EVALUATION OF THE BLOOD PRESSURE LOWERING ACTIVITY OF *ACALYPHA TORTA* (EUPHORBIACAE)

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE UNIVERSITY OF NIGERIA, NSUKKA, FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D) IN PHARMACOLOGICAL BIOCHEMISTRY

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APPROVAL

This work is an original investigation of the candidate Mrs Ezekwesili, C.N. and has not to the best of our knowledge been published in any journal or submitted in part or full for the award of any other diploma or degree of this or any other university. This thesis has been approved for the award of the degree of DOCTOR OF PHILOSOPHY in Pharmacological Biochemistry in the Department of Biochemistry of the University of Nigeria, Nsukka.

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DEDICATION

I dedicate this work to my GOD and my loving family – Mr C.C. Ezekwesili (my God given husband), Chinenye, Chukwudi, Chukwuebuka, Ifeoma and Akachukwu Ezekwesili

ABSTRACT

In this work the blood pressure lowering activity of *Acalypha torta* leaves was evaluated. Differential extraction using chloroform: methanol (2:1), ethanol and water as solvents yielded four extracts – upper methanol layer, 3.8% (w/w), lower chloroform layer, 5.3% (w/w), ethanol, 2.3% (w/w) and water, 3.8% (w/w) extracts. In anaesthetized normotensive cats only the ethanol extract produced a significant ($p<0.0001$) and dose-dependent fall in blood pressure. This action of the ethanol extract was antagonized by muscarinic receptor blocker (atropine, 20 µg/kg/body weight) and histamine blocker (promethazine, 20 µg/kg/ body weight). Results of acute toxicity studies with 200-8000 mg/kg. body weight of extract given i.p gave a median lethal dose (LD_{50}) of 562.30 mg/kg. body weight. The extract had no significant ($p<0.50$) effect on the levels of alanine transaminase and alkaline phosphatase at all the sub-actute doses (<50% LD_{50}) tested but dose-dependently increased the activity of aspartate transaminase. Histopathological changes were noted in the liver, spleen, brain, heart, kidney and lung. These included hepatocyte necrosis, follicular disorganization, inflammation, fibrosis and bronchial dilatation. The degree and frequency of these lesions indicated that the extract caused mild histopathological changes on these organs. The ethanol extract had vasodilatory effect by increasing the rate of flow of physiological fluid through the rat hind-quarters preparation by approximately 31%. It also antagonized adrenaline-induced contraction of isolated rabbit aortic strips showing possible relaxatory effect of the extract on vascular smooth muscles. The results also revealed that the extract had no effect on heart rate but reduced the force of contraction of the isolated rabbit heart by 71%. $CaCl_2$- induced contraction of the heart and platelet aggregation were significantly ($p<0.0001$) antagonized at 5.0mg/ml of the extract. Findings after 21-day treatment with 10 and 20mg/kg body weight of extract also showed significant ($p<0.0001$) reductions in the levels of serum triacylglycerol, total cholesterol and LDL-cholesterol within 14 days of treatment, whereas HDL-cholesterol level was increased. Increases in LDL-cholesterol level were observed in the treated groups after the 14th day, while HDL–cholesterol decreased. The concentrations of serum electrolytes (Na^+, K^+, Cl^- and HCO^-_3) were not affected. Analyses for the minerals of the ethanol extract of *A. torta* leaves revealed that they contained, (mg per 100 g) -Na (61.5± 0.96), K (354.0 ± 0.86), Ca (79.2 ± 1.29), Mg (68.8 ± 0.04), Cl^- (0.4 ± 0.02), HCO^-_3 (12.0 ± 0.04), CO_3^- (2.3 ± 0.05) and

SO_4^{2-} (0.2 ± 0.01). Bioactive substances such as flavonoids, cardiac glycosides, tannins, sterols and alkaloids were detected in whole *A. torta* leaves and the ethanol extract. Proximate analysis of the leaves gave moisture (42.0 ± 0.06 %), crude fibre (11.0 ± 0.01 %), crude protein (7.9 ± 0.02%), crude fat (20.3 ± 0.01%) and total carbohydrate (28.8 ± 0.02 %), whereas the ethanol extract contained moisture (69.7 ± 0.05 %), crude fibre (4.6 ± 0.02%), crude protein (2.9 ± 0.01 %), crude fat (13.6 ± 0.01 %) and total carbohydrate (3.1 ± 0.03%). Column chromatographic separation using silica gel (G60-200) as the stationary phase and chloroform: butanol; xylene (2:2:3) as the eluent yielded 67 fractions (1-67). Further elution with distilled water gave fractions 68-89. Thin-layer chromatography, concentrated the number of fractions to 13, A to M, when fractions with similar Rf valves were pooled together. The effects of each fraction (A- M) on blood pressure were tested in anaesthetized normotensive cat and only fraction L (77-80) caused a reduction in the blood pressure. Qualitative phytochemical study of fraction L also showed the presence of high levels of flavonoids, and cardiac glycosides, with traces of alkaloids and tannins. The LD_{50} of fraction L was found to be 1000 mg/kg. body weight.

Thus, ethanol extract of *A. torta* leaves possesses blood pressure lowering activity that may be mediated by dilation of the blood vessels and, or by depression of force of contraction of the heart.

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LIST OF ABBREVIATIONS

MAP	-	Mean Arterial Pressure
CO	-	Cardiac Output
TPR	-	Total Peripheral Resistance
ACTH	-	Adrenocorticotrophic hormone
ANP	-	Atrial Natriuretic peptide
ADH	-	Anti-diuretic hormone
ACE	-	Agiotensin- converting enzyme
ATI	-	Angiotensin I receptor
ALT	-	Alanine transaminase
AST	-	Aspartate transaminase
ALP	-	Alkaline phosphatase
TG	-	Triacylglycerol
CH	-	Cholesterol
PL	-	Phospholipids
FFA	-	Free fatty acids
CM	-	Chylomicrons
VLDL	-	Very – low-density – lipoproteins
LDL	-	Low density – lipoproteins
HDL	-	High – density – lipoproteins
LCAT	-	Lecittin – cholesteryl acyltransferase
LD_{50}	-	Median lethal dose
H.E	-	Haematoxylin and eosin
CMC	-	Carboxy methylcellulose
EE	-	Ethanol extract
WE	-	Water extract
UE	-	Upper layer extract

CHAPTER ONE: LITERATURE REVIEW

1.1 Introduction

The risks of raised blood pressure have been determined from several large-scale epidemiological surveys and hypertension has clearly been demonstrated to be a leading risk factor for stroke, coronary heart disease, heart failure and renal failure (Klag *et al.*, 1997; Hoel and Howard, 1997; Peggy, 1983; Afolabi, 2000 and Oladipo, 2000). It usually tends to cluster with other atherogenic risk factors such as hyperlipidaemia, glucose intolerance, obesity and hyperinsulinaemia which not only promote its occurrence, but also greatly influence its impact on cardiovascular diseases (Alper *et al.*, 2000).

However, the extent of damage caused by hypertension on the heart, vascular system and other target organs is a product of the degree of pressure elevation and duration of elevation (Materson, 1987). Severe hypertension induces these abnormalities within months to a few years. In contrast, mild hypertension may require decades in order to achieve measurable effects.

Antihypertensive therapy is therefore aimed at reducing morbidity and mortality due to fatal or nonfatal cardiovascular event and to prolong life, particularly in older persons, who comprise the majority of hypertensive patients. Over the past 50 years, there has been a progressive decline in cardiovascular mortality world wide, much of which is attributable to better control of blood pressure (Titilola, 2000). Surprisingly these dramatic improvements have recently declined, probably as a result of the reduction in the efficacy of the pharmaceutical agents and the resultant poor management of disease symptoms.

Although many drugs with proven pharmacological activity are available clinically to ameliorate cases of individuals with hypertension and other associated clinical symptoms (Brown and Goldstein, 1992), herbal medicines are growing in popularity in both developing and the developed world. In Africa, for example, up to 80% of the population depends on them, according to WHO estimates (Aschwanden, 2001). Also in India where the traditional Ayurvedic medicine employs over 1200 different herbs, herbal medicine is regularly used by about 65% of the population. It is estimated that 50% of Canadians, 75% of people in France and 85% of doctors in Japan utilize alternative

medicine, which often includes herbal remedies (Aschwanden, 2001). These herbal remedies offer a more widely available and more affordable alternative to pharmaceutical drugs (Marin-Bettolo, 1980).

Herbal remedies however natural, can cause serious illnesses, from allergy to liver or kidney malfunction, to cancer, ulcer, blindness and even death (Aschwanden, 2001). Perhaps the biggest problems with herbal medicines are a lack of standardization and of safety regulations (Green, 2001).

However, medicinal plants still represent a great deal of untapped reservoir of drugs and the structural diversity of hundreds of chemical constituents of these plants make a valuable source of novel lead compounds (Farnsworth, 1989). Therefore the growing popularity of these remedies is fuelled not only by the fact that many people believe they are safer, more natural than pharmaceuticals but also by increasing scientific interest in these herbal medicines. Natural product scientists are now intensifying efforts towards scientific evaluation of medicinal plants used in traditional remedies.

The *Euphorbiacae* is a great polymorphic family comprising a large number of plants of great economic importance. Nutritionally, the most useful as food are the *Manihot* genera. In the tropics, the non-poisonous tuberous roots of *Manihot palmata* may be eaten raw or cooked while the poisonous species, *Manihot utillisima* is usually cooked before eating as *tapioca*, cassava flour, garri and most recently, as cassava bread. Some of the genera are of medicinal values. For instance, the largest genera Euphorbia, typified by *Euphobia esula*, are sources of purgatives (Castor oil and croton oil), *Euphorbia hyberna* and *Euphorbia corollata* are antisyphillis and antiemetic drugs respectively (Bailey, 1960).

Acalypha torta belongs to the subfamily of *Euphorbiacae* known as *Acalypha* , a genus of over 200 species of shrubs distributed over the warmer regions of the world (Chittenden, 1956). Although *A. torta* serves as ornamental plant, its leaves are used in Nigeria for the management of hypertension (Mofunanya, Personal Communication). Inhibitory activities of the *Acalypha genus* against microorganisms have been reported (Hiremath.*et al.*, 1993; Alade and Irobi, 1993). The efficacy of *Acalypa torta* leaves as antibacterial has also been well documented in the literature (Caceres *et al.*, 1993 and

Irobi and Banso, 1994). This plant has also been found to possess antifungal and antimalarial actions (Irobi and Banso, 1994). The effects of leaf extracts of *Acalypha torta* on blood pressure were investigated using experimental animal models and plausible mode of action of the active extract studied to determine the claim that *A. torta* is an effective antihypertensive agent.

1.2 LITERATURE REVIEW

1.2.1 Hypertension

Hypertension (high blood pressure) is defined as a systolic blood pressure equal to or greater than 140 mmHg and or a diastolic pressure of 90 mmHg or above (Afolabi, 2000, Oladipo, 2000).

Using the diastolic pressure ranges hypertension may be categorized as mild (95-104), moderate (105-114) or severe (115 mmHg and above) (Nwodo, 2000). Hypertension has been proved to be the main risk factor in the development of such cardiovascular diseases as stroke, congestive heart failure, arteriosclerosis, renal insufficiency and myocardial infarction (Siegal, 1977; Peggy, 1983; Afolabi, 2000; Oladipo, 2000).

Cardiovascular disease and hypertension, in particular, is the major cause of increased morbidity and mortality in the developed countries and if not adequately checked the prevalence may be higher for the developing world (Aschwanden, 2001). Report of epidemiological survey conducted by the committee on non-communicable diseases in Nigeria revealed that over 3.33 million Nigerians aged 15years and above are hypertensive, with diastolic pressure of 95 mmHg and above. Consideration of diastolic blood pressure range of 90-94 mmHg (border line hypertension) increased the figure to 4.6 million. It was also recorded that approximately 20% of the adult population in Western industrialized countries are hypertensive (Bp>140/90 mmHg).

1.2.1.1 Classification

Hypertension is classified into two categories namely:

I. **Primary or Essential Hypertension:** This is defined as blood pressure elevation usually associated with ageing for which there is no obvious or evident initiating pathology. The rise in blood pressure is considered to be

progressive and incurable and account for the great bulk (over 95%) of cases of hypertension encountered clinically (Akubue, 2000).

II. **Secondary Hypertension:** The name secondary hypertension is restricted to blood pressure elevation resulting from renal or endocrine disease, aortic coarctation, pregnancy and certain drugs. The condition may be exacerbated by such dietary factors (e.g high salt intake) and obesity; this type of hypertension accounts for about 5% of hypertension cases. (Akubue, 2000; Guthrie, 1983). The kidneys secrete a number of substances that in a variety of ways (autocrine, paracrine or endocrine) are involved in the pathogenesis of hypertension (Afolabi, 2000).

Hypertension may also arise from imbalances between the vasoconstrictor and vasodilator mechanisms which include the renin-angiotensin system, renal prostaglandins, medullipin, platelet- activating factor and the kallikrein – kinin system.

1.2.1.2 The Concept of Human Blood Pressure Regulation and Causes of Hypertension

The regulation and maintenance of normal human blood pressure is a multifactorial process involving both physiological and biochemical mechanisms. Specifically, the human arterial blood pressure is a product of cardiac output and peripheral resistance (Oates and Brown , 2001) i.e

Mean Arterial Pressure (MAP) = Cardiac Output (CO)x
Total Peripheral Resistance (TPR)

The systolic blood pressure is maintained by the cardiac output (quantity of blood pumped by the heart per minute) which is a function of the force and rate of contraction of the heart, and the elasticity and distensibility of the conducting arteries, whereas the diastolic pressure depends on the total peripheral resistance (TPR) i.e. the resistance in the walls of the arterioles (Oates and Brown, 2001). These physiological processes involved in the regulation of blood pressure are acting under the influence of the complex biochemical mechanisms which may be categorized as Nervous or Humoral mechanisms.

Nervous Mechanism: In the nervous mechanisms, stimuli from both external and internal environment (such as higher cerebral centres, chemoreceptors and viscera) could cause the sympathetic nervous system to release its transmitters (adrenaline and noradrenaline). The adrenal medulla is also stimulated to release its own noradrenaline. Consequently, these transmitter substances by binding to and stimulating their β-adrenergic receptors cause increased vasoconstriction.

Humoral Mechanisms: The humoral mechanism is mainly concerned with renal sodium handling which plays a crucial role in the pathophysiology of hypertension (Guyton, 1991). This involves certain body hormones which actively contribute in the maintenance of plasma volume and cardiac output. These hormones include;

i. Anterior pituitary hormone- adrenocorticotrophic hormone (ACTH)
ii. Posterior pituitary hormone – Antidiuretic hormone (ADH)
iii. Mineralocorticoid (aldosterone) released from the adrenal cortex
iv. Plasma angiotensins II and III
v. Adrenal cortex corticosteroids which potentiate other pressor agents, and
vi. Atrial natriuretic peptide from the atrium (ANP).

Osmoreceptor cells in the hypothalamus monitor blood osmolarity through the pituitary peptide, antidiuretic hormone (vasopressin).

These sensors are also connected to the behavioural systems controlling thirst and salt craving (Afolabi, 2000).The action of ADH, on the other hand, is modulated by stretch receptors in the right atrium and the arterial tree which monitor total blood volume. Thus, there exists an interaction between the control of blood volume and blood osmolarity and this is where several other hormones are involved. Stretching the right atrium stimulates the release of atrialnatriuretic peptide (ANP), which promotes sodium excretion by the kidney, ultimately reducing blood volume and salt content.

Conversely, low salt concentration in the distal convoluted tubule, or inadequate perfusion of the kidney, both stimulate release of the enzyme renin from the renal tubular cells into the blood stream. This protease with short half-life cleaves the circulating protein, angiotensinogen (produced by the hepatocytes) to produce angiotensin I (AI). This is in turn broken down by the lung endothelial enzyme angiotensin-converting enzyme (ACE) to yield the more effective pressor substance named angiotensin II (AII)

(Akinola and Akinwale, 2000). When bound to ATI receptors present in the vascular tissue, AII exerts powerful vasoconstrictor effect on arteriolar smooth muscles thereby raising systemic blood pressure and restores kidney perfusion. Stimulation of ATI receptors in the adrenal cortex by angiotensin II causes vasoconstriction and production of the mineralocorticoid, aldosterone which increases sodium and water reabsorbtion from the kidney by binding to specific receptors in the kidney.

Increased activity of any of these mechanisms (involved in the regulation) would lead to increased vasoconstriction i.e. increased arteriolar tone with concomitant increase in the blood pressure. In otherwords, by expanding plasma volume and increasing cardiac output in the absence of any response to the normal negative feedback controls, these abnormalities ultimately result in the development of hypertension (Lifton, 1996).

Nervous **humoral**

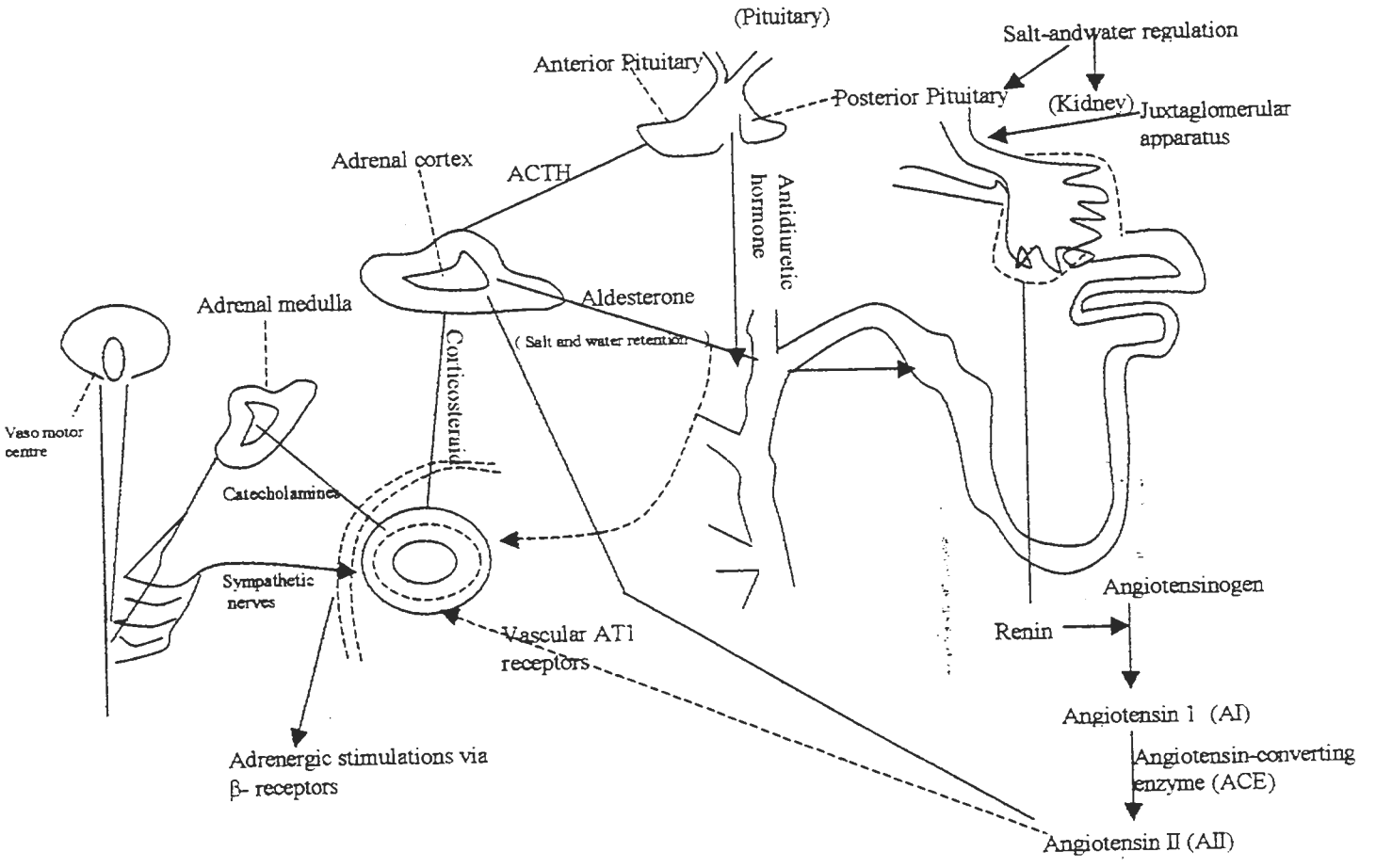

Fig 1: Regulation and maintenance of normal human blood pressure

1.2.1.2.1 Endocrine- Causes of Essential Hypertension

1. Renin – Angiotensin – Aldosterone:

Over activity of the renin-angiotensin-aldosterone system accounts for good percentage of secondary or essential hypertension and may arise from a variety of factors which include.

i. Overproduction of angiotensin due to renovascular disease, coarctation of the aorta or renal parenchyma disease which accounts for a significant number of cases of essential hypertension.

In women, cases of essential hypertension (about 0.2 - 1.0%) are due to over production of angiotensin by oestrogen containing pills. Oestrogens have been reported to cause a rise in blood pressure by enhancing hepatic synthesis and release of angiotensinogen into the plasma, ultimately leading to an increase in plasma level of angiotensin II and consequently aldosterone mediated fluid retention. Oestrogens also modulate the arterial wall smooth muscle tone (Akinola and Akinwale, 2000).

ii. Overproduction of Renin: Essential hypertension may also arise from excessive production of renin which may be of renal origin (juxtaglomerular cell tumours) or extra renal via the lung, urogenital and pancreatic cancers.

1.2.1.2.2 Mineralocorticoid Mediated Hypertension:

In this case, elevated levels of aldosterone and other mineralocorticoids but low level of renin are seen. These can be caused by several conditions including

i. Primary aldosteronism or Conn's syndrome
ii. Cushing's syndrome
iii. Congenital adrenal hyperplasia (due to 11-beta, and 17-α-hydroxylase deficiencies).
iv. Glucocorticoid resistance.

Excessive release of glucocorticoid into the system causes hypertension through a variety of mechanisms such as

i. Sodium shift to the extracellular space and consequent increase in plasma volume.
ii. Increased peripheral vascular resistance

iii. Increased cardiac output

iv. Increased production of renin – substrate.

1.2.1.2.3 Volume-Mediated Hypertension: This type of secondary hypertension may occur in Liddle's syndrome, Gordon's syndrome or in acromegaly.

In Liddle's syndrome there is a primary increase in collecting tubule reabsoption of sodium, increased potassium excretion leading to hypertension and hypokalaemia. The defect involves the sodium channels in the collecting tubules. These sodium channels are sensitive to aldosterone and amiloride. Aldosterone causes the channels to open thereby increasing sodium reabsorption whereas amiloride closes the channels (Blotero - Velez *et al.*, 1994).

In Gordon's syndrome there is increased reabsorption of chloride ion from the renal tubules causing hyperchloraemic metabolic acidosis.

Acromegaly results from increased secretion of growth hormone which triggers increased reabsorption and expansion of the extracellular space.
Also associated with this condition is increased sympathetic activity which also contributes to the hypertension.

1.2.1.2.4 Catecholamine – Mediated- Hypertension

This is hypertension induced by increased release of the catecholamines into the plasma. It may be caused by two conditions

i. Phaeochromocytomas (i.e tunour of the chromaffin tissue of adrenal medula). This tumour may be benign or malignant and causes secretion of noradrenaline, adrenaline, and rarely dopamine.

ii. Neuroblastomas (tumour of the sympathetic nervous system seen in children). They synthesize and release large amounts of catecholamine precursors and metabolites.

1.2.1.2.5 Miscellaneous: Miscellaneous causes of endocrine hypertension include hypercalcaemia (because calcium is one of the mediators of vascular smooth muscle contraction), hypoparathyroidism (parathyroid hormone receptors have vasodilatory effect), and hyperthyroidism. The mechanisms underlying the hypertensive effect of

hyperthyroidism are still unclear but are believed to be related to the inability of the vascular tree to accommodate the increased cardiac output, as well as the potentiation of catecholamine action by excess thyroxine. The action of thyroxine is linked to an increase in the number of beta-adrenergic receptors in some tissues, including the heart.

