

EFFECT OF FRACTION I OF *Abrus precatorius* SEED METHANOL
EXTRACT ON PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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TITLE PAGE

EFFECT OF FRACTION I OF *Abrus precatorius* SEED METHANOL EXTRACT ON
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CERTIFICATION

I certify that this research work titled "Effect of Fraction I of *Abrus Precatorius* Seed methanol extract on Paracetamol-Induced Liver Damage in Rats" was carried by Ukegbu Chimere Y, (PG/M.Sc./12/62883) under my supervision in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka.

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DEDICATION

This research work is dedicated to God, the Source of my strength, inspiration and wisdom.

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ABSTRACT

The aim of this study was to investigate the prophylactic and curative effects of an alkaloid-rich fraction of *Abrusprecatorius* seedchloroform-methanol extract on paracetamol-induced hepatotoxicity in rats. The percentage yield of the methanol extract of *Abrusprecatorius* seeds was found to be 2.08% w/w. Further purification of the extract using Sephadex gel G15 to get a purer sample was done. The fractions were spotted on a TLC plate and was spread with Drangendoff's reagent in which some fractions turned purple indicating the presence of alkaloids. The fractions that turned purple were pulled into a beaker and called fraction I which gave a percentage yield of 17.75% and was used in this study. The qualitative phytochemical analysis of fraction I revealed a wide range of phytochemicals such as alkaloids, flavonoids, saponins, glycosides, tannins and carbohydrates, steroids, terpenoids, and peptides which could be physiologically potent in ameliorating several diseases. The quantitative phytochemical analysis of fraction I of *Abrusprecatorius* seed methanol extract showed the presence of alkaloids (5480 ± 184 mg/100g), flavonoids (215 ± 97 mg/100g), saponins (2.98 ± 1.33 mg/100g) and tannins (6.4 ± 0.72 mg/100g). Hepatotoxicity was induced using paracetamol (2500 mg/kg b.w.) orally. For prophylactic treatment (hepato-protective), administration of extract was done for 7 days before paracetamol induction and collection of blood was done after 24 hours of administration. Curative treatment (hepato-curative) was done after paracetamol induction at day 0 and treatment was done for 14 days. Blood was collected on days 8 and 15 for the analyses. Prophylactic and curative treatments with fraction I of *Abrusprecatorius* methanol extract at the dose of 100 and 200mg/kg b.w for group 4 and 5 produced a significant decrease ($p < 0.05$) in the activities of the liver marker enzymes (ALP, AST, ALT) and bilirubin levels in a dose- and time-dependent manner compared to the paracetamol untreated group 2 (positive control). Groups 3, 4, and 5 treated with 100 mg/kg b.w. silymarin (standard hepato-protective and curative drug), 100mg/k.g. b.w. of fraction I and 200mg/k.g. b.w. of fraction I respectively before and after paracetamol induction caused a significant decrease ($p < 0.05$) in the serum urea and creatinine concentrations of both hepato-protective and hepato-curative groups compared to the positive control. Serum electrolyte concentrations showed a significant increase ($p < 0.05$) in the treated groups of both hepato-protective and curative when compared to the positive control. The MDA concentration decreased significantly ($p < 0.05$) in the treated groups and standard groups compared to the positive control after 24 hours (hepato-protective) and at day 8 and 15 (hepato-curative). Serum SOD activity of both protective and curative models, showed a dose- and time-dependent significant increase ($p < 0.05$) in the treated groups compared to the positive control. The haematological parameters of the rats treated with fraction I of *Abrusprecatorius* methanol extract at various doses showed a significant increase ($p < 0.05$) in the PCV levels, Hb concentration and RBC count compared to the positive control. A dose- and time-dependent significant decrease ($p < 0.05$) was observed in the WBC count of all treated groups (hepato-protective and hepato-curative) compared to the positive control. The test groups that received fraction I of *Abrusprecatorius* in both models showed a dose- and time-dependent effects on the biochemical markers used in the study similar to the standard drug. However, fraction I had more curative effect than protective but silymarin was more potent.

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CHAPTER ONE

1.0 INTRODUCTION

Plants, the first medicine of human being, have played a remarkable role in health care since the ancient times. Traditionally plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to the discovery of new drugs for a variety of diseases that threatens human health. Plants are the rich sources of organic compounds, many of which have been used for medicinal purposes. Medicinal plants are the plants whose parts (leaves, seeds, stems, roots, fruits, foliage etc), extracts, infusions, decoctions or powders are used in the treatment of different diseases of humans, plants and animals (Nweze *et al.*, 2004). There is a wide spectrum of trees, plants and shrubs whose seeds, roots, barks and leaves are used by humans throughout the globe due to their nutritional or medicinal value (Doughari *et al.*, 2009). The importance of herbs in the management of human ailments cannot be over emphasized.

Herbs play a major role in the management of various liver disorders along with other system associated diseases (Ebenyi *et al.*, 2012). Medicinal plants such as *Aloe vera*, *Eclipta alba*, *Phyllanthus niruri*, *Solanum Indicum*, *Maytenus emerginata* and *Aegle mameleoes* are well known for their hepato-protective effects (Parmar *et al.*, 2010). *Abrus precatorius* Linn is a leguminous plant of the Fabaceae family. Its seeds, roots and leaves are widely used for medicinal purposes in Africa and Asia (Yadava and Reddy, 2002). In Nigeria, the Igbos use the aqueous decoction of the seeds to treat a wide range of conditions including ulcer, infections, hypertension, diarrhoea, infarct and ogbanje (Nwodo and Alumanah, 1991).

1.1. General Description of *Abrus precatorius* Linn

Abrus precatorius, which belongs to the family of fabaceae is a plant that originated from Southeast Asia and now can be found in subtropical climate areas such as India, Sri Lanka, Thailand, the Philippine Islands, South China, Tropical Africa and the West Indies (Vavaprasad and Varahalarao, 2009). It is a slender, perennial climber that twines around trees, shrubs, and hedges. The leaves are pinnate and glabrous, with many leaflets (12 or more) arranged in pairs. Flowers are small and pale violet in colour with a short stalk, arranged in clusters. The plant is best known for its seeds, which are used as beads and in percussion instruments, and which are

toxic due to the presence of abrin. The plant is native to Indonesia and grows in tropical and subtropical areas of the world where it has been introduced. It has a tendency to become weedy and invasive where it has been introduced.

1.1.1 An Overview of *Abrus precatorius* Seed

The name *Abrus*, meaning beautiful or graceful, used to describe the appearance of the seed. The seed is found in a variety of colours such as black, brown, white and most commonly, red with a glossy appearance with the black band at the end that attaches to the plant. The *Abrus precatorius* seed is known by a variety of names that include jequirity (India), Crab's eye (Guam), Rosary pea (Egypt), Precatory peabean (USA), Indian Liquorice (Nigeria) and Giddee Giddee or Jumbie bead in Trinidad and Tobago (Hartley, 2010). The seeds of *Abrus precatorius* are much valued in native jewelry for their bright coloration. Most beans are black and red, suggesting a ladybug, though other colors are available. The Tamils use *Abrus* seeds of different colors. The red variety with black eye is the most common, but there are black, white and green varieties as well. The seeds of *Abrus precatorius* are very consistent in weight. Formerly Indians used these seeds to weigh gold using a measure called a Ratti, where 8 Ratti = 1 Masha; 12 Masha = 1 Tola (11.6 Grams) (Hartley, 2010).



Figure 1: *Abrus precatorius* seeds

(Source: Ali and Malek, 1996)

1.1.2 Taxonomy of *Abrus precatorius* Linn

Table 1: Taxonomic Hierarchy of *Abrus precatorius*

Kingdom	Plantae ĩ plantes, planta, vegetal, plants
Subkingdom	Viridaeplantae ĩ green plants
Infrakingdom	Streptophyta ĩ land plants
Division	Tracheophyta ĩ vascular plants, tracheophytes
Subdivision	Spermatophytina - spermatophytes, seed plants, phenerogames
Infradivision	Angiospermae ĩ flowering plants, angiosperms
Class	Magnoliopsida
Superorder	Rosanae
Order	Fabales
Family	Fabaceae ĩ peas, legumes
Subfamily	Faboideae
Genus	<i>Abrus</i>
Specie	<i>Abrus precatorius</i> Linn ĩ rosarypea, crab's eye, jeguerity, precatory bean, giddee giddee, Indian liquorice.

(Source; Ali and Malek, 1996)

1.1.3 Importance of *Abrus precatorius* Linn in Traditional Medicine

In the Ayurvedic medicine, leaves of *Abrus precatorius* are used as laxative, expectorant and aphrodisiac medicines known as Coqê eye. Seeds are said to be purgative, emetic, tonic, antiphlogistic, aphrodisiac and antiophthalmic. For the indigenous people they are potent phytomedicines, many of them in mixture with other plants. Their toxicity is underestimated (Anant and Maitreyi, 2012)

In Tanzania, traditional healers claim the competence in the treatment of epilepsy. *Abrus precatorius* can be found between 60 plants commonly used against this illness (Moshi *et al.*, 2005). Also in Zimbabwe, extracts of 58 plants popularly known to be effective against schistosomiasis were tested in vitro against excysted cysticercoids. Extracts of stem and root of *Abrus precatorius* were under the ten most effective samples (Ndamba and Nyazema, 1994).

In China, the herb of *Abrus precatorius* is used as a folk-medicine for the treatment of bronchitis, laryngitis and hepatitis. Because of their platelet inhibiting activity, abruquinones are suspected to be the active substances (Kuo *et al.*, 1995).

The leaf of *Abrus precatorius* has been used in Nigeria for the treatment of myriad of diseases including malaria, typhoid, cough, respiratory tract infections and hepatitis (Saganuwan and Onyeyili, 2010). The leaves are also considered useful in biliousness and in leucoderma, itching and other skin diseases. Its juice is employed as a cure for hoarseness, mixed with oil and applied to painful swellings. Dried leaves paste are used as a germicide to wounds in cattle. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. Usually seeds are of two types one is scarlet with black spot and the other variety is pure white and traditionally used against leucoderma, wounds, alopecia, asthma, tubercular glands, leprosy, fever, ulcer and tumor. Seeds of *Abrus precatorius* Linn are applied locally in sciatica, stiffness or shoulder joint and paralysis. It is useful in dysentery and skin diseases (Anant and Maitreyi, 2012).

Roots of *Abrus precatorius* Linn are used as diuretics and in the preparations prescribed for gonorrhoea, jaundice and haemoglobinuric bile. Some of the parts are used in night blindness,

inflamed gums, muscular pain and convulsion. It is also used for pain relief in groins, mucus in urine and grave land bone fracture in cattle (Anant and Maitreyi, 2012).

1.1.4 Pharmacological Uses of *Abrus precatorius*

The seed extract of *abrus precatorius* have also been shown to possess several pharmacological properties. It has been shown to have antifertility effect (Rao, 2007); ureterotonic effect and antidiarrhoeal effect (Nwodo, 1991). Aqueous extract of *A. precatorius* seeds has also been shown to exert antimicrobial activities (Desai and Sirsi, 1966).

It has been reported that Methanol extracts of *Abrus precatorius* exhibited antibacterial activity towards almost all the bacterial microorganisms (*Klebsilla pneumonia*, *staphylococcus aureus*, *streptococcus mitis* and *Micrococcus luteus*) used in the study (Varaprasad and Varahalarao, 2009). Also, *Abrus precatoriushas* Abortifacient effect (Sethiet *al.*, 1990); Agglutinin activity (Lin *et al.*, 1981); Cytotoxic activity (Desai *et al.*, 1966); Anti-inflammatory activity (Anam, 2001); Insecticidal activity (Hartzell and Wilcoxon, 1941); Hemagglutinin activity (Khan *et al.*, 1966); Spermicidal effect (Rajeshwari, 2011); Uterine relaxation effect and Uterine stimulant effect amongst many others (Nwodo and Botting, 1983). Although, *Abrus precatorius* has been shown to have so many pharmacological activities, the presence of toxic lectins in its seed limits its pharmacological utility.

1.1.5 Toxicity of *Abrus precatorius* Linn

Abrus precatorius beans are known as one of the most toxic plant parts worldwide. The human fatal dose is estimated as 0.1-1 µg/kg (Monago and Alumanah, 2005). The toxicity of the *Abrus* seed is associated with the presence of the toxic component, Abrin (a type of toxalbumin) which is a mixture of at least five lectins, abrin A ï D, and *Abrus*-agglutinin (Chaudhari *et al.*, 2012). The abrins consist of two peptide chains connected by a disulfide bridge.

Abrin A consists of an A-chain with N-glycosidase activity, which inhibits protein synthesis, and lectin-like B-chain responsible for binding with cell-surface receptors and penetrating of abrin-A molecule into the cell (Ohba and Morowaki, 2004). The relative molecular weight of abrin A and

C are around 64.000Da, that of two agglutinins 128.000Da (Hegde *et al.*, 1991). For further identification, the crystal structure was investigated. The abrin A crystals belong to the monoclinic space group P 2 (Tahirov, 1994). The sequence of amino acids of the B-chain in both abrin-A and abrin-B were elucidated by enzymatic digestion with trypsin. They consist of 268 amino acids and share 256 identical residues (Komira *et al.*, 1993). This chemical structure is assumed to be responsible for its toxic effects.

Abrins disarrange the protein biosynthesis by interfering with the 60 S-ribosomes of animal cells irreversibly. The toxicity of these abrin is variable, but they are the most potent toxins of the world, comparable with the botulinus toxin. The fifth of them, Abrus agglutinin, is not very toxic against cells, but it exhibits agglutination toward animal erythrocytes. Also it has been shown that Abrus agglutinin causes a total haemolysis in blood groups followed by a haemorrhagic gastroenteritis (Khan *et al.*, 1966).

Fatal incidents have been reported following ingestion of well-chewed seeds of *Abrus precatorius*. It has also been reported that poisoning has been experienced through a finger prick when stringing the seed. Symptoms of seed poisoning include severe gastroenteritis with pronounced nausea and vomiting, muscular weakness, tachycardia, cold sweat, bloody diarrhoea, dyspnoea, dehydration, loss of condition and recumbence (Anant and Maitreyi, 2012).

There is no physiological antidote. The treatment is essentially symptomatic. Since there is a long latent period associated with abrin poisoning, little value can be placed on induction of emesis or gastric lavage; these measures are useful only if ingestion has just occurred. Bismuth trisilicate may be given during poisoning by *Abrus precatorius* to reduce the level of gastrointestinal damage. If the emesis and/or diarrhoea become excessive, replacement fluids and electrolytes are advocated. If haemorrhage occurs, blood transfusion may be necessary (Khan *et al.*, 1966).

1.2.0 PHYTOCHEMICALS

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans (Mamta *et al.*, 2013). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from

environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Narasinga, 2003). Phytochemistry is the study of natural bioactive products found in plants that work with nutrients and dietary fibre to protect against diseases (Doughari *et al.*, 2009). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Mathai, 2000).

These phytochemicals are present in a variety of plants utilized as important components of both human and animal diets, and they are found in different parts of the plant which include; fruits, flower, bark seeds, root and stem (Tiwari *et al.*, 2011). They are chemical compounds formed during the plant normal metabolic processes. These chemicals are often referred to as 'secondary metabolites' of which there are several classes including alkaloids, flavonoids, glycosides, gums, coumarins, polysaccharides, phenols, tannins, terpenes and terpenoids .

1.2.1 Alkaloids

Alkaloids are natural products that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the 'alkaline' and it was used to describe any nitrogen-containing base (Mueller-Harvey and McAllan, 1992). These are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen.

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allopathically active chemicals (Molyneux *et al.*, 1996). The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, sardine), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, and vinblastine). These are just a few examples illustrating the great economic

importance of this group of plant constituents (Wink *et al.*, 1998). Some alkaloids have stimulant property as caffeine and nicotine, morphine is used as the analgesic and quinine as the antimalarial drug (Rao *et al.*, 1978).

1.2.2 Flavonoids

Flavonoids are an important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C₁₅ aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers (Kar, 2007). The compounds are derived from parent compounds known as flavans. They are organic compounds that have no direct involvement with the growth or development of plants, they are plant nutrients that when consumed in fruits and vegetables pose no toxic effect on humans, and are also beneficial to the human body. Flavonoids are poly-phenolic compounds that are ubiquitous in nature (Harborne and Baxter, 1999). More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960).

Flavonoids can be classified into five major sub groups, these include; flavones, flavonoids, flavanones, flavonols and anthocyanidines (Nijveldt *et al.*, 2001). Flavones are characterized by a planar structure because of a double bond in the central aromatic ring. Quercetin, one of the best described, is a member of this group. Quercetin is found in abundance in onions, apples, broccoli and berries. Flavonones are mainly found in citrus fruit, an example is naringin. Flavonoid is involved in scavenging of oxygen derived free radicals (Harborne and Baxter, 1999). It has been identified as a potent hypolipidemic agent in a number of studies (Tapas *et al.*, 2008). It has also been established that flavonoids from medicinal plants possess a high antioxidant potential due to their hydroxyl groups and protect more efficiently against free radical related diseases like arteriosclerosis (Kris-Etherton *et al.*, 2002).

1.2.3 Tannins

Tannins are polymerized phenols with defensive properties. Their name comes from their use in tanning, rawhides to produce leather. In tanning, collagen proteins are bound together with phenolic groups to increase the hide's resistance to water, microbes and heat (Hans-Walter and Fiona, 2005). Two categories of tannins that are of importance are the condensed and

hydrolysable tannins. Though widely distributed, their highest concentration is in the bark and galls of oaks (Hans-Walter and Fiona, 2005). They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group (Kar, 2007).

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity, and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996). One of their biological actions is to compete with proteins through non-specific forces such as hydrogen bonding and hydrophobic interactions, as well as by covalent bond formation (Haslam, 1996). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope, transport proteins etc.

1.2.4 Steroids

Sterols are triterpenes which are based on the cyclopentane hydrophenanthrene ring system (Harborne, 1998). Sterols were at one time considered to be animal substances (similar to sex hormones, bile acids, etc) but in recent years, an increasing number of such compounds have been detected in plant tissues. Sterols have essential functions in all eukaryotes. For example, free sterols are integral components of the membrane lipid bilayer where they play an important role in the regulation of membrane fluidity and permeability (Galm and Shen, 2007). While cholesterol is the major sterol in animals, a mixture of various sterols is present in higher plants, with sitosterol usually predominating. Sterols in plants are generally described as phytosterols with three known types occurring in higher plants: sitosterol (formerly known as β -sitosterol), stigmasterol and campesterol (Harborne, 1998).

1.2.5 Glycosides

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxyl (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemi-acetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colorless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phyto-constituents, found in the cell sap. Chemically, glycosides contain a carbohydrate

(glucose) and a non-carbohydrate part (aglycone or genin) (Kar, 2007). Glycosides are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action (Firn, 2010).

1.3 Acetaminophen (Paracetamol)

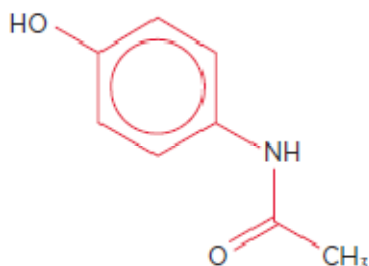


Fig 2: *N*-(4-hydroxyphenyl)acetamide (Acetaminophen) (Macintyre *et al.*, 2008).

Acetaminophen chemically known as *N*-acetyl-*p*-aminophenol, is a widely used analgesic and antipyretic agent with little anti-inflammatory effect (McDaid *et al.*, 2010). Acetaminophen is a white, odorless, crystalline powder with a slightly bitter taste. It has a molecular formula of $C_8H_9NO_2$ and molecular weight of 151.16 g. It is the most widely used drug for pain relief. Paracetamol is the International Non-proprietary Name (INN) and British Approved Name (BAN), while acetaminophen is the United States Adopted Name (USAN) and Japanese Adopted Name (JAN) (Macintyre *et al.*, 2008).

Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity (Macintyre *et al.*, 2008).

1.3.1 Pharmacokinetics

In order for increase effectiveness, paracetamol can be administered rectally, orally and intravenously. While all three mode of administration can achieve adequate plasma concentrations, there are differences in absorption and time to reach the plasma peak levels. With rectal administration, absorption can be unpredictable with bioavailability ranging from 24% to 98% varying with factors such as formulation of suppositories number used and the particle size of the paracetamol (McDaid *et al.*,2010). Oral bioavailability is dose dependant: with larger doses, the hepatic first pass effect is reduced due to overwhelming of the liver enzymatic capacity; and therefore, bioavailability is increased. In this case, bioavailability is inconsistent and in overall reduced, due to incomplete dissolution of the suppository in the rectum. The absorption rate through this route of administration is elongated.

The analgesic activity is attributable to the small fraction that penetrates into the brain(McDaid *et al.*,2010). Paracetamol given at therapeutic doses binds to plasma proteins at less than 20%. In case of intoxication, this proportion may increase to up to 50%(Huber *et al.*, 2009). Paracetamol is essentially metabolized in the liver by conjugation with glucuronic acid (55%) and sulfuric acid (35%). Hepatotoxic metabolites are produced in small amounts by the cytochrome P450 (isoenzyme CYP2E1). In the therapeutic plasma concentration range, this metabolite is detoxified by conjugation with glutathione(Macintyre *et al.*, 2008). In case of intoxication the amount of this toxic metabolite increases and outweighs the amount of available glutathione, which can lead to hepatic failure and renal tubular necrosis. Metabolites are excreted through the kidneys in the urine. Only 2-5% of the dose is excreted in an unchanged form in the urine. As a consequence of its short elimination half-life (1-3h), 24 hours after the ingestion of a single dose of paracetamol, 98% of the dose is eliminated(McDaid *et al.*,2010).

1.3.2 Metabolism of paracetamol.

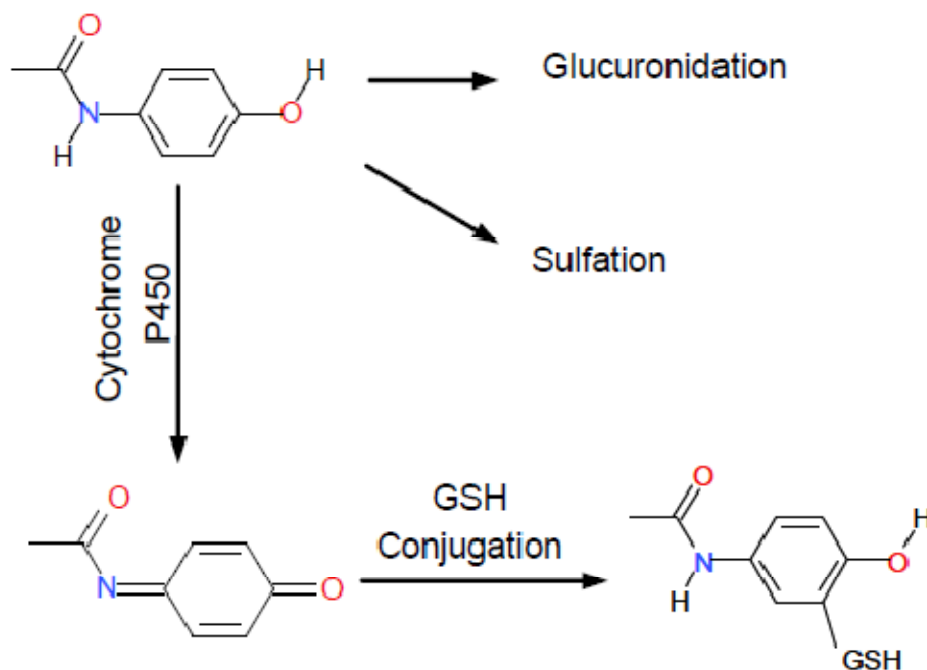


Fig. 2: Metabolism of paracetamol, Source: (Huber *et al.*, 2009)

Paracetamol is metabolised primarily in the liver through three metabolic pathways into toxic and non-toxic products. These pathways are glucuronidation, sulfation and N-hydroxylation (Huber *et al.*, 2009)

- Glucuronidation is believed to account for 40% to two-thirds of the metabolism of paracetamol.
- Sulfation (sulfate conjugation) may account for 20% to 40%.
- N-hydroxylation and rearrangement, then GSH conjugation, accounts for less than 15%. The hepatic cytochrome P450 enzyme system metabolises paracetamol, forming a minor yet significant alkylating toxic metabolite known as NAPQI (*N*-acetyl-*p*-benzo-quinone imine)(also known as N-acetylimidoquinone) NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione(Macintyre *et al.*, 2008).

1.3.3 Mechanism of action

Acetaminophen, also known as paracetamol, is a non-steroidal anti-inflammatory drug with potent antipyretic and analgesic actions but with very weak anti-inflammatory activity. When administered to humans, it reduces levels of prostaglandin metabolites in urine but does

not reduce synthesis of prostaglandins by blood platelets or by the stomach mucosa. Paracetamol has long been suspected of having a similar mechanism of action with aspirin due to their similarity in structure. Because acetaminophen is a weak inhibitor *in vitro* of both cyclooxygenase (COX) 1 and COX-2, the possibility exists that it inhibits a so far unidentified form of COX, perhaps COX-3 (Graham and Scott, 2005). In animal studies, COX enzymes in homogenates of different tissues vary in sensitivity to the inhibitory action of acetaminophen. This may be evidence that there are 12 isoforms of the enzyme. Recently, a variant of COX-2 induced with high concentrations of non-steroidal anti-inflammatory drugs was shown to be highly sensitive to inhibition by acetaminophen. Therefore COX-3 may be a product of the same gene that encodes COX-2, but have different molecular characteristics (Dong *et al.*, 2000).

Much investigation has centered on paracetamol inhibition of the COX enzyme because its analgesic and antipyretic effects are similar to those of aspirin, the archetype NSAID. However, paracetamol does not have significant anti-inflammatory activity nor does it inhibit production of the pro-clotting TXAs. Paracetamol does not appear to have a major effect peripherally, but its action appears to be mostly central. It seems reasonable to assume that although there may be some effect on COX enzyme, this effect is different from that seen with the NSAIDs (Graham and Scott, 2005).

1.3.4 Toxicity of Paracetamol

Hepatotoxicity is a direct liver injury caused by the toxic metabolite of acetaminophen N-acetyl-p-benzoquinone imine (NAPQI). Acetaminophen is considered a predictable hepatotoxin, where biochemical signs of liver damage will become apparent within 24 to 48 hours after the time of overdose and produce a dose-related centrilobular necrosis in the liver (Laura *et al.*, 2003).

When taken in therapeutic doses, greater than 90% of acetaminophen is metabolized to phenolic glucuronide and sulfate in the liver by glucuronyltransferases and sulfotransferases and subsequently excreted in the urine. Of the remaining acetaminophen, about 2% is excreted in the urine unchanged; approximately 5% to 10% is metabolized by cytochrome P450, mainly the enzyme CYP2E1, to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive, electrophilic molecule that causes harm by formation of covalent bonds with other intracellular proteins. This

reaction is prevented by conjugation with glutathione and subsequent reactions to generate a water-soluble product that is excreted into bile(Kanchana andSadiq, 2011).

After an overdose of paracetamol, elevated levels of the toxic NAPQI metabolite are generated, which extensively deplete hepatocellular GSH and covalently modify cellular proteins resulting in hepatocyte death (Galal *et al.*, 2012). With acetaminophen overdose, glucuronyltransferases and sulfotransferases are saturated, diverting the drug to be metabolized by cytochrome P450 and generating NAPQI in amounts that can deplete glutathione. If glutathione is not replenished, NAPQI will begin to accumulate in the hepatocytes.NAPQI can form covalent bonds with cellular proteinsand modify their structure and function resulting in inhibition of enzymatic activities (Prescott *et al.*,2006). Two of the enzymes that have been shown to be inhibited in paracetamol treated animals are glutathione peroxidase and thiol transferase. Inhibition of these enzymes renders the cell vulnerable to endogenous activated oxygen species with further oxidation of protein thiols (Prescott *et al.*,2006). Also this cellular disturbance leads to a decrease in calcium ATPase activities and an increase in levels of cytosolic calcium. Abnormal cellular calcium homeostasis can alter the permeability of the cell, causing the formation of blebs in the cellmembrane and loss of membrane integrity(Dong *et al.*, 2000).

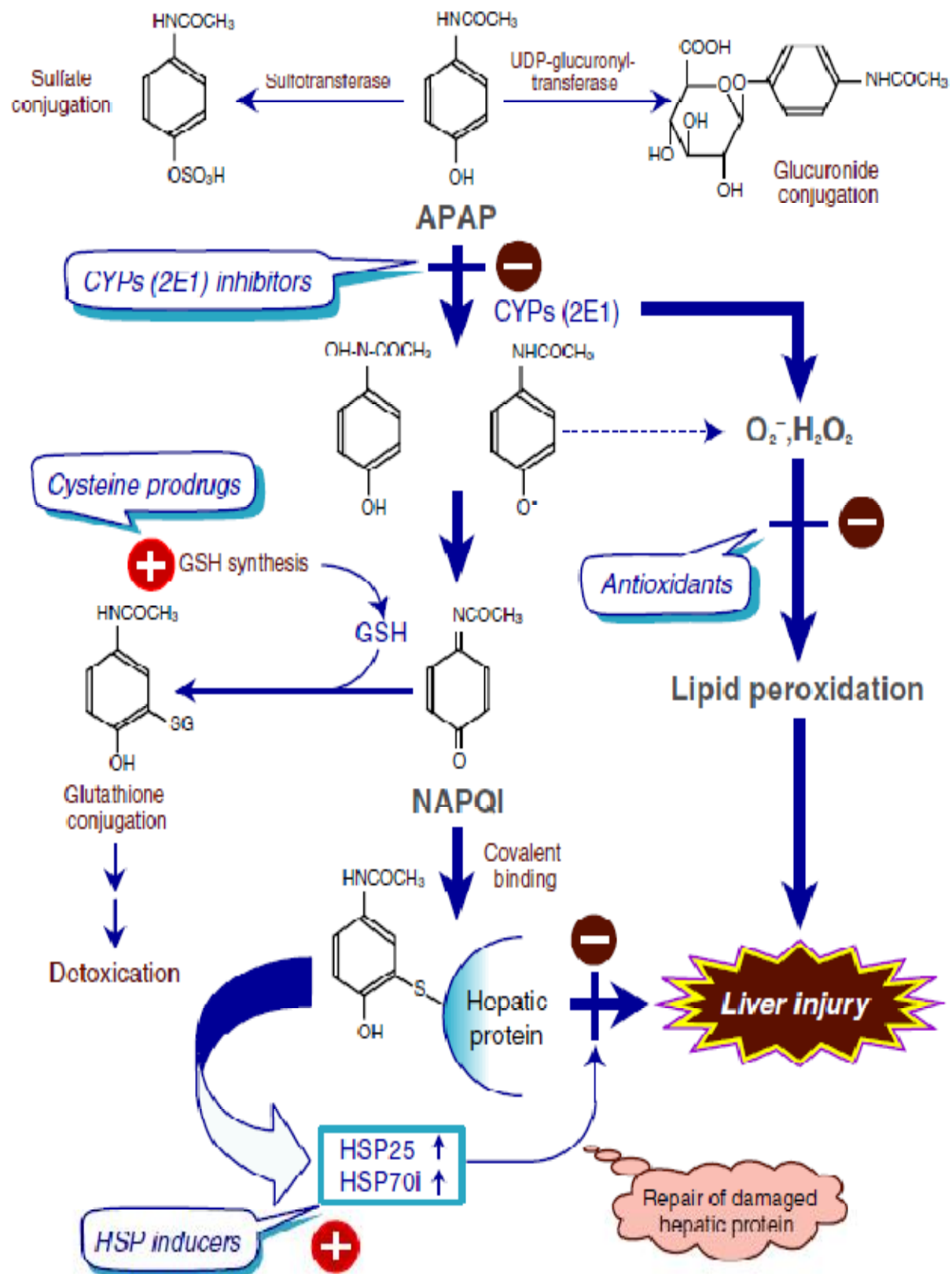


Fig 3; Toxic reactions of paracetamol (Source: Isao *et al.*, 2004)

1.4The Liver.

The liver is the largest internal organ of the body weighing approximately 1.5kg in adults. It is a vital organ present also in vertebrates and some other animals. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. The liver has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver is a highly specialized tissue that regulates a wide range of vital biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Song *et al.*, 2001).

1.4.1 Anatomy/Physiology of the Liver

The body of the liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44 to 1.66 kg, and is a soft, pinkish-brown, triangular organ (Cotran *et al.*, 2005). It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder. It is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries, which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. Lobules are the functional units of the liver (Rajiv *et al.*, 2012).

The liver plays a major role in metabolism which includes biosynthesis, degradation and storage of biochemical compounds. The various functions of the liver are carried out by the liver cells or hepatocytes. The liver performs over 500 metabolic functions, resulting in synthesis of products

that are released into the blood stream(e.g. glucose derived from glycogenesis, plasma proteins, clotting factors and urea),or that are excreted to the intestinal tract (bile) (Song *etal.*, 2001).

1.4.2 Liver Intoxication (Hepatotoxicity)

This implies a chemical ÷ driven liver damage. The liver plays a central role in transforming and cleaning chemicals and is susceptible to the toxicity from these agents. Certain mechanical agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ (Pablo *etal.*, 1992). Other chemical agents such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. The liver is a major organ for metabolism of foreign substances and also functionally interposed between the site of reabsorption and the systemic circulation. These conditions render the liver not only the most important organ for detoxification of foreign substances but also a major target of their toxicity. More than 1000 drugs have been associated with idiosyncratic hepatotoxicity (Chau, 2008). Moreover, drug ÷ induced hepatotoxicity contributes more than half of the cases of acute liver failure with paracetamol being the principal offending agent in western countries (Chau, 2008). Hepatotoxicity may be predictable or unpredictable. Predictable reactions are typical and occur with short latency (within a few days) after some threshold toxicity is reached. Paracetamol (acetaminophen) is a classic example. Conversely, idiosyncratic reactions occur with variable, sometimes prolonged latency (1 week to 1 year), with low incidence and may not be dose-related (Chau, 2008).

1.4.3 Mechanism of liver damage

Drugs continue to be taken off the market due to late discovery of hepatotoxicity. Due to its unique metabolism and close relationship with the gastro ÷ intestinal tract, the liver is susceptible to injury from drugs and other substances. Seventy-five percent (75%) of blood coming to the liver arrives directly from gastrointestinal organs and spleen via portal veins which bring drugs and xenobiotics in concentrated form (Song *etal.*, 2001). Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. For instance, in the case of paracetamol-induced liver damage, when the concentration of the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) generated from paracetamol metabolism exceeds that of the glutathione store, the toxic metabolite begins to react with other intracellular macromolecules

thereby causing damage to the liver cells (Galal *et al.*, 2012). Many other chemicals damage the mitochondria, an intracellular organelle that produces energy especially when their concentration exceeds that of the antioxidant level in the liver. Injury to the hepatocytes and bile duct could lead to accumulation of bile acids inside the liver. This promotes further liver damage.

1.5 Liver Function Tests

Liver function tests a broad range of normal functions performed by the liver. The diagnosis of liver disease depends upon a complete history, complete physical examination and evaluation of liver function test and further invasive and non-invasive tests (Rajiv *et al.*, 2012). The liver performs different kinds of biochemical, synthetic and excretory functions of the liver. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. Under normal circumstance, these enzymes are resided in the cells of the liver. But when the liver is injured these enzymes are spilled into the blood stream (Sultana *et al.*, 2004). Among the most sensitive and widely used of these liver enzymes are the aminotransferases (ALT). These enzymes are normally contained within liver cells. When the liver is injured, the cells spill the enzymes into the blood stream, raising the enzymes level in the blood and signaling their damage (Rajiv *et al.*, 2012).

1.5.1 Alkaline phosphatase (ALP)

Alkaline phosphatases are a family of zinc metalloenzymes, with a serine residue at the active centre; they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues (Thapa and Anuj, 2007). ALP is produced in the lower bile duct, bone and gut and is widely distributed in the body. In liver, alkaline phosphatase is found histo-chemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes. In liver, two distinct forms of alkaline phosphatase are also found but their precise roles are unknown. ALP is a hydrolase enzyme responsible for removing phosphate group from many types of molecules, including nucleotides, protein and alkaloids. Alkaline phosphatase lines the cells in the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. It is present in the bone and placenta, so it is higher in growing children (as their bones are being remodelled) and elderly patients with Paget's disease (Manson, 2004). Elevations occur as a result of both intrahepatic and extra-

hepatic obstruction to bile flow. ALP is also raised in cirrhosis and liver cancers, but levels can be within the reference range or with a slight increase in acute hepatitis. The normal range is 39-120IU/L.

1.5.2 Aspartate aminotransferase

Aspartate aminotransferase (AST) is more widely distributed than ALT. It is present in the liver, heart, kidneys, skeletal muscle and red blood cells. AST levels are raised in shock. It is less specific for liver disease and is not included in liver function profile by all laboratories because the enzyme is not localized in the liver. AST levels are also raised in pregnancy and after exercise. Ratios between ALT and AST are useful to physicians in assessing the etiology of liver enzyme abnormalities and also useful in differentiating between causes of liver damage (Manson, 2004). ALT exceeds AST in toxic hepatitis, chronic active hepatitis and cholestatic hepatitis. The ratio is characteristically elevated in alcoholic liver disease (Thapa and Anuj, 2007). The AST and ALT levels are increased to some extent in almost all liver diseases. The highest elevations occur in severe viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock.

1.5.3 Alanine aminotransferase

The enzyme ALT is present in high concentration in the liver. It is also found in cardiac and skeletal muscle (Manson, 2004). However, ALT is considered as a specific marker of hepatocellular damage because levels are generally only significantly raised in liver damage. ALT is also found in the heart and muscles in much lower concentrations; only marginal elevations occur in acute myocardial infarction. People with acute liver damage have particularly high ALT levels and those with chronic liver disease and obstructive jaundice have more modestly raised levels. Low ALT (and AST) levels suggest vitamin B6 deficiency. The levels of ALT abnormality are increased in conditions where cells of the liver have been inflamed or undergone cell death. As the hepatocytes are damaged, the ALT leaks into the blood stream leading to a rise in the serum level (Manson, 2004). Any form of hepatic cell damage can result in an elevation in the ALT. ALT is the most sensitive marker for liver cell damage (Manson, 2004). Elevations are often measured in multiples of the upper limit of normal (ULN). Reference range 5 to 40 IU/L (Reitman and Frankel, 1957).

1.5.4 Bilirubin

Bilirubin is an endogenous anion derived from haemoglobin degradation from the red blood cell (RBC). The classification of bilirubin into direct and indirect bilirubin is based on the original van der Beigh method of measuring bilirubin. Bilirubin is a yellow fluid produced in the liver when worn out red blood cells are broken down at the end of their 120 day lifespan (Manson, 2004). Bilirubin is a major product of hemoglobin. During splenic degradation of red blood cells, hemoglobin is separated out from iron and cell membrane components. Haemoglobin is transferred to the liver where it undergoes further metabolism in a process called conjugation. Conjugation allows hemoglobin to become more water soluble. The water insoluble bilirubin will be excreted into bile (Rajiv *et al.*, 2012). In the blood, unconjugated (or indirect) bilirubin is carried by albumin to the liver. It is conjugated to make it more water soluble, before it is excreted in bile. Conjugated bilirubin is also called direct bilirubin. The concentration of bilirubin in the serum therefore reflects the balance between the amount produced by erythrocyte destruction and that removed by the liver. As the liver becomes irritated, the total bilirubin may rise.

1.6 Kidney Function Tests

The kidney is bean shaped organ that serve several essential regulatory roles in vertebrate animals. They are essential in the urinary system and also serve homeostatic functions such as regulation of electrolytes, maintenance of acid base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove water soluble wastes, which are diverted into the urinary bladder. Kidney function tests are series laboratory tests that are done to ensure the functionality of the kidney. The tests include urea, creatinine and electrolytes (Bartels and Rohmen, 1972).

1.6.1 Urea

Urea or carbamide is an organic compound with the chemical formula $\text{CO}(\text{NH}_2)_2$. The molecule has two NH_2 groups joined by a carbonyl ($\text{C}=\text{O}$) functional group.

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates (Godfrey *et al.*, 1997). Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is then dissolved into the blood (in the reference range 10-

55mg/dl which is laboratory dependent) and further transported and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat.

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood that comes from urea. It is used as a marker of renal function, though it is inferior to other markers such as creatinine because blood urea levels are influenced by other factors such as diet and dehydration (Manson, 2004).

1.6.2 Creatinine

Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily measured by-product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply) (Godfrey *et al.*, 1997).

Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which correlates with the glomerular filtration rate (GFR) (Taylor, 1989). Blood creatinine levels may also be used alone to calculate the estimated GFR (eGFR). Men tend to have higher levels of creatinine than women because, in general, they have a greater mass of skeletal muscle. Measuring serum creatinine is a simple test, and it is the most commonly used indicator of renal function. A rise in blood creatinine level is observed only with marked damage to functioning nephrons. The reference range of serum creatinine is 1.2- 1.9 mg/dl (Bartels and Rohmen, 1972).

1.6.3 Sodium Ion

Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. The main source of body sodium is sodium chloride contained in ingested foods. Only about one-third of total body's sodium is contained in the skeleton since most of it is contained in the extracellular fluids (Tietz, 1976). Hyponatremia is

found in a variety of conditions including the following: severe polyuria, metabolic acidosis, Addison's disease, diarrhoea, and renal tubular disease. Hyponatremia (increased serum sodium level) is found in the following conditions: hyperadrenalism, severe dehydration, and diabetic coma after therapy with insulin, excess treatment with sodium salts (Henry *et al.*, 1974). Normal range is 100 – 130 mEq/L.

1.6.4 Potassium Ion

Potassium is the principle cation of the intracellular fluid. It is also an important constituent of the extracellular fluid due to its influence on muscle activity. Its intracellular function parallels that of extracellular function, namely influencing acid-base balance and osmotic pressure, including water retention (Henry *et al.*, 1974). Elevated levels of potassium (hyperkalemia) are often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels (hypokalemia) is associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex (Tietz, 1976). The reference range is 4 – 7 mEq/L.

1.6.5 Chloride Ion

Chloride, a major anion, is important in the maintenance of the cation/anion balance between intra- and extracellular fluids. This electrolyte is therefore essential to the control of proper hydration, osmotic pressure, and acid/base equilibrium. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction (Skeggs and Hochstrasser, 1964). The reference range is 70 – 95 mEq/L.

1.7 Antioxidants

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that involves the transfer of electrons from a substance to an oxidizing agent. Antioxidants are often reducing agents such as thiols, polyphenols or ascorbic acid (Seaver and Imlay, 2004). Antioxidants are intimately involved in the prevention of cellular damage which is the common pathway for a variety of diseases. Although, oxidation reactions are crucial for life, they can also be damaging, however insufficient levels of antioxidants or inhibition of the antioxidant enzymes (e.g. superoxide dismutase), causes oxidative stress which will

subsequently lead to inflammation and cellular damage. Antioxidants are widely used in dietary supplements and has been investigated for the prevention of diseases such as cancer, coronary heart disease and other sickness(Bjelakovic *et al.*,2007).

1.7.1 Superoxide Dismutase

Superoxide dismutases (SOD, EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. Superoxide is one of the main reactive oxygen species in the cell. Consequently, SOD serves a key antioxidant role. The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes(Hijora *et al.*, 2005).

Superoxide dismutase is an enzyme whose function is to protect against the potentially damaging activities of the superoxide radical generated by aerobic metabolic reactions. Two types of SOD have been found in all mammalian cells except erythrocytes. Cu, Zn-SOD was present in both the cytosol and the intermediate membrane space of the mitochondria, and Mn-SOD was present in the mitochondrial matrix (Bjelakovic *et al.*,2007).Non-sulfur Fe enzyme known as superoxide dismutase (SOD) catalyze disproportion of FeSOD is found in bacteria, especially the more primitive ones, the chloroplast of plants, a few protists and possibly eukaryotes. The homologous MnSODs and found in bacteria and mitochondria, and are believed to protect DNA from endogenous oxidative stress, whereas FeSOD may serve as a housekeeping enzyme and provide resistance to environmental oxidatives, SOD caused by the chemical progeny of O_2^- : H_2O_2 and OH. Atomic absorption spectroscopy reveals that SOD monomer contains one metal ion and no other cofactors (Bjelakovic *et al.*,2007).

1.8Lipid Peroxidation and Tissue Damage

Lipid peroxidation is a known mechanism of cellular injury in human and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from PUFA are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds which is the most abundant is malondialdehyde (MDA) (Sarka and Rautary, 2009).

Lipid peroxidation is one of the molecular mechanism for cell injury and is associated with a decrease of cellular antioxidants such as glutathione, superoxide dismutase (SOD) and catalase

(CAT) (Hijora *et al.*, 2005). Free radicals are released by activated leucocytes which cause peroxidation of membrane lipids. There is a rupture of the liposomal membranes, the release of lysosomal enzymes, necrosis of the cell and destruction of parenchymal tissue. All these processes culminate in an increase in serum MDA levels. Hence, increased serum MDA could be used as a marker for the free radical mediated destruction of liver parenchymal cells. Liver disease is accompanied by an increased production of free radicals. MDA has the ability to interact with lipoproteins and so has received particular attention in pharmacological studies (Sarkar and Rautava, 2009).

1.9 Haematology

Blood is a highly specialized fluid-like connective tissues, which circulate in a closed system of vessels as a liquid with red colour, but forms a solid phase out of the system, which we call plug or blood clot. Some people would also say that blood is the river of life simply because it connects to all the tissues of the body and these tissues needs the blood to survive. Haematology is the science that studies about the blood, blood transfusion, blood-forming tissues, and its structure, function, disease, and the convenience between structure and the function. Haematology also includes the study of etiology, diagnosis, treatment, and prevention of blood diseases that affect the production of blood and its components, such as, haemoglobin, blood proteins, and the mechanism of coagulation (Lewis *et al.*, 2002).

