JC, Knopf RF, Conn JW (1972) Failure of sulfonylureas to suppression plasma glucagon in man. *Diabetes,* 21: 216- 223.

Permert J, Larson J, Westermark GT, Herington MK, Christmanson L, Pour PM (1994) Islet amyliod polypeptide in patients with pancreatic cancer and diabetes. *N. Engl. J. Med.* 330**:** 313-318.

Polonsky K.S. (2012) ñThe past 200 years n diabetesò *New England Journal of medicine* **367** (14) 1332-1340

Polonsky KS, Sturis J, Bell GI (1996) Non-insulin dependent diabetes mellitus: a genetically programmed failure of the beta cell to compensate for insulin resistance *N. Eng. J. Med.* 334: 777-784.

Purdon C, Brusson M, Nyreen SL, Miles PDG, Halter J, Vranic M, Marliss EB (1993) The roles of insulin and catecholamines in the gluco-regulatory response during intense exercise and early recovery in insulin dependent diabetic and contend subjects. *J. Clin. Endocronl Metab*. 76: 566-573

Qaseem (2012) Oral pharmacologic treatment of type 2 diabetes mellitus: a clinical practice guideline from the American college of physicans. *Ann. Intern. Med*. 156 (3): 218-231.

Ramaiah SK (2011) Preclinical safety assessment: current gaps, challenges and approaches in identifying translatable biomarkers of drug-induced liver damage. *Clin. Laboratory med.,* 31: 161-172

Rand JS, Marshal R (2005) Understanding Feline and Canine Diabetes Mellitus Walthen *Focus Journal* 2: 55-57. Phyllanthus amarus

Rang HP, Dile MM, Riter JM, Moore RK (2003) Pharmacology (5^{th} ed). Churchill Livingstone, Edinburgh pp 385-393

Rees TD (1994) The diabetic dental patient. *Dental clinic of North America* 38 (3) 447-463

Reitman S, Frankel S (1957) "A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transminases". *American journal of clinical pathology*, 28**:**56-62.

Rena G, Pearson ER, Sakamoto K (2012) Molecular action and pharmacogenetics of metformin: current understanding of an old drug. *Diabetes management*. 2: 439-452.

Reutens AT, Prentice L, Atkins R (2008) The Epidemiology of Diabetic Kidney Disease,In: Ekoe J, (editor); the Epidemiology of Diabetes Mellitus, 2nd Edition. Chichester: JohnWiley & Sons Ltd. pp**.** 499-518.

Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de La Monte SM (2005) ñInsulin and Insulin-like growth factor expression and function deteriorate with progression of Alzheimerôs disease: like to brain reductions in acetylcholineò *Journal Alzheimer Dis.,* 8 (3) 247-68.

Rizvi SI, Zaid MA (2001) Intracellular reduced glutathione content in normal and type 2 Diabetic Erythrocytes: Effects of insulin and (-) epicatechin. *J physiol. Pharmacol*., 52 (3) 483-488

Robbins DC, Shoelson SE, Rubenstein AH, Tager, HS (1984) Familial hyperproinsulinaemia: two cohorts secreting indistinguishable type II intermediates of pro-insulin conversation. *J. Clino invest.,* 73: 714-719.

Rodrigues B, Cam MC, McNeil JH (1998) Metabolic disturbance in diabetic cardiomyopathy. *Mol. Cell Biochem.,* 180: 53-57.

Rossetti L, Giaccari A, De Fronzo RA (1990) Glucose toxicity. *Diabetes care*, 13: 610-630

Ross-Flanigan N (1999) ñDiabetes and periodontal disease: A Comple, two way connectionò http://www.umich.edu/2/urecord/9899/Jan25-99/gums.html.

Rother KI (2007) ñDiabetes treatment- Bridging the divideò *The New England Journal of Medicine,* 356 (15): 1499-501.

Rubino F, Gagner M (2002) Potential of surgery for curing type 2 diabetes mellitus *Ann-surg.,* 236 (5) 1: 554-559

Rush JW, Spriet LL (2001) Skeletal muscle glylogen phosphorylase a kinetics: effects of adenine nucleotide and faffeine. *J. Appl. Physiol.* 91: 2071-2278

Salmols E, Tyler JM, Mialhe P (1969) Suppression of pancreatic glucagon release by hypoglycemic sulfonylureas. *Lancet* 1: 174-176

Sanders JKM, Hunter B.K (1993) Modern NMR Spectoscopy 2nd edition, Oxford University Press, Oxford.

Saphieha P, Hammel D, Shao Z, Rivera JC, Zaniolo K, Joyal JS, Chemtob S (2010) Proliferative retinopatheis: angiogenesis that blinds. *Int. J. Biochem Cell Biol.,* 42:5-12.

Sato, H Genet, C Strehle A, Thomas, CLobstein, A, Wagner A Mioskowski C, Auwerx J, Saladin R (2007) Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea Biochem. Biophys. Res. Commun.,* 362**:** 793ï 798

Shah DK, Mon KM, Cabrera LM, Vahratain A, Kavourssi SK, Lebovic DI (2010) Thiazolidinediones decrease vascular endothelia growth factor (VEGF) production by human luteinized granulose cells in vitro *Fertil. Stril*. 93 (6): 2042- 2047

Shah N, Sattar A, Benanti M, Hollander S, Cheuk L (2006) Magnetic resonance spectroscopy as an imaging tool for cancer: a review of the literature. *The Journal of the American Osteopathic Association* 106 (1): 23-27

Shapiro AM, Ricordi C, Hering BJ (2006) International trial of the Edmonton protocol for Islet transplantation *N. Engl. J*. med. 355 (13) 1318-1330

Shen SW, Bressler R (1977) Clinical Pharmacology of oral antidiabetic agents. *N. Engl. J. Med*. 296- 497

Shoback (2011) Greenspanôs basic and Clinical Endocrinology (9th ed.) David g Gardner, Dolores (eds). New York: McGraw Hill Medical chapter 17 ISBN 0-07-162243-8]

Sigal RJ, Purdon C, Fisher SJ, Halter JB Vramic Marliss EB (1996) Hyperinsulinaemia prevents prolonged hyperglycemic after intense exercise in insulin- dependent diabetic subjects. J. Chin. *Endocrinol. Metab*. 79: 1049-1057

Sinha AK (1972) Colorimetric assay of catalase. Anal Biochm 47 (2):389-394

Smits P, Thieri T (1995) Cardiovascular effects of sulfonylurea derivatives. Implications for the treatment of NIDDM. *Diabetologia* 38: 116-121.

Solimena M, De Camilli P (1991) Autoimmunity to glutamic acid decarbotylase (GAD) in stiff-man syndrome and insulin-dependent diabetes mellitus. *Trends Nurosci*., 14: 452-457.

Soumyanath A (2005) Traditional medicine for modern times (1st ed). Taylor and Francis USA. Philadephia 282- 298

Standiford CJ, Vijans S,Hae MI, Harrison RV (2014) Management of type2 *Diabetes mellitus*. Guidelines for clinical care Ambulatory. Faculty Group practice. University of Michigan Health System, USA

Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type II *Diabetes mellitus* (MODY 4) linked to IPF1. *Nature Genetics* 117: 138-139.

Stone BE, VanThiel DH (1985) Diabetes mellitus and the liver. *Sem Liver Dis* 5:8-28,

Strata RJ, Alloway RR (1998) Pancreas transplantation for diabetes mellitus: a guide to recipient selection and optimum immunosupression *Bio Drugs* 10 (5) 347-357

Sturis J, Got Fredse CF, Romer J, Polin B, Ribol U, Brand CL, Wilken M, Wassermann K, Deacon CF, Carro RD, Knudsen LB (2003) GLP-1 derviative liraglutide in rats with bets-cell deficiencies: influence of metabolic state on beta-cell mass dynamics Br. *J. Pharmacol* 140: 123-132.

Swantson SK, Day C, Bailey CJ, Flatt PR (1990) Traditional plant treatment for diabetes studies in normal and streptozotocin diabetic mice. *Diabetologia* 33 (8): 462-464

Szkudelski T (2001) The mechanism of alloxan and streptozoticin action in B cells of the rat pancrens. *Physiol. Res*. 56 (6): 537-546.

Takeuchi M, Kikuchi S, Sasaki N (2004) ñInvolvement of advanced glycation end products in Alzheimerôs diseaseò *Curr Alzheimer res*. 1 (1): 39- 46

Tapondjou LA, David L, Beibam L, Sondengam F, Mohammad I, Choudhary AR, Fanie R, Hee-juhn P, Kyung-Tae L (2003) Saponins from *Cussonia bancoensis* and their inhibitory effects on nitric oxide production. *J. nat. production,* 66 (9): 1266-1269

Taylor SI (1992) Lilly lecture: molecular Mechanism of Insulin Resistance: Lessons from patients with mutations in the insulin receptor gene. *Diabetes,* 41: 1473-1490.

Tennant JR (2010) *Cussonnia arborea*. Hochst ex. A Pich. *Flora of Tropic Tast Africa* 10:621

Teodoro T, Zhang L, Alexander, T, Yue J, Volchuk A (2008) Oleanolic acid enhances insulin secretion in pancreatic beta cells. *Federation of European Biochemical Socities (FEBS)* 582 (9):1375-1380

Thrall MA, Weiser MG (2002) Haematology. In: Hendrix C. M. Ed. Laboratory Procedures for Veterinary Technicians, 4th edition, Mosby Inc, Missouri. Pp 29 - 74.

Tierney LM Mcphee SJ, Papadakis MA (2002) Current medical diagnosis and treatment. Lang medical Books/McGraw-Hill pp 1203-15

Tietz NW (1995) Clincal Guide to Laboratory Tests. 3rd edition. W.B Saunders Company, Philadelphia. pp 518 - 519

Tonascia, S., Meinert, C. L (1986). *Clinical trials: Design, conduct, and analysis*. New York: Oxford University Press.

Trease GE, Evans WC (1989) Textbook of pharmacognosy(14^{th} ed). W.B Saunders, London pp 40-47

Tsokos GC, Gorden P, Antonovych T, Wilson CB, Balow JE (1985) Lupus nephritis and other autoimmune features in patents with diabetes mellitus due to auto antibody to insulin receptors. *Ann intern. Med.* 102: 176-181.

Turban S, Stretton C, Drowin O (2012) Defining the contribution of AMPK and PKCs in the regulation of glucose uptake by metformin in Skeletal muscle cells. *J. Biol. Chem*., 287: 20088- 20099.

Twari AK and Rao JM (2002) Diabetes Mellitus and Multiple Therapeutic approaches of phytochemcials: present status and future prospects *Carri. Sci*., 83: 30-38.

Tyrbery B, Anderson A, Borg L (2001) Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta cell toxin alloxan. Gen Comp. *Endocrinol*. 122: 238-251

Vahl TP, Paty BW, Fuller BD, Prigeon RL, DAlessio DA (2003) Effects of GLP- 1- (7-36) NH2, GLP-1- (7-37) and GLP -1- (9-36) NH2 on intervenous glucose tolerance and glucose-induced insulin secretion in healthy humans. *J. Clin endocrimel metab*., 88: 1772- 1779

Van Staa TP, Patel D, Gallagher AM, de Bruin ML (2012) Glucose lowering agents and the patterns of risk for cancer: a study with the General practice Research Database and secondary care data. *Diabetologia.* 55: 654-665.

Vasudevan DM, Kumari S (2005) Text book of Biochemistry; Section B: General metabolism; 4th edi. medical publishers pp 106-110.

Venogopal PM, Prince PSM, Pari L (1998) Hypoglycemic activities of *Syzigium cumini* seeds effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology* 61:1-7.

Vinik Al, Fishwick DT, Pittenges G (2004) Advances in diabetes for the millennium: toward a cure for Diabetes. *Medscape General Medicine,* 6 (3 suppl.) 7:12

Von Mehring J, Minkowski O (1898) Diabetes mellitus Nach Pankreasexstirpation. *Arch Exp pathol. Pharmakol* 26:371-387

Wadkar KA, Magdum CS, Patil SS, Naikwade NS (2008) Antidiabetic potential and Indian medicinal plants. *J Herbal Med and Toxicol* 2: 45-50.

Walker M, Turnbull DM (1997) Mitochondrial related diabetes: A clinical perspective. *Diabetes. Med*. 14: 1007-1009.

Wallin B, Rosengren B, Shertyer HG, Camejo G (1993) Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microfilter plate; its use for evaluation of antioxidants. *Anal Biochm.* 288:10-15

Wasserman DH, Chenington AD (1996) Regulation of extramuscular fuel sources during exercise. In Handbook of physiology: Rowell LB, shepherded JT (eds). Columbia MD, Bermedia production

Weaver ML, Orwig BA, podriquez LC (2001) Pharmalokinethes and metabolism of neteglinide in humans. *Drug metab. DISPOS.* 29: 4156-421

Welihinda J, Arvidson G, Gylfe E, Hellman B, Karlsson E (1982) Biol MetLGer 41, 1229.

Weykamp, C.W; and Penders T. (1994) Effect of calibration on dispersion of glycohaemoglobin values determined by Laboratories using 21 methods. *Clin. Chem*. 40: 138-144.

WHO (1980) Report of the Expert Committee on Diabetes. WHO Technical Report Series no. 646. Geneva Switzerland.

WHO (1985) *Diabetes mellitus*: Report of a WHO study group. Technical Report Series 727, Geneva, Switzerland

WHO (1999) Definition, Diagnosis and Classification of *Diabetes mellitus* and its complicationsò, A Report of WHO consultation Department of Non-communicable disease surveillance Geneva, Switzerland.

WHO (2005). ñPreventing chronic diseases: A vital investmentò http://www.who.int/chp/chronic-disease-report/en/index/html.

WHO (2006) Definition and diagnosis of diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF consultation: WHO Geneva. Switzerland.

WHO (2011) the selection and use of essential medicine Report of WHO Expert Committee. 2011

Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes; estimates for the year 2000 and projection for 2030. *Diabetes care* 27 (5) 1047-1053.

Willis JA, Scott RS, Brown LJ, Forbes LV, Schmidli RS, Zimmet PZ (1996) Islet cell antibodies and antibodies against glutamic acid decarboxylase in newly diagnosed adult- onset diabetes mellitus. *Diabetes Res. Clin. Pract*. 33:89-97

Willson TM, Cobb JE, Cowon DJ, Wiethe RW, Cone ID, Prakash SR, Beck KD, Moore LB, Kliewer SA, Lehmann JM (1996) The structure-activity relationship between peroxisome proliferations activated receptor gamma agonism and the antilyperglycemic activity of thiazolidinediones. *J.Med. Chem*. 39: 665- 668

Wing RR, Blair EH, Bonomi P, Marcus MD, Watanabe R, Bergman RN (1994) Caloric restriction perse in a significant factor in improvements in glycaemic control and insulin sensitivity during weight loss in Obese NIDDM patients *Diabetes care*. 17:30-36

Wolfe RR, Kleins, Cararo F, Weber JM (1990) Role of triglyceride fatty acid cycle in controlling fat metabolism in humans during and after exercise *Am. J. Physiol*. 258: 382-389.

Wyngaarden JB, Smith LH, Bennett JC (1992) Cecil Textbook of Medicine. Philadephia: WB Saunders

Yajnik SC, Selgikar KM, Naik SS, Kanitkar SV Orshovh, Alberti KGMM (1992) The ketoacidosis-resistance in fibro- calculus -pancreatic- Diabetes. *Diabetes Res. Clin. Pract*. 15: 149-156

Yallow RS, Berson SA (2960) Immunoassay of endogenous plasma insulin in man. *The Journal of clinical Investigation* 39 (7): 1157-1175.

Yamagata K, Furuta, H, Oda N, Kaisaki PJ Menzel S, Cox NJ (1996) Mutation in the Hepatocyte nuclear factor. 4. Gene in maturity- onset diabetes of the young (MODY 1). Nature, 384: 438- 460

Yoshikawa, M and. Matsuda, H (2000) Antidiabetogenic activity of Oleanolic acid glycosides from medicinal food stuffs Bio Factors, 231ï 237

Yu J, Zhang Y, Sun S, Shen J, Qiu J, Yin X, Yin H, Jiang S (2006) Inhibitory effects of astragaloside IV on diabetic peripheral neuropathy in rats. *J Physiol Pharmacol*, 84: 579-587

Yusuf AB, Umar IA, Nok AJ (2012) Effects of methanol extract of *Vernonia amygdalina* leaf on survival and some biochemical parameters in acute Trypanosoma brucei brucei infection. *Afr. J. Biochem Res*. 6 (12): 150-158

Zhang Y, Jayaprakasam B, Seeram, NP, Olson LK, DeWitt D, Nair MG (2004) Insulin secretion and cyclooxygenase enzyme inhibition by Cabernet Sauvignon grape skin compounds *J. Agric. Food Chem*., 52:228ï 233

Zhou G, Meyers R, Li Y (2001) Role of AMP activated protein kinase in mechanism of metformin action *J. Clin invest*, 108: 1167-1174.

Zimmet PZ, Tuomi T, Mackay R, Rowley MJ, Konwles W, Cohen M (1994) Latent autoimmune diabetes mellitus in Adults (LADA): The role of antibodies to glutamic acid decarboxylase in Diagnosis and prediction of Insulin Dependency. *Diabetic Med.* 11: 299-303.

Zinman B, Gerich J, Buse JB, Lewin A, Schwartz S, Raskin P, Hale PM, Zdravkoric M, Blonde L (2009) Efficacy and safety of the human glucagon line peptide- 1 analog liraghitute in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD ï 4 met 4 TZD). *Diabetes care,* 32: 1224-1230.

Appendix 1: Effects of acute administration of *Cussonia arborea* root bark extract on Fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PRIND	1.00	3	73.3333	.57735	.33333	71.8991	74.7676	73.00	74.00
	2.00	3	73.3333	2.51661	1.45297	67.0817	79.5849	71.00	76.00
	3.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.00	75.00
	4.00	3	73.3333	.57735	.33333	71.8991	74.7676	73.00	74.00
	5.00	3	75.3333	1.52753	.88192	71.5388	79.1279	74.00	77.00
	6.00	3	75.3333	.57735	.33333	73.8991	76.7676	75.00	76.00
	Total	18	74.1111	1.45072	.34194	73.3897	74.8325	71.00	77.00
ZEROHR	1.00	3	326.3333	4.04145	2.33333	316.2938	336.3729	322.00	330.00
	2.00	3	333.3333	50.33223	29.05933	208.3011	458.3655	280.00	380.00
	3.00	3	316.0000	32.14032	18.55622	236.1590	395.8410	295.00	353.00
	4.00	3	328.0000	25.51470	14.73092	264.6180	391.3820	299.00	347.00
	5.00	3	343.3333	40.41452	23.33333	242.9381	443.7286	300.00	380.00
	6.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	Total	18	287.0000	101.38337	23.89629	236.5832	337.4168	74.00	380.00
ONEHR	1.00	3	265.6667	5.13160	2.96273	252.9191	278.4143	260.00	270.00
	2.00	3	285.6667	12.42310	7.17248	254.8060	316.5273	278.00	300.00
	3.00	3	301.0000	7.54983	4.35890	282.2452	319.7548	294.00	309.00
	4.00	3	240.0000	45.82576	26.45751	126.1625	353.8375	200.00	290.00
	5.00	3	332.0000	32.74141	18.90326	250.6658	413.3342	296.00	360.00
	6.00	3	74.3333	.57735	.33333	72.8991	75.7676	74.00	75.00
	Total	18	249.7778	88.19201	20.78706	205.9209	293.6346	74.00	360.00
THREEHR	1.00	3	160.3333	2.51661	1.45297	154.0817	166.5849	158.00	163.00
	2.00	3	183.3333	12.22020	7.05534	152.9767	213.6900	170.00	194.00
	3.00	3	278.6667	14.18920	8.19214	243.4187	313.9146	266.00	294.00
	4.00	3	160.0000	10.00000	5.77350	135.1586	184.8414	150.00	170.00
	5.00	3	315.6667	35.69781	20.61014	226.9884	404.3449	278.00	349.00
	6.00	3	75.6667	.57735	.33333	74.2324	77.1009	75.00	76.00
	Total	18	195.6111	83.50705	19.68280	154.0840	237.1382	75.00	349.00
SIXHR	1.00	3	96.6667	3.05505	1.76383	89.0775	104.2558	94.00	100.00
	2.00	3	164.6667	6.65833	3.84419	148.1265	181.2069	159.00	172.00
	3.00	3	231.0000	10.14889	5.85947	205.7888	256.2112	220.00	240.00
	4.00	3	98.6667	1.15470	.66667	95.7982	101.5351	98.00	100.00
	5.00	3	345.3333	33.24655	19.19491	262.7443	427.9224	310.00	376.00
	6.00	3	73.0000	1.00000	.57735	70.5159	75.4841	72.00	74.00
	Total	18	168.2222	98.68879	23.26117	119.1454	217.2990	72.00	376.00
TWNTFORH	1.00	3	91.0000	8.54400	4.93288	69.7755	112.2245	83.00	100.00
	2.00	3	142.6667	11.23981	6.48931	114.7454	170.5879	133.00	155.00
	3.00	3	193.0000	11.26943	6.50641	165.0052	220.9948	180.00	200.00
	4.00	3	98.0000	1.00000	.57735	95.5159	100.4841	97.00	99.00
	5.00	3	320.3333	20.50203	11.83685	269.4035	371.2632	300.00	341.00
	6.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	Total	18	153.3333	87.27104	20.56998	109.9345	196.7322	74.00	341.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PRIND	Between Groups	14.444	5	2.889	1.625	.227
	Within Groups	21.333	12	1.778		
	Total	35.778	17			
ZEROHR	Between Groups	163000.000	5	32600.000	33.333	.000
	Within Groups	11736.000	12	978.000		
	Total	174736.000	17			
ONEHR	Between Groups	125403.111	5	25080.622	44.130	.000
	Within Groups	6820.000	12	568.333		
	Total	132223.111	17			
THREEHR	Between Groups	115084.944	5	23016.989	79.751	.000
	Within Groups	3463.333	12	288.611		
	Total	118548.278	17			
SIXHR	Between Groups	163042.444	5	32608.489	154.746	.000
	Within Groups	2528.667	12	210.722		
	Total	165571.111	17			
TWNTFORH	Between Groups	127978.667	5	25595.733	205.131	.000
	Within Groups	1497.333	12	124.778		
	Total	129476.000	17			

Appendix 2: Effect of *Cussonia arborea* extract on FBG (mg/dl) of normoglycaemic rat (Oral glucose tolerance test)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FBSZEROHR	1.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.00	75.00
	2.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	3.00	3	76.3333	.57735	1.20333	74.8991	77.7676	76.00	77.00
	4.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	5.00	3	73.6667	.57735	.33333	72.2324	75.1009	73.00	74.00
	Total	15	74.8000	1.20712	.31168	74.1315	75.4685	73.00	77.00
THRTYMINS	1.00	3	151.0000	3.60555	2.08167	142.0433	159.9567	148.00	155.00
	2.00	3	147.6667	3.21455	1.85592	139.6813	155.6521	144.00	150.00
	3.00	3	152.3333	6.42910	3.71184	136.3626	168.3041	145.00	157.00
	4.00	3	120.6667	2.08167	1.57185	115.4955	125.8378	119.00	123.00
	5.00	3	158.0000	2.00000	1.15470	153.0317	162.9683	156.00	160.00
	Total	15	145.9333	13.90512	3.59029	138.2329	153.6337	119.00	160.00
SIXTYMNS	1.00	3	153.6667	12.66228	7.31057	122.2118	185.1215	144.00	168.00
	2.00	3	142.6667	2.51661	1.45297	136.4151	148.9183	140.00	145.00
	3.00	3	154.0000	5.29150	3.05505	140.8552	167.1448	148.00	158.00
	4.00	3	118.6667	3.21455	1.85592	110.6813	126.6521	115.00	121.00
	5.00	3	161.6667	4.93288	2.84800	149.4127	173.9206	156.00	165.00
	Total	15	146.1333	16.55668	4.27492	136.9645	155.3021	115.00	168.00
ONETWTY	1.00	3	107.3333	1.52753	.88192	103.5388	111.1279	106.00	109.00
	2.00	3	100.0000	1.00000	.57735	97.5159	102.4841	99.00	101.00
	3.00	3	105.3333	1.52753	.88192	101.5388	109.1279	104.00	107.00
	4.00	3	89.0000	2.64575	1.52753	82.4276	95.5724	87.00	92.00
	5.00	3	111.0000	1.00000	.57735	108.5159	113.4841	110.00	112.00
	Total	15	102.5333	8.03445	2.07449	98.0840	106.9827	87.00	112.00
ONEEIGHTY	1.00	3	99.0000	1.00000	.57735	96.5159	101.4841	98.00	100.00
	2.00	3	83.0000	6.08276	3.51188	67.8896	98.1104	79.00	90.00
	3.00	3	103.0000	2.00000	1.15470	98.0317	107.9683	101.00	105.00
	4.00	3	79.6667	2.51661	1.45297	73.4151	85.9183	77.00	82.00
	5.00	3	90.3333	1.52753	.88192	86.5388	94.1279	89.00	92.00
	Total	15	91.0000	9.65105	2.49189	85.6554	96.3446	77.00	105.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FBSZEROHR	Between Groups	13.067	4	3.267	4.455	.025
	Within Groups	7.333	10	.733		
	Total	20.400	14			
THRTYMINS	Between Groups	2560.933	4	640.233	43.852	.000
	Within Groups	146.000	10	14.600		
	Total	2706.933	14			
SIXTYMNS	Between Groups	3379.067	4	844.767	18.418	.000
	Within Groups	458.667	10	45.867		
	Total	3837.733	14			
ONETWTY	Between Groups	876.400	4	219.100	80.159	.000
	Within Groups	27.333	10	2.733		

	Total	903.733	14			
ONEEIGHTY	Between Groups	1202.667	4	300.667	29.671	.000
	Within Groups	101.333	10	10.133		
	Total	1304.000	14			

Appendix 3: Effects of chronic administration of *Cussonia arborea* root bark extract on Fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PRIND	1.00	3	78.0000	2.00000	1.15470	73.0317	82.9683	76.00	80.00
	2.00	3	79.3333	2.51661	1.45297	73.0817	85.5849	77.00	82.00
	3.00	3	78.3333	3.05505	1.76383	70.7442	85.9225	75.00	81.00
	4.00	3	77.6667	4.04145	2.33333	67.6271	87.7062	74.00	82.00
	5.00	3	77.6667	9.07377	5.23874	55.1262	100.2072	68.00	86.00
	6.00	3	76.3333	6.02771	3.48010	61.3597	91.3070	70.00	82.00
	Total	18	77.8889	4.36414	1.02864	75.7187	80.0591	68.00	86.00
ZEROHR	1.00	3	310.0000	26.45751	15.27525	244.2759	375.7241	290.00	340.00
	2.00	3	315.3333	17.47379	10.08850	271.9260	358.7406	296.00	330.00
	3.00	3	318.3333	28.44878	16.42491	247.6626	389.0040	299.00	351.00
	4.00	3	307.3333	12.70171	7.33333	275.7805	338.8861	300.00	322.00
	5.00	3	311.3333	16.65333	9.61480	269.9642	352.7025	298.00	330.00
	6.00	3	78.3333	7.02377	4.05518	60.8853	95.7813	71.00	85.00
	Total	18	273.4444	91.35594	21.53280	228.0142	318.8747	71.00	351.00
FOTEN	1.00	3	118.3333	7.63763	4.40959	99.3604	137.3062	110.00	125.00
	2.00	3	93.0000	11.26943	6.50641	65.0052	120.9948	80.00	100.00
	3.00	3	117.6667	11.23981	6.48931	89.7454	145.5879	108.00	130.00
	4.00	3	87.0000	2.64575	1.52753	80.4276	93.5724	85.00	90.00
	5.00	3	288.6667	12.05543	6.96020	258.7193	318.6140	276.00	300.00
	6.00	3	81.6667	7.09460	4.09607	64.0427	99.2906	74.00	88.00
	Total	18	131.0556	74.38412	17.53250	94.0652	168.0459	74.00	300.00
TWTEHT	1.00	3	127.0000	5.56776	3.21455	113.1689	140.8311	122.00	133.00
	2.00	3	94.6667	7.50555	4.33333	76.0218	113.3115	86.00	99.00
	3.00	3	127.3333	3.05505	1.76383	119.7442	134.9225	124.00	130.00
	4.00	3	87.6667	4.72582	2.72845	75.9271	99.4062	84.00	93.00
	5.00	3	292.6667	6.80686	3.92994	275.7575	309.5758	285.00	298.00
	6.00	3	77.6667	4.16333	2.40370	67.3244	88.0090	73.00	81.00
	Total	18	134.5000	75.44710	17.78305	96.9810	172.0190	73.00	298.00
FRTTYOO	1.00	3	113.6667	5.50757	3.17980	99.9851	127.3482	110.00	120.00
	2.00	3	80.0000	6.55744	3.78594	63.7104	96.2896	73.00	86.00
	3.00	3	118.0000	15.62050	9.01850	79.1965	156.8035	100.00	128.00
	4.00	3	80.0000	10.00000	5.77350	55.1586	104.8414	70.00	90.00
	5.00	3	303.6667	5.50757	3.17980	289.9851	317.3482	300.00	310.00
	6.00	3	82.6667	2.51661	1.45297	76.4151	88.9183	80.00	85.00
	Total	18	129.6667	82.01148	19.33029	88.8833	170.4500	70.00	310.00
FIFTTSX	1.00	3	111.6667	10.40833	6.00925	85.8109	137.5224	100.00	120.00
	2.00	3	79.0000	4.35890	2.51661	68.1719	89.8281	74.00	82.00
	3.00	3	117.0000	9.84886	5.68624	92.5341	141.4659	106.00	125.00
	4.00	3	76.3333	4.16333	2.40370	65.9910	86.6756	73.00	81.00
	5.00	3	295.6667	4.93288	2.84800	283.4127	307.9206	290.00	299.00
	6.00	3	76.6667	3.51188	2.02759	67.9427	85.3907	73.00	80.00
	Total	18	126.0556	80.11203	18.88259	86.2168	165.8943	73.00	299.00
SEVNTY	1.00	3	115.6667	5.03322	2.90593	103.1634	128.1699	111.00	121.00
	2.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.00	75.00
	3.00	3	119.6667	3.21455	1.85592	111.6813	127.6521	116.00	122.00
	4.00	3	73.6667	2.08167	1.20185	68.4955	78.8378	72.00	76.00