1.2.1.3 Therapeutics of Hypertension

Depending on the severity of the disease, treatment of hypertension may involve the non-pharmacological and, or pharmacological therapy.

1.2.1.3.1 Non- Pharmacological Approach

The goal of reducing blood pressure is to reduce morbidity and mortality from hypertension related target organ damage and the methods utilized in accomplishing this goal should not in themselves increase the risks for morbidity and mortality. The pressor effects of smoking cigarettes (Sharp *et al.*, 1990), excessive alcohol intake (Gary *et al.*, 1983; Criqui *et al.*, 1989; Moore *et al.*, 1990 and Klag *et al.*, 1990), caffeine use (Robertson *et al.*, 1978; Do-Un and Dimsdale, 1990 and Sharp and Benowitz, 1990), lack of physical activity (Bunker *et al.*, 1990), and high- salt consumption (Bulpitt, 1982; Khaw and Barrette-Conor, 1990 and Luft *et al.*, 1990) have been well documented. High fat diets (Peggy and George, 1983) and mental stress (Akubue, 2000 and Schnall *et al.*, 1990) have also been reported to contribute significantly to blood pressure elevations.

Non-pharmacological approach to therapy therefore involves modification of the patients life-style in order to achieve the desired goal. This usually entails complete abstinence from tobacco smoking, reduced intake of salt and high –dietary fats, (saturated fats and cholesterol), avoidance of alcohol, avoidance of excessive stress, maintenance of ideal body weight, reasonable physical exercise and adequate intake of potassium and perhaps magnesium (Final Report of the Subcommittee on Nonpharmacological Therapy, 1986).

This therapeutic model was mostly applied in the treatment of individuals with borderline hypertension (mean diastolic blood pressure in the range of 90-94 mmHg). But recently, it has been recommended that irrespective of whether drug treatment is eventually needed, all patients should be treated with nonpharmacologic therapy (Materson, 1987).

1.2.1.3.2 Pharmacological Therapy

Several pharmacologically active agents are now clinically available for effective management of hypertension. These drugs are classified into different categories based on their site and, or mechanism of actions as follows:

1.2.1.3.2.1 Diuretics

Diuretic drugs are those agents that promote urine secretion and alter Na^+ balance. Three classes of diuretic drugs now in use include thiazides, loop diuretics and potassium ion (K^+) sparing diuretics. Diuretics also reduce vascular resistance by decreasing interstitial fluid volume, smooth muscle Na^+ concentration, and intracellular Ca^{2+} concentration, thus reducing total peripheral resistance (Oates and Brown, 2001).

1.2.1.3.2.2 Sympatholytic Agents

These agents oppose the actions of the sympathetic nervous system resulting in overall reduction in the concentration of circulating catecholamines particularly adrenaline and noradrenaline. The sympatholytic drugs comprise the following: centrally acting agents such as α-methyldopa, and clonidine: adrenergic neuron blocking agents e.g guanadrel and reserpine: β-adrenergic antagonist e.g propranolol and metoprolol: α-adrenergic antagonists such as prazosin, phenoxybenzamine and phentolamine: mixed adrenergic antagonists e.g labetalol and carveditol.

1.2.1.3.2.3 Vasodilators

Vasolidators are vasoactive preparations that cause increases in the lumen of blood vessels and therefore increase the blood flow through these vessels with consequent fall in the blood pressure. There are two classes of vasodilators namely, arterial vasodilators e.g hydralazine and minoxidil: and arterial and venous vasodilators e.g niroprusside (Katzung, 1995).

1.2.1.3.2.4 Ca^{2+} Channel Blockers

Since intracellular free Ca^{2+} ions play crucial role in vascular smooth muscle contractility, inhibition of transmembrane Ca^{2+} ion movement by some agents have been found to cause reduction in blood pressure by relaxing arteriolar smooth muscle and

decreasing peripheral vascular resistance. Examples are verapamil, nifedipine and nimodipine (Oaetes and Brown, 2001; Lehmann *et al.* 1983).

1.2.1.3.2.5 Angiotensin- Converting Enzyme (ACE) Inhibitors

ACE inhibitors impair the synthesis of angiotensin II by inhibiting the action of renin the enzyme catalyzing the conversion of the angiotensin I to angiotensin II. Examples of available ACE inhibitors are captopril, analapril, benazepril and ramipril.

1.2.1.3.2.6 Angiotensin II – Receptor Antagonists

These are the non-peptide antagonists of angiotensin II receptors that are developed for clinical use. They include losartan, valartan and candesartan.

1.2.2 Low Blood Pressure

Low blood pressure (hypotension) is an abnormal condition characterized by blood pressure low enough to cause symptoms, such as dizziness and fainting (Berkow, 1999). Low blood pressure is commonly caused by overdose of certain medications such as antihypertensives, anti-anxiety agents, some antidepressants and narcotic analgesics. A steep fall in blood pressure may manifest in multiple-drug therapy of hypertension due to extensive depression of the vasopressor mechanisms

When the blood pressure is too low, the blood cannot supply enough oxygen and nutrients to the cells and properly remove wastes from them.

1.2.3 Euphorbiacae

Euphorbiacae is a great polymorphic family of over 207 genera and 4,000 species. These plants are widely distributed mainly in the tropics but some are also found in temperate regions. Morphologically, plants under this family vary greatly in size ranging from small herbs to shrubs or trees, with alternate, fleshy or cactus- like leaves that exude milky juice when cut. The flowers may be conspicuous or inconspicuous, monoecious or dioecious, regular or irregular. During fruiting, the fruits split into three portions leaving a central column and most often these fruits are dehiscent and berry – like, bearing albuminous seeds.

The family is of great economic importance. For instance, in medicine, the exudates of *Euphorbia esula. E. cyparissias, E. lathyras* etc are *purgatives; E. hyberna, Jatropha efficinalis*, *Croton* and *Stillinga sylvatica* are used for the treatment of syphilis, *E. corollata, E. Ipe cacuanhae* are emetic whereas *E. thymifolia* is used as a vermifuge (Bailey, 1960). Nutritionally, the most useful as food are the *Manihot* genera. In the tropics, the non-poisonous tuberous roots of *Manihot palmata* may be eating raw or cooked while the poisonous specie *Manihot utilisima* (bitter manioc) must be cooked before eating. This popular root is widely consumed as tapioca, cassava flour, or garri and as the most recently discovered cassava bread. The juice of *E. balcamifera*, is cooked and eaten as jelly and the popular almond seeds are from *Aleurites triloba.*

Over twenty genera of *Euphorbiacae* are cultivated purposely for ornamentals in homes and botanical gardens. These include *Acalypha, Aleurites, cadiaeum, Euphorbia and Ricirus* (Bailey and Bailey, 1959)

Fig. 1: Pictorial Representation of *Acalypha torta*

1.2.4 *Acalypha torta*

1.2.4.1 Morphological Description, Occurrence and Distribution

Acalypha torta is a medium sized ornamental garden herb or shrub with neither fragrance nor poisonous parts. It has thick, woody and green leafy stem. The plant has lengthy branched nodes which are alternately arranged in spiral form around the stem (Dutta, 1995). Axillary buds, give rise to the branches or greenish, inconspicuous and clustered flowers. The leaves are also adorned with serrated white margin (Chittenden, 1956).

A fully grown plant bears about 5 to 20 leaves on a branch and some appear yellowish sometimes due to such environmental factors as duration of sunlight and nutrient availability. The height of a mature plant is more than 2.7 m with a stem diameter of about 5-8 cm. It has a characteristic dicotyledonous taproot system with distinct arrangement of vascular tissue shown by a soft foamy tissue on viewing its transverse section (Dutta, 1995).

In seasonal occurrence, *Acalypha torta* is a perennial, evergreen plant since it does not shed all its leaves with the advent of the dry season. It emerges at the beginning of the rainy season and peaks in mid- May to Mid – June (Viggiani, 1995).

Although *Acalypha torta* is commonly found in West Africa, it is however not a local plant but an introduced breed. It is also largely found in Eastern and Southern parts of Nigeria, Ghana, Cameroun and Togo.

1.2.4.2 Medicinal Applications of *Acalypha Torta*

The leaves of *Acalypha torta*, commonly known as onuede (Igbo) and Me' itche (Hausa) have been alleged to possess diverse medicinal properties. In Igbo speaking areas of Nigeria, for instance, the leaves are used for the treatment of malaria, rheumatism, high blood pressure, chest pain and stomach upset. It is also used as a blood tonic. In such preparations the normal local extraction procedures ranging from boiling, maceration and chewing are adopted (Mofunanya, Personal communication). In the Yoruba speaking areas, water extract of *A. torta* has been used for decades for treating skin problems such as dermatitis and infanfile eczema (nla) (Jekayinfa *et al.*, 1997). Irobi and Banso (1994), reported the efficacy of alcoholic (ethanol & menthol) extracts of powdered *Acalypha torta* leaves against some anaerobic bacteria (*Clostridium perfringens, C. chauvoei,*

Bacteroides melaninogenicus and *B. fragilis.* The antifungal and antimalarial properties of the plant have also been reported (Irobi and Banso, 1994).

1.2.5 Pharmacologically Active Substances

The pharmacological or medicinal properties of many plants are attributed to their chemical constituents which include alkaloids, flavonoids, glycosides, saponins, tannins, sterols and triterpenes (Harborne, 1984 and Siems *et al.*, 1996).

1.2.5.1 Alkaloids

The alkaloids are naturally occurring amines (nitrogen-containing compounds), which are widely distributed throughout the plant kingdom. Most if not all, alkaloids exhibit basic properties and may be insoluble or sparingly soluble in water, but their salts are highly soluble in aqueous solvent. Alkaloids are known to possess appreciable pharmacological properties (Leung, 1980). For instance, quinine and quinidine from *Cinchona ledgeriana* have cardiac depressant properties, (Farnsworth, 1973) with quinidine being twice as active as quinine. Quinine also has antimalarial activity; berbamine, isotetrandine and hydroxyacanthine from *Beberis vulgaris* have hypotensive properties (Naidovich *et al.*, 1976). Likewise berberine from golden seal, aspidospermine and aspidosamine from white quebracho; and yarrow alkaloids from *Achillea millefolium* are reported to have blood pressure lowering activity (Preininger, 1975, Lyon, 1973, Kudrzycka- Bieloszabska and Glowniak, 1966).

1.2.5.2 Flavonoids

These are the largest group of naturally occurring phenols. They occur both in the free state and as glycosides. Structurally, flavonoids are 15-carbon compounds consisting of two aromatic rings A and B and an oxygen containing heterocyclic ring C. They are classified into several groups based on the oxidation level of ring C, as chalcones, flavanones, flavones, isoflavones and flavonols (collectively known as anthocyanin pigments).

Apart from the contribution of anthocyanins to flower colour and attraction of pollinators, some flavonoid compounds have been well recognized as antifungal, antiallergic, anti-inflammatory and antimicrobial agents (Ibrahim, 2002).

Some flavonoid compounds function as scavengers of free radicals and in the protection against uv radiation. They play important roles in plant growth and development, inhibiting the activity of certain enzymes and acting as regulators of hormonal transport (Dixon and Paiva, 1995). Certain flavonoids have also been implicated in plant-insect interactions, in which they act as feeding stimulants or feeding deterrants (Harborne and Grayer, 1994).

1.2.5.3 Glycosides

Glycosides are plant secondary metabolites containing a carbohydrate component linked by an ether bond to a noncarbohydrate nucleus (aglycone). Often the aglycone is released by the action of hydrolytic enzymes, which are separated from the glycosides in plant tissue, but the enzymes and glycosides come into intimate contact only when the plant tissue is disrupted.

The most important glycosides in humans and animals include saponins, cyanogenic glycosides, cardiac glycosides, tannins, glucosinolates and glycoalkaloids (Cheeke, 2002).

1.2.5.3.1 Cardiac Glycosides

Cardiac glycosides have a steroid aglycone nucleus, with a lactone group on the aglycone. They are commonly referred to as cardenolides because of their toxic effects on the heart. These compounds inhibit (Na^+ - K^+) ATPase in heart muscle, thereby

elevating Na^+ and intracellular Ca^{2+} via Na^+ - Ca^{2+} exchange. The increase in calcium concentration causes a more forceful contraction of the myocardium (Cheeke, 2002).

1.2.5.3.2 Cyanogenic Glycosides

Cyanogenic glycosides are glycosides of a sugar or sugars, with the aglycone containing cyanide. When they are hydrolysed by enzymic action they release HCN. HCN is a potent toxin, inhibiting the terminal respiratory enzymes cytochrome oxidase (Nelson and Cox, 2000). All plants produce cyanide, but in so-called cyanogenic plants a much higher concentration of cyanide occurs. Three main cyanogenic glycosides are amygdalin (found in almonds, and apples), linamarin (cassava and lima beans) and dhurrin. Inhibition of cytochrome oxidase blocks ATP formation and the tissues suffer energy deprivation.

1.2.5.4 Saponins

Saponins have surfactant or detergent properties because they form colloidal aqueous solutions that foam on shaking. They contain a lipid – soluble aglycone, linked by an ether bond to one or more water soluble carbohydrate side–chains. They have been employed as mild expectorants, anti-viral and anti-inflammatory agents for the treatment of rheumatoid arthritis. Some saponins are also known for their anthiulcerogenic activities (William, (1996).

1.2.5.5 Tannins

Tannins are naturally occurring non-volatile plant polyphenols with molecular weights greater than 500. Generally they appear as polymers of flavonoid units linked together by carbon-to – carbon bonds. They are heat- stable and possess astringent properties. Tannins interact with proteins and carbohydrates to form enzyme-resistant substrates. In addition, tannins interact with enzymes themselves causing interference with the digestibility of nutritional substances such as proteins (Deshpande and Salunkhe, 1982; Griffiths, 1986). Studies on the toxic effects of tannins also showed that these phytochemicals have damaging effect on the intestinal mucosa (Mitijavila *et al.*, 1977) and interfere with the absorption of iron (Garcia- Lopez *et al.*, 1990), glucose (Wetch *et al.*, 1989) and vitamine B_{12} (Carrera *et al.*, 1973).

On the other hand, tannins have been shown to have astringent and antitumour activities (Fong *et al.*, 1972; Bate-Smith, 1973). They also exhibit antiviral (Kucera and Herrman, 1967; Aizenman *et al.*, 1968) and antimicrobial (Sokolova *et al.*, 1969) properties.

1.2.6 The Heart

The heart is a roughly cone-shaped hollow muscular organ weighing about 255 g in women but heavier in men. It is located within the thoracic cavity in between the lungs, and a little more to the left than right. The heart is composed of three layers of tissues. The pericardium is a flexible, stretchable, two-layered sac that envelops the heart and functions to keep the heart in position, prevents the heart from overfilling with blood and protects it from chest infections. The myocardium is composed of specialized muscle tissue known as cardiac muscle (found exclusively in the heart), while the endocardium is a thin, smooth and glistening membrane consisting of flattened epithelial cells continuous with the lining of the blood vessels (Berkow *et al.*, 1997).

The primary functions of the heart are to supply oxygen and nutrients to the body and to rid the body of waste products. It is divided into a right and left sides by a partition of muscular tissue and endocardium known as septum and each side is further divided into an upper and a lower chamber by a valve (the right and left atrioventricular valves). These valves ensure unidirectional flow of blood from the upper chamber or atrium to the lower chamber or ventricle. Thus the heart has four chambers, the right and left atria and the right and left ventricles (Wilson, 1981).

During circulation, deoxygenated, carbondioxide-rich blood from the body flows through the two largest veins (the superior and inferior vena cavae) into the right atrium. When this chamber fills, it propels the blood into the right ventricle, which when full pumps the blood through the pulmonary valve into the pulmonary arteries that supply blood to the lungs. The blood then flows through tiny capillaries surrounding the air sacs in the lungs, releasing carbondioxide and absorbing oxygen, which is then exhaled. The oxygen-rich blood now flows through the pulmonary veins into the right atrium. This circuit is known as the pulmonary circulation. When the right atrium fills, the oxygen- rich blood is pumped into the left ventricle from where the blood is propelled through the aortic valve into the aorta, the largest artery in the body. The oxygen rich blood is then circulated to all parts of the body except the lungs (Berkow *et al.*, 1999).

1.2.7 The Vasculature

Blood vessels can be divided into five separate classes; arteries, arterioles, capillaries, venules and veins. The function of the vasculature is to transport blood to all tissues in order to supply oxygen and nutrients and to remove carbon dioxide and other waste products. The blood is also involved in heat distribution and temperature regulation. For the blood to perform these functions effectively, it must be supplied at an appropriate pressure. The blood vessels are therefore required to channel the flow of blood to those organs where the demand is greatest, inorder to maintain appropriate pressures under a range of physiological conditions. Arteries and arterioles have thick, smooth-muscle walls with elastic properties which are necessary in the dampening of the blood pressure surges resulting from the pulsatile nature of the cardiac output. Constriction of arteries and arterioles is also important in directing blood flow and blood pressure control as well. The degree of fluid and solute exchange through the capillaries is governed by the pressure of blood within them.

The primary function of the veins and venules is to transport deoxygenated blood from the organs and tissues back to the heart. Venoconstriction, therefore, causes increase in the pressure within the capillaries, thus increasing the movement of fluids into the tissues, with increase in the pressure of blood returning to the heart (venous return), and subsequent increases in cardiac output (Gard, 2001).

1.2.8 Cardiac Muscle

This kind of muscle is found exclusively in the heart. The cells (fibres) are about 14μm in diameter in a normal adult human heart, and are of varying lengths but are shorter than the smooth muscle fibres. Branches from each fibre fuse with similar branches from neighbouring cells and each has a central barrel shaped nucleus like the striped muscles (found in a skeletal muscle). The cardiac muscles have myofibrils and myofilaments and in spite of this, they are involuntary which means that they cannot be contracted at will. The fibres are arranged longitudinally and are surrounded by small amounts of connective tissue, a network of blood capillaries and has large number of mitochondria in its sarcoplasm (Majumdar, 1980).

There are three major types of cardiac muscle atrial muscle, ventricular muscle and specialized excitatory and conductive muscle fibres. Atrial and ventricular types of muscle have identical contraction pattern as the skeletal muscle except for the longer duration of contraction. In contrast, the specialized excitatory and conductive muscle fibres contract only feebly because they contain few contractile fibrils, but their contraction is usually rapid and therefore provides an excitatory and transmission system for rapid conduction of the cardiac excitatory signal throughout the heart (Jewel, 1978).

1.2.9 Smooth Muscles

This unstriped or involuntary muscle type forms the muscular sheets in the walls of stomach, intestine, blood vessels, uterus, urinary bladder, trachea etc. Smooth muscle fibres are long cells tapering at both ends. Commonly they are 20 to 200 μm long and 3-8 μm in diameter. The arrangement of the myofibrils and myofilaments in smooth muscle sarcoplasm is different from that found in the striped muscle fibres.

Each smooth muscle fibre is surrounded by a layer of glycoprotein. The cells have no sarcolemma, but a thin outer covering of cell membrane is usually present. At many points the cell membranes are in contact with each other forming numerous gap junctions through which ions flow freely from one cell to another (Majumdar, 1980; Gabella, 1984).

1.2.10 Excitation – Contraction Coupling Role of Calcium

Calcium ion, Ca^{2+}, is involved in the initiation of contraction of smooth muscle. Ca^{2+} enters through the voltage gate Ca^{2+} channels. In the smooth muscle, unlike skeletal muscle, it is necessary that the myosin be phosphorylated in order to activate ATPase. In other words, the contraction of vertebrate smooth muscle is regulated by phosphorylation of myosin. Once inside the cell cytosol, Ca^{2+} binds to calmodulin, and the resulting Ca^{2+}- calmodulin complex activates myosin light chain kinase. The activated myosin light chain kinase then catalyzes the phosphorylation of myosin.

At this stage, actin slides on myosin to produce contraction. Energy required for the contraction is provided by ATP hydrolysis to ADP. To cause relaxation of the smooth muscle contractile elements, it is necessary to remove the calcium ions. This removal is

achieved by a calcium pump that pumps the Ca^{2+} out of the smooth muscle fibre into the extracellular fluid or into the sarcoplasmic reticulum-Phosphoryl groups introduced by myosin light chain kinase are removed from myosin by a Ca^{2+}- independent phosphatase. This is mediated by cyclic adenosine monophosphate (cAMP). Contraction of smooth muscle is more sustained than that of skeletal muscle because this dephosphorylation is slower than the lowering of the cytosolic Ca^{2+} level of active transport by the ion into the sarcoplasmic reticulum (Stryer 1988).

1.2.11 Na^+ - K^+ ATPase

In most animals, high concentration of sodium ion (Na^+) and low concentration of K^+ are found in the extracellular medium whereas high concentration of potassium ion (K^+) and low concentration of Na^+ are found in the intracellular compartment.

These ionic gradients are generated by a specific transport system known as the Na^+ - K^+ pump. The active transport of Na^+ and K^+ is of great physiological significance in that the Na^+ - K^+ gradient generated in animal controls cell volume, renders nerves and muscle cells electrically excitable and also drives the active transport of sugars and amino acids. In a resting animal over 30% of the expended ATP is utilized to pump these ions.

Further research by Jens Skou leds to the discovery of an enzyme that hydrolyzes ATP only when Na^+ and K^+ are present, in addition to Mg^{2+} which is needed by all ATPases. This enzyme was therefore named the Na^+- K^+ ATPase and it is an integral part of the Na^+ - K^+ pump.

1.2.12 Transaminases

The transaminases constitute a group of enzymes which catalyse the interconversion of aminoacids and alpha-keto acids by transfer of amino groups. They are distributed in many organs but most abundantly in liver, heart and skeletal muscle. Several aminotransferases have been reported but alanine aminotransaferase (also called glutamate-pyruvate transaminase, GPT), E.C. 2.6.1.2,12 and aspartate aminotransferase (also called glutamate-oxaloacetate transaminase, GOT), E.C. 2.6.1.1 are important in the diagnosis of liver and heart damage or disease caused by exposure to toxic drugs and chemicals (Nsirim, 1999; Nelson and Cox, 2000). The activity of the transaminases is

localized both in the mitochondrial and soluble cytoplasmic fractions and there are good indications that the soluble enzymes and the mitochondrial enzymes are different proteins. For example, the soluble and mitochondrial aspartate transaminase (AST) have many different properties (Sheid *et al.,* 1965). The soluble and mitochondrial alanine transaminase (ALT) also appear to be different proteins with different pH–activity curves and the mitochondrial enzyme is less stable and has lower K_m than the soluble enzyme (Swick *et al.,* 1965.

Another interesting aspect of the aminotransferases is that the enzymes attacking the various aminoacids are not equally distributed between mitochondria and the soluble fraction (Kenney, 1962 and Litwack *et al.,* 1963).

1.2.13 Alkaline Phosphatases

These enzymes are classified as Alkaline phosphatases (E.C. 3.1.3.1) because of their ability to participate in hydrolase/ transferase reaction on a wide variety of phosphate containing compounds at alkaline pH.

The abundance of alkaline phosphatases in nature, including bacteria, plants and animals have been reported (McComb *et al.,* 1979) and this indicates that these enzymes are involved in fundamental biochemical processes. In man, high levels of alkaline phosphatase have been demonstrated in liver, bone, kidney, placenta and intestine and low but significant levels were found in other tissues (Torgny, 1984). They are plasma membrane enzymes, composed of monomers with molecular weights between 40 kd and 75 kd and the active enzymes are dependent on Zn^{2+} and Mg^{2+} ions.

1.2.14 Lipoproteins

The major lipids found in the human plasma are free fatty acids (FFA), triacylglycerols (TG), cholesterol (CH), and phospholipids (PL). Some of these lipids such as the saturated and unsaturated free fatty acids, cholesterol and phospholipids are also important constituents of the cellular membranes. The hydrophobic nature of these lipids demands that they be transported in the plasma bound with proteins. While most of the free fatty acids are carried by albumins, the other lipids circulate in the plasma as lipid-protein complexes known as the lipoproteins (Nelson and Cox, 2000).