1.9.1 Haemoglobin Count

Haemoglobin contains the red pigment that gives the red cells their colour and also carries oxygen from the lungs to the tissues and carries carbon dioxide (the waste products) from the tissues to the lungs. This test is primarily used to determine the presence of anaemia or, its reverse, polycythaemia, or to monitor a patient's response to treatment. Capillary blood or EDTA anti-coagulated venous blood can be used. The hemoglobin content in a solution may be estimated by several methods: by measurement of its colour, its power of combining with oxygen or carbon monoxide and by its iron content (Yared *et al.*, 2006).

Normal hemoglobin reference range: children 6 y ÷ 12 y 6-12 g/dl; Adult men 10-18 g/dl (Yared *et al.*, 2006). A low haemoglobin level means that less oxygen is being delivered round your body, leading to symptoms of anaemia such as fatigue, breathlessness, pallor, and palpitations. The patient may need to have a blood transfusion to help relieve these symptoms. In this case the

patient may need to have additional blood tests, in order to match the transfusion to your own blood as closely as possible(Cheesbrough, 2000).

1.9.2 Packed Cell Volume (PCV)

Packed cell volume is a measure of the proportion of blood volume that is occupied by red blood cells. It is normally about 45% in men and 40% in women(Yared *et al.*, 2006). It is considered an integral part of a person complete blood count results along with hemoglobin concentration, white blood cell count and platelet count.Low levels of PCV can be seen in the case of anemia, inflammation, kidney damage, malnutrition and pregnancy. However increase in PCV may be seen in myeloproliferative disorder, chronic obstructive pulmonary disease (COPD), capillary leak syndrome and anabolic androgenic steroid (AAS)(Lewis *et al.*, 2002).

1.9.3 Red Blood Cell (RBC) Count

Red blood cell count (RBC) measures the number of red cells in the blood.Red blood cells carry oxygen to the tissues and remove waste products from the body's tissues. These cells also contain hemoglobin. Red blood cells are measured in millions per cubic millimeter (mil/uL) of blood. The normal range for red blood cell count is $4.6 - 6.2 \times 10^6$ cells/ μ L (Lewis *et al.*,2002). A low count often accompanies anaemia, excess body fluid and blood loss. A high count is commonly seen in dehydration but could also mean some other complications such as polycythaemia, lung disease, alcoholism, smoking, kidney disease, dehydration, burns, sweating, diarrhea, carbon monoxide (co) exposure, etc while low RBC might indicate anaemia, sickle cell disease, cancer, peptic ulcer, lead poisoning, heavy menstrual bleeding etc depending on the aim of the test (Cheesbrough, 2000).

1.9.4 White Blood Cell (WBC) Count

White blood cell count (WBC) is a blood test carried out in the laboratory that measures the number of white blood cells per litre of blood. White cells protect against infection and allergies. High counts are seen during infection, after exercise and with stress. Low counts may be seen if there is suppression of the immune system. The normal range of WBC is $4.8- 10.0 \times 10^9$ /L (Yared *et al.*, 2006). An increase above the normal range could imply some other complications such as, leukemia, inflammation, tissue damage, stress, malnutrition, burns, lupus, kidney failure, rheumatoid arthritis, tuberculosis, thyroid gland problems while low WBC count might indicate

alcoholism, AIDS, enlarged spleen, viral infection, malaria, that the patient is undergoing chemotherapy, depending on the aim of the test.

1.10 Aim of the Study

This study is aimed at determining the possible effect of Fraction 1 of *Abrus precatorius* seed methanol extract on paracetamol-induced liver damage.

1.11 Specific Research Objectives

- To determine the median lethal dose (LD_{50}) of the extract.
- To fractionate the extract using Sephadex G15.
- To determine qualitatively and quantitatively the phytochemical constituents of the different fractions of *Abrus precatorius* seed methanol extract.
- Determination of the effect of Fraction 1 on liver marker enzymes.
- To determine the effect of Fraction 1 on some haematological parameters.
- To determine the effect of Fraction 1 on some kidney markers.
- To determine the effect of the Fraction 1 on some antioxidant enzymes.

CHAPTER TWO

2.1 Materials

2.1.1 Animals

The animals (Wistar albino rats) used for this study were between 3 and 7 weeks old weighing 70-120g. They were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. These animals were fed standard animal feed and water *ad libitum* and were acclimatized to laboratory conditions for 2 weeks.

2.1.2 Plant Materials

The seeds of *Abrus precatorius* Linn Fabaceae were collected from Igala Area of Kogi State and authenticated by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria.

2.1.3 Drugs

The drugs used for this study were purchased from Elofex Pharmaceutical Shop in Nsukka, Enugu State of Nigeria.

2.1.4 Instruments/Equipment

Equipment	Manufacturer
Centrifuge	Chikpas, England

Micropipette	Perfect, USA
Glass wares	Pyrex, England
Refrigerator	Thermocool, Germany
Microscope (B. brand specificity)	Sigma Aldrich, Germany
Spectrophotometer (unicotm UV-2101 PC)	Perfect, USA
Triple beam balance	Gallen Kanp, England
Chromatographic tank	Shandon, England
Water bath	Chikkpas, England
Heating magnetic stirrers	Perfect, USA
Improved Neubuer counting chamber	Gallen Kanp, England

2.1.5 Chemicals and Reagents

The chemicals and reagents used were of analytical grade, they include:

1% Thiobarturic acid	BDH England
2, 4-dinitrophenyl hydrazine	Merck Darmstadt, Germany
Absolute ethanol	BDH, England
Acetone	Sigma Aldrich, Germany
Aluminium chloride	BDH, England
Anticoagulant (EDTA, heparin)	Randox USA
Bismuth carbonate	BDH, England
Buffer	BDH, England
Butanol	Sigma, England
Chloroform	Sigma, England
Dichromate acetic acid	May and Baker, England
Distilled water	STC, UNN
Drangendorff's reagent	May and Baker, England
Ethyl acetate	BDH, England
Hydrochloric acid	May and Baker, England
Hydrogen peroxide	BDH, England
Lead acetate solution	Merck Darmstadt, Germany
Mayer's reagent	BDH, England
Methanol	Sigma, England

Picric acid	Merck Darmstadt, Germany
Potassium dichromate	Sigma Aldrich, Germany
Potassium hydroxide	Sigma Aldrich, Germany
Sephadex G-15	Sigma Aldrich, USA
Sodium chloride	BDH, England
Sodium hydroxide	May and Beakers, England
Sulphuric acid	May and Baker, England
Trichloroacetic acid	Sigma Aldrich, Germany
Tungstic acid/sodium tungstate	Merck Darmstadt, Germany
Turkey solution (20% glacial acetic acid)	Merck Darmstadt, Germany
Wagner's reagent	BDH, England

2.2 METHODS

2.2.1 Extraction of *Abrus precatorius* seeds

The seeds of *Abrus precatorius* were pulverized using a high speed grinder. Six hundred grammes (600g) of the crushed seeds were macerated in a mixture of 400 ml of methanol and 800 ml of chloroform for 24 hr. The macerates were filtered through Whatmann no 4 filter paper and the filtrate shaken with 0.2 volume water to obtain two layers (the upper methanol layer and lower chloroform). The upper methanol layer was collected and the extract concentrated using magnetic stirrer.

2.2.2 Determination of Extract Yield

The percentage yield of extract of *Abrus precatorius* was calculated by weighing the seeds before extraction and after concentration of the extract. It was calculated using the formula below:

$$\text{Percentage yield \%} = \frac{\text{Weight of extract} \times 100}{\text{Weight of seeds}} \times 100$$

2.2.3 Fractionation

Fractionation of the methanol extract of *Abrus precatorius* seeds was by gel filtration, using sephadex G₁₅ which was allowed to swell for 3hrs and packed in a column of height 27cm and

diameter 2.5cm. The extract was diluted with distilled water and introduced into the column and eluted with water. Fractions (elution) were then collected in test tubes labelled 1-50 of about 3ml each.

Absorbance reading of various fractions were read using spectrophotometer machine at a wavelength of 265nm. A plot of absorbance against the fraction was drawn to produce elution profile with different peaks of fraction range.

2.2.4 Thin Layer Chromatography

The Fractions were spotted on a TLC plate (precoated with silica gel) and was left to dry for about one hour. Afterward, it was inserted into the chromatographic tank (made up of butanol, acetic acid and water in ratio of 65:13:22 respectively which was allowed to equilibrate for one hour).

2.2.4 Visible Spectroscopy

After development of the plate, it was spread with Drangendoff's reagent. The fractions that turned purple were pulled into a beaker as fraction I while the other fractions that did not change colour were pulled together as fraction II. However fraction I was then concentrated and afterward, a given weight was dissolved in normal saline (stock solution) which was administered to the animals based on their body weight.

2.2.6 Determination of Fraction Yield

The percentage yield of fraction 1 of methanol extract of *Abrus precatorius* was calculated by weighing the extract before fractionation and after concentration of the fractions. It was calculated using the formula below:

$$\text{Percentage yield \%} = \frac{\text{Weight of fraction 1 after concentration} \times 100}{\text{Weight of fraction 1 before concentration}}$$

2.2.7 Qualitative Phytochemical Analysis of the different Fractions of *Abrus precatorius* Seed methanol Extract.

The phytochemical analysis of the seeds of *Abrus precatorius* were carried out according to the method of Harborne (1973) and Trease and Evans (2002) to identify its active constituents.

2.2.7.1 Test for alkaloids

A quantity of the sample (0.2g) was boiled with 5ml of 2% HCl on a steam bath. The mixture was filtered and 1ml of the filtrate was treated with 2 drops of dragendorff's reagent.

- (i) Dragendorff's reagent: An orange precipitate indicates the presence of alkaloids.
- (ii) Mayer's reagent: A creamy-white precipitate indicates the presence of alkaloids.
- (iii) Wagner's reagent: A reddish-brown precipitate indicates the presence of alkaloids.
- (iv) Picric acid (1%): A yellow precipitate indicates the presence of alkaloids.

2.2.7.2 Test for flavonoids

A measured weight, (0.2g) was heated with 10ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered, and the filtrate was used for the following tests.

- (i) Ammonium test: 4ml of the filtrate was shaken with 1ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicates the presence of flavonoids.
- (ii) Aluminium chloride test: 4ml of the filtrate was shaken with 1ml of 1% ammonium chloride solution and observed for light yellow colouration that indicates the presence of flavonoids

2.2.7.3 Test for saponins

The sample (0.1g) was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests.

- (i) Emulsion test: A quantity of the filtrate (1ml) was added to two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.
- (ii) Frothing test: A quality, 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

2.2.7.4 Test for tannins

A known weight, 2g was boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions.

- (i) Lead sub acetate solution: To 1ml of the filtrate, 3 drops of lead sub acetate solution was added. A gelatinous precipitate indicates the presence of tannins.
- (ii) Bromine water: To 1 ml of the filtrate was added 0.2 ml of bromine water and then observed for a pale brown precipitate.

(iii) Ferric chloride solution. 1ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

2.2.7.5 Test for carbohydrates

Test for carbohydrates was done using Molisch's test. Few drops of Molisch's reagent was added to 0.1 g of the extract dissolved in H₂O. This was followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. The mixture was allowed to stand for 2 minutes and then diluted with 5 ml of distilled H₂O. Formation of a red dull violet colour at the interface of the two layers indicates the presence of carbohydrates.

2.2.7.6 Test for terpenoids and steroids

Ethanol (9ml) was added to 1g of the sample and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath, and 5ml of hot water was added. The mixture was allowed to stand for 1 hour, and the waxy matter filtered off. The filtrate was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids.

Another 0.5ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicates the presence of terpenoids.

2.2.7.7 Test for peptides

Test for peptides was done by biuret test. 5 ml of sodium hydroxide was added to 0.1g of the sample and filtered. Few drops of 15% copper sulphate was added. A pink colour indicates the presence of peptides.

2.2.7.8 Test for glycosides

A quantity of the sample (2.0g) was mixed with 30ml of distilled water and 15ml of dilute sulphuric acid respectively and heated in a water bath for 5 minutes. The mixtures were filtered and the filtrates used for the test. To 5ml of each of the filtrate, 0.3ml of Fehling's solutions A and B was added until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides.

2.2.7.9 Test for resins

The seed methanol extract of *Abrus precatorius* Linn (0.2g) was extracted with 15ml of 95% ethanol. The alcohol extract was then poured into 20ml of distilled water in a beaker. The occurrence of a precipitate indicates the presence of resins.

2.2.7.10 Test for reducing sugars

A quantity, 0.1g of the sample was shaken vigorously with 5ml of distilled water and filtered. To the filtrate was added equal volumes of Fehling's solutions A and B and shaken vigorously. A brick-red precipitate indicates the presence of reducing sugars.

2.2.8 Quantitative Phytochemical Analysis of the different Fractions of *Abrus precatorius* Seed methanol Extract.

2.2.8.1 Alkaloid determination

The determination of alkaloid was as described by Harborne (1973). A portion (5g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid and ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the extract was concentrated on a water bath to one-quarter (1/4) of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.2.8.2 Determination of flavonoids

This was determined according to the method of Harborne (1973). A quantity, 5g of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through Whatman No. 1 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The solution was filtered into a weighed crucible. The filtrate was heated to dryness in an oven at 60°C. The dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

2.2.8.3 Determination of saponins

Determination of saponins was done by weighing 1g of the sample and macerating with 10ml of petroleum ether. The sample was decanted into a beaker and washed twice with 10 ml of normal saline and filter. The filtrate was allowed to evaporate to dryness. The residue was dissolve in 6 ml of ethanol. A known volume, 2ml of the solution was added to a test tube and 2 ml of chromogen solution was also added. The mixture was allowed to stand for 30 mins. The absorbance of the sample was read at 550 nm.

2.2.8.4Determination of tannins

Determination of tannins was done by weighing 1g of the sample and macerating with 50ml of methanol then filter. 0.3 ml of 0.1N ferric chloride in 0.1N HCL was added to 5ml of the filtrate. Also 0.3 ml of 0.0008M potassium ferricyanide and then shaken. The absorbance of the sample was read at 720 nm.

2.2.9Toxicological Studies (Acute Toxicity Test)

Acute toxicity (LD₅₀) of the methanol extract of *Abrus precatorius* was carried out by a modified method of Lorke (1983) to define the range of lethal dose and safe dose for the extract. A total of 15 swiss albino mice of either sex weighing 18-22g were used for this investigation. Swiss albino mice were starved of food for 18 hours but allowed to waterprior to the study and were grouped into five groups of three mice each. The animals were administered orally at the dose levels of 10, 100, 200, 400, and 700 mg/kg b.w.. The animals were then observed closely for 24 hrs for nervousness, dullness, in-cordiation and death.

2.2.10 Induction of Liver Damage

The induction of liver damage was according the method described by Mitchell *et al* (1973). Paracetamol was suspended in normal saline and administered orally at the dose of 2500 mg/kg b. w. For hepato-protective groups, administration of extract was done for 7 days before paracetamol induction and collection of blood was done after 24 hours of administration. However in the hepato-curative groups, curative treatments was done after paracetamol induction at day 0 and treatment was done for 14 days. Blood was collected at day 8 and 15 for the analyses.

2.2.11Experimental Design

A total of fifty (50) rats weighing 70-100g were used for the study. They were randomly divided into two sets (hepato-protective groups and hepato-curative groups) of five groups containing five rats per group based on the similarity of their weight. The route of administration (exposure) will be done orally.

Hepato-Protective Groups

Group 1: Received 5 ml/kg of normal saline (Negative control)

Group 2: Received paracetamol 2500 mg/kg b.w. only (Positive control)

Group 3: Received Silymarin (100 mg/kg b.w.) + paracetamol (Standard control)

Group 4: Received 100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol

Group 5: Received 200 mg/k.g. b.w. of fraction 1 the Abrus seed extract + paracetamol

Hepato-Curative Groups

Group 1: Received 5 ml/kg of normal saline (Negative control)

Group 2: Received paracetamol 2500 mg/kg b.w. only (Positive control)

Group 3: Received paracetamol + Silymarin (100 mg/kg b.w.) (Standard control)

Group 4: Received paracetamol + 100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract

Group 5: Received paracetamol + 200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract

2.2.12 Preparation of Sample Solutions

2.2.12.1 Preparation of normal saline

This was prepared by dissolving 0.9g of sodium chloride in 50ml of water and the volume is made up to 100ml of water.

2.2.12.2 Preparation of phosphate buffer

Saline about 200ml (pH 7.2)

Stock phosphate solution A	-	-	-	-	28ml
Stock phosphate solution B	-	-	-	-	72ml
Distilled water	-	-	-	-	100ml
Sodium chloride	-	-	-	-	1.7g

The phosphate solutions were mixed with water and 1.7g of sodium chloride was added and then mixed well to dissolve the salt. Then, pH meter was used to check the pH at temperature of 28°C.

2.2.12.3Preparation of stock solution

A known weight, 2.2g of fraction 1 of methanol extract of *Abrus precatorius* was dissolved in 30ml of normal saline.

2.2.12.4Preparation of drug solution

Paracetamol (4000 mg) was dissolved in 26ml of normal saline.

Silymarin (420 mg) was dissolved in 12ml of normal saline.

2.2.12.5Preparation of serum samples

Whole blood was collected from the animals in different groups through ocular puncture and introduced into two clean non-anti-coagulated and anti-coagulated blood sample containers. The blood samples were centrifuged at a speed of 300 rpm in order to get the supernatant (serum). The different serum gotten was used immediately for biochemical analysis.

2.2.13Assay of Alkaline Phosphate Activity

This was done using the QCA Commercial enzyme kit which is based on the phenolphthalein monophosphate method of Klein *et al.* (1960), Babson (1965) and Babson *et al.* (1966).

Principle

Serum alkaline phosphate hydrolyses a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns to pink colour that can be photometrically determined.

The concentrations in the reagent solution are;

2-Amino-2-methyl-1-propanol	-	-	-	7.9N
Phenolphthalein monophosphate	-	-	-	63mM
Na ₂ PO ₄	-	-	-	80mM
Stabilizers and preservatives				

Procedure

Distilled water (1ml) was pipetted into 2 sets of test tubes labelled SA sample and ST standard respectively. Then one drop of each of the chromogenic substrates was added to the distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37°C for 5min. A

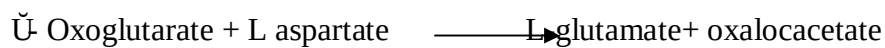
standard solution of 0.1M (alkaline phosphate) was added to the standard test tube (ST)only, followed by the addition of 0.1ml of the serum sample to the sample test tube (SA). The contents of the test tubes were mixed and incubated at 37⁰C for 20 min in a water bath. A colour developer (phenolphthalein monosulphate) (5.mle each) was added to both sets of test tubes. Absorbance of the sample against the blank (water) was read at a wave length of 550nm. The activity of alkaline phosphates in the serum was obtained from the formula below.

$$\frac{SAO.D}{STO.D} \times 30 = \text{U/L of Alkaline phosphates}$$

SAO.D = sample Optical Density
 STO.D= Standard Optical Density
 Normal values
 Adults= 9-35 U/L
 Children =35-100 U/L

2.2.14 Assay of Aspartate aminotransferase Activity

A Randox Commercial Enzyme Kit according to the method of Reitman and Frankel (1957) was used. This method is based on the principle that oxaloacetate is formed from the reaction below:



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

Reagents

Contents	Initial concentration of reagents		
Phosphate buffer	-	-	100mmol/,pH 7.4
L-Aspartate	-	-	100mmol/l
α -Oxoglutarate	-	-	2mmol/l
2, 4-dinitrophenyl hydrazine	-		2mmol/l
Sodium hydroxide solution	-		0.4mol/l

Measurement against Reagent Blank

The AST substrate phosphate buffer (0.5ml each) was pipette 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 added to the reagent blank (B). The entire reaction medium was well mixed and incubated for 30min in a water bath at 37°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 for exactly 20min at 25°C. Finally, 5.0ml of sodium hydroxide (0.4mol/l) solution was added to both the blank and the reagent test tubes respectively and thoroughly.

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

Measurement against Sample Blank

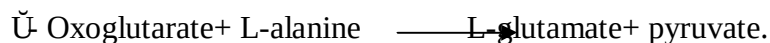
The AST substrate phosphate buffer (0.5ml each) was pipette into the sample blank (B) and sample (T) test tubes respectively. The serum sample (0.1ml) was added to the sample test (T) only and mixed immediately then incubated in a water bath for exactly 30min at 37°C. 2,4- Di-nitrophenylhydrazine was added to both the 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 20min at 25°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 and mixed thoroughly. Absorbance of the sample was read at a wavelength of 550nm against the sample blank after 5min.

Normal values in human, serum up to 30U/L

2.2.15 Assay of Alanine aminotransferase Activity

A Randox Commercial Enzyme Kit based on the methods of Reitman and Frankel (1957) was used.

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



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

Reagents

Contents

Initial concentrations of solutions

Buffer

Phosphate buffer	-	-	100mmol/l pH 7.4
L-alanine	-	-	200mmol/l
&-oxoglutarate	-	-	2.0mmol/l
2,4-dinitrophenyl hydrazine	-		2.0mmol/l
Sodium hydroxide solution	-		0.1mol/l

Procedure

Measurement against Sample Blank

The ALT substrate phosphate buffer (0.5ml each) was pitted 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 30min in a water bath at a temperature of 37⁰C. 2,4 ï dinitrophenyl hydrazine (0.5ml) was added to both test tubes labeled T (sample test) and B sample was (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 20min at 25⁰C. After which, 5.0ml of sodium hydroxide (NaOH) solution (0.4mol/l) was added to both test tubes and also mixed thoroughly. Absorbance of the sample was read at a wavelength of 550nm 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 were mixed and incubated for exactly 30min in a water bath at 37⁰C. Immediately after incubation, 2, 4- dinitrophenyl- 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 20min at 25⁰ C. Finally, 5.0ml of sodium hydroxide solution (0.4mol) was added to both blank and reagent test tubes respectively. Each was mixed thoroughly and absorbance of sample was read at a wavelength of 550nm against the reagent blank after 5min.

Normal Values in humans

Serum up to 20 U/L

2.2.16Determination of Total Bilirubin Concentration

A colorimetric method with a kit supplied by Randox was used using the method described by Jendrassik and Grof(1938).

Principle

Direct conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Reagent composition

Contents	Initial concentration of solution		
Sulphanilic acid	-	-	29mmol/L
Hydrochloric acid	-	-	0.17N
Sodium Nitrate	-	-	25mmol/L
Caffeine	-	-	0.26mol/L
Sodium benzoate	-	-	0.52mol/L
Tartrate	-	-	0.93mol/L
Sodium hydroxide	-	-	1.9N

Procedure

Reagent 1 (sulphanilic acid, hydrochloric acid,) 0.20ml, was pipetted into two different cuvettes labelled sample blank (B) and sample (A) respectively, then a drop (0.05ml) of reagent was introduced. Then a drop of 0.05ml of reagent was pipette into the cuvette containing sample (A) only. Afterwards, 1.0ml of reagent 3 (caffeine, sodium benzoate) was pipetted into the cuvettes containing samples B and A respectively. Serum sample (0.2ml) was then pipetted into both cuvettes, sample blank (B) and sample (A). Their contents were separately mixed and allowed to stand for 10min at 25°C.

This was followed by addition of 1ml of reagent 4 (Titrate, sodium hydroxide) into both cuvettes containing sample blank and sample. They were mixed and allowed to stand for 30min at 25°C. Finally, absorbance of bilirubin values were obtained using the calculation below:

Total bilirubin ($\mu\text{mol/L}$) = $184 \times A_{\text{TB}} (560 \text{ nm})$

Total bilirubin (mg/dl) = $10.8 \times A_{\text{TB}} (560\text{nm})$

Normal value in serum

Total bilirubin up to 1.7 $\mu\text{mol/L}$ OR up to 1 mg/Dl

2.2.17Determination of Serum Urea Concentration

The urea concentration was determined using the method described by Fawcett and Scott (1960).

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

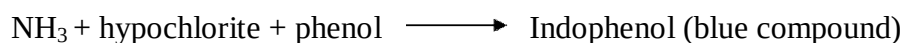
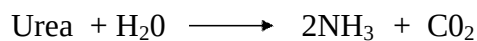


Table 2: Reagent composition of serum urea

Contents		Initial Concentration of Solutions
R1	EDTA	116mmol/l
	Sodium nitroprusside	6mmol/l
	Urease	1g/l
R2	Phenol(diluted)	120mmol/l
R3	Sodium hypochlorite (diluted)	27 mmol/l
	Sodium hydroxide	0.14 N

Table 3: Procedure for determination of serum urea

Pipette into three cuvettes	Blank	Standard	Sample
Sample	-----	-----	10µl
Standard	-----	10µl	-----
Distilled water	10µl	-----	-----
Reagent 1	100µl	100µl	100µl
Mix and incubate at 37°C for 10 mins			
Reagent 2	2.50ml	2.50ml	2.50ml
Reagent 3	2.50ml	2.50ml	2.50ml

Mix immediately and incubate at 37°C for 15 min

The absorbance of the sample (A_{sample}) and standard (A_{standard}) was read against the blank. The colour of the reaction is stable for at least 8 hours. Absorbance was read at 546 nm wavelength.

Calculation

$$\text{Serum Urea Concentration} = \frac{A_{\text{sample}} \times \text{Standard Concentration}}{A_{\text{standard}}}$$

Normal values

Serum: 1.7-9.1 mmol/l, 10-55 mg/dl

Urine: 333-583 mmol/24h, 20-35 g/24h

2.2.18Determination of Serum creatinine Concentration

Serum creatinine concentration was determined using the method described by Bartels and Rohmen (1972).

Principle

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

Table 4: Reagent composition of serum creatinine

Contents		Initial Concentration of Solutions
R1a	Picric Acid	35 mmol/l
R1b	Sodium hydroxide	0.32 mol/l
R2	Standard sample	50 mmol/l

Table 5: Procedure for the determination of serum Creatinine

Pipette into three cuvettes	Blank	Standard	Sample
Distilled water	50µl	-----	-----
Standard	-----	50µl	-----
Sample	-----	-----	50µl
Picric Acid	500µl	500µl	500µl
Mix and incubate at 37°C for 10 mins			
Sodium Hydroxide	2.00ml	2.00ml	2.00ml
Standard	2.00ml	2.00ml	2.00ml
Mix immediately and read after 30 seconds			

The absorbance of the sample (A_{sample}) and standard (A_{standard}) was read against the blank. Absorbance was read at 492 nm wavelength.

Calculation

$$\text{Serum Creatinine Concentration} = \frac{A_{\text{sample}} \times \text{Standard Concentration}}{A_{\text{standard}}}$$

Normal values

Serum: Men	53-97 $\mu\text{mol/l}$, (0.6-1.1 mg/dl)
Women	44-80 $\mu\text{mol/l}$, (0.5-0.9 mg/dl)
Urine: Men	8.84-13.3 mmol/24h
Women	11-15 g/24h

2.2.19 Determination of Serum Sodium Ion Concentration

Principle

Serum sodium ion concentration was determined by the method of Tietz, (1976) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Reagent composition

1. Filtrate Reagent: Uranyl acetate 2.1mM and Magnesium Acetate 20 mM in ethyl alcohol.
2. Acid Reagent: A diluted acetic acid.
3. Sodium Color Reagent: Potassium ferrocyanide, non-reactive stabilizers, fillers.
4. Sodium standard: Sodium chloride solution: 150 mEq/L of sodium.

Procedure

Filterate Preparation

1. Test tubes were labeled: blank, standard, control, patient.
2. A known volume, 1.0ml of filtrate reagent was added to all test tubes, 50 μl of the sample was also added to all test tubes and distilled water to the blank.
3. All the test tubes were shaken vigorously and mixed continuously for 3 min.
4. The tubes were centrifuged for 10 min at high speed (1,500G) and the supernatant fluids were collected for color development

Colour Development

1. The test tubes were labeled as described above.
2. A known volume, 1.0ml of acid reagent was added to all test tubes, 50µl of the supernatant of the tubes above were added to their respective tubes and 50µl of the color reagent was also added and mixed
3. The spectrophotometer was zeroed with distilled water at 550nm wavelength and the absorbance of all the test tubes was read.

Calculations

$$\frac{(\text{Abs. of Blank} - \text{Abs. of S}) \times \text{Conc. of STD (mEq/L)}}{(\text{Abs. of Blank} - \text{Abs. of STD})} = \text{Conc. of Sodium ion (mEq/L)}$$

Abs. = Absorbance, S = Sample, STD = Standard

Normal values

135 ÷ 155 mEq/L

2.2.20Determination of Serum Potassium Ion Concentration

Principle

Serum Potassium Ion concentration was determined by the method described by Tietz (1976) in which amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration.

Reagent composition

1. Potassium Reagent: Sodium tetraphenylboron 2.1 Mm
2. Potassium Standard: Equivalent to 4 mEq/L

Procedure

1. The test tubes were labeled blank, standard, control and patients and 0.01ml of potassium reagent was added to all test tubes.
2. 0.01mL of samples was added to respective tubes, mixed and allowed to sit at room temperature for 3 minutes.
3. After 3 minutes the samples were read spectrophotometrically at the wavelength of 500nm. The spectrophotometer was zeroed with the reagent blank.

Calculations

$$\frac{(\text{Abs. of Blank} - \text{Abs. of S}) \times \text{Conc. of STD (mEq/L)}}{(\text{Abs. of Blank} - \text{Abs. of STD})} = \text{Conc. of Potassium (mEq/L)}$$

Abs. = Absorbance, S = Sample, STD= Standard

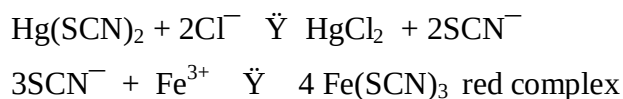
Normal values

3.4 ÷ 5.3 mEq/L

2.2.21Determination of Serum Chloride Ion Concentration

Principle

Serum chloride ion was determined by the method described by Skeggs and Hochstrasser (1964) in which chloride ion forms a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480nm. The intensity of the colour produced is directly proportional to chloride concentration.



Reagent composition

1. Chloride Reagent (Active Ingridients):

Mecuric Nitrate	-	-	0.058 mM
Mecuric Thiocyanate	-	-	1.75 Mm
Mecuric Chloride	-	-	0.74 mM
Ferric Nitrate	-	-	22.3 mM

2.Chloride Calibrator

Sodium chloride	-	-	100 mEq/L
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Procedure

1. The test tubes were labelled blank, calibrator and patients.
2. 1.5mL of Chloride Reagent was added to respective tubes; 0.01ml of calibrator was also added, mixed and allowed to sit at room temperature for 5 minutes.

3. After 5 minutes the samples were read spectrophotometrically at the wavelength of 480nm. The spectrophotometer was zeroed with the reagent blank.

Calculations

$$\frac{\text{Abs. of S} \times \text{Conc. of Calibrator (mEq/L)}}{\text{Abs. of Calibrator}} = \text{Conc. of Chloride ion (mEq/L)}$$

Abs. = Absorbance, S = Sample

Normal values

98- 106 mEq/L

2.2.22 Assay of Superoxide Dismutase (SOD) Activity

The activity of SOD was evaluated by the method of Xin *et al.* (1991). It is based on the inhibition of epinephrine auto-oxidation to adrenochrome in alkaline environment. Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent to a 4ml mixture of 0.3mm epinephrine, 1mm solution of Na₂CO₃, 0.3mm EDTA and 1ml of distilled water at a final volume of 6ml. The auto-oxidation was monitored spectrophotometrically at 480nm every 30secs for 5min. The experiment was repeated with 1.0ml of the serum. A graph of absorbance against time was plotted for each sample and initial rate of anti-oxidation was calculated. One unit of SOD activity was defined as the concentration of the enzyme in the sample that caused 50% reduction in the auto-oxidation of epinephrine. SOD activity was then calculated for each sample and expressed in lu/L.

2.2.23 Determination of Malondialdehyde

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

Principle

Malondialdehyde (MDA) reacts with thiobarbituric acid to form a red or pink coloured complex which, in acid solution, absorbs maximally at 532nm.



Reagent Preparation

1. **1.0% Thiobarbituric acid (TBA):** A known quantity, 1.0 g, thiobarbituric acid was dissolved in 83 ml of distilled water on warning. After complete dissolution the volume was made up to 100 ml with distilled water.

2. **25% Trichloroacetic acid (TCA):** A known quantity, 12.5 g, of trichloroacetic acid was dissolved in distilled water and made up to 50 ml in a volumetric flask with distilled water.

3. **Normal saline solution (NaCl):** A known quantity, 0.9 g, of NaCl was dissolved in 10 ml of distilled water and made up to 100 ml with distilled water.

Procedure

To 0.1 ml of plasma in test tube was added 0.45 ml of normal saline and mixed thoroughly before adding 0.5 ml of 25% trichloroacetic acid (TCA) and 0.5 ml of 1% thiobarbituric acid. The same volume of trichloroacetic acid, and saline was added to the blank. 0.1 ml of distilled water was also added to the blank instead of plasma. Then, the mixture was heated in a water bath at 95°C for 40 min. Turidity was removed by centrifugation. The mixture was allowed to cool before reading the absorbance of the clear supernatant against reagent blank at 532 nm. Thiobarbituric acid reacting substances were quantified as lipid peroxidation product by referring to a standard curve of (MDA) concentration (i. e. equivalent generated by acid hydrolysis of 1,1,3,3- tetraethoxypropane (TEP) prepared by serial dilution of a stock solution).

Table 6: Procedure for lipid peroxidation assay

	Blank	Test
Plasma	---	0.10ml
Distilled water	0.10 ml	---
Normal saline	0.45 ml	0.45 ml
25%TCA	0.50 ml	0.50 ml
1% TBA	0.50 ml	0.50ml

Then, the absorbance was taken at wavelengths 532nm and 600nm against a blank.

2.2.24Determination of Hemoglobin Concentration

Principle

Haemoglobin concentration was determined by the method described by Dacie and Lewis (1991) in which blood from EDTA is diluted in a Drabkin's solution containing potassium cyanide and

potassium ferricyanide. As a result, RBCs are hemolyzed and the haemoglobin is released. The released haemoglobin is oxidized in the following reaction.

Hemoglobin (Hgb) + ferricyanide $\rightarrow$ methemoglobin

Methemoglobin + cyanide $\rightarrow$ cyanmethemoglobin (or also called HiCN)

Absorbance of the HiCN solution is read in a spectrophotometer at 540 nm

Absorbance of the HiCN solution is compared with the reference HiCN standard solution

Procedure

The blood is diluted (EDTA) in Drabkin's solution by 1: 201 (20 μ L of blood in 4000 μ L). The tube is covered and inverted several times and the tube is left to stand for 5-10 minutes to ensure complete conversion. The HiCN solution is poured into a cuvette and read spectrophotometrically at the wavelength of 540 nm using the Drabkin's solution as blank.

HiCN is used as standard. Haemoglobin concentration is calculated using the following equation:

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Standard}} \times \text{Concentration of STD in mg/l} \times \frac{\text{Dilution factor}}{1000}$$

Result is expressed in g/dl

Normal range= 14- 20 g/dl

2.2.25 Determination of Packed Cell Volume (PCV)

This was done using standard technique as described by Ochei and Kolhartar (2008) Blood sample were collected into PCV tubes heparinized using capillary action. One end of the tube was sealed with plasticine and then centrifuged using the haematocrit centrifuge of 5 mins at 2500gram. The test result was read using a PCV haematocrit reader.

2.2.26 Determination of Red Blood Cell (RBC) Count

This was done using standard method as described Cheesbrough (2005). The blood sample was diluted in the ration of 1:20 with 10% Na_2CO_3 . The diluted sample was loaded into the Neubauer chamber with the aid of a Pasteur pipette. The RBC was counted form appropriate squares on the chamber under an electronic microscope.

2.2.27 Determination of Total White Blood Cell (WBC) Count

The white blood cell count was determined following the standards technique as described by Cheesbrough (2008). The blood sample was diluted 1:20 with Turks solution, which is 2% glacial acetic acid. The diluted sample was loaded into a Neubaer counting chamber with the aid of pasture pipette. The total WBC was calculated by counting the required number of squares on the counting chamber under a microscope.

2.3 Statistical Analysis

The results were expressed as means $\pm$ SD and tests of statistical significance were carried out using one way analysis of variance (ANOVA) with repeated measures. The Statistical Product for Service Solutions (SPSS), version 20 was used. P values < 0.05 will be considered significant.

CHAPTER THREE

RESULTS

3.1 Percentage Yield of Extract

As shown in table 7, the percentage yield of the seed methanol extract of *Abrus precatorius* was found to be 2.08%.

Table 7: Percentage Yield of Extract

Seeds (g)	yield after extraction (g)	% yield
601.42	12.51	2.08

3.2 Result of Acute Toxicity Studies (LD₅₀)

The acute toxicity test of the seed methanol extract of *Abrus precatorius* showed no death up to 700 mg/kg body weight. Table 8 shows the result of the acute toxicity (LD₅₀) test using a modified method of Lorke (1983).

Table 8: Acute Toxicity Studies (LD₅₀)

Phase 1	Dosage mg/kg body weight	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	200	0/3
Group 4	400	0/3
Group 5	700	2/3

3.3 Detection of Fraction

Figure 5 shows the absorbance reading of the different test tube fractions (1-50) of *Abrus precatorius* seed methanolextract at 265 nm fractionated using Sephadex gel G15 swollen packs and eluted with distilled water. The fractions were spotted on a TLC plate and was spread with Dragendorff's reagent in which some turned purple indicating the presence of alkaloids. The test tube fractions that turned purple were pulled into a beaker as fraction I, while the other test tube fractions in which there was no colour change were pulled together as fraction II.

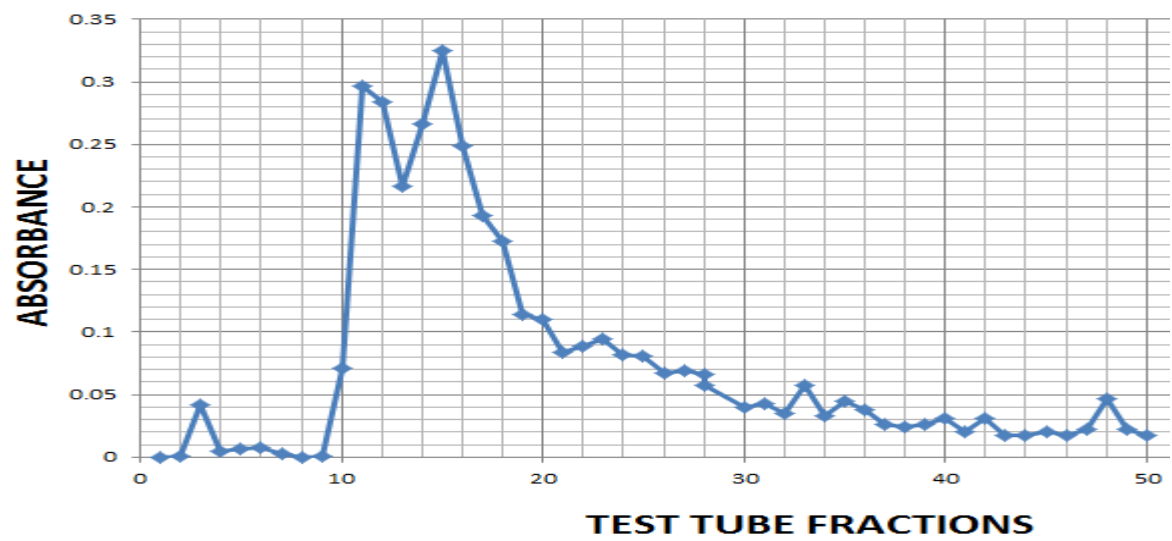


Fig.5: Spectrophotometer reading showing the absorbance level of the eluted fractions of *Abrus precatorius* seed methanol extract.

3.4 Percentage Yield of Fraction I and II

Table 9 shows the percentage yield of fraction 1 of *Abrus precatorius* after further purification with Sephadex gel G 15 and spraying with Dragendoff's reagent. The fractions that showed violet were collected together as fraction 1 and concentrated to give a dry weight of 2.22 g which is equal to 17.75 % of the initial weight of extract. Fraction II gave a dry weight of 1.54 g which is equal to 12.31 % of the initial weight of extract.

Table 9: Percentage Yield of Fraction I and II

Fractions	Extract (g)	Yield after fractionation of extract (g)	%Yield
I	12.51	2.22	17.75
I1	12.51	1.54	12.31

3.5 Qualitative Phytochemical Composition of Fractions I and II of *Abrus precatorius* Seed Extract

As shown in table 10, bioactive compounds such as alkaloids, flavonoids, saponins, glycosides, tannins and carbohydrates were found to be present in both fractions. Steroids, terpenoids, and peptides were found to be moderately present in only fraction 1, while glycoside resin and reducing sugars were all absent in both fractions of *Abrus precatorius* seed methanol extract.

Table 10: Qualitative Phytochemical Composition of Fractions I and II of *Abrus precatorius* Seed Extract

Phytochemicals	Fraction I	Fraction II
Alkaloids	+++	+++
Flavonoids	+++	+++
Saponin	+	+
Tannin	+	++
Carbohydrates	+	+
Steroids	+	-
Terpenoids	+	-
Peptides	+	-
Glycoside	-	-
Resin	-	-
Reducing sugar	-	-

Key: + Slightly present
 ++ Moderately present
 +++ Highly present
 - Not detected

3.6 Quantitative Phytochemical Composition of Fractions I and II of *Abrus precatorius* Seed Extract

Table 11 shows quantitatively the phytochemical composition of the different fractions of methanol extract of *Abrus precatorius* seed. Bioactive compounds such as alkaloids were found to be highest (5840 ± 184 ; 2000 ± 180 mg/100g) in both fractions compared to flavonoids (215 ± 97 ; 158 ± 17.6 mg/100g) and tannins (6.4 ± 0.72 ; 258 ± 45 mg/100g) that were moderately present in fractions I and II respectively. However saponins were found least (2.98 ± 1.33 ; 18.3 ± 2.43 mg/100g) in both fractions I and II respectively.

Table 11: Quantitative phytochemical Composition of Fractions I and II of *Abrus precatorius* seed extract in mg/100g.

Phytochemicals	Fraction I	Fraction II
Alkaloids	5840 $\pm$ 184	2000 $\pm$ 180
Flavonoids	215 $\pm$ 97	158 $\pm$ 17.6
Saponins	2.98 $\pm$ 1.33	18.3 $\pm$ 2.43
Tannins	6.4 $\pm$ 0.72	258 $\pm$ 45

Values indicate Mean $\pm$ S.D of n=3

3.7 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Alkaline Phosphatase (ALP) Activities in Paracetamol-Intoxicated Rats.

From fig. 6 after 24 hours, alkaline phosphatase (ALP) activities of group 2 rats (positive control) showed a significant increase ($p < 0.05$) when compared to the negative control (group 1) and the standard control (group 3). A non-significant difference ($p > 0.05$) was observed between the ALP activities of the test groups, however the test groups (group 4 and 5) showed a significant decrease ($p < 0.05$) when compared to the positive control. As observed in the hepatocurative groups at day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the activities of ALP between the test groups, but a significant increase ($p < 0.05$) was observed in the activities of ALP in the positive control when compared to the negative control and the standard control. However there was a significant decrease ($p < 0.05$) in the ALP activities of the test groups when compared to the positive control.

In the hepatocurative groups after 14 days treatment, it was observed that there was a significant increase ($p < 0.05$) in the ALP activities of the positive control groups when compared to the negative control and the standard control group. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant decrease ($p < 0.05$) when compared to the positive control.

0.05) in the ALP activities when compared to the positive control. Across the groups, groups 1, 2, 3, and 5 showed neither a significant increase nor decrease ($p \geq 0.05$) in 24 hours, day 8 and 15 respectively, while in group 4 there was a significant decrease ($p \leq 0.05$) in ALP activities at day 15 when compared to 24 hours. However in group 4 a non-significant difference ($p \geq 0.05$) was observed in ALP activities at day 8 when compared to day 15 and 24 hours.