	5.00	3	292.3333	12.42310	7.17248	261.4727	323.1940	278.00	300.00
	6.00	3	73.3333	2.08167	1.20185	68.1622	78.5045	71.00	75.00
	Total	18	124.7778	79.87605	18.82697	85.0564	164.4992	71.00	300.00
	1.00	3	108.6667	7.57188	4.37163	89.8571	127.4763	100.00	114.00
	2.00	3	82.3333	1.52753	.88192	78.5388	86.1279	81.00	84.00
	3.00	3	110.6667	8.38650	4.84195	89.8335	131.4999	101.00	116.00
EITYFOR	4.00	3	79.3333	2.08167	1.20185	74.1622	84.5045	77.00	81.00
	5.00	3	289.6667	8.50490	4.91031	268.5393	310.7940	281.00	298.00
	6.00	3	73.0000	1.00000	.57735	70.5159	75.4841	72.00	74.00
	Total	18	123.9444	77.83351	18.34553	85.2387	162.6501	72.00	298.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	14.444	5	2.889	.112	.987
PRIND	Within Groups	309.333	12	25.778		
	Total	323.778	17			
	Between Groups	137275.111	5	27455.022	71.539	.000
ZEROHR	Within Groups	4605.333	12	383.778		
	Total	141880.444	17			
	Between Groups	93032.278	5	18606.456	217.055	.000
FOTEN	Within Groups	1028.667	12	85.722		
	Total	94060.944	17			
	Between Groups	96403.167	5	19280.633	633.305	.000
TWTEHT	Within Groups	365.333	12	30.444		
	Total	96768.500	17			
	Between Groups	113432.000	5	22686.400	299.820	.000
FRTTYOO	Within Groups	908.000	12	75.667		
	Total	114340.000	17			
	Between Groups	108548.278	5	21709.656	467.993	.000
FIFTTSX	Within Groups	556.667	12	46.389		
	Total	109104.944	17			
	Between Groups	108063.778	5	21612.756	649.465	.000
SEVNTY	Within Groups	399.333	12	33.278		
	Total	108463.111	17			
	Between Groups	102571.611	5	20514.322	592.709	.000
EITYFOR	Within Groups	415.333	12	34.611		
	Total	102986.944	17			

Appendix 4: Effect of different fractions of *Cussonia arborea* on the fasting blood glucose (mg/dl) levels of alloxan induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	1.00	3	79.0000	2.64575	1.52753	72.4276	85.5724	76.00	81.00
	2.00	3	77.6667	4.93288	2.84800	65.4127	89.9206	72.00	81.00
	3.00	3	75.3333	3.78594	2.18581	65.9285	84.7381	71.00	78.00
	4.00	3	75.3333	2.30940	1.33333	69.5965	81.0702	74.00	78.00
	5.00	3	81.3333	4.04145	2.33333	71.2938	91.3729	77.00	85.00
Prindfbs	6.00	3	78.0000	8.71780	5.03322	56.3438	99.6562	72.00	88.00
	7.00	3	78.3333	7.23418	4.17665	60.3626	96.3040	70.00	83.00
	8.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	9.00	3	73.0000	4.35890	2.51661	62.1719	83.8281	70.00	78.00
	10.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.00	75.00
	Total	30	76.7000	4.59497	.83892	74.9842	78.4158	70.00	88.00
	1.00	3	293.0000	3.00000	1.73205	285.5476	300.4524	290.00	296.00
	2.00	3	307.0000	32.51154	18.77054	226.2369	387.7631	270.00	331.00
	3.00	3	282.0000	13.89244	8.02081	247.4893	316.5107	273.00	298.00
	4.00	3	295.3333	8.96289	5.17472	273.0683	317.5984	285.00	301.00
	5.00	3	290.3333	4.50925	2.60342	279.1317	301.5349	286.00	295.00
Postindfbs	6.00	3	294.3333	10.69268	6.17342	267.7713	320.8954	282.00	301.00
	7.00	3	284.0000	15.71623	9.07377	244.9587	323.0413	270.00	301.00
	8.00	3	287.6667	11.93035	6.88799	258.0300	317.3033	274.00	296.00
	9.00	3	273.3333	25.16611	14.52966	210.8172	335.8494	250.00	300.00
	10.00	3	76.0000	1.00000	.57735	73.5159	78.4841	75.00	77.00
	Total	30	268.3000	67.06721	12.24474	243.2567	293.3433	75.00	331.00
	1.00	3	286.3333	3.51188	2.02759	277.6093	295.0573	283.00	290.00
	2.00	3	211.3333	9.60902	5.54777	187.4632	235.2035	201.00	220.00
	3.00	3	237.0000	15.71623	9.07377	197.9587	276.0413	220.00	251.00
	4.00	3	278.0000	7.00000	4.04145	260.6110	295.3890	273.00	286.00
	5.00	3	279.6667	3.51188	2.02759	270.9427	288.3907	276.00	283.00
Onehrfbs	6.00	3	287.0000	14.73092	8.50490	250.4064	323.5936	271.00	300.00
	7.00	3	274.0000	22.53886	13.01281	218.0104	329.9896	260.00	300.00
	8.00	3	208.6667	6.11010	3.52767	193.4883	223.8450	202.00	214.00
	9.00	3	272.0000	17.52142	10.11599	228.4744	315.5256	255.00	290.00
	10.00	3	74.3333	1.52753	.88192	70.5388	78.1279	73.00	76.00
	Total	30	240.8333	64.16094	11.71413	216.8752	264.7914	73.00	300.00
	1.00	3	282.6667	8.32666	4.80740	261.9821	303.3512	276.00	292.00
	2.00	3	94.6667	10.11599	5.84047	69.5371	119.7962	83.00	101.00
	3.00	3	118.6667	12.05543	6.96020	88.7193	148.6140	106.00	130.00
	4.00	3	247.3333	40.99187	23.66667	145.5039	349.1628	200.00	271.00
	5.00	3	277.3333	2.51661	1.45297	271.0817	283.5849	275.00	280.00
Threehrfbs	6.00	3	279.3333	14.57166	8.41295	243.1353	315.5313	263.00	291.00
	7.00	3	269.6667	22.36813	12.91425	214.1011	325.2322	244.00	285.00
	8.00	3	85.6667	4.50925	2.60342	74.4651	96.8683	81.00	90.00
	9.00	3	255.3333	28.37840	16.38427	184.8375	325.8292	230.00	286.00
	10.00	3	75.3333	5.50757	3.17980	61.6518	89.0149	70.00	81.00
	Total	30	198.6000	89.79847	16.39488	165.0687	232.1313	70.00	292.00
Sixhrfbs	1.00	3	273.0000	20.22375	11.67619	222.7614	323.2386	250.00	288.00

2.00	3	78.0000	4.35890	2.51661	67.1719	88.8281	73.00	81.00
3.00	3	99.3333	10.50397	6.06447	73.2400	125.4266	89.00	110.00
4.00	3	244.3333	37.58102	21.69741	150.9769	337.6898	201.00	268.00
5.00	3	271.6667	8.14453	4.70225	251.4345	291.8988	266.00	281.00
6.00	3	267.0000	10.58301	6.11010	240.7104	293.2896	255.00	275.00
7.00	3	252.3333	45.39089	26.20645	139.5761	365.0906	200.00	281.00
8.00	3	80.3333	6.02771	3.48010	65.3597	95.3070	74.00	86.00
9.00	3	223.0000	4.35890	2.51661	212.1719	233.8281	220.00	228.00
10.00	3	76.0000	2.64575	1.52753	69.4276	82.5724	74.00	79.00
Total	30	186.5000	88.61764	16.17929	153.4096	219.5904	73.00	288.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
prindfbs	Between Groups	178.967	9	19.885	.918	.530
	Within Groups	433.333	20	21.667		
	Total	612.300	29			
postindfbs	Between Groups	125446.967	9	13938.552	55.806	.000
	Within Groups	4995.333	20	249.767		
	Total	130442.300	29			
onehrfbs	Between Groups	116412.833	9	12934.759	87.122	.000
	Within Groups	2969.333	20	148.467		
	Total	119382.167	29			
threehrfbs	Between Groups	226704.533	9	25189.393	70.512	.000
	Within Groups	7144.667	20	357.233		
	Total	233849.200	29			
sixhrfbs	Between Groups	219236.167	9	24359.574	57.294	.000
	Within Groups	8503.333	20	425.167		
	Total	227739.500	29			

Appendix 5: Effect of subfraction 2 of *Cussonia arborea* root bark extract on the fasting blood glucose (mg/dl) levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	1.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.00	76.00
	2.00	3	76.3333	1.52753	.88192	72.5388	80.1279	75.00	78.00
	3.00	3	75.4200	1.41746	.71437	71.8988	78.9412	74.26	77.00
Preindfbs	4.00	3	76.0000	2.64575	1.52753	69.4276	82.5724	74.00	79.00
	5.00	3	76.3333	1.52753	.88192	72.5388	80.1279	75.00	78.00
	6.00	3	76.0000	2.00000	1.15470	71.0317	80.9683	74.00	78.00
	Total	18	75.8478	1.56451	.36876	75.0698	76.6258	74.00	79.00
	1.00	3	310.0000	10.00000	5.77350	285.1586	334.8414	300.00	320.00
	2.00	3	313.3333	20.81666	12.01850	261.6219	365.0448	290.00	330.00
	3.00	3	333.3033	49.27713	28.45017	210.8921	455.7145	300.00	389.91
Postindfbs	4.00	3	313.3333	30.55050	17.63834	237.4417	389.2250	280.00	340.00
	5.00	3	300.0000	10.00000	5.77350	275.1586	324.8414	290.00	310.00
	6.00	3	74.6667	2.51661	1.45297	68.4151	80.9183	72.00	77.00
	Total	18	274.1061	94.85373	22.35724	226.9365	321.2758	72.00	389.91
	1.00	3	196.3333	5.50757	3.17980	182.6518	210.0149	190.00	200.00
	2.00	3	310.3333	16.74316	9.66667	268.7410	351.9256	291.00	320.00
	3.00	3	305.2333	5.56447	3.21265	291.4104	319.0562	299.00	309.70
Onehrfbs	4.00	3	197.0000	6.08276	3.51188	181.8896	212.1104	190.00	201.00
	5.00	3	295.6667	11.59023	6.69162	266.8749	324.4584	288.00	309.00
	6.00	3	74.6667	1.52753	.88192	70.8721	78.4612	73.00	76.00
	Total	18	229.8722	87.23187	20.56075	186.4928	273.2516	73.00	320.00
	1.00	3	76.3333	5.50757	3.17980	62.6518	90.0149	70.00	80.00
	2.00	3	297.0000	6.08276	3.51188	281.8896	312.1104	290.00	301.00
	3.00	3	289.9200	15.26759	8.81475	251.9932	327.8468	272.76	302.00
Threehrfbs	4.00	3	78.3333	2.51661	1.45297	72.0817	84.5849	76.00	81.00
	5.00	3	296.3333	5.50757	3.17980	282.6518	310.0149	290.00	300.00
	6.00	3	77.3333	5.50757	3.17980	63.6518	91.0149	71.00	81.00
	Total	18	185.8756	111.90806	26.37698	130.2250	241.5261	70.00	302.00
	1.00	3	79.6667	.57735	.33333	78.2324	81.1009	79.00	80.00
	2.00	3	298.6667	11.01514	6.35959	271.3035	326.0298	288.00	310.00
	3.00	3	291.5800	5.89145	3.40143	276.9448	306.2152	286.00	297.74
Sixhrfbs	4.00	3	77.0000	1.00000	.57735	74.5159	79.4841	76.00	78.00
	5.00	3	294.0000	6.92820	4.00000	276.7894	311.2106	286.00	298.00
	6.00	3	75.0000	2.64575	1.52753	68.4276	81.5724	73.00	78.00
	Total	18	185.9856	112.05719	26.41213	130.2608	241.7103	73.00	310.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
preindfbs	Between Groups	4.259	5	.852	.274	.919
	Within Groups	37.352	12	3.113		
	Total	41.611	17			
postindfbs	Between Groups	144950.453	5	28990.091	43.472	.000
	Within Groups	8002.472	12	666.873		
	Total	152952.925	17			
onehrfbs	Between Groups	128329.203	5	25665.841	298.847	.000
	Within Groups	1030.593	12	85.883		
	Total	129359.796	17			
threehrfbs	Between Groups	212163.154	5	42432.631	692.905	.000
	Within Groups	734.865	12	61.239		
	Total	212898.019	17			
sixhrfbs	Between Groups	213041.072	5	42608.214	1203.759	.000
	Within Groups	424.752	12	35.396		
	Total	213465.824	17			

Appendix 6: Glycosylated haemoglobin {HbA1c (%)} values of alloxan-induced diabetic rats treated with methanol root bark extract of *Cussonia arborea*

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FORTYTWO	1.00	3	7.3333	.49329	.28480	6.1079	8.5587	7.00	7.90
	2.00	3	5.4667	.05774	.03333	5.3232	5.6101	5.40	5.50
	3.00	3	7.3000	.45826	.26458	6.1616	8.4384	6.90	7.80
	4.00	3	5.4000	.40000	.23094	4.4063	6.3937	5.00	5.80
	5.00	3	7.7333	.20817	.12019	7.2162	8.2504	7.50	7.90
	6.00	3	5.0667	.05774	.03333	4.9232	5.2101	5.00	5.10
	Total	18	6.3833	1.15415	.27204	5.8094	6.9573	5.00	7.90
FIFTYSIX	1.00	3	7.7667	.15275	.08819	7.3872	8.1461	7.60	7.90
	2.00	3	5.8667	.05774	.03333	5.7232	6.0101	5.80	5.90
	3.00	3	7.6000	.20000	.11547	7.1032	8.0968	7.40	7.80
	4.00	3	5.9000	.10000	.05774	5.6516	6.1484	5.80	6.00
	5.00	3	9.1333	.15275	.08819	8.7539	9.5128	9.00	9.30
	6.00	3	5.1000	.10000	.05774	4.8516	5.3484	5.00	5.20
	Total	18	6.8944	1.43136	.33737	6.1826	7.6062	5.00	9.30
EIGHTYFOUR	1.00	3	8.0333	.23094	.13333	7.4596	8.6070	7.90	8.30
	2.00	3	5.9000	.10000	.05774	5.6516	6.1484	5.80	6.00
	3.00	3	8.0667	.05774	.03333	7.9232	8.2101	8.00	8.10
	4.00	3	5.9333	.05774	.03333	5.7899	6.0768	5.90	6.00
	5.00	3	10.8333	.41633	.24037	9.7991	11.8676	10.50	11.30
	6.00	3	5.2000	.10000	.05774	4.9516	5.4484	5.10	5.30
	Total	18	7.3278	1.97091	.46455	6.3477	8.3079	5.10	11.30

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FORTYTWO	Between Groups	21.318	5	4.264	38.566	.000
	Within Groups	1.327	12	.111		
	Total	22.645	17			
FIFTYSIX	Between Groups	34.609	5	6.922	377.558	.000
	Within Groups	.220	12	.018		
	Total	34.829	17			
EIGHTYFOUR	Between Groups	65.529	5	13.106	310.403	.000
	Within Groups	.507	12	.042		
	Total	66.036	17			

Appendix 7: Effects of chronic administration of *Cussonia arborea* root bark extract on other biochemical markers of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	1.00	3	91.9400	2.63536	1.52152	85.3934	98.4866	89.79	94.88
	2.00	3	71.4500	2.93312	1.69344	64.1637	78.7363	68.37	74.21
	3.00	3	111.6667	11.61190	6.70413	82.8211	140.5122	103.94	125.02
AST1	4.00	3	66.1667	5.44750	3.14511	52.6343	79.6990	62.77	72.45
	5.00	3	116.8533	9.19759	5.31023	94.0052	139.7014	107.12	125.40
	6.00	3	67.6000	6.58979	3.80462	51.2301	83.9699	61.16	74.33
	Total	18	87.6128	22.14681	5.22005	76.5994	98.6261	61.16	125.40
	1.00	3	88.9300	4.31675	2.49227	78.2066	99.6534	84.11	92.44
	2.00	3	78.4267	2.51198	1.45029	72.1866	84.6668	76.38	81.23
	3.00	3	126.3333	9.65845	5.57631	102.3404	150.3263	115.33	133.41
AST2	4.00	3	70.4033	3.48173	2.01018	61.7542	79.0524	67.38	74.21
	5.00	3	137.2333	7.47017	4.31290	118.6764	155.7903	130.25	145.11
	6.00	3	68.6267	6.64577	3.83694	52.1177	85.1357	62.04	75.33
	Total	18	94.9922	28.27714	6.66499	80.9303	109.0541	62.04	145.11
	1.00	3	78.2867	3.19052	1.84205	70.3610	86.2124	74.62	80.43
	2.00	3	76.8233	1.62057	.93563	72.7976	80.8490	75.32	78.54
	3.00	3	82.0267	1.85592	1.07152	77.4163	86.6370	80.55	84.11
AST3	4.00	3	77.4967	4.67665	2.70006	65.8792	89.1141	72.11	80.52
	5.00	3	85.6467	4.99890	2.88612	73.2287	98.0646	80.31	90.22
	6.00	3	72.2900	2.68922	1.55262	65.6096	78.9704	69.24	74.32
	Total	18	78.7617	5.18636	1.22244	76.1826	81.3408	69.24	90.22
	1.00	3	35.8233	.54930	.31714	34.4588	37.1879	35.19	36.17
	2.00	3	36.8533	2.57187	1.48487	30.4644	43.2422	33.98	38.94
	3.00	3	35.7300	1.84835	1.06715	31.1384	40.3216	33.63	37.11
ALT1	4.00	3	31.8600	.99242	.57297	29.3947	34.3253	30.83	32.81
	5.00	3	43.6867	4.74571	2.73994	31.8977	55.4757	38.34	47.40
	6.00	3	31.4400	1.16052	.67002	28.5571	34.3229	30.30	32.62
	Total	18	35.8989	4.62563	1.09027	33.5986	38.1992	30.30	47.40
	1.00	3	37.5267	1.06265	.61352	34.8869	40.1664	36.33	38.36
	2.00	3	32.9567	1.68797	.97455	28.7635	37.1498	31.11	34.42
	3.00	3	40.2533	1.34998	.77941	36.8998	43.6069	38.77	41.41
ALT2	4.00	3	32.0933	1.53027	.88350	28.2919	35.8947	30.44	33.46
	5.00	3	50.9967	1.26785	.73199	47.8472	54.1462	49.89	52.38
	6.00	3	31.9233	1.85877	1.07316	27.3059	36.5408	30.00	33.71
	Total	18	37.6250	7.02212	1.65513	34.1330	41.1170	30.00	52.38
	1.00	3	35.3233	.61011	.35225	33.8077	36.8389	34.62	35.71
	2.00	3	32.3200	.93984	.54262	29.9853	34.6547	31.44	33.31
	3.00	3	38.9900	1.05731	.61044	36.3635	41.6165	38.34	40.21
ALT3	4.00	3	32.9500	.53563	.30925	31.6194	34.2806	32.52	33.55
	5.00	3	51.3033	11.33832	6.54618	23.1374	79.4693	44.21	64.38
	6.00	3	32.6067	.81101	.46824	30.5920	34.6213	31.82	33.44
	Total	18	37.2489	7.93179	1.86954	33.3045	41.1933	31.44	64.38
	1.00	3	149.4867	1.80528	1.04228	145.0021	153.9712	147.70	151.31
CHOL1	2.00	3	141.2033	1.59500	.92088	137.2411	145.1655	139.61	142.80
	3.00	3	149.7000	2.41348	1.39343	143.7046	155.6954	146.93	151.35
	4.00	3	135.2267	5.68403	3.28168	121.1067	149.3466	128.81	139.63

	5.00	3	167.8633	.64810	.37418	166.2534	169.4733	167.12	168.31
	6.00	3	130.3700	6.98698	4.03394	113.0134	147.7266	125.60	138.39
	Total	18	145.6417	12.93809	3.04954	139.2077	152.0756	125.60	168.31
	1.00	3	148.8367	2.56067	1.47840	142.4756	155.1977	146.38	151.49
	2.00	3	135.3333	3.03045	1.74963	127.8053	142.8614	132.73	138.66
	3.00	3	162.7933	6.65616	3.84293	146.2585	179.3281	156.21	169.52
COL2	4.00	3	134.6800	3.00162	1.73298	127.2236	142.1364	131.41	137.31
	5.00	3	180.1100	2.87125	1.65772	172.9774	187.2426	177.56	183.22
	6.00	3	130.4133	5.11197	2.95140	117.7145	143.1122	126.55	136.21
	Total	18	148.6944	18.61599	4.38783	139.4369	157.9520	126.55	183.22
	1.00	3	142.9267	2.20360	1.27225	137.4526	148.4007	140.41	144.51
	2.00	3	133.0433	2.59620	1.49891	126.5940	139.4926	130.71	135.84
	3.00	3	153.4900	2.68967	1.55288	146.8085	160.1715	150.43	155.48
CHOL3	4.00	3	134.0833	2.44625	1.41234	128.0065	140.1602	131.41	136.21
	5.00	3	168.4733	.91577	.52872	166.1984	170.7482	167.58	169.41
	6.00	3	131.0033	2.09462	1.20933	125.8000	136.2067	129.22	133.31
	Total	18	143.8367	13.91566	3.27995	136.9166	150.7568	129.22	169.41
	1.00	3	173.3333	4.93288	2.84800	161.0794	185.5873	170.00	179.00
	2.00	3	165.0000	3.60555	2.08167	156.0433	173.9567	162.00	169.00
	3.00	3	184.6667	5.13160	2.96273	171.9191	197.4143	179.00	189.00
TRIG1	4.00	3	164.6667	2.08167	1.20185	159.4955	169.8378	163.00	167.00
	5.00	3	206.0000	2.00000	1.15470	201.0317	210.9683	204.00	208.00
	6.00	3	151.3333	.57735	.33333	149.8991	152.7676	151.00	152.00
	Total	18	174.1667	18.15700	4.27964	165.1374	183.1959	151.00	208.00
	1.00	3	171.3333	3.21455	1.85592	163.3479	179.3187	169.00	175.00
	2.00	3	151.6667	7.23418	4.17665	133.6960	169.6374	147.00	160.00
	3.00	3	197.6667	2.51661	1.45297	191.4151	203.9183	195.00	200.00
TRIG2	4.00	3	154.0000	3.60555	2.08167	145.0433	162.9567	150.00	157.00
	5.00	3	212.6667	3.05505	1.76383	205.0775	220.2558	210.00	216.00
	6.00	3	151.3333	1.15470	.66667	148.4649	154.2018	150.00	152.00
	Total	18	173.1111	24.96560	5.88445	160.6960	185.5262	147.00	216.00
	1.00	3	161.3333	3.21455	1.85592	153.3479	169.3187	159.00	165.00
	2.00	3	148.0000	1.00000	.57735	145.5159	150.4841	147.00	149.00
	3.00	3	187.6667	3.51188	2.02759	178.9427	196.3907	184.00	191.00
TRIG3	4.00	3	153.0000	2.64575	1.52753	146.4276	159.5724	150.00	155.00
	5.00	3	200.0000	1.00000	.57735	197.5159	202.4841	199.00	201.00
	6.00	3	152.0000	1.00000	.57735	149.5159	154.4841	151.00	153.00
	Total	18	167.0000	20.37299	4.80196	156.8687	177.1313	147.00	201.00
	1.00	3	52.0000	1.00000	.57735	49.5159	54.4841	51.00	53.00
	2.00	3	66.6667	5.68624	3.28295	52.5413	80.7921	62.00	73.00
	3.00	3	42.6667	1.15470	.66667	39.7982	45.5351	42.00	44.00
HDL1	4.00	3	62.3333	11.06044	6.38575	34.8577	89.8090	52.00	74.00
	5.00	3	30.3333	3.78594	2.18581	20.9285	39.7381	26.00	33.00
	6.00	3	55.6667	3.51188	2.02759	46.9427	64.3907	52.00	59.00
	Total	18	51.6111	13.37310	3.15207	44.9608	58.2614	26.00	74.00
	1.00	3	53.0000	2.64575	1.52753	46.4276	59.5724	50.00	55.00
	2.00	3	69.0000	5.56776	3.21455	55.1689	82.8311	64.00	75.00
	3.00	3	45.6667	2.08167	1.20185	40.4955	50.8378	44.00	48.00
HDL2	4.00	3	64.0000	6.55744	3.78594	47.7104	80.2896	57.00	70.00
	5.00	3	28.0000	4.35890	2.51661	17.1719	38.8281	23.00	31.00
	6.00	3	56.0000	2.64575	1.52753	49.4276	62.5724	53.00	58.00
	Total	18	52.6111	14.17180	3.34032	45.5636	59.6586	23.00	75.00
	1.00	3	52.0000	1.00000	.57735	49.5159	54.4841	51.00	53.00
	2.00	3	70.3333	3.21455	1.85592	62.3479	78.3187	68.00	74.00
HDL3	3.00	3	44.3333	1.15470	.66667	41.4649	47.2018	43.00	45.00
	4.00	3	64.3333	7.02377	4.05518	46.8853	81.7813	57.00	71.00

	5.00	3	29.6667	4.50925	2.60342	18.4651	40.8683	25.00	34.00
	6.00	3	56.3333	1.15470	.66667	53.4649	59.2018	55.00	57.00
	Total	18	52.8333	14.03462	3.30799	45.8541	59.8126	25.00	74.00
	1.00	3	34.6667	.98658	.56960	32.2159	37.1175	34.00	35.80
	2.00	3	30.3333	.50332	.29059	29.0830	31.5837	29.80	30.80
	3.00	3	36.9333	1.02632	.59255	34.3838	39.4829	35.80	37.80
VLDL1	4.00	3	28.9333	.41633	.24037	27.8991	29.9676	28.60	29.40
	5.00	3	41.2000	.40000	.23094	40.2063	42.1937	40.80	41.60
	6.00	3	31.2667	.90185	.52068	29.0263	33.5070	30.40	32.20
	Total	18	33.8889	4.39958	1.03699	31.7010	36.0768	28.60	41.60
	1.00	3	34.2667	.64291	.37118	32.6696	35.8637	33.80	35.00
	2.00	3	30.3333	1.44684	.83533	26.7392	33.9275	29.40	32.00
	3.00	3	39.5333	.50332	.29059	38.2830	40.7837	39.00	40.00
VLDL2	4.00	3	30.8000	.72111	.41633	29.0087	32.5913	30.00	31.40
	5.00	3	42.5333	.61101	.35277	41.0155	44.0512	42.00	43.20
	6.00	3	30.2667	.23094	.13333	29.6930	30.8404	30.00	30.40
	Total	18	34.6222	4.99312	1.17689	32.1392	37.1052	29.40	43.20
	1.00	3	32.2667	.64291	.37118	30.6696	33.8637	31.80	33.00
	2.00	3	29.6000	.20000	.11547	29.1032	30.0968	29.40	29.80
	3.00	3	37.5333	.70238	.40552	35.7885	39.2781	36.80	38.20
VLDL3	4.00	3	30.6000	.52915	.30551	29.2855	31.9145	30.00	31.00
	5.00	3	40.0000	.20000	.11547	39.5032	40.4968	39.80	40.20
	6.00	3	30.4000	.20000	.11547	29.9032	30.8968	30.20	30.60
	Total	18	33.4000	4.07460	.96039	31.3737	35.4263	29.40	40.20
	1.00	3	62.8333	3.23316	1.86667	54.8017	70.8650	59.90	66.30
	2.00	3	44.2000	6.93109	4.00167	26.9822	61.4178	36.20	48.40
	3.00	3	70.1000	2.10000	1.21244	64.8833	75.3167	67.70	71.60
LDL1	4.00	3	43.9333	13.30464	7.68144	10.8828	76.9839	33.80	59.00
	5.00	3	96.3333	3.70720	2.14035	87.1241	105.5425	93.90	100.60
	6.00	3	43.4333	10.66599	6.15801	16.9375	69.9291	34.40	55.20
	Total	18	60.1389	20.82369	4.90819	49.7835	70.4943	33.80	100.60
	1.00	3	61.0667	5.21664	3.01183	48.1078	74.0255	57.20	67.00
	2.00	3	40.5667	2.05508	1.18650	35.4616	45.6718	38.60	42.70
	3.00	3	77.1333	5.35288	3.09049	63.8360	90.4306	71.40	82.00
LDL2	4.00	3	39.6667	10.01665	5.78312	14.7839	64.5494	30.00	50.00
	5.00	3	108.6667	1.52753	.88192	104.8721	112.4612	107.00	110.00
	6.00	3	44.0000	7.81025	4.50925	24.5983	63.4017	39.00	53.00
	Total	18	61.8500	26.02782	6.13482	48.9067	74.7933	30.00	110.00
	1.00	3	58.6600	1.13248	.65383	55.8468	61.4732	57.61	59.86
	2.00	3	33.1100	1.45564	.84042	29.4940	36.7260	32.11	34.78
	3.00	3	71.6233	3.37189	1.94676	63.2471	79.9996	67.83	74.28
LDL3	4.00	3	39.1500	8.83871	5.10303	17.1934	61.1066	32.63	49.21
	5.00	3	98.8067	5.33841	3.08213	85.5453	112.0680	93.78	104.41
	6.00	3	46.2700	2.76697	1.59751	39.3965	53.1435	43.08	48.02
	Total	18	57.9367	23.19857	5.46796	46.4003	69.4730	32.11	104.41
	1.00	3	5.2700	.41146	.23756	4.2479	6.2921	4.84	5.66
	2.00	3	5.5767	.23245	.13421	4.9992	6.1541	5.40	5.84
	3.00	3	4.7933	.26839	.15496	4.1266	5.4601	4.49	5.00
TP1	4.00	3	5.1633	.15822	.09135	4.7703	5.5564	4.99	5.30
	5.00	3	4.3433	.30860	.17817	3.5767	5.1099	3.99	4.56
	6.00	3	5.4100	.25515	.14731	4.7762	6.0438	5.22	5.70
	Total	18	5.0928	.48682	.11475	4.8507	5.3349	3.99	5.84
	1.00	3	5.1367	.21221	.12252	4.6095	5.6638	4.90	5.31
	2.00	3	5.5933	.17898	.10333	5.1487	6.0379	5.44	5.79
TP2	3.00	3	4.8167	.16503	.09528	4.4067	5.2266	4.68	5.00
	4.00	3	5.2500	.38743	.22368	4.2876	6.2124	4.89	5.66