The proteins that are found in lipoproteins (known as apoproteins) vary in their molecular characteristics and are therefore of different types denoted by A, B,C, D, and E based on their structure and function.

Liporoteins are classified in order of increasing density as chylomicrons (CM), very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL), low density-lipoprotein (LDL) and high density lipoprotein (HDL). Chylomicrons contain triacylglycerols (85%), cholesterol (7%), phospholipids (6%) and apoproteins A,B,C and E (2%); VLDL contains triacylglycerols (60%), cholesterol (15%), phospholids (15%) and apoproteins B,C and E (10%); LDL contains TG(10%), CH (45%), PL (20%) and apoproteins B (25%); whereas HDL is composed of TG (3%), CH (20%), PL (27%) and apoproteins A,C and E (50%) (Nsirim, 1999).

Low density lipoprotein (LDL) is a small lipoprotein and accounts for most of the cholesterol in plasma. Because of its small size it can infiltrate tissues such as the arterial wall. In addition, LDL has proliferative effects on vascular smooth muscle cells and therefore may play a role in the pathogenesis of atherosclerosis and hypertension (Sachinidis *et al.*, 1989).

On entering the cell, it is slowly metabolized releasing the cholesterol part in the tissue. Thus cholesterol carried by LDL as LDL-cholesterol is called bad cholesterol because it increases the risk of a heart attack, arteriosclerosis and coronary artery disease(Goldstein *et al.*, 1973 and Keys, 1975).

Conversely, HDL derives cholesterol from tissue cells and other lipoproteins and esterifies it through the action of the enzyme lecithin-cholesteryl acyl transferase (LCAT). The HDL which is now rich in cholesteryl esters is taken up by the liver where the cholesterol enters the liver cholesterol pool leaving a small amount in the core of HDL. HDL-cholesterol, therefore lowers the risk of heart attack and is beneficial.

1.2.15 Rationale

Hypertension is prevalent not only in the Western or industrialized nations (Burt *et al.*, 1995) but also in the developing countries including Nigeria (Oladipo, 2000) and this disorder will continue to grow in importance as the population ages and developing

nations become more urbanized and industrialized. The clinical complications of hypertension could be diminished and life prolonged when the arterial blood pressure is lowered and maintained within the normal systolic/diastolic range by antihypertensive agents.

Herbal remedies are growing in popularity and scientific evidence of efficacy of these herbs is beginning to emerge from controlled preclinical and clinical trials. And of course, a number of commonly used pharmaceutical products are of botanical origin-aspirin, digitoxin, quinine and reserpine are well-known examples (Aschwanden, 2001).

A review of the herbal therapies used by women also revealed evidence of increased dependence on herbs for the management of symptoms of hypertension (Tesch, 2002). Like mistletoe, *Pavetta crassipes* and garlic, *Acalypha torta* is one of many herbs employed traditionally in the management of hypertension. Some pharmacologic activities of the *Acalyphs* such as their antimalarial, antibacterial and antifugal properties have been well documented (Hiremath *et al.*, 1993; Irobi and Banso, 1994; Jekayinfa *et al.*, 1997). The antihypertensive effect of *Acalypha torta* was investigated since there is no information on this in the literature searched.

Hypertension is a haemodynamic disorder in which increased arterial pressure may be associated with an increased cardiac output or increased total peripheral resistance. In most patients increased total peripheral resistance is the cardinal cause of increased arterial pressure (Alper *et al.*, 2002). The ability of *Acalypha torta* extract in reducing cardiac output and peripheral resistance was investigated.

Uncontrolled hypertension leads to thickening of blood vessels (arteriosclerosis) and this may result in fibrous plaque and thrombus formation which is probably responsible for ischaemia and infarction of the brain and kidneys in severe chronic cases (Alper *et al.*, 2002). This disorder also coexists with hyperlipidaemia, leading to the formation of lipid-rich atherosclerotic plaques eventually resulting in coronary and cerebrovascular events. Thus, the antithrombotic (anti-aggregatory) and hypolipidaemic potentials of this plant, *Acalypha torta,* were also investigated.

Perhaps the biggest problems with herbal medicines are lack of standardization and safety regulation since they could cause serious illnesses, from allergy to liver or kidney malfunction, cancer, and even death. Toxicological screening of *Acalypha torta* extract in experimental animal models was also carried out in this work. Serum enzyme assays also buttress other toxicological and histopathological investigations to give a complete safety evaluation.

1.2.16 Objectives

The objectives of this research study were therefore as follows:

1. To extract *Acalypha torta* leaves in various solvents to determine the disposition of the active components..
2. Intact animal preparation: determination of effects of the various extracts on the arterial blood pressure of normotensive cats.
3. Isolated tissue preparations: determination of the effects of the bioactive ethanol extract on selected tissues.
4. Toxicological studies of the ethanol extract.
   - (a). Acute toxicity in mice
   - (b). Sub-acute toxicity in rat norvegi (wistar albino rats).
     - (i) Assay of serum enzymes, ALT, AST and ALP
     - (ii) Histopathological examination of the liver, spleen, kidney, lungs, brain and heart.
5. To investigate the mechanism of action of the bioactive ethanol extract using
   - (a). Receptor – blocking study.
   - (b). Cardiovascular effects
     - (i). Effects on rat- hind-quaters preparation.
     - (ii). Effects on isolated rabbit arterial strips.
     - (iii). Effects on perfused rabbit heart.
     - (iv). Effects on $CaCl_2$- induced contraction of rabbit heart.
6. To determine other pharmacological activities of the ethanol extract
   - (a) Effect on isolated rabbit gut
   - (b). Effect on *in vitro* $CaCl_2$- induced platelet aggregations
   - (c) Effect on lipid profile of rabbit.
     - (i) Serum triacyglycerol level

(ii) Serum total cholesterol level

(iii) Serum LDL- cholesterol level

(iv) Serum HDL-cholesterol level

(d) Effect on serum electrolytes of rabbit.

(i) Serum cations, Na^+ and K^+.

(ii) Serum anions, Cl^- and HCO_3^-.

7. To determine the phytochemical and proximate composition of *A. torta* leaves and the bioactive ethanol extract of *A. torta* leaves

8. Determination of the mineral content of the bioactive ethanol extract

9. Isolation and phytochemical analysis of the active fraction from the ethanol extract.

10. Acute toxicity study of the active fraction to determine its median lethal dose (LD_{50}).

CHAPTER TWO: MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Plant Materials

Mature *Acalypha torta* leaves were collected from Abagana, Anambra State, Nigeria and authenticated in the Department of Botany, University of Nigeria, Nsukka by Mr. E.C. Ekekwe.

2.1.2 Animals

The following animals were used in the investigation. Adult cats were purchased from the main market in Obolo-Afor, Enugu State, Nigeria. Albino rabbits, were purchased from the main market in Awka, Anambra State, Nigeria. (Wistar Albino) rats and mice used for this work were obtained from the Animal House of the Department of Pharmacology, College of Medicine, University of Nigeria Teaching Hospital, Enugu.
All the experimental animals used were kept in the Biochemistry Departmental animal house for one to two weeks to acclimatize before use.

2.1.3 Chemicals

The following analytical chemicals were used: Sodium chloride, sodium hydroxide and sodium sulphate were obtained from Janssen Chimica, Belgium; methanol, ethanol, ethylacetate, formalin, aluminium chloride, glacial acetic acid, xylene, ferric chloride, copper sulphate and boric acid from BDH Ltd, Poole, England). Silica gel G60-200 (for column chromatography) chloroform, sulphuric acid, ammonia, hydrochloric acid, lead subacetate, bromine water, carbon tetrachloride, diethylether, ether, glucose, magnesium chloride butanol were supplied by May and Baker Ltd. Dagenham, England; Potassium chloride, calcium chloride, sodium hydrogen carbonate, sodium hydrogen phosphate, picric acid, perchloric acid (Riedel-De Haking seeles Hannover); Fehlings' solutions A and B ; Millons reagent, Molish reagent, anti-bumping chips, mixed indicator and silica gel G-60 (for thin layer chromagraphy were from E.Merk, Darmstadt, West Germany; Trisodium citrate was purchased from East Anglia Chemicals Hadleigh Ipswich, Suffolk; Histamine, Acetyl choline were from Sigma Chemical Company, U.S.A; Serum glutamine-oxaloacetate transaminase (SGOT) Kit, Serum glutamate-pyruvate-transaminase (SGPT) kit and serum alkaline phosphatase kit were made by Randox Laboratories Ltd, Ardmore, U.K; Serum triacylglycerol diagnostic kit, serum cholesterol

kit, serum LDL-cholesterol kit, serum HDL-cholesterol kit were produced by Biosystem S.A. CostaBrava, Spain; Silica gel 100-200 (for column chromatography) was purchased from BurgoyneBurbidges & Co. Mumbai, India. The following pharmaceuticals were also used: Atropine sulphate from Hamxmedica Ltd, U.K; Promethazine from Jinlingpharm. China; Adrenaline from PDH labs (PVT) Ltd. Lahore; Thiopental Sodium from Rotexmedica, Trittau, Germany and Heparin sodium mucous obtained from CP Pharmaceuticals Ltd, Wrexham, U.K; Carboxy methylcellulose was purchased from Philip Harris Biologicals Ltd, Weston.

2.1.4 Equipments and Glasswares

The equipments used include: Dissecting kit, plastic cannular, Brown-schuster myographic stand (C.F Palmer, London), Bulldog clip, three-way stop-cock, thermostatically controlled dissecting table, funnel, mercury manometer (C.F Palmer, London) writing lever, Brodie-startling kymographic drum (C.F Palmer, London) smoked paper, chromatographic tank, light microscope (Olympus), steam bath (GallenKamp), animal cages, aluminium dishes, oven, buchner funnel soxhlet extractor, kjehldal apparatus, flame photometer, organ bath, spectrophotometer (722 grating spectrophotometer), centrifuge (B-Bran Scientific and Instrument Company, England).

2.1.5 Reagents and Solutions for the Experiments

The reagent used in the study include;

Dragendorffs reagent

Mayers Reagent

Solutions for Blood Pressure Studies

1. Thiopentone: fifty milligrammes (50 mg) of thiopentone were dissolved in 10.0 ml of saline.
2. Normal saline: eight hundred and fifty milligrammes (850 mg) of NaCl were dissolved in 100 ml of distilled water.
3. Heparinized saline: two milliliters (2.0 ml) (10,0001.U) of heparin was made up to 100 ml with normal saline
4. Carboxymethylcellulose, CMC (1%): one grammes (1.0 g) of CMC was dissolved in 100ml of distilled water to obtain 1 % concentration.

5. Stock upper layer extract preparation: Two hundred milligrammess of the dried upper layer extract were dissolved in 4.0 ml of saline.
6. Stock ethanol extract preparation: two hundred milligrammes dissolved in 4.0ml of carboxymethylcellulose.
7. Stock water extract preparation: two hundred milligammes of the dried water extract were dissolved in 4.0 ml of saline to obtain the stock water extract.
8. Adrenaline standard: five hundred microgramme quantity of adrenaline was dissolved in 5.0 ml of saline to obtain 100 ug/ml adrenaline solution.
9. Histamine standard: ten milligrammes of histamine were dissolved in 10.0 ml of saline

Reagents for Acute Toxicity Study

1. Stock Ethanol extract preparation: three grammes of the dried ethanol extract were dissolved in 20.0 ml of normal saline.

Reagents For Sub-Acute Toxicity Study

1. Ethanol extract (1.0 g) was dissolved in 10.0 ml of normal saline to obtain the stock ethanol extract preparation.

Reagents for Dose-Response Study

1. Stock ethanol extract preparation was obtained by dissolving 400mg quantity of the dried ethanol extract in 3.0 ml of carboxymethylcellulose (1 %) solution.

Reagents for Aspartate Transaminase (AST) Activity Estimation

AST substrate contained

1. Phosphate buffer: 100 mmol/l, pH 7.4
   L-aspartate: 200 mmol/l
   α – Oxoglutarate: 2 mmol/l
2. 2,4-dinitrophenylhydrazine: 2 mmol/l

Reagents For Alanine Transaminase (ALT) Activity Estimation

ALT Substrate contained

1. Phosphate buffer: 100 mmol/l, pH 7.4
   L- alanine: 200 mmol/l
   α - oxoglutarate: 2 mol/l
2. 2,4-dinitrophenylhyrazine: 2 mmol/l

Reagents for Alkaline Phosphatase (ALP) Activity Estimation

ALP Substrate contains

1. Diethanolamine buffer: 1.0 mmol/l, pH 9.8
   $MgCl_2$: 0.5 mmol/l
2. Substrate:
   P-nitrophenylphosphate: 10 mmol/l

Reagent for Fixation

1. Formal saline (40%): Formaldehyde (40ml) was diluted to 100 ml with normal saline.

Reagents for Preparation of Slides

1. Paraffin, haematoxylin and eosin dyes
2. Ethanol, absolute, 90 % and 80 %
3. Chloroform

Reagents for Receptor Blocking Study

1. Stock histamine preparation: Histamine 100 µg was dissolved in 10.0 ml of normal saline
2. Stock acetylcholine preparation: Acetylcholine (100 µg) was dissolved in 10.0ml of normal saline
3. Stock atropine preparation: Atropine (1.0 mg) ampule was diluted to 10.0 ml with normal saline
4. Stock promethazine preparation: an ampule of promethazine (1.0 mg) was diluted to 10.0ml with normal saline.
5. Stock ethanol extract preparation: four hundred milligrammes of the dried ethanol extract was dissolved in 3.0ml of carboxymethylcellulose (1%) solution.

Reagents for Perfused Rat Hind- quarters Preparation Experiment

1. Potassium chloride (10 %): ten gramme quantity of KCl was dissolved in 100 ml of distilled water.
2. Calcium chloride (IM): $CaCl_2$ (110.99g) was dissolved in 1.0 L of distilled water.

3. Ringer-Locke solution contained the following chemicals in 5 litres of deionized water.

NaCl:	1 45.0 g
KCl:	2.1 g
Glucose:	5.0 g
$NaHCO_3$:	2.5 g
$CaCl_2$:	1.2 g

4. Stock ethanol extract preparation: The dried ethanol extract (400 mg) was dissolved in 3.0ml of Ringer-Locke solution.

Reagents for Isolated Rabbit Arterial Strips Experiment

1. Krebs solution contained the following s in 5 litres of deionized water.

NaCl:	69.0 g
KCl:	3.5 g
$MgSO_47H_2O$:	2.9 g
KH_2PO_4 :	1.6 g
Glucose:	20.0 g
$NaHCO_3$:	21.0 g
$CaCl_2$:	2.52 g
Aerating gas:	5% CO_2 in oxygen

2. Stock ethanol extract preparation: dried ethanol extract (200 mg) was dissolved in 4.0 ml of Krebs' solution.
3. Stock adrenaline preparation: one ampule of adrenaline (1.0 mg) was diluted to 10.0 ml with Krebs solution.

Reagents for Isolated Rabbit Heart Experiment

1. Ringer-Locke solution
2. Stock ethanol extract preparation: fifty milligramme quantity of dried ethanol extract (50 mg) was dissolved in 5.0 ml of Ringer-Locke solution
3. $CaCl_2$ (10%): ten grammes of $CaCl_2$ were dissolved in 100 ml of distilled water.

Reagents for Isolated Rabbit Gut Experiment

1. Tyrode solution contain in 1.0 litre of distilled water

NaCl:	8.0 g
KCl:	0.2 g
$MgCl_2$:	0.2 g
$NaHPO_4$:	0.05 g
$NaHPO_4$:	0.05 g
$NaHCO_3$:	1.0 g
Glucose:	1.0 g

2. Stock ethanol extract preparation was obtained by dissolving dried ethanol extract (200 mg) in 4.0 ml of Tyrode solution.

3. Acetylcholine: one hundred microgramme quantity of acetylcholine was dissolved in 10.0 ml of Tyrode solution.

4. Histamine: histamine dissolved in 10.0 ml of Tyrode solution.

Reagents for $CaCl_2$ – Induced Platelet Aggregation

1. Calcium chloride stock (0.1 M): calcium chloride (7.35 g) was dissolved in 100 ml of normal saline.
2. Sodium citrate (3.8%): sodium citrate (3.8 g) was dissolved in 100 ml of distilled water.
3. Stock ethanol extract preparation. ethanol extract (2.5 g) was dissolved in 2.0 ml of normal saline

Reagents for Serum Triacylglycerol Estimation

A.	Pipes:	45 mmol/l
	MgCl;	5 mmol/l
	4-chlorophenol:	6 mmol/l
	Lipase:	100 u/ml
	Glycerol-3-phosphate oxidase :	4 u/ml
	Peroxidase:	0.8 u/ml
	4-aminoantipyrine:	0.75 mmol/l
	ATP, pH 7.0:	0.9 mmol/l

S. Triacylglycerol standard: glycerol equivalent to 200 mg/dl triolein.

Reagents for Estimation of Serum Cholesterol

1. Acetic acid/Acetic anhydride (1:1 v/v)
2. Concentrated sulphuric acid
3. Working standard: contained 200 mg% of cholesterol in ethanol.

Reagents for the Estimation of Serum LDL-Cholesterol

1. Cholesterol estimation reagents
2. Polyvinyl sulphate: 3.0g/l
   Polyethyleneglycol: 3.0g/l

Reagent for HDL-Cholesterol Estimation

A.	Phosphotungstate:	0.4 mmol/l
	$MgCl_2$:	20.0 mmol/l
B.	Phosphate:	35 mmol/l
	Cholesterol esterase:	0.2 u/ml
	Cholesterol oxidase:	0.1 u/ml
	Peroxidase:	1.0 um/l
	4-aminoantipyrine:	0.5 mmol/l
	Sodium cholate:	0.5 mmol/l
	Dichlorophenolsulphonate:	4.0 mmol/l, pH 7.0
S.	HDL-Cholesterol standard:	cholesterol 15 mg/dl

Reagents for Serum Electrolyte Determination

1. Deionized water
2. Sodium standard equivalent to 4mEq/L
3. Potassium standard equivalent to 140 mEq/l
4. Mercurous thiocyanate: 1.0 mmol/l
5. Ferric nitrate: 10.0 mmol/l
6. Nitric acid: 62.0 mmol/l
7. Mercurous nitrate: 0.15 mmol/l
8. Chloride standard: 100 mEq/l

Reagents for Phytochemical and Proximate Analysis

1. Dragendorff's Reagent

   Bismuth subnitrate (0.6 g) was dissolved in concentrated HCl (2.0ml) and made up to 10.0ml with distilled water.

2. Mayer's Reagent

   Mercuric chloride (1.3 g) and potassium iodide (5.0 g) were dissolved in 100ml-distilled water.

3. Wagner's Reagent

   Potassium mercuric iodide solution

4 (a). Feh.ling's Solution A

   Copper sulphate crystals, 17 g, were dissolved in water and made up to 250 ml.

4(b). Fehling Solution B.

   Equal amounts (35 g) of sodium potassium tartarate and sodium hydroxide were dissolved in water and made up to 250 ml.

5. Mixture of equal volumes of A and B gave Fehling's solution.

6. Boric acid (2%) contained 2.0 g of boric acid dissolved in 100 ml of distilled water.

7. Aluminium chloride (1.0%): one gramme of aluminium chloride was dissolved in 100 ml of distilled water.

8. Sodium hydroxide (40 %) contained 40.0 g of sodium chloride dissolved in 100 ml of distilled water.

2.2 METHODS

2.2.1 Extraction Procedure

(a) Chloroform-methanol Extraction

Four hundred grammes (400g) of dried *Acalypha torta* leaves were ground into coarse powder and extracted by soaking in 5 vol. of chloroform: methanol (2:1), at room temperature for 24 hours with three changes of the solvents

The combined extract was passed through cheese cloth and then Whatman No. 1 filter paper. On addition of 0.2 vol. of distilled water, the extract yielded two layers, an upper aqueous methanol layer and a lower chloroform layer. The upper layer and lower layer were separated with the aid of a separating funnel. Each layer was evaporated to dryness to yield an upper layer extract and a lower layer extract.

(b) Alcohol Extraction

The residue from chloroform methanol extraction was dried and re-extracted in 5 vol . of ethanol, at room temperature, for 24hrs. Three changes of the solvent were made and the extracts were pooled together. The combined extract was filtered through cheese cloth and Whatman No. 1 filter paper. When evaporated to dryness, the filtrate yielded the ethanol extract.

(c) Water Extraction

The residue from alcohol extraction was dried and reextracted in 5 vol of water by following the same procedure outlined under alcohol extraction to obtain the dried water extract.

2.2.2 Determination of the Effect of the Extracts on Cat Blood Pressure

The effects of the upper layer, lower layer, ethanol and water extracts of *Acalypha torta* on normotensive cats were investigated using the method of Amos *et al* (2003). Male cats weighing between 1.5 and 2.0 kg were used for the study. Anaesthesia was induced in the animals by intraperitoneal administration of thiopentone (50 mg/kg body wt). Then the animals were laid on their back on the Brown-Schuster myographic stand (C.F Palmer, London), and the legs were fastened to the table by means of strong ropes, and the neck was shaved with sharp scissors. The longitudinal muscle in front of the trachea was exposed via a skin incision with a pair of scissors. The trachea was freed from the surrounding connective tissue using a curved iris forceps, and two ligatures passed round the trachea but not tied. A cut was made in the trachea to allow the insertion of a trachea cannula.

The proximal ligature was tied and pulled towards the head. This facilitated the insertion of the cannula and the distal ligature was then tied around the cannula to keep it properly aligned. The cannulation of the trachea was to allow for free respiration.

i. Cannulation of the Femoral Vein

Fur was cleared along the inguinal region close to the abdominal wall. The skin in this region was dissected, and the femoral vein was then exposed. By means of blunt dissection, the inguinal ligament was freed of connective tissue and all feeding venules to the femoral vein tied off. Following exposure of a suitable length of the femoral vein,

the blood flow was occluded by placing a bulldog clip at the proximal end. Two threads were carefully passed around the vein and half tied. The vein was then made to distend fully by using fingers to push blood towards the bulldog clip. The distal thread was firmly tied and pulled so as to stretch the vein slightly.

A small cut was made with a sharp pointed scissors not more than half way through the vein, as near to the tied thread (i.e the distal thread) and as far from the bulldog clip as possible. The cut was made at an angle of about 30 degrees so that there was a small flap which could be lifted up with a seeker while a fine plastic cannula (1.5 mm external diameter) was inserted and connected to a three way stop-cock which allowed for the injection of the drugs and saline. The proximal thread was tied around the cannula and the tubing connected to it was filled with heparinized saline (100 i.u/ml) before insertion. The essence of this procedure was to prevent blood clotting within the vessels and to carefully exclude air bubbles completely.

ii. Cannulation of the Carotid Artery

Then the carotid artery was located and cannulated as follows for the blood pressure recordings Two ligatures were passed round the cleared and exposed length of the carotid artery. The proximal thread (close to the head and away from the heart) was thereafter tied and when the artery became engorged with blood, the bulldog clip was placed at the end close to the heart. By incision and procedure similar to that described for venular cannulation, the carotid artery was prepared and prepared and connected to a mercury manometer through a three-way stop cock with a syringe filled with heparinised saline. All air bubbles in this system were flushed out with the heparinised saline and the arterial cannula shut off by means of the tap off the three-way stop-cock.

The pressure in the manometer was raised by means of the syringe to the expected blood pressure of the animal. The stop-cock was set so that the syringe was closed off and the arterial cannula was connected to the recording device. The bulldog clip was then removed.

iii. Recording Device Used

Changes in blood pressure of the animals were recorded with mercury manometer, a simple 'U' tube of about 5mm bore. It was a simple device that gave direct readings via

the pulsation of the mercury column. This was connected via a writing lever to a smoked paper on a Brodie-Startling Kymographic drum rotating at a speed of 16 mm per minute.

Prior to administration, upper layer and methanol extracts were dissolved in carboxymethyl cellulose solution (1%) whereas water extract and the standard or reference drugs used i.e. adrenaline (100 µg/kg body wt) and histamine (0.2 pg/kg body wt) were solubilized in normal saline. All the extracts and the standard agents were given intravenously and their effects compared with those of appropriate controls: cmc, methanol, chloroform, ethanol and distilled water.