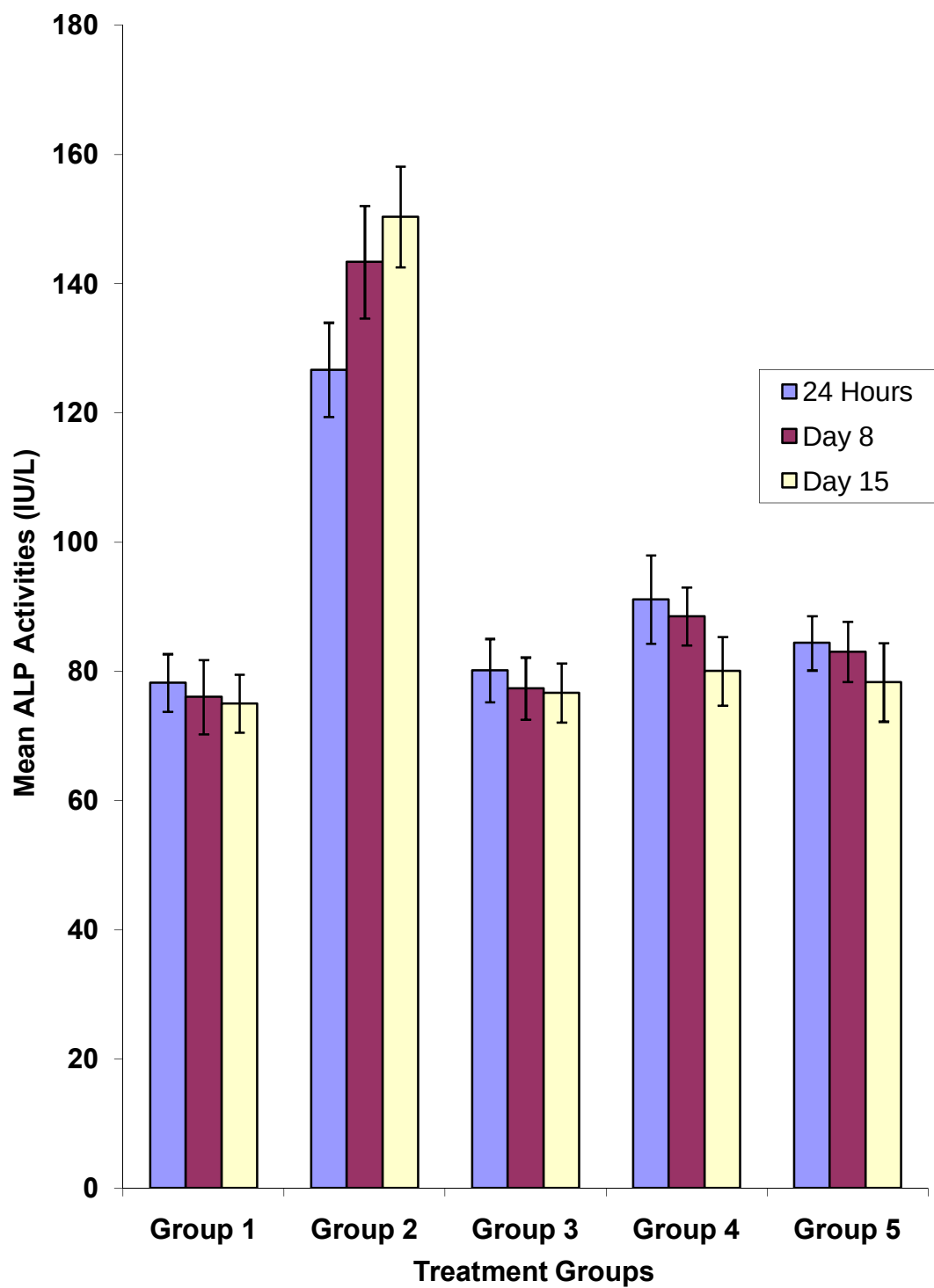


Fig. 6: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the alkaline phosphatase activities of paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)
Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)
Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)
Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)
Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.8 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Aspartate Aminotransferase (AST) Activities in Paracetamol-Intoxicated Rats.

As observed in figure 7, the hepato-protective groups (24 hours) showed a significant increase ($p < 0.05$) in the AST activities of the positive control group when compared to the negative control and the standard control. There was neither a significant increase nor decrease ($p < 0.05$) among the test groups, however the test groups showed a significant decrease ($p < 0.05$) in the AST activities when compared to the positive control. AST activities of group 2 rats (positive control) showed a significant increase ($p < 0.05$) when compared to the vehicle control group and the standard group in the hepato-curative groups after 7 days of treatment. A non-significant difference ($p < 0.05$) was observed between the AST activity of the test groups, however there was a significant decrease ($p < 0.05$) between the test groups and the positive control and a non-significant difference ($p < 0.05$) was observed between the test group and the standard control.

In the hepato-curative groups day 15, there was neither a significance increase nor decrease ($p < 0.05$) in the activities of AST between the test groups, but a significant increase ($p < 0.05$) was observed in the positive groups when compared to the negative control and the standard control. There was a significant decrease ($p < 0.05$) in the test groups when compared to the positive control group. In the group comparisons, groups 1, 3, 4 and 5 showed no significant difference ($p < 0.05$) in the activities of AST at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant increase ($p < 0.05$) in AST activities at day 15 when compared to day 15 and 24 hours.

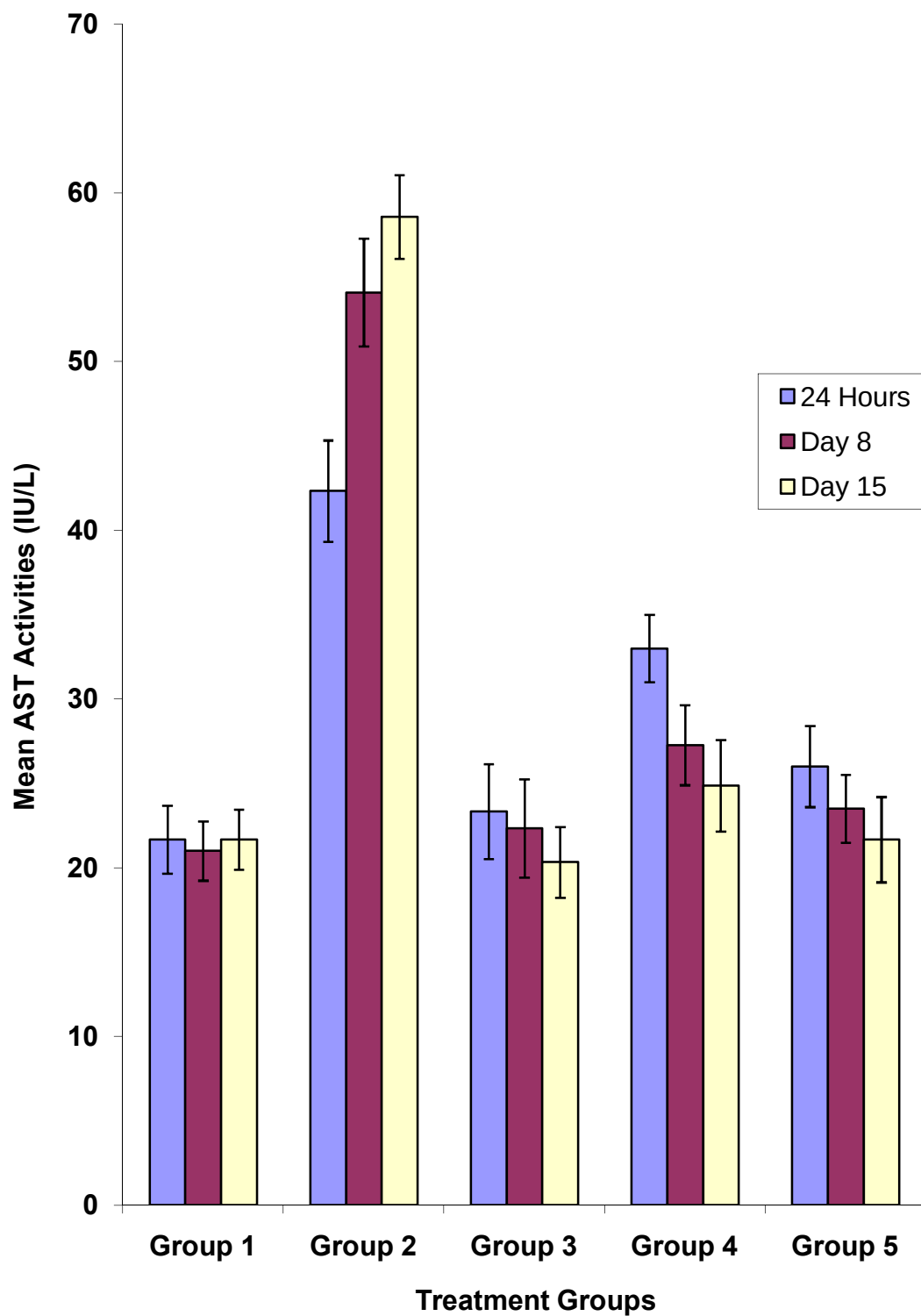


Fig. 7: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the aspartate aminotransferase activities in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.9 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Alanine Aminotransferase (ALT) Activities in Paracetamol-Intoxicated Rats.

From figure 8, In the hepato-protective groups (24 hours), alanine aminotransferase (ALT) activities of group 2 rats (positive control) showed a significant increase ($p < 0.05$) when compared to the vehicle control group and the standard group. A non-significant difference ($p > 0.05$) was observed between the ALT activities of the test groups, however the test groups were significantly ($p < 0.05$) lower than the positive control. As observed in the hepato-curative groups day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the activities of ALT between the test groups rather a significant increase ($p < 0.05$) was observed in the activities of ALT of the positive control when compared to the negative control and the standard control. There was an observed significant decrease ($p < 0.05$) in the test groups when compared to the positive control and a non-significant difference ($p > 0.05$) between the test groups and standard group.

In the hepato-curative groups day 15, it was observed that there was a significant increase ($p < 0.05$) in the ALT activities of the positive control groups when compared to the negative control and the standard control group. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant decrease ($p < 0.05$) in the ALT activities when compared to the positive control. Groups 1, 3, 4 and 5 showed a non-significant difference ($p > 0.05$) in the activities of ALT at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant increase ($p < 0.05$) in ALT levels at day 15 when compared to 24 hours. However in group 2, a non-significant difference ($p > 0.05$) was observed in ALT activities at day 8 when compared to 24 hours and day 15.

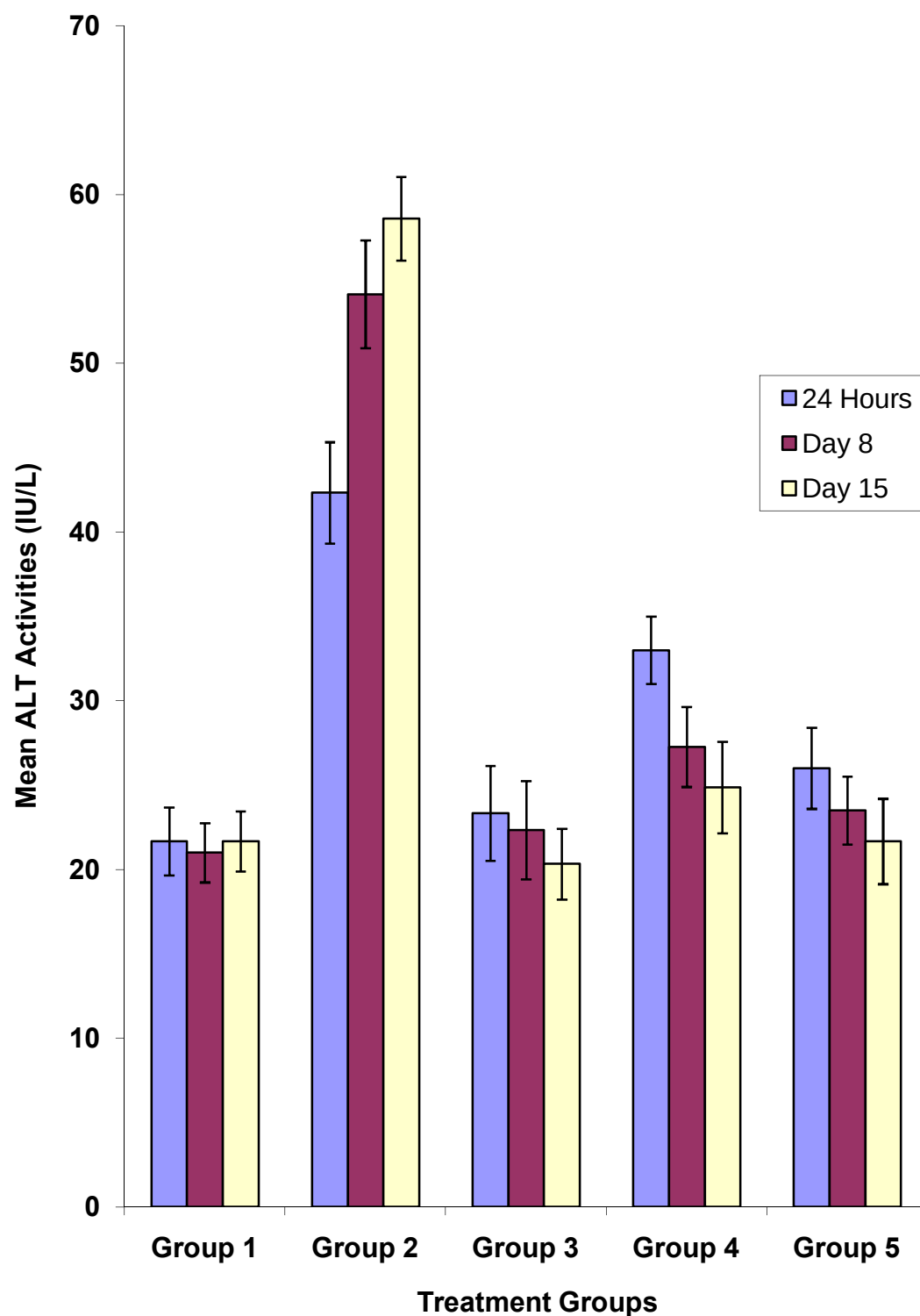


Fig. 2: Possible hepato- protective and curative effects of *Abrus precatorius* seed extract on the alanine aminotransferase activities in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)
Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)
Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)
Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)
Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.10 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Total Bilirubin Concentration in Paracetamol-Intoxicated Rats.

The results shown in figure 9 indicates that the total bilirubin concentration in the hepato-protective groups (24 hours) of group 2 rats (positive control) showed a significant increase ($p < 0.05$) when compared to the negative control and the standard control. A non-significant difference ($p > 0.05$) was observed between the total bilirubin concentrations of the test groups, however there was also no significant difference ($p > 0.05$) between the test groups and the standard control. In the hepato-curative groups day 8, the total bilirubin concentration of the positive control group increased significantly ($p < 0.05$) when compared to the negative control and the standard control. There was an observed significant decrease ($p < 0.05$) in the test groups when compared to the positive control. However there was a significant increase ($p < 0.05$) between the total bilirubin concentration of group 4 (Test group 100mg/kg b.w. of extract) rats when compared to negative and standard control and a non-significant difference ($p > 0.05$) was observed between group 5 (Test group 200mg/kg b.w. of extract) rats when compared to negative and standard control.

In the hepato-curative groups day 15, it was observed, a significant increase ($p < 0.05$) in the total bilirubin concentrations of the positive control group when compared to the negative control and the standard control. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups were significantly lower ($p < 0.05$) in the total bilirubin concentrations than the positive control. In group comparisons, there was a non-significant difference ($p > 0.05$) in the total bilirubin concentrations at 24 hours, day 8 and day 15 in group 1, 3, 4 and 5, while in group 2 there was a significant increase ($p < 0.05$) in the total bilirubin concentrations at day 15 when compared to day 8 and 24 hours.

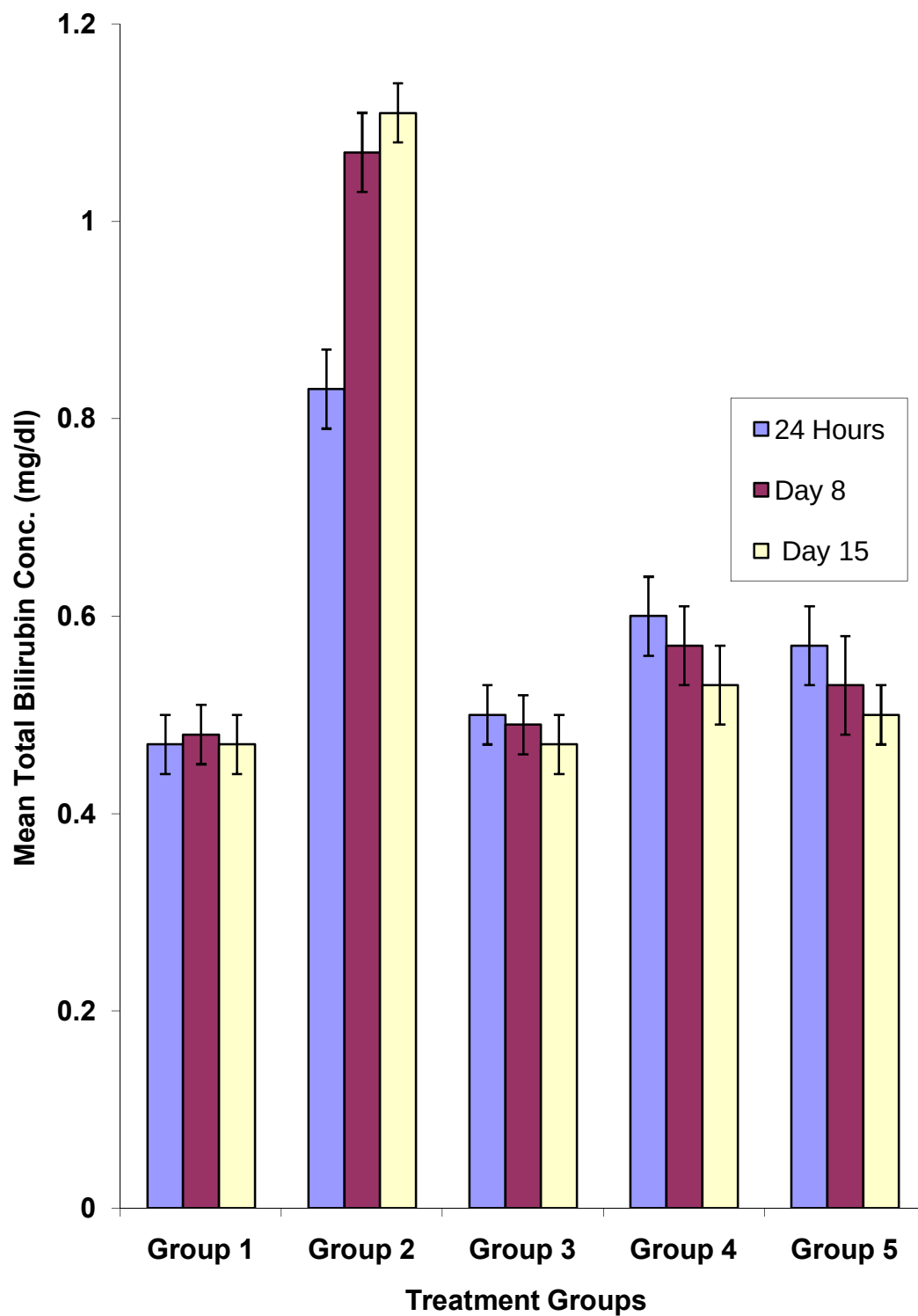


Fig. 9: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the total bilirubin concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.11 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Serum Urea Concentration in Paracetamol-Intoxicated Rats.

As observed in figure 10, the serum urea concentrations of group 2 rats (positive control) of the hepato-protective groups (24 hours) showed a significant increase ($p < 0.05$) when compared to the negative and the standard control groups. However there was neither a significant increase nor decrease ($p > 0.05$) between the serum urea concentrations of group 4 (test group 100mg/kg b.w. of extract) rats when compared to the positive control. A significant decrease ($p < 0.05$) was observed in the group 5 (Test group 200mg/kg b.w. of extract) rats when compared to the positive control. In the hepato-curative groups after treatment for 7 days, there was neither a significant increase nor decrease ($p > 0.05$) in the serum urea concentration between the test groups. A significant increase ($p < 0.05$) in the serum urea concentration was observed in the positive control when compared to the negative and the standard control groups, however there was an observed significant decrease ($p < 0.05$) in the test groups when compared to the positive control.

In the hepato-curative groups day 15, it was observed that there was a significant increase ($p < 0.05$) in the serum urea concentration of the positive control group when compared to the negative and the standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups when compared to the standard control. However the test groups showed a significant decrease ($P < 0.05$) in serum urea concentrations when compared to the positive control. Across the groups, group 1, 3, 4 and 5 showed a non-significant difference ($P > 0.05$) in the serum urea concentration at 24 hours, day 8 and day 15 respectively. However in group 2 there was a significant increase ($P < 0.05$) in serum urea concentrations at day 15 when compared to day 8 and 24 hours.

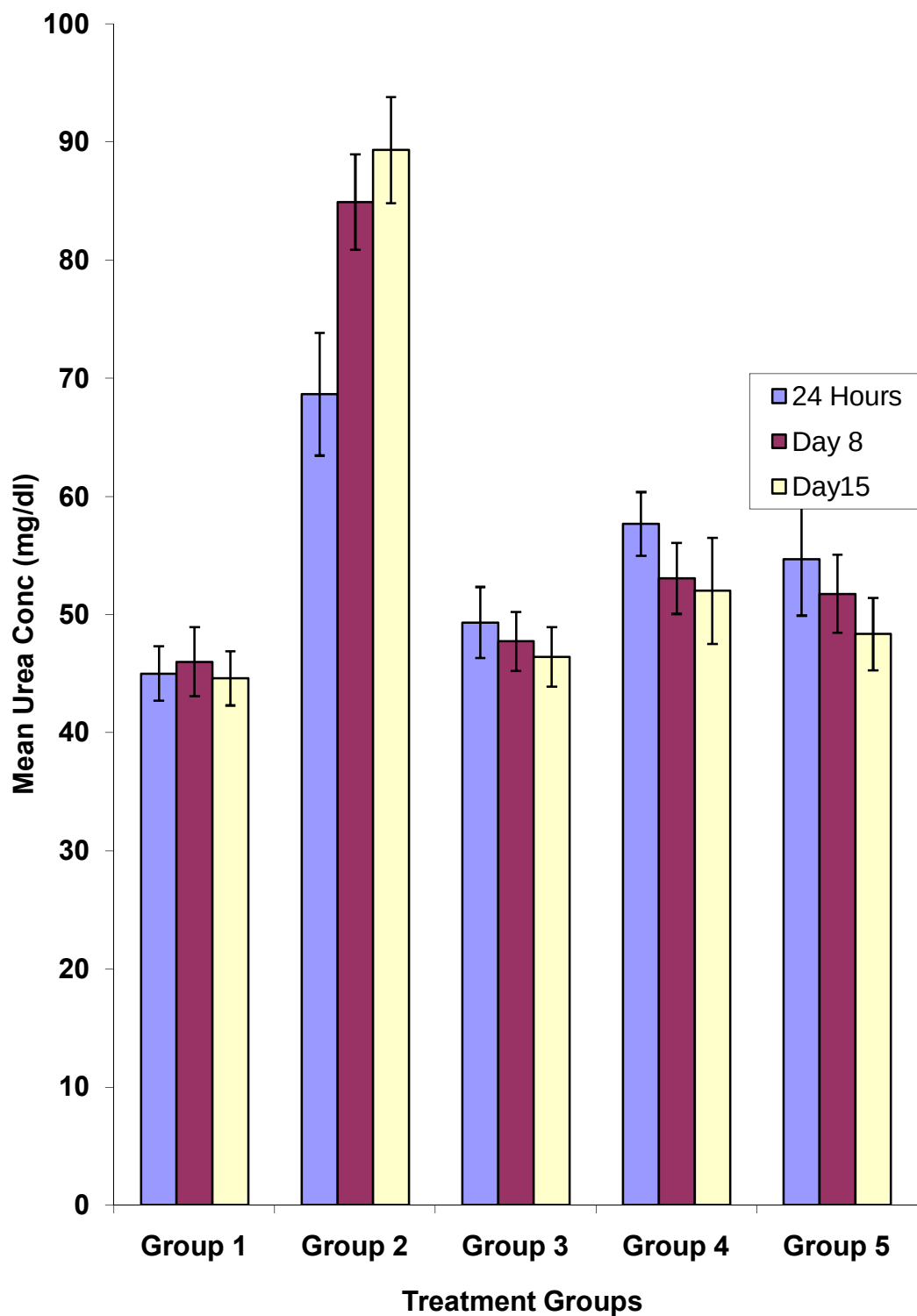


Fig. 10: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the serum urea concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.12 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on Serum Creatinine Concentration in Paracetamol-Intoxicated Rats.

The Serum Creatinine concentration of group 2 rats (positive control) of the hepato-protective groups (24 hours) showed a significant increase ($p < 0.05$) when compared to the negative and the standard control groups as shown in figure 11. However there was no significant difference ($p > 0.05$) between the serum creatinine concentrations of group 4 (test group 100mg/kg b.w. of extract) rats when compared to the positive control while a significant decrease ($p < 0.05$) was observed in the group 5 (Test group 200mg/kg b.w. of extract) rats when compared to the positive control. In the hepato-curative groups day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the serum creatinine concentration of the test groups, however the test groups decreased significantly ($p < 0.05$) when compared to the positive control. A significant increase ($p < 0.05$) in the concentration serum creatinine was observed in the positive groups when compared to the negative and standard control groups.

In the hepato-curative groups day 15, it was observed that there was a significant increase ($p < 0.05$) in the concentration of serum creatinine of the positive control when compared to the negative control and the standard control group, however a non-significant difference ($p > 0.05$) was observed between the test groups. The test groups showed a significant decrease ($p < 0.05$) in the serum creatinine concentrations when compared to the positive control. Across the groups, groups 1, 2, 3 and 4 showed no significant difference ($p > 0.05$) in the serum creatinine concentrations at 24 hours, day 8 and day 15 respectively. However in group 5 there was a significant decrease ($p < 0.05$) in the serum creatinine concentrations at day 15 when compared to 24 hours. However there was neither a significant increase nor decrease ($p > 0.05$) in the serum creatinine concentrations of day 8 when compared to day 15 and 24 hours in group 5 rats.

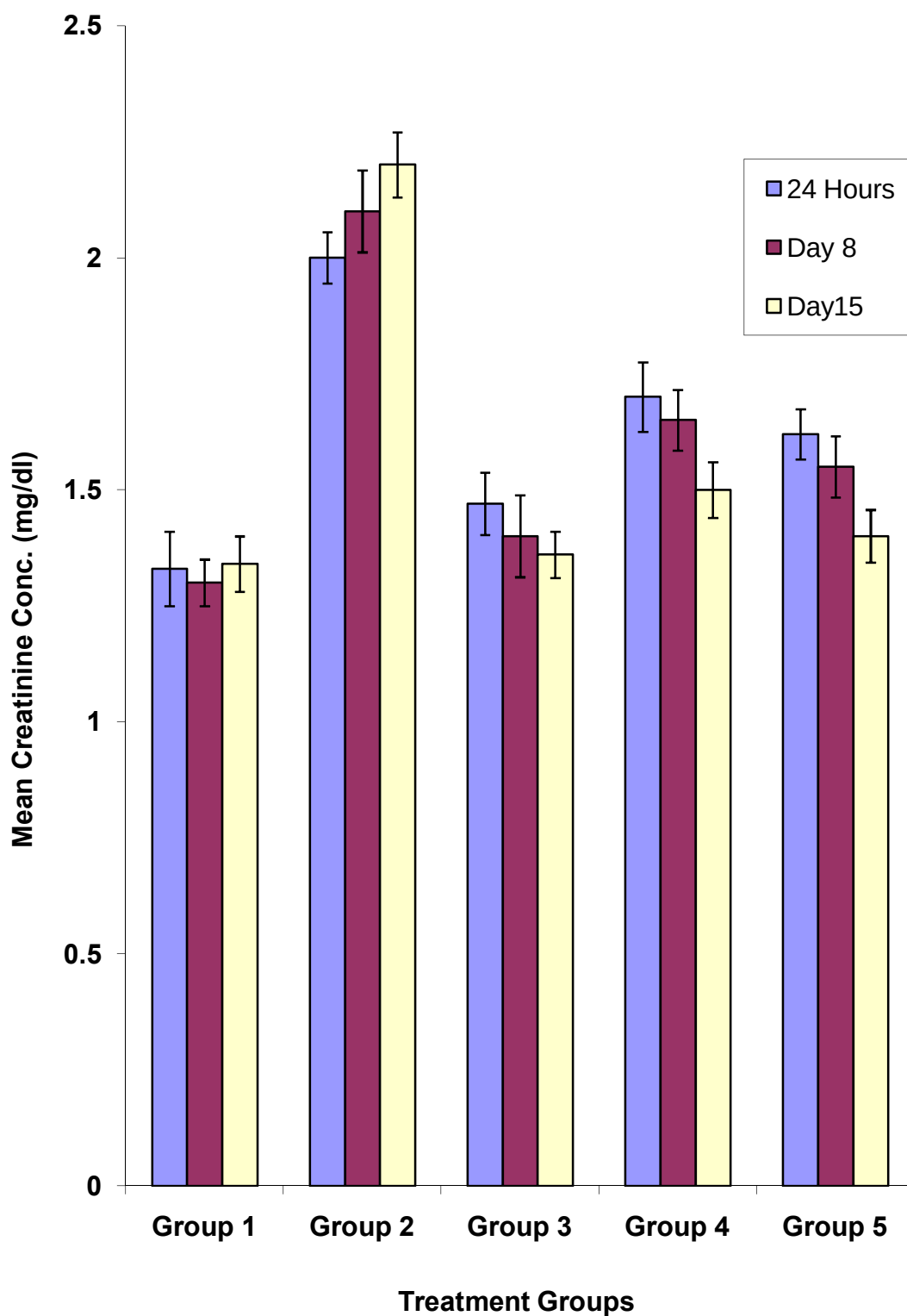


Fig. 11: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the creatinine concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.13 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Sodium Ion Concentration in Paracetamol-Intoxicated Rats.

The group 2 rats (positive control) of the hepato-protective groups (24 hours) showed a significant decrease ($p < 0.05$) in the Sodium ion concentration when compared to the negative and the standard control groups (figure 12). A non-significant difference ($p > 0.05$) was observed in the Sodium ion concentration of the test groups, but there was no significant difference ($p > 0.05$) between the sodium ion concentration of group 4 (test group 100mg/kg b.w. of extract) rats when compared to the positive control and a significant increase ($p < 0.05$) was observed between group 5 (Test group 200mg/kg b.w. of extract) rats when compared to the positive groups. As observed in the hepato-curative groups day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the sodium ion concentration of the test groups. However there was a significant increase ($p < 0.05$) in the sodium ion concentration of the test groups when compared to the positive control. A significant decrease ($p < 0.05$) in the sodium ion concentration was observed in the positive groups when compared to the negative and the standard control groups.

In the hepato-curative groups day 15, it was observed that the sodium ion concentration of the positive control groups decreased significantly ($p < 0.05$) when compared to the negative and standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant increase ($p < 0.05$) in the sodium ion concentration when compared to the positive control. Across the groups, groups 1, 3, 4 and 5 showed a non-significant difference ($p > 0.05$) in the sodium ion concentration at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant decrease ($p < 0.05$) in the sodium ion concentration at day 15 when compared to 24 hours. However there was neither a significant increase nor decrease ($p > 0.05$) in the sodium ion concentration at day 8 when compared to 24 hours.

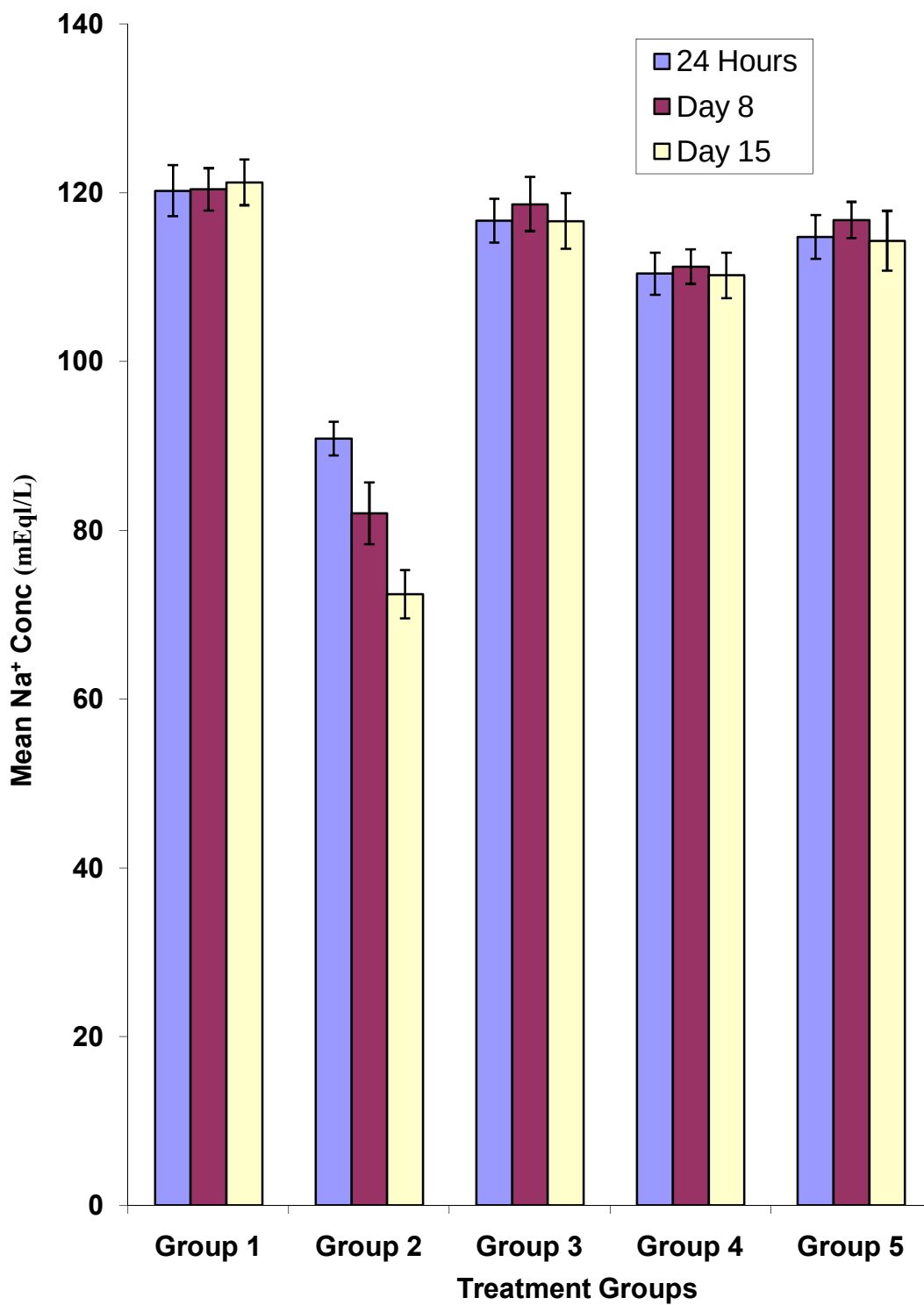


Fig. 12: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the sodium ion concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.14 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Potassium Ion Concentration in Paracetamol-Intoxicated Rats.

From figure 13, the hepato-protective groups (24 hours) showed a significant increase ($p < 0.05$) in the potassium ion concentration of group 2 rats (positive control) when compared to the negative and the standard control group. A non-significant difference ($p > 0.05$) was observed between the potassium ion concentrations of the test groups, however there was a significant decrease ($p < 0.05$) between the test groups and the positive control. In the hepato-curative groups day 8, a significant increase ($p < 0.05$) was observed in the potassium ion concentration of the positive groups when compared to the negative control and the standard control. There was neither a significant increase nor decrease ($p > 0.05$) in the potassium ion concentration between the test groups. There was a significant decrease ($p < 0.05$) in the test groups when compared to the positive control.

In the hepato-curative groups day 15, it was observed that there was a significant increase ($p < 0.05$) in the potassium ion concentration of the positive control groups when compared to the negative and the standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups, however the test groups showed a significant decrease ($p < 0.05$) in the potassium ion concentration when compared to the positive control. Between groups 1, 3, and 5 there was no significant difference ($p > 0.05$) in the potassium ion concentration at 24 hours, day 8 and day 15 respectively. Also as observed in group 2 and group 4 there was no significant difference ($p > 0.05$) in the potassium ion concentration at day 15 when compared to day 8 and 24 hours.

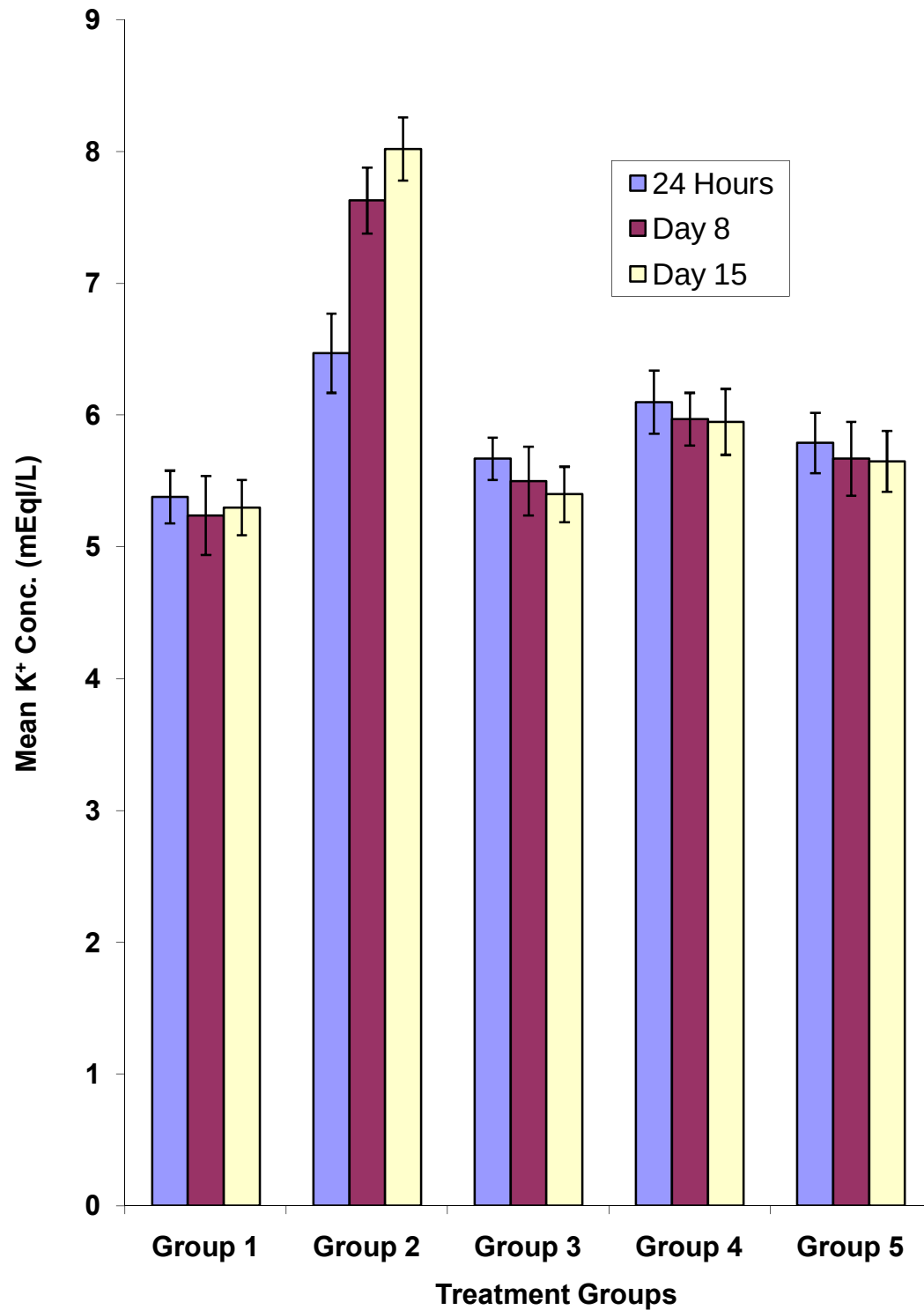


Fig. 13: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the potassium ion concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.15 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Chloride Ion Concentration in Paracetamol-Intoxicated Rats.

From figure 14, in the hepato-protective groups (24 hours) the chloride ion concentration of group 2 rats (positive control) was significantly lower ($p < 0.05$) than that of the negative and the standard control groups. A non-significant difference ($p > 0.05$) was observed between the chloride ion concentrations of the test groups, however there was a significant increase ($p < 0.05$) between the test groups and the positive control. In the hepato-curative groups day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the chloride ion concentrations between the test groups. However a significant decrease ($p < 0.05$) was observed in the chloride ion concentrations of the positive control when compared to the negative and standard control groups, but the test groups showed a significant increase ($p < 0.05$) in the chloride ion concentrations when compared to the positive control.

In the hepato-curative groups day 15, it was observed that there was a significant decrease ($p < 0.05$) in the chloride ion concentrations of positive control group when compared to the negative and the standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant increase ($p < 0.05$) in the chloride ion concentrations when compared to the positive control. Across the groups, groups 1, 3, 4 and 5 showed no significant difference ($p > 0.05$) in the chloride ion concentrations at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant decrease ($p < 0.05$) in the chloride ion concentrations at 24 hours when compared to day 15.

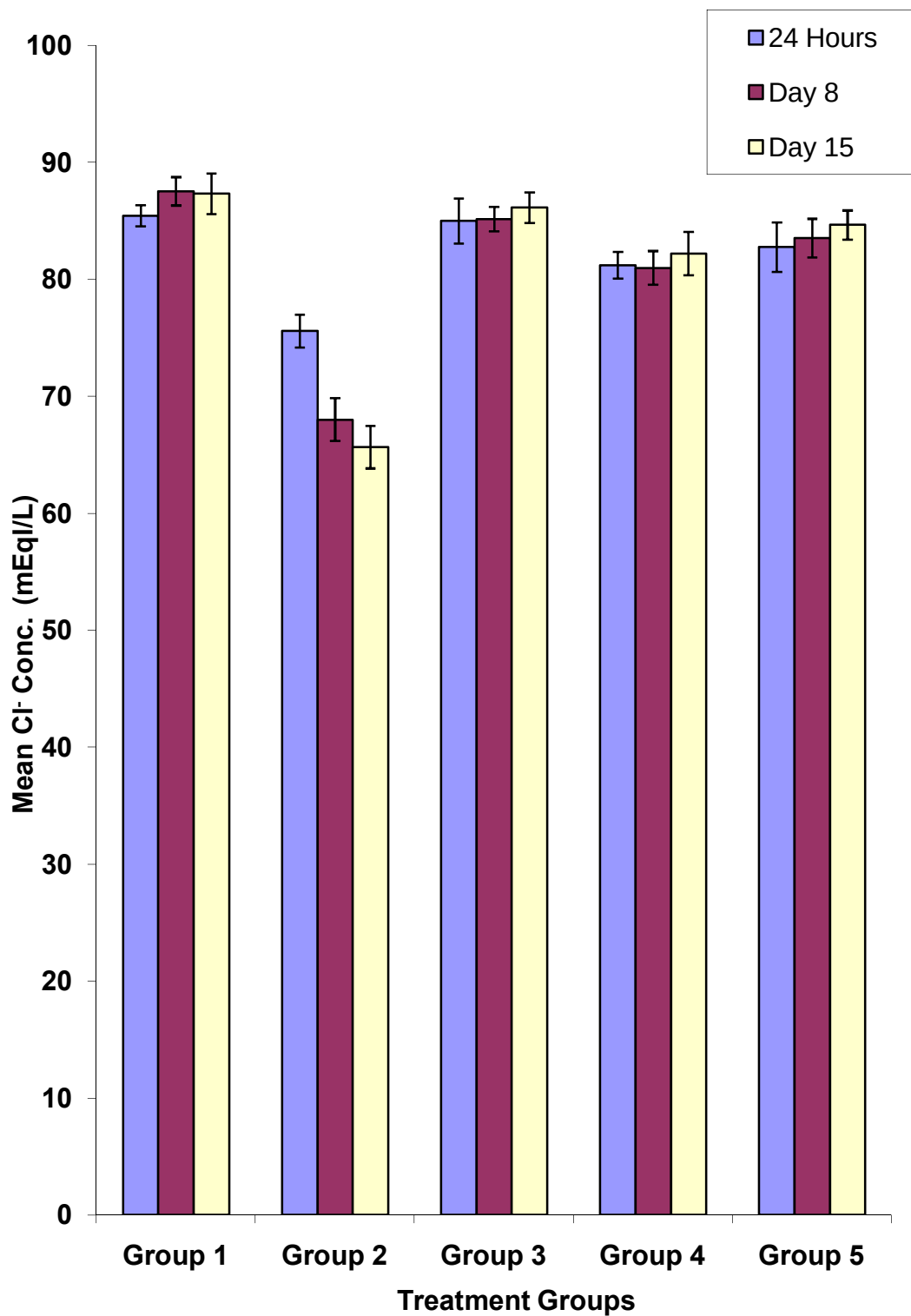


Fig. 14: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the chloride ion concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.16 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Superoxide Dismutase(SOD) Activities in Paracetamol-Intoxicated Rats.

The SOD activity in group 2 rats (positive control) of hepato-protective groups (24 hours) showed a significant decrease ($p < 0.05$) when compared to the negative and the standard control groups as shown in figure 15. A non-significant difference ($p > 0.05$) was observed between the SOD activity of the test groups, however there was a significant increase ($p < 0.05$) between the test groups and the positive control. At day 8 (hepato-curative groups), there was neither a significant increase nor decrease ($p > 0.05$) in the activities of SOD between the test groups. However a significant decrease ($p < 0.05$) in the activity of SOD was observed in the positive control when compared to the negative and standard control. There was also a significant increase ($p < 0.05$) in the test groups when compared to the positive control.

In the hepato-curative groups day 15, it was observed, a significant decrease ($p < 0.05$) in the SOD activity of the positive control when compared to the negative and standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant increase ($p < 0.05$) in the SOD activity when compared to the positive control. Across the groups, groups 1, 3, 4, and 5 showed no significant difference ($p > 0.05$) in the SOD activity at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant decrease ($p < 0.05$) in the SOD activity at day 15 when compared to day 8 and 24 hours.