	5.00	3	4.0000	.01000	.00577	3.9752	4.0248	3.99	4.01
	6.00	3	5.6133	.21197	.12238	5.0868	6.1399	5.42	5.84
	Total	18	5.0683	.59626	.14054	4.7718	5.3648	3.99	5.84
	1.00	3	3.9667	.06658	.03844	3.8013	4.1321	3.89	4.01
	2.00	3	5.4367	.46004	.26560	4.2939	6.5795	4.98	5.90
	3.00	3	3.9500	.06083	.03512	3.7989	4.1011	3.88	3.99
TP3	4.00	3	5.0133	.03215	.01856	4.9335	5.0932	4.99	5.05
	5.00	3	3.3067	.26858	.15506	2.6395	3.9738	3.00	3.50
	6.00	3	5.6967	.23629	.13642	5.1097	6.2836	5.43	5.88
	Total	18	4.5617	.91974	.21678	4.1043	5.0190	3.00	5.90
	1.00	3	2.6033	.44792	.25861	1.4906	3.7160	2.26	3.11
	2.00	3	2.7000	.26287	.15177	2.0470	3.3530	2.51	3.00
	3.00	3	2.8667	.23965	.13836	2.2713	3.4620	2.59	3.01
ALB1	4.00	3	2.5967	.37099	.21419	1.6751	3.5183	2.27	3.00
	5.00	3	2.5900	.50587	.29206	1.3334	3.8466	2.01	2.94
	6.00	3	2.8833	.56862	.32830	1.4708	4.2959	2.25	3.35
	Total	18	2.7067	.37321	.08797	2.5211	2.8923	2.01	3.35
	1.00	3	2.8933	.10504	.06064	2.6324	3.1543	2.79	3.00
	2.00	3	3.0000	.01000	.00577	2.9752	3.0248	2.99	3.01
	3.00	3	2.5867	.50846	.29356	1.3236	3.8498	2.00	2.90
ALB2	4.00	3	3.0400	.06083	.03512	2.8889	3.1911	3.00	3.11
	5.00	3	2.3367	.29160	.16836	1.6123	3.0611	2.00	2.51
	6.00	3	3.3400	.24331	.14048	2.7356	3.9444	3.06	3.50
	Total	18	2.8661	.40080	.09447	2.6668	3.0654	2.00	3.50
	1.00	3	2.1800	.26058	.15044	1.5327	2.8273	2.01	2.48
	2.00	3	3.0400	.06083	.03512	2.8889	3.1911	3.00	3.11
	3.00	3	1.6967	.28219	.16292	.9957	2.3977	1.50	2.02
ALB3	4.00	3	3.0467	.06028	.03480	2.8969	3.1964	2.99	3.11
	5.00	3	1.8700	.32512	.18771	1.0624	2.6776	1.50	2.11
	6.00	3	3.3167	.23629	.13642	2.7297	3.9036	3.05	3.50
	Total	18	2.5250	.67856	.15994	2.1876	2.8624	1.50	3.50
	1.00	3	2.6667	.64291	.37118	1.0696	4.2637	2.20	3.40
	2.00	3	2.8767	.03215	.01856	2.7968	2.9565	2.84	2.90
	3.00	3	1.9267	.06429	.03712	1.7670	2.0864	1.88	2.00
GLOB1	4.00	3	2.5667	.42336	.24443	1.5150	3.6184	2.20	3.03
	5.00	3	2.5267	.38657	.22318	1.5664	3.4870	2.26	2.97
	6.00	3	1.7533	.19732	.11392	1.2632	2.2435	1.62	1.98
	Total	18	2.3861	.51574	.12156	2.1296	2.6426	1.62	3.40
	1.00	3	2.2433	.11547	.06667	1.9565	2.5302	2.11	2.31
	2.00	3	2.5933	.18583	.10729	2.1317	3.0550	2.44	2.80
	3.00	3	2.2300	.50110	.28931	.9852	3.4748	1.78	2.77
GLOB2	4.00	3	2.2100	.40361	.23302	1.2074	3.2126	1.88	2.66
	5.00	3	1.6633	.28290	.16333	.9606	2.3661	1.50	1.99
	6.00	3	2.2733	.16773	.09684	1.8567	2.6900	2.08	2.38
	Total	18	2.2022	.38294	.09026	2.0118	2.3927	1.50	2.80
	1.00	3	1.7867	.22502	.12991	1.2277	2.3456	1.53	1.95
	2.00	3	2.3967	.41102	.23730	1.3756	3.4177	1.97	2.79
	3.00	3	2.2533	.25968	.14993	1.6083	2.8984	1.97	2.48
GLOB3	4.00	3	1.9667	.06658	.03844	1.8013	2.1321	1.89	2.01
	5.00	3	1.4300	.50863	.29366	.1665	2.6935	.89	1.90
	6.00	3	2.3800	.10000	.05774	2.1316	2.6284	2.28	2.48
	Total	18	2.0356	.44124	.10400	1.8161	2.2550	.89	2.79
	1.00	3	.9367	.09866	.05696	.6916	1.1817	.87	1.05
	2.00	3	1.0267	.37005	.21365	.1074	1.9459	.66	1.40
AG1	3.00	3	1.4867	.12055	.06960	1.1872	1.7861	1.36	1.60
	4.00	3	1.0433	.30567	.17648	.2840	1.8027	.75	1.36

	5.00	3	1.5100	.42790	.24705	.4470	2.5730	1.02	1.81
	6.00	3	1.2533	.24007	.13860	.6570	1.8497	.98	1.43
	Total	18	1.2094	.33469	.07889	1.0430	1.3759	.66	1.81
	1.00	3	1.1667	.05774	.03333	1.0232	1.3101	1.10	1.20
	2.00	3	1.3000	.00000	.00000	1.3000	1.3000	1.30	1.30
	3.00	3	1.2000	.45826	.26458	.0616	2.3384	.70	1.60
AG2	4.00	3	1.4000	.26458	.15275	.7428	2.0572	1.10	1.60
	5.00	3	1.4667	.40415	.23333	.4627	2.4706	1.00	1.70
	6.00	3	1.5000	.20000	.11547	1.0032	1.9968	1.30	1.70
	Total	18	1.3389	.27255	.06424	1.2034	1.4744	.70	1.70
	1.00	3	1.2900	.21633	.12490	.7526	1.8274	1.11	1.53
	2.00	3	1.2467	.32347	.18676	.4431	2.0502	1.05	1.62
	3.00	3	.7667	.22301	.12875	.2127	1.3207	.60	1.02
AG3	4.00	3	1.5533	.08386	.04842	1.3450	1.7617	1.50	1.65
	5.00	3	1.4967	.80308	.46366	-.4983	3.4916	.79	2.37
	6.00	3	52.2167	88.14696	50.89167	-166.7525	271.1859	1.28	154.00
	Total	18	9.7617	35.99949	8.48516	-8.1405	27.6638	.60	154.00
	1.00	3	13.6033	.62692	.36195	12.0460	15.1607	12.88	13.99
	2.00	3	12.4400	.77156	.44546	10.5233	14.3567	11.55	12.92
	3.00	3	14.7100	.84859	.48993	12.6020	16.8180	13.75	15.36
BUN1	4.00	3	12.1533	1.13359	.65448	9.3373	14.9693	11.35	13.45
	5.00	3	16.5533	1.55918	.90019	12.6801	20.4265	14.85	17.91
	6.00	3	12.1467	.50817	.29339	10.8843	13.4090	11.56	12.45
	Total	18	13.6011	1.84519	.43491	12.6835	14.5187	11.35	17.91
	1.00	3	14.5233	.50738	.29294	13.2629	15.7837	13.99	15.00
	2.00	3	13.2967	.62067	.35834	11.7548	14.8385	12.88	14.01
	3.00	3	16.8567	.23965	.13836	16.2613	17.4520	16.58	17.00
BUN2	4.00	3	13.5667	1.07449	.62036	10.8975	16.2359	12.44	14.58
	5.00	3	20.4533	.43776	.25274	19.3659	21.5408	19.99	20.86
	6.00	3	12.1500	.25120	.14503	11.5260	12.7740	12.00	12.44
	Total	18	15.1411	2.90370	.68441	13.6971	16.5851	12.00	20.86
	1.00	3	14.9567	1.37158	.79188	11.5495	18.3639	13.44	16.11
	2.00	3	13.5700	.63222	.36501	11.9995	15.1405	13.00	14.25
	3.00	3	18.3300	.66506	.38397	16.6779	19.9821	17.66	18.99
BUN3	4.00	3	13.7767	1.11500	.64375	11.0068	16.5465	12.66	14.89
	5.00	3	27.2433	3.76835	2.17566	17.8822	36.6044	24.41	31.52
	6.00	3	12.9500	.42320	.24434	11.8987	14.0013	12.56	13.40
	Total	18	16.8044	5.33736	1.25803	14.1502	19.4586	12.56	31.52
	1.00	3	.7033	.01528	.00882	.6654	.7413	.69	.72
	2.00	3	.7200	.02646	.01528	.6543	.7857	.70	.75
	3.00	3	.7733	.03786	.02186	.6793	.8674	.73	.80
CREAT1	4.00	3	.7267	.05686	.03283	.5854	.8679	.68	.79
	5.00	3	.8200	.04359	.02517	.7117	.9283	.77	.85
	6.00	3	.6233	.03512	.02028	.5361	.7106	.59	.66
	Total	18	.7278	.07026	.01656	.6928	.7627	.59	.85
	1.00	3	.8000	.01000	.00577	.7752	.8248	.79	.81
	2.00	3	.7333	.01528	.00882	.6954	.7713	.72	.75
	3.00	3	.8367	.04509	.02603	.7247	.9487	.79	.88
CREAT2	4.00	3	.7167	.01528	.00882	.6787	.7546	.70	.73
	5.00	3	.9833	.07638	.04410	.7936	1.1731	.90	1.05
	6.00	3	.6667	.01528	.00882	.6287	.7046	.65	.68
	Total	18	.7894	.11053	.02605	.7345	.8444	.65	1.05
	1.00	3	.8200	.02646	.01528	.7543	.8857	.79	.84
	2.00	3	.6933	.01528	.00882	.6554	.7313	.68	.71
CREAT3	3.00	3	.8000	.01000	.00577	.7752	.8248	.79	.81
	4.00	3	.7100	.01000	.00577	.6852	.7348	.70	.72

	5.00	3	1.1233	.08083	.04667	.9225	1.3241	1.05	1.21
	6.00	3	.6667	.00577	.00333	.6523	.6810	.66	.67
	Total	18	.8022	.16123	.03800	.7220	.8824	.66	1.21
	1.00	3	.6733	.15695	.09062	.2834	1.0632	.55	.85
	2.00	3	.7800	.01732	.01000	.7370	.8230	.77	.80
	3.00	3	.9667	.06807	.03930	.7976	1.1358	.89	1.02
TBIL1	4.00	3	.6367	.17010	.09821	.2141	1.0592	.47	.81
	5.00	3	1.6067	.20232	.11681	1.1041	2.1093	1.48	1.84
	6.00	3	.5067	.08327	.04807	.2998	.7135	.44	.60
	Total	18	.8617	.38861	.09160	.6684	1.0549	.44	1.84
	1.00	3	.9900	.01000	.00577	.9652	1.0148	.98	1.00
	2.00	3	.8700	.02646	.01528	.8043	.9357	.84	.89
	3.00	3	1.1500	.13892	.08021	.8049	1.4951	.99	1.24
TBIL2	4.00	3	.8033	.03215	.01856	.7235	.8832	.78	.84
	5.00	3	1.7100	.16093	.09292	1.3102	2.1098	1.58	1.89
	6.00	3	.5433	.03786	.02186	.4493	.6374	.50	.57
	Total	18	1.0111	.38096	.08979	.8217	1.2006	.50	1.89
	1.00	3	.9167	.03055	.01764	.8408	.9926	.89	.95
	2.00	3	.8100	.02646	.01528	.7443	.8757	.79	.84
	3.00	3	1.0633	.12702	.07333	.7478	1.3789	.99	1.21
TBIL3	4.00	3	.8200	.04359	.02517	.7117	.9283	.79	.87
	5.00	3	1.9233	.06658	.03844	1.7579	2.0887	1.88	2.00
	6.00	3	.5667	.00577	.00333	.5523	.5810	.56	.57
	Total	18	1.0167	.44738	.10545	.7942	1.2391	.56	2.00
	1.00	3	.1733	.06110	.03528	.0216	.3251	.12	.24
	2.00	3	.1700	.01000	.00577	.1452	.1948	.16	.18
	3.00	3	.2067	.09452	.05457	-.0281	.4415	.10	.28
CONBIL1	4.00	3	.1567	.05033	.02906	.0316	.2817	.11	.21
	5.00	3	.3767	.11930	.06888	.0803	.6730	.28	.51
	6.00	3	.2300	.04583	.02646	.1162	.3438	.18	.27
	Total	18	.2189	.09815	.02313	.1701	.2677	.10	.51
	1.00	3	.3600	.01000	.00577	.3352	.3848	.35	.37
	2.00	3	.4567	.05859	.03383	.3111	.6022	.39	.50
	3.00	3	.3400	.01000	.00577	.3152	.3648	.33	.35
CONBIL2	4.00	3	.3900	.05568	.03215	.2517	.5283	.34	.45
	5.00	3	.3567	.03512	.02028	.2694	.4439	.32	.39
	6.00	3	.4467	.01528	.00882	.4087	.4846	.43	.46
	Total	18	.3917	.05576	.01314	.3639	.4194	.32	.50
	1.00	3	.3567	.00577	.00333	.3423	.3710	.35	.36
	2.00	3	.4700	.01000	.00577	.4452	.4948	.46	.48
	3.00	3	.3500	.01000	.00577	.3252	.3748	.34	.36
CONBIL3	4.00	3	.3733	.02082	.01202	.3216	.4250	.35	.39
	5.00	3	.3300	.02000	.01155	.2803	.3797	.31	.35
	6.00	3	.3433	.02082	.01202	.2916	.3950	.32	.36
	Total	18	.3706	.04952	.01167	.3459	.3952	.31	.48
	1.00	3	.6100	.01000	.00577	.5852	.6348	.60	.62
	2.00	3	.5000	.09644	.05568	.2604	.7396	.43	.61
	3.00	3	.7600	.14177	.08185	.4078	1.1122	.65	.92
UNCONBL1	4.00	3	.4800	.14422	.08327	.1217	.8383	.32	.60
	5.00	3	1.2300	.25632	.14799	.5933	1.8667	.99	1.50
	6.00	3	.2767	.04726	.02728	.1593	.3941	.24	.33
	Total	18	.6428	.33061	.07793	.4784	.8072	.24	1.50
	1.00	3	.4133	.03215	.01856	.3335	.4932	.39	.45
UNCONBL2	2.00	3	.6300	.01000	.00577	.6052	.6548	.62	.64
	3.00	3	.8100	.13077	.07550	.4852	1.1348	.66	.90
	4.00	3	.4133	.08505	.04910	.2021	.6246	.33	.50

	5.00	3	1.3867	.13577	.07839	1.0494	1.7239	1.26	1.53
	6.00	3	.0967	.04509	.02603	-.0153	.2087	.05	.14
	Total	18	.6250	.42296	.09969	.4147	.8353	.05	1.53
	1.00	3	.3400	.03606	.02082	.2504	.4296	.31	.38
	2.00	3	.5600	.03000	.01732	.4855	.6345	.53	.59
	3.00	3	.7133	.11846	.06839	.4191	1.0076	.64	.85
UNCONBIL3	4.00	3	.4467	.03055	.01764	.3708	.5226	.42	.48
	5.00	3	1.5933	.06807	.03930	1.4242	1.7624	1.54	1.67
	6.00	3	.2233	.02309	.01333	.1660	.2807	.21	.25
	Total	18	.6461	.46708	.11009	.4138	.8784	.21	1.67
	1.00	3	1.9400	.14422	.08327	1.5817	2.2983	1.82	2.10
	2.00	3	2.5667	.11719	.06766	2.2756	2.8578	2.48	2.70
	3.00	3	3.1233	.29143	.16826	2.3994	3.8473	2.85	3.43
AA1	4.00	3	2.0733	.12662	.07311	1.7588	2.3879	1.96	2.21
	5.00	3	1.9433	.22942	.13246	1.3734	2.5132	1.68	2.10
	6.00	3	2.9133	.32716	.18889	2.1006	3.7260	2.61	3.26
	Total	18	2.4267	.52027	.12263	2.1679	2.6854	1.68	3.43
	1.00	3	2.3833	.20257	.11695	1.8801	2.8865	2.22	2.61
	2.00	3	2.3633	.04726	.02728	2.2459	2.4807	2.31	2.40
	3.00	3	3.1400	.30000	.17321	2.3948	3.8852	2.84	3.44
AA2	4.00	3	2.1900	.03464	.02000	2.1039	2.2761	2.15	2.21
	5.00	3	2.6900	.14731	.08505	2.3241	3.0559	2.60	2.86
	6.00	3	2.1433	.08083	.04667	1.9425	2.3441	2.07	2.23
	Total	18	2.4850	.37771	.08903	2.2972	2.6728	2.07	3.44
	1.00	3	2.3767	.10970	.06333	2.1042	2.6492	2.29	2.50
	2.00	3	2.2167	.11372	.06566	1.9342	2.4992	2.09	2.31
	3.00	3	2.1000	.01732	.01000	2.0570	2.1430	2.09	2.12
AA3	4.00	3	2.3500	.14731	.08505	1.9841	2.7159	2.19	2.48
	5.00	3	1.8900	.14000	.08083	1.5422	2.2378	1.73	1.99
	6.00	3	2.2167	.12741	.07356	1.9002	2.5332	2.07	2.30
	Total	18	2.1917	.19485	.04593	2.0948	2.2886	1.73	2.50
	1.00	3	18.8933	.70437	.40667	17.1436	20.6431	18.40	19.70
	2.00	3	17.6867	1.08247	.62496	14.9977	20.3757	16.50	18.62
	3.00	3	19.0167	.36747	.21216	18.1038	19.9295	18.78	19.44
BUNCREAT1	4.00	3	16.8333	2.61653	1.51065	10.3335	23.3332	14.75	19.77
	5.00	3	20.1600	1.04843	.60531	17.5556	22.7644	19.28	21.32
	6.00	3	19.5533	1.83895	1.06172	14.9851	24.1215	17.52	21.10
	Total	18	18.6906	1.69402	.39928	17.8481	19.5330	14.75	21.32
	1.00	3	18.1333	.97125	.56075	15.7206	20.5461	17.30	19.20
	2.00	3	18.1333	.40415	.23333	17.1294	19.1373	17.70	18.50
	3.00	3	20.1667	1.17189	.67659	17.2555	23.0778	19.30	21.50
BUNCREAT2	4.00	3	18.9467	1.69898	.98091	14.7262	23.1672	17.04	20.30
	5.00	3	20.8667	1.19304	.68880	17.9030	23.8303	19.90	22.20
	6.00	3	18.2333	.55076	.31798	16.8652	19.6015	17.60	18.60
	Total	18	19.0800	1.43253	.33765	18.3676	19.7924	17.04	22.20
	1.00	3	19.5800	1.18773	.68574	16.6295	22.5305	18.84	20.95
	2.00	3	18.2700	2.10029	1.21260	13.0526	23.4874	16.19	20.39
	3.00	3	22.9133	.54592	.31519	21.5572	24.2695	22.35	23.44
BUNCREAT3	4.00	3	19.4100	1.73701	1.00286	15.0950	23.7250	17.83	21.27
	5.00	3	24.2033	2.05469	1.18627	19.0992	29.3075	21.99	26.05
	6.00	3	19.4200	.51215	.29569	18.1477	20.6923	19.03	20.00
	Total	18	20.6328	2.54446	.59974	19.3674	21.8981	16.19	26.05

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.

	Between Groups	7722.021	5	1544.404	30.078	.000
AST1	Within Groups	616.161	12	51.347		
	Total	8338.182	17			
	Between Groups	13132.503	5	2626.501	68.422	.000
AST2	Within Groups	460.644	12	38.387		
	Total	13593.147	17			
	Between Groups	316.587	5	63.317	5.401	.008
AST3	Within Groups	140.684	12	11.724		
	Total	457.271	17			
	Between Groups	293.367	5	58.673	10.005	.001
ALT1	Within Groups	70.372	12	5.864		
	Total	363.739	17			
	Between Groups	811.863	5	162.373	73.777	.000
ALT2	Within Groups	26.410	12	2.201		
	Total	838.273	17			
	Between Groups	805.774	5	161.155	7.332	.002
ALT3	Within Groups	263.751	12	21.979		
	Total	1069.525	17			
	Between Groups	2659.354	5	531.871	34.250	.000
CHOL1	Within Groups	186.348	12	15.529		
	Total	2845.702	17			
	Between Groups	5684.577	5	1136.915	65.952	.000
COL2	Within Groups	206.862	12	17.239		
	Total	5891.439	17			
	Between Groups	3231.895	5	646.379	129.101	.000
CHOL3	Within Groups	60.081	12	5.007		
	Total	3291.976	17			
	Between Groups	5459.833	5	1091.967	90.578	.000
TRIG1	Within Groups	144.667	12	12.056		
	Total	5604.500	17			
	Between Groups	10410.444	5	2082.089	134.812	.000
TRIG2	Within Groups	185.333	12	15.444		
	Total	10595.778	17			
	Between Groups	6990.667	5	1398.133	256.800	.000
TRIG3	Within Groups	65.333	12	5.444		
	Total	7056.000	17			
	Between Groups	2672.944	5	534.589	17.464	.000
HDL1	Within Groups	367.333	12	30.611		
	Total	3040.278	17			
	Between Groups	3191.611	5	638.322	34.401	.000
HDL2	Within Groups	222.667	12	18.556		
	Total	3414.278	17			
	Between Groups	3181.167	5	636.233	45.626	.000
HDL3	Within Groups	167.333	12	13.944		
	Total	3348.500	17			
	Between Groups	322.204	5	64.441	112.834	.000
VLDL1	Within Groups	6.853	12	.571		
	Total	329.058	17			
	Between Groups	416.418	5	83.284	134.812	.000
VLDL2	Within Groups	7.413	12	.618		
	Total	423.831	17			
	Between Groups	279.627	5	55.925	256.800	.000
VLDL3	Within Groups	2.613	12	.218		
	Total	282.240	17			
LDL1	Between Groups	6636.796	5	1327.359	21.676	.000

	Within Groups	734.847	12	61.237		
	Total	7371.643	17			
	Between Groups	11069.092	5	2213.818	59.363	.000
LDL2	Within Groups	447.513	12	37.293		
	Total	11516.605	17			
	Between Groups	8890.855	5	1778.171	82.675	.000
LDL3	Within Groups	258.097	12	21.508		
	Total	9148.952	17			
	Between Groups	3.067	5	.613	7.657	.002
TP1	Within Groups	.961	12	.080		
	Total	4.029	17			
	Between Groups	5.445	5	1.089	21.821	.000
TP2	Within Groups	.599	12	.050		
	Total	6.044	17			
	Between Groups	13.683	5	2.737	47.079	.000
TP3	Within Groups	.698	12	.058		
	Total	14.381	17			
	Between Groups	.280	5	.056	.322	.891
ALB1	Within Groups	2.088	12	.174		
	Total	2.368	17			
	Between Groups	1.896	5	.379	5.447	.008
ALB2	Within Groups	.835	12	.070		
	Total	2.731	17			
	Between Groups	7.195	5	1.439	27.288	.000
ALB3	Within Groups	.633	12	.053		
	Total	7.828	17			
	Between Groups	2.950	5	.590	4.503	.015
GLOB1	Within Groups	1.572	12	.131		
	Total	4.522	17			
	Between Groups	1.353	5	.271	2.848	.064
GLOB2	Within Groups	1.140	12	.095		
	Total	2.493	17			
	Between Groups	2.190	5	.438	4.691	.013
GLOB3	Within Groups	1.120	12	.093		
	Total	3.310	17			
	Between Groups	.914	5	.183	2.213	.121
AG1	Within Groups	.991	12	.083		
	Total	1.904	17			
	Between Groups	.289	5	.058	.714	.625
AG2	Within Groups	.973	12	.081		
	Total	1.263	17			
	Between Groups	6489.895	5	1297.979	1.002	.457
AG3	Within Groups	15541.481	12	1295.123		
	Total	22031.376	17			
	Between Groups	46.515	5	9.303	9.822	.001
BUN1	Within Groups	11.365	12	.947		
	Total	57.880	17			
	Between Groups	139.116	5	27.823	79.142	.000
BUN2	Within Groups	4.219	12	.352		
	Total	143.335	17			
	Between Groups	447.593	5	89.519	29.277	.000
BUN3	Within Groups	36.692	12	3.058		
	Total	484.285	17			
	Between Groups	.066	5	.013	9.130	.001
CREAT1	Within Groups	.017	12	.001		
	Total	.084	17			

	Between Groups	.190	5	.038	26.358	.000
CREAT2	Within Groups	.017	12	.001		
	Total	.208	17			
	Between Groups	.427	5	.085	66.469	.000
CREAT3	Within Groups	.015	12	.001		
	Total	.442	17			
	Between Groups	2.355	5	.471	26.563	.000
TBIL1	Within Groups	.213	12	.018		
	Total	2.567	17			
	Between Groups	2.370	5	.474	58.686	.000
TBIL2	Within Groups	.097	12	.008		
	Total	2.467	17			
	Between Groups	3.354	5	.671	166.790	.000
TBIL3	Within Groups	.048	12	.004		
	Total	3.403	17			
	Between Groups	.101	5	.020	3.813	.027
CONBIL1	Within Groups	.063	12	.005		
	Total	.164	17			
	Between Groups	.036	5	.007	5.334	.008
CONBIL2	Within Groups	.016	12	.001		
	Total	.053	17			
	Between Groups	.039	5	.008	30.956	.000
CONBIL3	Within Groups	.003	12	.000		
	Total	.042	17			
	Between Groups	1.622	5	.324	16.459	.000
UNCONBL1	Within Groups	.236	12	.020		
	Total	1.858	17			
	Between Groups	2.949	5	.590	77.052	.000
UNCONBL2	Within Groups	.092	12	.008		
	Total	3.041	17			
	Between Groups	3.664	5	.733	196.880	.000
UNCONBIL3	Within Groups	.045	12	.004		
	Total	3.709	17			
	Between Groups	4.011	5	.802	16.308	.000
AA1	Within Groups	.590	12	.049		
	Total	4.602	17			
	Between Groups	2.100	5	.420	15.488	.000
AA2	Within Groups	.325	12	.027		
	Total	2.425	17			
	Between Groups	.480	5	.096	6.954	.003
AA3	Within Groups	.166	12	.014		
	Total	.645	17			
	Between Groups	22.525	5	4.505	2.059	.142
BUNCREAT1	Within Groups	26.260	12	2.188		
	Total	48.785	17			
	Between Groups	20.700	5	4.140	3.502	.035
BUNCREAT2	Within Groups	14.186	12	1.182		
	Total	34.886	17			
	Between Groups	82.821	5	16.564	7.296	.002
BUNCREAT3	Within Groups	27.242	12	2.270		
	Total	110.063	17			