2.2.3 Dose-Response Study

The essence of this experiment was to find out if the blood pressure lowering effect of the ethanol extract of *Acalypha torta* varied with the administered dose. Male cats weighing between 1.8-2.1kg were used. After anaesthesia and cannulations of the trachea, carotid artery and femoral vein were done as described in 2.2.2 above, varying doses of the ethanol extract (2, 4, 6, 8, 10 and 20 mg/kg body wt) were then administered via the three-way stop-cock and the changes in the blood pressure of the animals were recorded using the recording device. Temperature was maintained at 37$\pm$ 1^{0}C by means of a thermostatically controlled dissecting table.

2.2.4 Acute-Toxicity Study

A 24-hr acute toxicity study was carried out to determine the risk of acute intoxication by the ethanol extract of *Acalypha torta*. This study was carried out by the method of Lorke, 1983.

Adult albino mice weighing (16-35 g) were used. Male and female were used to access the possible influence of sex variation in the acute toxicity of this extract. All the 42 mice used were starved overnight i.e. for 18 hours prior to intraperitoneal administration of the extract. The animals were divided into seven dosage groups (corresponding to seven cages) required to establish the lethal dose (LD_{50}). The grouping was done according to the weights of the animals and all the animals were allowed free access to both water and animal feed (Guinea grower's mash). Mice in groups 1-6 received 200, 500, 1000, 2000, 4000 and 8000 g/kg body wt. of extracts respectively whereas group 7 animals received

normal saline (1 ml/kg body wt.) and served as the control group. The various groups of mice were housed in separate animal cages. After the administration of extract, the animals were observed for 24 hours for any behavioural changes and deaths. Changes monitored included irregular or staggering movement (ataxia), aggression, drowsiness, twitching, clumsiness, loss of appetite, respiration rate, analgesia, paralysis and convulsion.

2.2.5 Sub-Acute Toxicity Study

Sub-acute toxicity studies were also carried out to ascertain the effect of doses below 50% of the median lethal dose (LD_{50}) of the ethanol extract within a short-term period of 28 days. Fifty mature Wistar albino rats of both sexes were used. Normal saline and extract preparations were administered intraperitoneally to the appropriate groups of ten rats each. The treatment groups were designed as follows:

Group A - received 50 mg/kg body wt of ethanol extract daily for 28 days.
Group B - received 100 mg/kg body wt of ethanol extract daily for 28 days
Group C - received 200 mg/kg body wt. of ethanol extract daily for 28 days
Group D - received 100 mg/kg body wt of ethanol extract once i.e for 1 day only
Group E - received 1 ml/kg body wt of normal saline (control) daily for 28 days.

The animals were weighed daily and two rats were randomly selected from each group and sacrificed on days 1, 3, 7, 14 and 28 of treatment. Blood samples were collected through cardiac puncture and sera obtained by centrifuging at 10,000 rpm for 5-10 min for enzyme assays.

2.2.5.1 Aspartate Transaminase (AST) Assay

Aspartate transaminase (AST) or serum glutamate-oxaloacetate transaminse (SGOT) activity was assayed with Randox Diagnostic Kit according to the method of Reitman and Frankel, 1957.

The basic principle of the assay method is that oxaloacetic acid which is formed from the reaction of α-oxoglutarate with L-aspartate is decarboxylated to pyruvate .

Pyruvate then reacts with 2,4-dinitrophenylhydrazine in alkaline medium to forr hydrazone. The amount of phenylhydrazone formed is proportional to the aspar transaminase activity.

α- oxoglutarate + L-aspartate → L-glutamate + oxaloacetic acid

ASSAY PROCEDURE: The substrate (0.5 ml) was warmed for 5 min at 37^{0}C and ml of serum was added to each tube and mixed. The tubes were then incubated at 3 for 30minutes. One millimolar solution (0.5 ml) of 2,4-dinitrophenylhydrazine v added, mixed thoroughly and allowed to stand at room temperature for 20 minut Following this, 0.5 ml of sodium hyroxide (0.5 N) was then added to each tube a allowed to stand for 5 minutes. At the end of this period, absorbances were read agains reagent blank at 550 nm. AST activities in i μ/L were obtained from tables in the kit.

2.2.5.2 Alanine Transaminase (ALT) Assay

Alanine transaminase or serum glutamate-pyruvate transaminase (SGPT) activity w also estimated using Randox Diagnostic Kit according to the method of Reitman a Frankel, 1957. The principle is that pyruvic acid, produced when α-oxoglutarate reac with L-alanine, is decarboxylated and then reacts with 2,4-dinitrophenyhydrazine alkaline medium to give a hyrazone which is proportional to the ALT activity.

α- Oxoglutarate + L-alanine SGPT → L-glutamate + pyruvate

ASSAY PROCEDURE: Exactly 0.5ml of the substrate was warmed for 5 min at 37^0 and 0.1 ml of serum sample (0.1ml) was added to each tube and mixed. The tubes we incubated at 37^{0}C for 30 minutes and 1.0 ml of 2, 4-dinitrophenylhydrazine (1.0 mM added to each. They were mixed thoroughly and allowed to stand at room temperatu for 20 minutes. 0.5 N NaOH. To each tube (0.5 ml) was then added and allowed to star for 5 minutes. Absorbances at 550 nm were then read against a reagent blank. AL activities in 1i/L were obtained from the table provided in the kit.

2.2.5.3 Alkaline Phosphatase Assay

Serum alkaline phosphatase activity was assayed according to the standard colorimetri method of Reitman and Frankel, 1957 using Randox Diagnostic kit. The principle is that

P-Nitrophenylphosphate + H_2O $\xrightarrow[ALP]{}$ Phosphate + P-nitrophenol-

ASSAY PROCEDURE

One milliliter of p-nitrophenylphosphate and 0.2 ml of sera were pipetted into each tube and mixed at 30^0C. Absorbances were read against air at 405nm for initial minute. It was then read again after 1, 2 and 3 minutes. Alkaline phosphatase activities in I.μ/L were obtained from the range of values, between 73-207, I.μ/L given in the kit.

2.2.5.4 Histopathological Method

Sections of some vital organs, liver, kidney, brain, lungs, heart and spleen were carefully collected from the rats *post mortem* and fixed in 10% formal saline for at least 72hours. The sections were trimmed, processed and stained with haematoxylin and eosin (H.E) stains for microscopic examination. The processing procedure is as outlined below.

Step 1: Dehydration- The tissues were dehydrated in ascending grades of alcohol for not less than 5 hours.

80% ethanol for 2 hours

90% ethanol for 1 ½ hours

100% absolute ethanol for 1 ½ hours

Step 2: Clearing - The tissues were cleared using chloroform. They were immersed in chloroform overnight.

Step 3: Paraffin Wax Infiltration – Molten paraffin was used and two changes were made after 1 ½ hours in each bath maintained at 57^0C – 60^0C.

Step 4: Embedding - Molten paraffin wax was also used for the embedding.

Step 5: Staining – After embedding, the tissues were sectioned and stained with haematoxylin and eosin (H.E).

2.2.6 Receptor – Blocking Activity

The ability of the extract to block certain receptors was investigated using a modified method of Amos *et al.*, 2003.

After anaesthetizing the cat with thiopentone (50 mg/kg wt.) cannulation of the trache carotid artery and fermoral vein was done according to 2:2:2 and the animal w prepared for experimentation. Cholinergic and histamine receptor blockades we established by the injection of their specific antagonists- atropine (20 pg/kg body wt) a promethazine (20 μg/kg body wt.) respectively.

Adrenergic receptors (α and β) blockades were not studied due to unavailability of the specific blockers. As soon as the animals arterial pressure stabilized, the blockers wer administered slowly and carefully excluding all air bubbles. The extract (20 mg/kg w was then given immediately.

2.2.7 Experiment on the Perfused Rat-Hinquarters Preparation

This experiment was designed to define the effect of *Acalypha torta* extract on bloo vessels i.e whether it is dilating or constricting the peripheral blood vessels.

The method was according to Hutchings *et al.*, (1980). Rats of either sex weighing between 150g and 210g were used for the experiment. The animals were killed by stunning and then bled. The abdominal cavity was dissected longitudinally starting from the sternum down to the anus. The viscera were then removed exposing the dark dorsal vein, which was then cannulated. The body wall and vertebral column were transected above the point of cannulation and the cannula filled with Ringer – Locke solution and connected to the perfusion system by means of a fine rubber tubing. The hind-quarters preparation was placed on wire mesh on a plastic funnel to facilitate collection of the fluid with a measuring cylinder. The method employed here is a modification of Thorpe's. The physiological fluid was constantly aerated with 5 % CO_2 in O_2 and the pressure at which the physiological fluid and extract perfused the vessels was kept constant. The flow rate was calibrated by recording the volume of fluid dropped at 5 min interval until a constant or near constant volume was obtained. The composition (g/l) of the perfusion fluid (Ringer-Locke solution) used was NaCl, 9.00; 0.42; $NaHCO_3$, 0.50; $CaCl_2$, 0.24; Glucose, 1.00; The equilibration time was about 30 min. Alteration of flow rate caused by four doses, (13.3- 59.9) mg/ml of the ethanol extract of *Acalypha torta* was then recorded.

2.2.8 Studies on Isolated Rabbit Aortic Strips

The method used was according to Furchgott and Bhadrakom (1953).

An adult male rabbit weighing 1.98kg was killed by a blow on the head. The throat was cut to open up the chest thus exposing the heart . The aorta was cut through, as near the heart as possible, and dissected free for as long a distance as possible. It was transferred to a dish containing Krebs solution and cut spirally so as to produce a continuous strip, about 4mm wide and 3 to 4cm long. A thread was then attached at each end and the preparation was mounted in Krebs solution at 37^0C aerated with a mixture of oxygen (95%) and carbon dioxide (5%). One end of the strip was attached to a fixed pin in the bath and the other to a lever writing on a smoked drum.. The load on the lever was 3.5g. The preparation was allowed 30 minutes to settle down after dissection. The drug-tissue contact time of 2 minutes was allowed throughout the experiment and 8 minute interval was allowed between additions. The composition of Krebs solution used was NaCl, 69 g; KCL, 3.5 g; $MgSO_4$. $7H_2O$, 2.9 g; KH_2PO_4, 1.6 g ; Glucose, 20 g; $NaHCO_3$, 21 g; and $CaCl_2$, 2.52 g. The effect of 10.mg. of *Acalypha torta* extract was first studied and then viability of the tissue confirmed by the injection of different doses, 5.0, 30.0 and 60.0 µg of adrenaline standard. The effects of adrenaline (60 µg) in the presence of two doses, 10.0 mg and 20.0mg. of *A.torta* extract were then recorded. In both cases the extract was allowed 2 minutes of incubation with the tissue before the injection of adrenaline. The drum speed of 4.0 mm per minute was maintained throughout the work.

2.2.9. Studies on Rabbit Heart Preparation

Having seen the effects of the ethanol extract of *Acalypha torta* on cat arterial blood pressure, peripheral blood vessels and arterial preparations, it was considered pertinent to also investigate its effects on rabbit whole heart. A modified method of Amos *et al.*, (2003) was employed. Male rabbits weighing between 1.5 and 2.0kg were used for this work. They were killed by stunning and blood allowed to drain out from a sharp slit on the throat. The heart was immediately removed with some portion of the aorta and immersed in ice-cold Ringer-Locke solution maintained at 0^0C. The heart preparation was squeezed severally in the Ringer-Locke solution to remove as much blood as possible. The aorta was located and dissected free and all other vessels connected to the heart were trimmed off. The aorta was cut below the point where it bifurcates and the heart was then transferred to the perfusion apparatus where the aorta was tied to exclude

air bubbles. The perfusion fluid was Ringer-Locke solution in a reservoir maintained at a constant pressure. An appropriate concentration of the extract (2.0 – 5.0 mg) was then introduced through the rubber tubing connecting the reservoir to the cannula perfusing the heart.

The entire set up was maintained at a temperature of 37^0C by water circulated from a thermostat. This experiment would allow the aortic valve to close by the pressure of the perfusing fluid, which passes only through the coronary vessels and escapes from the inferior vena cava. Threads were attached to the ventricles by a hook through a pulley system which was connected via a spring lever to a kymograph to record the size of the contractions on a rotating drum. Equilibration time of not less than 30 minutes was allowed before the extract was introduced and allowed to perfuse the heart. The effects of $CaCl_2$ (3.0 and 6.0 mg) were studied. The effect of the extract on response to calcium chloride was also investigated.

2.2.10 Studies on Intestinal Smooth Muscle

Considering the fact that *Acalypha torta* leaves are taken orally, the effect of the extract on the intestinal smooth muscle was also investigated. Isolated rabbit gut was used. Male rabbits weighing approximately 2.0 kg were fasted overnight and then killed by stunning and bleeding. In each case the abdomen was opened and the caecum lifted forward. The ileum was found joined to the back of the caecum. Lengths, 2.0-3 cm segments of the gut were then cut and placed in a dish containing Tyrode solution of the following composition in g/l; NaCl, 8.0; KCl, 0.2; MgCl, 0.2; $NaHPO_4$, 0.05; $NaHCO_3$, 1.0; and glucose, 1.0. The solution was supplied with air from an aerator. A thread was attached at each end by inserting a needle from the inside of the gut outwards. One thread was tied to a fixed pin in the organ bath aerated at about 37^0C, and the other to a lever which had a frontal-writing point.

The load on the lever was about 1g and the magnification of the contraction was tenfold. Response following varying doses of the extract, 2.5 -10.0 mg, histamine, 0.002 µg and acetylcholine, 0.002 µg were recorded. Drug-tissue contact time of 2 minutes was maintained except for acetylcholine which lasted for 10 minutes. The tissue was washed thrice between additions of drugs.

2.2.11 Platelet Aggregatory Activity Study

The method used was a modification (Nwodo, 1981) of that of Born and Cross (1963). Human blood samples were obtained from healthy male volunteers who had not taken any drug for at least one week. Blood samples were drawn by venipuncture into plastic sample tubes containing 0.1 vol., 3.8% trisodium citrate and centrifuged at 300rpm for 15 minutes. The supernatants were drawn out and used as platelet-rich plasma (PRP). Reaction medium (2.5ml) containing normal saline (2.0ml) and PRP (0.5ml) served as the control. Aggregation of platelets was induced by the addition of 4mM $CaCl_2$ (0.1ml). The absorbance at 600nm was monitored for 5 minutes using spectrophotometer. Varying concentrations of the extract, 5-15mg/ml final concentrations, were then included in the medium and allowed 15 seconds incubation with platelet-rich-plasma. Aggregation was then induced and monitored as before. Appropriate blanks containing the extract but without PRP were used.

2.2.12 Determination of Mineral Levels of the Ethanol extract of *A. torta*

The involvement of high levels of some mineral elements particularly sodium ion (Na^+) as a predisposing factor to high blood pressure. The mineral content of the extract was therefore analysed to ascertain the extent to which this extract furnishes these mineral elements into the blood. Atomic absorption spectrophotometry and flame photometry were used for the analyses according to the method of AOAC (1970)

FLAME PHOTOMETERY

Five hundred milligramme quantities of sample were ashed for 3 hours in a muffle furnace and wet digested with perchloric acid (50.0 ml) and the digests were used for the analysis. The flame photometer was switched on and allowed to warm up for 15 minutes before the compressed air pump and butane gas cylinder were switched on. The equipment was then ignited and adjusted until the blue cone appeared to allow for complete combustion. Deionized water was used to zero the equipment before the readings for the standard solutions, 1.0 - 4.0 ppm, and extract were taken. A graph of concentration (ppm) against absorbance was plotted.

Atomic Absorption Spectrophotometry

A two-gramme quantity of sample was ashed in a muffle furnace at 450^0C. The weight of the ash was determined and 10.0 ml of HCl (6.0 mm) added. The content of the

crucible was evaporated to dryness on a hot plate at a low heat until the residue was dry. The dry residue was moistened with 2.0 ml of HCL (36 %w/w). The crucible was then covered with a watch glass and gently boiled for 2 minutes. About 10.0 ml of distilled water was added and the solution boiled again. Crucible was removed, washed with distilled water and the washings diluted to 50.0 ml. This was then filtered through whatman No 1 filter paper. The first few millilitres of the filtrate were discarded and the rest retained for the determinations.

2.2.13 Investigation of the Effect of Ethanol Extract of *Acalypha torta* on the Lipid Profile and Serum Electrolytes in Rabbits

This study was carried out using nine adult healthy looking male rabbits. The animals were weighed and divided into three groups of three rabbits each according to their body weights. They were maintained on standard growers mash (Guinea animal feed) and vegetables. Free access to drinkable water was also allowed throughout the period of study. The control group of rabbits were given a daily dose of normal saline, 1 ml/kg body wt of animals, whereas two different doses of the ethanol extract of *Acalypha torta,* 10.0 and 20.0 mg/kg body wt.., were administered daily to the test groups (T_1 and T_2) respectively. All administrations were intraperitoneally for twenty-one days (3weeks). Blood samples were collected via the ear lobes of the animals on days 1, 7, 14 and 21. After clotting the blood samples were centrifuged at 3,000 rpm and the supernatant sera samples were used for lipid profile and electrolyte determinations.

2.2.13.1 Determination of Serum Cholesterol

Total serum cholesterol was determined according to the method Richmond, (1973). Biosystems diagnostic kit was used.

Principle: The principle of this estimation procedure is that cholestrol reacts with acetic anhydride and concentrated sulphuric acid to give a green colour. The intensity of the colour produced which is measured colorimetrically at 570nm and the concentration is proportional to the amount of cholesterol in the sample.

Procedure: One hundred microlitre (0.1ml) of serum was mixed with 2.5 ml of acetic acid/acetic anhydride mixture and allowed to stand at a temperature of 20^0C for 10 minutes. Concentrated sulphuric acid (0.5 ml) was then added. After each addition, the content of each tube was mixed immediately and cooled by immersing in water for 10

minutes. Distilled water, 0.1 ml, (Blank) and cholesterol standard were treated likewise and absorbance at 570 nm were read. The cholesterol concentrations of the sera were calculated using the formular:

$$\text{Cholesterol (mmol/L)} = \frac{\text{T-B x 5.7}}{\text{S- B}}$$

Where

T = absorbance for test

B = absorbance for blank

S = absorbance for standard

5.7 = concentration of standard.

2.2.13.2 Determination of LDL-Cholesterol

Assman's (1984) method of determination was employed using Biosystems diagnostic kit for cholesterol – LDL estimation.

Principle of the method

Low-density lipoproteins (LDL) in the sample (serum) precipitate with the addition of polyvinylsulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is measured spectrophotometrically by means of the coupled reactions outlined below:

$$\text{Cholesterol} + \tfrac{1}{2}O_2 + H_2O \xrightarrow{\text{Cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure: Four hundred microlitres (0.4 ml) of serum samples were pipetted in labelled centrifuge tubes and mixed thoroughly with 0.2 ml of reagent (A). The tub were allowed to stand undisturbed for 15 minutes at room temperature. The mixtu were centrifuged at 4000 rpm for 15 minutes and the supernatant carefully collect Aliquots of supernatants (20 µl) were then mixed thoroughly with the reagent cholesterol kit) and The incubated at room temperature for 30 minutes. A 20 µl - quar of the cholesterol standard was treated like the sample and the absorbance of the stan and sample were measured at 500 nm against the blank (distilled water).

Calculations:

The cholesterol concentration of the supernatants were calculated using the general formular:

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 5.18 \times 1.5$$

Where 5.18 = concentration of standard

1.5 = sample dilution factor

The LDL –Cholesterol concentration in the sample was calculated as follows.

LDL – Cholesterol = Total cholesterol – cholesterol in supernatant

2.2.13.3 Determination of Serum HDL-Cholesterol

Grove's method (1979) for HDL-cholesterol estimation was adopted. Biosystems diagnostic kit was used for the analysis.

Principle of the Method

The fundamental principle is that very-low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with the introduction of phosphotungstate and magnesium ions (Grove, 1979; Burstein *et al.*, 1980). The resulting supernatant after centrifugation contains the high density lipoproteins (HDL). The HDL-cholesterol in the supernatant is then measured spectrophotometrically by means of the coupled reaction described below:

$$\text{Cholesterol esters} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{Cholesterol Oxidase}} \text{cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{DCFS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Assay Procedure: Aliquots of serum sample (0.2 ml) were mixed thoroughly with reagent A and allowed to stand or 10 minutes at room temperature. The tubes were centrifuged at 4000 rpm for 10 minutes and the supernatants carefully collected with the aid of a micropipette. An amount (50µl) of supernatant was thoroughly mixed with 1.0

ml of reagent B. HDL – cholesterol standard and distilled water (blank) were treated as the sample. The tubes were thereafter incubated for 30 minutes at room temperature. Absorbances of the sample and standard were then measured at 500 nm.

Calculations

The HDL-cholesterol concentration of the samples were calculated using the formular;

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 1.36 = \text{HDL- cholesterol (mmol/L)}$$

Where 1.36 is the concentration of the standard

2.2.13.4 Determination of Serum Triacylglycerol

The quantitative method of Bucalo and David, (1973) was used to determine the triacylglycerol levels of the experimental animals.

Principle of the method: The principle behind this experimental procedure is that when the triacylglycerols contained in the sample undergo the coupled reactions described below a coloured complex is generated which can then be measured spectrophotometrically.

$$\text{Triacylglycerols} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol kinase}} \text{Glycerol} -3 -P + ADP$$

$$\text{Glycerol} - 3\text{-}P + O_2 \xrightarrow{\text{G-3-P Oxidase}} \text{Dihydroxyacetone-P} + H_2O_2$$

$$2H_2O_2 + 4\text{–Aminoantipyrine} + 4\text{- Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + 4H_2O$$

Procedure: Into labeled test tubes were pipetted 10 µl of serum samples and 1.0 ml aliquots of reagent A. The same volume of the standard solution was treated as the sample. After thorough mixing the test tubes were incubated at room temperature for 15 minutes. The absorbance of the standard and sample were then recorded at 500 nm against the Blank (1.0 ml of reagent).

CALCULATIONS

The triacylglycerol concentration in the samples were calculated using the general formular:

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 2.26 = \text{mmo/l triacylglycerols}$$

Where 2.26 = concentration of the standard

2.2.14 Determination of Serum Potassium and Sodium (Flame Photometry)

Principle: The principle is that, potassium or sodium is finely sprayed under carefully controlled conditions into a burner, the flame de-solvates the solution leaving solids (salts) which dissociate to give neutral ground state atoms. Some of these atoms were excited in the flame thus moving into a higher state. When these excited atoms fall back to their ground states light of characteristic wavelengths is emitted (590 nm for sodium and for potassium). This light then passes through suitable filter into a photosensitive element and the amount of current produced is measured. This is proportional to the amount of sodium or potassium present originally in the sample.

2.2.15 Determination of Serum Chloride Levels

The colorimetric method of Schoenfeld and Loewell, (1964) was used.

Principle: Chlorides react with mercurous thiocyanate releasing thiocyanate ions which form, together with trivalent iron, a coloured compound whose colour intensity is proportional to chlorine concentration.

Procedure: Ten microlitre of serum sample was mixed with 1000 µl of reagent R1 containing mercurous thiocyanate 1.0 ml/l, ferric nitrate, 10.0 mmol/l, nitric acid, 62.0 mmol/l and mercurous nitrate, 0.15 mmol/l. Chloride standard 10 µl and blank (distilled water) were given the same treatment as the sample. The tubes were then incubated for 5 minutes after 37^0C. The absorbance of sample and standard was then measured against the reagent blank.

Calculations

$$\text{Chloride (mEq/l) or mmol/l} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Where 100 = concentration of standard.

2.2.16 Determination of Serum Bicarbonate Levels

The titrimetric method of Vanslyke and Cullen, (1917) was adopted. Unhaemolysed blood samples were centrifuged and the sera carefully collected.