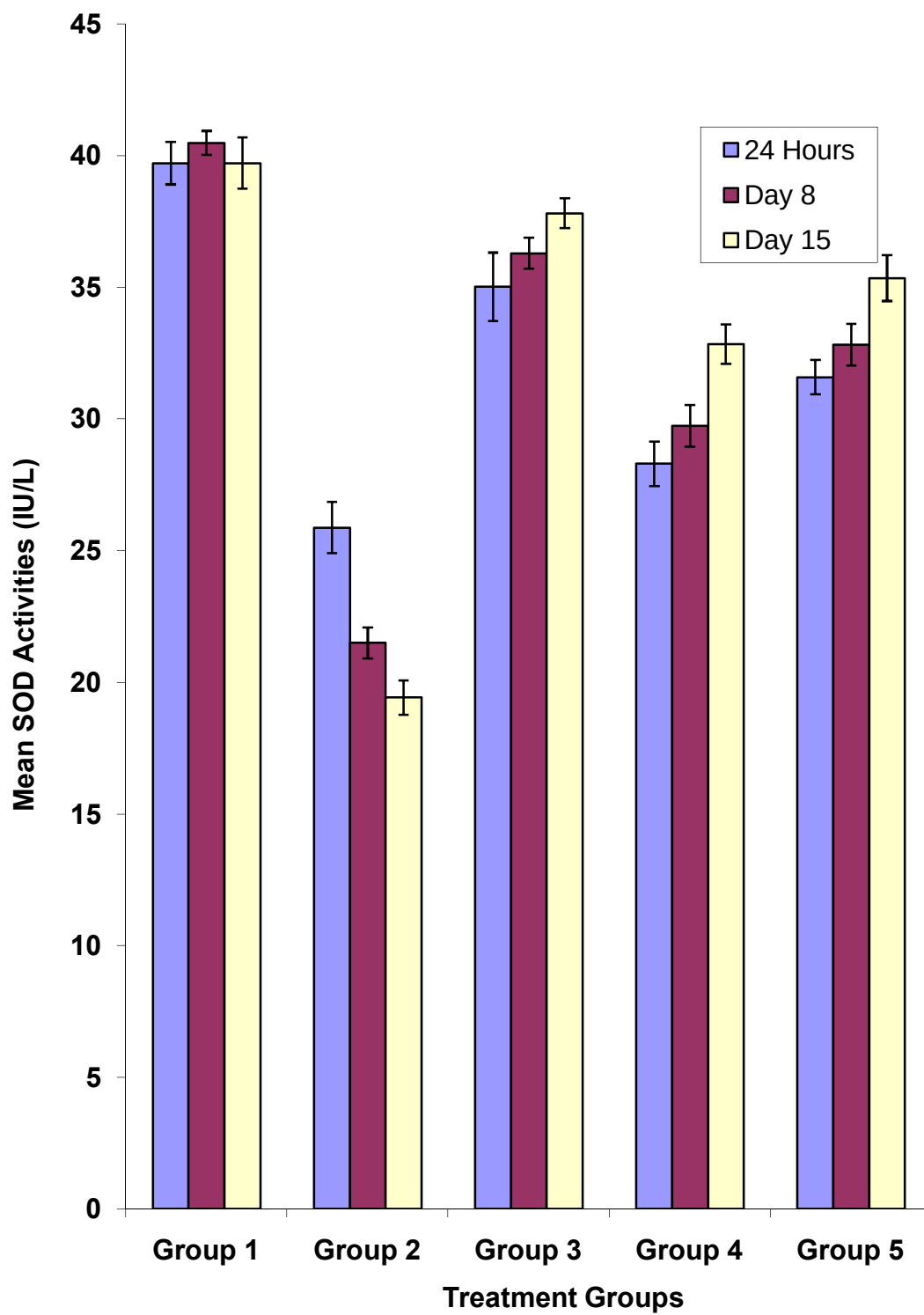


Fig. 15: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the superoxide dismutase activities in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.17 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Malondialdehyde (MDA) Concentration in Paracetamol-Intoxicated Rats.

As observed in figure. 16, the group 2 rats (positive control) of the hepato-protective groups (24 hours) showed a significant increase ($P < 0.05$) in MDA concentration when compared to the negative and standard control groups. A non-significant difference ($P > 0.05$) was observed in the MDA concentration of the test groups, however there was also a non-significant difference ($P > 0.05$) between the test groups and the standard control group. In the hepato-curative groups day 8, there was a significant decrease ($P < 0.05$) in the test groups when compared to the positive control. However there was neither a significant increase nor decrease ($P > 0.05$) in the concentration of MDA between the test groups. A significant increase ($P < 0.05$) in the MDA concentration was observed in the positive control when compared to the negative and standard control groups.

It was observed that there was a significant increase ($P < 0.05$) in the MDA concentrations of the positive control when compared to the negative and standard control groups in the hepato-curative groups at day 15. A non-significant difference ($P > 0.05$) was observed between the test groups. However the test groups showed a significant decrease ($P < 0.05$) in the MDA concentration when compared to the positive control. Groups 1, 3, 4 and 5 showed no significant difference ($P > 0.05$) in the MDA concentrations at 24 hours, day 8 and day 15 respectively, while in group 2 there was a significant increase ($P < 0.05$) in the MDA concentration at day 15 when compared to 24 hours. However there was no significant difference ($P > 0.05$) in the MDA concentrations at day 8 when compared to day 15 and 24 hours as seen in group 2.

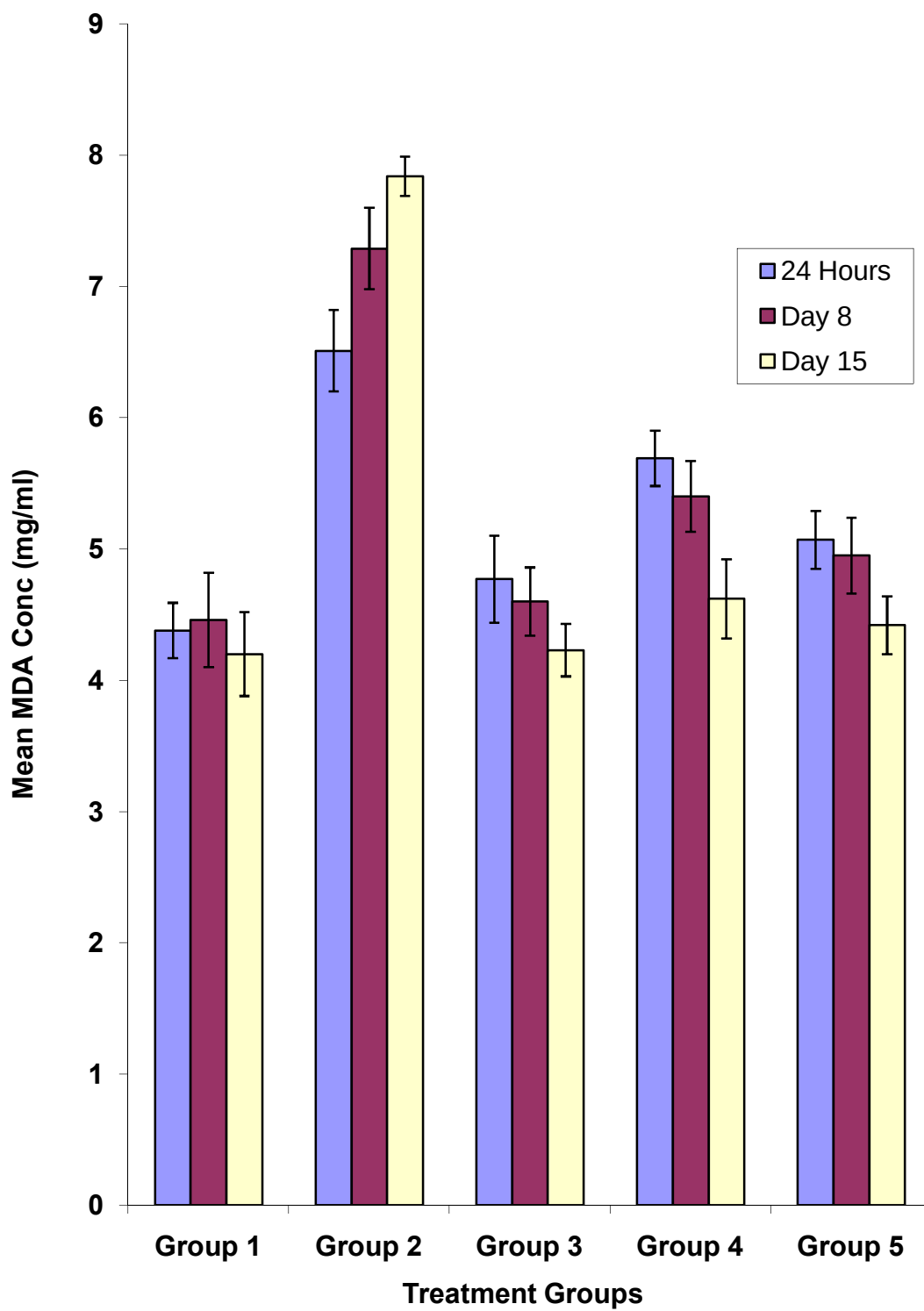


Fig. 16: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the malondialdehyde concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.18 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Haemoglobin Concentration in Paracetamol-Intoxicated Rats.

From figure 17, the hepato-protective groups (24 hours) showed a significant decrease ($p < 0.05$) in the haemoglobin concentration of group 2 rats (positive control) when compared to the negative and standard control groups. There was an observed significant increase ($p < 0.05$) in the haemoglobin concentration of test groups when compared to the positive control. A non-significant difference ($p > 0.05$) was observed in the haemoglobin concentration of the test groups, however there was no significant difference ($p > 0.05$) between the test groups and the standard control. There was neither a significant increase nor decrease ($p > 0.05$) in the haemoglobin concentration between the test groups of the hepato-curative groups at day 8. However there was a significant decrease ($p < 0.05$) in the haemoglobin concentration of the positive control when compared to the negative and standard control. There was an observed significant increase ($p < 0.05$) in the haemoglobin concentration of test groups when compared to the positive control.

In the hepato-curative groups day 15, it was observed that there was a significant decrease ($p < 0.05$) in the haemoglobin concentration of the positive control when compared to the negative and standard control groups, however a non-significant difference ($p > 0.05$) was observed between the test groups. The test groups showed a significant increase ($p < 0.05$) in the haemoglobin concentration when compared to the positive control. Groups 1, 3, and 5 showed no significant difference ($p > 0.05$) in their haemoglobin concentrations at 24 hours, day 8 and day 15 respectively. Also as seen in group 2 and 4 there was a non-significant difference ($p > 0.05$) in the haemoglobin concentration at day 15 when compared to day 8 and 24 hours respectively.

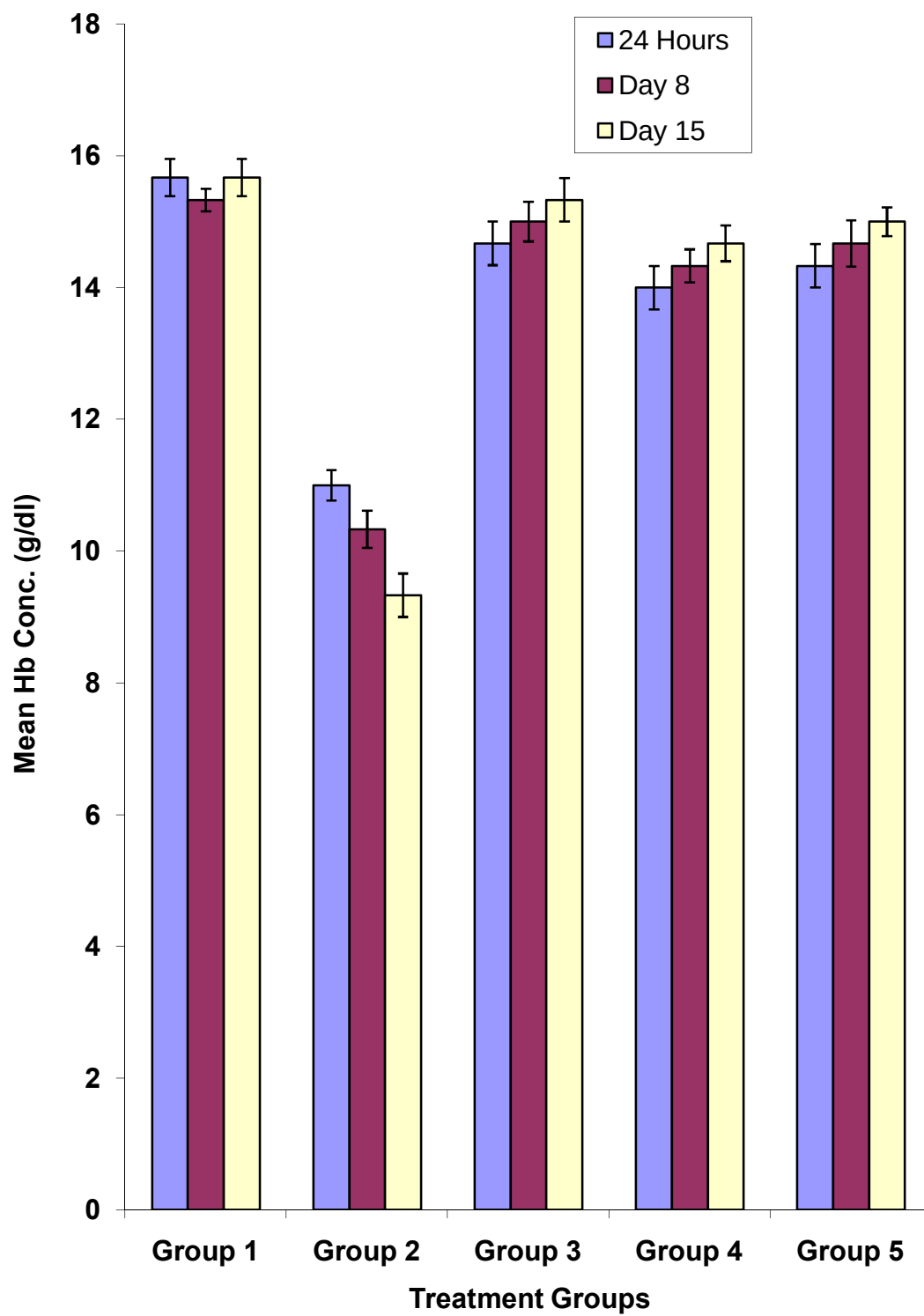


Fig. 17: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the haemoglobin concentration in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.19 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Packed Cell Volume (PCV) Concentration in Paracetamol-Intoxicated Rats.

The hepato-protective groups (24 hours) showed a significant decrease ($p < 0.05$) in the packed cell volume (PCV) concentration of group 2 rats (positive control) when compared to the normal and standard control groups as seen in figure 18. A non-significant difference ($p > 0.05$) was observed in the PCV concentration of the test groups, however the test groups showed a significant decrease ($p < 0.05$) when compared to the standard control group. In the hepato-curative groups day 8, there was a significant increase ($p < 0.05$) in the test groups when compared to the positive control. However there was no observed significant difference ($p > 0.05$) in the PCV concentration of the test groups. A significant decrease ($p < 0.05$) in the PCV concentration was observed in the positive groups when compared to the negative and the standard control groups.

The hepato-curative groups showed a significant decrease ($p < 0.05$) in the PCV concentration of the positive control when compared to the negative and the standard control at day 15. A non-significant difference ($p > 0.05$) was observed between the test groups when compared to the standard control. However the test groups showed a significant increase ($p < 0.05$) in the PCV concentration when compared to the positive control. Groups 1, 3, and 5 showed a non-significant difference ($p > 0.05$) in their PCV concentrations at 24 hours, 7 days and 14 days respectively. Also as seen in group 2 and 4 there was no significant difference ($p > 0.05$) in the PCV concentration at day 15 when compared to day 8 and 24 hours respectively.

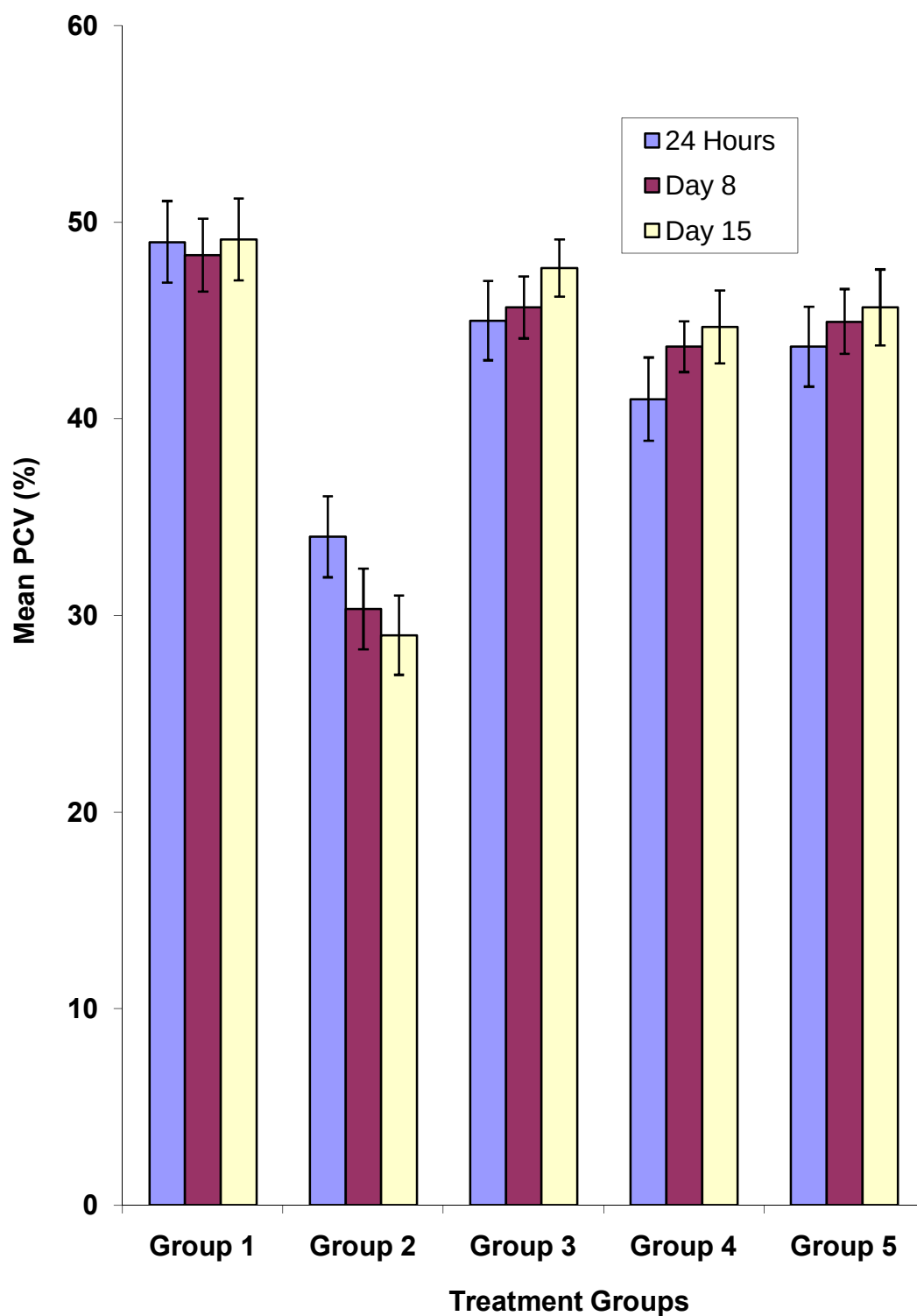


Fig. 18: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the packed cell volume activities of paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.20 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the Red Blood Cell Count (RBC) in Paracetamol-Intoxicated Rats.

From figure 19, the hepato-protective groups (24 hours) showed a significant difference ($p < 0.05$) in the red blood cell count (RBC) of group 2 rats (positive control) when compared to the negative and the standard control groups. A non-significant difference ($p > 0.05$) was observed between the RBC of the test groups, however the test groups showed a significant increase ($p < 0.05$) when compared to the positive control. In the hepato-curative groups day 8, there was neither a significant increase nor decrease ($p > 0.05$) in the RBC between the test groups, rather a significant decrease ($p < 0.05$) was observed in the RBC of the positive groups when compared to the negative and the standard control groups. There was an observed significant increase ($p < 0.05$) in the test groups when compared to the positive control.

The hepato-curative groups showed a significant decrease ($p < 0.05$) in the RBC of the positive control when compared to the negative and the standard control groups at day 15. A non-significant difference ($p > 0.05$) was observed between the test groups when compared to the standard control group. However the RBC of the test groups increased significantly ($p < 0.05$) when compared to the positive control. Across the groups, groups 1, 3, and 5 showed a non-significant difference ($p > 0.05$) in the RBC concentrations at 24 hours, day 8 and day 15 respectively. Also in group 2 and 4 there was neither a significant increase nor decrease ($p > 0.05$) in the RBC concentration at day 15 when compared to day 8 and 24 hours respectively.

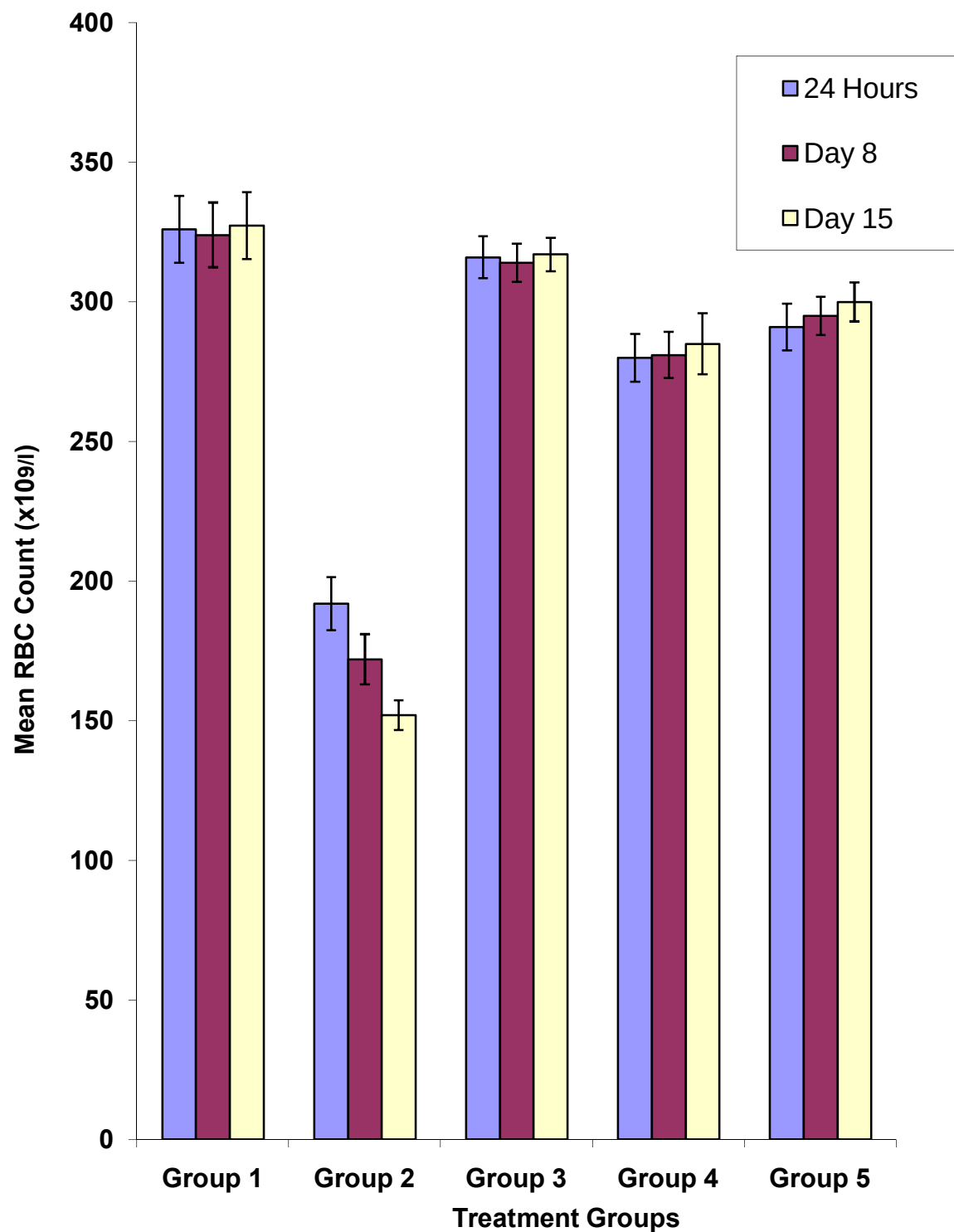


Fig. 19: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the red blood cell count in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)

Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)

Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)

Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

3.21 Effect of Fraction 1 of *Abrus precatorius* Seed Methanol Extract on the White Blood Cell Count (WBC) in Paracetamol-Intoxicated Rats.

In figure 20, the hepato-protective groups (24 hours) showed a significant increase ($p < 0.05$) in the WBC of group 2 rats (positive control) when compared to the negative and standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups, however there was a significant decrease ($p < 0.05$) in the WBC of the test groups when compared to the positive control. The hepato-curative groups showed a significant increase ($p < 0.05$) in the WBC of the positive control when compared to the negative and standard control groups at day 8. A non-significant difference ($p > 0.05$) was observed among the test groups when compared to the standard control group. However the test groups showed a significant decrease ($p < 0.05$) in the WBC when compared to the positive control.

In the hepato-curative groups day 15, it was observed, a significant increase ($p < 0.05$) in the WBC of the positive control groups when compared to the negative and standard control groups. A non-significant difference ($p > 0.05$) was observed between the test groups. However the test groups showed a significant decrease ($p < 0.05$) in the WBC when compared to the positive control. It is seen in figure 20 that groups 1, 3, and 5 showed a non-significant difference ($p > 0.05$) in their WBC at 24 hours, day 8 and day 15 respectively. Also in group 2 and 4 there was neither a significant increase nor decrease ($p > 0.05$) in the WBC at day 15 when compared to day 8 and 24 hours respectively.

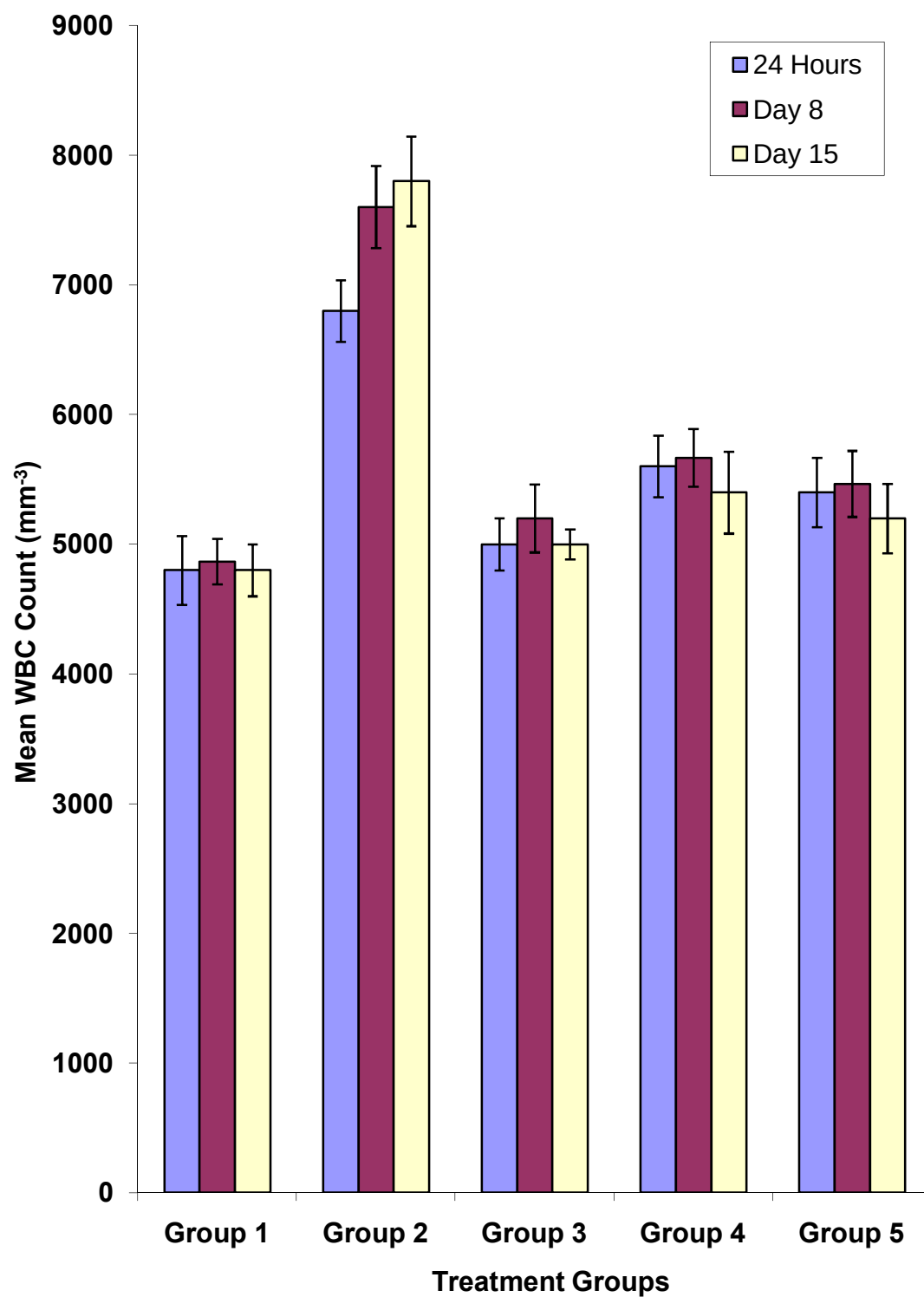


Fig. 20: Possible hepato- protective and curative effects of *Abrus precatorius* seed fraction on the white blood cell count in paracetamol-intoxicated rats

Group 1 = Negative control (5 ml/kg of normal saline)
Group 2 = Positive control (paracetamol 2500 mg/kg b.w.)
Group 3 = Standard control (Silymarin 100 mg/kg b.w. + paracetamol)
Group 4 = Test group (100 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)
Group 5 = Test group (200 mg/k.g. b.w. of fraction 1 of the Abrus seed extract + paracetamol)

CHAPTER FOUR

DISCUSSION

The liver is a key organ regulating homeostasis within the body by various functions. The liver occupies the pivotal position in the body, plays an essential role in drug and xenobiotic metabolism and also in maintaining the biological equilibrium of the organism (Kumar *et al.*, 2010). The role played by the liver in the removal of toxic substances from the portal circulation makes it susceptible to persistence attack by offending foreign compounds (xenobiotic) culminating in liver dysfunction (Vidhya and Mettilda, 2009). However hepatotoxicity is one of very common ailment resulting from serious debilities ranging from severe metabolic disorders to mortality (Kanchana and Sadiq, 2011), and conventional drugs used in its treatment are often inadequate. It is therefore necessary to search for alternative remedies for the treatment of liver diseases, especially drug-induced liver diseases.

Paracetamol, a commonly used analgesic, is considered safe at therapeutic doses. However, an overdose of paracetamol causes severe hepatotoxicity and necrosis in both humans and experimental animals (Garba *et al.*, 2009). At therapeutic levels, paracetamol is primarily metabolized in the liver by glucuronidation and sulphation; however, a small proportion undergoes cytochrome P450 (CYP450)-mediated bioactivation to N-acetyl-p-benzoquinimine (NAPQI), which is rapidly quenched by glutathione (GSH) (Kanchana and Sadiq, 2011). After an overdose of paracetamol, elevated levels of the toxic NAPQI metabolite are generated, which extensively deplete hepatocellular GSH and covalently oxidizes tissue macromolecules such as lipids and -SH groups of cellular proteins resulting in altered homeostasis and hepatocyte death (Galal *et al.*, 2012).

The choice of *Abrus precatorius* for this research work was based on the numerous pharmacological properties of *Abrus precatorius*, some of which include; antifertility effect (Rao, 2007); ureterotonic effect and antidiarrhoeal effect (Nwodo, 1991), anti-inflammatory

activity (Anam, 2001); spermicidal effect (Rajeshwari, 2011); uterine relaxation effect and uterine stimulant effect (Nwodo and Botting, 1983) amongst many others. The findings of this study are based on the hepato-protective ability and possible hepato-curative ability of fraction I of *Abrus precatorius* seed methanol extract on paracetamol-induced liver damage. In this study, the potential effect of fraction I of *Abrus precatorius* seed methanol extract on some liver marker enzymes, serum electrolytes and other biochemical parameters that could serve as indicators of liver damage were investigated.

The percentage yield of methanol extract of *Abrus precatorius* seeds was found to be 2.08% w/w. The acute toxicity test of the seed methanol extract of *Abrus precatorius* showed acute toxicity at the dose of 700 mg/k.g. b.w. Further purification of the extract using Sephadex gel G15 to get a purer sample was done which gave a percentage yield of 17.75% for fraction I and was used in this study.

Preliminary phytochemical analysis of fraction I showed the presence of some active agents as alkaloids, flavonoids, saponin, steroids, terpenoids etc as major compounds that could contribute to its medicinal properties. Alkaloids and flavonoids were found to be highly present while tannins were found to be slightly present in the fraction. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010) while flavonoids have been shown to have hepato-protective and hepato-curative capacity (Seevola *et al.*, 1984; Wegner and Fintelmann, 1999). Also the hepato-protective and hepato-curative effects of the standard drug, silymarin, used in this study have been shown to be due to the flavonolignan (polyphenolic fraction) extracted from the seeds of *Silybum marianum* plant (Pandey and Sahni, 2011). This probably suggests that the fraction may have the ability to scavenge free radicals due to the presence of alkaloids and flavonoids which are the chief sources of antioxidant in plants.

The quantitative phytochemical analysis of fraction I of *Abrus precatorius* seed methanol extract revealed the following phytochemicals in increasing measure: tannins, saponins, flavonoids and alkaloids and these phytochemicals could be physiologically potent in ameliorating several diseases. These phytochemicals have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone mechanisms and antibacterial and antiviral effects.

(Mamta *et al.*, 2013). The phytochemicals identified in fraction I of *Abrus precatorius* seed extract such as alkaloids, flavonoids, tannins, saponins etc all of which are known to have some therapeutic properties such as anti-microbial, anti-inflammatory and hepato-protective activities, which make the plant medicinally valuable (Mamta *et al.*, 2013). Flavonoids and alkaloids are potential antioxidant and probably exhibit free radical scavenging properties (Kris-Etherton *et al.*, 2002). Scavenging of reactive oxygen species, superoxide and hydroxyl radicals by these phytochemicals decreases the risk of oxidative damage to the tissues; contributing to the rapid and efficient hepato-protective and curative effects.

In this study, a significant increase ($p < 0.05$) was observed in the activities of AST, ALT, ALP and bilirubin level of the paracetamol group (group 2) when compared to the treated groups in both hepato-protective and hepato-curative models. This trend shows that the paracetamol affected the liver cells; thereby causing the enzymes to leak into circulation. However, treatment with fraction 1 of *Abrus precatorius* seed methanol extract at the dose of 100 and 200 mg/kg for groups 4 and 5 caused a significant decrease ($p < 0.05$) in the elevated activities of AST, ALT, ALP and bilirubin level in a dose- and time-dependent manner in both models which is a possible evidence that the extract was able to ameliorate the paracetamol-induced hepatocellular damage and may contain compounds that can be useful in the treatment of liver damage. The reversal of increased serum enzymes in acetaminophen-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity of the extract. This is in agreement with the commonly accepted view that serum activities of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987).

Possible mechanism that may be responsible for the protective and curative effects of the extract could be as a result of its antioxidant effect or by its action as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes (Ranju *et al.*, 2009). Also, its ability to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). Thus, by trapping oxygen related free radicals, the fraction could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipid peroxidative processes (Gupta, 2006).

Effective control of ALP activity and bilirubin concentration points towards an early improvement in the secretory mechanism of the hepatic cells. The efficacy of any hepato-protective or hepato-curative drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and the plant extract decreased acetaminophen induced elevated enzyme activities in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. This is in line with the work of Battu and Kumar(2009), where the hepato-protective activity of hydroalcoholic seedextract of *Abrus precatorius* on paracetamol-induced liver damage in rats.

Serum urea and creatinine are kidney markers used to test for the functionality of the kidney in a diseased state. Urea is a product of protein metabolism that should be excreted through the urine. Creatinine is produced in the body in proportion to body mass. In this result, administration of the toxicant resulted to a significant increase($p < 0.05$) in the serum urea and creatinine levels which could be as a result of kidney damage or increase in breakdown of intracellular membrane proteins or increase in the breakdown of muscle mass or creatinine phosphate in the untreated groups (positive control). However, in the groups treated with the extract (groups 4 and 5) and the standard drug (group 3), there was a marked reduction in serum urea and creatinine levels, which shows that the extract could aid in restoring the kidney functionality, probably by reviving the cells of the kidney. This result is in line with the works of (Ezeonwu and Dahiru, 2013) which suggest that serum urea and creatinine levels reverse back to normal at the healing of the hepatocytes in the case of a liver damage.

The level of serum electrolytes(Na^+ and Cl^-) showed a significant decrease ($p' 0.05$) in the positive control when compared to the treated groups (groups 3, 4 and 5) of both hepato-protective and curative groups. The loss in electrolytes could be as a result of impairment in the kidney function or insensitivity to the antidiuretic, aldosterone and parathyroid hormone in maintaining the electrolyte balance of the system. However, potassium ion concentration showed a time-dependent significant increase ($p' 0.05$) in the groups that received only the toxicant. This could be as a result of renal failure (Henry *et al.*, 1974) or metabolic acidosis which may be caused by the formation of mercapuric acid a compound formed by the reaction of the toxic metabolite of acetaminophen N-acetyl-p-benzoquinone imine (NAPQI) with

glutathione. However, treatment with fraction 1 of *Abrus Precatorius* seed methanol extract was able to maintain the electrolyte balance of the body in a dose- and time-dependent manner. This shows that the extract contains some active biochemical compounds that have the capacity to revive the non-functional/dead cells of the kidney and could be effective in the case of kidney damage.

Activity of serum superoxide dismutase (SOD) is the most sensitive enzymatic index in liver injury caused by ROS and oxidative stress. It has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Decrease in the activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury (Curtis and Mortiz, 1972). In the present study, it was observed that treatment with the extract caused a significant increase ($p < 0.05$) in hepatic SOD activity; thus, reduces reactive free radical induced oxidative damage to liver. This is in agreement with the work done by Ranjua *et al* (2009), where the *in-vitro* antioxidative activity of phenolic and flavonoid compounds extracted from seeds of *Abrus precatorius* was determined.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to paracetamol administration. The significant increase ($p < 0.05$) in serum MDA concentration suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. However, treatment with fraction 1 of *Abrus precatorius* seed methanol extract caused a significant decrease ($p < 0.05$) in the serum MDA concentration. This shows that the extract was able to prevent preoxidative damage and further damage of intracellular macromolecules in the system. Hence, the mechanism of the hepato-protective and hepato-curative effects of the extract could be attributed to the antioxidant effect. This is in line with the work done by Muthuswamy *et al*, (2012) where the Anticataractic and antioxidant activities of *Abrus precatorius* Linn against calcium-induced cataractogenesis were determined using goat lenses.

Haematological parameters such as HB, PCV, RBC showed significant decreases ($p < 0.05$) in groups that received only the toxicant. This could be as a result of haemolysis or inability of the kidney to produce erythropoietin (a hormone that stimulates the production of red blood cells) as a result of kidney failure. However, treatment with the extract showed a dose- and time-

dependent significant increase($p < 0.05$) in the haematological parameters when compared to the untreated group. However, the extract was able to maintain the haematological parameters within the normal range when compared to the negative control. This shows that the extract could have the ability to regenerate red cells, prevent haemolysis and maintain the blood level in the body. This is in agreement with the work done by Raghunath *et al.*, (2009) where the contraceptive effect of oil extract of seeds of *Abrus precatorius* (L) in male albino rats was evaluated.

In the present study, a significant increase ($p < 0.05$) in the white blood cell count (WBC) was observed in the group that received only the toxicant. This could be as a result of infestation, anaemia, infections, tissue damage inflammation or the body in its mechanism fighting the foreign compounds. However treatment with the extract and the standard drug caused a significant decrease($p < 0.05$) in the WBC count when compared to the positive control group. A non-significant difference ($p > 0.05$) was observed between the negative control groups, standard control groups and the test groups. By this result, it could be deduced that there was no anaemic condition, infection or tissue damage among these groups. The fraction 1 of the extract was able to maintain the WBC count in the test groups. This is in line with the work that was done by Anbu *et al.* (2011) where the anticancer activity of petroleum ether extract of *Abrus precatorius* on Ehrlich Ascitis carcinoma in mice was evaluated.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by acetaminophen. Treatment with different doses of Fraction 1 of *Abrus precatorius* seed methanol extract produced only mild degenerative changes and absence of centrilobular necrosis in a dose- and time-dependent manner, indicating its hepato-protective and hepato-curative efficiency. Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of fraction 1 of *Abrus Precatorius* seed methanol extract on acetaminophen induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme activities in addition to free radicals scavenging action.

4.2 CONCLUSION

In conclusion, the results of this study have demonstrated that overdose of paracetamol at a dose of 2500 mg / kg b.w. could be dangerous to the liver. From this findings, the Fraction 1 of *Abrus precatorius* methanol extract was able prevent liver damage in the paracetamol-intoxicated rats;

thereby, enhancing the synthesis of antioxidant, reduce lipid peroxidation, prevent leakage of liver enzymes into system, and also improve haematological parameters in a dose-dependent manner, hence its use as antioxidants, hepato-protective and hepato-curative agents may have scientific bases. The present study also highlights that the extract possesses a high antioxidant activity which can enhance the body defense mechanism in conditions of oxidative stress and as a potent therapeutic agent in the management of liver disorders.

4.3 SUGGESTION FOR FURTHER STUDIES

- I. There is need to use the fraction II of *Abrus precatorius* chloroform-methanol extract in a similar study to compare their potency.
- II. Further studies regarding the isolation and characterization of the active compounds that could be responsible for the hepato-protective and hepato-curative activity of *Abrus precatorius* seed is recommended.
- III. There is need to determine the structure and activity relationship of the active agent(s)

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APPENDICES

Appendices 1

Preparation of Reagents

5% (W/V) Ferric Chloride Solution

A quantity of ferric chloride (5.0 g) was dissolved and made up to 100 ml with distilled water.

Ammonium Solution

A quantity, of the stock concentration ammonium solution (187.5 ml) was diluted in 31.25 ml of distilled water and then made up to 500 ml with distilled water.

Aluminium Chloride Solution

Aluminium chloride (0.5 g) was dissolved and made up to 100 ml with distilled water.

Lead Sub Acetate Solution

15 % lead acetate was mixed with 20 ml of absolute ethanol and made up to 100 ml with distilled water.

Wagner's Reagent

A known quantity of iodine crystals (2.0 g) and potassium iodide (3.0 g) were dissolved in minimum amount of water and made up to 100 ml with distilled water.

Mayer's Reagent

13.5 g of mercuric chloride was dissolved in 50 ml of distilled water. Also, 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. The two solutions were mixed and the volume made up to 100 ml with distilled water.

Dragendorff's Reagent

A known quantity of bismuth carbonate (0.85 g) was dissolved in 100 ml of glacial acetic acid and 40 ml of distilled water to give solution A. Another solution called B was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water. Both solutions were mixed to give a stock solution.

Molisch Reagent

A quantity, (1.0 g) of α -naphthol was dissolved in 100 ml of absolute ethanol.

2% (v/v) Hydrochloric Acid

A known quantity of concentrated hydrochloric acid (2.0 ml) was diluted with minimum volume distilled water and made up to 100 ml with distilled water.

1% (W/V) Picric Acid

A known quantity of picric acid (1.0 g) was dissolved in 100 ml of water.

Normal Saline

Normal saline was prepared by dissolving 0.9 g of sodium chloride in distilled water and made up to 100 ml.

25 % Trichloroacetic Acid (TCA)

25.0 g of TCA was dissolved in 0.3% NaOH and made up to 100 ml with NaOH.

1 % Thiobarbituric Acid (TBA)

A quantity of TBA (1.0 g) was dissolved in distilled water and made up to 100 ml.