Appendix 8: Effects of chronic administration of *Cussonia arborea* root bark extract on the in vivo antioxidants and haematological indices of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MDA1	1.00	3	6.6000	.20000	.11547	6.1032	7.0968	6.40	6.80
	2.00	3	5.7333	.15275	.08819	5.3539	6.1128	5.60	5.90
	3.00	3	7.2667	.55076	.31798	5.8985	8.6348	6.90	7.90
	4.00	3	5.1333	.15275	.08819	4.7539	5.5128	5.00	5.30
	5.00	3	7.3333	.15275	.08819	6.9539	7.7128	7.20	7.50
	6.00	3	4.3333	.15275	.08819	3.9539	4.7128	4.20	4.50
	Total	18	6.0667	1.15860	.27308	5.4905	6.6428	4.20	7.90
MDA2	1.00	3	6.1000	.17321	.10000	5.6697	6.5303	6.00	6.30
	2.00	3	4.2667	.11547	.06667	3.9798	4.5535	4.20	4.40
	3.00	3	6.7333	.20817	.12019	6.2162	7.2504	6.50	6.90
	4.00	3	4.8000	.26458	.15275	4.1428	5.4572	4.50	5.00
	5.00	3	9.2000	.10000	.05774	8.9516	9.4484	9.10	9.30
	6.00	3	4.3000	.10000	.05774	4.0516	4.5484	4.20	4.40
	Total	18	5.9000	1.79115	.42218	5.0093	6.7907	4.20	9.30
MDA3	1.00	3	6.1000	.43589	.25166	5.0172	7.1828	5.80	6.60
	2.00	3	4.5333	.11547	.06667	4.2465	4.8202	4.40	4.60
	3.00	3	9.2667	1.16762	.67412	6.3661	12.1672	8.00	10.30
	4.00	3	4.7000	.10000	.05774	4.4516	4.9484	4.60	4.80
	5.00	3	10.3000	.10000	.05774	10.0516	10.5484	10.20	10.40
	6.00	3	4.5000	.10000	.05774	4.2516	4.7484	4.40	4.60
	Total	18	6.5667	2.46386	.58074	5.3414	7.7919	4.40	10.40
SOD1	1.00	3	.2967	.00577	.00333	.2823	.3110	.29	.30
	2.00	3	.3867	.00577	.00333	.3723	.4010	.38	.39
	3.00	3	.2897	.00950	.00549	.2661	.3133	.28	.30
	4.00	3	.3333	.02517	.01453	.2708	.3958	.31	.36
	5.00	3	.2863	.00473	.00273	.2746	.2981	.28	.29
	6.00	3	.5700	.01000	.00577	.5452	.5948	.56	.58
	Total	18	.3604	.10334	.02436	.3091	.4118	.28	.58
SOD2	1.00	3	.2933	.00577	.00333	.2790	.3077	.29	.30
	2.00	3	.3967	.00577	.00333	.3823	.4110	.39	.40
	3.00	3	.2800	.01000	.00577	.2552	.3048	.27	.29
	4.00	3	.3700	.03000	.01732	.2955	.4445	.34	.40
	5.00	3	.2000	.01000	.00577	.1752	.2248	.19	.21
	6.00	3	.5733	.00577	.00333	.5590	.5877	.57	.58
	Total	18	.3522	.12163	.02867	.2917	.4127	.19	.58
SOD3	1.00	3	.3733	.03055	.01764	.2974	.4492	.34	.40
	2.00	3	.6167	.05774	.03333	.4732	.7601	.55	.65
	3.00	3	.2800	.01000	.00577	.2552	.3048	.27	.29
	4.00	3	.5900	.01000	.00577	.5652	.6148	.58	.60
	5.00	3	.1567	.00577	.00333	.1423	.1710	.15	.16
	6.00	3	.5967	.00577	.00333	.5823	.6110	.59	.60
	Total	18	.4356	.18382	.04333	.3441	.5270	.15	.65
GSH1	1.00	3	135.6667	3.05505	1.76383	128.0775	143.2558	133.00	139.00
	2.00	3	144.3333	.57735	.33333	142.8991	145.7676	144.00	145.00
	3.00	3	135.6667	4.50925	2.60342	124.4651	146.8683	131.00	140.00
	4.00	3	142.0000	2.00000	1.15470	137.0317	146.9683	140.00	144.00

	5.00	3	121.0000	1.00000	.57735	118.5159	123.4841	120.00	122.00
	6.00	3	163.0000	2.64575	1.52753	156.4276	169.5724	160.00	165.00
	Total	18	140.2778	13.13641	3.09628	133.7452	146.8104	120.00	165.00
	1.00	3	140.3333	1.52753	.88192	136.5388	144.1279	139.00	142.00
	2.00	3	161.0000	1.00000	.57735	158.5159	163.4841	160.00	162.00
	3.00	3	137.6667	1.52753	.88192	133.8721	141.4612	136.00	139.00
GSH2	4.00	3	162.0000	2.64575	1.52753	155.4276	168.5724	159.00	164.00
	5.00	3	112.3333	2.51661	1.45297	106.0817	118.5849	110.00	115.00
	6.00	3	166.0000	2.64575	1.52753	159.4276	172.5724	163.00	168.00
	Total	18	146.5556	19.39443	4.57131	136.9109	156.2002	110.00	168.00
	1.00	3	142.0000	2.64575	1.52753	135.4276	148.5724	140.00	145.00
	2.00	3	167.3333	.57735	.33333	165.8991	168.7676	167.00	168.00
	3.00	3	138.0000	1.00000	.57735	135.5159	140.4841	137.00	139.00
GSH3	4.00	3	163.3333	2.88675	1.66667	156.1622	170.5044	160.00	165.00
	5.00	3	99.0000	1.00000	.57735	96.5159	101.4841	98.00	100.00
	6.00	3	165.6667	.57735	.33333	164.2324	167.1009	165.00	166.00
	Total	18	145.8889	24.65023	5.81012	133.6306	158.1472	98.00	168.00
	1.00	3	2.6133	.11504	.06642	2.3276	2.8991	2.50	2.73
	2.00	3	3.8067	.35921	.20739	2.9143	4.6990	3.41	4.11
	3.00	3	1.9633	.05508	.03180	1.8265	2.1001	1.90	2.00
CAT1	4.00	3	3.3500	.14107	.08145	2.9996	3.7004	3.22	3.50
	5.00	3	1.4000	.00000	.00000	1.4000	1.4000	1.40	1.40
	6.00	3	5.3563	.05450	.03147	5.2209	5.4917	5.32	5.42
	Total	18	3.0816	1.34045	.31595	2.4150	3.7482	1.40	5.42
	1.00	3	2.7133	.15044	.08686	2.3396	3.0871	2.54	2.81
	2.00	3	4.9200	.05292	.03055	4.7886	5.0514	4.88	4.98
	3.00	3	2.2967	.06658	.03844	2.1313	2.4621	2.22	2.34
CAT2	4.00	3	4.3300	.58026	.33501	2.8886	5.7714	3.99	5.00
	5.00	3	.6933	.52253	.30168	-.6047	1.9914	.09	1.00
	6.00	3	5.7433	.05132	.02963	5.6159	5.8708	5.70	5.80
	Total	18	3.4494	1.78696	.42119	2.5608	4.3381	.09	5.80
	1.00	3	3.6200	.54745	.31607	2.2601	4.9799	2.99	3.98
	2.00	3	5.8167	.05508	.03180	5.6799	5.9535	5.78	5.88
	3.00	3	2.4300	.51740	.29872	1.1447	3.7153	1.99	3.00
CAT3	4.00	3	4.5900	.55073	.31796	3.2219	5.9581	4.20	5.22
	5.00	3	.8733	.12014	.06936	.5749	1.1718	.75	.99
	6.00	3	5.7567	.05033	.02906	5.6316	5.8817	5.71	5.81
	Total	18	3.8478	1.85857	.43807	2.9235	4.7720	.75	5.88
	1.00	3	5.8100	.06083	.03512	5.6589	5.9611	5.77	5.88
	2.00	3	6.6100	.26851	.15503	5.9430	7.2770	6.32	6.85
	3.00	3	4.9233	.06658	.03844	4.7579	5.0887	4.88	5.00
RBC1	4.00	3	6.7267	.05859	.03383	6.5811	6.8722	6.66	6.77
	5.00	3	4.8333	.15275	.08819	4.4539	5.2128	4.70	5.00
	6.00	3	7.4500	.05000	.02887	7.3258	7.5742	7.40	7.50
	Total	18	6.0589	.99510	.23455	5.5640	6.5537	4.70	7.50
	1.00	3	5.1833	.90185	.52068	2.9430	7.4237	4.25	6.05
	2.00	3	6.9633	.06351	.03667	6.8056	7.1211	6.89	7.00
	3.00	3	5.3100	.49427	.28537	4.0822	6.5378	4.88	5.85
RBC2	4.00	3	6.9600	.06083	.03512	6.8089	7.1111	6.89	7.00
	5.00	3	3.8667	.11547	.06667	3.5798	4.1535	3.80	4.00
	6.00	3	7.7667	.15275	.08819	7.3872	8.1461	7.60	7.90
	Total	18	6.0083	1.41831	.33430	5.3030	6.7136	3.80	7.90
	1.00	3	6.9333	.05774	.03333	6.7899	7.0768	6.90	7.00
	2.00	3	7.5333	.05774	.03333	7.3899	7.6768	7.50	7.60
RBC3	3.00	3	6.4133	.45446	.26238	5.2844	7.5423	6.00	6.90
	4.00	3	7.4667	.05774	.03333	7.3232	7.6101	7.40	7.50

	5.00	3	3.6000	.17321	.10000	3.1697	4.0303	3.50	3.80
	6.00	3	7.6333	.05774	.03333	7.4899	7.7768	7.60	7.70
	Total	18	6.5967	1.45536	.34303	5.8729	7.3204	3.50	7.70
	1.00	3	11.6267	.18556	.10713	11.1657	12.0876	11.45	11.82
	2.00	3	13.0333	.45092	.26034	11.9132	14.1535	12.60	13.50
	3.00	3	10.9967	.58969	.34046	9.5318	12.4615	10.33	11.45
HB1	4.00	3	12.5933	.35726	.20626	11.7059	13.4808	12.33	13.00
	5.00	3	10.6067	.60252	.34787	9.1099	12.1034	10.21	11.30
	6.00	3	14.1333	.32146	.18559	13.3348	14.9319	13.90	14.50
	Total	18	12.1650	1.30709	.30808	11.5150	12.8150	10.21	14.50
	1.00	3	11.8033	.30665	.17704	11.0416	12.5651	11.45	12.00
	2.00	3	13.9333	.05774	.03333	13.7899	14.0768	13.90	14.00
	3.00	3	10.9900	.69656	.40216	9.2596	12.7204	10.21	11.55
HB2	4.00	3	13.4900	.14177	.08185	13.1378	13.8422	13.33	13.60
	5.00	3	10.0667	.51316	.29627	8.7919	11.3414	9.50	10.50
	6.00	3	14.1667	.28868	.16667	13.4496	14.8838	14.00	14.50
	Total	18	12.4083	1.63138	.38452	11.5971	13.2196	9.50	14.50
	1.00	3	13.4667	.80829	.46667	11.4588	15.4746	13.00	14.40
	2.00	3	14.1000	.26458	.15275	13.4428	14.7572	13.90	14.40
	3.00	3	13.2667	.55076	.31798	11.8985	14.6348	12.90	13.90
HB3	4.00	3	14.1333	.32146	.18559	13.3348	14.9319	13.90	14.50
	5.00	3	8.8333	.76376	.44096	6.9360	10.7306	8.00	9.50
	6.00	3	14.3333	.28868	.16667	13.6162	15.0504	14.00	14.50
	Total	18	13.0222	2.01977	.47606	12.0178	14.0266	8.00	14.50
	1.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00	35.00
	2.00	3	37.3333	.57735	.33333	35.8991	38.7676	37.00	38.00
	3.00	3	33.0000	1.00000	.57735	30.5159	35.4841	32.00	34.00
PCV1	4.00	3	36.3333	1.52753	.88192	32.5388	40.1279	35.00	38.00
	5.00	3	28.3333	1.52753	.88192	24.5388	32.1279	27.00	30.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00	42.00
	Total	18	35.0556	4.13695	.97509	32.9983	37.1128	27.00	42.00
	1.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00	35.00
	2.00	3	38.6667	.57735	.33333	37.2324	40.1009	38.00	39.00
	3.00	3	32.0000	.00000	.00000	32.0000	32.0000	32.00	32.00
PCV2	4.00	3	38.3333	.57735	.33333	36.8991	39.7676	38.00	39.00
	5.00	3	27.6667	.57735	.33333	26.2324	29.1009	27.00	28.00
	6.00	3	41.3333	1.52753	.88192	37.5388	45.1279	40.00	43.00
	Total	18	35.3889	4.77911	1.12645	33.0123	37.7655	27.00	43.00
	1.00	3	36.0000	1.00000	.57735	33.5159	38.4841	35.00	37.00
	2.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00	41.00
	3.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00	35.00
PCV3	4.00	3	39.3333	.57735	.33333	37.8991	40.7676	39.00	40.00
	5.00	3	24.3333	.57735	.33333	22.8991	25.7676	24.00	25.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00	42.00
	Total	18	35.9444	5.92574	1.39671	32.9976	38.8912	24.00	42.00
	1.00	3	58.6667	1.52753	.88192	54.8721	62.4612	57.00	60.00
	2.00	3	56.0000	1.73205	1.00000	51.6973	60.3027	55.00	58.00
	3.00	3	67.0000	3.00000	1.73205	59.5476	74.4524	64.00	70.00
MCV1	4.00	3	54.0000	2.00000	1.15470	49.0317	58.9683	52.00	56.00
	5.00	3	58.3333	3.21455	1.85592	50.3479	66.3187	56.00	62.00
	6.00	3	54.6667	1.52753	.88192	50.8721	58.4612	53.00	56.00
	Total	18	58.1111	4.84936	1.14301	55.6996	60.5226	52.00	70.00
	1.00	3	67.3333	12.05543	6.96020	37.3860	97.2807	56.00	80.00
	2.00	3	55.3333	1.15470	.66667	52.4649	58.2018	54.00	56.00
MCV2	3.00	3	61.3333	5.68624	3.28295	47.2079	75.4587	55.00	66.00
	4.00	3	55.0000	1.00000	.57735	52.5159	57.4841	54.00	56.00

	5.00	3	71.3333	1.52753	.88192	67.5388	75.1279	70.00	73.00
	6.00	3	53.3333	1.15470	.66667	50.4649	56.2018	52.00	54.00
	Total	18	60.6111	8.35350	1.96894	56.4570	64.7652	52.00	80.00
	1.00	3	51.9333	1.15902	.66916	49.0542	54.8125	50.70	53.00
	2.00	3	54.5333	1.00167	.57831	52.0451	57.0216	53.50	55.50
	3.00	3	53.4667	2.54231	1.46780	47.1512	59.7821	50.70	55.70
MCV3	4.00	3	52.5667	.51316	.29627	51.2919	53.8414	52.00	53.00
	5.00	3	67.4000	1.12694	.65064	64.6005	70.1995	66.10	68.10
	6.00	3	54.9000	1.76918	1.02144	50.5051	59.2949	53.00	56.50
	Total	18	55.8000	5.58580	1.31659	53.0222	58.5778	50.70	68.10
	1.00	3	33.8667	.20817	.12019	33.3496	34.3838	33.70	34.10
	2.00	3	34.9000	.72111	.41633	33.1087	36.6913	34.10	35.50
	3.00	3	33.4667	2.65581	1.53333	26.8693	40.0641	30.40	35.00
MCHC1	4.00	3	34.6667	.50332	.29059	33.4163	35.9170	34.20	35.20
	5.00	3	34.2667	6.38462	3.68616	18.4064	50.1269	26.90	38.20
	6.00	3	34.5000	1.63707	.94516	30.4333	38.5667	33.10	36.30
	Total	18	34.2778	2.50730	.59098	33.0309	35.5246	26.90	38.20
	1.00	3	34.3667	.86217	.49777	32.2249	36.5084	33.60	35.30
	2.00	3	36.0333	.51316	.29627	34.7586	37.3081	35.60	36.60
	3.00	3	34.0333	2.68390	1.54955	27.3661	40.7005	31.00	36.10
MCHC2	4.00	3	35.2000	.36056	.20817	34.3043	36.0957	34.90	35.60
	5.00	3	36.4000	2.50000	1.44338	30.1897	42.6103	33.90	38.90
	6.00	3	34.4333	1.62583	.93868	30.3945	38.4721	33.00	36.20
	Total	18	35.0778	1.68961	.39824	34.2376	35.9180	31.00	38.90
	1.00	3	37.0000	3.46410	2.00000	28.3947	45.6053	35.00	41.00
	2.00	3	34.3333	1.15470	.66667	31.4649	37.2018	33.00	35.00
	3.00	3	38.0000	1.73205	1.00000	33.6973	42.3027	37.00	40.00
MCHC3	4.00	3	35.3333	.57735	.33333	33.8991	36.7676	35.00	36.00
	5.00	3	36.0000	2.64575	1.52753	29.4276	42.5724	33.00	38.00
	6.00	3	35.0000	1.00000	.57735	32.5159	37.4841	34.00	36.00
	Total	18	35.9444	2.12747	.50145	34.8865	37.0024	33.00	41.00
	1.00	3	19.9667	.55076	.31798	18.5985	21.3348	19.40	20.50
	2.00	3	19.7333	.85049	.49103	17.6206	21.8461	18.90	20.60
	3.00	3	22.3000	1.15326	.66583	19.4352	25.1648	21.10	23.40
MCH1	4.00	3	18.7333	.45092	.26034	17.6132	19.8535	18.30	19.20
	5.00	3	21.9333	1.55027	.89505	18.0823	25.7844	20.40	23.50
	6.00	3	18.6333	.65064	.37565	17.0171	20.2496	18.00	19.30
	Total	18	20.2167	1.67411	.39459	19.3842	21.0492	18.00	23.50
	1.00	3	23.4000	4.65725	2.68887	11.8307	34.9693	18.90	28.20
	2.00	3	20.0333	.23094	.13333	19.4596	20.6070	19.90	20.30
	3.00	3	20.7333	1.87705	1.08372	16.0705	25.3962	19.60	22.90
MCH2	4.00	3	19.3667	.25166	.14530	18.7415	19.9918	19.10	19.60
	5.00	3	25.8333	1.04083	.60093	23.2478	28.4189	25.00	27.00
	6.00	3	18.2000	.43589	.25166	17.1172	19.2828	17.70	18.50
	Total	18	21.2611	3.19819	.75382	19.6707	22.8515	17.70	28.20
	1.00	3	19.3667	1.25033	.72188	16.2607	22.4727	18.50	20.80
	2.00	3	18.7000	.45826	.26458	17.5616	19.8384	18.30	19.20
	3.00	3	20.7333	1.68622	.97354	16.5445	24.9221	18.80	21.90
MCH3	4.00	3	18.9000	.36056	.20817	18.0043	19.7957	18.60	19.30
	5.00	3	24.0000	1.73205	1.00000	19.6973	28.3027	22.00	25.00
	6.00	3	18.7667	.57735	.33333	17.3324	20.2009	18.10	19.10
	Total	18	20.0778	2.17108	.51173	18.9981	21.1574	18.10	25.00
	1.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10	7.20
WBC1	2.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00	7.20
	3.00	3	7.0333	.05774	.03333	6.8899	7.1768	7.00	7.10
	4.00	3	6.9333	.05774	.03333	6.7899	7.0768	6.90	7.00

	5.00	3	7.0000	.10000	.05774	6.7516	7.2484	6.90	7.10
	6.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00	7.20
	Total	18	7.0500	.09852	.02322	7.0010	7.0990	6.90	7.20
	1.00	3	7.1667	.05774	.03333	7.0232	7.3101	7.10	7.20
	2.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10	7.20
	3.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10	7.20
WBC2	4.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00	7.20
	5.00	3	6.9000	.10000	.05774	6.6516	7.1484	6.80	7.00
	6.00	3	7.2667	.11547	.06667	6.9798	7.5535	7.20	7.40
	Total	18	7.1167	.13394	.03157	7.0501	7.1833	6.80	7.40
	1.00	3	7.2000	.10000	.05774	6.9516	7.4484	7.10	7.30
	2.00	3	7.2667	.15275	.08819	6.8872	7.6461	7.10	7.40
	3.00	3	6.9667	.20817	.12019	6.4496	7.4838	6.80	7.20
WBC3	4.00	3	6.9667	.11547	.06667	6.6798	7.2535	6.90	7.10
	5.00	3	6.9000	.10000	.05774	6.6516	7.1484	6.80	7.00
	6.00	3	7.2667	.15275	.08819	6.8872	7.6461	7.10	7.40
	Total	18	7.0944	.19844	.04677	6.9958	7.1931	6.80	7.40
	1.00	3	57.6667	1.15470	.66667	54.7982	60.5351	57.00	59.00
	2.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00	59.00
	3.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00	60.00
LYMPH1	4.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00	59.00
	5.00	3	56.0000	1.00000	.57735	53.5159	58.4841	55.00	57.00
	6.00	3	58.0000	1.00000	.57735	55.5159	60.4841	57.00	59.00
	Total	18	57.8889	1.23140	.29024	57.2765	58.5012	55.00	60.00
	1.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00	60.00
	2.00	3	59.3333	1.15470	.66667	56.4649	62.2018	58.00	60.00
	3.00	3	60.0000	1.00000	.57735	57.5159	62.4841	59.00	61.00
LYMPH2	4.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00	60.00
	5.00	3	61.3333	2.08167	1.20185	56.1622	66.5045	59.00	63.00
	6.00	3	58.0000	1.00000	.57735	55.5159	60.4841	57.00	59.00
	Total	18	59.4444	1.50381	.35445	58.6966	60.1923	57.00	63.00
	1.00	3	58.3333	1.15470	.66667	55.4649	61.2018	57.00	59.00
	2.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00	60.00
	3.00	3	57.3333	.57735	.33333	55.8991	58.7676	57.00	58.00
LYMPH3	4.00	3	57.6667	.57735	.33333	56.2324	59.1009	57.00	58.00
	5.00	3	57.0000	1.00000	.57735	54.5159	59.4841	56.00	58.00
	6.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00	59.00
	Total	18	57.9444	.99836	.23532	57.4480	58.4409	56.00	60.00
	1.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00	41.00
	2.00	3	41.3333	1.15470	.66667	38.4649	44.2018	40.00	42.00
	3.00	3	40.0000	1.00000	.57735	37.5159	42.4841	39.00	41.00
NEUT1	4.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00	41.00
	5.00	3	43.0000	1.00000	.57735	40.5159	45.4841	42.00	44.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00	42.00
	Total	18	41.1111	1.23140	.29024	40.4988	41.7235	39.00	44.00
	1.00	3	39.3333	.57735	.33333	37.8991	40.7676	39.00	40.00
	2.00	3	39.6667	.57735	.33333	38.2324	41.1009	39.00	40.00
	3.00	3	39.0000	1.00000	.57735	36.5159	41.4841	38.00	40.00
NEUT2	4.00	3	39.6667	.57735	.33333	38.2324	41.1009	39.00	40.00
	5.00	3	37.3333	2.51661	1.45297	31.0817	43.5849	35.00	40.00
	6.00	3	40.6667	1.15470	.66667	37.7982	43.5351	40.00	42.00
	Total	18	39.2778	1.48742	.35059	38.5381	40.0175	35.00	42.00
	1.00	3	40.0000	1.00000	.57735	37.5159	42.4841	39.00	41.00
	2.00	3	40.6667	1.15470	.66667	37.7982	43.5351	40.00	42.00
NEUT3	3.00	3	41.3333	.57735	.33333	39.8991	42.7676	41.00	42.00
	4.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00	42.00

	5.00	3	41.6667	.57735	.33333	40.2324	43.1009	41.00	42.00
	6.00	3	40.3333	.57735	.33333	38.8991	41.7676	40.00	41.00
	Total	18	40.8333	.92355	.21768	40.3741	41.2926	39.00	42.00
	1.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	2.00	3	.0667	.05774	.03333	-.0768	.2101	.00	.10
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
BASO1	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	5.00	3	.0667	.11547	.06667	-.2202	.3535	.00	.20
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0389	.06077	.01432	.0087	.0691	.00	.20
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
BASO2	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	5.00	3	.0667	.05774	.03333	-.0768	.2101	.00	.10
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0278	.04609	.01086	.0049	.0507	.00	.10
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
BASO3	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	5.00	3	.0333	.05774	.03333	-.1101	.1768	.00	.10
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0222	.04278	.01008	.0009	.0435	.00	.10
	1.00	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	2.00	3	.7333	.11547	.06667	.4465	1.0202	.60	.80
	3.00	3	.7000	.10000	.05774	.4516	.9484	.60	.80
EOS1	4.00	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	5.00	3	.8000	.10000	.05774	.5516	1.0484	.70	.90
	6.00	3	.7667	.05774	.03333	.6232	.9101	.70	.80
	Total	18	.7444	.07838	.01847	.7055	.7834	.60	.90
	1.00	3	.7000	.10000	.05774	.4516	.9484	.60	.80
	2.00	3	.9667	.37859	.21858	.0262	1.9071	.70	1.40
	3.00	3	.7000	.10000	.05774	.4516	.9484	.60	.80
EOS2	4.00	3	.9000	.26458	.15275	.2428	1.5572	.70	1.20
	5.00	3	.8667	.11547	.06667	.5798	1.1535	.80	1.00
	6.00	3	1.0000	.43589	.25166	-.0828	2.0828	.70	1.50
	Total	18	.8556	.25718	.06062	.7277	.9835	.60	1.50
	1.00	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	2.00	3	.6667	.11547	.06667	.3798	.9535	.60	.80
	3.00	3	.6000	.10000	.05774	.3516	.8484	.50	.70
EOS3	4.00	3	1.0333	.37859	.21858	.0929	1.9738	.60	1.30
	5.00	3	.5667	.05774	.03333	.4232	.7101	.50	.60
	6.00	3	1.0000	.34641	.20000	.1395	1.8605	.80	1.40
	Total	18	.7667	.26568	.06262	.6345	.8988	.50	1.40
	1.00	3	.2333	.05774	.03333	.0899	.3768	.20	.30
	2.00	3	.2000	.10000	.05774	-.0484	.4484	.10	.30
	3.00	3	.2667	.05774	.03333	.1232	.4101	.20	.30
MONO1	4.00	3	.2333	.05774	.03333	.0899	.3768	.20	.30
	5.00	3	.1333	.05774	.03333	-.0101	.2768	.10	.20
	6.00	3	.2333	.05774	.03333	.0899	.3768	.20	.30
	Total	18	.2167	.07071	.01667	.1815	.2518	.10	.30
	1.00	3	.3000	.10000	.05774	.0516	.5484	.20	.40
	2.00	3	.3333	.23094	.13333	-.2404	.9070	.20	.60
MONO2	3.00	3	.2667	.05774	.03333	.1232	.4101	.20	.30
	4.00	3	.4000	.34641	.20000	-.4605	1.2605	.20	.80

	5.00	3	.4000	.43589	.25166	-.6828	1.4828	.10	.90
	6.00	3	.3333	.15275	.08819	-.0461	.7128	.20	.50
	Total	18	.3389	.22265	.05248	.2282	.4496	.10	.90
	1.00	3	.2667	.05774	.03333	.1232	.4101	.20	.30
	2.00	3	.3000	.10000	.05774	.0516	.5484	.20	.40
	3.00	3	.3667	.05774	.03333	.2232	.5101	.30	.40
MONO3	4.00	3	.6000	.17321	.10000	.1697	1.0303	.40	.70
	5.00	3	.3667	.05774	.03333	.2232	.5101	.30	.40
	6.00	3	.3667	.20817	.12019	-.1504	.8838	.20	.60
	Total	18	.3778	.15168	.03575	.3023	.4532	.20	.70

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	21.947	5	4.389	60.311	.000
MDA1	Within Groups	.873	12	.073		
	Total	22.820	17			
	Between Groups	54.187	5	10.837	368.060	.000
MDA2	Within Groups	.353	12	.029		
	Total	54.540	17			
	Between Groups	100.007	5	20.001	75.162	.000
MDA3	Within Groups	3.193	12	.266		
	Total	103.200	17			
	Between Groups	.180	5	.036	236.297	.000
SOD1	Within Groups	.002	12	.000		
	Total	.182	17			
	Between Groups	.249	5	.050	249.111	.000
SOD2	Within Groups	.002	12	.000		
	Total	.252	17			
	Between Groups	.565	5	.113	149.659	.000
SOD3	Within Groups	.009	12	.001		
	Total	.574	17			
	Between Groups	2849.611	5	569.922	81.417	.000
GSH1	Within Groups	84.000	12	7.000		
	Total	2933.611	17			
	Between Groups	6342.444	5	1268.489	292.728	.000
GSH2	Within Groups	52.000	12	4.333		
	Total	6394.444	17			
	Between Groups	10293.778	5	2058.756	686.252	.000
GSH3	Within Groups	36.000	12	3.000		
	Total	10329.778	17			
	Between Groups	30.209	5	6.042	215.562	.000
CAT1	Within Groups	.336	12	.028		
	Total	30.546	17			
	Between Groups	53.000	5	10.600	99.030	.000
CAT2	Within Groups	1.284	12	.107		
	Total	54.285	17			
	Between Groups	56.942	5	11.388	76.715	.000
CAT3	Within Groups	1.781	12	.148		
	Total	58.723	17			
	Between Groups	16.615	5	3.323	182.080	.000
RBC1	Within Groups	.219	12	.018		
	Total	16.834	17			