Principle: The method is based on the fact that when dilute HCl is in contact with the plasma or serum, carbondioxide (CO_2) is released. Titration of the resultant mixture against sodium hydroxide (NaOH) using phenol red as indicator gives a faint pink colour on complete neutralization. The amount of NaOH (titre) is proportional to the concentration of bicarbonate ion (HCO_3^-) present in the sample

Procedure: In a universal container was added serum sample (0.2 ml), carbondioxide free deionized water (2.0 ml), 0.0I N HCL (1.0 ml) and 2 drops of phenol red indicator. After addition the container was whirled round to release the carbon dioxide. The resulting solution was then titrated against 0.01N sodium hydroxide to a faintpink colour end point.

Calculation: The amounts of bicarbonate ions in the sera were calculated using the formular.

$$\frac{1 - \text{Titre}}{2} \times 100 = \text{mmol/l } HCO_3^-$$

Where 1 = volume of hydrochloric acid used, 2 is = constant

2.2.17 Phytochemical Analysis Procedures

The same phytochemical and proximate analytical techniques were used for the determination of phytochemical and proximate composition of whole *Acalypha torta* leaves, ethanol extract of *Acalypha torta* and the bioactive fraction (L) from the ethanol extract.

2.2.17.1 Test for Alkaloids

Two hundred milligramme quality of sample was boiled with 5.0 ml of 2% HCl in a steam bath. The mixture was then filtered through Whatman No 1 filter paper and 1.0 ml samples of the filtrate treated with 2 drops of the following reagents:

(i) Dragendorffs' reagent

(ii) Mayers' reagent

(iii) Wagners' reagent

Colour changes were observed and recorded

2.2.17.2 Test for Flavonoids

To 0.2 g sample was added 10.0 ml of ethylacetate and heated in boiling water for 3 minutes. The mixture was filtered and the filtrate used for the following tests:

(i) Four hundred microlitres (0.4 ml) of filtrate were shaken with 1.0 ml of 1% Aluminium chloride solution and observed for light yellow coloration in ethylacetate layer.

(ii) Another 0.4 ml quantity of the filtrate was shaken with 1.0 ml of dilute ammonia. The layers were allowed to separate and were observed for a yellow colouration.

2.2.17.3 Test for Glycosides

Two hundred milligrammes of the sample was boiled with 10.0 ml of dilute sulphuric acid for 15 minutes in a water bath. The mixture was cooled and neutralized with 5.0% solution of NaOH, using litmus paper to detect complete neutralization. Ten millilitres of a 1:1 mixture of fehlings solutions A and B were added and the mixture heated again. The formation of a brick red precipitate is indicative of the presence of glycosides.

2.2.17.4 Test for Cardiac Glycosides

One hundred milligrammes of the sample and 5.0 ml of chloroform were warmed in a water bath and decanted. The solution was evaporated to dryness and residue dissolved in 3.0 ml of glacial acetic acid containing a drop of ferric chloride. The solution was then poured gently into a second tube containing concentrated sulphuric acid and observed for a reddish brown ring at the surface.

2.2.17.5 Test For Anthracene Glycosides

A quantity (0.1 g) of the sample was added to a mixture of dilute sulphuric acid (5.0 ml) and ferric chloride (5.0 ml) solutions. The resulting mixture was boiled for 5 minutes cooled and filtered into a 50.0 ml. separating funnel. The filtrate was shaken with an equal volume of carbon tetrachloride and the lower organic layer carefully separated into a test tube. Five millilitres volume of dilute ammonia solution was then added gently with shaking and observed for a pink colouration in the ammonia layer.

2.2.17.6 Test for O - and C - glycosides

Two hundred milligramme quantity of the sample was boiled with 5.0 ml of distilled water in a boiling water bath for 5 minutes. It was cooled, filtered and the filtrate treated with 25% hydrochloric acid (5.0 ml). The solution was heated again for 15 minutes and then cooled with diethylether (10.0 ml) in a separating funnel. Both phases were examined for the presence of a purple - red colour in the aqueous layer.

The combined ether extract was shaken with 3.5% dilute ammonia solution (5.0 ml) and the mixture observed. The acid phase was treated with 0.5 g of ferric chloride, heated in a water bath for 30 minutes, and then cooled. The resulting solution was extracted with chloroform (10.0 ml), washed with distilled water (5.0 ml) and then 3.5% dilute ammonia solution (3.0 ml) was added and observed for a dark red precipitate.

2.2.17.7 Test for Saponins

One hundred milligrammes of the sample was boiled with 5.0 ml of distilled water for 5 minutes. The mixture was filtered while hot and filtrate used for the following tests:

(i) Emulsion Test: one hundred microlitres of the filtrate was shaken with 2 drops of olive oil and observed for the formation of emulsion.

(ii) Frothing test: The filtrate (0.1ml) was diluted with 4.0 ml of distilled water. The diluted sample was shaken vigorously and then observed, on standing, for stable froth.

2.2.17.8 Test for Tannins

Two hundred milligrammes of the sample were boiled with 5.0 ml of 45% ethanol for 5 minutes. The mixture was cooled, filtered and the filtrate used for the following tests.

(i) Lead subacetate test – To 1ml of filtrate was added 3 drops of lead subacetate solution and observed for a gelatinuous precipitate.

(ii) Bromine Water Test: The filtrate (1.0ml) was mixed with 0.5 ml of bromine water and observed for a pale brown precipitate.

2.2.17.9 Test for Sterols and Triterpenes

Two grammes of the sample were extracted by refluxing with 25.0 ml of chloroform for 1 hr and the chloroform extract was used for the following tests:

(i) Moleschott Test - Two milliliters of the (2.0 ml) of chloroform extract were evaporated to dryness in a water bath. The residue was redissolved in 5 parts of conc.

H_2SO_4 and one part of water. A dark green colour indicates the presence of sterols and triterpenes.

(ii) Salkowskis' Test – Two millilitres of the chloroform extract was concentrated and conc. H_2SO_4 (1.0ml) was gently added. A brown colour at the interface indicated the presence of sterol and triterpenes.

2.2.17.10 Test for Proteins

To 0.1 g of the sample was added 5.0 ml of distilled water, allowed to stand for 3 hr. Then it was boiled and shaken with 0.1 ml of millon's reagent. A pinkish precipitate indicated the presence of proteins.

2.2.17.11 Test for Carbohydrates

A quantity (0.1 g) of the sample was shaken vigorously with distilled water and filtered. To the aqueous filtrate was added four drops of Molish reagent followed by vigorous shaking. Concentrated sulphuric acid (1.0 ml) was carefully added to form a layer below the aqueous solution and a brown ring at the interface indicated the presence of carbohydrates.

2.2.17.12 Test for Reducing Sugars

Two hundred milligrammes of the sample were extracted with 5.0ml of distilled water by warming for 5minutes. A 1:1 mixture of Fehlings solutions A and B was added until the mixture was alkaline. The mixture was then heated in a water bath for 2 minutes and observed for a brick-red precipitate

2.2.18 Proximate Analysis Methods

2.2.18.1 Determination of Moisture Content

Five grammes of fresh sample were weighed out in covered, flat aluminium dishes and dried to constant weight at 100^0C in an oven filled with controlled ventilation. The moisture content was then determined using the following application:

Calculation:

$$\text{Moisture content\%} = \frac{(\text{wt.of fresh sample}) - (\text{wt. of dry sample})}{\text{Wt. of fresh sample}} \times \frac{100}{1}$$

2.2.18.2 Determination of Crude Fibre Content

Two grammes quantity of the sample were weighed out into a 500 ml.beaker. Two millilitres of H_2SO_4 was added to the sample and the beaker placed under the condenser and boiled for 30 minutes. Distilled water was used to maintain the volume. It was filtered using Whatman No-1 filter paper in a buchner funnel and washed well with boiled water. The residue was then transferred back to a beaker and hot NaOH solution (200 ml) added to it. The beaker was returned under the condenser and boiled for another 30 minutes. At the end it was filtered through porous crucible and washed with boiling water, 1% HCL and again boiling water. This residue was finally washed twice with ethanol,dried overnight at 100^0C, cooled and weighed.

Calculation : Percentage crude fibre content was calculated using the formular

$$\text{Crude Fibre (\%)} = \frac{(\text{wt. of crucible + dried residue}) - (\text{wt. of crucible + ashed residue})}{\text{wt of sample}} \times \frac{100}{1}$$

2.2.18.3 Determination of Crude Fat

Two grammes of the sample were extracted using soxhlet extractor. The sample was weighed into the extraction thimble and the thimble fixed inside the soxhlet apparatus. Dry solvent flask containing 200 ml of ether was positioned beneath it and connected to the condenser. The heating rate was adjusted and ether reclaimed using the apparatus. Remaining ether was completely evaporated by boiling in a water bath and drying the flask at 105^0C for 30 minutes. Sample was cooled in a dessicator and weighed.

Calculation: To calculate the total fat %, the following formular was applied:

$$\text{crude fat (\%)} = \frac{\text{(wt. of fat)}}{\text{wt of sample}} \times \frac{100}{1}$$

2.2.18.4 Determination of Crude Protein

The total nitrogen (crude protein) was determined using Kjehldal method.

Digestion: One gramme of the sample was introduced into the bottom of a 500 ml. Kjehldal flask. Concentrated H_2SO_4 (20.0 ml) was added and mixed gently by swirling

under tap water. Three grammes of Kjehdal catalyst (Na_2SO_4 (110.0 g) + $CuSO_4$ (1.0 g) was introduced into the flask and entire mixture was boiled gently in the Kjehdal flask in a fume cupboard until a clear green solution was obtained. The digest mixture was made up to 100 ml with distilled water.

Distillation of the digest mixture: The sample chamber of the distillation apparatus was flushed with deionized water. The digest was poured into a sample chamber through the connecting funnel. The flask was then washed with distilled water (5.0 ml) and poured into the chamber. Ten millimitres of NaOH (40%) was poured into the distillation apparatus and heated for 25 minutes. The nitrogen contained in the sample reacted with NaOH liberating ammonia. The liberated ammonia was absorbed in boric acid (5.0 ml) containing one drop of mixed indicator. This distillate was then titrated with standard hydrochloric acid (0.1 N HCl) to end point and the value of the titre was recorded.

Calculation

$$\% \text{ Nitrogen} = \frac{1.4 \times \text{Xdilution factor}}{\text{Original wt of sample (mg)}} \times \frac{100}{1}$$

$$\% \text{ Protein} = \frac{1.4 \times \text{x dilution factor}}{\text{Original wt of sample (mg)}} \times \frac{100 \times 6.25}{1}$$

i.e % Nitrogen x 6.25

where x is the titre value

2.2.18.5 Determination of Total Ash

The crucibles were thoroughly washed dried and placed in a hot air-circulation oven for 2 hr and then cooled to room temperature in a dessicators. The empty crucibles were placed in the muffle furnace to burn off all organic matter and also to stabilize the weight of the crucibles at the temperature range of 550-600^{0}C. They were placed in the dessicator to cool to room temperature. Two grammes of defatted samples were accurately weighed into the crucibles and ashed in the muffle furnace at 600^{0}C for 3 hr. They were then transferred into the dessicator to cool to room temperature and were weighed. Calculation of percentage ash was done using the formular below.

$$\% \text{ Ash} = \frac{\text{wt of (Crucible + Ash)} - \text{wt of crucible}}{\text{wt of sample}} \times \frac{100}{1}$$

2.2.18.6 Determination of Total Carbohydrates

This was conveniently done using the difference method. The total carbohydrate was obtained as 100% less the sum total of % fat, % ash, % moisture, % crude fibre and % protein. In other words,

Total carbohydrate = 100 – (% Fat + %Ash+% Crude+% Protein+% Crude Fibre +% Moisture).

2.2.19 Fractionation of the pharmacologically active ethanol extract

2.2.19.1 Column Chromatography

A glass chromatographic column (4cm x 70cm) was uniformly packed with the stationary phase (silica gel G- 60-200). The sample was concentrated to 30.0 ml and layered on the top of the column and eluted with –chloroform: butanol:xylene(2:2:3). Eluates were collected in fractions of 5.0 ml aliquots at the flow rate of 40 drops per minute. After 67 fractions, then the column was again eluted with water at the same flow rate for a further 22 fractions.

2.2.19.2 Thin-Layer Chromatography

The number of fractions (89) obtained using column chromatography was thinned down by the application of thin-layer chromatography.

A slurry was prepared by suspending 35.0g of gel in 100.0ml of distilled water and a uniform layer (0.25mm thick) was spread on plates of glass supports with the aid of a spreading machine. The coated plates were then activated by heating them at a temperature of 60^0C or 45 minutes. Fractions were spoted on siligal G-60 thin layer chromatoplates, air dried and developed in chloroform-butanol-xylene (2:2:3). By comparison of Rfs, the fractions were grouped into A – M. They were pooled and evaporated to dryness.

2.2.19.3 Determination of The Effects of Fractions A To M on Cat Blood Pressure

A male cat weighing 2.30 kg was used. Anaesthesia was induced with thiopentone, 50.0 mg/kg. body weight. and the animal cannulated as described in 2:2:2. After a resting period of 30 minutes the fractions were solubilized in 1.0% carboxymethylcellulose

(CMC) and 0.5 ml/kg.wt. of each fraction was administered to the animal through the three-way cock. The effects of the fractions A to M were then recorded by the aid of the recording device, kymograph.

2.2.20 Determination of The Lethal Dose (LD_{50}) of Fraction L

Acute toxicity of the bioactive fraction (L) was determined by a modification of the method described in 2.2.4. Forty-eight adult albino mice weighing between 39.0 to 49.6 g were used. They were divided into eight groups (A-H) of six animals each based on their body weights and housed in separate animal cages. Animals in control group A received 1ml /kg body weight. of normal saline whereas the treated groups, B-H, were given different doses (100, 200, 500, 1000, 2000, 4000 and 8000 mg/kg. body wt. respectively) of fraction L. The animals were then allowed free access to water and feed and were observed for 24 hr for changes in behaviour and deaths

2.2.21 Analysis of The Phytochemicals Present in Fraction L

Phytochemical constituents of fraction L were analysed following the steps outlined in 2.2.13.1 to 2.2.13.8.

2.2.21 Statistical Methods

Statistical analysis of all the data was done using ANOVA and Bonferroni's multiple comparison test.

CHAPTER THREE: RESULTS

3.1 EXTRACTION

Extraction with chloroform: methanol (2:1) yielded two layers- the upper (methanol) layer and the lower (chloroform) layer. After evaporation, the yields of the four extracts obtained were, upper layer (3.8% i.e 3.8 g per 100 g of pulverized dry leaves), lower layer extract (5.3%), ethanol extract (2.3%) and water extract (3.8%).

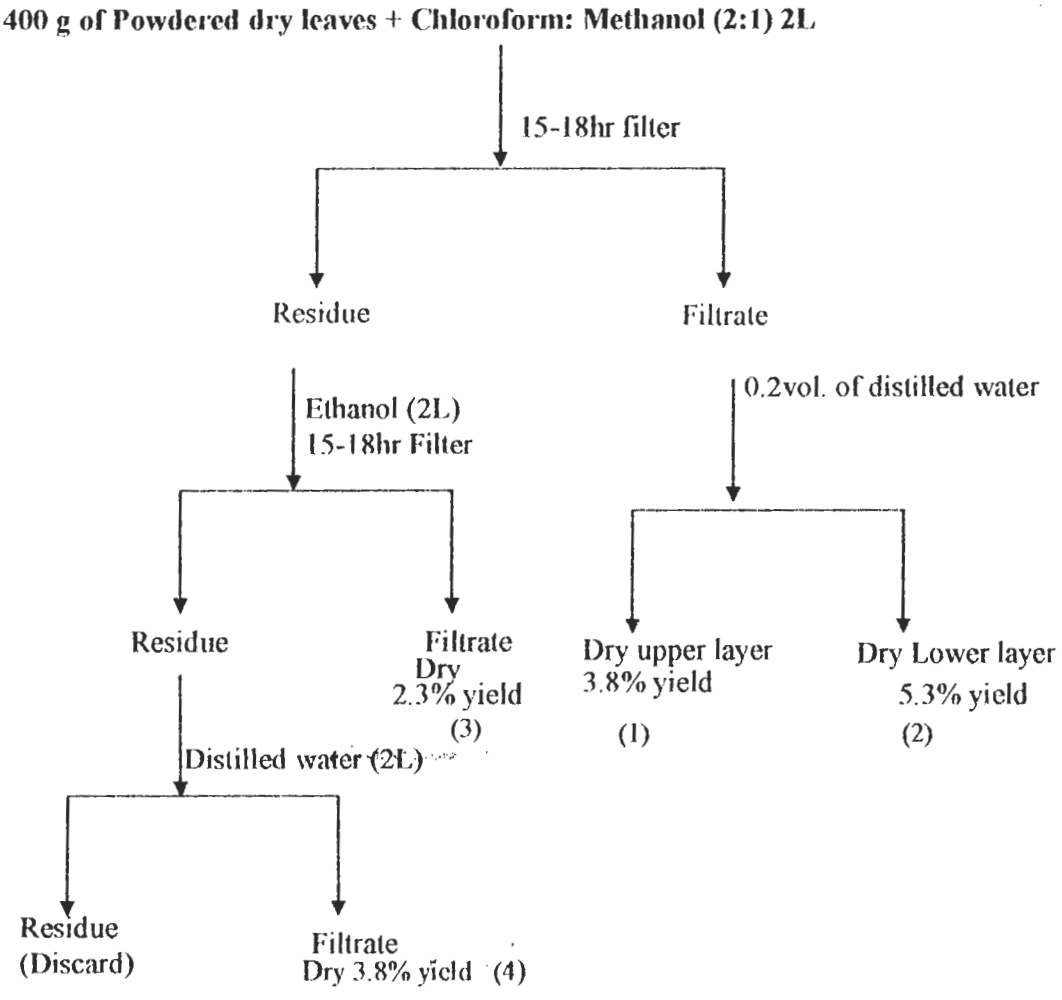

Fig. 3 – Percentage yields after extraction.

3.2 RESULTS OF BLOOD PRESSURE STUDIES

3.2.1 Effect of Upper (Methanol) Layer, Ethanol and water Extracts on Cat Blood Pressure.

Fig 4 shows that the upper (methanol) layer extract (12.5 mg/kg. body weight) has no effect on the arterial blood pressure of normotensive cats. Water extract (WE), at the

blood pressure of the cat by 35.0± 1.24 mmHg. The observed increase in blood pressure was higher than that (24.0 ± 1.50mmHg) obtained, with adrenaline standard (100 µg/kg). Like histamine (100 µg/kg.wt), ethanol extract (EE) of Acalypha torta at 12.5 mg/kg body weight caused an average reduction of 41.0± 2.35 in the arterial blood pressure of normotensive cats. This action was also seen to be immediate and not sustained.

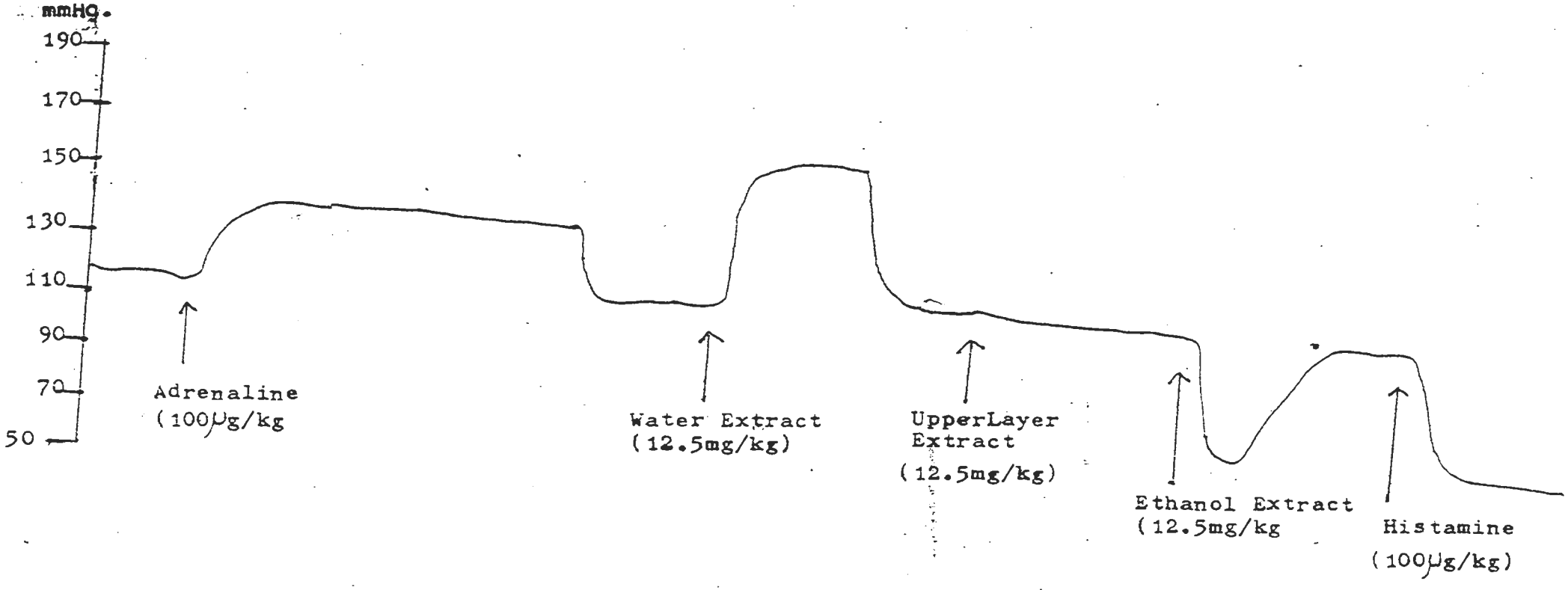

Fig. 4: Effect of upper (methanol) layer, ethanol and water extracts of Acalypha torta leaves on the blood pressure of anaesthetized normotensive cat

3.2.2 Result of Dose-Response Study

The effects observed following intravenous administration of varying doses of the ethanol extract (2.0 – 20.0 mg/kg.wt.) revealed that the blood pressure lowering action of EE was dose – dependent. Minimum activity of 5.33± 1.76 mmHg (app. 3.6% decrease) was obtained at a dose of 2.0 mg/kg.wt of extract and the percentage reduction increased progressively with dose (see Figs 5&6), whereas maximal effect was recorded at a dose of 20.0 mg/kg.wt which lowered the arterial blood pressure of the normotensive cat by an average of 86.33±12.91mmHg, i.e 58.33%.

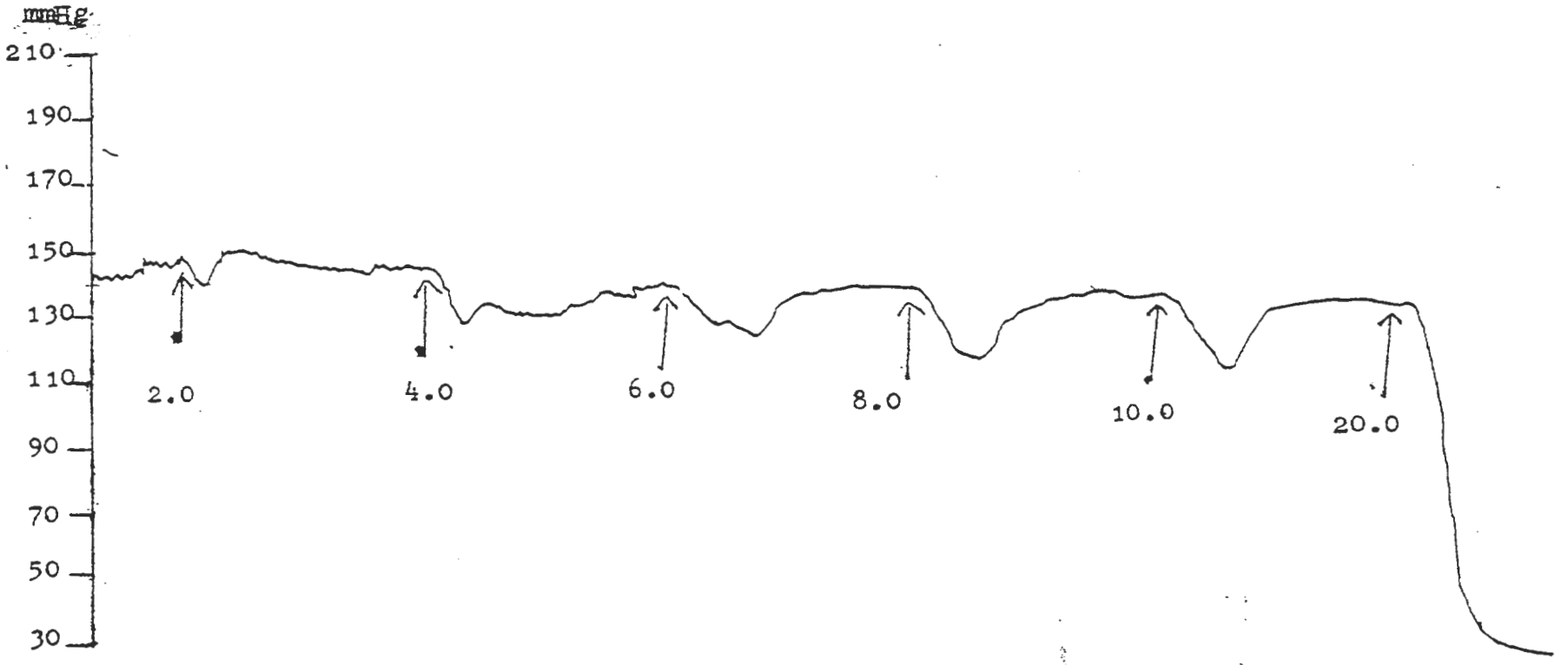

Fig. 5: Effect of varying doses (mg/kg.body wt.) of ethanol extract of A. torta on the blood pressure of anaesthetized normotensive cat.