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	Negative Control	3	78.2100	2.53077	1.46114	71.9232	84.4968	75.29	79.77
	Positive Control	3	126.6600	27.52611	15.89221	58.2813	195.0387	101.63	156.14
	Standard Control	3	80.1000	5.96516	3.44399	65.2817	94.9183	73.29	84.40
	100 mg/kg b.w. of Fraction1	3	91.1000	6.39446	3.69184	75.2153	106.9847	86.49	98.40
	200 mg/kg b.w. of Fraction 1	3	84.3500	5.88125	3.39554	69.7402	98.9598	78.00	89.61
	Total	15	92.0840	21.59277	5.57523	80.1263	104.0417	73.29	156.14
AST	Negative Control	3	38.6667	2.88675	1.66667	31.4956	45.8378	37.00	42.00
	Positive Control	3	68.0000	1.00000	.57735	65.5159	70.4841	67.00	69.00
	Standard Control	3	41.6667	6.02771	3.48010	26.6930	56.6403	36.00	48.00
	100 mg/kg b.w. of Fraction1	3	52.6667	4.72582	2.72845	40.9271	64.4062	49.00	58.00
	200 mg/kg b.w. of Fraction 1	3	48.6667	4.72582	2.72845	36.9271	60.4062	45.00	54.00
	Total	15	49.9333	11.25336	2.90560	43.7014	56.1652	36.00	69.00
ALT	Negative Control	3	21.6667	3.21455	1.85592	13.6813	29.6521	18.00	24.00
	Positive Control	3	42.3333	12.50333	7.21880	11.2733	73.3933	30.00	55.00
	Standard Control	3	23.3333	5.03322	2.90593	10.8301	35.8366	18.00	28.00
	100 mg/kg b.w. of Fraction1	3	33.0000	5.00000	2.88675	20.5793	45.4207	28.00	38.00
	200 mg/kg b.w. of Fraction 1	3	26.3333	5.13160	2.96273	13.5857	39.0809	22.00	32.00
	Total	15	29.3333	9.80282	2.53108	23.9047	34.7620	18.00	55.00

TBIL	Negative Control	3	.4667	.05774	.03333	.3232	.6101	.40	.50
	Positive Control	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	Standard Control	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	100 mg/kg b.w. of Fraction1	3	.6000	.10000	.05774	.3516	.8484	.50	.70
	200 mg/kg b.w. of Fraction 1	3	.5667	.11547	.06667	.2798	.8535	.50	.70
	Total	15	.5733	.11629	.03003	.5089	.6377	.40	.80
UREA	Negative Control	3	45.0000	5.19615	3.00000	32.0920	57.9080	42.00	51.00
	Positive Control	3	68.0000	6.00000	3.46410	53.0952	82.9048	62.00	74.00
	Standard Control	3	49.3333	2.51661	1.45297	43.0817	55.5849	47.00	52.00
	100 mg/kg b.w. of Fraction1	3	57.6667	8.08290	4.66667	37.5876	77.7457	49.00	65.00
	200 mg/kg b.w. of Fraction 1	3	54.6667	8.32666	4.80740	33.9821	75.3512	48.00	64.00
	Total	15	54.9333	9.75754	2.51939	49.5298	60.3369	42.00	74.00
CREAT ININE	Negative Control	3	1.3000	.36056	.20817	.4043	2.1957	.90	1.60
	Positive Control	3	2.0000	.10000	.05774	1.7516	2.2484	1.90	2.10
	Standard Control	3	1.4667	.20817	.12019	.9496	1.9838	1.30	1.70
	100 mg/kg b.w. of Fraction1	3	1.7000	.00000	.00000	1.7000	1.7000	1.70	1.70
	200 mg/kg b.w. of Fraction 1	3	1.6333	.11547	.06667	1.3465	1.9202	1.50	1.70
	Total	15	1.6200	.29568	.07635	1.4563	1.7837	.90	2.10
Sodium	Negative Control	3	120.2500	7.72075	4.45758	101.0706	139.4294	111.35	125.15
	Positive Control	3	90.8700	6.18297	3.56974	75.5107	106.2293	85.86	97.78
	Standard Control	3	116.6733	22.04948	12.73027	61.8994	171.4473	92.87	136.40

	100 mg/kg b.w. of Fraction1	3	110.4167	8.03638	4.63980	90.4532	130.3801	101.25	116.25
	200 mg/kg b.w. of Fraction 1	3	114.7800	2.00192	1.15581	109.8069	119.7531	112.50	116.25
	Total	15	110.5980	14.43107	3.72609	102.6063	118.5897	85.86	136.40
Potassi um	Negative Control	3	5.3767	1.27064	.73361	2.2202	8.5331	4.13	6.67
	Positive Control	3	6.4667	1.20500	.69571	3.4733	9.4601	5.26	7.67
	Standard Control	3	5.6667	.63721	.36789	4.0838	7.2496	4.97	6.22
	100 mg/kg b.w. of Fraction1	3	6.1033	1.00600	.58081	3.6043	8.6024	4.95	6.80
	200 mg/kg b.w. of Fraction 1	3	5.7900	.59195	.34176	4.3195	7.2605	5.11	6.19
	Total	15	5.8807	.91703	.23677	5.3728	6.3885	4.13	7.67
Chlorid e	Negative Control	3	85.4467	4.98559	2.87843	73.0618	97.8316	79.72	88.82
	Positive Control	3	79.6000	4.85370	2.80228	67.5427	91.6573	76.00	85.12
	Standard Control	3	85.0000	5.38070	3.10655	71.6336	98.3664	79.02	89.45
	100 mg/kg b.w. of Fraction1	3	81.2333	3.32861	1.92177	72.9646	89.5021	77.39	83.19
	200 mg/kg b.w. of Fraction 1	3	84.6767	1.89740	1.09547	79.9633	89.3901	82.69	86.47
	Total	15	83.1913	4.35873	1.12542	80.7776	85.6051	76.00	89.45
MOD	Negative Control	3	39.7200	1.55936	.90030	35.8463	43.5937	38.78	41.52
	Positive Control	3	25.8800	1.16550	.67290	22.9847	28.7753	24.60	26.88
	Standard Control	3	35.0200	1.62613	.93885	30.9805	39.0595	33.43	36.68
	100 mg/kg b.w. of Fraction1	3	28.3000	5.96165	3.44196	13.4904	43.1096	21.69	33.27
	200 mg/kg b.w. of Fraction 1	3	31.5867	.96173	.55526	29.1976	33.9757	30.48	32.22
	Total	15	32.1013	5.64263	1.45692	28.9766	35.2261	21.69	41.52

SOD	Negative Control	3	4.3800	1.06226	.61330	1.7412	7.0188	3.50	5.56
	Positive Control	3	6.5133	.51965	.30002	5.2225	7.8042	6.16	7.11
	Standard Control	3	4.4667	.95133	.54925	2.1034	6.8299	3.67	5.52
	100 mg/kg b.w. of Fraction1	3	5.6933	.57012	.32916	4.2771	7.1096	5.23	6.33
	200 mg/kg b.w. of Fraction 1	3	5.0733	.72418	.41810	3.2744	6.8723	4.24	5.55
	Total	15	5.2253	1.06501	.27499	4.6355	5.8151	3.50	7.11
HB	Negative Control	3	15.6667	1.52753	.88192	11.8721	19.4612	14.00	17.00
	Positive Control	3	11.0000	2.64575	1.52753	4.4276	17.5724	9.00	14.00
	Standard Control	3	14.6667	1.15470	.66667	11.7982	17.5351	14.00	16.00
	100 mg/kg b.w. of Fraction1	3	14.0000	2.00000	1.15470	9.0317	18.9683	12.00	16.00
	200 mg/kg b.w. of Fraction 1	3	14.3333	.57735	.33333	12.8991	15.7676	14.00	15.00
	Total	15	13.9333	2.18654	.56456	12.7225	15.1442	9.00	17.00
PCV	Negative Control	3	49.0000	3.60555	2.08167	40.0433	57.9567	46.00	53.00
	Positive Control	3	34.0000	5.29150	3.05505	20.8552	47.1448	30.00	40.00
	Standard Control	3	45.0000	4.35890	2.51661	34.1719	55.8281	42.00	50.00
	100 mg/kg b.w. of Fraction1	3	41.0000	4.35890	2.51661	30.1719	51.8281	38.00	46.00
	200 mg/kg b.w. of Fraction 1	3	43.6667	3.51188	2.02759	34.9427	52.3907	40.00	47.00
	Total	15	42.5333	6.30042	1.62676	39.0443	46.0224	30.00	53.00
RBC	Negative Control	3	326.0000	52.42137	30.26549	195.7781	456.2219	282.00	384.00
	Positive Control	3	192.0000	66.81317	38.57460	26.0269	357.9731	132.00	264.00
	Standard Control	3	316.0000	18.33030	10.58301	270.4650	361.5350	300.00	336.00

100 mg/kg b.w. of Fraction1	3	280.0000	24.97999	14.42221	217.9463	342.0537	252.00	300.00
200 mg/kg b.w. of Fraction 1	3	291.3333	26.10236	15.07021	226.4915	356.1752	262.00	312.00
Total	15	281.0667	60.67650	15.66661	247.4651	314.6682	132.00	384.00
WBC Negative Control	3	4300.000 0	458.25757	264.57513	3161.6251	5438.3749	3900.00	4800.00
Positive Control	3	6800.000 0	600.00000	346.41016	5309.5174	8290.4826	6200.00	7400.00
Standard Control	3	5000.000 0	400.00000	230.94011	4006.3449	5993.6551	4600.00	5400.00
100 mg/kg b.w. of Fraction1	3	5600.000 0	200.00000	115.47005	5103.1725	6096.8275	5400.00	5800.00
200 mg/kg b.w. of Fraction 1	3	5400.000 0	200.00000	115.47005	4903.1725	5896.8275	5200.00	5600.00
Total	15	5420.000 0	915.11123	236.28070	4913.2283	5926.7717	3900.00	7400.00

Appendix 2b: Hepatoprotective- Post Hoc Tests

Multiple Comparisons

Dependent Variable (I) Groups (J) Groups							95% Confidence Interval	
				Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	Negative Control	Positive Control	-48.45000*	10.80218	.001	-72.5188	-24.3812
			Standard Control	-1.89000	10.80218	.865	-25.9588	22.1788

			100 mg/kg b.w. of Fraction1	-12.89000	10.80218	.260	-36.9588	11.1788
			200 mg/kg b.w. of Fraction 1	-6.14000	10.80218	.582	-30.2088	17.9288
		Positive Control	Negative Control	48.45000*	10.80218	.001	24.3812	72.5188
			Standard Control	46.56000*	10.80218	.002	22.4912	70.6288
			100 mg/kg b.w. of Fraction1	35.56000*	10.80218	.008	11.4912	59.6288
			200 mg/kg b.w. of Fraction 1	42.31000*	10.80218	.003	18.2412	66.3788
		Standard Control	Negative Control	1.89000	10.80218	.865	-22.1788	25.9588
			Positive Control	-46.56000*	10.80218	.002	-70.6288	-22.4912
			100 mg/kg b.w. of Fraction1	-11.00000	10.80218	.333	-35.0688	13.0688
			200 mg/kg b.w. of Fraction 1	-4.25000	10.80218	.702	-28.3188	19.8188
		100 mg/kg b.w. of Fraction1	Negative Control	12.89000	10.80218	.260	-11.1788	36.9588
			Positive Control	-35.56000*	10.80218	.008	-59.6288	-11.4912
			Standard Control	11.00000	10.80218	.333	-13.0688	35.0688
			200 mg/kg b.w. of Fraction 1	6.75000	10.80218	.546	-17.3188	30.8188
		200 mg/kg b.w. of Fraction 1	Negative Control	6.14000	10.80218	.582	-17.9288	30.2088
			Positive Control	-42.31000*	10.80218	.003	-66.3788	-18.2412
			Standard Control	4.25000	10.80218	.702	-19.8188	28.3188
			100 mg/kg b.w. of Fraction1	-6.75000	10.80218	.546	-30.8188	17.3188
AST	LSD	Negative Control	Positive Control	-29.33333*	3.47051	.000	-37.0661	-21.6006
			Standard Control	-3.00000	3.47051	.408	-10.7328	4.7328

			100 mg/kg b.w. of Fraction1	-14.00000*	3.47051	.002	-21.7328	-6.2672
			200 mg/kg b.w. of Fraction 1	-10.00000*	3.47051	.016	-17.7328	-2.2672
		Positive Control	Negative Control	29.33333*	3.47051	.000	21.6006	37.0661
			Standard Control	26.33333*	3.47051	.000	18.6006	34.0661
			100 mg/kg b.w. of Fraction1	15.33333*	3.47051	.001	7.6006	23.0661
			200 mg/kg b.w. of Fraction 1	19.33333*	3.47051	.000	11.6006	27.0661
		Standard Control	Negative Control	3.00000	3.47051	.408	-4.7328	10.7328
			Positive Control	-26.33333*	3.47051	.000	-34.0661	-18.6006
			100 mg/kg b.w. of Fraction1	-11.00000*	3.47051	.010	-18.7328	-3.2672
			200 mg/kg b.w. of Fraction 1	-7.00000	3.47051	.071	-14.7328	.7328
		100 mg/kg b.w. of Fraction1	Negative Control	14.00000*	3.47051	.002	6.2672	21.7328
			Positive Control	-15.33333*	3.47051	.001	-23.0661	-7.6006
			Standard Control	11.00000*	3.47051	.010	3.2672	18.7328
			200 mg/kg b.w. of Fraction 1	4.00000	3.47051	.276	-3.7328	11.7328
		200 mg/kg b.w. of Fraction 1	Negative Control	10.00000*	3.47051	.016	2.2672	17.7328
			Positive Control	-19.33333*	3.47051	.000	-27.0661	-11.6006
			Standard Control	7.00000	3.47051	.071	-.7328	14.7328
			100 mg/kg b.w. of Fraction1	-4.00000	3.47051	.276	-11.7328	3.7328
ALT	LSD	Negative Control	Positive Control	-20.66667*	5.69600	.005	-33.3582	-7.9752
			Standard Control	-1.66667	5.69600	.776	-14.3582	11.0248
			100 mg/kg b.w. of Fraction1	-11.33333	5.69600	.075	-24.0248	1.3582

			200 mg/kg b.w. of Fraction 1	-4.66667	5.69600	.432	-17.3582	8.0248
		Positive Control	Negative Control	20.66667*	5.69600	.005	7.9752	33.3582
			Standard Control	19.00000*	5.69600	.008	6.3085	31.6915
			100 mg/kg b.w. of Fraction1	9.33333	5.69600	.132	-3.3582	22.0248
			200 mg/kg b.w. of Fraction 1	16.00000*	5.69600	.019	3.3085	28.6915
		Standard Control	Negative Control	1.66667	5.69600	.776	-11.0248	14.3582
			Positive Control	-19.00000*	5.69600	.008	-31.6915	-6.3085
			100 mg/kg b.w. of Fraction1	-9.66667	5.69600	.121	-22.3582	3.0248
			200 mg/kg b.w. of Fraction 1	-3.00000	5.69600	.610	-15.6915	9.6915
		100 mg/kg b.w. of Fraction1	Negative Control	11.33333	5.69600	.075	-1.3582	24.0248
			Positive Control	-9.33333	5.69600	.132	-22.0248	3.3582
			Standard Control	9.66667	5.69600	.121	-3.0248	22.3582
			200 mg/kg b.w. of Fraction 1	6.66667	5.69600	.269	-6.0248	19.3582
		200 mg/kg b.w. of Fraction 1	Negative Control	4.66667	5.69600	.432	-8.0248	17.3582
			Positive Control	-16.00000*	5.69600	.019	-28.6915	-3.3085
			Standard Control	3.00000	5.69600	.610	-9.6915	15.6915
			100 mg/kg b.w. of Fraction1	-6.66667	5.69600	.269	-19.3582	6.0248
TBIL	LSD	Negative Control	Positive Control	-.26667*	.06325	.002	-.4076	-.1257
			Standard Control	-.03333	.06325	.610	-.1743	.1076
			100 mg/kg b.w. of Fraction1	-.13333	.06325	.061	-.2743	.0076
			200 mg/kg b.w. of Fraction 1	-.10000	.06325	.145	-.2409	.0409

				Positive Control	Negative Control	.26667 [*]	.06325	.002	.1257	.4076
					Standard Control	.23333 [*]	.06325	.004	.0924	.3743
					100 mg/kg b.w. of Fraction1	.13333	.06325	.061	-.0076	.2743
					200 mg/kg b.w. of Fraction 1	.16667 [*]	.06325	.025	.0257	.3076
				Standard Control	Negative Control	.03333	.06325	.610	-.1076	.1743
					Positive Control	-.23333 [*]	.06325	.004	-.3743	-.0924
					100 mg/kg b.w. of Fraction1	-.10000	.06325	.145	-.2409	.0409
					200 mg/kg b.w. of Fraction 1	-.06667	.06325	.317	-.2076	.0743
				100 mg/kg b.w. of Fraction1	Negative Control	.13333	.06325	.061	-.0076	.2743
					Positive Control	-.13333	.06325	.061	-.2743	.0076
					Standard Control	.10000	.06325	.145	-.0409	.2409
					200 mg/kg b.w. of Fraction 1	.03333	.06325	.610	-.1076	.1743
				200 mg/kg b.w. of Fraction 1	Negative Control	.10000	.06325	.145	-.0409	.2409
					Positive Control	-.16667 [*]	.06325	.025	-.3076	-.0257
					Standard Control	.06667	.06325	.317	-.0743	.2076
					100 mg/kg b.w. of Fraction1	-.03333	.06325	.610	-.1743	.1076
UREA	LSD	Negative Control	Positive Control	-23.00000 [*]	5.21536	.001	-34.6206	-11.3794		
			Standard Control	-4.33333	5.21536	.425	-15.9539	7.2872		
			100 mg/kg b.w. of Fraction1	-12.66667 [*]	5.21536	.036	-24.2872	-1.0461		
			200 mg/kg b.w. of Fraction 1	-9.66667	5.21536	.094	-21.2872	1.9539		
				Positive Control	Negative Control	23.00000 [*]	5.21536	.001	11.3794	34.6206

				Standard Control	18.66667*	5.21536	.005	7.0461	30.2872
				100 mg/kg b.w. of Fraction1	10.33333	5.21536	.076	-1.2872	21.9539
				200 mg/kg b.w. of Fraction 1	13.33333*	5.21536	.029	1.7128	24.9539
				Standard Control Negative Control	4.33333	5.21536	.425	-7.2872	15.9539
				Positive Control	-18.66667*	5.21536	.005	-30.2872	-7.0461
				100 mg/kg b.w. of Fraction1	-8.33333	5.21536	.141	-19.9539	3.2872
				200 mg/kg b.w. of Fraction 1	-5.33333	5.21536	.331	-16.9539	6.2872
				100 mg/kg b.w. of Negative Control Fraction1	12.66667*	5.21536	.036	1.0461	24.2872
				Positive Control	-10.33333	5.21536	.076	-21.9539	1.2872
				Standard Control	8.33333	5.21536	.141	-3.2872	19.9539
				200 mg/kg b.w. of Fraction 1	3.00000	5.21536	.578	-8.6206	14.6206
				200 mg/kg b.w. of Negative Control Fraction 1	9.66667	5.21536	.094	-1.9539	21.2872
				Positive Control	-13.33333*	5.21536	.029	-24.9539	-1.7128
				Standard Control	5.33333	5.21536	.331	-6.2872	16.9539
				100 mg/kg b.w. of Fraction1	-3.00000	5.21536	.578	-14.6206	8.6206
CREATI NINE	LSD	Negative Control	Positive Control		-.70000*	.16193	.002	-1.0608	-.3392
			Standard Control		-.16667	.16193	.328	-.5275	.1941
			100 mg/kg b.w. of Fraction1		-.40000*	.16193	.033	-.7608	-.0392
			200 mg/kg b.w. of Fraction 1		-.33333	.16193	.067	-.6941	.0275
		Positive Control	Negative Control		.70000*	.16193	.002	.3392	1.0608
			Standard Control		.53333*	.16193	.008	.1725	.8941

				100 mg/kg b.w. of Fraction1	.30000	.16193	.094	-.0608	.6608	
				200 mg/kg b.w. of Fraction 1	.36667 [*]	.16193	.047	.0059	.7275	
				Standard Control	Negative Control	.16667	.16193	.328	-.1941	.5275
					Positive Control	-.53333 [*]	.16193	.008	-.8941	-.1725
					100 mg/kg b.w. of Fraction1	-.23333	.16193	.180	-.5941	.1275
					200 mg/kg b.w. of Fraction 1	-.16667	.16193	.328	-.5275	.1941
				100 mg/kg b.w. of Fraction1	Negative Control	.40000 [*]	.16193	.033	.0392	.7608
					Positive Control	-.30000	.16193	.094	-.6608	.0608
					Standard Control	.23333	.16193	.180	-.1275	.5941
					200 mg/kg b.w. of Fraction 1	.06667	.16193	.689	-.2941	.4275
				200 mg/kg b.w. of Fraction 1	Negative Control	.33333	.16193	.067	-.0275	.6941
					Positive Control	-.36667 [*]	.16193	.047	-.7275	-.0059
					Standard Control	.16667	.16193	.328	-.1941	.5275
					100 mg/kg b.w. of Fraction1	-.06667	.16193	.689	-.4275	.2941
Sodium	LSD	Negative Control	Positive Control	29.38000 [*]	9.32816	.010	8.5956	50.1644		
			Standard Control	3.57667	9.32816	.709	-17.2078	24.3611		
			100 mg/kg b.w. of Fraction1	9.83333	9.32816	.317	-10.9511	30.6178		
			200 mg/kg b.w. of Fraction 1	5.47000	9.32816	.571	-15.3144	26.2544		
	Positive Control	Negative Control	-29.38000 [*]	9.32816	.010	-50.1644	-8.5956			
		Standard Control	-25.80333 [*]	9.32816	.020	-46.5878	-5.0189			
		100 mg/kg b.w. of Fraction1	-19.54667	9.32816	.063	-40.3311	1.2378			

200 mg/kg b.w. of Fraction 1				-23.91000*	9.32816	.028	-44.6944	-3.1256
Standard Control	Negative Control			-3.57667	9.32816	.709	-24.3611	17.2078
	Positive Control			25.80333*	9.32816	.020	5.0189	46.5878
	100 mg/kg b.w. of Fraction1			6.25667	9.32816	.518	-14.5278	27.0411
	200 mg/kg b.w. of Fraction 1			1.89333	9.32816	.843	-18.8911	22.6778
100 mg/kg b.w. of Fraction1	Negative Control			-9.83333	9.32816	.317	-30.6178	10.9511
	Positive Control			19.54667	9.32816	.063	-1.2378	40.3311
	Standard Control			-6.25667	9.32816	.518	-27.0411	14.5278
	200 mg/kg b.w. of Fraction 1			-4.36333	9.32816	.650	-25.1478	16.4211
200 mg/kg b.w. of Fraction 1	Negative Control			-5.47000	9.32816	.571	-26.2544	15.3144
	Positive Control			23.91000*	9.32816	.028	3.1256	44.6944
	Standard Control			-1.89333	9.32816	.843	-22.6778	18.8911
	100 mg/kg b.w. of Fraction1			4.36333	9.32816	.650	-16.4211	25.1478
Potassium LSD	Negative Control	Positive Control		-1.09000	.80291	.204	-2.8790	.6990
		Standard Control		-.29000	.80291	.725	-2.0790	1.4990
		100 mg/kg b.w. of Fraction1		-.72667	.80291	.387	-2.5157	1.0623
		200 mg/kg b.w. of Fraction 1		-.41333	.80291	.618	-2.2023	1.3757
	Positive Control	Negative Control		1.09000	.80291	.204	-.6990	2.8790
		Standard Control		.80000	.80291	.343	-.9890	2.5890
		100 mg/kg b.w. of Fraction1		.36333	.80291	.661	-1.4257	2.1523
		200 mg/kg b.w. of Fraction 1		.67667	.80291	.419	-1.1123	2.4657

	Standard Control	Negative Control		.29000	.80291	.725	-1.4990	2.0790
		Positive Control		-.80000	.80291	.343	-2.5890	.9890
		100 mg/kg b.w. of Fraction1		-.43667	.80291	.598	-2.2257	1.3523
		200 mg/kg b.w. of Fraction 1		-.12333	.80291	.881	-1.9123	1.6657
	100 mg/kg b.w. of Fraction1	Negative Control		.72667	.80291	.387	-1.0623	2.5157
		Positive Control		-.36333	.80291	.661	-2.1523	1.4257
		Standard Control		.43667	.80291	.598	-1.3523	2.2257
		200 mg/kg b.w. of Fraction 1		.31333	.80291	.705	-1.4757	2.1023
	200 mg/kg b.w. of Fraction 1	Negative Control		.41333	.80291	.618	-1.3757	2.2023
		Positive Control		-.67667	.80291	.419	-2.4657	1.1123
		Standard Control		.12333	.80291	.881	-1.6657	1.9123
		100 mg/kg b.w. of Fraction1		-.31333	.80291	.705	-2.1023	1.4757
Chloride	LSD	Negative Control	Positive Control	5.84667	3.50326	.126	-1.9591	13.6524
			Standard Control	.44667	3.50326	.901	-7.3591	8.2524
			100 mg/kg b.w. of Fraction1	4.21333	3.50326	.257	-3.5924	12.0191
			200 mg/kg b.w. of Fraction 1	.77000	3.50326	.830	-7.0357	8.5757
	Positive Control	Negative Control		-5.84667	3.50326	.126	-13.6524	1.9591
		Standard Control		-5.40000	3.50326	.154	-13.2057	2.4057
		100 mg/kg b.w. of Fraction1		-1.63333	3.50326	.651	-9.4391	6.1724
		200 mg/kg b.w. of Fraction 1		-5.07667	3.50326	.178	-12.8824	2.7291
	Standard Control	Negative Control		-.44667	3.50326	.901	-8.2524	7.3591

				Positive Control	5.40000	3.50326	.154	-2.4057	13.2057
				100 mg/kg b.w. of Fraction1	3.76667	3.50326	.308	-4.0391	11.5724
				200 mg/kg b.w. of Fraction 1	.32333	3.50326	.928	-7.4824	8.1291
				100 mg/kg b.w. of Negative Control Fraction1	-4.21333	3.50326	.257	-12.0191	3.5924
				Positive Control	1.63333	3.50326	.651	-6.1724	9.4391
				Standard Control	-3.76667	3.50326	.308	-11.5724	4.0391
				200 mg/kg b.w. of Fraction 1	-3.44333	3.50326	.349	-11.2491	4.3624
				200 mg/kg b.w. of Negative Control Fraction 1	-.77000	3.50326	.830	-8.5757	7.0357
				Positive Control	5.07667	3.50326	.178	-2.7291	12.8824
				Standard Control	-.32333	3.50326	.928	-8.1291	7.4824
				100 mg/kg b.w. of Fraction1	3.44333	3.50326	.349	-4.3624	11.2491
MOD	LSD	Negative Control	Positive Control		13.84000*	2.39167	.000	8.5110	19.1690
			Standard Control		4.70000	2.39167	.078	-.6290	10.0290
			100 mg/kg b.w. of Fraction1		11.42000*	2.39167	.001	6.0910	16.7490
			200 mg/kg b.w. of Fraction 1		8.13333*	2.39167	.007	2.8044	13.4623
		Positive Control	Negative Control		-13.84000*	2.39167	.000	-19.1690	-8.5110
			Standard Control		-9.14000*	2.39167	.003	-14.4690	-3.8110
			100 mg/kg b.w. of Fraction1		-2.42000	2.39167	.335	-7.7490	2.9090
			200 mg/kg b.w. of Fraction 1		-5.70667*	2.39167	.038	-11.0356	-.3777
		Standard Control	Negative Control		-4.70000	2.39167	.078	-10.0290	.6290
			Positive Control		9.14000*	2.39167	.003	3.8110	14.4690

			100 mg/kg b.w. of Fraction1	6.72000*	2.39167	.018	1.3910	12.0490
			200 mg/kg b.w. of Fraction 1	3.43333	2.39167	.182	-1.8956	8.7623
			100 mg/kg b.w. of Negative Control Fraction1	-11.42000*	2.39167	.001	-16.7490	-6.0910
			Positive Control	2.42000	2.39167	.335	-2.9090	7.7490
			Standard Control	-6.72000*	2.39167	.018	-12.0490	-1.3910
			200 mg/kg b.w. of Fraction 1	-3.28667	2.39167	.199	-8.6156	2.0423
			200 mg/kg b.w. of Negative Control Fraction 1	-8.13333*	2.39167	.007	-13.4623	-2.8044
			Positive Control	5.70667*	2.39167	.038	.3777	11.0356
			Standard Control	-3.43333	2.39167	.182	-8.7623	1.8956
			100 mg/kg b.w. of Fraction1	3.28667	2.39167	.199	-2.0423	8.6156
SOD	LSD	Negative Control	Positive Control	-2.13333*	.64838	.008	-3.5780	-.6887
			Standard Control	-.08667	.64838	.896	-1.5313	1.3580
			100 mg/kg b.w. of Fraction1	-1.31333	.64838	.070	-2.7580	.1313
			200 mg/kg b.w. of Fraction 1	-.69333	.64838	.310	-2.1380	.7513
		Positive Control	Negative Control	2.13333*	.64838	.008	.6887	3.5780
			Standard Control	2.04667*	.64838	.010	.6020	3.4913
			100 mg/kg b.w. of Fraction1	.82000	.64838	.235	-.6247	2.2647
			200 mg/kg b.w. of Fraction 1	1.44000	.64838	.051	-.0047	2.8847
		Standard Control	Negative Control	.08667	.64838	.896	-1.3580	1.5313
			Positive Control	-2.04667*	.64838	.010	-3.4913	-.6020
			100 mg/kg b.w. of Fraction1	-1.22667	.64838	.088	-2.6713	.2180

200 mg/kg b.w. of Fraction 1				- .60667	.64838	.371	-2.0513	.8380
100 mg/kg b.w. of Negetive Control Fraction1				1.31333	.64838	.070	-.1313	2.7580
Positive Control				-.82000	.64838	.235	-2.2647	.6247
Standard Control				1.22667	.64838	.088	-.2180	2.6713
200 mg/kg b.w. of Fraction 1				.62000	.64838	.361	-.8247	2.0647
200 mg/kg b.w. of Negetive Control Fraction 1				.69333	.64838	.310	-.7513	2.1380
Positive Control				-1.44000	.64838	.051	-2.8847	.0047
Standard Control				.60667	.64838	.371	-.8380	2.0513
100 mg/kg b.w. of Fraction1				-.62000	.64838	.361	-2.0647	.8247
HB	LSD	Negetive Control	Positive Control	4.66667*	1.41421	.008	1.5156	7.8177
			Standard Control	1.00000	1.41421	.496	-2.1511	4.1511
			100 mg/kg b.w. of Fraction1	1.66667	1.41421	.266	-1.4844	4.8177
			200 mg/kg b.w. of Fraction 1	1.33333	1.41421	.368	-1.8177	4.4844
		Positive Control	Negetive Control	-4.66667*	1.41421	.008	-7.8177	-1.5156
			Standard Control	-3.66667*	1.41421	.027	-6.8177	-.5156
			100 mg/kg b.w. of Fraction1	-3.00000	1.41421	.060	-6.1511	.1511
			200 mg/kg b.w. of Fraction 1	-3.33333*	1.41421	.040	-6.4844	-.1823
		Standard Control	Negetive Control	-1.00000	1.41421	.496	-4.1511	2.1511
			Positive Control	3.66667*	1.41421	.027	.5156	6.8177
			100 mg/kg b.w. of Fraction1	.66667	1.41421	.647	-2.4844	3.8177
			200 mg/kg b.w. of Fraction 1	.33333	1.41421	.818	-2.8177	3.4844

100 mg/kg b.w. of Negative Control Fraction1				-1.66667	1.41421	.266	-4.8177	1.4844
Positive Control				3.00000	1.41421	.060	-.1511	6.1511
Standard Control				-.66667	1.41421	.647	-3.8177	2.4844
200 mg/kg b.w. of Fraction 1				-.33333	1.41421	.818	-3.4844	2.8177
200 mg/kg b.w. of Negative Control Fraction 1				-1.33333	1.41421	.368	-4.4844	1.8177
Positive Control				3.33333*	1.41421	.040	.1823	6.4844
Standard Control				-.33333	1.41421	.818	-3.4844	2.8177
100 mg/kg b.w. of Fraction1				.33333	1.41421	.818	-2.8177	3.4844
PCV	LSD	Negative Control	Positive Control	15.00000*	3.48967	.002	7.2245	22.7755
			Standard Control	4.00000	3.48967	.278	-3.7755	11.7755
			100 mg/kg b.w. of Fraction1	8.00000*	3.48967	.045	.2245	15.7755
			200 mg/kg b.w. of Fraction 1	5.33333	3.48967	.157	-2.4421	13.1088
		Positive Control	Negative Control	-15.00000*	3.48967	.002	-22.7755	-7.2245
			Standard Control	-11.00000*	3.48967	.010	-18.7755	-3.2245
			100 mg/kg b.w. of Fraction1	-7.00000	3.48967	.073	-14.7755	.7755
			200 mg/kg b.w. of Fraction 1	-9.66667*	3.48967	.020	-17.4421	-1.8912
		Standard Control	Negative Control	-4.00000	3.48967	.278	-11.7755	3.7755
			Positive Control	11.00000*	3.48967	.010	3.2245	18.7755
			100 mg/kg b.w. of Fraction1	4.00000	3.48967	.278	-3.7755	11.7755
			200 mg/kg b.w. of Fraction 1	1.33333	3.48967	.710	-6.4421	9.1088
		100 mg/kg b.w. of Negative Control		-8.00000*	3.48967	.045	-15.7755	-.2245

Fraction1			Positive Control	7.00000	3.48967	.073	-.7755	14.7755		
			Standard Control	-4.00000	3.48967	.278	-11.7755	3.7755		
			200 mg/kg b.w. of Fraction 1	-2.66667	3.48967	.462	-10.4421	5.1088		
200 mg/kg b.w. of Fraction 1			Negative Control	-5.33333	3.48967	.157	-13.1088	2.4421		
			Positive Control	9.66667*	3.48967	.020	1.8912	17.4421		
			Standard Control	-1.33333	3.48967	.710	-9.1088	6.4421		
			100 mg/kg b.w. of Fraction1	2.66667	3.48967	.462	-5.1088	10.4421		
RBC	LSD	Negative Control	Positive Control	134.00000*	34.35760	.003	57.4465	210.5535		
			Standard Control	10.00000	34.35760	.777	-66.5535	86.5535		
			100 mg/kg b.w. of Fraction1	46.00000	34.35760	.210	-30.5535	122.5535		
			200 mg/kg b.w. of Fraction 1	34.66667	34.35760	.337	-41.8868	111.2202		
		Positive Control	Negative Control	-134.00000*	34.35760	.003	-210.5535	-57.4465		
			Standard Control	-124.00000*	34.35760	.005	-200.5535	-47.4465		
			100 mg/kg b.w. of Fraction1	-88.00000*	34.35760	.028	-164.5535	-11.4465		
			200 mg/kg b.w. of Fraction 1	-99.33333*	34.35760	.016	-175.8868	-22.7798		
		Standard Control	Negative Control	-10.00000	34.35760	.777	-86.5535	66.5535		
			Positive Control	124.00000*	34.35760	.005	47.4465	200.5535		
			100 mg/kg b.w. of Fraction1	36.00000	34.35760	.319	-40.5535	112.5535		
			200 mg/kg b.w. of Fraction 1	24.66667	34.35760	.489	-51.8868	101.2202		
		100 mg/kg b.w. of Fraction1			Negative Control	-46.00000	34.35760	.210	-122.5535	30.5535
					Positive Control	88.00000*	34.35760	.028	11.4465	164.5535

				Standard Control	-36.00000	34.35760	.319	-112.5535	40.5535
				200 mg/kg b.w. of Fraction 1	-11.33333	34.35760	.748	-87.8868	65.2202
				200 mg/kg b.w. of Negative Control Fraction 1	-34.66667	34.35760	.337	-111.2202	41.8868
				Positive Control	99.33333*	34.35760	.016	22.7798	175.8868
				Standard Control	-24.66667	34.35760	.489	-101.2202	51.8868
				100 mg/kg b.w. of Fraction1	11.33333	34.35760	.748	-65.2202	87.8868
WBC	LSD	Negative Control	Positive Control	-2500.00000*	328.63353	.000	-3232.2411	-1767.7589	
			Standard Control	-700.00000	328.63353	.059	-1432.2411	32.2411	
			100 mg/kg b.w. of Fraction1	-1300.00000*	328.63353	.003	-2032.2411	-567.7589	
			200 mg/kg b.w. of Fraction 1	-1100.00000*	328.63353	.007	-1832.2411	-367.7589	
		Positive Control	Negative Control	2500.00000*	328.63353	.000	1767.7589	3232.2411	
			Standard Control	1800.00000*	328.63353	.000	1067.7589	2532.2411	
			100 mg/kg b.w. of Fraction1	1200.00000*	328.63353	.004	467.7589	1932.2411	
			200 mg/kg b.w. of Fraction 1	1400.00000*	328.63353	.002	667.7589	2132.2411	
		Standard Control	Negative Control	700.00000	328.63353	.059	-32.2411	1432.2411	
			Positive Control	-1800.00000*	328.63353	.000	-2532.2411	-1067.7589	
			100 mg/kg b.w. of Fraction1	-600.00000	328.63353	.098	-1332.2411	132.2411	
			200 mg/kg b.w. of Fraction 1	-400.00000	328.63353	.251	-1132.2411	332.2411	
		100 mg/kg b.w. of Fraction1	Negative Control	1300.00000*	328.63353	.003	567.7589	2032.2411	
			Positive Control	-1200.00000*	328.63353	.004	-1932.2411	-467.7589	
			Standard Control	600.00000	328.63353	.098	-132.2411	1332.2411	

200 mg/kg b.w. of Fraction 1	200.00000	328.63353	.556	-532.2411	932.2411
200 mg/kg b.w. of Negative Control Fraction 1	1100.00000*	328.63353	.007	367.7589	1832.2411
Positive Control	-1400.00000*	328.63353	.002	-2132.2411	-667.7589
Standard Control	400.00000	328.63353	.251	-332.2411	1132.2411
100 mg/kg b.w. of Fraction1	-200.00000	328.63353	.556	-932.2411	532.2411

*. The mean difference is significant at the 0.05 level.

Appendix 2c: Hepatocurative day 8- Descriptives

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	Negative Control	3	76.0000	5.29150	3.05505	62.8552	89.1448	72.00	82.00
	Positive Control	3	133.3333	22.03028	12.71919	78.6071	188.0596	112.00	156.00
	Standard Control	3	77.3333	6.42910	3.71184	61.3626	93.3041	70.00	82.00
	100 mg/kg b.w. of Fraction 1	4	88.5000	5.00000	2.50000	80.5439	96.4561	82.00	94.00
	200 mg/kg b.w. of Fraction 1	4	83.0000	9.30949	4.65475	68.1865	97.8135	72.00	94.00
	Total	17	90.9412	22.82671	5.53629	79.2048	102.6776	70.00	156.00
AST	Negative Control	3	39.3333	7.63763	4.40959	20.3604	58.3062	31.00	46.00
	Positive Control	3	75.3333	4.16333	2.40370	64.9910	85.6756	72.00	80.00
	Standard Control	3	40.3333	6.11010	3.52767	25.1550	55.5117	35.00	47.00

	100 mg/kg b.w. of Fraction 1	4	48.0000	16.49242	8.24621	21.7569	74.2431	34.00	66.00
	200 mg/kg b.w. of Fraction 1	4	46.0000	5.16398	2.58199	37.7830	54.2170	40.00	52.00
	Total	17	49.4706	15.29754	3.71020	41.6053	57.3359	31.00	80.00
ALT	Negative Control	3	21.0000	2.34681	1.35493	15.1702	26.8298	18.30	22.55
	Positive Control	3	50.0900	5.58724	3.22579	36.2105	63.9695	45.67	56.37
	Standard Control	3	22.3300	4.87673	2.81558	10.2155	34.4445	18.73	27.88
	100 mg/kg b.w. of Fraction 1	4	27.2500	6.42035	3.21018	17.0338	37.4662	18.20	32.22
	200 mg/kg b.w. of Fraction 1	4	23.5000	5.85072	2.92536	14.1902	32.8098	18.00	31.69
	Total	17	28.4271	11.55116	2.80157	22.4880	34.3661	18.00	56.37
TBIL	Negative Control	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	Positive Control	3	1.0667	.15275	.08819	.6872	1.4461	.90	1.20
	Standard Control	3	.5000	.10000	.05774	.2516	.7484	.40	.60
	100 mg/kg b.w. of Fraction 1	4	.5750	.09574	.04787	.4227	.7273	.50	.70
	200 mg/kg b.w. of Fraction 1	4	.5250	.05000	.02500	.4454	.6046	.50	.60
	Total	17	.6235	.22784	.05526	.5064	.7407	.40	1.20
Urea	Negative Control	3	46.0000	4.31753	2.49273	35.2747	56.7253	42.21	50.70
	Positive Control	3	75.9300	2.35578	1.36011	70.0779	81.7821	73.54	78.25
	Standard Control	3	47.7267	10.27502	5.93229	22.2021	73.2512	39.09	59.09
	100 mg/kg b.w. of Fraction 1	4	53.0500	5.95958	2.97979	43.5670	62.5330	47.63	61.52
	200 mg/kg b.w. of Fraction 1	4	51.7525	8.59346	4.29673	38.0784	65.4266	41.27	62.25
	Total	17	54.5988	12.14028	2.94445	48.3569	60.8408	39.09	78.25

Creatinine	Negative Control	3	1.3000	.26458	.15275	.6428	1.9572	1.00	1.50
	Positive Control	3	2.0333	.15275	.08819	1.6539	2.4128	1.90	2.20
	Standard Control	3	1.4000	.20000	.11547	.9032	1.8968	1.20	1.60
	100 mg/kg b.w. of Fraction 1	4	1.6500	.12910	.06455	1.4446	1.8554	1.50	1.80
	200 mg/kg b.w. of Fraction 1	4	1.5500	.12910	.06455	1.3446	1.7554	1.40	1.70
	Total	17	1.5882	.28914	.07013	1.4396	1.7369	1.00	2.20
Sodium	Negative Control	3	120.4100	10.86000	6.27002	93.4323	147.3877	109.55	131.27
	Positive Control	3	82.0000	4.63006	2.67317	70.4983	93.5017	77.25	86.50
	Standard Control	3	118.6600	5.32375	3.07367	105.4351	131.8849	112.52	121.99
	100 mg/kg b.w. of Fraction 1	4	111.2500	7.23419	3.61709	99.7388	122.7612	102.40	120.12
	200 mg/kg b.w. of Fraction 1	4	116.7800	9.18843	4.59421	102.1592	131.4008	105.16	127.64
	Total	17	110.3135	15.48440	3.75552	102.3522	118.2749	77.25	131.27
Potassium	Negative Control	3	5.2433	.92500	.53405	2.9455	7.5412	4.32	6.17
	Positive Control	3	7.1300	1.18710	.68537	4.1811	10.0789	5.81	8.11
	Standard Control	3	5.4700	.46872	.27062	4.3056	6.6344	4.94	5.83
	100 mg/kg b.w. of Fraction 1	4	5.9700	.97423	.48712	4.4198	7.5202	4.76	6.97
	200 mg/kg b.w. of Fraction 1	4	5.6700	.51942	.25971	4.8435	6.4965	5.19	6.37
	Total	17	5.8876	.97601	.23672	5.3858	6.3895	4.32	8.11
Chloride	Negative Control	3	87.5500	1.05000	.60622	84.9417	90.1583	86.50	88.60
	Positive Control	3	72.0167	1.43117	.82628	68.4615	75.5719	70.90	73.63
	Standard Control	3	85.1800	3.75533	2.16814	75.8512	94.5088	81.18	88.63

	100 mg/kg b.w. of Fraction 1	4	81.0000	7.39888	3.69944	69.2267	92.7733	75.45	91.81
	200 mg/kg b.w. of Fraction 1	4	85.5500	7.83791	3.91896	73.0781	98.0219	74.53	92.90
	Total	17	82.3788	7.31758	1.77477	78.6165	86.1412	70.90	92.90
SOD	Negative Control	3	40.4900	5.98320	3.45440	25.6269	55.3531	33.78	45.27
	Positive Control	3	23.5033	.74460	.42990	21.6536	25.3530	22.66	24.07
	Standard Control	3	36.3033	5.19217	2.99770	23.4053	49.2014	32.61	42.24
	100 mg/kg b.w. of Fraction 1	4	29.7400	1.63030	.81515	27.1458	32.3342	27.70	31.60
	200 mg/kg b.w. of Fraction 1	4	32.8250	2.98844	1.49422	28.0697	37.5803	29.42	36.14
	Total	17	32.4206	6.47066	1.56937	29.0937	35.7475	22.66	45.27
MDA	Negative Control	3	4.4633	.59467	.34333	2.9861	5.9406	4.12	5.15
	Positive Control	3	6.7900	.84285	.48662	4.6962	8.8838	5.91	7.59
	Standard Control	3	4.6000	.50685	.29263	3.3409	5.8591	4.12	5.13
	100 mg/kg b.w. of Fraction 1	4	5.4025	.73781	.36890	4.2285	6.5765	4.64	6.39
	200 mg/kg b.w. of Fraction 1	4	4.9475	.58762	.29381	4.0125	5.8825	4.24	5.52
	Total	17	5.2329	1.00012	.24256	4.7187	5.7472	4.12	7.59
HB	Negative Control	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00
	Positive Control	3	10.3333	1.52753	.88192	6.5388	14.1279	9.00	12.00
	Standard Control	3	15.0000	1.00000	.57735	12.5159	17.4841	14.00	16.00
	100 mg/kg b.w. of Fraction 1	4	14.0000	.81650	.40825	12.7008	15.2992	13.00	15.00
	200 mg/kg b.w. of Fraction 1	4	14.2500	.50000	.25000	13.4544	15.0456	14.00	15.00
	Total	17	13.8235	1.91165	.46364	12.8406	14.8064	9.00	16.00

PCV	Negative Control	3	48.3333	3.21455	1.85592	40.3479	56.3187	46.00	52.00
	Positive Control	3	30.3333	4.50925	2.60342	19.1317	41.5349	26.00	35.00
	Standard Control	3	45.6667	2.08167	1.20185	40.4955	50.8378	44.00	48.00
	100 mg/kg b.w. of Fraction 1	4	42.7500	7.27438	3.63719	31.1748	54.3252	36.00	53.00
	200 mg/kg b.w. of Fraction 1	4	44.2500	3.59398	1.79699	38.5312	49.9688	41.00	49.00
	Total	17	42.4118	7.31487	1.77412	38.6508	46.1727	26.00	53.00
RBC	Negative Control	3	324.0000	52.30679	30.19934	194.0627	453.9373	288.00	384.00
	Positive Control	3	172.0000	18.33030	10.58301	126.4650	217.5350	156.00	192.00
	Standard Control	3	314.0000	27.05550	15.62050	246.7904	381.2096	288.00	342.00
	100 mg/kg b.w. of Fraction 1	4	281.0000	24.73863	12.36932	241.6353	320.3647	252.00	312.00
	200 mg/kg b.w. of Fraction 1	4	295.0000	23.40940	11.70470	257.7504	332.2496	268.00	324.00
	Total	17	278.4706	59.27702	14.37679	247.9932	308.9480	156.00	384.00
WBC	Negative Control	3	4466.666 7	305.5050 5	176.38342	3707.750 1	5225.583 3	4200.0 0	4800.00
	Positive Control	3	7200.000 0	1000.000 00	577.35027	4715.862 3	9684.137 7	6200.0 0	8200.00
	Standard Control	3	5200.000 0	400.0000 0	230.94011	4206.344 9	6193.655 1	4800.0 0	5600.00
	100 mg/kg b.w. of Fraction 1	4	5650.000 0	378.5938 9	189.29694	5047.572 6	6252.427 4	5400.0 0	6200.00
	200 mg/kg b.w. of Fraction 1	4	5450.000 0	341.5650 3	170.78251	4906.493 8	5993.506 2	5000.0 0	5800.00
	Total	17	5588.235 3	980.9958 9	237.92645	5083.853 7	6092.616 8	4200.0 0	8200.00