	Between Groups	31.993	5	6.399	34.837	.000
RBC2	Within Groups	2.204	12	.184		
	Total	34.197	17			
	Between Groups	35.508	5	7.102	170.528	.000
RBC3	Within Groups	.500	12	.042		
	Total	36.007	17			
	Between Groups	26.685	5	5.337	27.149	.000
HB1	Within Groups	2.359	12	.197		
	Total	29.044	17			
	Between Groups	43.345	5	8.669	54.791	.000
HB2	Within Groups	1.899	12	.158		
	Total	45.244	17			
	Between Groups	65.758	5	13.152	43.920	.000
HB3	Within Groups	3.593	12	.299		
	Total	69.351	17			
	Between Groups	276.278	5	55.256	45.209	.000
PCV1	Within Groups	14.667	12	1.222		
	Total	290.944	17			
	Between Groups	380.944	5	76.189	124.673	.000
PCV2	Within Groups	7.333	12	.611		
	Total	388.278	17			
	Between Groups	590.278	5	118.056	212.500	.000
PCV3	Within Groups	6.667	12	.556		
	Total	596.944	17			
	Between Groups	337.778	5	67.556	13.075	.000
MCV1	Within Groups	62.000	12	5.167		
	Total	399.778	17			
	Between Groups	818.944	5	163.789	5.351	.008
MCV2	Within Groups	367.333	12	30.611		
	Total	1186.278	17			
	Between Groups	503.473	5	100.695	44.842	.000
MCV3	Within Groups	26.947	12	2.246		
	Total	530.420	17			
	Between Groups	4.244	5	.849	.099	.990
MCHC1	Within Groups	102.627	12	8.552		
	Total	106.871	17			
	Between Groups	14.064	5	2.813	.979	.469
MCHC2	Within Groups	34.467	12	2.872		
	Total	48.531	17			
	Between Groups	27.611	5	5.522	1.343	.311
MCHC3	Within Groups	49.333	12	4.111		
	Total	76.944	17			
	Between Groups	36.872	5	7.374	8.214	.001
MCH1	Within Groups	10.773	12	.898		
	Total	47.645	17			
	Between Groups	120.676	5	24.135	5.443	.008
MCH2	Within Groups	53.207	12	4.434		
	Total	173.883	17			
	Between Groups	63.971	5	12.794	9.501	.001
MCH3	Within Groups	16.160	12	1.347		
	Total	80.131	17			
	Between Groups	.085	5	.017	2.550	.085
WBC1	Within Groups	.080	12	.007		
	Total	.165	17			
WBC2	Between Groups	.218	5	.044	6.046	.005
	Within Groups	.087	12	.007		

	Total	.305	17			
	Between Groups	.423	5	.085	4.114	.021
WBC3	Within Groups	.247	12	.021		
	Total	.669	17			
	Between Groups	15.778	5	3.156	3.787	.027
LYMPH1	Within Groups	10.000	12	.833		
	Total	25.778	17			
	Between Groups	19.111	5	3.822	2.372	.102
LYMPH2	Within Groups	19.333	12	1.611		
	Total	38.444	17			
	Between Groups	8.278	5	1.656	2.292	.111
LYMPH3	Within Groups	8.667	12	.722		
	Total	16.944	17			
	Between Groups	15.778	5	3.156	3.787	.027
NEUT1	Within Groups	10.000	12	.833		
	Total	25.778	17			
	Between Groups	18.278	5	3.656	2.269	.114
NEUT2	Within Groups	19.333	12	1.611		
	Total	37.611	17			
	Between Groups	5.833	5	1.167	1.615	.230
NEUT3	Within Groups	8.667	12	.722		
	Total	14.500	17			
	Between Groups	.009	5	.002	.425	.823
BASO1	Within Groups	.053	12	.004		
	Total	.063	17			
	Between Groups	.009	5	.002	.850	.541
BASO2	Within Groups	.027	12	.002		
	Total	.036	17			
	Between Groups	.004	5	.001	.400	.840
BASO3	Within Groups	.027	12	.002		
	Total	.031	17			
	Between Groups	.018	5	.004	.492	.776
EOS1	Within Groups	.087	12	.007		
	Total	.104	17			
	Between Groups	.251	5	.050	.690	.640
EOS2	Within Groups	.873	12	.073		
	Total	1.124	17			
	Between Groups	.613	5	.123	2.509	.089
EOS3	Within Groups	.587	12	.049		
	Total	1.200	17			
	Between Groups	.032	5	.006	1.425	.284
MONO1	Within Groups	.053	12	.004		
	Total	.085	17			
	Between Groups	.043	5	.009	.128	.983
MONO2	Within Groups	.800	12	.067		
	Total	.843	17			
	Between Groups	.204	5	.041	2.629	.079
MONO3	Within Groups	.187	12	.016		
	Total	.391	17			

Appendix 9: Weekly weight (g) changes associated with treatment of alloxan-induced diabetic rats with *Cussonia arborea* extract

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	1.00	3	100.4667	.55076	.31798	99.0985	101.8348	100.10	101.10
	2.00	3	101.3333	2.30940	1.33333	95.5965	107.0702	100.00	104.00
	3.00	3	102.0000	1.00000	.57735	99.5159	104.4841	101.00	103.00
WKZERO	4.00	3	101.6667	2.88675	1.66667	94.4956	108.8378	100.00	105.00
	5.00	3	102.0000	1.00000	.57735	99.5159	104.4841	101.00	103.00
	6.00	3	101.6667	2.88675	1.66667	94.4956	108.8378	100.00	105.00
	Total	18	101.5222	1.77484	.41833	100.6396	102.4048	100.00	105.00
	1.00	3	102.0000	1.00000	.57735	99.5159	104.4841	101.00	103.00
	2.00	3	101.3333	1.52753	.88192	97.5388	105.1279	100.00	103.00
	3.00	3	102.0000	2.00000	1.15470	97.0317	106.9683	100.00	104.00
WKONE	4.00	3	102.3333	3.21455	1.85592	94.3479	110.3187	100.00	106.00
	5.00	3	98.0000	2.64575	1.52753	91.4276	104.5724	95.00	100.00
	6.00	3	106.3333	1.52753	.88192	102.5388	110.1279	105.00	108.00
	Total	18	102.0000	3.06786	.72310	100.4744	103.5256	95.00	108.00
	1.00	3	102.3333	.57735	.33333	100.8991	103.7676	102.00	103.00
	2.00	3	104.0000	1.00000	.57735	101.5159	106.4841	103.00	105.00
	3.00	3	101.6667	1.15470	.66667	98.7982	104.5351	101.00	103.00
WKTWO	4.00	3	107.0000	2.00000	1.15470	102.0317	111.9683	105.00	109.00
	5.00	3	92.0000	2.00000	1.15470	87.0317	96.9683	90.00	94.00
	6.00	3	117.0000	6.08276	3.51188	101.8896	132.1104	110.00	121.00
	Total	18	104.0000	7.99264	1.88388	100.0254	107.9746	90.00	121.00
	1.00	3	104.6667	1.52753	.88192	100.8721	108.4612	103.00	106.00
	2.00	3	110.6667	2.08167	1.20185	105.4955	115.8378	109.00	113.00
	3.00	3	104.0000	1.00000	.57735	101.5159	106.4841	103.00	105.00
WKTHREE	4.00	3	112.0000	2.64575	1.52753	105.4276	118.5724	110.00	115.00
	5.00	3	90.0000	1.00000	.57735	87.5159	92.4841	89.00	91.00
	6.00	3	128.3333	4.72582	2.72845	116.5938	140.0729	123.00	132.00
	Total	18	108.2778	11.97451	2.82242	102.3230	114.2326	89.00	132.00
	1.00	3	106.0000	4.35890	2.51661	95.1719	116.8281	101.00	109.00
	2.00	3	119.0000	2.64575	1.52753	112.4276	125.5724	116.00	121.00
	3.00	3	106.3333	4.04145	2.33333	96.2938	116.3729	102.00	110.00
WKFOUR	4.00	3	119.0000	4.35890	2.51661	108.1719	129.8281	114.00	122.00
	5.00	3	87.0000	1.00000	.57735	84.5159	89.4841	86.00	88.00
	6.00	3	137.6667	2.08167	1.20185	132.4955	142.8378	136.00	140.00
	Total	18	112.5000	16.23087	3.82565	104.4286	120.5714	86.00	140.00
	1.00	3	108.3333	2.08167	1.20185	103.1622	113.5045	106.00	110.00
	2.00	3	124.3333	5.85947	3.38296	109.7776	138.8891	120.00	131.00
	3.00	3	109.0000	2.00000	1.15470	104.0317	113.9683	107.00	111.00
WKFIVE	4.00	3	122.3333	3.21455	1.85592	114.3479	130.3187	120.00	126.00
	5.00	3	85.6667	1.52753	.88192	81.8721	89.4612	84.00	87.00
	6.00	3	153.3333	4.16333	2.40370	142.9910	163.6756	150.00	158.00
	Total	18	117.1667	21.30244	5.02103	106.5732	127.7601	84.00	158.00
	1.00	3	110.3333	2.30940	1.33333	104.5965	116.0702	109.00	113.00
WKSIX	2.00	3	128.6667	2.08167	1.20185	123.4955	133.8378	127.00	131.00
	3.00	3	111.6667	2.08167	1.20185	106.4955	116.8378	110.00	114.00
	4.00	3	127.6667	1.15470	.66667	124.7982	130.5351	127.00	129.00

	5.00	3	84.0000	1.00000	.57735	81.5159	86.4841	83.00	85.00
	6.00	3	173.6667	1.52753	.88192	169.8721	177.4612	172.00	175.00
	Total	18	122.6667	28.00000	6.59966	108.7426	136.5907	83.00	175.00
	1.00	3	116.6667	2.51661	1.45297	110.4151	122.9183	114.00	119.00
	2.00	3	133.0000	1.00000	.57735	130.5159	135.4841	132.00	134.00
	3.00	3	118.0000	2.00000	1.15470	113.0317	122.9683	116.00	120.00
WKSEVEN	4.00	3	132.6667	2.51661	1.45297	126.4151	138.9183	130.00	135.00
	5.00	3	80.3333	.57735	.33333	78.8991	81.7676	80.00	81.00
	6.00	3	182.6667	3.05505	1.76383	175.0775	190.2558	180.00	186.00
	Total	18	127.2222	31.29660	7.37668	111.6588	142.7857	80.00	186.00
	1.00	3	121.3333	1.15470	.66667	118.4649	124.2018	120.00	122.00
	2.00	3	135.3333	2.51661	1.45297	129.0817	141.5849	133.00	138.00
	3.00	3	122.0000	1.00000	.57735	119.5159	124.4841	121.00	123.00
WKEIGHT	4.00	3	135.6667	1.52753	.88192	131.8721	139.4612	134.00	137.00
	5.00	3	80.6667	.57735	.33333	79.2324	82.1009	80.00	81.00
	6.00	3	193.3333	3.05505	1.76383	185.7442	200.9225	190.00	196.00
	Total	18	131.3889	34.24847	8.07244	114.3575	148.4203	80.00	196.00
	1.00	3	123.6667	3.21455	1.85592	115.6813	131.6521	120.00	126.00
	2.00	3	144.0000	2.00000	1.15470	139.0317	148.9683	142.00	146.00
	3.00	3	125.3333	1.52753	.88192	121.5388	129.1279	124.00	127.00
WKNINE	4.00	3	141.3333	1.52753	.88192	137.5388	145.1279	140.00	143.00
	5.00	3	82.0000	1.00000	.57735	79.5159	84.4841	81.00	83.00
	6.00	3	200.0000	1.00000	.57735	197.5159	202.4841	199.00	201.00
	Total	18	136.0556	36.09868	8.50854	118.1041	154.0070	81.00	201.00
	1.00	3	127.6667	1.52753	.88192	123.8721	131.4612	126.00	129.00
	2.00	3	144.6667	.57735	.33333	143.2324	146.1009	144.00	145.00
	3.00	3	127.0000	1.00000	.57735	124.5159	129.4841	126.00	128.00
WKTEN	4.00	3	146.0000	1.00000	.57735	143.5159	148.4841	145.00	147.00
	5.00	3	80.6667	.57735	.33333	79.2324	82.1009	80.00	81.00
	6.00	3	212.3333	2.08167	1.20185	207.1622	217.5045	210.00	214.00
	Total	18	139.7222	40.15529	9.46469	119.7535	159.6910	80.00	214.00
	1.00	3	129.6667	1.52753	.88192	125.8721	133.4612	128.00	131.00
	2.00	3	153.6667	2.08167	1.20185	148.4955	158.8378	152.00	156.00
	3.00	3	128.3333	.57735	.33333	126.8991	129.7676	128.00	129.00
WKELEVEN	4.00	3	152.6667	2.51661	1.45297	146.4151	158.9183	150.00	155.00
	5.00	3	78.3333	1.15470	.66667	75.4649	81.2018	77.00	79.00
	6.00	3	244.6667	5.03322	2.90593	232.1634	257.1699	240.00	250.00
	Total	18	147.8889	51.45174	12.12729	122.3025	173.4752	77.00	250.00
	1.00	3	134.3333	2.08167	1.20185	129.1622	139.5045	132.00	136.00
	2.00	3	175.0000	4.35890	2.51661	164.1719	185.8281	170.00	178.00
	3.00	3	132.0000	2.64575	1.52753	125.4276	138.5724	130.00	135.00
WKTWELVE	4.00	3	161.3333	1.52753	.88192	157.5388	165.1279	160.00	163.00
	5.00	3	77.3333	.57735	.33333	75.8991	78.7676	77.00	78.00
	6.00	3	249.6667	8.96289	5.17472	227.4016	271.9317	244.00	260.00
	Total	18	154.9444	53.90457	12.70543	128.1383	181.7506	77.00	260.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WKZERO	Between Groups	4.944	5	.989	.244	.935
	Within Groups	48.607	12	4.051		
	Total	53.551	17			
WKONE	Between Groups	106.000	5	21.200	4.711	.013
	Within Groups	54.000	12	4.500		
	Total	160.000	17			
WKTWO	Between Groups	990.667	5	198.133	24.940	.000
	Within Groups	95.333	12	7.944		
	Total	1086.000	17			
WKTHREE	Between Groups	2361.611	5	472.322	74.577	.000
	Within Groups	76.000	12	6.333		
	Total	2437.611	17			
WKFOUR	Between Groups	4345.167	5	869.033	78.213	.000
	Within Groups	133.333	12	11.111		
	Total	4478.500	17			
WKFIVE	Between Groups	7569.167	5	1513.833	124.995	.000
	Within Groups	145.333	12	12.111		
	Total	7714.500	17			
WKSIX	Between Groups	13290.667	5	2658.133	854.400	.000
	Within Groups	37.333	12	3.111		
	Total	13328.000	17			
WKSEVEN	Between Groups	16596.444	5	3319.289	728.624	.000
	Within Groups	54.667	12	4.556		
	Total	16651.111	17			
WKEIGHT	Between Groups	19898.944	5	3979.789	1155.423	.000
	Within Groups	41.333	12	3.444		
	Total	19940.278	17			
WKNINE	Between Groups	22110.944	5	4422.189	1263.483	.000
	Within Groups	42.000	12	3.500		
	Total	22152.944	17			
WKTEN	Between Groups	27392.944	5	5478.589	3521.950	.000
	Within Groups	18.667	12	1.556		
	Total	27411.611	17			
WKELEVEN	Between Groups	44923.778	5	8984.756	1347.713	.000
	Within Groups	80.000	12	6.667		
	Total	45003.778	17			
WKTWELVE	Between Groups	49170.278	5	9834.056	520.626	.000
	Within Groups	226.667	12	18.889		
	Total	49396.944	17			

Appendix 1: Effects of **acute administration** of *Cussonia arborea* root bark extract on **Fasting blood glucose (mg/dl)** levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minim
						Lower Bound	Upper Bound	
PRIND	1.00	3	73.3333	.57735	.33333	71.8991	74.7676	7
	2.00	3	73.3333	2.51661	1.45297	67.0817	79.5849	7
	3.00	3	74.0000	1.00000	.57735	71.5159	76.4841	7
	4.00	3	73.3333	.57735	.33333	71.8991	74.7676	7
	5.00	3	75.3333	1.52753	.88192	71.5388	79.1279	7
	6.00	3	75.3333	.57735	.33333	73.8991	76.7676	7
	Total	18	74.1111	1.45072	.34194	73.3897	74.8325	7
ZEROHR	1.00	3	326.3333	4.04145	2.33333	316.2938	336.3729	32
	2.00	3	333.3333	50.33223	29.05933	208.3011	458.3655	28
	3.00	3	316.0000	32.14032	18.55622	236.1590	395.8410	29
	4.00	3	328.0000	25.51470	14.73092	264.6180	391.3820	29
	5.00	3	343.3333	40.41452	23.33333	242.9381	443.7286	30
	6.00	3	75.0000	1.00000	.57735	72.5159	77.4841	7
	Total	18	287.0000	101.38337	23.89629	236.5832	337.4168	7
ONEHR	1.00	3	265.6667	5.13160	2.96273	252.9191	278.4143	26
	2.00	3	285.6667	12.42310	7.17248	254.8060	316.5273	27
	3.00	3	301.0000	7.54983	4.35890	282.2452	319.7548	29
	4.00	3	240.0000	45.82576	26.45751	126.1625	353.8375	20
	5.00	3	332.0000	32.74141	18.90326	250.6658	413.3342	29
	6.00	3	74.3333	.57735	.33333	72.8991	75.7676	7
	Total	18	249.7778	88.19201	20.78706	205.9209	293.6346	7
THREEHR	1.00	3	160.3333	2.51661	1.45297	154.0817	166.5849	15
	2.00	3	183.3333	12.22020	7.05534	152.9767	213.6900	17
	3.00	3	278.6667	14.18920	8.19214	243.4187	313.9146	26
	4.00	3	160.0000	10.00000	5.77350	135.1586	184.8414	15
	5.00	3	315.6667	35.69781	20.61014	226.9884	404.3449	27
	6.00	3	75.6667	.57735	.33333	74.2324	77.1009	7
	Total	18	195.6111	83.50705	19.68280	154.0840	237.1382	7
SIXHR	1.00	3	96.6667	3.05505	1.76383	89.0775	104.2558	9
	2.00	3	164.6667	6.65833	3.84419	148.1265	181.2069	15
	3.00	3	231.0000	10.14889	5.85947	205.7888	256.2112	22
	4.00	3	98.6667	1.15470	.66667	95.7982	101.5351	9
	5.00	3	345.3333	33.24655	19.19491	262.7443	427.9224	31
	6.00	3	73.0000	1.00000	.57735	70.5159	75.4841	7
	Total	18	168.2222	98.68879	23.26117	119.1454	217.2990	7
TWNTFORH	1.00	3	91.0000	8.54400	4.93288	69.7755	112.2245	8
	2.00	3	142.6667	11.23981	6.48931	114.7454	170.5879	13
	3.00	3	193.0000	11.26943	6.50641	165.0052	220.9948	18
	4.00	3	98.0000	1.00000	.57735	95.5159	100.4841	9
	5.00	3	320.3333	20.50203	11.83685	269.4035	371.2632	30
	6.00	3	75.0000	1.00000	.57735	72.5159	77.4841	7
	Total	18	153.3333	87.27104	20.56998	109.9345	196.7322	7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PRIND	Between Groups	14.444	5	2.889	1.625	.227
	Within Groups	21.333	12	1.778		
	Total	35.778	17			
ZEROHR	Between Groups	163000.000	5	32600.000	33.333	.000
	Within Groups	11736.000	12	978.000		
	Total	174736.000	17			
ONEHR	Between Groups	125403.111	5	25080.622	44.130	.000
	Within Groups	6820.000	12	568.333		
	Total	132223.111	17			
THREEHR	Between Groups	115084.944	5	23016.989	79.751	.000
	Within Groups	3463.333	12	288.611		
	Total	118548.278	17			
SIXHR	Between Groups	163042.444	5	32608.489	154.746	.000
	Within Groups	2528.667	12	210.722		
	Total	165571.111	17			
TWNTFORH	Between Groups	127978.667	5	25595.733	205.131	.000
	Within Groups	1497.333	12	124.778		
	Total	129476.000	17			

Appendix 2: Effect of *Cussonia arborea* extract on FBG (**mg/dl**) of normoglycaemic rat (**Oral glucose tolerance test**)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Mini
						Lower Bound	Upper Bound	
FBSZEROHR	1.00	3	74.0000	1.00000	.57735	71.5159	76.4841	
	2.00	3	75.0000	1.00000	.57735	72.5159	77.4841	
	3.00	3	76.3333	.57735	1.20333	74.8991	77.7676	
	4.00	3	75.0000	1.00000	.57735	72.5159	77.4841	
	5.00	3	73.6667	.57735	.33333	72.2324	75.1009	
	Total	15	74.8000	1.20712	.31168	74.1315	75.4685	
THRTYMINS	1.00	3	151.0000	3.60555	2.08167	142.0433	159.9567	1
	2.00	3	147.6667	3.21455	1.85592	139.6813	155.6521	1
	3.00	3	152.3333	6.42910	3.71184	136.3626	168.3041	1
	4.00	3	120.6667	2.08167	1.57185	115.4955	125.8378	1
	5.00	3	158.0000	2.00000	1.15470	153.0317	162.9683	1
	Total	15	145.9333	13.90512	3.59029	138.2329	153.6337	1
SIXTYMNS	1.00	3	153.6667	12.66228	7.31057	122.2118	185.1215	1
	2.00	3	142.6667	2.51661	1.45297	136.4151	148.9183	1
	3.00	3	154.0000	5.29150	3.05505	140.8552	167.1448	1
	4.00	3	118.6667	3.21455	1.85592	110.6813	126.6521	1
	5.00	3	161.6667	4.93288	2.84800	149.4127	173.9206	1
	Total	15	146.1333	16.55668	4.27492	136.9645	155.3021	1

ONETWTY	1.00	3	107.3333	1.52753	.88192	103.5388	111.1279	1
	2.00	3	100.0000	1.00000	.57735	97.5159	102.4841	
	3.00	3	105.3333	1.52753	.88192	101.5388	109.1279	1
	4.00	3	89.0000	2.64575	1.52753	82.4276	95.5724	
	5.00	3	111.0000	1.00000	.57735	108.5159	113.4841	1
	Total	15	102.5333	8.03445	2.07449	98.0840	106.9827	
ONEEIGHTY	1.00	3	99.0000	1.00000	.57735	96.5159	101.4841	
	2.00	3	83.0000	6.08276	3.51188	67.8896	98.1104	
	3.00	3	103.0000	2.00000	1.15470	98.0317	107.9683	1
	4.00	3	79.6667	2.51661	1.45297	73.4151	85.9183	
	5.00	3	90.3333	1.52753	.88192	86.5388	94.1279	
	Total	15	91.0000	9.65105	2.49189	85.6554	96.3446	

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FBSZEROHR	Between Groups	13.067	4	3.267	4.455	.025
	Within Groups	7.333	10	.733		
	Total	20.400	14			
THRTYMINS	Between Groups	2560.933	4	640.233	43.852	.000
	Within Groups	146.000	10	14.600		
	Total	2706.933	14			
SIXTYMNS	Between Groups	3379.067	4	844.767	18.418	.000
	Within Groups	458.667	10	45.867		
	Total	3837.733	14			
ONETWTY	Between Groups	876.400	4	219.100	80.159	.000
	Within Groups	27.333	10	2.733		
	Total	903.733	14			
ONEEIGHTY	Between Groups	1202.667	4	300.667	29.671	.000
	Within Groups	101.333	10	10.133		
	Total	1304.000	14			

Appendix 3: Effects of **chronic administration** of *Cussonia arborea* root bark extract on **Fasting blood glucose (mg/dl)** levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
PRIND	1.00	3	78.0000	2.00000	1.15470	73.0317	82.9683	76.0
	2.00	3	79.3333	2.51661	1.45297	73.0817	85.5849	77.0
	3.00	3	78.3333	3.05505	1.76383	70.7442	85.9225	75.0
	4.00	3	77.6667	4.04145	2.33333	67.6271	87.7062	74.0

	5.00	3	77.6667	9.07377	5.23874	55.1262	100.2072	68.0
	6.00	3	76.3333	6.02771	3.48010	61.3597	91.3070	70.0
	Total	18	77.8889	4.36414	1.02864	75.7187	80.0591	68.0
	1.00	3	310.0000	26.45751	15.27525	244.2759	375.7241	290.0
	2.00	3	315.3333	17.47379	10.08850	271.9260	358.7406	296.0
	3.00	3	318.3333	28.44878	16.42491	247.6626	389.0040	299.0
ZEROHR	4.00	3	307.3333	12.70171	7.33333	275.7805	338.8861	300.0
	5.00	3	311.3333	16.65333	9.61480	269.9642	352.7025	298.0
	6.00	3	78.3333	7.02377	4.05518	60.8853	95.7813	71.0
	Total	18	273.4444	91.35594	21.53280	228.0142	318.8747	71.0
	1.00	3	118.3333	7.63763	4.40959	99.3604	137.3062	110.0
	2.00	3	93.0000	11.26943	6.50641	65.0052	120.9948	80.0
	3.00	3	117.6667	11.23981	6.48931	89.7454	145.5879	108.0
FOTEN	4.00	3	87.0000	2.64575	1.52753	80.4276	93.5724	85.0
	5.00	3	288.6667	12.05543	6.96020	258.7193	318.6140	276.0
	6.00	3	81.6667	7.09460	4.09607	64.0427	99.2906	74.0
	Total	18	131.0556	74.38412	17.53250	94.0652	168.0459	74.0
	1.00	3	127.0000	5.56776	3.21455	113.1689	140.8311	122.0
	2.00	3	94.6667	7.50555	4.33333	76.0218	113.3115	86.0
	3.00	3	127.3333	3.05505	1.76383	119.7442	134.9225	124.0
TWTEHT	4.00	3	87.6667	4.72582	2.72845	75.9271	99.4062	84.0
	5.00	3	292.6667	6.80686	3.92994	275.7575	309.5758	285.0
	6.00	3	77.6667	4.16333	2.40370	67.3244	88.0090	73.0
	Total	18	134.5000	75.44710	17.78305	96.9810	172.0190	73.0
	1.00	3	113.6667	5.50757	3.17980	99.9851	127.3482	110.0
	2.00	3	80.0000	6.55744	3.78594	63.7104	96.2896	73.0
	3.00	3	118.0000	15.62050	9.01850	79.1965	156.8035	100.0
FRTTYOO	4.00	3	80.0000	10.00000	5.77350	55.1586	104.8414	70.0
	5.00	3	303.6667	5.50757	3.17980	289.9851	317.3482	300.0
	6.00	3	82.6667	2.51661	1.45297	76.4151	88.9183	80.0
	Total	18	129.6667	82.01148	19.33029	88.8833	170.4500	70.0
	1.00	3	111.6667	10.40833	6.00925	85.8109	137.5224	100.0
	2.00	3	79.0000	4.35890	2.51661	68.1719	89.8281	74.0
	3.00	3	117.0000	9.84886	5.68624	92.5341	141.4659	106.0
FIFTTSX	4.00	3	76.3333	4.16333	2.40370	65.9910	86.6756	73.0
	5.00	3	295.6667	4.93288	2.84800	283.4127	307.9206	290.0
	6.00	3	76.6667	3.51188	2.02759	67.9427	85.3907	73.0
	Total	18	126.0556	80.11203	18.88259	86.2168	165.8943	73.0
	1.00	3	115.6667	5.03322	2.90593	103.1634	128.1699	111.0
	2.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.0
	3.00	3	119.6667	3.21455	1.85592	111.6813	127.6521	116.0
SEVNTY	4.00	3	73.6667	2.08167	1.20185	68.4955	78.8378	72.0
	5.00	3	292.3333	12.42310	7.17248	261.4727	323.1940	278.0
	6.00	3	73.3333	2.08167	1.20185	68.1622	78.5045	71.0
	Total	18	124.7778	79.87605	18.82697	85.0564	164.4992	71.0
	1.00	3	108.6667	7.57188	4.37163	89.8571	127.4763	100.0
	2.00	3	82.3333	1.52753	.88192	78.5388	86.1279	81.0
	3.00	3	110.6667	8.38650	4.84195	89.8335	131.4999	101.0
EITYFOR	4.00	3	79.3333	2.08167	1.20185	74.1622	84.5045	77.0
	5.00	3	289.6667	8.50490	4.91031	268.5393	310.7940	281.0
	6.00	3	73.0000	1.00000	.57735	70.5159	75.4841	72.0
	Total	18	123.9444	77.83351	18.34553	85.2387	162.6501	72.0

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PRIND	Between Groups	14.444	5	2.889	.112	.987
	Within Groups	309.333	12	25.778		
	Total	323.778	17			
ZEROHR	Between Groups	137275.111	5	27455.022	71.539	.000
	Within Groups	4605.333	12	383.778		
	Total	141880.444	17			
FOTEN	Between Groups	93032.278	5	18606.456	217.055	.000
	Within Groups	1028.667	12	85.722		
	Total	94060.944	17			
TWTEHT	Between Groups	96403.167	5	19280.633	633.305	.000
	Within Groups	365.333	12	30.444		
	Total	96768.500	17			
FRTTYOO	Between Groups	113432.000	5	22686.400	299.820	.000
	Within Groups	908.000	12	75.667		
	Total	114340.000	17			
FIFTTSX	Between Groups	108548.278	5	21709.656	467.993	.000
	Within Groups	556.667	12	46.389		
	Total	109104.944	17			
SEVNTY	Between Groups	108063.778	5	21612.756	649.465	.000
	Within Groups	399.333	12	33.278		
	Total	108463.111	17			
EITYFOR	Between Groups	102571.611	5	20514.322	592.709	.000
	Within Groups	415.333	12	34.611		
	Total	102986.944	17			

Appendix 4: Effect of **different fractions** of *Cussonia arborea* on the **fasting blood glucose (mg/dl)** levels of alloxan induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
Prindfbs	1.00	3	79.0000	2.64575	1.52753	72.4276	85.5724	76.
	2.00	3	77.6667	4.93288	2.84800	65.4127	89.9206	72.
	3.00	3	75.3333	3.78594	2.18581	65.9285	84.7381	71.
	4.00	3	75.3333	2.30940	1.33333	69.5965	81.0702	74.
	5.00	3	81.3333	4.04145	2.33333	71.2938	91.3729	77.
	6.00	3	78.0000	8.71780	5.03322	56.3438	99.6562	72.
	7.00	3	78.3333	7.23418	4.17665	60.3626	96.3040	70.
	8.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.
	9.00	3	73.0000	4.35890	2.51661	62.1719	83.8281	70.
	10.00	3	74.0000	1.00000	.57735	71.5159	76.4841	73.
	Total	30	76.7000	4.59497	.83892	74.9842	78.4158	70.