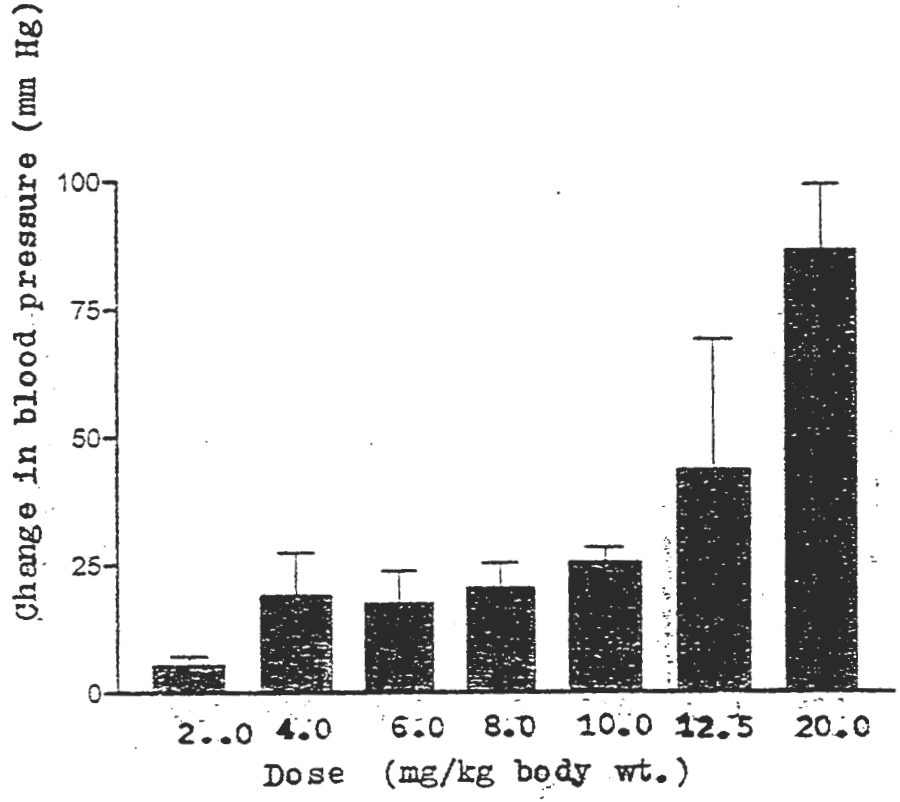

Fig.6: Effect of varying doses of ethanol extract of *Acalypha torta* on the blood pressure of anaesthetised Cat. The effect is given as the average for each dose of the drug in three animals ± SEM.

3.2.3 Control Experiment

Result of the control experiment (Fig. 7) shows that the different solvents chloroform, methanol, ethanol, carboxymethylcellulose, water and normal saline used for the extraction and blood pressure study has no marked effect on the cat arterial blood pressure. While chloroform and methanol caused slight increases of 2.0 and 1.0mmHg respectively, ethanol, carboxymethylcellulose, water and normal saline had no effect on the arterial pressure of normotensive cats.

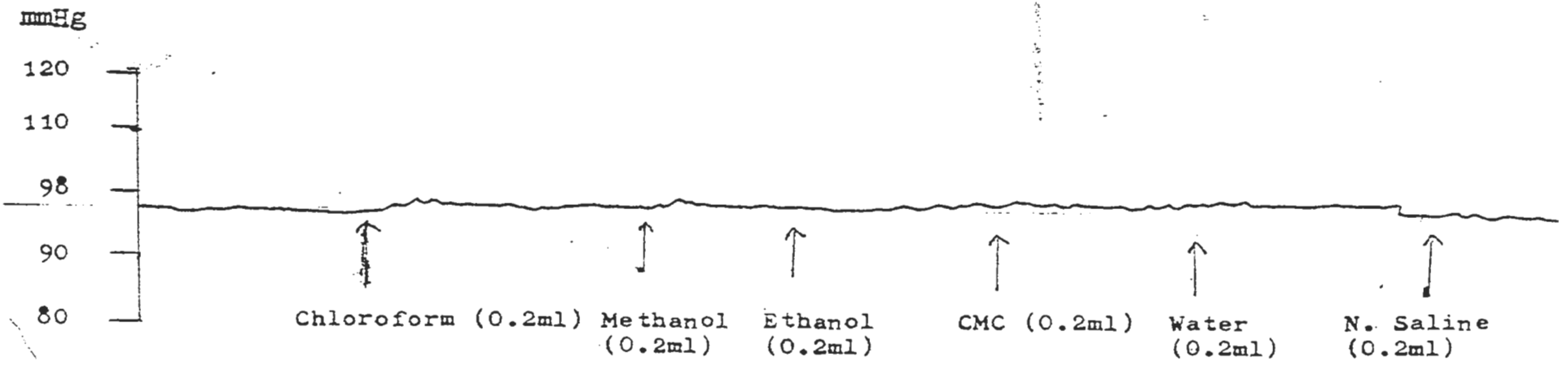

Fig. 7: Effect of the various solvents used on the blood pressure of anaesthetized normotensive cat.

3.3 TOXICITY STUDIES

3.3.1 Acute Toxicity Tests With Mice

From the plot of percentage mortality versus logarithm of dose of extract (Fig.8), the median lethal dose, LD_{50}, of the ethanol extract of *A. torta* was determined to be 562.30 mg/kg.body wt. No lethality was recorded in the control group.

In group 2 mice that received 200 mg/kg. body weight of extract, no aggression, tremor, twitching, and no change in respiration rate were observed . Drowsiness and reduced appetite were noted but the animals were reasonably alert. One death was recorded by the sixth hour after administration. By the 20th hour the remaining mice had regained their normal reflexes together with improved appetite.

In group 3, treated with 500 mg/kg body weight of extract, immediately after injection, all animals were calm, dull and clustered together. Within 8 hr they exhibited drowsiness, one had increase in respiration and later died. Within 23 hr two other mice died, and generally there was loss of appetite. Thus a total of three deaths were recorded within 24 hr.

For group 4 animals receiving (1000 mg/kg doses), no change in behaviour was observed immediately after administration. Within 2-3 hr there was reduced appetite, drowsiness and clustering, but the feeding habit was restored by the 6th hr. Between 15 – 24 hr, five animals died peacefully without twitching, tremor or convulsion.

Among group 5 mice administered 2000 mg. kg (body weight) of extract all the mice became weak and clustered together immediately after treatment, with little increase in respiratory rate. Appetite was also reduced and one of the animals died within hr. Between 18 and 20 hr the remaining five mice died peacefully.

In groups 6 and 7 that received 4000 and 8000 mg/kg.body weight of extract respectively, there was severe convulsion immediately after treatment and all the animals died within 20-30 min of administration.

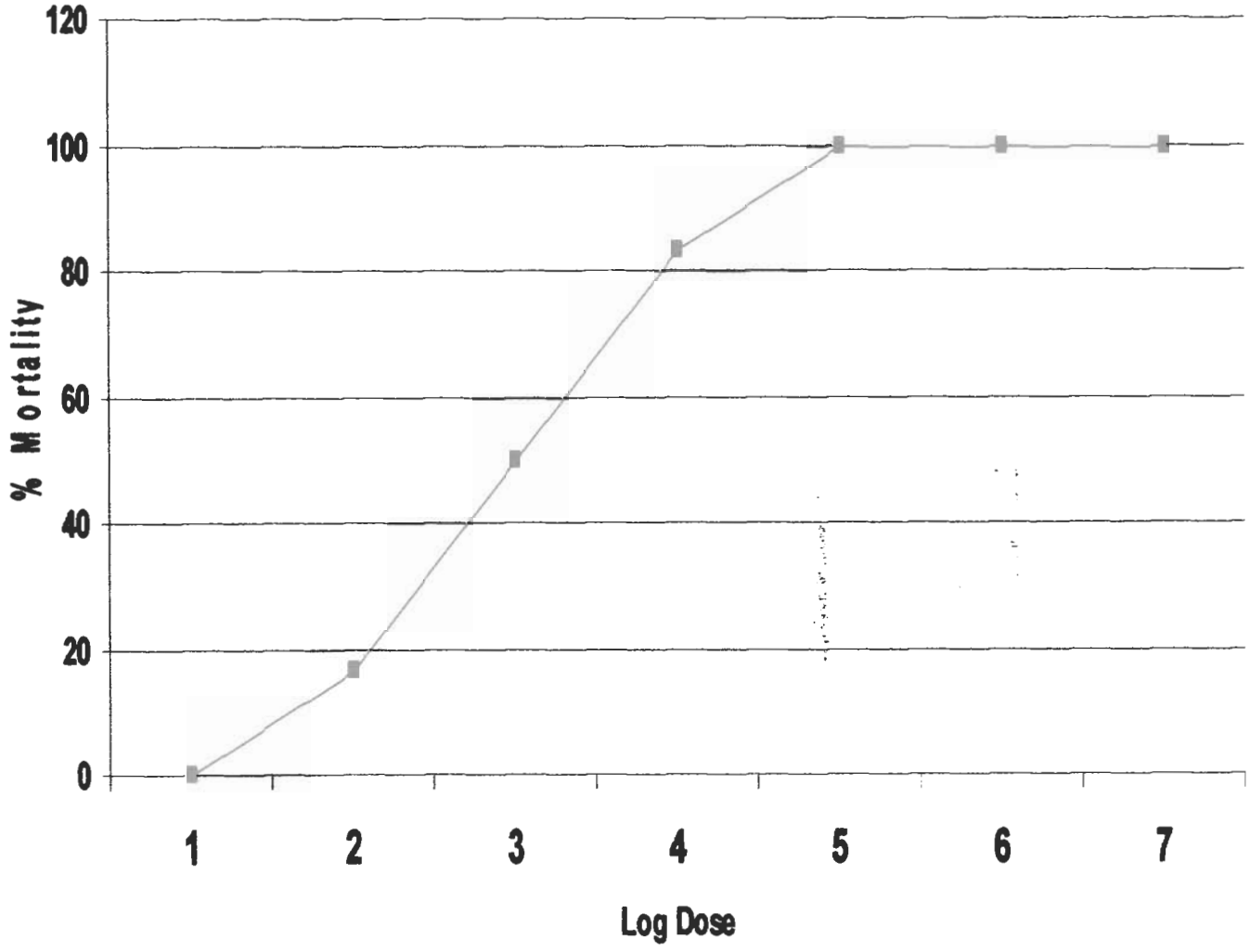

Fig. 8: A plot of percentage mortality against log of dose of ethanol extract of *A. torta* after a 24hr-acute toxicity study in mice

3.3.2 Sub-acute Toxicity Studies in Rats

3.3.2.1 Conditions of the Animals During the Experiment

Day 1: All the animals were agitated at the onset of the experiment and the effect was more in the groups, A, B, C and D, treated with the extract.

Day 3: General and slight enlargement of the internal organs particularly the liver was noticed. Reduced intake of food was obvious. The effect was more in group C which received daily dose of 200 mg/kg. body weight of the extract.

Day 7: All the organs still maintained their normal colours and slight increase in size. Rats in groups A-C still had diminished appetite whereas increased ingestion of food was seen in groups D and E.

Day 14: The animals in groups D and E had assumed their normal feeding habit. Groups A and B animals had slightly enhanced appetite, while poor feeding habit was still noticeable in group C and they appeared weak. Normal weights of the organs had been restored but the livers and spleens were darker in colour for those in groups A, B and C.

Day 28: The animals in groups D and E appeared relatively normal. Those in groups A and B showed slight recovery of appetite. One of the animals in group B died on the 17^{th} day, while all the group C rats died on the 19^{th} day of treatment. The livers and spleens of those in groups A and B were still darker in colour, while organs of animals in D and E were generally normal.

3.3.2.2 Effect of Extract on Mean Body Weight

As shown in Fig. 9, persistent and significant reductions in the body weights of the treated animals were observed throughout the period of study. This effect was more in all the animals that were on daily dose of the extract irrespective of the size of the dose. By the 28^{th} day the body weight had decreased by 32.5% and 33.7% in the groups treated with daily doses of 50 and 100 mg/kg. body weight respectively. For the group that received the highest dose (200 mg/kg body weight), the body weight reduced by 40.3% on the 21^{st} day of the experiment. For the group that received a single dose of 100 mg/kg body weight of the extract, increase in body weight (from 124 ± 0.62 to 132.4 ± 0.64) was recorded by the 14^{th} day. In the control rats gradual increase in weight was observed throughout the period.

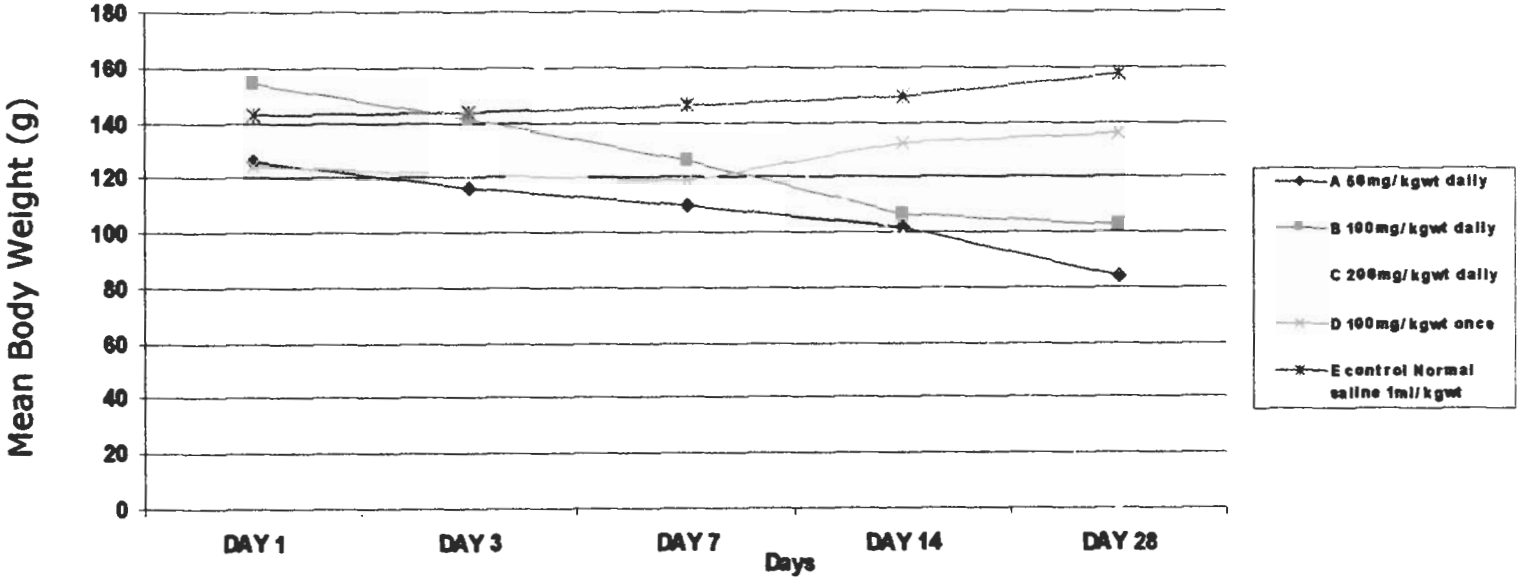

Fig. 9: Effect of ethanol extract of *A. torta* on mean body weight of rats

3.3.2.3 Effect of Extract on Serum Alkaline Phosphatase Activity

When compared with the control animals, the extract did not have any significant effect on the activity of alkaline phosphatase. (Fig.10). Although there were fluctuations, normal alkaline phosphatase activity was maintained in all the groups throughout the 28days of the study.

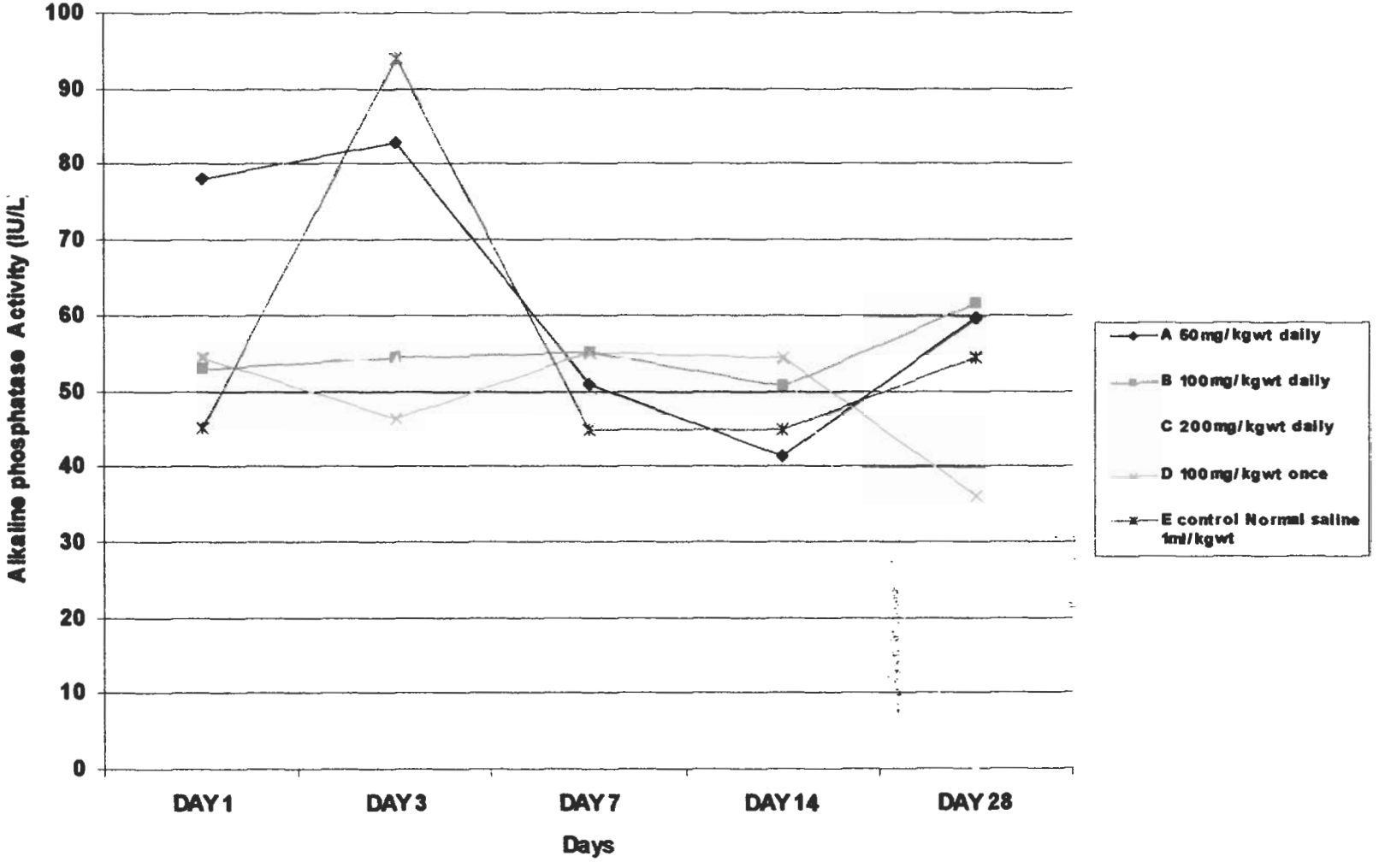

Fig. 10: Effect of Ethanol Extract of *A. torta* on serum alkaline phosphatase activity

3.3.2.4 Effect of Extract on Serum Alanine Transaminase Activity

Fig. 11 shows that the extract caused no significant influence on the serum alanine transaminase activity of the treated animals when compared to the control animals. This activity was also maintained within the normal range observed in the control (untreated) animals.

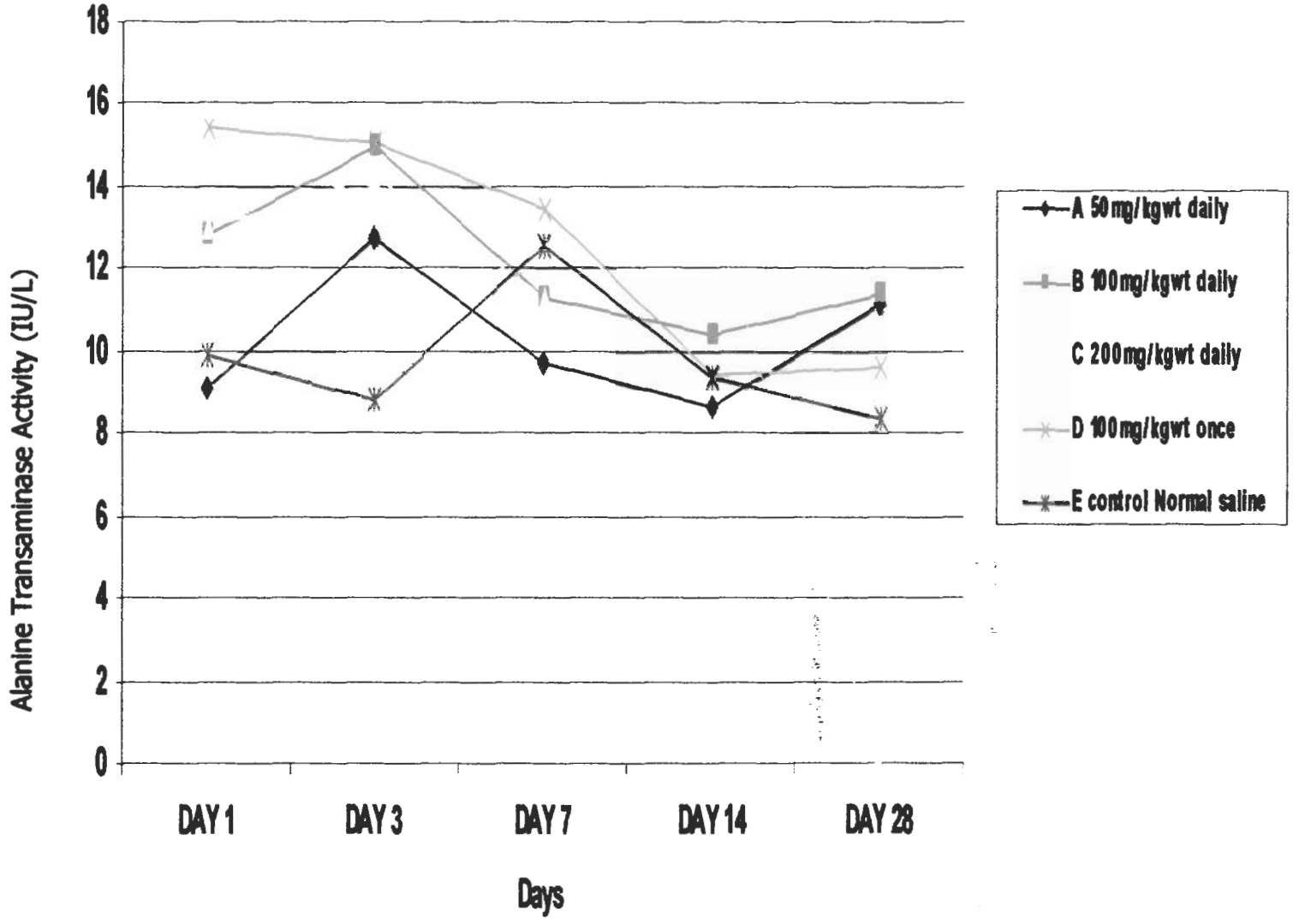

Fig. 11: Effect of ethanol extract of *A. torta* on serum alanine transaminase activity

3.3.2.5 Effect of Extract on Serum Aspartate Transaminase Activity

Significant ($p<0.0001$) increases in aspartate transaminase activity were observed in all the groups (treated and untreated) on the 7th day of the experiment. i.e. 52.54%, 27.17%, 34.25% and 32.86% in the groups treated with 50, 100, 200 mg/kg body weight of extract, and control group respectively. But by the 25th day, normal activity had been restored in the groups that received 50 mg/kg body weight daily, 100 mg/kg body weight once and control group whereas consistent increases were recorded in the groups that were given higher doses (100 and 200 mg/kg body weight) of the extract (see Fig. 12).