Appendix 2d: Hepato-curative day 8- Post Hoc Tests

Multiple Comparisons

Dependent Variable (I) Groups (J) Groups							95% Confidence Interval	
				Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	Negative Control	Positive Control	-57.33333 [*]	8.95772	.000	-76.8505	-37.8161
			Standard Control	-1.33333	8.95772	.884	-20.8505	18.1839
			100 mg/kg b.w. of Fraction 1	-12.50000	8.37918	.162	-30.7567	5.7567
			200 mg/kg b.w. of Fraction 1	-7.00000	8.37918	.420	-25.2567	11.2567
		Positive Control	Negative Control	57.33333 [*]	8.95772	.000	37.8161	76.8505
			Standard Control	56.00000 [*]	8.95772	.000	36.4828	75.5172
			100 mg/kg b.w. of Fraction 1	44.83333 [*]	8.37918	.000	26.5767	63.0900
			200 mg/kg b.w. of Fraction 1	50.33333 [*]	8.37918	.000	32.0767	68.5900
		Standard Control	Negative Control	1.33333	8.95772	.884	-18.1839	20.8505
			Positive Control	-56.00000 [*]	8.95772	.000	-75.5172	-36.4828
			100 mg/kg b.w. of Fraction 1	-11.16667	8.37918	.207	-29.4233	7.0900
			200 mg/kg b.w. of Fraction 1	-5.66667	8.37918	.512	-23.9233	12.5900
		100 mg/kg b.w. of Fraction 1	Negative Control	12.50000	8.37918	.162	-5.7567	30.7567
			Positive Control	-44.83333 [*]	8.37918	.000	-63.0900	-26.5767
			Standard Control	11.16667	8.37918	.207	-7.0900	29.4233

			200 mg/kg b.w. of Fraction 1	5.50000	7.75761	.492	-11.4024	22.4024
			200 mg/kg b.w. of Negative Control Fraction 1	7.00000	8.37918	.420	-11.2567	25.2567
			Positive Control	-50.33333*	8.37918	.000	-68.5900	-32.0767
			Standard Control	5.66667	8.37918	.512	-12.5900	23.9233
			100 mg/kg b.w. of Fraction 1	-5.50000	7.75761	.492	-22.4024	11.4024
AST	LSD	Negative Control	Positive Control	-36.00000*	7.89515	.001	-53.2020	-18.7980
			Standard Control	-1.00000	7.89515	.901	-18.2020	16.2020
			100 mg/kg b.w. of Fraction 1	-8.66667	7.38523	.263	-24.7577	7.4244
			200 mg/kg b.w. of Fraction 1	-6.66667	7.38523	.384	-22.7577	9.4244
		Positive Control	Negative Control	36.00000*	7.89515	.001	18.7980	53.2020
			Standard Control	35.00000*	7.89515	.001	17.7980	52.2020
			100 mg/kg b.w. of Fraction 1	27.33333*	7.38523	.003	11.2423	43.4244
			200 mg/kg b.w. of Fraction 1	29.33333*	7.38523	.002	13.2423	45.4244
		Standard Control	Negative Control	1.00000	7.89515	.901	-16.2020	18.2020
			Positive Control	-35.00000*	7.89515	.001	-52.2020	-17.7980
			100 mg/kg b.w. of Fraction 1	-7.66667	7.38523	.320	-23.7577	8.4244
			200 mg/kg b.w. of Fraction 1	-5.66667	7.38523	.458	-21.7577	10.4244
		100 mg/kg b.w. of Fraction 1	Negative Control	8.66667	7.38523	.263	-7.4244	24.7577
			Positive Control	-27.33333*	7.38523	.003	-43.4244	-11.2423
			Standard Control	7.66667	7.38523	.320	-8.4244	23.7577
			200 mg/kg b.w. of Fraction 1	2.00000	6.83740	.775	-12.8974	16.8974

200 mg/kg b.w. of Negative Control Fraction 1				6.66667	7.38523	.384	-9.4244	22.7577
Positive Control				-29.33333*	7.38523	.002	-45.4244	-13.2423
Standard Control				5.66667	7.38523	.458	-10.4244	21.7577
100 mg/kg b.w. of Fraction 1				-2.00000	6.83740	.775	-16.8974	12.8974
ALT	LSD	Negative Control	Positive Control	-29.09000*	4.39299	.000	-38.6615	-19.5185
			Standard Control	-1.33000	4.39299	.767	-10.9015	8.2415
			100 mg/kg b.w. of Fraction 1	-6.25000	4.10926	.154	-15.2033	2.7033
			200 mg/kg b.w. of Fraction 1	-2.50000	4.10926	.554	-11.4533	6.4533
		Positive Control	Negative Control	29.09000*	4.39299	.000	19.5185	38.6615
			Standard Control	27.76000*	4.39299	.000	18.1885	37.3315
			100 mg/kg b.w. of Fraction 1	22.84000*	4.10926	.000	13.8867	31.7933
			200 mg/kg b.w. of Fraction 1	26.59000*	4.10926	.000	17.6367	35.5433
		Standard Control	Negative Control	1.33000	4.39299	.767	-8.2415	10.9015
			Positive Control	-27.76000*	4.39299	.000	-37.3315	-18.1885
			100 mg/kg b.w. of Fraction 1	-4.92000	4.10926	.254	-13.8733	4.0333
			200 mg/kg b.w. of Fraction 1	-1.17000	4.10926	.781	-10.1233	7.7833
		100 mg/kg b.w. of Fraction 1	Negative Control	6.25000	4.10926	.154	-2.7033	15.2033
			Positive Control	-22.84000*	4.10926	.000	-31.7933	-13.8867
			Standard Control	4.92000	4.10926	.254	-4.0333	13.8733
			200 mg/kg b.w. of Fraction 1	3.75000	3.80444	.344	-4.5392	12.0392
		200 mg/kg b.w. of Negative Control		2.50000	4.10926	.554	-6.4533	11.4533

Fraction 1			Positive Control	-26.59000*	4.10926	.000	-35.5433	-17.6367	
			Standard Control	1.17000	4.10926	.781	-7.7833	10.1233	
			100 mg/kg b.w. of Fraction 1	-3.75000	3.80444	.344	-12.0392	4.5392	
TBIL	LSD	Negative Control	Positive Control	-.56667*	.07515	.000	-.7304	-.4029	
			Standard Control	.00000	.07515	1.000	-.1637	.1637	
			100 mg/kg b.w. of Fraction 1	-.07500	.07030	.307	-.2282	.0782	
			200 mg/kg b.w. of Fraction 1	-.02500	.07030	.728	-.1782	.1282	
	Positive Control	Negative Control		.56667*	.07515	.000	.4029	.7304	
				.56667*	.07515	.000	.4029	.7304	
				.49167*	.07030	.000	.3385	.6448	
				.54167*	.07030	.000	.3885	.6948	
	Standard Control	Negative Control		.00000	.07515	1.000	-.1637	.1637	
			Positive Control	-.56667*	.07515	.000	-.7304	-.4029	
			100 mg/kg b.w. of Fraction 1	-.07500	.07030	.307	-.2282	.0782	
			200 mg/kg b.w. of Fraction 1	-.02500	.07030	.728	-.1782	.1282	
	100 mg/kg b.w. of Fraction 1	Negative Control		.07500	.07030	.307	-.0782	.2282	
			Positive Control	-.49167*	.07030	.000	-.6448	-.3385	
			Standard Control	.07500	.07030	.307	-.0782	.2282	
			200 mg/kg b.w. of Fraction 1	.05000	.06509	.457	-.0918	.1918	
	200 mg/kg b.w. of			Negative Control	.02500	.07030	.728	-.1282	.1782

Fraction 1				Positive Control	-.54167*	.07030	.000	-.6948	-.3885
				Standard Control	.02500	.07030	.728	-.1282	.1782
				100 mg/kg b.w. of Fraction 1	-.05000	.06509	.457	-.1918	.0918
Urea	LSD	Negative Control	Positive Control		-29.93000*	5.71366	.000	-42.3790	-17.4810
			Standard Control		-1.72667	5.71366	.768	-14.1757	10.7223
			100 mg/kg b.w. of Fraction 1		-7.05000	5.34464	.212	-18.6950	4.5950
			200 mg/kg b.w. of Fraction 1		-5.75250	5.34464	.303	-17.3975	5.8925
		Positive Control	Negative Control		29.93000*	5.71366	.000	17.4810	42.3790
			Standard Control		28.20333*	5.71366	.000	15.7543	40.6523
			100 mg/kg b.w. of Fraction 1		22.88000*	5.34464	.001	11.2350	34.5250
			200 mg/kg b.w. of Fraction 1		24.17750*	5.34464	.001	12.5325	35.8225
		Standard Control	Negative Control		1.72667	5.71366	.768	-10.7223	14.1757
			Positive Control		-28.20333*	5.71366	.000	-40.6523	-15.7543
			100 mg/kg b.w. of Fraction 1		-5.32333	5.34464	.339	-16.9683	6.3216
			200 mg/kg b.w. of Fraction 1		-4.02583	5.34464	.466	-15.6708	7.6191
		100 mg/kg b.w. of Fraction 1	Negative Control		7.05000	5.34464	.212	-4.5950	18.6950
			Positive Control		-22.88000*	5.34464	.001	-34.5250	-11.2350
			Standard Control		5.32333	5.34464	.339	-6.3216	16.9683
			200 mg/kg b.w. of Fraction 1		1.29750	4.94817	.798	-9.4836	12.0786
		200 mg/kg b.w. of Fraction 1	Negative Control		5.75250	5.34464	.303	-5.8925	17.3975
			Positive Control		-24.17750*	5.34464	.001	-35.8225	-12.5325

				Standard Control	4.02583	5.34464	.466	-7.6191	15.6708
				100 mg/kg b.w. of Fraction 1	-1.29750	4.94817	.798	-12.0786	9.4836
Creatinine e	LSD	Negative Control	Positive Control		-.73333*	.14272	.000	-1.0443	-.4224
			Standard Control		-.10000	.14272	.497	-.4110	.2110
			100 mg/kg b.w. of Fraction 1		-.35000*	.13351	.022	-.6409	-.0591
			200 mg/kg b.w. of Fraction 1		-.25000	.13351	.086	-.5409	.0409
		Positive Control	Negative Control		.73333*	.14272	.000	.4224	1.0443
			Standard Control		.63333*	.14272	.001	.3224	.9443
			100 mg/kg b.w. of Fraction 1		.38333*	.13351	.014	.0924	.6742
			200 mg/kg b.w. of Fraction 1		.48333*	.13351	.004	.1924	.7742
		Standard Control	Negative Control		.10000	.14272	.497	-.2110	.4110
			Positive Control		-.63333*	.14272	.001	-.9443	-.3224
			100 mg/kg b.w. of Fraction 1		-.25000	.13351	.086	-.5409	.0409
			200 mg/kg b.w. of Fraction 1		-.15000	.13351	.283	-.4409	.1409
	100 mg/kg b.w. of Fraction 1	Negative Control			.35000*	.13351	.022	.0591	.6409
			Positive Control		-.38333*	.13351	.014	-.6742	-.0924
			Standard Control		.25000	.13351	.086	-.0409	.5409
			200 mg/kg b.w. of Fraction 1		.10000	.12360	.434	-.1693	.3693
	200 mg/kg b.w. of Fraction 1	Negative Control			.25000	.13351	.086	-.0409	.5409
			Positive Control		-.48333*	.13351	.004	-.7742	-.1924
			Standard Control		.15000	.13351	.283	-.1409	.4409

			100 mg/kg b.w. of Fraction 1	-.10000	.12360	.434	-.3693	.1693
Sodium	LSD	Negative Control	Positive Control	38.41000*	6.43653	.000	24.3860	52.4340
			Standard Control	1.75000	6.43653	.790	-12.2740	15.7740
			100 mg/kg b.w. of Fraction 1	9.16000	6.02082	.154	-3.9582	22.2782
			200 mg/kg b.w. of Fraction 1	3.63000	6.02082	.558	-9.4882	16.7482
		Positive Control	Negative Control	-38.41000*	6.43653	.000	-52.4340	-24.3860
			Standard Control	-36.66000*	6.43653	.000	-50.6840	-22.6360
			100 mg/kg b.w. of Fraction 1	-29.25000*	6.02082	.000	-42.3682	-16.1318
			200 mg/kg b.w. of Fraction 1	-34.78000*	6.02082	.000	-47.8982	-21.6618
		Standard Control	Negative Control	-1.75000	6.43653	.790	-15.7740	12.2740
			Positive Control	36.66000*	6.43653	.000	22.6360	50.6840
			100 mg/kg b.w. of Fraction 1	7.41000	6.02082	.242	-5.7082	20.5282
			200 mg/kg b.w. of Fraction 1	1.88000	6.02082	.760	-11.2382	14.9982
		100 mg/kg b.w. of Fraction 1	Negative Control	-9.16000	6.02082	.154	-22.2782	3.9582
			Positive Control	29.25000*	6.02082	.000	16.1318	42.3682
			Standard Control	-7.41000	6.02082	.242	-20.5282	5.7082
			200 mg/kg b.w. of Fraction 1	-5.53000	5.57420	.341	-17.6751	6.6151
		200 mg/kg b.w. of Fraction 1	Negative Control	-3.63000	6.02082	.558	-16.7482	9.4882
			Positive Control	34.78000*	6.02082	.000	21.6618	47.8982
			Standard Control	-1.88000	6.02082	.760	-14.9982	11.2382
			100 mg/kg b.w. of Fraction 1	5.53000	5.57420	.341	-6.6151	17.6751

Potassium	LSD	Negative Control	Positive Control	-1.88667*	.69225	.018	-3.3950	-.3784
			Standard Control	-.22667	.69225	.749	-1.7350	1.2816
			100 mg/kg b.w. of Fraction 1	-.72667	.64754	.284	-2.1375	.6842
			200 mg/kg b.w. of Fraction 1	-.42667	.64754	.522	-1.8375	.9842
		Positive Control	Negative Control	1.88667*	.69225	.018	.3784	3.3950
			Standard Control	1.66000*	.69225	.034	.1517	3.1683
			100 mg/kg b.w. of Fraction 1	1.16000	.64754	.098	-.2509	2.5709
			200 mg/kg b.w. of Fraction 1	1.46000*	.64754	.044	.0491	2.8709
		Standard Control	Negative Control	.22667	.69225	.749	-1.2816	1.7350
			Positive Control	-1.66000*	.69225	.034	-3.1683	-.1517
			100 mg/kg b.w. of Fraction 1	-.50000	.64754	.455	-1.9109	.9109
			200 mg/kg b.w. of Fraction 1	-.20000	.64754	.763	-1.6109	1.2109
		100 mg/kg b.w. of Fraction 1	Negative Control	.72667	.64754	.284	-.6842	2.1375
			Positive Control	-1.16000	.64754	.098	-2.5709	.2509
			Standard Control	.50000	.64754	.455	-.9109	1.9109
			200 mg/kg b.w. of Fraction 1	.30000	.59951	.626	-1.0062	1.6062
		200 mg/kg b.w. of Fraction 1	Negative Control	.42667	.64754	.522	-.9842	1.8375
			Positive Control	-1.46000*	.64754	.044	-2.8709	-.0491
			Standard Control	.20000	.64754	.763	-1.2109	1.6109
			100 mg/kg b.w. of Fraction 1	-.30000	.59951	.626	-1.6062	1.0062
Chloride	LSD	Negative Control	Positive Control	15.53333*	4.61300	.006	5.4825	25.5842

				Standard Control	2.37000	4.61300	.617	-7.6809	12.4209
				100 mg/kg b.w. of Fraction 1	6.55000	4.31506	.155	-2.8517	15.9517
				200 mg/kg b.w. of Fraction 1	2.00000	4.31506	.651	-7.4017	11.4017
				Positive Control	-15.53333*	4.61300	.006	-25.5842	-5.4825
				Standard Control	-13.16333*	4.61300	.015	-23.2142	-3.1125
				100 mg/kg b.w. of Fraction 1	-8.98333	4.31506	.059	-18.3850	.4184
				200 mg/kg b.w. of Fraction 1	-13.53333*	4.31506	.009	-22.9350	-4.1316
				Standard Control	-2.37000	4.61300	.617	-12.4209	7.6809
				Positive Control	13.16333*	4.61300	.015	3.1125	23.2142
				100 mg/kg b.w. of Fraction 1	4.18000	4.31506	.352	-5.2217	13.5817
				200 mg/kg b.w. of Fraction 1	-.37000	4.31506	.933	-9.7717	9.0317
				100 mg/kg b.w. of Fraction 1	-6.55000	4.31506	.155	-15.9517	2.8517
				Positive Control	8.98333	4.31506	.059	-.4184	18.3850
				Standard Control	-4.18000	4.31506	.352	-13.5817	5.2217
				200 mg/kg b.w. of Fraction 1	-4.55000	3.99497	.277	-13.2543	4.1543
				200 mg/kg b.w. of Fraction 1	-2.00000	4.31506	.651	-11.4017	7.4017
				Positive Control	13.53333*	4.31506	.009	4.1316	22.9350
				Standard Control	.37000	4.31506	.933	-9.0317	9.7717
				100 mg/kg b.w. of Fraction 1	4.55000	3.99497	.277	-4.1543	13.2543
SOD	LSD	Negative Control	Positive Control		16.98667*	2.99434	.000	10.4626	23.5108
			Standard Control		4.18667	2.99434	.187	-2.3374	10.7108

		100 mg/kg b.w. of Fraction 1	10.75000*	2.80095	.002	4.6473	16.8527	
		200 mg/kg b.w. of Fraction 1	7.66500*	2.80095	.018	1.5623	13.7677	
Positive Control	Negative Control	-16.98667*	2.99434	.000	-23.5108	-10.4626		
	Standard Control	-12.80000*	2.99434	.001	-19.3241	-6.2759		
	100 mg/kg b.w. of Fraction 1	-6.23667*	2.80095	.046	-12.3394	-.1339		
	200 mg/kg b.w. of Fraction 1	-9.32167*	2.80095	.006	-15.4244	-3.2189		
Standard Control	Negative Control	-4.18667	2.99434	.187	-10.7108	2.3374		
	Positive Control	12.80000*	2.99434	.001	6.2759	19.3241		
	100 mg/kg b.w. of Fraction 1	6.56333*	2.80095	.037	.4606	12.6661		
	200 mg/kg b.w. of Fraction 1	3.47833	2.80095	.238	-2.6244	9.5811		
100 mg/kg b.w. of Fraction 1	Negative Control	-10.75000*	2.80095	.002	-16.8527	-4.6473		
	Positive Control	6.23667*	2.80095	.046	.1339	12.3394		
	Standard Control	-6.56333*	2.80095	.037	-12.6661	-.4606		
	200 mg/kg b.w. of Fraction 1	-3.08500	2.59318	.257	-8.7350	2.5650		
200 mg/kg b.w. of Fraction 1	Negative Control	-7.66500*	2.80095	.018	-13.7677	-1.5623		
	Positive Control	9.32167*	2.80095	.006	3.2189	15.4244		
	Standard Control	-3.47833	2.80095	.238	-9.5811	2.6244		
	100 mg/kg b.w. of Fraction 1	3.08500	2.59318	.257	-2.5650	8.7350		
MDA	LSD	Negative Control	Positive Control	-2.32667*	.54318	.001	-3.5102	-1.1432
			Standard Control	-.13667	.54318	.806	-1.3202	1.0468
			100 mg/kg b.w. of Fraction 1	-.93917	.50810	.089	-2.0462	.1679

			200 mg/kg b.w. of Fraction 1		-.48417	.50810	.359	-1.5912	.6229
	Positive Control		Negative Control		2.32667*	.54318	.001	1.1432	3.5102
			Standard Control		2.19000*	.54318	.002	1.0065	3.3735
			100 mg/kg b.w. of Fraction 1		1.38750*	.50810	.018	.2804	2.4946
			200 mg/kg b.w. of Fraction 1		1.84250*	.50810	.003	.7354	2.9496
	Standard Control		Negative Control		.13667	.54318	.806	-1.0468	1.3202
			Positive Control		-2.19000*	.54318	.002	-3.3735	-1.0065
			100 mg/kg b.w. of Fraction 1		-.80250	.50810	.140	-1.9096	.3046
			200 mg/kg b.w. of Fraction 1		-.34750	.50810	.507	-1.4546	.7596
	100 mg/kg b.w. of Fraction 1		Negative Control		.93917	.50810	.089	-.1679	2.0462
			Positive Control		-1.38750*	.50810	.018	-2.4946	-.2804
			Standard Control		.80250	.50810	.140	-.3046	1.9096
			200 mg/kg b.w. of Fraction 1		.45500	.47041	.353	-.5699	1.4799
	200 mg/kg b.w. of Fraction 1		Negative Control		.48417	.50810	.359	-.6229	1.5912
			Positive Control		-1.84250*	.50810	.003	-2.9496	-.7354
			Standard Control		.34750	.50810	.507	-.7596	1.4546
			100 mg/kg b.w. of Fraction 1		-.45500	.47041	.353	-1.4799	.5699
HB	LSD	Negative Control	Positive Control		5.00000*	.74846	.000	3.3693	6.6307
			Standard Control		.33333	.74846	.664	-1.2974	1.9641
			100 mg/kg b.w. of Fraction 1		1.33333	.70012	.081	-.1921	2.8588
			200 mg/kg b.w. of Fraction 1		1.08333	.70012	.148	-.4421	2.6088

PCV	LSD	Positive Control	Negative Control	-5.00000*	.74846	.000	-6.6307	-3.3693
			Standard Control	-4.66667*	.74846	.000	-6.2974	-3.0359
			100 mg/kg b.w. of Fraction 1	-3.66667*	.70012	.000	-5.1921	-2.1412
			200 mg/kg b.w. of Fraction 1	-3.91667*	.70012	.000	-5.4421	-2.3912
		Standard Control	Negative Control	-.33333	.74846	.664	-1.9641	1.2974
			Positive Control	4.66667*	.74846	.000	3.0359	6.2974
			100 mg/kg b.w. of Fraction 1	1.00000	.70012	.179	-.5254	2.5254
			200 mg/kg b.w. of Fraction 1	.75000	.70012	.305	-.7754	2.2754
		100 mg/kg b.w. of Fraction 1	Negative Control	-1.33333	.70012	.081	-2.8588	.1921
			Positive Control	3.66667*	.70012	.000	2.1412	5.1921
			Standard Control	-1.00000	.70012	.179	-2.5254	.5254
			200 mg/kg b.w. of Fraction 1	-.25000	.64818	.706	-1.6623	1.1623
		200 mg/kg b.w. of Fraction 1	Negative Control	-1.08333	.70012	.148	-2.6088	.4421
			Positive Control	3.91667*	.70012	.000	2.3912	5.4421
			Standard Control	-.75000	.70012	.305	-2.2754	.7754
			100 mg/kg b.w. of Fraction 1	.25000	.64818	.706	-1.1623	1.6623
		Negative Control	Positive Control	18.00000*	3.85501	.001	9.6007	26.3993
			Standard Control	2.66667	3.85501	.502	-5.7327	11.0660
			100 mg/kg b.w. of Fraction 1	5.58333	3.60603	.148	-2.2735	13.4402
			200 mg/kg b.w. of Fraction 1	4.08333	3.60603	.280	-3.7735	11.9402
		Positive Control	Negative Control	-18.00000*	3.85501	.001	-26.3993	-9.6007

				Standard Control	-15.33333 [*]	3.85501	.002	-23.7327	-6.9340
				100 mg/kg b.w. of Fraction 1	-12.41667 [*]	3.60603	.005	-20.2735	-4.5598
				200 mg/kg b.w. of Fraction 1	-13.91667 [*]	3.60603	.002	-21.7735	-6.0598
				Standard Control	-2.66667	3.85501	.502	-11.0660	5.7327
				Positive Control	15.33333 [*]	3.85501	.002	6.9340	23.7327
				100 mg/kg b.w. of Fraction 1	2.91667	3.60603	.434	-4.9402	10.7735
				200 mg/kg b.w. of Fraction 1	1.41667	3.60603	.701	-6.4402	9.2735
				100 mg/kg b.w. of Fraction 1	-5.58333	3.60603	.148	-13.4402	2.2735
				Positive Control	12.41667 [*]	3.60603	.005	4.5598	20.2735
				Standard Control	-2.91667	3.60603	.434	-10.7735	4.9402
				200 mg/kg b.w. of Fraction 1	-1.50000	3.33854	.661	-8.7740	5.7740
				200 mg/kg b.w. of Fraction 1	-4.08333	3.60603	.280	-11.9402	3.7735
				Positive Control	13.91667 [*]	3.60603	.002	6.0598	21.7735
				Standard Control	-1.41667	3.60603	.701	-9.2735	6.4402
				100 mg/kg b.w. of Fraction 1	1.50000	3.33854	.661	-5.7740	8.7740
RBC	LSD	Negative Control	Positive Control	152.00000 [*]	24.81935	.000	97.9233	206.0767	
			Standard Control	10.00000	24.81935	.694	-44.0767	64.0767	
			100 mg/kg b.w. of Fraction 1	43.00000	23.21637	.089	-7.5841	93.5841	
			200 mg/kg b.w. of Fraction 1	29.00000	23.21637	.235	-21.5841	79.5841	
		Positive Control	Negative Control	-152.00000 [*]	24.81935	.000	-206.0767	-97.9233	
			Standard Control	-142.00000 [*]	24.81935	.000	-196.0767	-87.9233	

			100 mg/kg b.w. of Fraction 1	-109.00000*	23.21637	.001	-159.5841	-58.4159
			200 mg/kg b.w. of Fraction 1	-123.00000*	23.21637	.000	-173.5841	-72.4159
		Standard Control	Negative Control	-10.00000	24.81935	.694	-64.0767	44.0767
			Positive Control	142.00000*	24.81935	.000	87.9233	196.0767
			100 mg/kg b.w. of Fraction 1	33.00000	23.21637	.181	-17.5841	83.5841
			200 mg/kg b.w. of Fraction 1	19.00000	23.21637	.429	-31.5841	69.5841
		100 mg/kg b.w. of Fraction 1	Negative Control	-43.00000	23.21637	.089	-93.5841	7.5841
			Positive Control	109.00000*	23.21637	.001	58.4159	159.5841
			Standard Control	-33.00000	23.21637	.181	-83.5841	17.5841
			200 mg/kg b.w. of Fraction 1	-14.00000	21.49419	.527	-60.8318	32.8318
		200 mg/kg b.w. of Fraction 1	Negative Control	-29.00000	23.21637	.235	-79.5841	21.5841
			Positive Control	123.00000*	23.21637	.000	72.4159	173.5841
			Standard Control	-19.00000	23.21637	.429	-69.5841	31.5841
			100 mg/kg b.w. of Fraction 1	14.00000	21.49419	.527	-32.8318	60.8318
WBC	LSD	Negative Control	Positive Control	-2733.33333*	427.30854	.000	-3664.3587	-1802.3080
			Standard Control	-733.33333	427.30854	.112	-1664.3587	197.6920
			100 mg/kg b.w. of Fraction 1	-1183.33333*	399.71054	.012	-2054.2278	-312.4389
			200 mg/kg b.w. of Fraction 1	-983.33333*	399.71054	.030	-1854.2278	-112.4389
		Positive Control	Negative Control	2733.33333*	427.30854	.000	1802.3080	3664.3587
			Standard Control	2000.00000*	427.30854	.001	1068.9747	2931.0253
			100 mg/kg b.w. of Fraction 1	1550.00000*	399.71054	.002	679.1055	2420.8945

	200 mg/kg b.w. of Fraction 1	1750.00000*	399.71054	.001	879.1055	2620.8945
Standard Control	Negative Control	733.33333	427.30854	.112	-197.6920	1664.3587
	Positive Control	-2000.00000*	427.30854	.001	-2931.0253	-1068.9747
	100 mg/kg b.w. of Fraction 1	-450.00000	399.71054	.282	-1320.8945	420.8945
	200 mg/kg b.w. of Fraction 1	-250.00000	399.71054	.543	-1120.8945	620.8945
100 mg/kg b.w. of Fraction 1	Negative Control	1183.33333*	399.71054	.012	312.4389	2054.2278
	Positive Control	-1550.00000*	399.71054	.002	-2420.8945	-679.1055
	Standard Control	450.00000	399.71054	.282	-420.8945	1320.8945
	200 mg/kg b.w. of Fraction 1	200.00000	370.06006	.599	-606.2916	1006.2916
200 mg/kg b.w. of Fraction 1	Negative Control	983.33333*	399.71054	.030	112.4389	1854.2278
	Positive Control	-1750.00000*	399.71054	.001	-2620.8945	-879.1055
	Standard Control	250.00000	399.71054	.543	-620.8945	1120.8945
	100 mg/kg b.w. of Fraction 1	-200.00000	370.06006	.599	-1006.2916	606.2916

*. The mean difference is significant at the 0.05 level.

Appendix 2e: Hepatocurative day 15- Descriptives

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	Negative Control	3	75.0000	3.00000	1.73205	67.5476	82.4524	72.00	78.00
	Positive Control	3	165.3333	8.32666	4.80740	144.6488	186.0179	156.00	172.00
	Standard Control	3	76.6667	2.88675	1.66667	69.4956	83.8378	75.00	80.00
	100 mg/kg b.w. of Fraction 1	3	80.0000	2.00000	1.15470	75.0317	84.9683	78.00	82.00
	200 mg/kg b.w. of Fraction 1	3	78.0000	2.00000	1.15470	73.0317	82.9683	76.00	80.00
	Total	15	95.0000	36.62552	9.45667	74.7175	115.2825	72.00	172.00
AST	Negative Control	3	37.6667	2.51661	1.45297	31.4151	43.9183	35.00	40.00
	Positive Control	3	89.3333	2.51661	1.45297	83.0817	95.5849	87.00	92.00
	Standard Control	3	39.6667	4.04145	2.33333	29.6271	49.7062	36.00	44.00
	100 mg/kg b.w. of Fraction 1	3	45.6667	1.52753	.88192	41.8721	49.4612	44.00	47.00
	200 mg/kg b.w. of Fraction 1	3	41.3333	3.05505	1.76383	33.7442	48.9225	38.00	44.00
	Total	15	50.7333	20.30646	5.24311	39.4880	61.9787	35.00	92.00
ALT	Negative Control	3	19.6667	5.15493	2.97620	6.8611	32.4722	15.00	25.20
	Positive Control	3	68.5300	9.79521	5.65527	44.1974	92.8626	62.78	79.84
	Standard Control	3	20.3333	6.08714	3.51441	5.2120	35.4546	15.60	27.20
	100 mg/kg b.w. of Fraction 1	3	24.8667	3.05341	1.76289	17.2816	32.4518	21.90	28.00

	200 mg/kg b.w. of Fraction 1	3	21.6667	2.37136	1.36910	15.7759	27.5574	19.70	24.30
	Total	15	31.0127	20.13418	5.19862	19.8627	42.1626	15.00	79.84
TBIL	Negative Control	3	.4667	.05774	.03333	.3232	.6101	.40	.50
	Positive Control	3	1.4667	.11547	.06667	1.1798	1.7535	1.40	1.60
	Standard Control	3	.4667	.11547	.06667	.1798	.7535	.40	.60
	100 mg/kg b.w. of Fraction 1	3	.5333	.05774	.03333	.3899	.6768	.50	.60
	200 mg/kg b.w. of Fraction 1	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	Total	15	.6867	.41034	.10595	.4594	.9139	.40	1.60
Urea	Negative Control	3	44.6000	5.88037	3.39503	29.9924	59.2076	38.87	50.62
	Positive Control	3	92.3300	6.22831	3.59592	76.8580	107.8020	87.95	99.46
	Standard Control	3	46.4100	4.89993	2.82898	34.2379	58.5821	41.70	51.48
	100 mg/kg b.w. of Fraction 1	3	52.0000	10.45802	6.03794	26.0208	77.9792	41.40	62.31
	200 mg/kg b.w. of Fraction 1	3	48.3400	7.82316	4.51671	28.9062	67.7738	42.11	57.12
	Total	15	56.7360	19.59810	5.06021	45.8829	67.5891	38.87	99.46
Creatinine	Negative Control	3	1.2667	.40415	.23333	.2627	2.2706	.90	1.70
	Positive Control	3	2.2000	.40000	.23094	1.2063	3.1937	1.80	2.60
	Standard Control	3	1.3667	.41633	.24037	.3324	2.4009	.90	1.70
	100 mg/kg b.w. of Fraction 1	3	1.5000	.17321	.10000	1.0697	1.9303	1.40	1.70
	200 mg/kg b.w. of Fraction 1	3	1.4000	.10000	.05774	1.1516	1.6484	1.30	1.50
	Total	15	1.5467	.44379	.11459	1.3009	1.7924	.90	2.60
Sodium	Negative Control	3	121.2200	2.88520	1.66577	114.0528	128.3872	118.44	124.20

	Positive Control	3	72.4500	3.39510	1.96016	64.0161	80.8839	68.72	75.36
	Standard Control	3	119.6633	7.88084	4.55000	100.0862	139.2404	111.51	127.24
	100 mg/kg b.w. of Fraction 1	3	113.2133	7.02557	4.05621	95.7609	130.6658	106.24	120.29
	200 mg/kg b.w. of Fraction 1	3	117.3233	4.33936	2.50533	106.5438	128.1029	112.36	120.40
	Total	15	108.7740	19.56205	5.05090	97.9409	119.6071	68.72	127.24
Potassium	Negative Control	3	5.3000	.30000	.17321	4.5548	6.0452	5.00	5.60
	Positive Control	3	8.0200	.84923	.49031	5.9104	10.1296	7.50	9.00
	Standard Control	3	5.5000	.43589	.25166	4.4172	6.5828	5.20	6.00
	100 mg/kg b.w. of Fraction 1	3	5.9500	.58949	.34034	4.4856	7.4144	5.45	6.60
	200 mg/kg b.w. of Fraction 1	3	5.6500	.63836	.36856	4.0642	7.2358	4.95	6.20
	Total	15	6.0840	1.14151	.29474	5.4519	6.7161	4.95	9.00
Chloride	Negative Control	3	87.3300	1.58370	.91435	83.3959	91.2641	86.02	89.09
	Positive Control	3	65.6600	2.46441	1.42283	59.5381	71.7819	63.78	68.45
	Standard Control	3	86.1600	5.45025	3.14670	72.6208	99.6992	79.98	90.28
	100 mg/kg b.w. of Fraction 1	3	82.2300	2.00007	1.15474	77.2615	87.1985	80.22	84.22
	200 mg/kg b.w. of Fraction 1	3	86.6700	3.30927	1.91061	78.4493	94.8907	84.20	90.43
	Total	15	81.6100	8.89587	2.29690	76.6836	86.5364	63.78	90.43
SOD	Negative Control	3	39.7233	5.49105	3.17026	26.0828	53.3639	34.60	45.52
	Positive Control	3	19.4167	2.08725	1.20508	14.2316	24.6017	17.14	21.24
	Standard Control	3	37.8233	4.34591	2.50911	27.0275	48.6192	34.33	42.69
	100 mg/kg b.w. of Fraction 1	3	32.8400	4.93717	2.85047	20.5754	45.1046	27.14	35.78

	200 mg/kg b.w. of Fraction 1	3	35.3500	1.85518	1.07109	30.7415	39.9585	33.58	37.28
	Total	15	33.0307	8.18526	2.11343	28.4978	37.5635	17.14	45.52
MDA	Negative Control	3	4.2000	.54945	.31723	2.8351	5.5649	3.82	4.83
	Positive Control	3	7.8433	.25423	.14678	7.2118	8.4749	7.55	8.00
	Standard Control	3	4.2333	.48911	.28239	3.0183	5.4484	3.67	4.55
	100 mg/kg b.w. of Fraction 1	3	4.6200	.52735	.30447	3.3100	5.9300	4.23	5.22
	200 mg/kg b.w. of Fraction 1	3	4.4200	.41797	.24132	3.3817	5.4583	3.95	4.75
	Total	15	5.0633	1.49848	.38691	4.2335	5.8932	3.67	8.00
HB	Negative Control	3	15.6667	1.15470	.66667	12.7982	18.5351	15.00	17.00
	Positive Control	3	9.3333	.57735	.33333	7.8991	10.7676	9.00	10.00
	Standard Control	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00
	100 mg/kg b.w. of Fraction 1	3	14.6667	.57735	.33333	13.2324	16.1009	14.00	15.00
	200 mg/kg b.w. of Fraction 1	3	15.0000	1.00000	.57735	12.5159	17.4841	14.00	16.00
	Total	15	14.0000	2.53546	.65465	12.5959	15.4041	9.00	17.00
PCV	Negative Control	3	49.0000	2.64575	1.52753	42.4276	55.5724	47.00	52.00
	Positive Control	3	25.0000	4.35890	2.51661	14.1719	35.8281	20.00	28.00
	Standard Control	3	47.6667	2.51661	1.45297	41.4151	53.9183	45.00	50.00
	100 mg/kg b.w. of Fraction 1	3	44.6667	2.51661	1.45297	38.4151	50.9183	42.00	47.00
	200 mg/kg b.w. of Fraction 1	3	45.6667	1.52753	.88192	41.8721	49.4612	44.00	47.00
	Total	15	42.4000	9.45516	2.44131	37.1639	47.6361	20.00	52.00
RBC	Negative Control	3	327.3333	66.16142	38.19831	162.9793	491.6874	252.00	376.00

	Positive Control	3	142.0000	4.35890	2.51661	131.1719	152.8281	137.00	145.00
	Standard Control	3	317.0000	25.63201	14.79865	253.3266	380.6734	293.00	344.00
	100 mg/kg b.w. of Fraction 1	3	285.0000	23.89561	13.79613	225.6400	344.3600	264.00	311.00
	200 mg/kg b.w. of Fraction 1	3	300.0000	30.19934	17.43560	224.9807	375.0193	272.00	332.00
	Total	15	274.2667	76.44556	19.73816	231.9325	316.6008	137.00	376.00
WBC	Negative Control	3	4200.000 0	346.41016	200.0000 0	3339.469 5	5060.5305	3800.00	4400.00
	Positive Control	3	7800.000 0	600.00000	346.4101 6	6309.517 4	9290.4826	7200.00	8400.00
	Standard Control	3	5000.000 0	200.00000	115.4700 5	4503.172 5	5496.8275	4800.00	5200.00
	100 mg/kg b.w. of Fraction 1	3	5400.000 0	721.11026	416.3332 0	3608.662 8	7191.3372	4800.00	6200.00
	200 mg/kg b.w. of Fraction 1	3	5200.000 0	400.00000	230.9401 1	4206.344 9	6193.6551	4800.00	5600.00
	Total	15	5520.000 0	1319.7402 3	340.7554 6	4789.152 2	6250.8478	3800.00	8400.00

Appendix 2f: Hepatocurative day 15-Post Hoc Tests

Multiple Comparisons

Dependent Variable (I) Groups (J) Groups							95% Confidence Interval	
				Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	Negative Control	Positive Control	-90.33333 [*]	3.55278	.000	-98.2494	-82.4173
			Standard Control	-1.66667	3.55278	.649	-9.5827	6.2494

				100 mg/kg b.w. of Fraction 1	-5.00000	3.55278	.190	-12.9161	2.9161	
				200 mg/kg b.w. of Fraction 1	-3.00000	3.55278	.418	-10.9161	4.9161	
Positive Control	Negative Control				90.33333 [*]	3.55278	.000	82.4173	98.2494	
	Standard Control				88.66667 [*]	3.55278	.000	80.7506	96.5827	
	100 mg/kg b.w. of Fraction 1				85.33333 [*]	3.55278	.000	77.4173	93.2494	
	200 mg/kg b.w. of Fraction 1				87.33333 [*]	3.55278	.000	79.4173	95.2494	
	Standard Control				1.66667	3.55278	.649	-6.2494	9.5827	
	Positive Control				-88.66667 [*]	3.55278	.000	-96.5827	-80.7506	
Standard Control	100 mg/kg b.w. of Fraction 1				-3.33333	3.55278	.370	-11.2494	4.5827	
	200 mg/kg b.w. of Fraction 1				-1.33333	3.55278	.715	-9.2494	6.5827	
	100 mg/kg b.w. of Fraction 1				5.00000	3.55278	.190	-2.9161	12.9161	
	Positive Control				-85.33333 [*]	3.55278	.000	-93.2494	-77.4173	
	Standard Control				3.33333	3.55278	.370	-4.5827	11.2494	
	200 mg/kg b.w. of Fraction 1				2.00000	3.55278	.586	-5.9161	9.9161	
200 mg/kg b.w. of Fraction 1	Negative Control				3.00000	3.55278	.418	-4.9161	10.9161	
	Positive Control				-87.33333 [*]	3.55278	.000	-95.2494	-79.4173	
	Standard Control				1.33333	3.55278	.715	-6.5827	9.2494	
	100 mg/kg b.w. of Fraction 1				-2.00000	3.55278	.586	-9.9161	5.9161	
	Positive Control				-51.66667 [*]	2.32857	.000	-56.8550	-46.4783	
	Standard Control				-2.00000	2.32857	.411	-7.1884	3.1884	
AST	LSD	100 mg/kg b.w. of Fraction 1				-8.00000 [*]	2.32857	.006	-13.1884	-2.8116
		200 mg/kg b.w. of Fraction 1				-3.66667	2.32857	.146	-8.8550	1.5217
		Positive Control				51.66667 [*]	2.32857	.000	46.4783	56.8550
		Standard Control				49.66667 [*]	2.32857	.000	44.4783	54.8550

				100 mg/kg b.w. of Fraction 1	43.66667 [*]	2.32857	.000	38.4783	48.8550
				200 mg/kg b.w. of Fraction 1	48.00000 [*]	2.32857	.000	42.8116	53.1884
				Standard Control Negative Control	2.00000	2.32857	.411	-3.1884	7.1884
				Positive Control	-49.66667 [*]	2.32857	.000	-54.8550	-44.4783
				100 mg/kg b.w. of Fraction 1	-6.00000 [*]	2.32857	.028	-11.1884	-.8116
				200 mg/kg b.w. of Fraction 1	-1.66667	2.32857	.491	-6.8550	3.5217
				100 mg/kg b.w. of Negative Control Fraction 1	8.00000 [*]	2.32857	.006	2.8116	13.1884
				Positive Control	-43.66667 [*]	2.32857	.000	-48.8550	-38.4783
				Standard Control	6.00000 [*]	2.32857	.028	.8116	11.1884
				200 mg/kg b.w. of Fraction 1	4.33333	2.32857	.092	-.8550	9.5217
				200 mg/kg b.w. of Negative Control Fraction 1	3.66667	2.32857	.146	-1.5217	8.8550
				Positive Control	-48.00000 [*]	2.32857	.000	-53.1884	-42.8116
				Standard Control	1.66667	2.32857	.491	-3.5217	6.8550
				100 mg/kg b.w. of Fraction 1	-4.33333	2.32857	.092	-9.5217	.8550
ALT	LSD	Negative Control	Positive Control		-48.86333 [*]	4.82382	.000	-59.6115	-38.1152
			Standard Control		-.66667	4.82382	.893	-11.4148	10.0815
			100 mg/kg b.w. of Fraction 1		-5.20000	4.82382	.306	-15.9481	5.5481
			200 mg/kg b.w. of Fraction 1		-2.00000	4.82382	.687	-12.7481	8.7481
		Positive Control	Negative Control		48.86333 [*]	4.82382	.000	38.1152	59.6115
			Standard Control		48.19667 [*]	4.82382	.000	37.4485	58.9448
			100 mg/kg b.w. of Fraction 1		43.66333 [*]	4.82382	.000	32.9152	54.4115
			200 mg/kg b.w. of Fraction 1		46.86333 [*]	4.82382	.000	36.1152	57.6115
	Standard Control	Negative Control			.66667	4.82382	.893	-10.0815	11.4148
			Positive Control		-48.19667 [*]	4.82382	.000	-58.9448	-37.4485

				100 mg/kg b.w. of Fraction 1	-4.53333	4.82382	.369	-15.2815	6.2148
				200 mg/kg b.w. of Fraction 1	-1.33333	4.82382	.788	-12.0815	9.4148
				100 mg/kg b.w. of Negative Control Fraction 1	5.20000	4.82382	.306	-5.5481	15.9481
				Positive Control	-43.66333 [*]	4.82382	.000	-54.4115	-32.9152
				Standard Control	4.53333	4.82382	.369	-6.2148	15.2815
				200 mg/kg b.w. of Fraction 1	3.20000	4.82382	.522	-7.5481	13.9481
				200 mg/kg b.w. of Negative Control Fraction 1	2.00000	4.82382	.687	-8.7481	12.7481
				Positive Control	-46.86333 [*]	4.82382	.000	-57.6115	-36.1152
				Standard Control	1.33333	4.82382	.788	-9.4148	12.0815
				100 mg/kg b.w. of Fraction 1	-3.20000	4.82382	.522	-13.9481	7.5481
TBIL	LSD	Negative Control	Positive Control		-1.00000 [*]	.06667	.000	-1.1485	-.8515
			Standard Control		.00000	.06667	1.000	-.1485	.1485
			100 mg/kg b.w. of Fraction 1		-.06667	.06667	.341	-.2152	.0819
			200 mg/kg b.w. of Fraction 1		-.03333	.06667	.628	-.1819	.1152
		Positive Control	Negative Control		1.00000 [*]	.06667	.000	.8515	1.1485
			Standard Control		1.00000 [*]	.06667	.000	.8515	1.1485
			100 mg/kg b.w. of Fraction 1		.93333 [*]	.06667	.000	.7848	1.0819
			200 mg/kg b.w. of Fraction 1		.96667 [*]	.06667	.000	.8181	1.1152
		Standard Control	Negative Control		.00000	.06667	1.000	-.1485	.1485
			Positive Control		-1.00000 [*]	.06667	.000	-1.1485	-.8515
			100 mg/kg b.w. of Fraction 1		-.06667	.06667	.341	-.2152	.0819
			200 mg/kg b.w. of Fraction 1		-.03333	.06667	.628	-.1819	.1152
		100 mg/kg b.w. of Negative Control Fraction 1			.06667	.06667	.341	-.0819	.2152
		Positive Control			-.93333 [*]	.06667	.000	-1.0819	-.7848