	1.00	3	293.0000	3.00000	1.73205	285.5476	300.4524	290.
	2.00	3	307.0000	32.51154	18.77054	226.2369	387.7631	270.
	3.00	3	282.0000	13.89244	8.02081	247.4893	316.5107	273.
	4.00	3	295.3333	8.96289	5.17472	273.0683	317.5984	285.
	5.00	3	290.3333	4.50925	2.60342	279.1317	301.5349	286.
Postindfbs	6.00	3	294.3333	10.69268	6.17342	267.7713	320.8954	282.
	7.00	3	284.0000	15.71623	9.07377	244.9587	323.0413	270.
	8.00	3	287.6667	11.93035	6.88799	258.0300	317.3033	274.
	9.00	3	273.3333	25.16611	14.52966	210.8172	335.8494	250.
	10.00	3	76.0000	1.00000	.57735	73.5159	78.4841	75.
	Total	30	268.3000	67.06721	12.24474	243.2567	293.3433	75.
	1.00	3	286.3333	3.51188	2.02759	277.6093	295.0573	283.
	2.00	3	211.3333	9.60902	5.54777	187.4632	235.2035	201.
	3.00	3	237.0000	15.71623	9.07377	197.9587	276.0413	220.
	4.00	3	278.0000	7.00000	4.04145	260.6110	295.3890	273.
	5.00	3	279.6667	3.51188	2.02759	270.9427	288.3907	276.
Onehrfbs	6.00	3	287.0000	14.73092	8.50490	250.4064	323.5936	271.
	7.00	3	274.0000	22.53886	13.01281	218.0104	329.9896	260.
	8.00	3	208.6667	6.11010	3.52767	193.4883	223.8450	202.
	9.00	3	272.0000	17.52142	10.11599	228.4744	315.5256	255.
	10.00	3	74.3333	1.52753	.88192	70.5388	78.1279	73.
	Total	30	240.8333	64.16094	11.71413	216.8752	264.7914	73.
	1.00	3	282.6667	8.32666	4.80740	261.9821	303.3512	276.
	2.00	3	94.6667	10.11599	5.84047	69.5371	119.7962	83.
	3.00	3	118.6667	12.05543	6.96020	88.7193	148.6140	106.
	4.00	3	247.3333	40.99187	23.66667	145.5039	349.1628	200.
	5.00	3	277.3333	2.51661	1.45297	271.0817	283.5849	275.
Threehrfbs	6.00	3	279.3333	14.57166	8.41295	243.1353	315.5313	263.
	7.00	3	269.6667	22.36813	12.91425	214.1011	325.2322	244.
	8.00	3	85.6667	4.50925	2.60342	74.4651	96.8683	81.
	9.00	3	255.3333	28.37840	16.38427	184.8375	325.8292	230.
	10.00	3	75.3333	5.50757	3.17980	61.6518	89.0149	70.
	Total	30	198.6000	89.79847	16.39488	165.0687	232.1313	70.
	1.00	3	273.0000	20.22375	11.67619	222.7614	323.2386	250.
	2.00	3	78.0000	4.35890	2.51661	67.1719	88.8281	73.
	3.00	3	99.3333	10.50397	6.06447	73.2400	125.4266	89.
	4.00	3	244.3333	37.58102	21.69741	150.9769	337.6898	201.
	5.00	3	271.6667	8.14453	4.70225	251.4345	291.8988	266.
Sixhrfbs	6.00	3	267.0000	10.58301	6.11010	240.7104	293.2896	255.
	7.00	3	252.3333	45.39089	26.20645	139.5761	365.0906	200.
	8.00	3	80.3333	6.02771	3.48010	65.3597	95.3070	74.
	9.00	3	223.0000	4.35890	2.51661	212.1719	233.8281	220.
	10.00	3	76.0000	2.64575	1.52753	69.4276	82.5724	74.
	Total	30	186.5000	88.61764	16.17929	153.4096	219.5904	73.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
prindfbs	Between Groups	178.967	9	19.885	.918	.530
	Within Groups	433.333	20	21.667		
	Total	612.300	29			
postindfbs	Between Groups	125446.967	9	13938.552	55.806	.000
	Within Groups	4995.333	20	249.767		
	Total	130442.300	29			
onehrfbs	Between Groups	116412.833	9	12934.759	87.122	.000
	Within Groups	2969.333	20	148.467		
	Total	119382.167	29			
threehrfbs	Between Groups	226704.533	9	25189.393	70.512	.000
	Within Groups	7144.667	20	357.233		
	Total	233849.200	29			
sixhrfbs	Between Groups	219236.167	9	24359.574	57.294	.000
	Within Groups	8503.333	20	425.167		
	Total	227739.500	29			

Appendix 5:Effect of **subfraction 2** of *Cussonia arborea* root bark extract on the **fasting blood glucose (mg/dl)** levels of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
Preindfbs	1.00	3	75.0000	1.00000	.57735	72.5159	77.4841	74.
	2.00	3	76.3333	1.52753	.88192	72.5388	80.1279	75.
	3.00	3	75.4200	1.41746	.71437	71.8988	78.9412	74.
	4.00	3	76.0000	2.64575	1.52753	69.4276	82.5724	74.
	5.00	3	76.3333	1.52753	.88192	72.5388	80.1279	75.
	6.00	3	76.0000	2.00000	1.15470	71.0317	80.9683	74.
	Total	18	75.8478	1.56451	.36876	75.0698	76.6258	74.
Postindfbs	1.00	3	310.0000	10.00000	5.77350	285.1586	334.8414	300.
	2.00	3	313.3333	20.81666	12.01850	261.6219	365.0448	290.
	3.00	3	333.3033	49.27713	28.45017	210.8921	455.7145	300.
	4.00	3	313.3333	30.55050	17.63834	237.4417	389.2250	280.
	5.00	3	300.0000	10.00000	5.77350	275.1586	324.8414	290.

	6.00	3	74.6667	2.51661	1.45297	68.4151	80.9183	72.
	Total	18	274.1061	94.85373	22.35724	226.9365	321.2758	72.
	1.00	3	196.3333	5.50757	3.17980	182.6518	210.0149	190.
	2.00	3	310.3333	16.74316	9.66667	268.7410	351.9256	291.
	3.00	3	305.2333	5.56447	3.21265	291.4104	319.0562	299.
Onehrfbs	4.00	3	197.0000	6.08276	3.51188	181.8896	212.1104	190.
	5.00	3	295.6667	11.59023	6.69162	266.8749	324.4584	288.
	6.00	3	74.6667	1.52753	.88192	70.8721	78.4612	73.
	Total	18	229.8722	87.23187	20.56075	186.4928	273.2516	73.
	1.00	3	76.3333	5.50757	3.17980	62.6518	90.0149	70.
	2.00	3	297.0000	6.08276	3.51188	281.8896	312.1104	290.
	3.00	3	289.9200	15.26759	8.81475	251.9932	327.8468	272.
Threehrfbs	4.00	3	78.3333	2.51661	1.45297	72.0817	84.5849	76.
	5.00	3	296.3333	5.50757	3.17980	282.6518	310.0149	290.
	6.00	3	77.3333	5.50757	3.17980	63.6518	91.0149	71.
	Total	18	185.8756	111.90806	26.37698	130.2250	241.5261	70.
	1.00	3	79.6667	.57735	.33333	78.2324	81.1009	79.
	2.00	3	298.6667	11.01514	6.35959	271.3035	326.0298	288.
	3.00	3	291.5800	5.89145	3.40143	276.9448	306.2152	286.
Sixhrfbs	4.00	3	77.0000	1.00000	.57735	74.5159	79.4841	76.
	5.00	3	294.0000	6.92820	4.00000	276.7894	311.2106	286.
	6.00	3	75.0000	2.64575	1.52753	68.4276	81.5724	73.
	Total	18	185.9856	112.05719	26.41213	130.2608	241.7103	73.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	4.259	5	.852	.274	.919
preindfbs	Within Groups	37.352	12	3.113		
	Total	41.611	17			
	Between Groups	144950.453	5	28990.091	43.472	.000
postindfbs	Within Groups	8002.472	12	666.873		
	Total	152952.925	17			
	Between Groups	128329.203	5	25665.841	298.847	.000
onehrfbs	Within Groups	1030.593	12	85.883		
	Total	129359.796	17			
	Between Groups	212163.154	5	42432.631	692.905	.000
threehrfbs	Within Groups	734.865	12	61.239		
	Total	212898.019	17			
	Between Groups	213041.072	5	42608.214	1203.759	.000
sixhrfbs	Within Groups	424.752	12	35.396		

Total	213465.824	17			

Appendix 6: Glycosylated haemoglobin {HbA1c (%)} values of alloxan-induced diabetic rats treated with methanol root bark extract of *Cussonia arborea*

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minir
						Lower Bound	Upper Bound	
FORTYTWO	1.00	3	7.3333	.49329	.28480	6.1079	8.5587	
	2.00	3	5.4667	.05774	.03333	5.3232	5.6101	
	3.00	3	7.3000	.45826	.26458	6.1616	8.4384	
	4.00	3	5.4000	.40000	.23094	4.4063	6.3937	
	5.00	3	7.7333	.20817	.12019	7.2162	8.2504	
	6.00	3	5.0667	.05774	.03333	4.9232	5.2101	
	Total	18	6.3833	1.15415	.27204	5.8094	6.9573	
FIFTYSIX	1.00	3	7.7667	.15275	.08819	7.3872	8.1461	
	2.00	3	5.8667	.05774	.03333	5.7232	6.0101	
	3.00	3	7.6000	.20000	.11547	7.1032	8.0968	
	4.00	3	5.9000	.10000	.05774	5.6516	6.1484	
	5.00	3	9.1333	.15275	.08819	8.7539	9.5128	
	6.00	3	5.1000	.10000	.05774	4.8516	5.3484	
	Total	18	6.8944	1.43136	.33737	6.1826	7.6062	
EIGHTYFOUR	1.00	3	8.0333	.23094	.13333	7.4596	8.6070	
	2.00	3	5.9000	.10000	.05774	5.6516	6.1484	
	3.00	3	8.0667	.05774	.03333	7.9232	8.2101	
	4.00	3	5.9333	.05774	.03333	5.7899	6.0768	
	5.00	3	10.8333	.41633	.24037	9.7991	11.8676	
	6.00	3	5.2000	.10000	.05774	4.9516	5.4484	
	Total	18	7.3278	1.97091	.46455	6.3477	8.3079	

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FORTYTWO	Between Groups	21.318	5	4.264	38.566	.000
	Within Groups	1.327	12	.111		
	Total	22.645	17			
FIFTYSIX	Between Groups	34.609	5	6.922	377.558	.000
	Within Groups	.220	12	.018		
	Total	34.829	17			
EIGHTYFOUR	Between Groups	65.529	5	13.106	310.403	.000

	Within Groups	.507	12	.042		
	Total	66.036	17			

Appendix 7: Effects of chronic administration of *Cussonia arborea* root bark extract on some biochemical markers of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minim
						Lower Bound	Upper Bound	
	1.00	3	91.9400	2.63536	1.52152	85.3934	98.4866	
	2.00	3	71.4500	2.93312	1.69344	64.1637	78.7363	
	3.00	3	111.6667	11.61190	6.70413	82.8211	140.5122	1
AST1	4.00	3	66.1667	5.44750	3.14511	52.6343	79.6990	
	5.00	3	116.8533	9.19759	5.31023	94.0052	139.7014	1
	6.00	3	67.6000	6.58979	3.80462	51.2301	83.9699	
	Total	18	87.6128	22.14681	5.22005	76.5994	98.6261	
	1.00	3	88.9300	4.31675	2.49227	78.2066	99.6534	
	2.00	3	78.4267	2.51198	1.45029	72.1866	84.6668	
	3.00	3	126.3333	9.65845	5.57631	102.3404	150.3263	1
AST2	4.00	3	70.4033	3.48173	2.01018	61.7542	79.0524	
	5.00	3	137.2333	7.47017	4.31290	118.6764	155.7903	1
	6.00	3	68.6267	6.64577	3.83694	52.1177	85.1357	
	Total	18	94.9922	28.27714	6.66499	80.9303	109.0541	
	1.00	3	78.2867	3.19052	1.84205	70.3610	86.2124	
	2.00	3	76.8233	1.62057	.93563	72.7976	80.8490	
	3.00	3	82.0267	1.85592	1.07152	77.4163	86.6370	
AST3	4.00	3	77.4967	4.67665	2.70006	65.8792	89.1141	
	5.00	3	85.6467	4.99890	2.88612	73.2287	98.0646	
	6.00	3	72.2900	2.68922	1.55262	65.6096	78.9704	
	Total	18	78.7617	5.18636	1.22244	76.1826	81.3408	
	1.00	3	35.8233	.54930	.31714	34.4588	37.1879	
	2.00	3	36.8533	2.57187	1.48487	30.4644	43.2422	
	3.00	3	35.7300	1.84835	1.06715	31.1384	40.3216	
ALT1	4.00	3	31.8600	.99242	.57297	29.3947	34.3253	
	5.00	3	43.6867	4.74571	2.73994	31.8977	55.4757	
	6.00	3	31.4400	1.16052	.67002	28.5571	34.3229	
	Total	18	35.8989	4.62563	1.09027	33.5986	38.1992	
	1.00	3	37.5267	1.06265	.61352	34.8869	40.1664	
	2.00	3	32.9567	1.68797	.97455	28.7635	37.1498	
	3.00	3	40.2533	1.34998	.77941	36.8998	43.6069	
ALT2	4.00	3	32.0933	1.53027	.88350	28.2919	35.8947	
	5.00	3	50.9967	1.26785	.73199	47.8472	54.1462	
	6.00	3	31.9233	1.85877	1.07316	27.3059	36.5408	
	Total	18	37.6250	7.02212	1.65513	34.1330	41.1170	
	1.00	3	35.3233	.61011	.35225	33.8077	36.8389	
	2.00	3	32.3200	.93984	.54262	29.9853	34.6547	
ALT3	3.00	3	38.9900	1.05731	.61044	36.3635	41.6165	
	4.00	3	32.9500	.53563	.30925	31.6194	34.2806	
	5.00	3	51.3033	11.33832	6.54618	23.1374	79.4693	

	6.00	3	32.6067	.81101	.46824	30.5920	34.6213	
	Total	18	37.2489	7.93179	1.86954	33.3045	41.1933	
	1.00	3	149.4867	1.80528	1.04228	145.0021	153.9712	1
	2.00	3	141.2033	1.59500	.92088	137.2411	145.1655	1
	3.00	3	149.7000	2.41348	1.39343	143.7046	155.6954	1
CHOL1	4.00	3	135.2267	5.68403	3.28168	121.1067	149.3466	1
	5.00	3	167.8633	.64810	.37418	166.2534	169.4733	1
	6.00	3	130.3700	6.98698	4.03394	113.0134	147.7266	1
	Total	18	145.6417	12.93809	3.04954	139.2077	152.0756	1
	1.00	3	148.8367	2.56067	1.47840	142.4756	155.1977	1
	2.00	3	135.3333	3.03045	1.74963	127.8053	142.8614	1
	3.00	3	162.7933	6.65616	3.84293	146.2585	179.3281	1
COL2	4.00	3	134.6800	3.00162	1.73298	127.2236	142.1364	1
	5.00	3	180.1100	2.87125	1.65772	172.9774	187.2426	1
	6.00	3	130.4133	5.11197	2.95140	117.7145	143.1122	1
	Total	18	148.6944	18.61599	4.38783	139.4369	157.9520	1
	1.00	3	142.9267	2.20360	1.27225	137.4526	148.4007	1
	2.00	3	133.0433	2.59620	1.49891	126.5940	139.4926	1
	3.00	3	153.4900	2.68967	1.55288	146.8085	160.1715	1
CHOL3	4.00	3	134.0833	2.44625	1.41234	128.0065	140.1602	1
	5.00	3	168.4733	.91577	.52872	166.1984	170.7482	1
	6.00	3	131.0033	2.09462	1.20933	125.8000	136.2067	1
	Total	18	143.8367	13.91566	3.27995	136.9166	150.7568	1
	1.00	3	173.3333	4.93288	2.84800	161.0794	185.5873	1
	2.00	3	165.0000	3.60555	2.08167	156.0433	173.9567	1
	3.00	3	184.6667	5.13160	2.96273	171.9191	197.4143	1
TRIG1	4.00	3	164.6667	2.08167	1.20185	159.4955	169.8378	1
	5.00	3	206.0000	2.00000	1.15470	201.0317	210.9683	2
	6.00	3	151.3333	.57735	.33333	149.8991	152.7676	1
	Total	18	174.1667	18.15700	4.27964	165.1374	183.1959	1
	1.00	3	171.3333	3.21455	1.85592	163.3479	179.3187	1
	2.00	3	151.6667	7.23418	4.17665	133.6960	169.6374	1
	3.00	3	197.6667	2.51661	1.45297	191.4151	203.9183	1
TRIG2	4.00	3	154.0000	3.60555	2.08167	145.0433	162.9567	1
	5.00	3	212.6667	3.05505	1.76383	205.0775	220.2558	2
	6.00	3	151.3333	1.15470	.66667	148.4649	154.2018	1
	Total	18	173.1111	24.96560	5.88445	160.6960	185.5262	1
	1.00	3	161.3333	3.21455	1.85592	153.3479	169.3187	1
	2.00	3	148.0000	1.00000	.57735	145.5159	150.4841	1
	3.00	3	187.6667	3.51188	2.02759	178.9427	196.3907	1
TRIG3	4.00	3	153.0000	2.64575	1.52753	146.4276	159.5724	1
	5.00	3	200.0000	1.00000	.57735	197.5159	202.4841	1
	6.00	3	152.0000	1.00000	.57735	149.5159	154.4841	1
	Total	18	167.0000	20.37299	4.80196	156.8687	177.1313	1
	1.00	3	52.0000	1.00000	.57735	49.5159	54.4841	
	2.00	3	66.6667	5.68624	3.28295	52.5413	80.7921	
	3.00	3	42.6667	1.15470	.66667	39.7982	45.5351	
HDL1	4.00	3	62.3333	11.06044	6.38575	34.8577	89.8090	
	5.00	3	30.3333	3.78594	2.18581	20.9285	39.7381	
	6.00	3	55.6667	3.51188	2.02759	46.9427	64.3907	
	Total	18	51.6111	13.37310	3.15207	44.9608	58.2614	
	1.00	3	53.0000	2.64575	1.52753	46.4276	59.5724	
	2.00	3	69.0000	5.56776	3.21455	55.1689	82.8311	
HDL2	3.00	3	45.6667	2.08167	1.20185	40.4955	50.8378	
	4.00	3	64.0000	6.55744	3.78594	47.7104	80.2896	
	5.00	3	28.0000	4.35890	2.51661	17.1719	38.8281	

	6.00	3	56.0000	2.64575	1.52753	49.4276	62.5724	
	Total	18	52.6111	14.17180	3.34032	45.5636	59.6586	
	1.00	3	52.0000	1.00000	.57735	49.5159	54.4841	
	2.00	3	70.3333	3.21455	1.85592	62.3479	78.3187	
	3.00	3	44.3333	1.15470	.66667	41.4649	47.2018	
HDL3	4.00	3	64.3333	7.02377	4.05518	46.8853	81.7813	
	5.00	3	29.6667	4.50925	2.60342	18.4651	40.8683	
	6.00	3	56.3333	1.15470	.66667	53.4649	59.2018	
	Total	18	52.8333	14.03462	3.30799	45.8541	59.8126	
	1.00	3	34.6667	.98658	.56960	32.2159	37.1175	
	2.00	3	30.3333	.50332	.29059	29.0830	31.5837	
	3.00	3	36.9333	1.02632	.59255	34.3838	39.4829	
VLDL1	4.00	3	28.9333	.41633	.24037	27.8991	29.9676	
	5.00	3	41.2000	.40000	.23094	40.2063	42.1937	
	6.00	3	31.2667	.90185	.52068	29.0263	33.5070	
	Total	18	33.8889	4.39958	1.03699	31.7010	36.0768	
	1.00	3	34.2667	.64291	.37118	32.6696	35.8637	
	2.00	3	30.3333	1.44684	.83533	26.7392	33.9275	
	3.00	3	39.5333	.50332	.29059	38.2830	40.7837	
VLDL2	4.00	3	30.8000	.72111	.41633	29.0087	32.5913	
	5.00	3	42.5333	.61101	.35277	41.0155	44.0512	
	6.00	3	30.2667	.23094	.13333	29.6930	30.8404	
	Total	18	34.6222	4.99312	1.17689	32.1392	37.1052	
	1.00	3	32.2667	.64291	.37118	30.6696	33.8637	
	2.00	3	29.6000	.20000	.11547	29.1032	30.0968	
	3.00	3	37.5333	.70238	.40552	35.7885	39.2781	
VLDL3	4.00	3	30.6000	.52915	.30551	29.2855	31.9145	
	5.00	3	40.0000	.20000	.11547	39.5032	40.4968	
	6.00	3	30.4000	.20000	.11547	29.9032	30.8968	
	Total	18	33.4000	4.07460	.96039	31.3737	35.4263	
	1.00	3	62.8333	3.23316	1.86667	54.8017	70.8650	
	2.00	3	44.2000	6.93109	4.00167	26.9822	61.4178	
	3.00	3	70.1000	2.10000	1.21244	64.8833	75.3167	
LDL1	4.00	3	43.9333	13.30464	7.68144	10.8828	76.9839	
	5.00	3	96.3333	3.70720	2.14035	87.1241	105.5425	
	6.00	3	43.4333	10.66599	6.15801	16.9375	69.9291	
	Total	18	60.1389	20.82369	4.90819	49.7835	70.4943	
	1.00	3	61.0667	5.21664	3.01183	48.1078	74.0255	
	2.00	3	40.5667	2.05508	1.18650	35.4616	45.6718	
	3.00	3	77.1333	5.35288	3.09049	63.8360	90.4306	
LDL2	4.00	3	39.6667	10.01665	5.78312	14.7839	64.5494	
	5.00	3	108.6667	1.52753	.88192	104.8721	112.4612	1
	6.00	3	44.0000	7.81025	4.50925	24.5983	63.4017	
	Total	18	61.8500	26.02782	6.13482	48.9067	74.7933	
	1.00	3	58.6600	1.13248	.65383	55.8468	61.4732	
	2.00	3	33.1100	1.45564	.84042	29.4940	36.7260	
	3.00	3	71.6233	3.37189	1.94676	63.2471	79.9996	
LDL3	4.00	3	39.1500	8.83871	5.10303	17.1934	61.1066	
	5.00	3	98.8067	5.33841	3.08213	85.5453	112.0680	
	6.00	3	46.2700	2.76697	1.59751	39.3965	53.1435	
	Total	18	57.9367	23.19857	5.46796	46.4003	69.4730	
	1.00	3	5.2700	.41146	.23756	4.2479	6.2921	
	2.00	3	5.5767	.23245	.13421	4.9992	6.1541	
TP1	3.00	3	4.7933	.26839	.15496	4.1266	5.4601	
	4.00	3	5.1633	.15822	.09135	4.7703	5.5564	
	5.00	3	4.3433	.30860	.17817	3.5767	5.1099	

	6.00	3	5.4100	.25515	.14731	4.7762	6.0438
	Total	18	5.0928	.48682	.11475	4.8507	5.3349
	1.00	3	5.1367	.21221	.12252	4.6095	5.6638
	2.00	3	5.5933	.17898	.10333	5.1487	6.0379
	3.00	3	4.8167	.16503	.09528	4.4067	5.2266
TP2	4.00	3	5.2500	.38743	.22368	4.2876	6.2124
	5.00	3	4.0000	.01000	.00577	3.9752	4.0248
	6.00	3	5.6133	.21197	.12238	5.0868	6.1399
	Total	18	5.0683	.59626	.14054	4.7718	5.3648
	1.00	3	3.9667	.06658	.03844	3.8013	4.1321
	2.00	3	5.4367	.46004	.26560	4.2939	6.5795
	3.00	3	3.9500	.06083	.03512	3.7989	4.1011
TP3	4.00	3	5.0133	.03215	.01856	4.9335	5.0932
	5.00	3	3.3067	.26858	.15506	2.6395	3.9738
	6.00	3	5.6967	.23629	.13642	5.1097	6.2836
	Total	18	4.5617	.91974	.21678	4.1043	5.0190
	1.00	3	2.6033	.44792	.25861	1.4906	3.7160
	2.00	3	2.7000	.26287	.15177	2.0470	3.3530
	3.00	3	2.8667	.23965	.13836	2.2713	3.4620
ALB1	4.00	3	2.5967	.37099	.21419	1.6751	3.5183
	5.00	3	2.5900	.50587	.29206	1.3334	3.8466
	6.00	3	2.8833	.56862	.32830	1.4708	4.2959
	Total	18	2.7067	.37321	.08797	2.5211	2.8923
	1.00	3	2.8933	.10504	.06064	2.6324	3.1543
	2.00	3	3.0000	.01000	.00577	2.9752	3.0248
	3.00	3	2.5867	.50846	.29356	1.3236	3.8498
ALB2	4.00	3	3.0400	.06083	.03512	2.8889	3.1911
	5.00	3	2.3367	.29160	.16836	1.6123	3.0611
	6.00	3	3.3400	.24331	.14048	2.7356	3.9444
	Total	18	2.8661	.40080	.09447	2.6668	3.0654
	1.00	3	2.1800	.26058	.15044	1.5327	2.8273
	2.00	3	3.0400	.06083	.03512	2.8889	3.1911
	3.00	3	1.6967	.28219	.16292	.9957	2.3977
ALB3	4.00	3	3.0467	.06028	.03480	2.8969	3.1964
	5.00	3	1.8700	.32512	.18771	1.0624	2.6776
	6.00	3	3.3167	.23629	.13642	2.7297	3.9036
	Total	18	2.5250	.67856	.15994	2.1876	2.8624
	1.00	3	2.6667	.64291	.37118	1.0696	4.2637
	2.00	3	2.8767	.03215	.01856	2.7968	2.9565
	3.00	3	1.9267	.06429	.03712	1.7670	2.0864
GLOB1	4.00	3	2.5667	.42336	.24443	1.5150	3.6184
	5.00	3	2.5267	.38657	.22318	1.5664	3.4870
	6.00	3	1.7533	.19732	.11392	1.2632	2.2435
	Total	18	2.3861	.51574	.12156	2.1296	2.6426
	1.00	3	2.2433	.11547	.06667	1.9565	2.5302
	2.00	3	2.5933	.18583	.10729	2.1317	3.0550
	3.00	3	2.2300	.50110	.28931	.9852	3.4748
GLOB2	4.00	3	2.2100	.40361	.23302	1.2074	3.2126
	5.00	3	1.6633	.28290	.16333	.9606	2.3661
	6.00	3	2.2733	.16773	.09684	1.8567	2.6900
	Total	18	2.2022	.38294	.09026	2.0118	2.3927
	1.00	3	1.7867	.22502	.12991	1.2277	2.3456
	2.00	3	2.3967	.41102	.23730	1.3756	3.4177
GLOB3	3.00	3	2.2533	.25968	.14993	1.6083	2.8984
	4.00	3	1.9667	.06658	.03844	1.8013	2.1321
	5.00	3	1.4300	.50863	.29366	.1665	2.6935

	6.00	3	2.3800	.10000	.05774	2.1316	2.6284
	Total	18	2.0356	.44124	.10400	1.8161	2.2550
	1.00	3	.9367	.09866	.05696	.6916	1.1817
	2.00	3	1.0267	.37005	.21365	.1074	1.9459
	3.00	3	1.4867	.12055	.06960	1.1872	1.7861
AG1	4.00	3	1.0433	.30567	.17648	.2840	1.8027
	5.00	3	1.5100	.42790	.24705	.4470	2.5730
	6.00	3	1.2533	.24007	.13860	.6570	1.8497
	Total	18	1.2094	.33469	.07889	1.0430	1.3759
	1.00	3	1.1667	.05774	.03333	1.0232	1.3101
	2.00	3	1.3000	.00000	.00000	1.3000	1.3000
	3.00	3	1.2000	.45826	.26458	.0616	2.3384
AG2	4.00	3	1.4000	.26458	.15275	.7428	2.0572
	5.00	3	1.4667	.40415	.23333	.4627	2.4706
	6.00	3	1.5000	.20000	.11547	1.0032	1.9968
	Total	18	1.3389	.27255	.06424	1.2034	1.4744
	1.00	3	1.2900	.21633	.12490	.7526	1.8274
	2.00	3	1.2467	.32347	.18676	.4431	2.0502
	3.00	3	.7667	.22301	.12875	.2127	1.3207
AG3	4.00	3	1.5533	.08386	.04842	1.3450	1.7617
	5.00	3	1.4967	.80308	.46366	-.4983	3.4916
	6.00	3	52.2167	88.14696	50.89167	-166.7525	271.1859
	Total	18	9.7617	35.99949	8.48516	-8.1405	27.6638
	1.00	3	13.6033	.62692	.36195	12.0460	15.1607
	2.00	3	12.4400	.77156	.44546	10.5233	14.3567
	3.00	3	14.7100	.84859	.48993	12.6020	16.8180
BUN1	4.00	3	12.1533	1.13359	.65448	9.3373	14.9693
	5.00	3	16.5533	1.55918	.90019	12.6801	20.4265
	6.00	3	12.1467	.50817	.29339	10.8843	13.4090
	Total	18	13.6011	1.84519	.43491	12.6835	14.5187
	1.00	3	14.5233	.50738	.29294	13.2629	15.7837
	2.00	3	13.2967	.62067	.35834	11.7548	14.8385
	3.00	3	16.8567	.23965	.13836	16.2613	17.4520
BUN2	4.00	3	13.5667	1.07449	.62036	10.8975	16.2359
	5.00	3	20.4533	.43776	.25274	19.3659	21.5408
	6.00	3	12.1500	.25120	.14503	11.5260	12.7740
	Total	18	15.1411	2.90370	.68441	13.6971	16.5851
	1.00	3	14.9567	1.37158	.79188	11.5495	18.3639
	2.00	3	13.5700	.63222	.36501	11.9995	15.1405
	3.00	3	18.3300	.66506	.38397	16.6779	19.9821
BUN3	4.00	3	13.7767	1.11500	.64375	11.0068	16.5465
	5.00	3	27.2433	3.76835	2.17566	17.8822	36.6044
	6.00	3	12.9500	.42320	.24434	11.8987	14.0013
	Total	18	16.8044	5.33736	1.25803	14.1502	19.4586
	1.00	3	.7033	.01528	.00882	.6654	.7413
	2.00	3	.7200	.02646	.01528	.6543	.7857
	3.00	3	.7733	.03786	.02186	.6793	.8674
CREAT1	4.00	3	.7267	.05686	.03283	.5854	.8679
	5.00	3	.8200	.04359	.02517	.7117	.9283
	6.00	3	.6233	.03512	.02028	.5361	.7106
	Total	18	.7278	.07026	.01656	.6928	.7627
	1.00	3	.8000	.01000	.00577	.7752	.8248
	2.00	3	.7333	.01528	.00882	.6954	.7713
CREAT2	3.00	3	.8367	.04509	.02603	.7247	.9487
	4.00	3	.7167	.01528	.00882	.6787	.7546
	5.00	3	.9833	.07638	.04410	.7936	1.1731