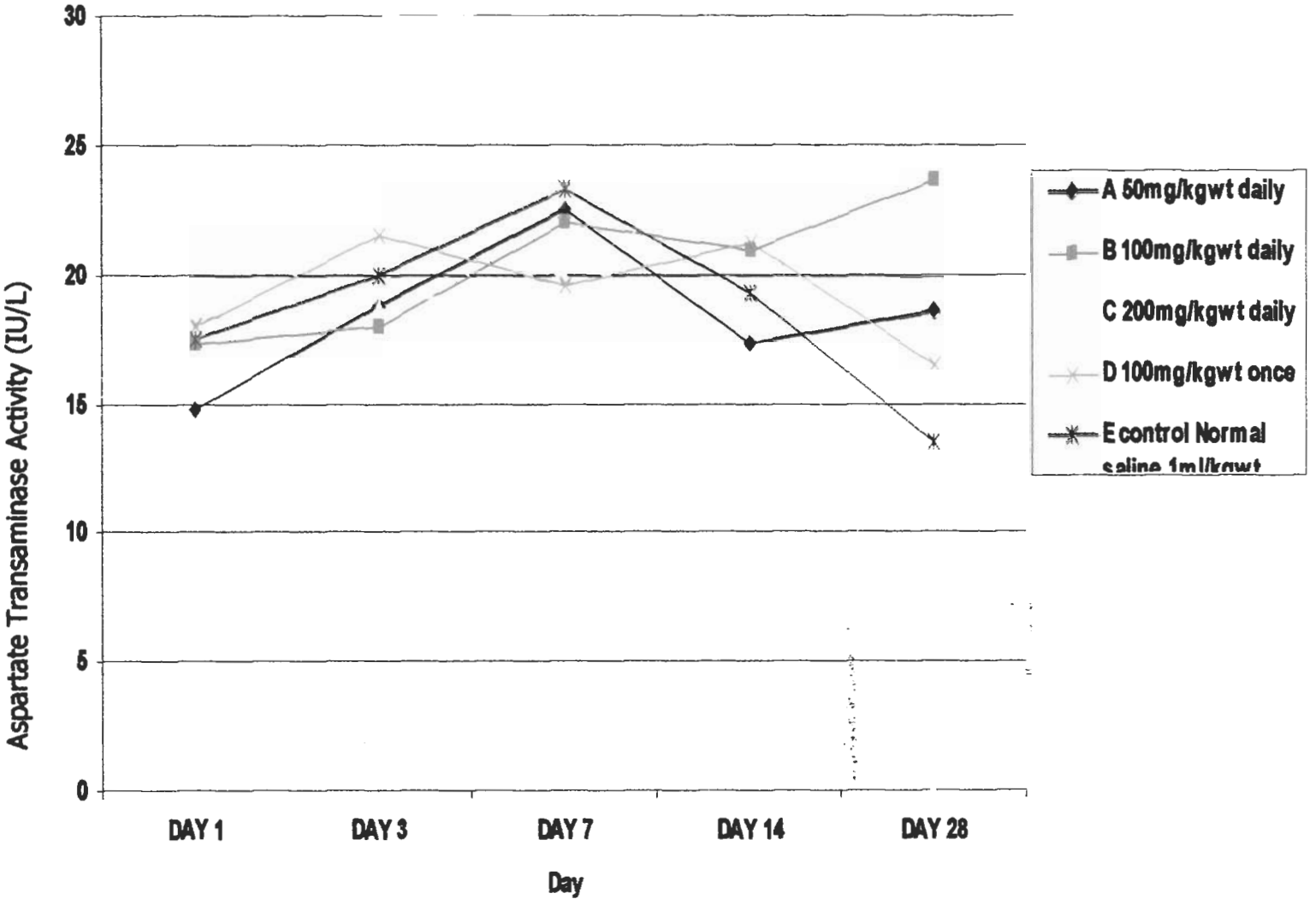

Fig. 12: Effect of Ethanol Extract of *A. torta* on serum aspartate transaminase activity

3.3.2.6 Histopathological Evaluation

LIVER: The histological changes found in the liver included prominent necrosis and sinusoidal dilation (in the rats dosed 100 mg/kg daily for 28 days). There were pyknosis and hyperchromasia with mononuclear cell infiltration (in group A which received 50 mg/kg body weight of extract) indicating evidence of hepatitis. Normal liver architecture was noted in groups E (control) and D (100 mg/kg body weight of extract once).

SPLEEN

The photomicrograph of the splenic section of rats dosed 50 mg/kg body weight (group A) showed vascular congestion and follicular disorganization. This change was also noted with daily dose of 100 mg/kg.body weight of extract. There were haemorrhagic areas and stromal infiltration of chronic inflammatory cells. Single dose treatment with 100 mg/kg.body weight of extract did not alter the normal architecture of any of the organs examined when compared to that of control animals.

BRAIN

A cross section of the brain of rats in group A (50 mg/kg body weight) displayed evidence of mild fibrosis in some parts. It also showed nuclear eosinophilia and chromatolysis, though these changes were mild. These same changes were seen in group B which received 100 mg/kg body weight of extract for 28 days. The nuclei were deeply stained (i.e. nuclear hyperchromasia).

HEART

The histological changes of the heart section of rats dosed 50 and 100 mg/kg.body weight respectively showed ventricular wall fibrosis with lymphoplasmacytic cells and perinuclear haloes.

KIDNEY

There were tubular necrosis and hyalinization in the kidney section of rats dosed 50 mg/kg body weight of the extract (group A). In group B dosed 100 mg/kg body weight

daily glomerular degeneration was noted. Droplets of hyaline materials were seen in the tubules.

LUNG

Lung section revealed arterial hyalinization and attenuation in rats dosed 50 mg/kg body weight daily for 28 days. Lymphocytes were noted stromally. The photomicrograph the lung of rats of the 100 mg/kg body weight group displayed architecture of vascular rupture and congestion. Bronchial dilatation was also noted.

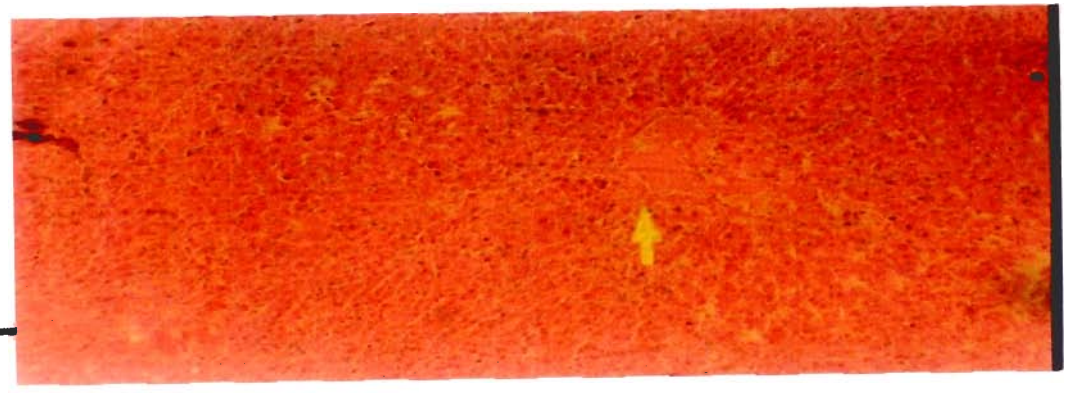

Plate 1: Liver section of the control group of rats showing normal architecture(H&E Stain X 100)

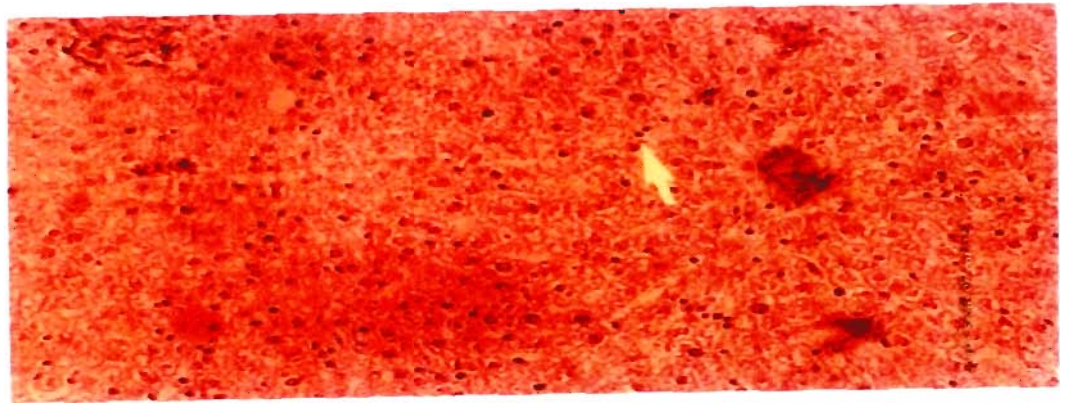

Plate 2: Liver section of rats dosed 50mg/kg wt/day showing hyperchromasia and mononuclear cell infiltration (H&E Stain, ×400)

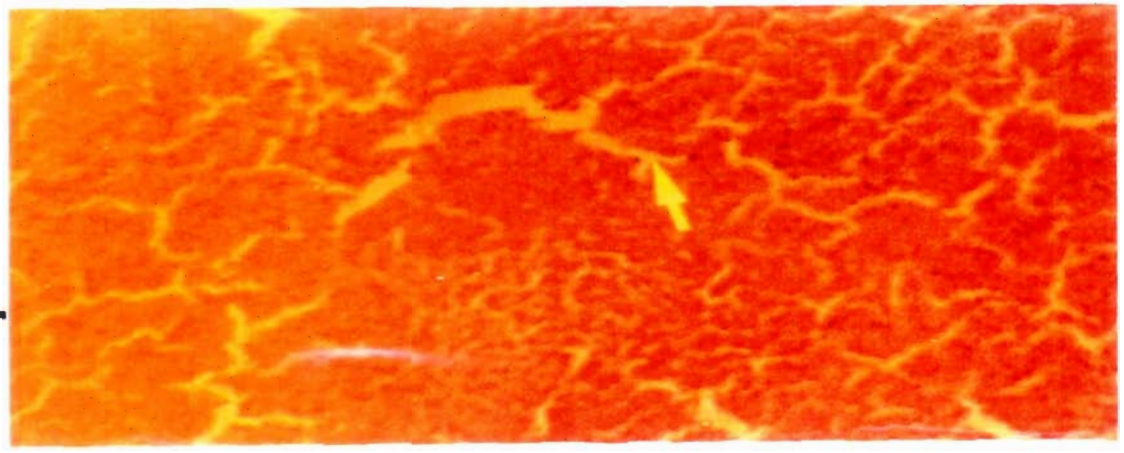

Plate 3: Liver section of rats treated with 100mg/kg wt/day showing necrosis and sinusoidal dilation. (H&E Stain, × 100).

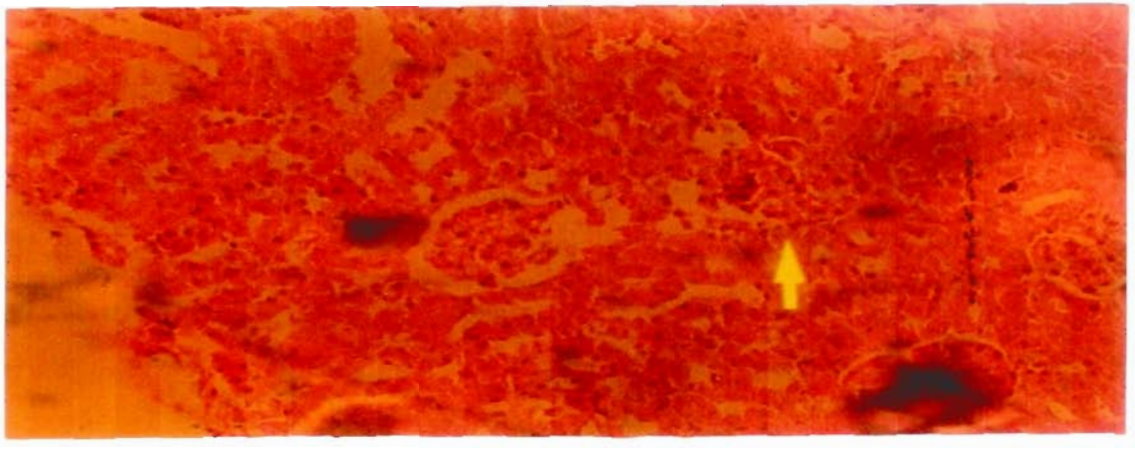

Plate 4:Kidney section of control group showing normal architecture. (H&E Stain, ×400).

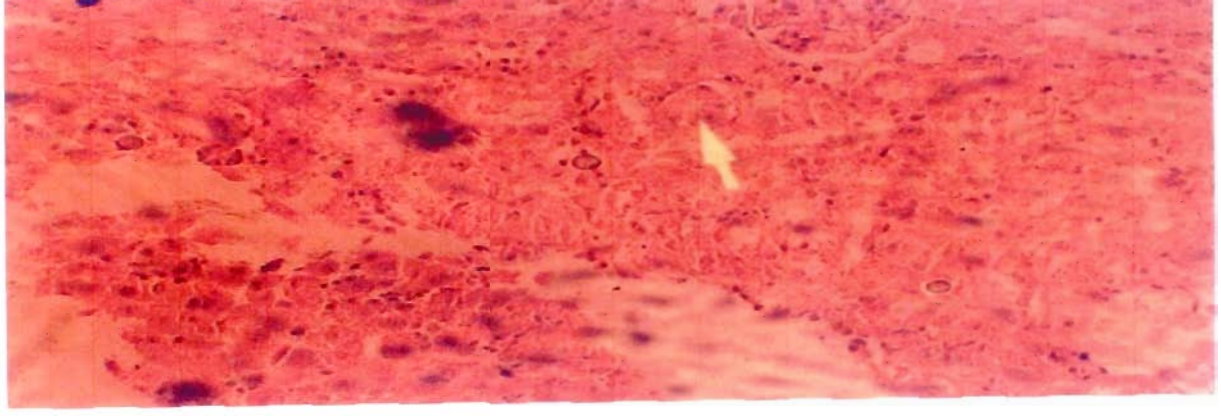

Plate 5: Kidney section of rats dosed 50mg/kg Wt./ days showing tubular necrosis and Hyalinization. (H&E Stain, × 400).

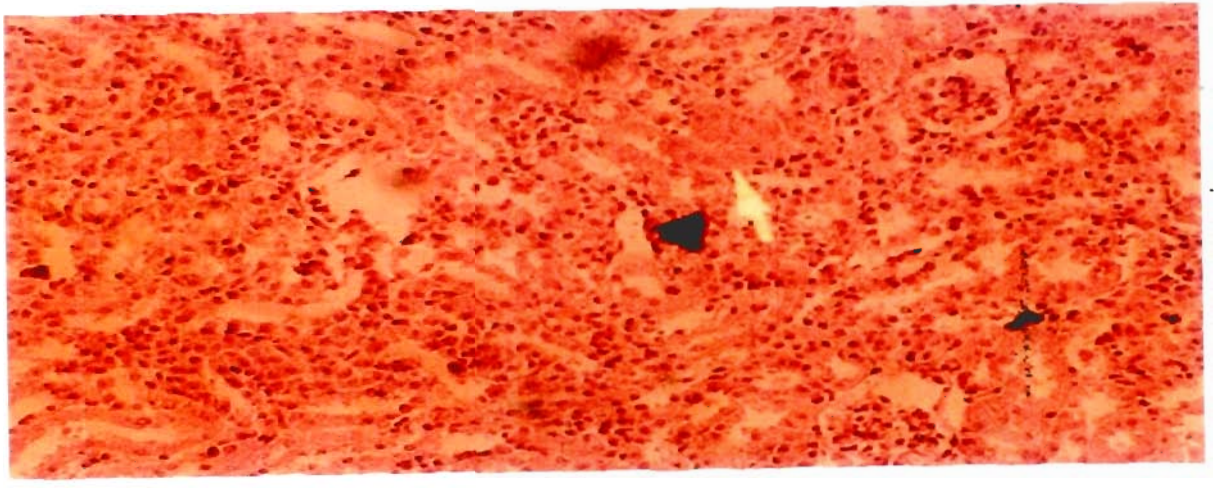

Plate 6: Kidney section of rats dosed 100mg/kg Wt./days showing glomerular degeneration (arrowed) . (H&E Stain, × 100).

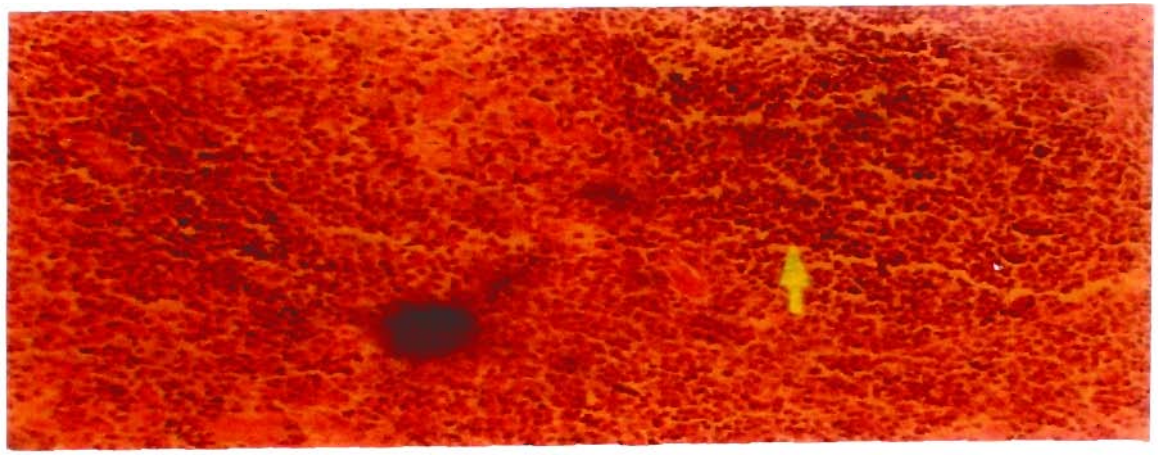

Plate 7: Splenic section of control rat (H&E Stain, × 400)

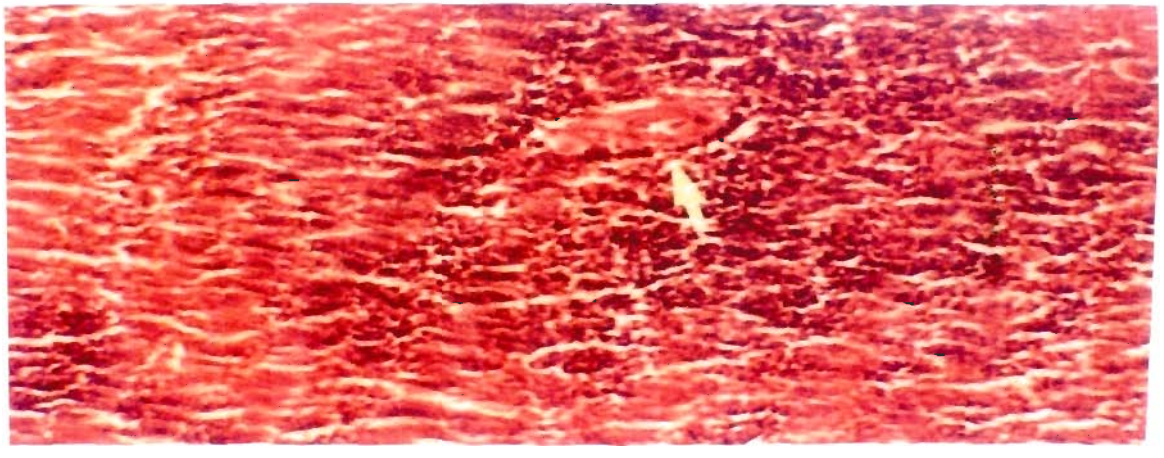

Plate 8: Splenic section of rats given 50mg/kg wt./day showing vascular congestion and follicular disorganization (H&E Stain, ×100)

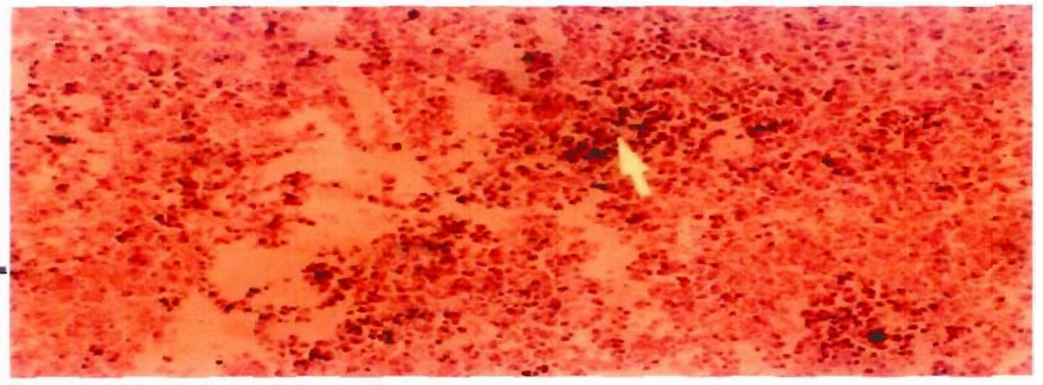

Plate 9: Splenic section of rats dosed 100mg/kg.wt/day also showing follicular disorganisation and chronic inflammatory cells (arrowed).(H&E Stain, ×100).

Plate 10: Heart section of control rats (H&E Stain, ×400).

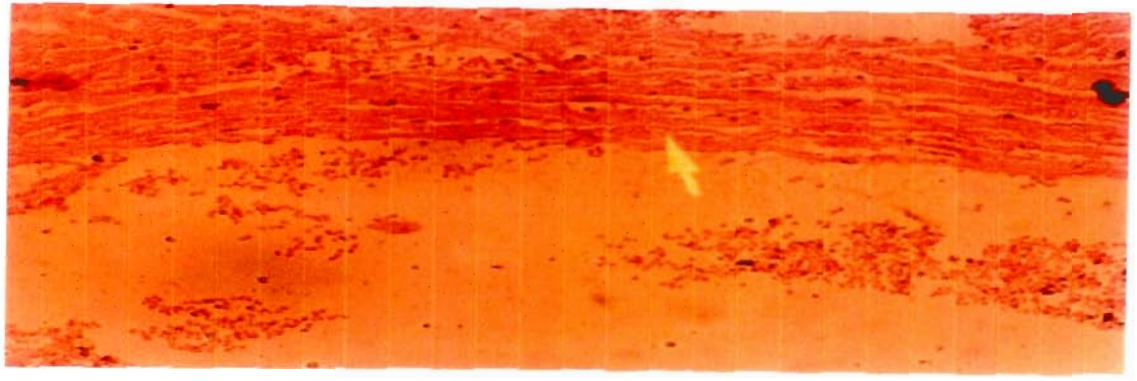

Plate 11: Heart section of rats dosed 50 and 100mg/kg Wt/day showing ventricular wall fibrosis (See Arrow) (H&E Stain, ×400).