Standard Control				.06667	.06667	.341	-.0819	.2152
200 mg/kg b.w. of Fraction 1				.03333	.06667	.628	-.1152	.1819
200 mg/kg b.w. of Negative Control Fraction 1				.03333	.06667	.628	-.1152	.1819
Positive Control				-.96667*	.06667	.000	-1.1152	-.8181
Standard Control				.03333	.06667	.628	-.1152	.1819
100 mg/kg b.w. of Fraction 1				-.03333	.06667	.628	-.1819	.1152
Urea	LSD	Negative Control	Positive Control	-47.73000*	5.97720	.000	-61.0480	-34.4120
			Standard Control	-1.81000	5.97720	.768	-15.1280	11.5080
			100 mg/kg b.w. of Fraction 1	-7.40000	5.97720	.244	-20.7180	5.9180
			200 mg/kg b.w. of Fraction 1	-3.74000	5.97720	.546	-17.0580	9.5780
		Positive Control	Negative Control	47.73000*	5.97720	.000	34.4120	61.0480
			Standard Control	45.92000*	5.97720	.000	32.6020	59.2380
			100 mg/kg b.w. of Fraction 1	40.33000*	5.97720	.000	27.0120	53.6480
			200 mg/kg b.w. of Fraction 1	43.99000*	5.97720	.000	30.6720	57.3080
		Standard Control	Negative Control	1.81000	5.97720	.768	-11.5080	15.1280
			Positive Control	-45.92000*	5.97720	.000	-59.2380	-32.6020
			100 mg/kg b.w. of Fraction 1	-5.59000	5.97720	.372	-18.9080	7.7280
			200 mg/kg b.w. of Fraction 1	-1.93000	5.97720	.753	-15.2480	11.3880
		100 mg/kg b.w. of Fraction 1	Negative Control	7.40000	5.97720	.244	-5.9180	20.7180
			Positive Control	-40.33000*	5.97720	.000	-53.6480	-27.0120
			Standard Control	5.59000	5.97720	.372	-7.7280	18.9080
			200 mg/kg b.w. of Fraction 1	3.66000	5.97720	.554	-9.6580	16.9780
		200 mg/kg b.w. of Fraction 1	Negative Control	3.74000	5.97720	.546	-9.5780	17.0580
			Positive Control	-43.99000*	5.97720	.000	-57.3080	-30.6720

				Standard Control	1.93000	5.97720	.753	-11.3880	15.2480
				100 mg/kg b.w. of Fraction 1	-3.66000	5.97720	.554	-16.9780	9.6580
Creatinin e	LSD	Negative Control	Positive Control		-.93333 [*]	.26750	.006	-1.5294	-.3373
			Standard Control		-.10000	.26750	.716	-.6960	.4960
			100 mg/kg b.w. of Fraction 1		-.23333	.26750	.404	-.8294	.3627
			200 mg/kg b.w. of Fraction 1		-.13333	.26750	.629	-.7294	.4627
		Positive Control	Negative Control		.93333 [*]	.26750	.006	.3373	1.5294
			Standard Control		.83333 [*]	.26750	.011	.2373	1.4294
			100 mg/kg b.w. of Fraction 1		.70000 [*]	.26750	.026	.1040	1.2960
			200 mg/kg b.w. of Fraction 1		.80000 [*]	.26750	.014	.2040	1.3960
		Standard Control	Negative Control		.10000	.26750	.716	-.4960	.6960
			Positive Control		-.83333 [*]	.26750	.011	-1.4294	-.2373
			100 mg/kg b.w. of Fraction 1		-.13333	.26750	.629	-.7294	.4627
			200 mg/kg b.w. of Fraction 1		-.03333	.26750	.903	-.6294	.5627
		100 mg/kg b.w. of Fraction 1	Negative Control		.23333	.26750	.404	-.3627	.8294
			Positive Control		-.70000 [*]	.26750	.026	-1.2960	-.1040
			Standard Control		.13333	.26750	.629	-.4627	.7294
			200 mg/kg b.w. of Fraction 1		.10000	.26750	.716	-.4960	.6960
		200 mg/kg b.w. of Fraction 1	Negative Control		.13333	.26750	.629	-.4627	.7294
			Positive Control		-.80000 [*]	.26750	.014	-1.3960	-.2040
			Standard Control		.03333	.26750	.903	-.5627	.6294
			100 mg/kg b.w. of Fraction 1		-.10000	.26750	.716	-.6960	.4960
Sodium	LSD	Negative Control	Positive Control	48.77000 [*]	4.47433	.000	38.8006	58.7394	
			Standard Control	1.55667	4.47433	.735	-8.4128	11.5261	

		100 mg/kg b.w. of Fraction 1	8.00667	4.47433	.104	-1.9628	17.9761
		200 mg/kg b.w. of Fraction 1	3.89667	4.47433	.404	-6.0728	13.8661
Positive Control	Negative Control		-48.77000*	4.47433	.000	-58.7394	-38.8006
	Standard Control		-47.21333*	4.47433	.000	-57.1828	-37.2439
	100 mg/kg b.w. of Fraction 1		-40.76333*	4.47433	.000	-50.7328	-30.7939
	200 mg/kg b.w. of Fraction 1		-44.87333*	4.47433	.000	-54.8428	-34.9039
Standard Control	Negative Control		-1.55667	4.47433	.735	-11.5261	8.4128
	Positive Control		47.21333*	4.47433	.000	37.2439	57.1828
	100 mg/kg b.w. of Fraction 1		6.45000	4.47433	.180	-3.5194	16.4194
	200 mg/kg b.w. of Fraction 1		2.34000	4.47433	.612	-7.6294	12.3094
100 mg/kg b.w. of Fraction 1	Negative Control		-8.00667	4.47433	.104	-17.9761	1.9628
	Positive Control		40.76333*	4.47433	.000	30.7939	50.7328
	Standard Control		-6.45000	4.47433	.180	-16.4194	3.5194
	200 mg/kg b.w. of Fraction 1		-4.11000	4.47433	.380	-14.0794	5.8594
200 mg/kg b.w. of Fraction 1	Negative Control		-3.89667	4.47433	.404	-13.8661	6.0728
	Positive Control		44.87333*	4.47433	.000	34.9039	54.8428
	Standard Control		-2.34000	4.47433	.612	-12.3094	7.6294
	100 mg/kg b.w. of Fraction 1		4.11000	4.47433	.380	-5.8594	14.0794
Potassiu m	LSD	Negative Control	-2.72000*	.48390	.000	-3.7982	-1.6418
		Standard Control	-.20000	.48390	.688	-1.2782	.8782
		100 mg/kg b.w. of Fraction 1	-.65000	.48390	.209	-1.7282	.4282
		200 mg/kg b.w. of Fraction 1	-.35000	.48390	.486	-1.4282	.7282
	Positive Control	Negative Control	2.72000*	.48390	.000	1.6418	3.7982
		Standard Control	2.52000*	.48390	.000	1.4418	3.5982

				100 mg/kg b.w. of Fraction 1	2.07000 [*]	.48390	.002	.9918	3.1482
				200 mg/kg b.w. of Fraction 1	2.37000 [*]	.48390	.001	1.2918	3.4482
				Standard Control Negative Control	.20000	.48390	.688	-.8782	1.2782
				Positive Control	-2.52000 [*]	.48390	.000	-3.5982	-1.4418
				100 mg/kg b.w. of Fraction 1	-.45000	.48390	.374	-1.5282	.6282
				200 mg/kg b.w. of Fraction 1	-.15000	.48390	.763	-1.2282	.9282
				100 mg/kg b.w. of Fraction 1 Negative Control	.65000	.48390	.209	-.4282	1.7282
				Positive Control	-2.07000 [*]	.48390	.002	-3.1482	-.9918
				Standard Control	.45000	.48390	.374	-.6282	1.5282
				200 mg/kg b.w. of Fraction 1	.30000	.48390	.549	-.7782	1.3782
				200 mg/kg b.w. of Fraction 1 Negative Control	.35000	.48390	.486	-.7282	1.4282
				Positive Control	-2.37000 [*]	.48390	.001	-3.4482	-1.2918
				Standard Control	.15000	.48390	.763	-.9282	1.2282
				100 mg/kg b.w. of Fraction 1	-.30000	.48390	.549	-1.3782	.7782
Chloride	LSD	Negative Control	Positive Control		21.67000 [*]	2.66429	.000	15.7336	27.6064
			Standard Control		1.17000	2.66429	.670	-4.7664	7.1064
			100 mg/kg b.w. of Fraction 1		5.10000	2.66429	.085	-.8364	11.0364
			200 mg/kg b.w. of Fraction 1		.66000	2.66429	.809	-5.2764	6.5964
	Positive Control	Negative Control			-21.67000 [*]	2.66429	.000	-27.6064	-15.7336
			Standard Control		-20.50000 [*]	2.66429	.000	-26.4364	-14.5636
			100 mg/kg b.w. of Fraction 1		-16.57000 [*]	2.66429	.000	-22.5064	-10.6336
			200 mg/kg b.w. of Fraction 1		-21.01000 [*]	2.66429	.000	-26.9464	-15.0736
	Standard Control	Negative Control			-1.17000	2.66429	.670	-7.1064	4.7664
			Positive Control		20.50000 [*]	2.66429	.000	14.5636	26.4364

				100 mg/kg b.w. of Fraction 1	3.93000	2.66429	.171	-2.0064	9.8664
				200 mg/kg b.w. of Fraction 1	-.51000	2.66429	.852	-6.4464	5.4264
				100 mg/kg b.w. of Negative Control Fraction 1	-5.10000	2.66429	.085	-11.0364	.8364
				Positive Control	16.57000 [*]	2.66429	.000	10.6336	22.5064
				Standard Control	-3.93000	2.66429	.171	-9.8664	2.0064
				200 mg/kg b.w. of Fraction 1	-4.44000	2.66429	.127	-10.3764	1.4964
				200 mg/kg b.w. of Negative Control Fraction 1	-.66000	2.66429	.809	-6.5964	5.2764
				Positive Control	21.01000 [*]	2.66429	.000	15.0736	26.9464
				Standard Control	.51000	2.66429	.852	-5.4264	6.4464
				100 mg/kg b.w. of Fraction 1	4.44000	2.66429	.127	-1.4964	10.3764
SOD	LSD	Negative Control	Positive Control		20.30667 [*]	3.29064	.000	12.9747	27.6387
			Standard Control		1.90000	3.29064	.576	-5.4320	9.2320
			100 mg/kg b.w. of Fraction 1		6.88333	3.29064	.063	-.4487	14.2153
			200 mg/kg b.w. of Fraction 1		4.37333	3.29064	.213	-2.9587	11.7053
		Positive Control	Negative Control		-20.30667 [*]	3.29064	.000	-27.6387	-12.9747
			Standard Control		-18.40667 [*]	3.29064	.000	-25.7387	-11.0747
			100 mg/kg b.w. of Fraction 1		-13.42333 [*]	3.29064	.002	-20.7553	-6.0913
			200 mg/kg b.w. of Fraction 1		-15.93333 [*]	3.29064	.001	-23.2653	-8.6013
		Standard Control	Negative Control		-1.90000	3.29064	.576	-9.2320	5.4320
			Positive Control		18.40667 [*]	3.29064	.000	11.0747	25.7387
			100 mg/kg b.w. of Fraction 1		4.98333	3.29064	.161	-2.3487	12.3153
			200 mg/kg b.w. of Fraction 1		2.47333	3.29064	.470	-4.8587	9.8053
		100 mg/kg b.w. of Negative Control Fraction 1			-6.88333	3.29064	.063	-14.2153	.4487
		Positive Control			13.42333 [*]	3.29064	.002	6.0913	20.7553

				Standard Control	-4.98333	3.29064	.161	-12.3153	2.3487
				200 mg/kg b.w. of Fraction 1	-2.51000	3.29064	.463	-9.8420	4.8220
				200 mg/kg b.w. of Negative Control Fraction 1	-4.37333	3.29064	.213	-11.7053	2.9587
				Positive Control	15.93333 [*]	3.29064	.001	8.6013	23.2653
				Standard Control	-2.47333	3.29064	.470	-9.8053	4.8587
				100 mg/kg b.w. of Fraction 1	2.51000	3.29064	.463	-4.8220	9.8420
MDA	LSD	Negative Control	Positive Control		-3.64333 [*]	.37569	.000	-4.4804	-2.8062
			Standard Control		-.03333	.37569	.931	-.8704	.8038
			100 mg/kg b.w. of Fraction 1		-.42000	.37569	.290	-1.2571	.4171
			200 mg/kg b.w. of Fraction 1		-.22000	.37569	.571	-1.0571	.6171
		Positive Control	Negative Control		3.64333 [*]	.37569	.000	2.8062	4.4804
			Standard Control		3.61000 [*]	.37569	.000	2.7729	4.4471
			100 mg/kg b.w. of Fraction 1		3.22333 [*]	.37569	.000	2.3862	4.0604
			200 mg/kg b.w. of Fraction 1		3.42333 [*]	.37569	.000	2.5862	4.2604
		Standard Control	Negative Control		.03333	.37569	.931	-.8038	.8704
			Positive Control		-3.61000 [*]	.37569	.000	-4.4471	-2.7729
			100 mg/kg b.w. of Fraction 1		-.38667	.37569	.328	-1.2238	.4504
			200 mg/kg b.w. of Fraction 1		-.18667	.37569	.630	-1.0238	.6504
		100 mg/kg b.w. of Fraction 1	Negative Control		.42000	.37569	.290	-.4171	1.2571
			Positive Control		-3.22333 [*]	.37569	.000	-4.0604	-2.3862
			Standard Control		.38667	.37569	.328	-.4504	1.2238
			200 mg/kg b.w. of Fraction 1		.20000	.37569	.606	-.6371	1.0371
		200 mg/kg b.w. of Fraction 1	Negative Control		.22000	.37569	.571	-.6171	1.0571
			Positive Control		-3.42333 [*]	.37569	.000	-4.2604	-2.5862

Standard Control				.18667	.37569	.630	-.6504	1.0238
100 mg/kg b.w. of Fraction 1				-.20000	.37569	.606	-1.0371	.6371
HB	LSD	Negative Control	Positive Control	6.33333 ⁺	.66667	.000	4.8479	7.8188
			Standard Control	.33333	.66667	.628	-1.1521	1.8188
			100 mg/kg b.w. of Fraction 1	1.00000	.66667	.165	-.4854	2.4854
			200 mg/kg b.w. of Fraction 1	.66667	.66667	.341	-.8188	2.1521
		Positive Control	Negative Control	-6.33333 ⁺	.66667	.000	-7.8188	-4.8479
			Standard Control	-6.00000 ⁺	.66667	.000	-7.4854	-4.5146
			100 mg/kg b.w. of Fraction 1	-5.33333 ⁺	.66667	.000	-6.8188	-3.8479
			200 mg/kg b.w. of Fraction 1	-5.66667 ⁺	.66667	.000	-7.1521	-4.1812
		Standard Control	Negative Control	-.33333	.66667	.628	-1.8188	1.1521
			Positive Control	6.00000 ⁺	.66667	.000	4.5146	7.4854
			100 mg/kg b.w. of Fraction 1	.66667	.66667	.341	-.8188	2.1521
			200 mg/kg b.w. of Fraction 1	.33333	.66667	.628	-1.1521	1.8188
		100 mg/kg b.w. of Fraction 1	Negative Control	-1.00000	.66667	.165	-2.4854	.4854
			Positive Control	5.33333 ⁺	.66667	.000	3.8479	6.8188
			Standard Control	-.66667	.66667	.341	-2.1521	.8188
			200 mg/kg b.w. of Fraction 1	-.33333	.66667	.628	-1.8188	1.1521
		200 mg/kg b.w. of Fraction 1	Negative Control	-.66667	.66667	.341	-2.1521	.8188
			Positive Control	5.66667 ⁺	.66667	.000	4.1812	7.1521
			Standard Control	-.33333	.66667	.628	-1.8188	1.1521
			100 mg/kg b.w. of Fraction 1	.33333	.66667	.628	-1.1521	1.8188
PCV	LSD	Negative Control	Positive Control	24.00000 ⁺	2.33809	.000	18.7904	29.2096
			Standard Control	1.33333	2.33809	.581	-3.8763	6.5429

			100 mg/kg b.w. of Fraction 1	4.33333	2.33809	.094	-.8763	9.5429
			200 mg/kg b.w. of Fraction 1	3.33333	2.33809	.184	-1.8763	8.5429
		Positive Control	Negative Control	-24.00000*	2.33809	.000	-29.2096	-18.7904
			Standard Control	-22.66667*	2.33809	.000	-27.8763	-17.4571
			100 mg/kg b.w. of Fraction 1	-19.66667*	2.33809	.000	-24.8763	-14.4571
			200 mg/kg b.w. of Fraction 1	-20.66667*	2.33809	.000	-25.8763	-15.4571
		Standard Control	Negative Control	-1.33333	2.33809	.581	-6.5429	3.8763
			Positive Control	22.66667*	2.33809	.000	17.4571	27.8763
			100 mg/kg b.w. of Fraction 1	3.00000	2.33809	.228	-2.2096	8.2096
			200 mg/kg b.w. of Fraction 1	2.00000	2.33809	.412	-3.2096	7.2096
		100 mg/kg b.w. of Negative Control Fraction 1		-4.33333	2.33809	.094	-9.5429	.8763
			Positive Control	19.66667*	2.33809	.000	14.4571	24.8763
			Standard Control	-3.00000	2.33809	.228	-8.2096	2.2096
			200 mg/kg b.w. of Fraction 1	-1.00000	2.33809	.678	-6.2096	4.2096
		200 mg/kg b.w. of Negative Control Fraction 1		-3.33333	2.33809	.184	-8.5429	1.8763
			Positive Control	20.66667*	2.33809	.000	15.4571	25.8763
			Standard Control	-2.00000	2.33809	.412	-7.2096	3.2096
			100 mg/kg b.w. of Fraction 1	1.00000	2.33809	.678	-4.2096	6.2096
RBC	LSD	Negative Control	Positive Control	185.33333*	29.5213	.000	119.5556	251.111
			Standard Control	10.33333	29.5213	.734	-55.4444	76.1110
			100 mg/kg b.w. of Fraction 1	42.33333	29.5217	.182	-23.4444	108.110
			200 mg/kg b.w. of Fraction 1	27.33333	29.5213	.376	-38.4444	93.1110
		Positive Control	Negative Control	-185.33333*	29.52137	.000	-251.1110	-119.5556
			Standard Control	-175.00000*	29.52137	.000	-240.7777	-109.2223

				100 mg/kg b.w. of Fraction 1	-143.00000 [*]	29.52137	.001	-208.7777	-77.2223	
				200 mg/kg b.w. of Fraction 1	-158.00000 [*]	29.52137	.000	-223.7777	-92.2223	
				Standard Control	Negative Control	-10.33333	29.52137	.734	-76.1110	55.4444
					Positive Control	175.00000 [*]	29.52137	.000	109.2223	240.7777
					100 mg/kg b.w. of Fraction 1	32.00000	29.52137	.304	-33.7777	97.7777
					200 mg/kg b.w. of Fraction 1	17.00000	29.52137	.577	-48.7777	82.7777
				100 mg/kg b.w. of Fraction 1	Negative Control	-42.33333	29.52137	.182	-108.1110	23.4444
					Positive Control	143.00000 [*]	29.52137	.001	77.2223	208.7777
					Standard Control	-32.00000	29.52137	.304	-97.7777	33.7777
					200 mg/kg b.w. of Fraction 1	-15.00000	29.52137	.622	-80.7777	50.7777
				200 mg/kg b.w. of Fraction 1	Negative Control	-27.33333	29.52137	.376	-93.1110	38.4444
					Positive Control	158.00000 [*]	29.52137	.000	92.2223	223.7777
					Standard Control	-17.00000	29.52137	.577	-82.7777	48.7777
					100 mg/kg b.w. of Fraction 1	15.00000	29.52137	.622	-50.7777	80.7777
WBC	LSD	Negative Control	Positive Control		-3600.00000 [*]	400.00000	.000	-4491.2555	-2708.7445	
				Standard Control		-800.00000	400.00000	.073	-1691.2555	91.2555
				100 mg/kg b.w. of Fraction 1		-1200.00000 [*]	400.00000	.013	-2091.2555	-308.7445
				200 mg/kg b.w. of Fraction 1		-1000.00000 [*]	400.00000	.031	-1891.2555	-108.7445
		Positive Control	Negative Control		3600.00000 [*]	400.00000	.000	2708.7445	4491.2555	
				Standard Control		2800.00000 [*]	400.00000	.000	1908.7445	3691.2555
				100 mg/kg b.w. of Fraction 1		2400.00000 [*]	400.00000	.000	1508.7445	3291.2555
				200 mg/kg b.w. of Fraction 1		2600.00000 [*]	400.00000	.000	1708.7445	3491.2555
		Standard Control	Negative Control		800.00000	400.00000	.073	-91.2555	1691.2555	
				Positive Control		-2800.00000 [*]	400.00000	.000	-3691.2555	-1908.7445

100 mg/kg b.w. of Fraction 1	-400.00000	400.00000	.341	-1291.2555	491.2555
200 mg/kg b.w. of Fraction 1	-200.00000	400.00000	.628	-1091.2555	691.2555
100 mg/kg b.w. of Negative Control Fraction 1	1200.00000*	400.00000	.013	308.7445	2091.2555
Positive Control	-2400.00000*	400.00000	.000	-3291.2555	-1508.7445
Standard Control	400.00000	400.00000	.341	-491.2555	1291.2555
200 mg/kg b.w. of Fraction 1	200.00000	400.00000	.628	-691.2555	1091.2555
200 mg/kg b.w. of Negative Control Fraction 1	1000.00000*	400.00000	.031	108.7445	1891.2555
Positive Control	-2600.00000*	400.00000	.000	-3491.2555	-1708.7445
Standard Control	200.00000	400.00000	.628	-691.2555	1091.2555
100 mg/kg b.w. of Fraction 1	-200.00000	400.00000	.628	-1091.2555	691.2555

*. The mean difference is significant at the 0.05 level.

Appendix 2g: Group 1 Comparisons - Descriptive

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	After 24 hrs	3	78.2100	2.53077	1.46114	71.9232	84.4968	75.29	79.77
	After 7 days	3	76.0000	5.29150	3.05505	62.8552	89.1448	72.00	82.00
	After 14 days	3	75.0000	3.00000	1.73205	67.5476	82.4524	72.00	78.00
	Total	9	76.4033	3.58814	1.19605	73.6452	79.1614	72.00	82.00

AST	After 24 hrs	3	38.6667	2.88675	1.66667	31.4956	45.8378	37.00	42.00
	After 7 days	3	39.3333	7.63763	4.40959	20.3604	58.3062	31.00	46.00
	After 14 days	3	37.6667	2.51661	1.45297	31.4151	43.9183	35.00	40.00
	Total	9	38.5556	4.33333	1.44444	35.2247	41.8865	31.00	46.00
ALT	After 24 hrs	3	21.6667	3.21455	1.85592	13.6813	29.6521	18.00	24.00
	After 7 days	3	21.0000	2.34681	1.35493	15.1702	26.8298	18.30	22.55
	After 14 days	3	19.6667	5.15493	2.97620	6.8611	32.4722	15.00	25.20
	Total	9	20.7778	3.37362	1.12454	18.1846	23.3710	15.00	25.20
TBIL	After 24 hrs	3	.4667	.05774	.03333	.3232	.6101	.40	.50
	After 7 days	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	After 14 days	3	.4667	.05774	.03333	.3232	.6101	.40	.50
	Total	9	.4778	.04410	.01470	.4439	.5117	.40	.50
Urea	After 24 hrs	3	45.0000	5.19615	3.00000	32.0920	57.9080	42.00	51.00
	After 7 days	3	46.0000	4.31753	2.49273	35.2747	56.7253	42.21	50.70
	After 14 days	3	44.6000	5.88037	3.39503	29.9924	59.2076	38.87	50.62
	Total	9	45.2000	4.52161	1.50720	41.7244	48.6756	38.87	51.00
Creatinine	After 24 hrs	3	1.3000	.36056	.20817	.4043	2.1957	.90	1.60
	After 7 days	3	1.3000	.26458	.15275	.6428	1.9572	1.00	1.50
	After 14 days	3	1.2667	.40415	.23333	.2627	2.2706	.90	1.70
	Total	9	1.2889	.30185	.10062	1.0569	1.5209	.90	1.70
Sodium	After 24 hrs	3	120.2500	7.72075	4.45758	101.0706	139.4294	111.35	125.15

	After 7 days	3	120.4100	10.86000	6.27002	93.4323	147.3877	109.55	131.27
	After 14 days	3	121.2200	2.88520	1.66577	114.0528	128.3872	118.44	124.20
	Total	9	120.6267	6.83164	2.27721	115.3754	125.8779	109.55	131.27
Potassium	After 24 hrs	3	5.3767	1.27064	.73361	2.2202	8.5331	4.13	6.67
	After 7 days	3	5.2433	.92500	.53405	2.9455	7.5412	4.32	6.17
	After 14 days	3	5.3000	.30000	.17321	4.5548	6.0452	5.00	5.60
	Total	9	5.3067	.80212	.26737	4.6901	5.9232	4.13	6.67
Chloride	After 24 hrs	3	85.4467	4.98559	2.87843	73.0618	97.8316	79.72	88.82
	After 7 days	3	87.5500	1.05000	.60622	84.9417	90.1583	86.50	88.60
	After 14 days	3	87.3300	1.58370	.91435	83.3959	91.2641	86.02	89.09
	Total	9	86.7756	2.84940	.94980	84.5853	88.9658	79.72	89.09
SOD	After 24 hrs	3	39.7200	1.55936	.90030	35.8463	43.5937	38.78	41.52
	After 7 days	3	40.4900	5.98320	3.45440	25.6269	55.3531	33.78	45.27
	After 14 days	3	39.7233	5.49105	3.17026	26.0828	53.3639	34.60	45.52
	Total	9	39.9778	4.15248	1.38416	36.7859	43.1697	33.78	45.52
MDA	After 24 hrs	3	4.3800	1.06226	.61330	1.7412	7.0188	3.50	5.56
	After 7 days	3	4.4633	.59467	.34333	2.9861	5.9406	4.12	5.15
	After 14 days	3	4.2000	.54945	.31723	2.8351	5.5649	3.82	4.83
	Total	9	4.3478	.67792	.22597	3.8267	4.8689	3.50	5.56
HB	After 24 hrs	3	15.6667	1.52753	.88192	11.8721	19.4612	14.00	17.00
	After 7 days	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00

	After 14 days	3	15.6667	1.15470	.66667	12.7982	18.5351	15.00	17.00
	Total	9	15.5556	1.01379	.33793	14.7763	16.3348	14.00	17.00
PCV	After 24 hrs	3	49.0000	3.60555	2.08167	40.0433	57.9567	46.00	53.00
	After 7 days	3	48.3333	3.21455	1.85592	40.3479	56.3187	46.00	52.00
	After 14 days	3	49.0000	2.64575	1.52753	42.4276	55.5724	47.00	52.00
	Total	9	48.7778	2.77389	.92463	46.6456	50.9100	46.00	53.00
RBC	After 24 hrs	3	326.0000	52.42137	30.26549	195.7781	456.2219	282.00	384.00
	After 7 days	3	324.0000	52.30679	30.19934	194.0627	453.9373	288.00	384.00
	After 14 days	3	327.3333	66.16142	38.19831	162.9793	491.6874	252.00	376.00
	Total	9	325.7778	49.67338	16.55779	287.5954	363.9601	252.00	384.00
WBC	After 24 hrs	3	4300.0000	458.25757	264.57513	3161.6251	5438.3749	3900.00	4800.00
	After 7 days	3	4466.6667	305.50505	176.38342	3707.7501	5225.5833	4200.00	4800.00
	After 14 days	3	4200.0000	346.41016	200.00000	3339.4695	5060.5305	3800.00	4400.00
	Total	9	4322.2222	345.60736	115.20245	4056.5649	4587.8796	3800.00	4800.00

Post Hoc Tests

Multiple Comparisons

Dependent Variable						95% Confidence Interval	
			Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Variable	(I) TIME	(J) TIME					

ALP	LSD	After 24 hrs	After 7 days	2.21000	3.10572	.503	-5.3894	9.8094
			After 14 days	3.21000	3.10572	.341	-4.3894	10.8094
		After 7 days	After 24 hrs	-2.21000	3.10572	.503	-9.8094	5.3894
			After 14 days	1.00000	3.10572	.758	-6.5994	8.5994
		After 14 days	After 24 hrs	-3.21000	3.10572	.341	-10.8094	4.3894
			After 7 days	-1.00000	3.10572	.758	-8.5994	6.5994
AST	LSD	After 24 hrs	After 7 days	-.66667	4.02768	.874	-10.5220	9.1887
			After 14 days	1.00000	4.02768	.812	-8.8554	10.8554
		After 7 days	After 24 hrs	.66667	4.02768	.874	-9.1887	10.5220
			After 14 days	1.66667	4.02768	.693	-8.1887	11.5220
		After 14 days	After 24 hrs	-1.00000	4.02768	.812	-10.8554	8.8554
			After 7 days	-1.66667	4.02768	.693	-11.5220	8.1887
ALT	LSD	After 24 hrs	After 7 days	.66667	3.07008	.835	-6.8455	8.1789
			After 14 days	2.00000	3.07008	.539	-5.5122	9.5122
		After 7 days	After 24 hrs	-.66667	3.07008	.835	-8.1789	6.8455
			After 14 days	1.33333	3.07008	.679	-6.1789	8.8455
		After 14 days	After 24 hrs	-2.00000	3.07008	.539	-9.5122	5.5122
			After 7 days	-1.33333	3.07008	.679	-8.8455	6.1789
TBIL	LSD	After 24 hrs	After 7 days	-.03333	.03849	.420	-.1275	.0608
			After 14 days	.00000	.03849	1.000	-.0942	.0942
		After 7 days	After 24 hrs	.03333	.03849	.420	-.0608	.1275

After 14 days				.03333	.03849	.420	-.0608	.1275
After 14 days After 24 hrs				.00000	.03849	1.000	-.0942	.0942
After 7 days				-.03333	.03849	.420	-.1275	.0608
Urea	LSD	After 24 hrs	After 7 days	-1.00000	4.22216	.821	-11.3312	9.3312
		After 14 days		.40000	4.22216	.928	-9.9312	10.7312
		After 7 days	After 24 hrs	1.00000	4.22216	.821	-9.3312	11.3312
		After 14 days		1.40000	4.22216	.751	-8.9312	11.7312
		After 14 days	After 24 hrs	-.40000	4.22216	.928	-10.7312	9.9312
		After 7 days		-1.40000	4.22216	.751	-11.7312	8.9312
Creatinine	LSD	After 24 hrs	After 7 days	.00000	.28415	1.000	-.6953	.6953
		After 14 days		.03333	.28415	.910	-.6620	.7286
		After 7 days	After 24 hrs	.00000	.28415	1.000	-.6953	.6953
		After 14 days		.03333	.28415	.910	-.6620	.7286
		After 14 days	After 24 hrs	-.03333	.28415	.910	-.7286	.6620
		After 7 days		-.03333	.28415	.910	-.7286	.6620
Sodium	LSD	After 24 hrs	After 7 days	-.16000	6.42692	.981	-15.8861	15.5661
		After 14 days		-.97000	6.42692	.885	-16.6961	14.7561
		After 7 days	After 24 hrs	.16000	6.42692	.981	-15.5661	15.8861
		After 14 days		-.81000	6.42692	.904	-16.5361	14.9161
		After 14 days	After 24 hrs	.97000	6.42692	.885	-14.7561	16.6961
		After 7 days		.81000	6.42692	.904	-14.9161	16.5361

Potassium	LSD	After 24 hrs	After 7 days	.13333	.75427	.866	-1.7123	1.9790
			After 14 days	.07667	.75427	.922	-1.7690	1.9223
		After 7 days	After 24 hrs	-.13333	.75427	.866	-1.9790	1.7123
			After 14 days	-.05667	.75427	.943	-1.9023	1.7890
		After 14 days	After 24 hrs	-.07667	.75427	.922	-1.9223	1.7690
			After 7 days	.05667	.75427	.943	-1.7890	1.9023
Chloride	LSD	After 24 hrs	After 7 days	-2.10333	2.51514	.435	-8.2577	4.0510
			After 14 days	-1.88333	2.51514	.482	-8.0377	4.2710
		After 7 days	After 24 hrs	2.10333	2.51514	.435	-4.0510	8.2577
			After 14 days	.22000	2.51514	.933	-5.9343	6.3743
		After 14 days	After 24 hrs	1.88333	2.51514	.482	-4.2710	8.0377
			After 7 days	-.22000	2.51514	.933	-6.3743	5.9343
SOD	LSD	After 24 hrs	After 7 days	-.77000	3.89820	.850	-10.3086	8.7686
			After 14 days	-.00333	3.89820	.999	-9.5419	9.5352
		After 7 days	After 24 hrs	.77000	3.89820	.850	-8.7686	10.3086
			After 14 days	.76667	3.89820	.851	-8.7719	10.3052
		After 14 days	After 24 hrs	.00333	3.89820	.999	-9.5352	9.5419
			After 7 days	-.76667	3.89820	.851	-10.3052	8.7719
MDA	LSD	After 24 hrs	After 7 days	-.08333	.62963	.899	-1.6240	1.4573
			After 14 days	.18000	.62963	.785	-1.3606	1.7206
		After 7 days	After 24 hrs	.08333	.62963	.899	-1.4573	1.6240

				After 14 days	.26333	.62963	.690	-1.2773	1.8040
				After 14 days After 24 hrs	-.18000	.62963	.785	-1.7206	1.3606
				After 7 days	-.26333	.62963	.690	-1.8040	1.2773
HB	LSD	After 24 hrs	After 7 days	.33333	.94281	.736	-1.9736	2.6403	
		After 14 days		.00000	.94281	1.000	-2.3070	2.3070	
		After 7 days	After 24 hrs	-.33333	.94281	.736	-2.6403	1.9736	
		After 14 days		-.33333	.94281	.736	-2.6403	1.9736	
		After 14 days	After 24 hrs	.00000	.94281	1.000	-2.3070	2.3070	
		After 7 days		.33333	.94281	.736	-1.9736	2.6403	
PCV	LSD	After 24 hrs	After 7 days	.66667	2.59629	.806	-5.6862	7.0196	
		After 14 days		.00000	2.59629	1.000	-6.3529	6.3529	
		After 7 days	After 24 hrs	-.66667	2.59629	.806	-7.0196	5.6862	
		After 14 days		-.66667	2.59629	.806	-7.0196	5.6862	
		After 14 days	After 24 hrs	.00000	2.59629	1.000	-6.3529	6.3529	
		After 7 days		.66667	2.59629	.806	-5.6862	7.0196	
RBC	LSD	After 24 hrs	After 7 days	2.00000	46.81247	.967	-112.5460	116.5460	
		After 14 days		-1.33333	46.81247	.978	-115.8793	113.2127	
		After 7 days	After 24 hrs	-2.00000	46.81247	.967	-116.5460	112.5460	
		After 14 days		-3.33333	46.81247	.946	-117.8793	111.2127	
		After 14 days	After 24 hrs	1.33333	46.81247	.978	-113.2127	115.8793	
		After 7 days		3.33333	46.81247	.946	-111.2127	117.8793	

WBC	LSD	After 24 hrs	After 7 days	-166.66667	306.71497	.606	-917.1712	583.8378
			After 14 days	100.00000	306.71497	.755	-650.5045	850.5045
		After 7 days	After 24 hrs	166.66667	306.71497	.606	-583.8378	917.1712
			After 14 days	266.66667	306.71497	.418	-483.8378	1017.1712
		After 14 days	After 24 hrs	-100.00000	306.71497	.755	-850.5045	650.5045
			After 7 days	-266.66667	306.71497	.418	-1017.1712	483.8378

Appendix 2h: Group 2 Comparisons - Descriptive

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	After 24 hrs	3	126.6600	27.52611	15.89221	58.2813	195.0387	101.63	156.14
	After 7 days	3	133.3333	22.03028	12.71919	78.6071	188.0596	112.00	156.00
	After 14 days	3	165.3333	8.32666	4.80740	144.6488	186.0179	156.00	172.00
	Total	9	141.7756	25.46779	8.48926	122.1993	161.3518	101.63	172.00

AST	After 24 hrs	3	68.0000	1.00000	.57735	65.5159	70.4841	67.00	69.00
	After 7 days	3	75.3333	4.16333	2.40370	64.9910	85.6756	72.00	80.00
	After 14 days	3	89.3333	2.51661	1.45297	83.0817	95.5849	87.00	92.00
	Total	9	77.5556	9.70967	3.23656	70.0920	85.0191	67.00	92.00
ALT	After 24 hrs	3	42.3333	12.50333	7.21880	11.2733	73.3933	30.00	55.00
	After 7 days	3	50.0900	5.58724	3.22579	36.2105	63.9695	45.67	56.37
	After 14 days	3	68.5300	9.79521	5.65527	44.1974	92.8626	62.78	79.84
	Total	9	53.6511	14.37645	4.79215	42.6004	64.7018	30.00	79.84
TBIL	After 24 hrs	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	After 7 days	3	1.0667	.15275	.08819	.6872	1.4461	.90	1.20
	After 14 days	3	1.4667	.11547	.06667	1.1798	1.7535	1.40	1.60
	Total	9	1.0889	.33333	.11111	.8327	1.3451	.70	1.60
Urea	After 24 hrs	3	68.0000	6.00000	3.46410	53.0952	82.9048	62.00	74.00
	After 7 days	3	75.9300	2.35578	1.36011	70.0779	81.7821	73.54	78.25
	After 14 days	3	92.3300	6.22831	3.59592	76.8580	107.8020	87.95	99.46
	Total	9	78.7533	11.64301	3.88100	69.8037	87.7029	62.00	99.46
Creatinine	After 24 hrs	3	2.0000	.10000	.05774	1.7516	2.2484	1.90	2.10
	After 7 days	3	2.0333	.15275	.08819	1.6539	2.4128	1.90	2.20
	After 14 days	3	2.2000	.40000	.23094	1.2063	3.1937	1.80	2.60
	Total	9	2.0778	.23863	.07954	1.8944	2.2612	1.80	2.60
Sodium	After 24 hrs	3	90.8700	6.18297	3.56974	75.5107	106.2293	85.86	97.78

	After 7 days	3	82.0000	4.63006	2.67317	70.4983	93.5017	77.25	86.50
	After 14 days	3	72.4500	3.39510	1.96016	64.0161	80.8839	68.72	75.36
	Total	9	81.7733	9.02470	3.00823	74.8363	88.7103	68.72	97.78
Potassium	After 24 hrs	3	6.4667	1.20500	.69571	3.4733	9.4601	5.26	7.67
	After 7 days	3	7.1300	1.18710	.68537	4.1811	10.0789	5.81	8.11
	After 14 days	3	8.0200	.84923	.49031	5.9104	10.1296	7.50	9.00
	Total	9	7.2056	1.16242	.38747	6.3120	8.0991	5.26	9.00
Chloride	After 24 hrs	3	79.6000	4.85370	2.80228	67.5427	91.6573	76.00	85.12
	After 7 days	3	72.0167	1.43117	.82628	68.4615	75.5719	70.90	73.63
	After 14 days	3	65.6600	2.46441	1.42283	59.5381	71.7819	63.78	68.45
	Total	9	72.4256	6.66706	2.22235	67.3008	77.5503	63.78	85.12
SOD	After 24 hrs	3	25.8800	1.16550	.67290	22.9847	28.7753	24.60	26.88
	After 7 days	3	23.5033	.74460	.42990	21.6536	25.3530	22.66	24.07
	After 14 days	3	19.4167	2.08725	1.20508	14.2316	24.6017	17.14	21.24
	Total	9	22.9333	3.09562	1.03187	20.5538	25.3128	17.14	26.88
MDA	After 24 hrs	3	6.5133	.51965	.30002	5.2225	7.8042	6.16	7.11
	After 7 days	3	6.7900	.84285	.48662	4.6962	8.8838	5.91	7.59
	After 14 days	3	7.8433	.25423	.14678	7.2118	8.4749	7.55	8.00
	Total	9	7.0489	.79413	.26471	6.4385	7.6593	5.91	8.00
HB	After 24 hrs	3	11.0000	2.64575	1.52753	4.4276	17.5724	9.00	14.00
	After 7 days	3	10.3333	1.52753	.88192	6.5388	14.1279	9.00	12.00

	After 14 days	3	9.3333	.57735	.33333	7.8991	10.7676	9.00	10.00
	Total	9	10.2222	1.71594	.57198	8.9032	11.5412	9.00	14.00
PCV	After 24 hrs	3	34.0000	5.29150	3.05505	20.8552	47.1448	30.00	40.00
	After 7 days	3	30.3333	4.50925	2.60342	19.1317	41.5349	26.00	35.00
	After 14 days	3	25.0000	4.35890	2.51661	14.1719	35.8281	20.00	28.00
	Total	9	29.7778	5.67401	1.89134	25.4163	34.1392	20.00	40.00
RBC	After 24 hrs	3	192.0000	66.81317	38.57460	26.0269	357.9731	132.00	264.00
	After 7 days	3	172.0000	18.33030	10.58301	126.4650	217.5350	156.00	192.00
	After 14 days	3	142.0000	4.35890	2.51661	131.1719	152.8281	137.00	145.00
	Total	9	168.6667	40.98475	13.66158	137.1630	200.1703	132.00	264.00
WBC	After 24 hrs	3	6800.0000	600.00000	346.41016	5309.5174	8290.4826	6200.00	7400.00
	After 7 days	3	7200.0000	1000.00000	577.35027	4715.8623	9684.1377	6200.00	8200.00
	After 14 days	3	7800.0000	600.00000	346.41016	6309.5174	9290.4826	7200.00	8400.00
	Total	9	7266.6667	787.40079	262.46693	6661.4168	7871.9165	6200.00	8400.00

Post Hoc Tests

Multiple Comparisons

Dependent Variable	(I) TIME	(J) TIME				95% Confidence Interval	
			Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound

ALP	LSD	After 24 hrs	After 7 days	-6.67333	17.07730	.709	-48.4600	35.1133
			After 14 days	-38.67333	17.07730	.064	-80.4600	3.1133
		After 7 days	After 24 hrs	6.67333	17.07730	.709	-35.1133	48.4600
			After 14 days	-32.00000	17.07730	.110	-73.7866	9.7866
		After 14 days	After 24 hrs	38.67333	17.07730	.064	-3.1133	80.4600
			After 7 days	32.00000	17.07730	.110	-9.7866	73.7866
AST	LSD	After 24 hrs	After 7 days	-7.33333*	2.34126	.020	-13.0622	-1.6045
			After 14 days	-21.33333*	2.34126	.000	-27.0622	-15.6045
		After 7 days	After 24 hrs	7.33333*	2.34126	.020	1.6045	13.0622
			After 14 days	-14.00000*	2.34126	.001	-19.7288	-8.2712
		After 14 days	After 24 hrs	21.33333*	2.34126	.000	15.6045	27.0622
			After 7 days	14.00000*	2.34126	.001	8.2712	19.7288
ALT	LSD	After 24 hrs	After 7 days	-7.75667	7.93721	.366	-27.1783	11.6650
			After 14 days	-26.19667*	7.93721	.016	-45.6183	-6.7750
		After 7 days	After 24 hrs	7.75667	7.93721	.366	-11.6650	27.1783
			After 14 days	-18.44000	7.93721	.059	-37.8616	.9816
		After 14 days	After 24 hrs	26.19667*	7.93721	.016	6.7750	45.6183
			After 7 days	18.44000	7.93721	.059	-.9816	37.8616
TBIL	LSD	After 24 hrs	After 7 days	-.33333*	.09428	.012	-.5640	-.1026
			After 14 days	-.73333*	.09428	.000	-.9640	-.5026
		After 7 days	After 24 hrs	.33333*	.09428	.012	.1026	.5640

				After 14 days	-.40000*	.09428	.005	-.6307	-.1693
				After 14 days After 24 hrs	.73333*	.09428	.000	.5026	.9640
				After 7 days	.40000*	.09428	.005	.1693	.6307
Urea	LSD	After 24 hrs	After 7 days	-7.93000	4.22536	.110	-18.2691	2.4091	
		After 14 days		-24.33000*	4.22536	.001	-34.6691	-13.9909	
		After 7 days	After 24 hrs	7.93000	4.22536	.110	-2.4091	18.2691	
		After 14 days		-16.40000*	4.22536	.008	-26.7391	-6.0609	
		After 14 days	After 24 hrs	24.33000*	4.22536	.001	13.9909	34.6691	
		After 7 days		16.40000*	4.22536	.008	6.0609	26.7391	
Creatinine	LSD	After 24 hrs	After 7 days	-.03333	.20728	.878	-.5405	.4739	
		After 14 days		-.20000	.20728	.372	-.7072	.3072	
		After 7 days	After 24 hrs	.03333	.20728	.878	-.4739	.5405	
		After 14 days		-.16667	.20728	.452	-.6739	.3405	
		After 14 days	After 24 hrs	.20000	.20728	.372	-.3072	.7072	
		After 7 days		.16667	.20728	.452	-.3405	.6739	
Sodium	LSD	After 24 hrs	After 7 days	8.87000	3.97753	.067	-.8627	18.6027	
		After 14 days		18.42000*	3.97753	.004	8.6873	28.1527	
		After 7 days	After 24 hrs	-8.87000	3.97753	.067	-18.6027	.8627	
		After 14 days		9.55000	3.97753	.053	-.1827	19.2827	
		After 14 days	After 24 hrs	-18.42000*	3.97753	.004	-28.1527	-8.6873	
		After 7 days		-9.55000	3.97753	.053	-19.2827	.1827	

Potassium	LSD	After 24 hrs	After 7 days	-.66333	.89224	.485	-2.8466	1.5199
			After 14 days	-1.55333	.89224	.132	-3.7366	.6299
		After 7 days	After 24 hrs	.66333	.89224	.485	-1.5199	2.8466
			After 14 days	-.89000	.89224	.357	-3.0732	1.2932
		After 14 days	After 24 hrs	1.55333	.89224	.132	-.6299	3.7366
			After 7 days	.89000	.89224	.357	-1.2932	3.0732
Chloride	LSD	After 24 hrs	After 7 days	7.58333*	2.65330	.029	1.0909	14.0757
			After 14 days	13.94000*	2.65330	.002	7.4476	20.4324
		After 7 days	After 24 hrs	-7.58333*	2.65330	.029	-14.0757	-1.0909
			After 14 days	6.35667	2.65330	.054	-.1357	12.8491
		After 14 days	After 24 hrs	-13.94000*	2.65330	.002	-20.4324	-7.4476
			After 7 days	-6.35667	2.65330	.054	-12.8491	.1357
SOD	LSD	After 24 hrs	After 7 days	2.37667	1.18035	.091	-.5115	5.2649
			After 14 days	6.46333*	1.18035	.002	3.5751	9.3515
		After 7 days	After 24 hrs	-2.37667	1.18035	.091	-5.2649	.5115
			After 14 days	4.08667*	1.18035	.013	1.1985	6.9749
		After 14 days	After 24 hrs	-6.46333*	1.18035	.002	-9.3515	-3.5751
			After 7 days	-4.08667*	1.18035	.013	-6.9749	-1.1985
MDA	LSD	After 24 hrs	After 7 days	-.27667	.48191	.587	-1.4559	.9025
			After 14 days	-1.33000*	.48191	.033	-2.5092	-.1508
		After 7 days	After 24 hrs	.27667	.48191	.587	-.9025	1.4559

				After 14 days	-1.05333	.48191	.071	-2.2325	.1259
				After 14 days After 24 hrs	1.33000*	.48191	.033	.1508	2.5092
				After 7 days	1.05333	.48191	.071	-.1259	2.2325
HB	LSD	After 24 hrs	After 7 days	.66667	1.46566	.665	-2.9197	4.2530	
		After 14 days		1.66667	1.46566	.299	-1.9197	5.2530	
		After 7 days	After 24 hrs	-.66667	1.46566	.665	-4.2530	2.9197	
		After 14 days		1.00000	1.46566	.521	-2.5863	4.5863	
		After 14 days	After 24 hrs	-1.66667	1.46566	.299	-5.2530	1.9197	
		After 7 days		-1.00000	1.46566	.521	-4.5863	2.5863	
PCV	LSD	After 24 hrs	After 7 days	3.66667	3.86820	.380	-5.7985	13.1318	
		After 14 days		9.00000	3.86820	.059	-.4651	18.4651	
		After 7 days	After 24 hrs	-3.66667	3.86820	.380	-13.1318	5.7985	
		After 14 days		5.33333	3.86820	.217	-4.1318	14.7985	
		After 14 days	After 24 hrs	-9.00000	3.86820	.059	-18.4651	.4651	
		After 7 days		-5.33333	3.86820	.217	-14.7985	4.1318	
RBC	LSD	After 24 hrs	After 7 days	20.00000	32.72444	.564	-60.0738	100.0738	
		After 14 days		50.00000	32.72444	.177	-30.0738	130.0738	
		After 7 days	After 24 hrs	-20.00000	32.72444	.564	-100.0738	60.0738	
		After 14 days		30.00000	32.72444	.395	-50.0738	110.0738	
		After 14 days	After 24 hrs	-50.00000	32.72444	.177	-130.0738	30.0738	
		After 7 days		-30.00000	32.72444	.395	-110.0738	50.0738	

WBC	LSD	After 24 hrs	After 7 days	-400.00000	618.24123	.542	-1912.7818	1112.7818
			After 14 days	-1000.00000	618.24123	.157	-2512.7818	512.7818
		After 7 days	After 24 hrs	400.00000	618.24123	.542	-1112.7818	1912.7818
			After 14 days	-600.00000	618.24123	.369	-2112.7818	912.7818
		After 14 days	After 24 hrs	1000.00000	618.24123	.157	-512.7818	2512.7818
			After 7 days	600.00000	618.24123	.369	-912.7818	2112.7818

*. The mean difference is significant at the 0.05 level.