	6.00	3	.6667	.01528	.00882	.6287	.7046
	Total	18	.7894	.11053	.02605	.7345	.8444
	1.00	3	.8200	.02646	.01528	.7543	.8857
	2.00	3	.6933	.01528	.00882	.6554	.7313
	3.00	3	.8000	.01000	.00577	.7752	.8248
CREAT3	4.00	3	.7100	.01000	.00577	.6852	.7348
	5.00	3	1.1233	.08083	.04667	.9225	1.3241
	6.00	3	.6667	.00577	.00333	.6523	.6810
	Total	18	.8022	.16123	.03800	.7220	.8824
	1.00	3	.6733	.15695	.09062	.2834	1.0632
	2.00	3	.7800	.01732	.01000	.7370	.8230
	3.00	3	.9667	.06807	.03930	.7976	1.1358
TBIL1	4.00	3	.6367	.17010	.09821	.2141	1.0592
	5.00	3	1.6067	.20232	.11681	1.1041	2.1093
	6.00	3	.5067	.08327	.04807	.2998	.7135
	Total	18	.8617	.38861	.09160	.6684	1.0549
	1.00	3	.9900	.01000	.00577	.9652	1.0148
	2.00	3	.8700	.02646	.01528	.8043	.9357
	3.00	3	1.1500	.13892	.08021	.8049	1.4951
TBIL2	4.00	3	.8033	.03215	.01856	.7235	.8832
	5.00	3	1.7100	.16093	.09292	1.3102	2.1098
	6.00	3	.5433	.03786	.02186	.4493	.6374
	Total	18	1.0111	.38096	.08979	.8217	1.2006
	1.00	3	.9167	.03055	.01764	.8408	.9926
	2.00	3	.8100	.02646	.01528	.7443	.8757
	3.00	3	1.0633	.12702	.07333	.7478	1.3789
TBIL3	4.00	3	.8200	.04359	.02517	.7117	.9283
	5.00	3	1.9233	.06658	.03844	1.7579	2.0887
	6.00	3	.5667	.00577	.00333	.5523	.5810
	Total	18	1.0167	.44738	.10545	.7942	1.2391
	1.00	3	.1733	.06110	.03528	.0216	.3251
	2.00	3	.1700	.01000	.00577	.1452	.1948
	3.00	3	.2067	.09452	.05457	-.0281	.4415
CONBIL1	4.00	3	.1567	.05033	.02906	.0316	.2817
	5.00	3	.3767	.11930	.06888	.0803	.6730
	6.00	3	.2300	.04583	.02646	.1162	.3438
	Total	18	.2189	.09815	.02313	.1701	.2677
	1.00	3	.3600	.01000	.00577	.3352	.3848
	2.00	3	.4567	.05859	.03383	.3111	.6022
	3.00	3	.3400	.01000	.00577	.3152	.3648
CONBIL2	4.00	3	.3900	.05568	.03215	.2517	.5283
	5.00	3	.3567	.03512	.02028	.2694	.4439
	6.00	3	.4467	.01528	.00882	.4087	.4846
	Total	18	.3917	.05576	.01314	.3639	.4194
	1.00	3	.3567	.00577	.00333	.3423	.3710
	2.00	3	.4700	.01000	.00577	.4452	.4948
	3.00	3	.3500	.01000	.00577	.3252	.3748
CONBIL3	4.00	3	.3733	.02082	.01202	.3216	.4250
	5.00	3	.3300	.02000	.01155	.2803	.3797
	6.00	3	.3433	.02082	.01202	.2916	.3950
	Total	18	.3706	.04952	.01167	.3459	.3952
	1.00	3	.6100	.01000	.00577	.5852	.6348
	2.00	3	.5000	.09644	.05568	.2604	.7396
UNCONBL1	3.00	3	.7600	.14177	.08185	.4078	1.1122
	4.00	3	.4800	.14422	.08327	.1217	.8383
	5.00	3	1.2300	.25632	.14799	.5933	1.8667

	6.00	3	.2767	.04726	.02728	.1593	.3941
	Total	18	.6428	.33061	.07793	.4784	.8072
	1.00	3	.4133	.03215	.01856	.3335	.4932
	2.00	3	.6300	.01000	.00577	.6052	.6548
	3.00	3	.8100	.13077	.07550	.4852	1.1348
UNCONBL2	4.00	3	.4133	.08505	.04910	.2021	.6246
	5.00	3	1.3867	.13577	.07839	1.0494	1.7239
	6.00	3	.0967	.04509	.02603	-.0153	.2087
	Total	18	.6250	.42296	.09969	.4147	.8353
	1.00	3	.3400	.03606	.02082	.2504	.4296
	2.00	3	.5600	.03000	.01732	.4855	.6345
	3.00	3	.7133	.11846	.06839	.4191	1.0076
UNCONBIL3	4.00	3	.4467	.03055	.01764	.3708	.5226
	5.00	3	1.5933	.06807	.03930	1.4242	1.7624
	6.00	3	.2233	.02309	.01333	.1660	.2807
	Total	18	.6461	.46708	.11009	.4138	.8784
	1.00	3	1.9400	.14422	.08327	1.5817	2.2983
	2.00	3	2.5667	.11719	.06766	2.2756	2.8578
	3.00	3	3.1233	.29143	.16826	2.3994	3.8473
AA1	4.00	3	2.0733	.12662	.07311	1.7588	2.3879
	5.00	3	1.9433	.22942	.13246	1.3734	2.5132
	6.00	3	2.9133	.32716	.18889	2.1006	3.7260
	Total	18	2.4267	.52027	.12263	2.1679	2.6854
	1.00	3	2.3833	.20257	.11695	1.8801	2.8865
	2.00	3	2.3633	.04726	.02728	2.2459	2.4807
	3.00	3	3.1400	.30000	.17321	2.3948	3.8852
AA2	4.00	3	2.1900	.03464	.02000	2.1039	2.2761
	5.00	3	2.6900	.14731	.08505	2.3241	3.0559
	6.00	3	2.1433	.08083	.04667	1.9425	2.3441
	Total	18	2.4850	.37771	.08903	2.2972	2.6728
	1.00	3	2.3767	.10970	.06333	2.1042	2.6492
	2.00	3	2.2167	.11372	.06566	1.9342	2.4992
	3.00	3	2.1000	.01732	.01000	2.0570	2.1430
AA3	4.00	3	2.3500	.14731	.08505	1.9841	2.7159
	5.00	3	1.8900	.14000	.08083	1.5422	2.2378
	6.00	3	2.2167	.12741	.07356	1.9002	2.5332
	Total	18	2.1917	.19485	.04593	2.0948	2.2886
	1.00	3	18.8933	.70437	.40667	17.1436	20.6431
	2.00	3	17.6867	1.08247	.62496	14.9977	20.3757
	3.00	3	19.0167	.36747	.21216	18.1038	19.9295
BUNCREAT1	4.00	3	16.8333	2.61653	1.51065	10.3335	23.3332
	5.00	3	20.1600	1.04843	.60531	17.5556	22.7644
	6.00	3	19.5533	1.83895	1.06172	14.9851	24.1215
	Total	18	18.6906	1.69402	.39928	17.8481	19.5330
	1.00	3	18.1333	.97125	.56075	15.7206	20.5461
	2.00	3	18.1333	.40415	.23333	17.1294	19.1373
	3.00	3	20.1667	1.17189	.67659	17.2555	23.0778
BUNCREAT2	4.00	3	18.9467	1.69898	.98091	14.7262	23.1672
	5.00	3	20.8667	1.19304	.68880	17.9030	23.8303
	6.00	3	18.2333	.55076	.31798	16.8652	19.6015
	Total	18	19.0800	1.43253	.33765	18.3676	19.7924
	1.00	3	19.5800	1.18773	.68574	16.6295	22.5305
	2.00	3	18.2700	2.10029	1.21260	13.0526	23.4874
BUNCREAT3	3.00	3	22.9133	.54592	.31519	21.5572	24.2695
	4.00	3	19.4100	1.73701	1.00286	15.0950	23.7250

	5.00	3	24.2033	2.05469	1.18627	19.0992	29.3075
	6.00	3	19.4200	.51215	.29569	18.1477	20.6923
	Total	18	20.6328	2.54446	.59974	19.3674	21.8981

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AST1	Between Groups	7722.021	5	1544.404	30.078	.000
	Within Groups	616.161	12	51.347		
	Total	8338.182	17			
AST2	Between Groups	13132.503	5	2626.501	68.422	.000
	Within Groups	460.644	12	38.387		
	Total	13593.147	17			
AST3	Between Groups	316.587	5	63.317	5.401	.008
	Within Groups	140.684	12	11.724		
	Total	457.271	17			
ALT1	Between Groups	293.367	5	58.673	10.005	.001
	Within Groups	70.372	12	5.864		
	Total	363.739	17			
ALT2	Between Groups	811.863	5	162.373	73.777	.000
	Within Groups	26.410	12	2.201		
	Total	838.273	17			
ALT3	Between Groups	805.774	5	161.155	7.332	.002
	Within Groups	263.751	12	21.979		
	Total	1069.525	17			
CHOL1	Between Groups	2659.354	5	531.871	34.250	.000
	Within Groups	186.348	12	15.529		
	Total	2845.702	17			
COL2	Between Groups	5684.577	5	1136.915	65.952	.000
	Within Groups	206.862	12	17.239		
	Total	5891.439	17			
CHOL3	Between Groups	3231.895	5	646.379	129.101	.000
	Within Groups	60.081	12	5.007		
	Total	3291.976	17			
TRIG1	Between Groups	5459.833	5	1091.967	90.578	.000
	Within Groups	144.667	12	12.056		
	Total	5604.500	17			
TRIG2	Between Groups	10410.444	5	2082.089	134.812	.000
	Within Groups	185.333	12	15.444		
	Total	10595.778	17			
TRIG3	Between Groups	6990.667	5	1398.133	256.800	.000
	Within Groups	65.333	12	5.444		
	Total	7056.000	17			
HDL1	Between Groups	2672.944	5	534.589	17.464	.000
	Within Groups	367.333	12	30.611		
	Total	3040.278	17			
HDL2	Between Groups	3191.611	5	638.322	34.401	.000
	Within Groups	222.667	12	18.556		
	Total	3414.278	17			
HDL3	Between Groups	3181.167	5	636.233	45.626	.000
	Within Groups	167.333	12	13.944		
	Total	3348.500	17			
VLDL1	Between Groups	322.204	5	64.441	112.834	.000
	Within Groups	6.853	12	.571		

	Total	329.058	17			
	Between Groups	416.418	5	83.284	134.812	.000
VLDL2	Within Groups	7.413	12	.618		
	Total	423.831	17			
	Between Groups	279.627	5	55.925	256.800	.000
VLDL3	Within Groups	2.613	12	.218		
	Total	282.240	17			
	Between Groups	6636.796	5	1327.359	21.676	.000
LDL1	Within Groups	734.847	12	61.237		
	Total	7371.643	17			
	Between Groups	11069.092	5	2213.818	59.363	.000
LDL2	Within Groups	447.513	12	37.293		
	Total	11516.605	17			
	Between Groups	8890.855	5	1778.171	82.675	.000
LDL3	Within Groups	258.097	12	21.508		
	Total	9148.952	17			
	Between Groups	3.067	5	.613	7.657	.002
TP1	Within Groups	.961	12	.080		
	Total	4.029	17			
	Between Groups	5.445	5	1.089	21.821	.000
TP2	Within Groups	.599	12	.050		
	Total	6.044	17			
	Between Groups	13.683	5	2.737	47.079	.000
TP3	Within Groups	.698	12	.058		
	Total	14.381	17			
	Between Groups	.280	5	.056	.322	.891
ALB1	Within Groups	2.088	12	.174		
	Total	2.368	17			
	Between Groups	1.896	5	.379	5.447	.008
ALB2	Within Groups	.835	12	.070		
	Total	2.731	17			
	Between Groups	7.195	5	1.439	27.288	.000
ALB3	Within Groups	.633	12	.053		
	Total	7.828	17			
	Between Groups	2.950	5	.590	4.503	.015
GLOB1	Within Groups	1.572	12	.131		
	Total	4.522	17			
	Between Groups	1.353	5	.271	2.848	.064
GLOB2	Within Groups	1.140	12	.095		
	Total	2.493	17			
	Between Groups	2.190	5	.438	4.691	.013
GLOB3	Within Groups	1.120	12	.093		
	Total	3.310	17			
	Between Groups	.914	5	.183	2.213	.121
AG1	Within Groups	.991	12	.083		
	Total	1.904	17			
	Between Groups	.289	5	.058	.714	.625
AG2	Within Groups	.973	12	.081		
	Total	1.263	17			
	Between Groups	6489.895	5	1297.979	1.002	.457
AG3	Within Groups	15541.481	12	1295.123		
	Total	22031.376	17			
	Between Groups	46.515	5	9.303	9.822	.001
BUN1	Within Groups	11.365	12	.947		
	Total	57.880	17			
BUN2	Between Groups	139.116	5	27.823	79.142	.000

	Within Groups	4.219	12	.352		
	Total	143.335	17			
	Between Groups	447.593	5	89.519	29.277	.000
BUN3	Within Groups	36.692	12	3.058		
	Total	484.285	17			
	Between Groups	.066	5	.013	9.130	.001
CREAT1	Within Groups	.017	12	.001		
	Total	.084	17			
	Between Groups	.190	5	.038	26.358	.000
CREAT2	Within Groups	.017	12	.001		
	Total	.208	17			
	Between Groups	.427	5	.085	66.469	.000
CREAT3	Within Groups	.015	12	.001		
	Total	.442	17			
	Between Groups	2.355	5	.471	26.563	.000
TBIL1	Within Groups	.213	12	.018		
	Total	2.567	17			
	Between Groups	2.370	5	.474	58.686	.000
TBIL2	Within Groups	.097	12	.008		
	Total	2.467	17			
	Between Groups	3.354	5	.671	166.790	.000
TBIL3	Within Groups	.048	12	.004		
	Total	3.403	17			
	Between Groups	.101	5	.020	3.813	.027
CONBIL1	Within Groups	.063	12	.005		
	Total	.164	17			
	Between Groups	.036	5	.007	5.334	.008
CONBIL2	Within Groups	.016	12	.001		
	Total	.053	17			
	Between Groups	.039	5	.008	30.956	.000
CONBIL3	Within Groups	.003	12	.000		
	Total	.042	17			
	Between Groups	1.622	5	.324	16.459	.000
UNCONBL1	Within Groups	.236	12	.020		
	Total	1.858	17			
	Between Groups	2.949	5	.590	77.052	.000
UNCONBL2	Within Groups	.092	12	.008		
	Total	3.041	17			
	Between Groups	3.664	5	.733	196.880	.000
UNCONBIL3	Within Groups	.045	12	.004		
	Total	3.709	17			
	Between Groups	4.011	5	.802	16.308	.000
AA1	Within Groups	.590	12	.049		
	Total	4.602	17			
	Between Groups	2.100	5	.420	15.488	.000
AA2	Within Groups	.325	12	.027		
	Total	2.425	17			
	Between Groups	.480	5	.096	6.954	.003
AA3	Within Groups	.166	12	.014		
	Total	.645	17			
	Between Groups	22.525	5	4.505	2.059	.142
BUNCREAT1	Within Groups	26.260	12	2.188		
	Total	48.785	17			
	Between Groups	20.700	5	4.140	3.502	.035
BUNCREAT2	Within Groups	14.186	12	1.182		
	Total	34.886	17			

	Between Groups	82.821	5	16.564	7.296	.002
BUNCREAT3	Within Groups	27.242	12	2.270		
	Total	110.063	17			

Appendix 8: Effects of chronic administration of *Cussonia arborea* root bark extract on the i*n vivo* antioxidants and haematological indices of alloxan-induced diabetic rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
	1.00	3	6.6000	.20000	.11547	6.1032	7.0968	6.40
	2.00	3	5.7333	.15275	.08819	5.3539	6.1128	5.60
	3.00	3	7.2667	.55076	.31798	5.8985	8.6348	6.90
MDA1	4.00	3	5.1333	.15275	.08819	4.7539	5.5128	5.00
	5.00	3	7.3333	.15275	.08819	6.9539	7.7128	7.20
	6.00	3	4.3333	.15275	.08819	3.9539	4.7128	4.20
	Total	18	6.0667	1.15860	.27308	5.4905	6.6428	4.20
	1.00	3	6.1000	.17321	.10000	5.6697	6.5303	6.00
	2.00	3	4.2667	.11547	.06667	3.9798	4.5535	4.20
	3.00	3	6.7333	.20817	.12019	6.2162	7.2504	6.50
MDA2	4.00	3	4.8000	.26458	.15275	4.1428	5.4572	4.50
	5.00	3	9.2000	.10000	.05774	8.9516	9.4484	9.10
	6.00	3	4.3000	.10000	.05774	4.0516	4.5484	4.20
	Total	18	5.9000	1.79115	.42218	5.0093	6.7907	4.20
	1.00	3	6.1000	.43589	.25166	5.0172	7.1828	5.80
	2.00	3	4.5333	.11547	.06667	4.2465	4.8202	4.40
	3.00	3	9.2667	1.16762	.67412	6.3661	12.1672	8.00
MDA3	4.00	3	4.7000	.10000	.05774	4.4516	4.9484	4.60
	5.00	3	10.3000	.10000	.05774	10.0516	10.5484	10.20
	6.00	3	4.5000	.10000	.05774	4.2516	4.7484	4.40
	Total	18	6.5667	2.46386	.58074	5.3414	7.7919	4.40
	1.00	3	.2967	.00577	.00333	.2823	.3110	.29
	2.00	3	.3867	.00577	.00333	.3723	.4010	.38
	3.00	3	.2897	.00950	.00549	.2661	.3133	.28
SOD1	4.00	3	.3333	.02517	.01453	.2708	.3958	.31
	5.00	3	.2863	.00473	.00273	.2746	.2981	.28
	6.00	3	.5700	.01000	.00577	.5452	.5948	.56
	Total	18	.3604	.10334	.02436	.3091	.4118	.28
	1.00	3	.2933	.00577	.00333	.2790	.3077	.29
	2.00	3	.3967	.00577	.00333	.3823	.4110	.39
	3.00	3	.2800	.01000	.00577	.2552	.3048	.27
SOD2	4.00	3	.3700	.03000	.01732	.2955	.4445	.34
	5.00	3	.2000	.01000	.00577	.1752	.2248	.19
	6.00	3	.5733	.00577	.00333	.5590	.5877	.57
	Total	18	.3522	.12163	.02867	.2917	.4127	.19
	1.00	3	.3733	.03055	.01764	.2974	.4492	.34
	2.00	3	.6167	.05774	.03333	.4732	.7601	.55
	3.00	3	.2800	.01000	.00577	.2552	.3048	.27
SOD3	4.00	3	.5900	.01000	.00577	.5652	.6148	.58
	5.00	3	.1567	.00577	.00333	.1423	.1710	.15
	6.00	3	.5967	.00577	.00333	.5823	.6110	.59
	Total	18	.4356	.18382	.04333	.3441	.5270	.15
GSH1	1.00	3	135.6667	3.05505	1.76383	128.0775	143.2558	133.00

	2.00	3	144.3333	.57735	.33333	142.8991	145.7676	144.00
	3.00	3	135.6667	4.50925	2.60342	124.4651	146.8683	131.00
	4.00	3	142.0000	2.00000	1.15470	137.0317	146.9683	140.00
	5.00	3	121.0000	1.00000	.57735	118.5159	123.4841	120.00
	6.00	3	163.0000	2.64575	1.52753	156.4276	169.5724	160.00
	Total	18	140.2778	13.13641	3.09628	133.7452	146.8104	120.00
	1.00	3	140.3333	1.52753	.88192	136.5388	144.1279	139.00
	2.00	3	161.0000	1.00000	.57735	158.5159	163.4841	160.00
	3.00	3	137.6667	1.52753	.88192	133.8721	141.4612	136.00
GSH2	4.00	3	162.0000	2.64575	1.52753	155.4276	168.5724	159.00
	5.00	3	112.3333	2.51661	1.45297	106.0817	118.5849	110.00
	6.00	3	166.0000	2.64575	1.52753	159.4276	172.5724	163.00
	Total	18	146.5556	19.39443	4.57131	136.9109	156.2002	110.00
	1.00	3	142.0000	2.64575	1.52753	135.4276	148.5724	140.00
	2.00	3	167.3333	.57735	.33333	165.8991	168.7676	167.00
	3.00	3	138.0000	1.00000	.57735	135.5159	140.4841	137.00
GSH3	4.00	3	163.3333	2.88675	1.66667	156.1622	170.5044	160.00
	5.00	3	99.0000	1.00000	.57735	96.5159	101.4841	98.00
	6.00	3	165.6667	.57735	.33333	164.2324	167.1009	165.00
	Total	18	145.8889	24.65023	5.81012	133.6306	158.1472	98.00
	1.00	3	2.6133	.11504	.06642	2.3276	2.8991	2.50
	2.00	3	3.8067	.35921	.20739	2.9143	4.6990	3.41
	3.00	3	1.9633	.05508	.03180	1.8265	2.1001	1.90
CAT1	4.00	3	3.3500	.14107	.08145	2.9996	3.7004	3.22
	5.00	3	1.4000	.00000	.00000	1.4000	1.4000	1.40
	6.00	3	5.3563	.05450	.03147	5.2209	5.4917	5.32
	Total	18	3.0816	1.34045	.31595	2.4150	3.7482	1.40
	1.00	3	2.7133	.15044	.08686	2.3396	3.0871	2.54
	2.00	3	4.9200	.05292	.03055	4.7886	5.0514	4.88
	3.00	3	2.2967	.06658	.03844	2.1313	2.4621	2.22
CAT2	4.00	3	4.3300	.58026	.33501	2.8886	5.7714	3.99
	5.00	3	.6933	.52253	.30168	-.6047	1.9914	.09
	6.00	3	5.7433	.05132	.02963	5.6159	5.8708	5.70
	Total	18	3.4494	1.78696	.42119	2.5608	4.3381	.09
	1.00	3	3.6200	.54745	.31607	2.2601	4.9799	2.99
	2.00	3	5.8167	.05508	.03180	5.6799	5.9535	5.78
	3.00	3	2.4300	.51740	.29872	1.1447	3.7153	1.99
CAT3	4.00	3	4.5900	.55073	.31796	3.2219	5.9581	4.20
	5.00	3	.8733	.12014	.06936	.5749	1.1718	.75
	6.00	3	5.7567	.05033	.02906	5.6316	5.8817	5.71
	Total	18	3.8478	1.85857	.43807	2.9235	4.7720	.75
	1.00	3	5.8100	.06083	.03512	5.6589	5.9611	5.77
	2.00	3	6.6100	.26851	.15503	5.9430	7.2770	6.32
	3.00	3	4.9233	.06658	.03844	4.7579	5.0887	4.88
RBC1	4.00	3	6.7267	.05859	.03383	6.5811	6.8722	6.66
	5.00	3	4.8333	.15275	.08819	4.4539	5.2128	4.70
	6.00	3	7.4500	.05000	.02887	7.3258	7.5742	7.40
	Total	18	6.0589	.99510	.23455	5.5640	6.5537	4.70
	1.00	3	5.1833	.90185	.52068	2.9430	7.4237	4.25
	2.00	3	6.9633	.06351	.03667	6.8056	7.1211	6.89
	3.00	3	5.3100	.49427	.28537	4.0822	6.5378	4.88
RBC2	4.00	3	6.9600	.06083	.03512	6.8089	7.1111	6.89
	5.00	3	3.8667	.11547	.06667	3.5798	4.1535	3.80
	6.00	3	7.7667	.15275	.08819	7.3872	8.1461	7.60
	Total	18	6.0083	1.41831	.33430	5.3030	6.7136	3.80
RBC3	1.00	3	6.9333	.05774	.03333	6.7899	7.0768	6.90

	2.00	3	7.5333	.05774	.03333	7.3899	7.6768	7.50
	3.00	3	6.4133	.45446	.26238	5.2844	7.5423	6.00
	4.00	3	7.4667	.05774	.03333	7.3232	7.6101	7.40
	5.00	3	3.6000	.17321	.10000	3.1697	4.0303	3.50
	6.00	3	7.6333	.05774	.03333	7.4899	7.7768	7.60
	Total	18	6.5967	1.45536	.34303	5.8729	7.3204	3.50
	1.00	3	11.6267	.18556	.10713	11.1657	12.0876	11.45
	2.00	3	13.0333	.45092	.26034	11.9132	14.1535	12.60
	3.00	3	10.9967	.58969	.34046	9.5318	12.4615	10.33
HB1	4.00	3	12.5933	.35726	.20626	11.7059	13.4808	12.33
	5.00	3	10.6067	.60252	.34787	9.1099	12.1034	10.21
	6.00	3	14.1333	.32146	.18559	13.3348	14.9319	13.90
	Total	18	12.1650	1.30709	.30808	11.5150	12.8150	10.21
	1.00	3	11.8033	.30665	.17704	11.0416	12.5651	11.45
	2.00	3	13.9333	.05774	.03333	13.7899	14.0768	13.90
	3.00	3	10.9900	.69656	.40216	9.2596	12.7204	10.21
HB2	4.00	3	13.4900	.14177	.08185	13.1378	13.8422	13.33
	5.00	3	10.0667	.51316	.29627	8.7919	11.3414	9.50
	6.00	3	14.1667	.28868	.16667	13.4496	14.8838	14.00
	Total	18	12.4083	1.63138	.38452	11.5971	13.2196	9.50
	1.00	3	13.4667	.80829	.46667	11.4588	15.4746	13.00
	2.00	3	14.1000	.26458	.15275	13.4428	14.7572	13.90
	3.00	3	13.2667	.55076	.31798	11.8985	14.6348	12.90
HB3	4.00	3	14.1333	.32146	.18559	13.3348	14.9319	13.90
	5.00	3	8.8333	.76376	.44096	6.9360	10.7306	8.00
	6.00	3	14.3333	.28868	.16667	13.6162	15.0504	14.00
	Total	18	13.0222	2.01977	.47606	12.0178	14.0266	8.00
	1.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00
	2.00	3	37.3333	.57735	.33333	35.8991	38.7676	37.00
	3.00	3	33.0000	1.00000	.57735	30.5159	35.4841	32.00
PCV1	4.00	3	36.3333	1.52753	.88192	32.5388	40.1279	35.00
	5.00	3	28.3333	1.52753	.88192	24.5388	32.1279	27.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00
	Total	18	35.0556	4.13695	.97509	32.9983	37.1128	27.00
	1.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00
	2.00	3	38.6667	.57735	.33333	37.2324	40.1009	38.00
	3.00	3	32.0000	.00000	.00000	32.0000	32.0000	32.00
PCV2	4.00	3	38.3333	.57735	.33333	36.8991	39.7676	38.00
	5.00	3	27.6667	.57735	.33333	26.2324	29.1009	27.00
	6.00	3	41.3333	1.52753	.88192	37.5388	45.1279	40.00
	Total	18	35.3889	4.77911	1.12645	33.0123	37.7655	27.00
	1.00	3	36.0000	1.00000	.57735	33.5159	38.4841	35.00
	2.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00
	3.00	3	34.3333	.57735	.33333	32.8991	35.7676	34.00
PCV3	4.00	3	39.3333	.57735	.33333	37.8991	40.7676	39.00
	5.00	3	24.3333	.57735	.33333	22.8991	25.7676	24.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00
	Total	18	35.9444	5.92574	1.39671	32.9976	38.8912	24.00
	1.00	3	58.6667	1.52753	.88192	54.8721	62.4612	57.00
	2.00	3	56.0000	1.73205	1.00000	51.6973	60.3027	55.00
	3.00	3	67.0000	3.00000	1.73205	59.5476	74.4524	64.00
MCV1	4.00	3	54.0000	2.00000	1.15470	49.0317	58.9683	52.00
	5.00	3	58.3333	3.21455	1.85592	50.3479	66.3187	56.00
	6.00	3	54.6667	1.52753	.88192	50.8721	58.4612	53.00
	Total	18	58.1111	4.84936	1.14301	55.6996	60.5226	52.00
MCV2	1.00	3	67.3333	12.05543	6.96020	37.3860	97.2807	56.00

	2.00	3	55.3333	1.15470	.66667	52.4649	58.2018	54.00
	3.00	3	61.3333	5.68624	3.28295	47.2079	75.4587	55.00
	4.00	3	55.0000	1.00000	.57735	52.5159	57.4841	54.00
	5.00	3	71.3333	1.52753	.88192	67.5388	75.1279	70.00
	6.00	3	53.3333	1.15470	.66667	50.4649	56.2018	52.00
	Total	18	60.6111	8.35350	1.96894	56.4570	64.7652	52.00
	1.00	3	51.9333	1.15902	.66916	49.0542	54.8125	50.70
	2.00	3	54.5333	1.00167	.57831	52.0451	57.0216	53.50
	3.00	3	53.4667	2.54231	1.46780	47.1512	59.7821	50.70
MCV3	4.00	3	52.5667	.51316	.29627	51.2919	53.8414	52.00
	5.00	3	67.4000	1.12694	.65064	64.6005	70.1995	66.10
	6.00	3	54.9000	1.76918	1.02144	50.5051	59.2949	53.00
	Total	18	55.8000	5.58580	1.31659	53.0222	58.5778	50.70
	1.00	3	33.8667	.20817	.12019	33.3496	34.3838	33.70
	2.00	3	34.9000	.72111	.41633	33.1087	36.6913	34.10
	3.00	3	33.4667	2.65581	1.53333	26.8693	40.0641	30.40
MCHC1	4.00	3	34.6667	.50332	.29059	33.4163	35.9170	34.20
	5.00	3	34.2667	6.38462	3.68616	18.4064	50.1269	26.90
	6.00	3	34.5000	1.63707	.94516	30.4333	38.5667	33.10
	Total	18	34.2778	2.50730	.59098	33.0309	35.5246	26.90
	1.00	3	34.3667	.86217	.49777	32.2249	36.5084	33.60
	2.00	3	36.0333	.51316	.29627	34.7586	37.3081	35.60
	3.00	3	34.0333	2.68390	1.54955	27.3661	40.7005	31.00
MCHC2	4.00	3	35.2000	.36056	.20817	34.3043	36.0957	34.90
	5.00	3	36.4000	2.50000	1.44338	30.1897	42.6103	33.90
	6.00	3	34.4333	1.62583	.93868	30.3945	38.4721	33.00
	Total	18	35.0778	1.68961	.39824	34.2376	35.9180	31.00
	1.00	3	37.0000	3.46410	2.00000	28.3947	45.6053	35.00
	2.00	3	34.3333	1.15470	.66667	31.4649	37.2018	33.00
	3.00	3	38.0000	1.73205	1.00000	33.6973	42.3027	37.00
MCHC3	4.00	3	35.3333	.57735	.33333	33.8991	36.7676	35.00
	5.00	3	36.0000	2.64575	1.52753	29.4276	42.5724	33.00
	6.00	3	35.0000	1.00000	.57735	32.5159	37.4841	34.00
	Total	18	35.9444	2.12747	.50145	34.8865	37.0024	33.00
	1.00	3	19.9667	.55076	.31798	18.5985	21.3348	19.40
	2.00	3	19.7333	.85049	.49103	17.6206	21.8461	18.90
	3.00	3	22.3000	1.15326	.66583	19.4352	25.1648	21.10
MCH1	4.00	3	18.7333	.45092	.26034	17.6132	19.8535	18.30
	5.00	3	21.9333	1.55027	.89505	18.0823	25.7844	20.40
	6.00	3	18.6333	.65064	.37565	17.0171	20.2496	18.00
	Total	18	20.2167	1.67411	.39459	19.3842	21.0492	18.00
	1.00	3	23.4000	4.65725	2.68887	11.8307	34.9693	18.90
	2.00	3	20.0333	.23094	.13333	19.4596	20.6070	19.90
	3.00	3	20.7333	1.87705	1.08372	16.0705	25.3962	19.60
MCH2	4.00	3	19.3667	.25166	.14530	18.7415	19.9918	19.10
	5.00	3	25.8333	1.04083	.60093	23.2478	28.4189	25.00
	6.00	3	18.2000	.43589	.25166	17.1172	19.2828	17.70
	Total	18	21.2611	3.19819	.75382	19.6707	22.8515	17.70
	1.00	3	19.3667	1.25033	.72188	16.2607	22.4727	18.50
	2.00	3	18.7000	.45826	.26458	17.5616	19.8384	18.30
	3.00	3	20.7333	1.68622	.97354	16.5445	24.9221	18.80
MCH3	4.00	3	18.9000	.36056	.20817	18.0043	19.7957	18.60
	5.00	3	24.0000	1.73205	1.00000	19.6973	28.3027	22.00
	6.00	3	18.7667	.57735	.33333	17.3324	20.2009	18.10
	Total	18	20.0778	2.17108	.51173	18.9981	21.1574	18.10
WBC1	1.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10

	2.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00
	3.00	3	7.0333	.05774	.03333	6.8899	7.1768	7.00
	4.00	3	6.9333	.05774	.03333	6.7899	7.0768	6.90
	5.00	3	7.0000	.10000	.05774	6.7516	7.2484	6.90
	6.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00
	Total	18	7.0500	.09852	.02322	7.0010	7.0990	6.90
	1.00	3	7.1667	.05774	.03333	7.0232	7.3101	7.10
	2.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10
	3.00	3	7.1333	.05774	.03333	6.9899	7.2768	7.10
WBC2	4.00	3	7.1000	.10000	.05774	6.8516	7.3484	7.00
	5.00	3	6.9000	.10000	.05774	6.6516	7.1484	6.80
	6.00	3	7.2667	.11547	.06667	6.9798	7.5535	7.20
	Total	18	7.1167	.13394	.03157	7.0501	7.1833	6.80
	1.00	3	7.2000	.10000	.05774	6.9516	7.4484	7.10
	2.00	3	7.2667	.15275	.08819	6.8872	7.6461	7.10
	3.00	3	6.9667	.20817	.12019	6.4496	7.4838	6.80
WBC3	4.00	3	6.9667	.11547	.06667	6.6798	7.2535	6.90
	5.00	3	6.9000	.10000	.05774	6.6516	7.1484	6.80
	6.00	3	7.2667	.15275	.08819	6.8872	7.6461	7.10
	Total	18	7.0944	.19844	.04677	6.9958	7.1931	6.80
	1.00	3	57.6667	1.15470	.66667	54.7982	60.5351	57.00
	2.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00
	3.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00
LYMPH1	4.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00
	5.00	3	56.0000	1.00000	.57735	53.5159	58.4841	55.00
	6.00	3	58.0000	1.00000	.57735	55.5159	60.4841	57.00
	Total	18	57.8889	1.23140	.29024	57.2765	58.5012	55.00
	1.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00
	2.00	3	59.3333	1.15470	.66667	56.4649	62.2018	58.00
	3.00	3	60.0000	1.00000	.57735	57.5159	62.4841	59.00
LYMPH2	4.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00
	5.00	3	61.3333	2.08167	1.20185	56.1622	66.5045	59.00
	6.00	3	58.0000	1.00000	.57735	55.5159	60.4841	57.00
	Total	18	59.4444	1.50381	.35445	58.6966	60.1923	57.00
	1.00	3	58.3333	1.15470	.66667	55.4649	61.2018	57.00
	2.00	3	59.0000	1.00000	.57735	56.5159	61.4841	58.00
	3.00	3	57.3333	.57735	.33333	55.8991	58.7676	57.00
LYMPH3	4.00	3	57.6667	.57735	.33333	56.2324	59.1009	57.00
	5.00	3	57.0000	1.00000	.57735	54.5159	59.4841	56.00
	6.00	3	58.3333	.57735	.33333	56.8991	59.7676	58.00
	Total	18	57.9444	.99836	.23532	57.4480	58.4409	56.00
	1.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00
	2.00	3	41.3333	1.15470	.66667	38.4649	44.2018	40.00
	3.00	3	40.0000	1.00000	.57735	37.5159	42.4841	39.00
NEUT1	4.00	3	40.6667	.57735	.33333	39.2324	42.1009	40.00
	5.00	3	43.0000	1.00000	.57735	40.5159	45.4841	42.00
	6.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00
	Total	18	41.1111	1.23140	.29024	40.4988	41.7235	39.00
	1.00	3	39.3333	.57735	.33333	37.8991	40.7676	39.00
	2.00	3	39.6667	.57735	.33333	38.2324	41.1009	39.00
	3.00	3	39.0000	1.00000	.57735	36.5159	41.4841	38.00
NEUT2	4.00	3	39.6667	.57735	.33333	38.2324	41.1009	39.00
	5.00	3	37.3333	2.51661	1.45297	31.0817	43.5849	35.00
	6.00	3	40.6667	1.15470	.66667	37.7982	43.5351	40.00
	Total	18	39.2778	1.48742	.35059	38.5381	40.0175	35.00
NEUT3	1.00	3	40.0000	1.00000	.57735	37.5159	42.4841	39.00

	2.00	3	40.6667	1.15470	.66667	37.7982	43.5351	40.00
	3.00	3	41.3333	.57735	.33333	39.8991	42.7676	41.00
	4.00	3	41.0000	1.00000	.57735	38.5159	43.4841	40.00
	5.00	3	41.6667	.57735	.33333	40.2324	43.1009	41.00
	6.00	3	40.3333	.57735	.33333	38.8991	41.7676	40.00
	Total	18	40.8333	.92355	.21768	40.3741	41.2926	39.00
	1.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	2.00	3	.0667	.05774	.03333	-.0768	.2101	.00
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00
BASO1	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	5.00	3	.0667	.11547	.06667	-.2202	.3535	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00
	Total	18	.0389	.06077	.01432	.0087	.0691	.00
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00
	2.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00
BASO2	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	5.00	3	.0667	.05774	.03333	-.0768	.2101	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00
	Total	18	.0278	.04609	.01086	.0049	.0507	.00
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00
	2.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	3.00	3	.0333	.05774	.03333	-.1101	.1768	.00
BASO3	4.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	5.00	3	.0333	.05774	.03333	-.1101	.1768	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00
	Total	18	.0222	.04278	.01008	.0009	.0435	.00
	1.00	3	.7333	.05774	.03333	.5899	.8768	.70
	2.00	3	.7333	.11547	.06667	.4465	1.0202	.60
	3.00	3	.7000	.10000	.05774	.4516	.9484	.60
EOS1	4.00	3	.7333	.05774	.03333	.5899	.8768	.70
	5.00	3	.8000	.10000	.05774	.5516	1.0484	.70
	6.00	3	.7667	.05774	.03333	.6232	.9101	.70
	Total	18	.7444	.07838	.01847	.7055	.7834	.60
	1.00	3	.7000	.10000	.05774	.4516	.9484	.60
	2.00	3	.9667	.37859	.21858	.0262	1.9071	.70
	3.00	3	.7000	.10000	.05774	.4516	.9484	.60
EOS2	4.00	3	.9000	.26458	.15275	.2428	1.5572	.70
	5.00	3	.8667	.11547	.06667	.5798	1.1535	.80
	6.00	3	1.0000	.43589	.25166	-.0828	2.0828	.70
	Total	18	.8556	.25718	.06062	.7277	.9835	.60
	1.00	3	.7333	.05774	.03333	.5899	.8768	.70
	2.00	3	.6667	.11547	.06667	.3798	.9535	.60
	3.00	3	.6000	.10000	.05774	.3516	.8484	.50
EOS3	4.00	3	1.0333	.37859	.21858	.0929	1.9738	.60
	5.00	3	.5667	.05774	.03333	.4232	.7101	.50
	6.00	3	1.0000	.34641	.20000	.1395	1.8605	.80
	Total	18	.7667	.26568	.06262	.6345	.8988	.50
	1.00	3	.2333	.05774	.03333	.0899	.3768	.20
	2.00	3	.2000	.10000	.05774	-.0484	.4484	.10
	3.00	3	.2667	.05774	.03333	.1232	.4101	.20
MONO1	4.00	3	.2333	.05774	.03333	.0899	.3768	.20
	5.00	3	.1333	.05774	.03333	-.0101	.2768	.10
	6.00	3	.2333	.05774	.03333	.0899	.3768	.20
	Total	18	.2167	.07071	.01667	.1815	.2518	.10
MONO2	1.00	3	.3000	.10000	.05774	.0516	.5484	.20

	2.00	3	.3333	.23094	.13333	-.2404	.9070	.20
	3.00	3	.2667	.05774	.03333	.1232	.4101	.20
	4.00	3	.4000	.34641	.20000	-.4605	1.2605	.20
	5.00	3	.4000	.43589	.25166	-.6828	1.4828	.10
	6.00	3	.3333	.15275	.08819	-.0461	.7128	.20
	Total	18	.3389	.22265	.05248	.2282	.4496	.10
	1.00	3	.2667	.05774	.03333	.1232	.4101	.20
	2.00	3	.3000	.10000	.05774	.0516	.5484	.20
	3.00	3	.3667	.05774	.03333	.2232	.5101	.30
MONO3	4.00	3	.6000	.17321	.10000	.1697	1.0303	.40
	5.00	3	.3667	.05774	.03333	.2232	.5101	.30
	6.00	3	.3667	.20817	.12019	-.1504	.8838	.20
	Total	18	.3778	.15168	.03575	.3023	.4532	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	21.947	5	4.389	60.311	.000
MDA1	Within Groups	.873	12	.073		
	Total	22.820	17			
	Between Groups	54.187	5	10.837	368.060	.000
MDA2	Within Groups	.353	12	.029		
	Total	54.540	17			
	Between Groups	100.007	5	20.001	75.162	.000
MDA3	Within Groups	3.193	12	.266		
	Total	103.200	17			
	Between Groups	.180	5	.036	236.297	.000
SOD1	Within Groups	.002	12	.000		
	Total	.182	17			
	Between Groups	.249	5	.050	249.111	.000
SOD2	Within Groups	.002	12	.000		
	Total	.252	17			
	Between Groups	.565	5	.113	149.659	.000
SOD3	Within Groups	.009	12	.001		
	Total	.574	17			
	Between Groups	2849.611	5	569.922	81.417	.000
GSH1	Within Groups	84.000	12	7.000		
	Total	2933.611	17			
	Between Groups	6342.444	5	1268.489	292.728	.000
GSH2	Within Groups	52.000	12	4.333		
	Total	6394.444	17			
	Between Groups	10293.778	5	2058.756	686.252	.000
GSH3	Within Groups	36.000	12	3.000		
	Total	10329.778	17			
	Between Groups	30.209	5	6.042	215.562	.000
CAT1	Within Groups	.336	12	.028		
	Total	30.546	17			
	Between Groups	53.000	5	10.600	99.030	.000
CAT2	Within Groups	1.284	12	.107		
	Total	54.285	17			
	Between Groups	56.942	5	11.388	76.715	.000
CAT3	Within Groups	1.781	12	.148		
	Total	58.723	17			

	Between Groups	16.615	5	3.323	182.080	.000
RBC1	Within Groups	.219	12	.018		
	Total	16.834	17			
	Between Groups	31.993	5	6.399	34.837	.000
RBC2	Within Groups	2.204	12	.184		
	Total	34.197	17			
	Between Groups	35.508	5	7.102	170.528	.000
RBC3	Within Groups	.500	12	.042		
	Total	36.007	17			
	Between Groups	26.685	5	5.337	27.149	.000
HB1	Within Groups	2.359	12	.197		
	Total	29.044	17			
	Between Groups	43.345	5	8.669	54.791	.000
HB2	Within Groups	1.899	12	.158		
	Total	45.244	17			
	Between Groups	65.758	5	13.152	43.920	.000
HB3	Within Groups	3.593	12	.299		
	Total	69.351	17			
	Between Groups	276.278	5	55.256	45.209	.000
PCV1	Within Groups	14.667	12	1.222		
	Total	290.944	17			
	Between Groups	380.944	5	76.189	124.673	.000
PCV2	Within Groups	7.333	12	.611		
	Total	388.278	17			
	Between Groups	590.278	5	118.056	212.500	.000
PCV3	Within Groups	6.667	12	.556		
	Total	596.944	17			
	Between Groups	337.778	5	67.556	13.075	.000
MCV1	Within Groups	62.000	12	5.167		
	Total	399.778	17			
	Between Groups	818.944	5	163.789	5.351	.008
MCV2	Within Groups	367.333	12	30.611		
	Total	1186.278	17			
	Between Groups	503.473	5	100.695	44.842	.000
MCV3	Within Groups	26.947	12	2.246		
	Total	530.420	17			
	Between Groups	4.244	5	.849	.099	.990
MCHC1	Within Groups	102.627	12	8.552		
	Total	106.871	17			
	Between Groups	14.064	5	2.813	.979	.469
MCHC2	Within Groups	34.467	12	2.872		
	Total	48.531	17			
	Between Groups	27.611	5	5.522	1.343	.311
MCHC3	Within Groups	49.333	12	4.111		
	Total	76.944	17			
	Between Groups	36.872	5	7.374	8.214	.001
MCH1	Within Groups	10.773	12	.898		
	Total	47.645	17			
	Between Groups	120.676	5	24.135	5.443	.008
MCH2	Within Groups	53.207	12	4.434		
	Total	173.883	17			
	Between Groups	63.971	5	12.794	9.501	.001
MCH3	Within Groups	16.160	12	1.347		
	Total	80.131	17			
WBC1	Between Groups	.085	5	.017	2.550	.085
	Within Groups	.080	12	.007		

	Total	.165	17			
	Between Groups	.218	5	.044	6.046	.005
WBC2	Within Groups	.087	12	.007		
	Total	.305	17			
	Between Groups	.423	5	.085	4.114	.021
WBC3	Within Groups	.247	12	.021		
	Total	.669	17			
	Between Groups	15.778	5	3.156	3.787	.027
LYMPH1	Within Groups	10.000	12	.833		
	Total	25.778	17			
	Between Groups	19.111	5	3.822	2.372	.102
LYMPH2	Within Groups	19.333	12	1.611		
	Total	38.444	17			
	Between Groups	8.278	5	1.656	2.292	.111
LYMPH3	Within Groups	8.667	12	.722		
	Total	16.944	17			
	Between Groups	15.778	5	3.156	3.787	.027
NEUT1	Within Groups	10.000	12	.833		
	Total	25.778	17			
	Between Groups	18.278	5	3.656	2.269	.114
NEUT2	Within Groups	19.333	12	1.611		
	Total	37.611	17			
	Between Groups	5.833	5	1.167	1.615	.230
NEUT3	Within Groups	8.667	12	.722		
	Total	14.500	17			
	Between Groups	.009	5	.002	.425	.823
BASO1	Within Groups	.053	12	.004		
	Total	.063	17			
	Between Groups	.009	5	.002	.850	.541
BASO2	Within Groups	.027	12	.002		
	Total	.036	17			
	Between Groups	.004	5	.001	.400	.840
BASO3	Within Groups	.027	12	.002		
	Total	.031	17			
	Between Groups	.018	5	.004	.492	.776
EOS1	Within Groups	.087	12	.007		
	Total	.104	17			
	Between Groups	.251	5	.050	.690	.640
EOS2	Within Groups	.873	12	.073		
	Total	1.124	17			
	Between Groups	.613	5	.123	2.509	.089
EOS3	Within Groups	.587	12	.049		
	Total	1.200	17			
	Between Groups	.032	5	.006	1.425	.284
MONO1	Within Groups	.053	12	.004		
	Total	.085	17			
	Between Groups	.043	5	.009	.128	.983
MONO2	Within Groups	.800	12	.067		
	Total	.843	17			
	Between Groups	.204	5	.041	2.629	.079
MONO3	Within Groups	.187	12	.016		
	Total	.391	17			

Appendix 9: Weekly weight (**g**) changes associated with treatment of alloxan-induced diabetic rats with *Cussonia arborea* extract

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minim
						Lower Bound	Upper Bound	
	1.00	3	100.4667	.55076	.31798	99.0985	101.8348	10
	2.00	3	101.3333	2.30940	1.33333	95.5965	107.0702	10
	3.00	3	102.0000	1.00000	.57735	99.5159	104.4841	10
WKZERO	4.00	3	101.6667	2.88675	1.66667	94.4956	108.8378	10
	5.00	3	102.0000	1.00000	.57735	99.5159	104.4841	10
	6.00	3	101.6667	2.88675	1.66667	94.4956	108.8378	10
	Total	18	101.5222	1.77484	.41833	100.6396	102.4048	10
	1.00	3	102.0000	1.00000	.57735	99.5159	104.4841	10
	2.00	3	101.3333	1.52753	.88192	97.5388	105.1279	10
	3.00	3	102.0000	2.00000	1.15470	97.0317	106.9683	10
WKONE	4.00	3	102.3333	3.21455	1.85592	94.3479	110.3187	10
	5.00	3	98.0000	2.64575	1.52753	91.4276	104.5724	9
	6.00	3	106.3333	1.52753	.88192	102.5388	110.1279	10
	Total	18	102.0000	3.06786	.72310	100.4744	103.5256	9
	1.00	3	102.3333	.57735	.33333	100.8991	103.7676	10
	2.00	3	104.0000	1.00000	.57735	101.5159	106.4841	10
	3.00	3	101.6667	1.15470	.66667	98.7982	104.5351	10
WKTWO	4.00	3	107.0000	2.00000	1.15470	102.0317	111.9683	10
	5.00	3	92.0000	2.00000	1.15470	87.0317	96.9683	9
	6.00	3	117.0000	6.08276	3.51188	101.8896	132.1104	11
	Total	18	104.0000	7.99264	1.88388	100.0254	107.9746	9
	1.00	3	104.6667	1.52753	.88192	100.8721	108.4612	10
	2.00	3	110.6667	2.08167	1.20185	105.4955	115.8378	10
	3.00	3	104.0000	1.00000	.57735	101.5159	106.4841	10
WKTHREE	4.00	3	112.0000	2.64575	1.52753	105.4276	118.5724	11
	5.00	3	90.0000	1.00000	.57735	87.5159	92.4841	8
	6.00	3	128.3333	4.72582	2.72845	116.5938	140.0729	12
	Total	18	108.2778	11.97451	2.82242	102.3230	114.2326	8
	1.00	3	106.0000	4.35890	2.51661	95.1719	116.8281	10
	2.00	3	119.0000	2.64575	1.52753	112.4276	125.5724	11
	3.00	3	106.3333	4.04145	2.33333	96.2938	116.3729	10
WKFOUR	4.00	3	119.0000	4.35890	2.51661	108.1719	129.8281	11
	5.00	3	87.0000	1.00000	.57735	84.5159	89.4841	8
	6.00	3	137.6667	2.08167	1.20185	132.4955	142.8378	13
	Total	18	112.5000	16.23087	3.82565	104.4286	120.5714	8
	1.00	3	108.3333	2.08167	1.20185	103.1622	113.5045	10
	2.00	3	124.3333	5.85947	3.38296	109.7776	138.8891	12
	3.00	3	109.0000	2.00000	1.15470	104.0317	113.9683	10
WKFIVE	4.00	3	122.3333	3.21455	1.85592	114.3479	130.3187	12
	5.00	3	85.6667	1.52753	.88192	81.8721	89.4612	8
	6.00	3	153.3333	4.16333	2.40370	142.9910	163.6756	15
	Total	18	117.1667	21.30244	5.02103	106.5732	127.7601	8
	1.00	3	110.3333	2.30940	1.33333	104.5965	116.0702	10
WKSIX	2.00	3	128.6667	2.08167	1.20185	123.4955	133.8378	12
	3.00	3	111.6667	2.08167	1.20185	106.4955	116.8378	11

	4.00	3	127.6667	1.15470	.66667	124.7982	130.5351	12
	5.00	3	84.0000	1.00000	.57735	81.5159	86.4841	8
	6.00	3	173.6667	1.52753	.88192	169.8721	177.4612	17
	Total	18	122.6667	28.00000	6.59966	108.7426	136.5907	8
	1.00	3	116.6667	2.51661	1.45297	110.4151	122.9183	11
	2.00	3	133.0000	1.00000	.57735	130.5159	135.4841	13
	3.00	3	118.0000	2.00000	1.15470	113.0317	122.9683	11
WKSEVEN	4.00	3	132.6667	2.51661	1.45297	126.4151	138.9183	13
	5.00	3	80.3333	.57735	.33333	78.8991	81.7676	8
	6.00	3	182.6667	3.05505	1.76383	175.0775	190.2558	18
	Total	18	127.2222	31.29660	7.37668	111.6588	142.7857	8
	1.00	3	121.3333	1.15470	.66667	118.4649	124.2018	12
	2.00	3	135.3333	2.51661	1.45297	129.0817	141.5849	13
	3.00	3	122.0000	1.00000	.57735	119.5159	124.4841	12
WKEIGHT	4.00	3	135.6667	1.52753	.88192	131.8721	139.4612	13
	5.00	3	80.6667	.57735	.33333	79.2324	82.1009	8
	6.00	3	193.3333	3.05505	1.76383	185.7442	200.9225	19
	Total	18	131.3889	34.24847	8.07244	114.3575	148.4203	8
	1.00	3	123.6667	3.21455	1.85592	115.6813	131.6521	12
	2.00	3	144.0000	2.00000	1.15470	139.0317	148.9683	14
	3.00	3	125.3333	1.52753	.88192	121.5388	129.1279	12
WKNINE	4.00	3	141.3333	1.52753	.88192	137.5388	145.1279	14
	5.00	3	82.0000	1.00000	.57735	79.5159	84.4841	8
	6.00	3	200.0000	1.00000	.57735	197.5159	202.4841	19
	Total	18	136.0556	36.09868	8.50854	118.1041	154.0070	8
	1.00	3	127.6667	1.52753	.88192	123.8721	131.4612	12
	2.00	3	144.6667	.57735	.33333	143.2324	146.1009	14
	3.00	3	127.0000	1.00000	.57735	124.5159	129.4841	12
WKTEN	4.00	3	146.0000	1.00000	.57735	143.5159	148.4841	14
	5.00	3	80.6667	.57735	.33333	79.2324	82.1009	8
	6.00	3	212.3333	2.08167	1.20185	207.1622	217.5045	21
	Total	18	139.7222	40.15529	9.46469	119.7535	159.6910	8
	1.00	3	129.6667	1.52753	.88192	125.8721	133.4612	12
	2.00	3	153.6667	2.08167	1.20185	148.4955	158.8378	15
	3.00	3	128.3333	.57735	.33333	126.8991	129.7676	12
WKELEVEN	4.00	3	152.6667	2.51661	1.45297	146.4151	158.9183	15
	5.00	3	78.3333	1.15470	.66667	75.4649	81.2018	7
	6.00	3	244.6667	5.03322	2.90593	232.1634	257.1699	24
	Total	18	147.8889	51.45174	12.12729	122.3025	173.4752	7
	1.00	3	134.3333	2.08167	1.20185	129.1622	139.5045	13
	2.00	3	175.0000	4.35890	2.51661	164.1719	185.8281	17
	3.00	3	132.0000	2.64575	1.52753	125.4276	138.5724	13
WKTWELVE	4.00	3	161.3333	1.52753	.88192	157.5388	165.1279	16
	5.00	3	77.3333	.57735	.33333	75.8991	78.7676	7
	6.00	3	249.6667	8.96289	5.17472	227.4016	271.9317	24
	Total	18	154.9444	53.90457	12.70543	128.1383	181.7506	7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WKZERO	Between Groups	4.944	5	.989	.244	.935
	Within Groups	48.607	12	4.051		
	Total	53.551	17			
WKONE	Between Groups	106.000	5	21.200	4.711	.013
	Within Groups	54.000	12	4.500		
	Total	160.000	17			
WKTWO	Between Groups	990.667	5	198.133	24.940	.000
	Within Groups	95.333	12	7.944		
	Total	1086.000	17			
WKTHREE	Between Groups	2361.611	5	472.322	74.577	.000
	Within Groups	76.000	12	6.333		
	Total	2437.611	17			
WKFOUR	Between Groups	4345.167	5	869.033	78.213	.000
	Within Groups	133.333	12	11.111		
	Total	4478.500	17			
WKFIVE	Between Groups	7569.167	5	1513.833	124.995	.000
	Within Groups	145.333	12	12.111		
	Total	7714.500	17			
WKSIX	Between Groups	13290.667	5	2658.133	854.400	.000
	Within Groups	37.333	12	3.111		
	Total	13328.000	17			
WKSEVEN	Between Groups	16596.444	5	3319.289	728.624	.000
	Within Groups	54.667	12	4.556		
	Total	16651.111	17			
WKEIGHT	Between Groups	19898.944	5	3979.789	1155.423	.000
	Within Groups	41.333	12	3.444		
	Total	19940.278	17			
WKNINE	Between Groups	22110.944	5	4422.189	1263.483	.000
	Within Groups	42.000	12	3.500		
	Total	22152.944	17			
WKTEN	Between Groups	27392.944	5	5478.589	3521.950	.000
	Within Groups	18.667	12	1.556		
	Total	27411.611	17			
WKELEVEN	Between Groups	44923.778	5	8984.756	1347.713	.000
	Within Groups	80.000	12	6.667		
	Total	45003.778	17			
WKTWELVE	Between Groups	49170.278	5	9834.056	520.626	.000
	Within Groups	226.667	12	18.889		
	Total	49396.944	17			