Appendix 2i: Group 3 Comparisons - Descriptive

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	After 24 hrs	3	80.1000	5.96516	3.44399	65.2817	94.9183	73.29	84.40
	After 7 days	3	77.3333	6.42910	3.71184	61.3626	93.3041	70.00	82.00

	After 14 days	3	76.6667	2.88675	1.66667	69.4956	83.8378	75.00	80.00
	Total	9	78.0333	4.87835	1.62612	74.2835	81.7832	70.00	84.40
AST	After 24 hrs	3	41.6667	6.02771	3.48010	26.6930	56.6403	36.00	48.00
	After 7 days	3	40.3333	6.11010	3.52767	25.1550	55.5117	35.00	47.00
	After 14 days	3	39.6667	4.04145	2.33333	29.6271	49.7062	36.00	44.00
	Total	9	40.5556	4.82470	1.60823	36.8470	44.2642	35.00	48.00
ALT	After 24 hrs	3	23.3333	5.03322	2.90593	10.8301	35.8366	18.00	28.00
	After 7 days	3	22.3300	4.87673	2.81558	10.2155	34.4445	18.73	27.88
	After 14 days	3	20.3333	6.08714	3.51441	5.2120	35.4546	15.60	27.20
	Total	9	21.9989	4.82612	1.60871	18.2892	25.7086	15.60	28.00
TBIL	After 24 hrs	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	After 7 days	3	.5000	.10000	.05774	.2516	.7484	.40	.60
	After 14 days	3	.4667	.11547	.06667	.1798	.7535	.40	.60
	Total	9	.4889	.07817	.02606	.4288	.5490	.40	.60
Urea	After 24 hrs	3	49.3333	2.51661	1.45297	43.0817	55.5849	47.00	52.00
	After 7 days	3	47.7267	10.27502	5.93229	22.2021	73.2512	39.09	59.09
	After 14 days	3	46.4100	4.89993	2.82898	34.2379	58.5821	41.70	51.48
	Total	9	47.8233	5.96551	1.98850	43.2378	52.4088	39.09	59.09
Creatinine	After 24 hrs	3	1.4667	.20817	.12019	.9496	1.9838	1.30	1.70
	After 7 days	3	1.4000	.20000	.11547	.9032	1.8968	1.20	1.60
	After 14 days	3	1.3667	.41633	.24037	.3324	2.4009	.90	1.70

	Total	9	1.4111	.25712	.08571	1.2135	1.6088	.90	1.70
Sodium	After 24 hrs	3	116.6733	22.04948	12.73027	61.8994	171.4473	92.87	136.40
	After 7 days	3	118.6600	5.32375	3.07367	105.4351	131.8849	112.52	121.99
	After 14 days	3	119.6633	7.88084	4.55000	100.0862	139.2404	111.51	127.24
	Total	9	118.3322	12.07866	4.02622	109.0477	127.6167	92.87	136.40
Potassium	After 24 hrs	3	5.6667	.63721	.36789	4.0838	7.2496	4.97	6.22
	After 7 days	3	5.4700	.46872	.27062	4.3056	6.6344	4.94	5.83
	After 14 days	3	5.5000	.43589	.25166	4.4172	6.5828	5.20	6.00
	Total	9	5.5456	.46082	.15361	5.1913	5.8998	4.94	6.22
Chloride	After 24 hrs	3	85.0000	5.38070	3.10655	71.6336	98.3664	79.02	89.45
	After 7 days	3	85.1800	3.75533	2.16814	75.8512	94.5088	81.18	88.63
	After 14 days	3	86.1600	5.45025	3.14670	72.6208	99.6992	79.98	90.28
	Total	9	85.4467	4.29909	1.43303	82.1421	88.7512	79.02	90.28
SOD	After 24 hrs	3	35.0200	1.62613	.93885	30.9805	39.0595	33.43	36.68
	After 7 days	3	36.3033	5.19217	2.99770	23.4053	49.2014	32.61	42.24
	After 14 days	3	37.8233	4.34591	2.50911	27.0275	48.6192	34.33	42.69
	Total	9	36.3822	3.68775	1.22925	33.5476	39.2169	32.61	42.69
MDA	After 24 hrs	3	4.4667	.95133	.54925	2.1034	6.8299	3.67	5.52
	After 7 days	3	4.6000	.50685	.29263	3.3409	5.8591	4.12	5.13
	After 14 days	3	4.2333	.48911	.28239	3.0183	5.4484	3.67	4.55
	Total	9	4.4333	.61329	.20443	3.9619	4.9047	3.67	5.52

HB	After 24 hrs	3	14.6667	1.15470	.66667	11.7982	17.5351	14.00	16.00
	After 7 days	3	15.0000	1.00000	.57735	12.5159	17.4841	14.00	16.00
	After 14 days	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00
	Total	9	15.0000	.86603	.28868	14.3343	15.6657	14.00	16.00
PCV	After 24 hrs	3	45.0000	4.35890	2.51661	34.1719	55.8281	42.00	50.00
	After 7 days	3	45.6667	2.08167	1.20185	40.4955	50.8378	44.00	48.00
	After 14 days	3	47.6667	2.51661	1.45297	41.4151	53.9183	45.00	50.00
	Total	9	46.1111	2.97676	.99225	43.8230	48.3993	42.00	50.00
RBC	After 24 hrs	3	316.0000	18.33030	10.58301	270.4650	361.5350	300.00	336.00
	After 7 days	3	314.0000	27.05550	15.62050	246.7904	381.2096	288.00	342.00
	After 14 days	3	317.0000	25.63201	14.79865	253.3266	380.6734	293.00	344.00
	Total	9	315.6667	20.80865	6.93622	299.6717	331.6616	288.00	344.00
WBC	After 24 hrs	3	5000.0000	400.00000	230.94011	4006.3449	5993.6551	4600.00	5400.00
	After 7 days	3	5200.0000	400.00000	230.94011	4206.3449	6193.6551	4800.00	5600.00
	After 14 days	3	5000.0000	200.00000	115.47005	4503.1725	5496.8275	4800.00	5200.00
	Total	9	5066.6667	316.22777	105.40926	4823.5925	5309.7408	4600.00	5600.00

Post Hoc Tests

Multiple Comparisons

Dependent	(I) TIME	(J) TIME	95% Confidence Interval
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Variable				Mean Difference (I- J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	After 24 hrs	After 7 days	2.76667	4.35252	.548	-7.8836	13.4169
			After 14 days	3.43333	4.35252	.460	-7.2169	14.0836
		After 7 days	After 24 hrs	-2.76667	4.35252	.548	-13.4169	7.8836
			After 14 days	.66667	4.35252	.883	-9.9836	11.3169
		After 14 days	After 24 hrs	-3.43333	4.35252	.460	-14.0836	7.2169
			After 7 days	-.66667	4.35252	.883	-11.3169	9.9836
AST	LSD	After 24 hrs	After 7 days	1.33333	4.47214	.776	-9.6096	12.2763
			After 14 days	2.00000	4.47214	.670	-8.9429	12.9429
		After 7 days	After 24 hrs	-1.33333	4.47214	.776	-12.2763	9.6096
			After 14 days	.66667	4.47214	.886	-10.2763	11.6096
		After 14 days	After 24 hrs	-2.00000	4.47214	.670	-12.9429	8.9429
			After 7 days	-.66667	4.47214	.886	-11.6096	10.2763
ALT	LSD	After 24 hrs	After 7 days	1.00333	4.37592	.826	-9.7042	11.7108
			After 14 days	3.00000	4.37592	.519	-7.7075	13.7075
		After 7 days	After 24 hrs	-1.00333	4.37592	.826	-11.7108	9.7042
			After 14 days	1.99667	4.37592	.664	-8.7108	12.7042
		After 14 days	After 24 hrs	-3.00000	4.37592	.519	-13.7075	7.7075
			After 7 days	-1.99667	4.37592	.664	-12.7042	8.7108
TBIL	LSD	After 24 hrs	After 7 days	.00000	.07201	1.000	-.1762	.1762

				After 14 days	.03333	.07201	.660	-.1429	.2095
				After 7 days After 24 hrs	.00000	.07201	1.000	-.1762	.1762
				After 14 days	.03333	.07201	.660	-.1429	.2095
				After 14 days After 24 hrs	-.03333	.07201	.660	-.2095	.1429
				After 7 days	-.03333	.07201	.660	-.2095	.1429
Urea	LSD	After 24 hrs	After 7 days		1.60667	5.49583	.780	-11.8411	15.0545
			After 14 days		2.92333	5.49583	.614	-10.5245	16.3711
		After 7 days	After 24 hrs		-1.60667	5.49583	.780	-15.0545	11.8411
			After 14 days		1.31667	5.49583	.819	-12.1311	14.7645
		After 14 days	After 24 hrs		-2.92333	5.49583	.614	-16.3711	10.5245
			After 7 days		-1.31667	5.49583	.819	-14.7645	12.1311
Creatinine	LSD	After 24 hrs	After 7 days		.06667	.23882	.790	-.5177	.6510
			After 14 days		.10000	.23882	.690	-.4844	.6844
		After 7 days	After 24 hrs		-.06667	.23882	.790	-.6510	.5177
			After 14 days		.03333	.23882	.894	-.5510	.6177
		After 14 days	After 24 hrs		-.10000	.23882	.690	-.6844	.4844
			After 7 days		-.03333	.23882	.894	-.6177	.5510
Sodium	LSD	After 24 hrs	After 7 days		-1.98667	11.31989	.866	-29.6854	25.7121
			After 14 days		-2.99000	11.31989	.801	-30.6888	24.7088
		After 7 days	After 24 hrs		1.98667	11.31989	.866	-25.7121	29.6854
			After 14 days		-1.00333	11.31989	.932	-28.7021	26.6954

After 14 days After 24 hrs				2.99000	11.31989	.801	-24.7088	30.6888
After 7 days				1.00333	11.31989	.932	-26.6954	28.7021
Potassium	LSD	After 24 hrs	After 7 days	.19667	.42576	.660	-.8451	1.2385
		After 14 days		.16667	.42576	.709	-.8751	1.2085
		After 7 days	After 24 hrs	-.19667	.42576	.660	-1.2385	.8451
		After 14 days		-.03000	.42576	.946	-1.0718	1.0118
		After 14 days	After 24 hrs	-.16667	.42576	.709	-1.2085	.8751
		After 7 days		.03000	.42576	.946	-1.0118	1.0718
Chloride	LSD	After 24 hrs	After 7 days	-.18000	4.02104	.966	-10.0191	9.6591
		After 14 days		-1.16000	4.02104	.783	-10.9991	8.6791
		After 7 days	After 24 hrs	.18000	4.02104	.966	-9.6591	10.0191
		After 14 days		-.98000	4.02104	.816	-10.8191	8.8591
		After 14 days	After 24 hrs	1.16000	4.02104	.783	-8.6791	10.9991
		After 7 days		.98000	4.02104	.816	-8.8591	10.8191
SOD	LSD	After 24 hrs	After 7 days	-1.28333	3.28261	.709	-9.3156	6.7489
		After 14 days		-2.80333	3.28261	.426	-10.8356	5.2289
		After 7 days	After 24 hrs	1.28333	3.28261	.709	-6.7489	9.3156
		After 14 days		-1.52000	3.28261	.660	-9.5523	6.5123
		After 14 days	After 24 hrs	2.80333	3.28261	.426	-5.2289	10.8356
		After 7 days		1.52000	3.28261	.660	-6.5123	9.5523
MDA	LSD	After 24 hrs	After 7 days	-.13333	.55801	.819	-1.4987	1.2321

				After 14 days	.23333	.55801	.690	-1.1321	1.5987
				After 7 days After 24 hrs	.13333	.55801	.819	-1.2321	1.4987
				After 14 days	.36667	.55801	.535	-.9987	1.7321
				After 14 days After 24 hrs	-.23333	.55801	.690	-1.5987	1.1321
				After 7 days	-.36667	.55801	.535	-1.7321	.9987
HB	LSD	After 24 hrs	After 7 days	-.33333	.76980	.680	-2.2170	1.5503	
			After 14 days	-.66667	.76980	.420	-2.5503	1.2170	
		After 7 days	After 24 hrs	.33333	.76980	.680	-1.5503	2.2170	
			After 14 days	-.33333	.76980	.680	-2.2170	1.5503	
		After 14 days	After 24 hrs	.66667	.76980	.420	-1.2170	2.5503	
			After 7 days	.33333	.76980	.680	-1.5503	2.2170	
PCV	LSD	After 24 hrs	After 7 days	-.66667	2.56760	.804	-6.9494	5.6160	
			After 14 days	-2.66667	2.56760	.339	-8.9494	3.6160	
		After 7 days	After 24 hrs	.66667	2.56760	.804	-5.6160	6.9494	
			After 14 days	-2.00000	2.56760	.466	-8.2827	4.2827	
		After 14 days	After 24 hrs	2.66667	2.56760	.339	-3.6160	8.9494	
			After 7 days	2.00000	2.56760	.466	-4.2827	8.2827	
RBC	LSD	After 24 hrs	After 7 days	2.00000	19.57890	.922	-45.9078	49.9078	
			After 14 days	-1.00000	19.57890	.961	-48.9078	46.9078	
		After 7 days	After 24 hrs	-2.00000	19.57890	.922	-49.9078	45.9078	
			After 14 days	-3.00000	19.57890	.883	-50.9078	44.9078	

After 14 days After 24 hrs				1.00000	19.57890	.961	-46.9078	48.9078
After 7 days				3.00000	19.57890	.883	-44.9078	50.9078
WBC	LSD	After 24 hrs	After 7 days	-200.00000	282.84271	.506	-892.0912	492.0912
		After 14 days		.00000	282.84271	1.000	-692.0912	692.0912
		After 7 days	After 24 hrs	200.00000	282.84271	.506	-492.0912	892.0912
		After 14 days		200.00000	282.84271	.506	-492.0912	892.0912
		After 14 days	After 24 hrs	.00000	282.84271	1.000	-692.0912	692.0912
		After 7 days		-200.00000	282.84271	.506	-892.0912	492.0912

Appendix 2j: Group 4 Comparisons - Descriptive

Descriptives

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALP	After 24 hrs	3	91.1000	6.39446	3.69184	75.2153	106.9847	86.49	98.40

	After 7 days	4	88.5000	5.00000	2.50000	80.5439	96.4561	82.00	94.00
	After 14 days	3	80.0000	2.00000	1.15470	75.0317	84.9683	78.00	82.00
	Total	10	86.7300	6.41595	2.02890	82.1403	91.3197	78.00	98.40
AST	After 24 hrs	3	52.6667	4.72582	2.72845	40.9271	64.4062	49.00	58.00
	After 7 days	4	48.0000	16.49242	8.24621	21.7569	74.2431	34.00	66.00
	After 14 days	3	45.6667	1.52753	.88192	41.8721	49.4612	44.00	47.00
	Total	10	48.7000	10.23122	3.23539	41.3810	56.0190	34.00	66.00
ALT	After 24 hrs	3	33.0000	5.00000	2.88675	20.5793	45.4207	28.00	38.00
	After 7 days	4	27.2500	6.42035	3.21018	17.0338	37.4662	18.20	32.22
	After 14 days	3	24.8667	3.05341	1.76289	17.2816	32.4518	21.90	28.00
	Total	10	28.2600	5.75748	1.82067	24.1413	32.3787	18.20	38.00
TBIL	After 24 hrs	3	.6000	.10000	.05774	.3516	.8484	.50	.70
	After 7 days	4	.5750	.09574	.04787	.4227	.7273	.50	.70
	After 14 days	3	.5333	.05774	.03333	.3899	.6768	.50	.60
	Total	10	.5700	.08233	.02603	.5111	.6289	.50	.70
Urea	After 24 hrs	3	57.6667	8.08290	4.66667	37.5876	77.7457	49.00	65.00
	After 7 days	4	53.0500	5.95958	2.97979	43.5670	62.5330	47.63	61.52
	After 14 days	3	52.0000	10.45802	6.03794	26.0208	77.9792	41.40	62.31
	Total	10	54.1200	7.54067	2.38457	48.7257	59.5143	41.40	65.00
Creatinine	After 24 hrs	3	1.7000	.00000	.00000	1.7000	1.7000	1.70	1.70
	After 7 days	4	1.6500	.12910	.06455	1.4446	1.8554	1.50	1.80

	After 14 days	3	1.5000	.17321	.10000	1.0697	1.9303	1.40	1.70
	Total	10	1.6200	.13984	.04422	1.5200	1.7200	1.40	1.80
Sodium	After 24 hrs	3	110.4167	8.03638	4.63980	90.4532	130.3801	101.25	116.25
	After 7 days	4	111.2500	7.23419	3.61709	99.7388	122.7612	102.40	120.12
	After 14 days	3	113.2133	7.02557	4.05621	95.7609	130.6658	106.24	120.29
	Total	10	111.5890	6.64482	2.10128	106.8356	116.3424	101.25	120.29
Potassium	After 24 hrs	3	6.1033	1.00600	.58081	3.6043	8.6024	4.95	6.80
	After 7 days	4	5.9700	.97423	.48712	4.4198	7.5202	4.76	6.97
	After 14 days	3	5.9500	.58949	.34034	4.4856	7.4144	5.45	6.60
	Total	10	6.0040	.78948	.24965	5.4392	6.5688	4.76	6.97
Chloride	After 24 hrs	3	81.2333	3.32861	1.92177	72.9646	89.5021	77.39	83.19
	After 7 days	4	81.0000	7.39888	3.69944	69.2267	92.7733	75.45	91.81
	After 14 days	3	82.2300	2.00007	1.15474	77.2615	87.1985	80.22	84.22
	Total	10	81.4390	4.68051	1.48011	78.0908	84.7872	75.45	91.81
SOD	After 24 hrs	3	28.3000	5.96165	3.44196	13.4904	43.1096	21.69	33.27
	After 7 days	4	29.7400	1.63030	.81515	27.1458	32.3342	27.70	31.60
	After 14 days	3	32.8400	4.93717	2.85047	20.5754	45.1046	27.14	35.78
	Total	10	30.2380	4.22135	1.33491	27.2182	33.2578	21.69	35.78
MDA	After 24 hrs	3	5.6933	.57012	.32916	4.2771	7.1096	5.23	6.33
	After 7 days	4	5.4025	.73781	.36890	4.2285	6.5765	4.64	6.39
	After 14 days	3	4.6200	.52735	.30447	3.3100	5.9300	4.23	5.22

	Total	10	5.2550	.72361	.22882	4.7374	5.7726	4.23	6.39
HB	After 24 hrs	3	14.0000	2.00000	1.15470	9.0317	18.9683	12.00	16.00
	After 7 days	4	14.0000	.81650	.40825	12.7008	15.2992	13.00	15.00
	After 14 days	3	14.6667	.57735	.33333	13.2324	16.1009	14.00	15.00
	Total	10	14.2000	1.13529	.35901	13.3879	15.0121	12.00	16.00
PCV	After 24 hrs	3	41.0000	4.35890	2.51661	30.1719	51.8281	38.00	46.00
	After 7 days	4	42.7500	7.27438	3.63719	31.1748	54.3252	36.00	53.00
	After 14 days	3	44.6667	2.51661	1.45297	38.4151	50.9183	42.00	47.00
	Total	10	42.8000	5.05085	1.59722	39.1868	46.4132	36.00	53.00
RBC	After 24 hrs	3	280.0000	24.97999	14.42221	217.9463	342.0537	252.00	300.00
	After 7 days	4	281.0000	24.73863	12.36932	241.6353	320.3647	252.00	312.00
	After 14 days	3	285.0000	23.89561	13.79613	225.6400	344.3600	264.00	311.00
	Total	10	281.9000	21.77894	6.88711	266.3203	297.4797	252.00	312.00
WBC	After 24 hrs	3	5600.0000	200.00000	115.47005	5103.1725	6096.8275	5400.00	5800.00
	After 7 days	4	5650.0000	378.59389	189.29694	5047.5726	6252.4274	5400.00	6200.00
	After 14 days	3	5400.0000	721.11026	416.33320	3608.6628	7191.3372	4800.00	6200.00
	Total	10	5560.0000	429.98708	135.97385	5252.4058	5867.5942	4800.00	6200.00

Post Hoc Tests

Multiple Comparisons

Dependent Variable (I) TIME (J) TIME							95% Confidence Interval	
				Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	After 24 hrs	After 7 days	2.60000	3.70561	.506	-6.1624	11.3624
			After 14 days	11.10000*	3.96146	.026	1.7326	20.4674
		After 7 days	After 24 hrs	-2.60000	3.70561	.506	-11.3624	6.1624
			After 14 days	8.50000	3.70561	.055	-.2624	17.2624
		After 14 days	After 24 hrs	-11.10000*	3.96146	.026	-20.4674	-1.7326
			After 7 days	-8.50000	3.70561	.055	-17.2624	.2624
AST	LSD	After 24 hrs	After 7 days	4.66667	8.49183	.600	-15.4133	24.7466
			After 14 days	7.00000	9.07814	.466	-14.4664	28.4664
		After 7 days	After 24 hrs	-4.66667	8.49183	.600	-24.7466	15.4133
			After 14 days	2.33333	8.49183	.791	-17.7466	22.4133
		After 14 days	After 24 hrs	-7.00000	9.07814	.466	-28.4664	14.4664
			After 7 days	-2.33333	8.49183	.791	-22.4133	17.7466
ALT	LSD	After 24 hrs	After 7 days	5.75000	4.00322	.194	-3.7161	15.2161
			After 14 days	8.13333	4.27962	.099	-1.9864	18.2530
		After 7 days	After 24 hrs	-5.75000	4.00322	.194	-15.2161	3.7161
			After 14 days	2.38333	4.00322	.570	-7.0828	11.8494
		After 14 days	After 24 hrs	-8.13333	4.27962	.099	-18.2530	1.9864
			After 7 days	-2.38333	4.00322	.570	-11.8494	7.0828

TBIL	LSD	After 24 hrs	After 7 days	.02500	.06719	.721	-.1339	.1839
			After 14 days	.06667	.07182	.384	-.1032	.2365
		After 7 days	After 24 hrs	-.02500	.06719	.721	-.1839	.1339
			After 14 days	.04167	.06719	.555	-.1172	.2005
		After 14 days	After 24 hrs	-.06667	.07182	.384	-.2365	.1032
			After 7 days	-.04167	.06719	.555	-.2005	.1172
Urea	LSD	After 24 hrs	After 7 days	4.61667	6.16412	.478	-9.9592	19.1925
			After 14 days	5.66667	6.58972	.418	-9.9156	21.2489
		After 7 days	After 24 hrs	-4.61667	6.16412	.478	-19.1925	9.9592
			After 14 days	1.05000	6.16412	.870	-13.5258	15.6258
		After 14 days	After 24 hrs	-5.66667	6.58972	.418	-21.2489	9.9156
			After 7 days	-1.05000	6.16412	.870	-15.6258	13.5258
Creatinine	LSD	After 24 hrs	After 7 days	.05000	.09574	.618	-.1764	.2764
			After 14 days	.20000	.10235	.092	-.0420	.4420
		After 7 days	After 24 hrs	-.05000	.09574	.618	-.2764	.1764
			After 14 days	.15000	.09574	.161	-.0764	.3764
		After 14 days	After 24 hrs	-.20000	.10235	.092	-.4420	.0420
			After 7 days	-.15000	.09574	.161	-.3764	.0764
Sodium	LSD	After 24 hrs	After 7 days	-.83333	5.66336	.887	-14.2251	12.5584
			After 14 days	-2.79667	6.05439	.658	-17.1130	11.5197
		After 7 days	After 24 hrs	.83333	5.66336	.887	-12.5584	14.2251

				After 14 days	-1.96333	5.66336	.739	-15.3551	11.4284
				After 14 days After 24 hrs	2.79667	6.05439	.658	-11.5197	17.1130
				After 7 days	1.96333	5.66336	.739	-11.4284	15.3551
Potassium	LSD	After 24 hrs	After 7 days	.13333	.68108	.850	-1.4772	1.7438	
		After 14 days		.15333	.72811	.839	-1.5684	1.8750	
		After 7 days	After 24 hrs	-.13333	.68108	.850	-1.7438	1.4772	
		After 14 days		.02000	.68108	.977	-1.5905	1.6305	
		After 14 days	After 24 hrs	-.15333	.72811	.839	-1.8750	1.5684	
		After 7 days		-.02000	.68108	.977	-1.6305	1.5905	
Chloride	LSD	After 24 hrs	After 7 days	.23333	4.02482	.955	-9.2839	9.7505	
		After 14 days		-.99667	4.30271	.823	-11.1710	9.1776	
		After 7 days	After 24 hrs	-.23333	4.02482	.955	-9.7505	9.2839	
		After 14 days		-1.23000	4.02482	.769	-10.7472	8.2872	
		After 14 days	After 24 hrs	.99667	4.30271	.823	-9.1776	11.1710	
		After 7 days		1.23000	4.02482	.769	-8.2872	10.7472	
SOD	LSD	After 24 hrs	After 7 days	-1.44000	3.26353	.672	-9.1570	6.2770	
		After 14 days		-4.54000	3.48886	.234	-12.7898	3.7098	
		After 7 days	After 24 hrs	1.44000	3.26353	.672	-6.2770	9.1570	
		After 14 days		-3.10000	3.26353	.374	-10.8170	4.6170	
		After 14 days	After 24 hrs	4.54000	3.48886	.234	-3.7098	12.7898	
		After 7 days		3.10000	3.26353	.374	-4.6170	10.8170	

MDA	LSD	After 24 hrs	After 7 days	.29083	.48643	.569	-.8594	1.4411
			After 14 days	1.07333	.52001	.078	-.1563	2.3030
		After 7 days	After 24 hrs	-.29083	.48643	.569	-1.4411	.8594
			After 14 days	.78250	.48643	.152	-.3677	1.9327
		After 14 days	After 24 hrs	-1.07333	.52001	.078	-2.3030	.1563
			After 7 days	-.78250	.48643	.152	-1.9327	.3677
HB	LSD	After 24 hrs	After 7 days	.00000	.94281	1.000	-2.2294	2.2294
			After 14 days	-.66667	1.00791	.529	-3.0500	1.7167
		After 7 days	After 24 hrs	.00000	.94281	1.000	-2.2294	2.2294
			After 14 days	-.66667	.94281	.502	-2.8961	1.5627
		After 14 days	After 24 hrs	.66667	1.00791	.529	-1.7167	3.0500
			After 7 days	.66667	.94281	.502	-1.5627	2.8961
PCV	LSD	After 24 hrs	After 7 days	-1.75000	4.17749	.688	-11.6282	8.1282
			After 14 days	-3.66667	4.46592	.439	-14.2269	6.8936
		After 7 days	After 24 hrs	1.75000	4.17749	.688	-8.1282	11.6282
			After 14 days	-1.91667	4.17749	.660	-11.7949	7.9615
		After 14 days	After 24 hrs	3.66667	4.46592	.439	-6.8936	14.2269
			After 7 days	1.91667	4.17749	.660	-7.9615	11.7949
RBC	LSD	After 24 hrs	After 7 days	-1.00000	18.76610	.959	-45.3748	43.3748
			After 14 days	-5.00000	20.06181	.810	-52.4386	42.4386
		After 7 days	After 24 hrs	1.00000	18.76610	.959	-43.3748	45.3748

After 14 days				-4.00000	18.76610	.837	-48.3748	40.3748
After 14 days After 24 hrs				5.00000	20.06181	.810	-42.4386	52.4386
After 7 days				4.00000	18.76610	.837	-40.3748	48.3748
WBC	LSD	After 24 hrs	After 7 days	-50.00000	359.39764	.893	-899.8404	799.8404
		After 14 days		200.00000	384.21224	.619	-708.5176	1108.5176
		After 7 days	After 24 hrs	50.00000	359.39764	.893	-799.8404	899.8404
		After 14 days		250.00000	359.39764	.509	-599.8404	1099.8404
		After 14 days	After 24 hrs	-200.00000	384.21224	.619	-1108.5176	708.5176
		After 7 days		-250.00000	359.39764	.509	-1099.8404	599.8404

*. The mean difference is significant at the 0.05 level.

Appendix 2k: Group 5 Comparisons - Descriptive

Descriptives

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum

ALP	After 24 hrs	3	84.3500	5.88125	3.39554	69.7402	98.9598	78.00	89.61
	After 7 days	4	83.0000	9.30949	4.65475	68.1865	97.8135	72.00	94.00
	After 14 days	3	78.0000	2.00000	1.15470	73.0317	82.9683	76.00	80.00
	Total	10	81.9050	6.71363	2.12304	77.1024	86.7076	72.00	94.00
AST	After 24 hrs	3	48.6667	4.72582	2.72845	36.9271	60.4062	45.00	54.00
	After 7 days	4	46.0000	5.16398	2.58199	37.7830	54.2170	40.00	52.00
	After 14 days	3	41.3333	3.05505	1.76383	33.7442	48.9225	38.00	44.00
	Total	10	45.4000	5.01553	1.58605	41.8121	48.9879	38.00	54.00
ALT	After 24 hrs	3	26.3333	5.13160	2.96273	13.5857	39.0809	22.00	32.00
	After 7 days	4	23.5000	5.85072	2.92536	14.1902	32.8098	18.00	31.69
	After 14 days	3	21.6667	2.37136	1.36910	15.7759	27.5574	19.70	24.30
	Total	10	23.8000	4.71254	1.49024	20.4288	27.1712	18.00	32.00
TBIL	After 24 hrs	3	.5667	.11547	.06667	.2798	.8535	.50	.70
	After 7 days	4	.5250	.05000	.02500	.4454	.6046	.50	.60
	After 14 days	3	.5000	.00000	.00000	.5000	.5000	.50	.50
	Total	10	.5300	.06749	.02134	.4817	.5783	.50	.70
Urea	After 24 hrs	3	54.6667	8.32666	4.80740	33.9821	75.3512	48.00	64.00
	After 7 days	4	51.7525	8.59346	4.29673	38.0784	65.4266	41.27	62.25
	After 14 days	3	48.3400	7.82316	4.51671	28.9062	67.7738	42.11	57.12
	Total	10	51.6030	7.76604	2.45584	46.0475	57.1585	41.27	64.00
Creatinine	After 24 hrs	3	1.6333	.11547	.06667	1.3465	1.9202	1.50	1.70

	After 7 days	4	1.5500	.12910	.06455	1.3446	1.7554	1.40	1.70
	After 14 days	3	1.4000	.10000	.05774	1.1516	1.6484	1.30	1.50
	Total	10	1.5300	.14181	.04485	1.4286	1.6314	1.30	1.70
Sodium	After 24 hrs	3	114.7800	2.00192	1.15581	109.8069	119.7531	112.50	116.25
	After 7 days	4	116.7800	9.18843	4.59421	102.1592	131.4008	105.16	127.64
	After 14 days	3	117.3233	4.33936	2.50533	106.5438	128.1029	112.36	120.40
	Total	10	116.3430	5.86830	1.85572	112.1451	120.5409	105.16	127.64
Potassium	After 24 hrs	3	5.7900	.59195	.34176	4.3195	7.2605	5.11	6.19
	After 7 days	4	5.6700	.51942	.25971	4.8435	6.4965	5.19	6.37
	After 14 days	3	5.6500	.63836	.36856	4.0642	7.2358	4.95	6.20
	Total	10	5.7000	.51214	.16195	5.3336	6.0664	4.95	6.37
Chloride	After 24 hrs	3	84.6767	1.89740	1.09547	79.9633	89.3901	82.69	86.47
	After 7 days	4	85.5500	7.83791	3.91896	73.0781	98.0219	74.53	92.90
	After 14 days	3	86.6700	3.30927	1.91061	78.4493	94.8907	84.20	90.43
	Total	10	85.6240	4.93736	1.56133	82.0920	89.1560	74.53	92.90
SOD	After 24 hrs	3	31.5867	.96173	.55526	29.1976	33.9757	30.48	32.22
	After 7 days	4	32.8250	2.98844	1.49422	28.0697	37.5803	29.42	36.14
	After 14 days	3	35.3500	1.85518	1.07109	30.7415	39.9585	33.58	37.28
	Total	10	33.2110	2.53340	.80113	31.3987	35.0233	29.42	37.28
MDA	After 24 hrs	3	5.0733	.72418	.41810	3.2744	6.8723	4.24	5.55
	After 7 days	4	4.9475	.58762	.29381	4.0125	5.8825	4.24	5.52

	After 14 days	3	4.4200	.41797	.24132	3.3817	5.4583	3.95	4.75
	Total	10	4.8270	.59360	.18771	4.4024	5.2516	3.95	5.55
HB	After 24 hrs	3	14.3333	.57735	.33333	12.8991	15.7676	14.00	15.00
	After 7 days	4	14.2500	.50000	.25000	13.4544	15.0456	14.00	15.00
	After 14 days	3	15.0000	1.00000	.57735	12.5159	17.4841	14.00	16.00
	Total	10	14.5000	.70711	.22361	13.9942	15.0058	14.00	16.00
PCV	After 24 hrs	3	43.6667	3.51188	2.02759	34.9427	52.3907	40.00	47.00
	After 7 days	4	44.2500	3.59398	1.79699	38.5312	49.9688	41.00	49.00
	After 14 days	3	45.6667	1.52753	.88192	41.8721	49.4612	44.00	47.00
	Total	10	44.5000	2.87711	.90982	42.4418	46.5582	40.00	49.00
RBC	After 24 hrs	3	291.3333	26.10236	15.07021	226.4915	356.1752	262.00	312.00
	After 7 days	4	295.0000	23.40940	11.70470	257.7504	332.2496	268.00	324.00
	After 14 days	3	300.0000	30.19934	17.43560	224.9807	375.0193	272.00	332.00
	Total	10	295.4000	23.43881	7.41200	278.6329	312.1671	262.00	332.00
WBC	After 24 hrs	3	5400.0000	200.00000	115.47005	4903.1725	5896.8275	5200.00	5600.00
	After 7 days	4	5450.0000	341.56503	170.78251	4906.4938	5993.5062	5000.00	5800.00
	After 14 days	3	5200.0000	400.00000	230.94011	4206.3449	6193.6551	4800.00	5600.00
	Total	10	5360.0000	309.83867	97.97959	5138.3548	5581.6452	4800.00	5800.00

Post Hoc Tests

Multiple Comparisons

Dependent Variable (I) TIME (J) TIME							95% Confidence Interval	
				Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
ALP	LSD	After 24 hrs	After 7 days	1.35000	5.30077	.806	-11.1843	13.8843
			After 14 days	6.35000	5.66676	.299	-7.0498	19.7498
		After 7 days	After 24 hrs	-1.35000	5.30077	.806	-13.8843	11.1843
			After 14 days	5.00000	5.30077	.377	-7.5343	17.5343
		After 14 days	After 24 hrs	-6.35000	5.66676	.299	-19.7498	7.0498
			After 7 days	-5.00000	5.30077	.377	-17.5343	7.5343
AST	LSD	After 24 hrs	After 7 days	2.66667	3.45607	.466	-5.5056	10.8390
			After 14 days	7.33333	3.69470	.088	-1.4032	16.0699
		After 7 days	After 24 hrs	-2.66667	3.45607	.466	-10.8390	5.5056
			After 14 days	4.66667	3.45607	.219	-3.5056	12.8390
		After 14 days	After 24 hrs	-7.33333	3.69470	.088	-16.0699	1.4032
			After 7 days	-4.66667	3.45607	.219	-12.8390	3.5056
ALT	LSD	After 24 hrs	After 7 days	2.83333	3.72610	.472	-5.9775	11.6442
			After 14 days	4.66667	3.98337	.280	-4.7525	14.0858
		After 7 days	After 24 hrs	-2.83333	3.72610	.472	-11.6442	5.9775
			After 14 days	1.83333	3.72610	.638	-6.9775	10.6442
		After 14 days	After 24 hrs	-4.66667	3.98337	.280	-14.0858	4.7525

			After 7 days	-1.83333	3.72610	.638	-10.6442	6.9775
TBIL	LSD	After 24 hrs	After 7 days	.04167	.05336	.460	-.0845	.1678
			After 14 days	.06667	.05704	.281	-.0682	.2016
		After 7 days	After 24 hrs	-.04167	.05336	.460	-.1678	.0845
			After 14 days	.02500	.05336	.654	-.1012	.1512
		After 14 days	After 24 hrs	-.06667	.05704	.281	-.2016	.0682
			After 7 days	-.02500	.05336	.654	-.1512	.1012
Urea	LSD	After 24 hrs	After 7 days	2.91417	6.34175	.660	-12.0817	17.9100
			After 14 days	6.32667	6.77961	.382	-9.7046	22.3579
		After 7 days	After 24 hrs	-2.91417	6.34175	.660	-17.9100	12.0817
			After 14 days	3.41250	6.34175	.607	-11.5833	18.4083
		After 14 days	After 24 hrs	-6.32667	6.77961	.382	-22.3579	9.7046
			After 7 days	-3.41250	6.34175	.607	-18.4083	11.5833
Creatinine	LSD	After 24 hrs	After 7 days	.08333	.08975	.384	-.1289	.2956
			After 14 days	.23333*	.09595	.045	.0064	.4602
		After 7 days	After 24 hrs	-.08333	.08975	.384	-.2956	.1289
			After 14 days	.15000	.08975	.139	-.0622	.3622
		After 14 days	After 24 hrs	-.23333*	.09595	.045	-.4602	-.0064
			After 7 days	-.15000	.08975	.139	-.3622	.0622
Sodium	LSD	After 24 hrs	After 7 days	-2.00000	4.99130	.701	-13.8026	9.8026
			After 14 days	-2.54333	5.33593	.648	-15.1608	10.0741

		After 7 days	After 24 hrs	2.00000	4.99130	.701	-9.8026	13.8026
			After 14 days	-.54333	4.99130	.916	-12.3459	11.2592
		After 14 days	After 24 hrs	2.54333	5.33593	.648	-10.0741	15.1608
			After 7 days	.54333	4.99130	.916	-11.2592	12.3459
Potassium	LSD	After 24 hrs	After 7 days	.12000	.44019	.793	-.9209	1.1609
			After 14 days	.14000	.47058	.775	-.9728	1.2528
		After 7 days	After 24 hrs	-.12000	.44019	.793	-1.1609	.9209
			After 14 days	.02000	.44019	.965	-1.0209	1.0609
		After 14 days	After 24 hrs	-.14000	.47058	.775	-1.2528	.9728
			After 7 days	-.02000	.44019	.965	-1.0609	1.0209
Chloride	LSD	After 24 hrs	After 7 days	-.87333	4.21704	.842	-10.8451	9.0984
			After 14 days	-1.99333	4.50821	.672	-12.6536	8.6669
		After 7 days	After 24 hrs	.87333	4.21704	.842	-9.0984	10.8451
			After 14 days	-1.12000	4.21704	.798	-11.0917	8.8517
		After 14 days	After 24 hrs	1.99333	4.50821	.672	-8.6669	12.6536
			After 7 days	1.12000	4.21704	.798	-8.8517	11.0917
SOD	LSD	After 24 hrs	After 7 days	-1.23833	1.72060	.495	-5.3069	2.8302
			After 14 days	-3.76333	1.83940	.080	-8.1128	.5862
		After 7 days	After 24 hrs	1.23833	1.72060	.495	-2.8302	5.3069
			After 14 days	-2.52500	1.72060	.186	-6.5936	1.5436
		After 14 days	After 24 hrs	3.76333	1.83940	.080	-.5862	8.1128

			After 7 days	2.52500	1.72060	.186	-1.5436	6.5936
MDA	LSD	After 24 hrs	After 7 days	.12583	.45038	.788	-.9392	1.1908
			After 14 days	.65333	.48148	.217	-.4852	1.7919
		After 7 days	After 24 hrs	-.12583	.45038	.788	-1.1908	.9392
			After 14 days	.52750	.45038	.280	-.5375	1.5925
		After 14 days	After 24 hrs	-.65333	.48148	.217	-1.7919	.4852
			After 7 days	-.52750	.45038	.280	-1.5925	.5375
HB	LSD	After 24 hrs	After 7 days	.08333	.53359	.880	-1.1784	1.3451
			After 14 days	-.66667	.57044	.281	-2.0155	.6822
		After 7 days	After 24 hrs	-.08333	.53359	.880	-1.3451	1.1784
			After 14 days	-.75000	.53359	.203	-2.0117	.5117
		After 14 days	After 24 hrs	.66667	.57044	.281	-.6822	2.0155
			After 7 days	.75000	.53359	.203	-.5117	2.0117
PCV	LSD	After 24 hrs	After 7 days	-.58333	2.38193	.814	-6.2157	5.0490
			After 14 days	-2.00000	2.54639	.458	-8.0213	4.0213
		After 7 days	After 24 hrs	.58333	2.38193	.814	-5.0490	6.2157
			After 14 days	-1.41667	2.38193	.571	-7.0490	4.2157
		After 14 days	After 24 hrs	2.00000	2.54639	.458	-4.0213	8.0213
			After 7 days	1.41667	2.38193	.571	-4.2157	7.0490
RBC	LSD	After 24 hrs	After 7 days	-3.66667	20.06379	.860	-51.1100	43.7767
			After 14 days	-8.66667	21.44909	.698	-59.3857	42.0524

				After 7 days	After 24 hrs	3.66667	20.06379	.860	-43.7767	51.1100
					After 14 days	-5.00000	20.06379	.810	-52.4433	42.4433
				After 14 days	After 24 hrs	8.66667	21.44909	.698	-42.0524	59.3857
					After 7 days	5.00000	20.06379	.810	-42.4433	52.4433
WBC	LSD	After 24 hrs	After 7 days	-50.00000	250.00000	.847	-641.1561	541.1561		
			After 14 days	200.00000	267.26124	.479	-431.9724	831.9724		
		After 7 days	After 24 hrs	50.00000	250.00000	.847	-541.1561	641.1561		
			After 14 days	250.00000	250.00000	.351	-341.1561	841.1561		
		After 14 days	After 24 hrs	-200.00000	267.26124	.479	-831.9724	431.9724		
			After 7 days	-250.00000	250.00000	.351	-841.1561	341.1561		

*. The mean difference is significant at the 0.05 level.