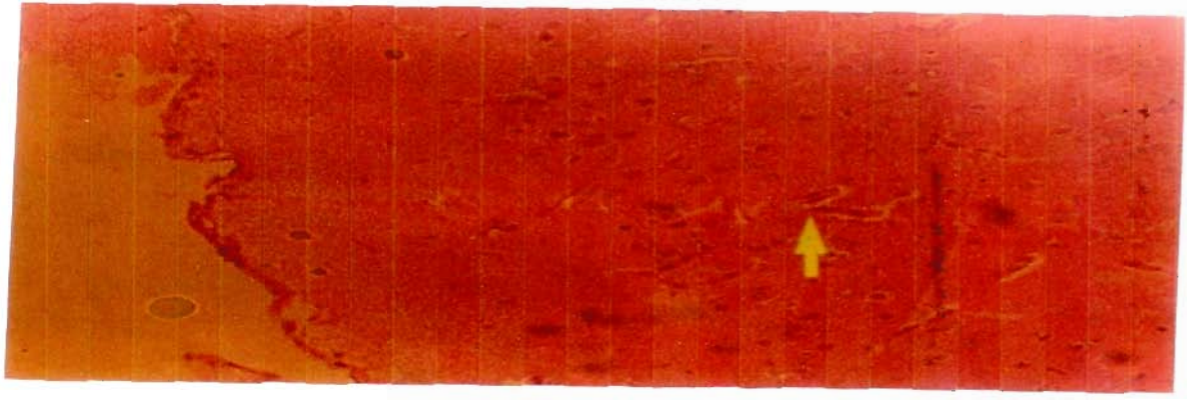

Plate 12: Brain section of control rats.
(H&E Stain, × 400).

Plate 13: Brain section of rats given 50mg/kg wt./day showing mild fibrosis and nuclear eosinophilia. (H&E Stain, ×100)

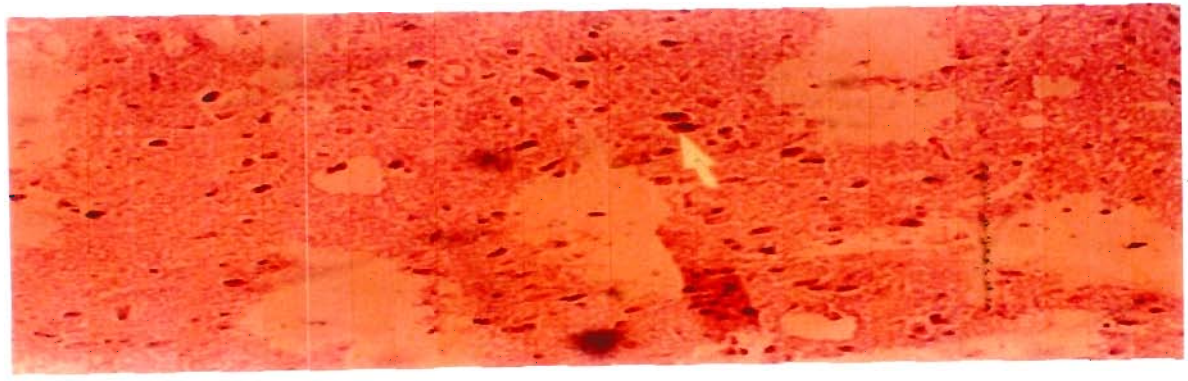

Plate 14: Brain section of rat dosed 100mg/kg wt./day showing nuclear hyperchromasia and eosinophilia. (H&E Stain, ×400).

Plate 15: Lung section of the control rat (H&E Stain, ×100).

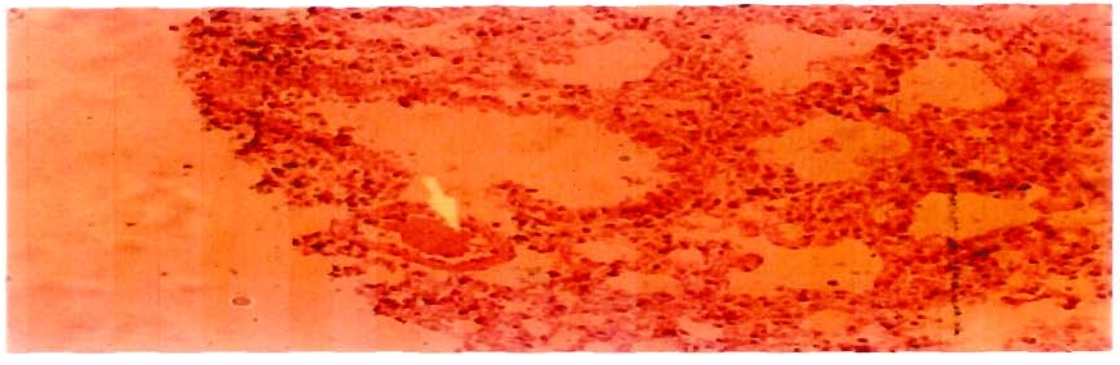

Plate 16: Lung section of rats treated with 50mg/kg wt./day showing arterial hyalinization (H&E Stain, ×100).

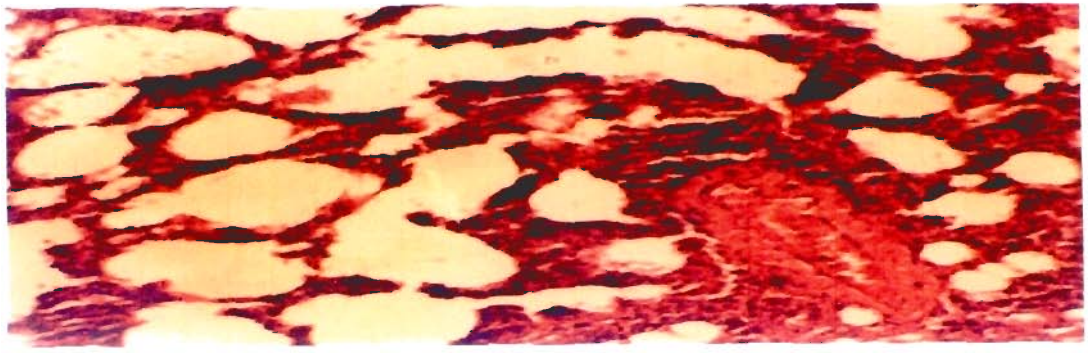

Plate 17: Lung section of rats treated with 100mg/kg wt. showing bronchial dilation and arterial congestion. (H&E Stain, ×400).

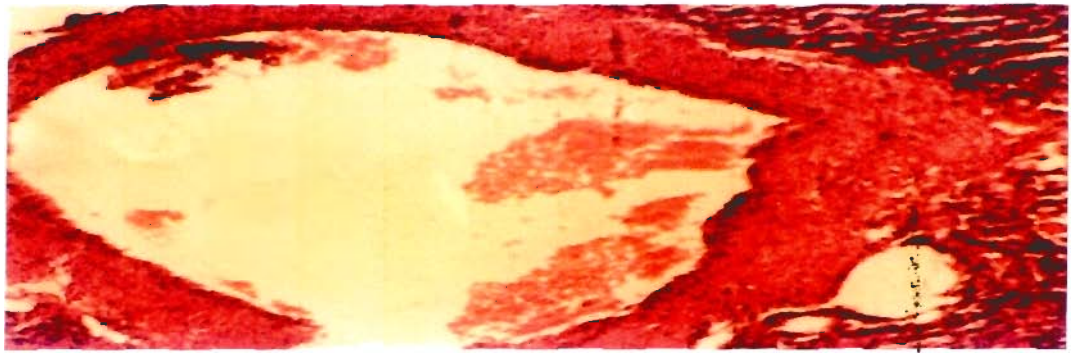

Plate 18: Lung section of rats dosed 100mg/kg wt. showing vascular wall rupture. (H.E. Stain × 400)

3.4.0 Mechanism of Action

3.4.1 Receptor Blocking Study

Fig. 13A shows that the blood pressure-lowering effect of the extract was completely abolished in the presence of promethazine (20 μg/kg). The average change in blood pressure was moved from –16 mmHg (caused by extract, 20 mg/kg body weight, alone) to + 1.0 mmHg (caused by promethazine, 20 μg/kg + extract, 20 mg/kg body weight).

In the study on cholinergic transmission, Fig. 13B, it was also observed that the ability of the extract to reduce blood pressure was blocked in the presence of the cholinergic receptor blocker, atropine. The change in blood pressure (mmHg) was from –22 (caused by extract, 20 mg/kg body weight, alone) to +4.0 (caused by atropine, 20 μg/kg + extract, 20 mg/kg).

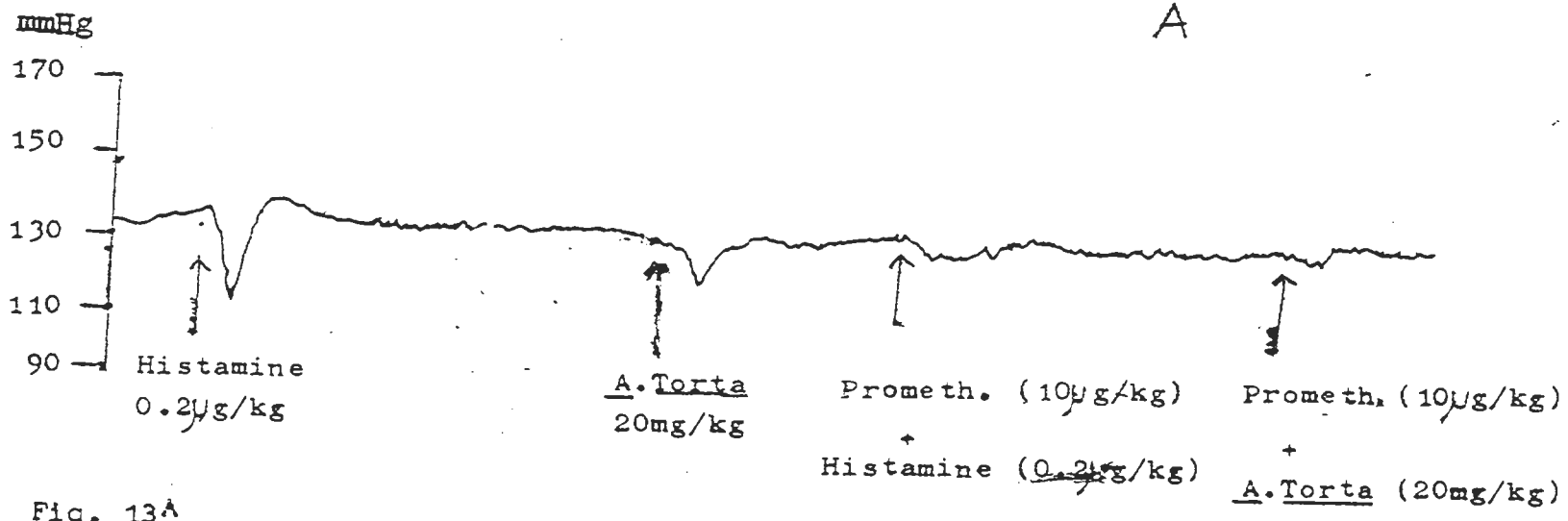

Fig. 13[A] Effect of promethazine on the blood pressure lowering action of ethanol extract of A. torta.

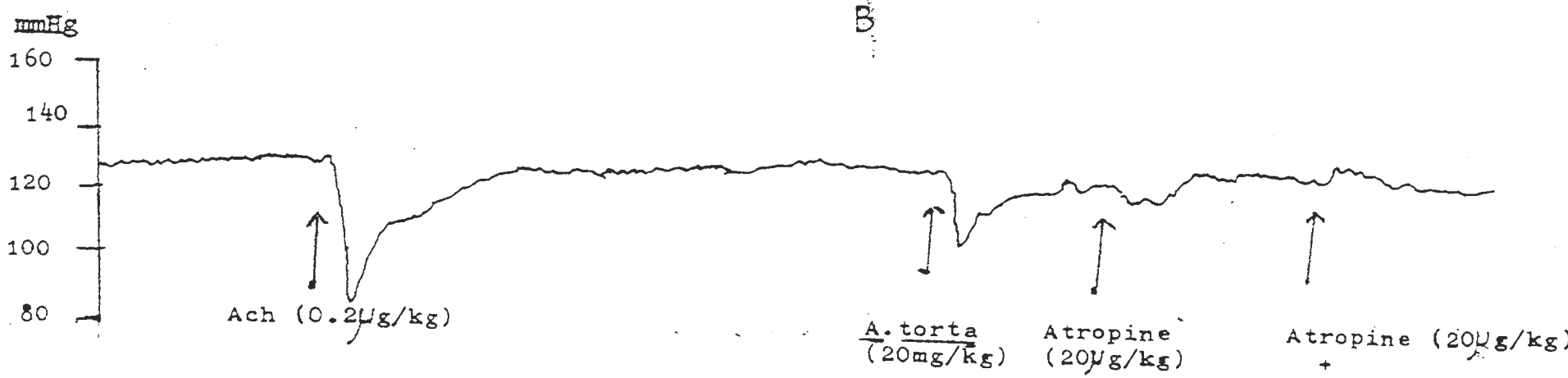

FIg. 13[B]: Effect of atropine on the blood pressure lowering action of ethanol extract of A. torta.

3.4.2 Effect of Extract on Perfused Rat Hind-Quarters Preparation

Results presented in Fig. 14 revealed that the ethanolic extract of *A. torta* increased the flow rate of physiological fluid in the rat hind-quarters preparation. The magnitude of the increase was found to vary with the concentration of the extract. Minimal increase of 4.15% $\pm$ 0.48% was obtained at 13.30 mg/ml extract concentration, while maximum increase of 30.98 $\pm$ 3.06 was caused by 39.90 mg/ml of extract.

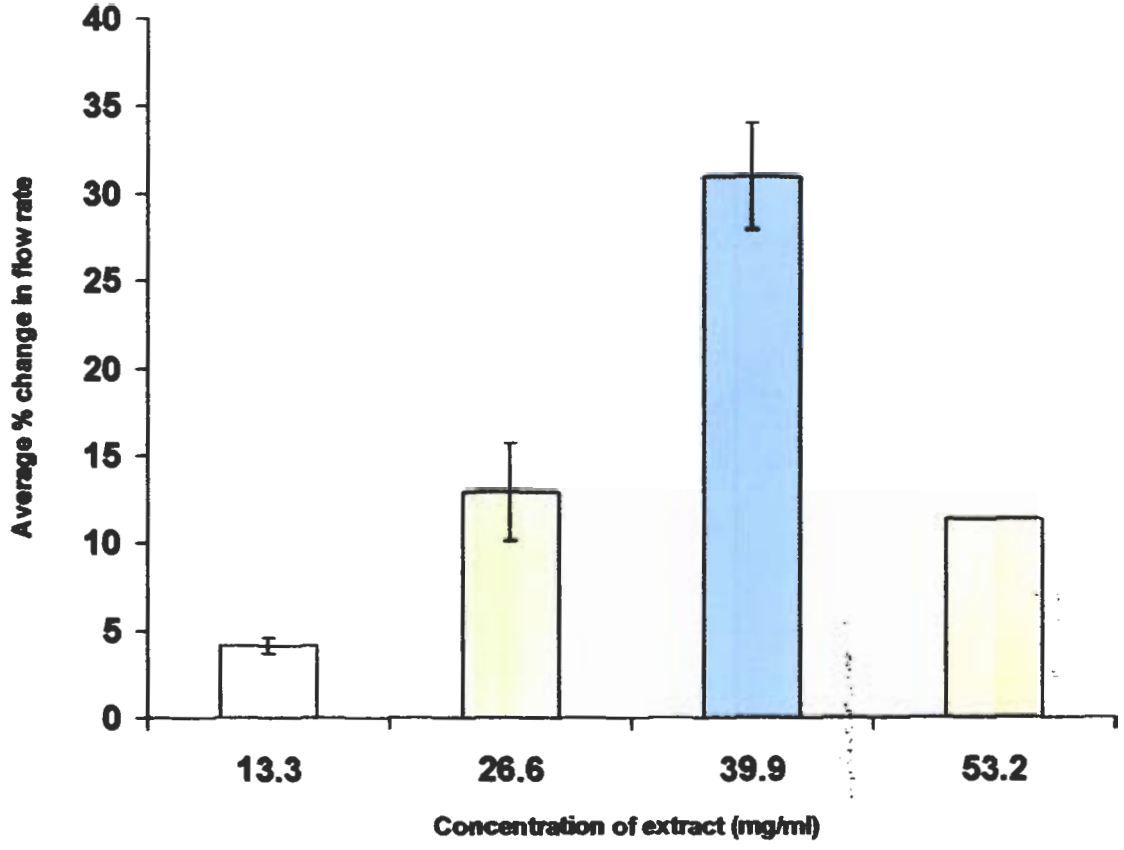

Fig. 14: Effect of *A. torta* extract on the flow rate of fluid through the rat hind quarters preparation

3.4.3 Effect of Extract on Isolated Rabbit Arterial Strips

From tracings in Fig. 15 it was obvious that adrenaline,60 μg, induced contraction of rabbit arterial preparation. Incubation of the arterial strips with *A. torta* extract (10.0 mg), 2.0 minutes, before the injection of adrenaline (60 μg) caused a reduction of 16.7% in adrenaline-induced contraction. A higher dose, (20 mg) of extract diminished adrenaline-induced contraction of arterial strips by 33.3% within 20.0 minutes, and this action was completely abolished after 20.0 minutes.

Fig. 15: Effect of ethanol extract of *A. torta* on adrenaline - induced contraction of rabbit arterial strips.

3.4.4 Effect of Extract on Isolated Rabbit Heart Preparation

Fig. 16A shows the tracings of the contraction of perfused rabbit heart. It is evident that the extract had no effect on the rate of contraction of the perfused rabbit heart but the force of contraction was greatly reduced. The normal height of contraction was 7.0 mm but when 5.0 mg of extract was introduced an immediate reduction in the height of contraction to 2.0 mm (i.e. 71% reduction) was observed. This response was also found to be concentration – dependent (fig. 16B).

Calcium chloride (3.0 & 6.0 mg) induced abnormally rapid action of the heart. This action of $CaCl_2$ was also completely abolished by 5.0 mg of *A. torta* extract.

Fig. 16A: Tracings showing the effect of ethanol extract of *A. torta* on contraction of perfused rabbit heart.

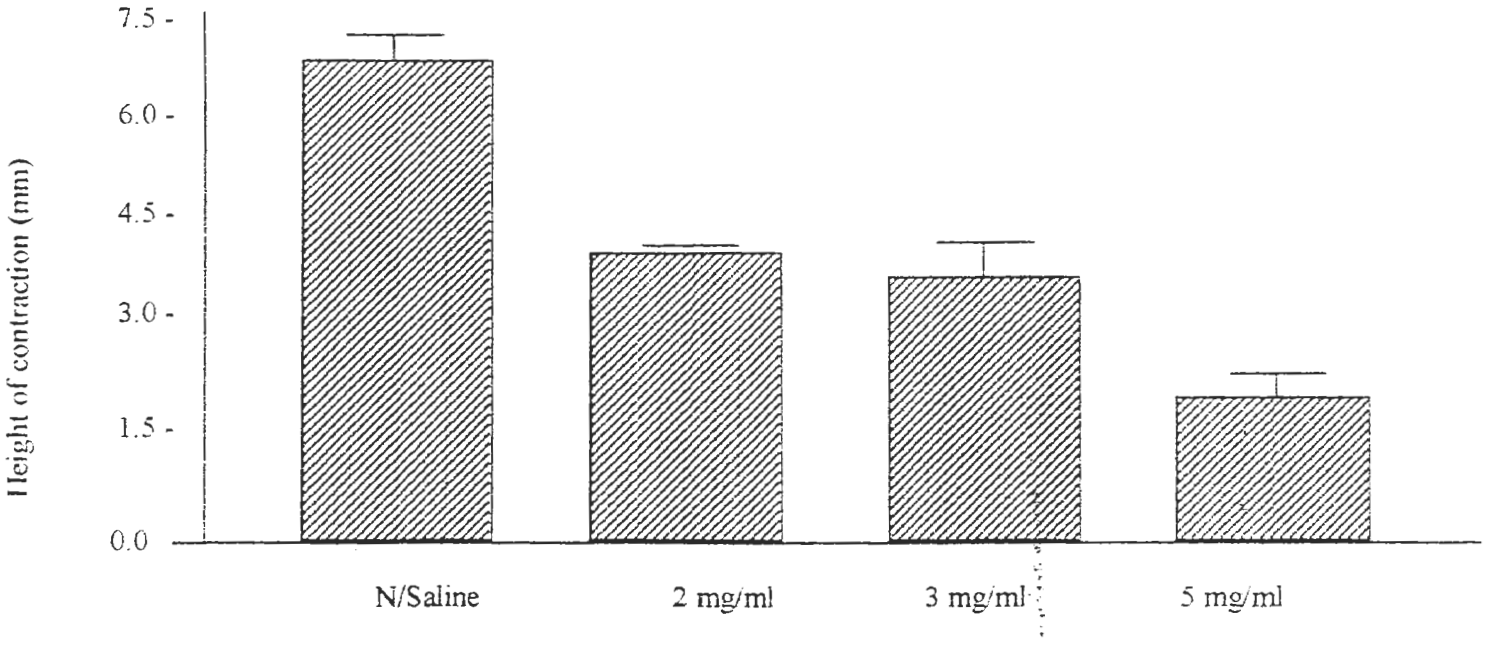

Fig. 16: Effect of ethanol extract of *A. torta* on the force of contraction of isolated rabbit heart.

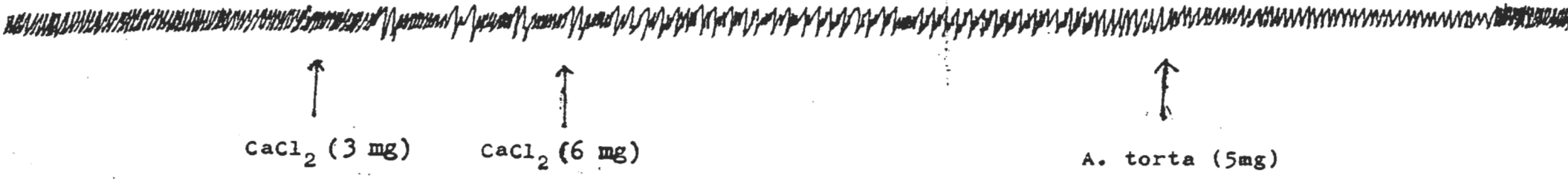

Fig. 16^B: Effect of ethanol extract of A. torta on calcium chloride - induced perturbation of rabbit heart contraction.

3.5.0 OTHER PHARMACOLOGICAL ACTIVITIES OF THE EXTRACT

3.5.1 Effect of Extract on Isolated Rabbit Gut

Fig.17 shows spontaneous contraction of the rabbit gut in the absence of drugs. The average height of contraction on stabilization was 0.80 ± 0.03 cm. Following the introduction of *A. torta* extract, 2.5 mg, the average height of contraction was doubled to 1.7 ± 0.4 cm. Further increases in the concentration of the extract to 5.0, 7.5 and 10.0 mg did not cause any change in the magnitude of the response to the extract. Histamine, 0.002 µg, abolished this spontaneous contraction of isolated rabbit gut, whereas acetylcholine, 0.002 µg, induced prolonged contraction of this tissue.

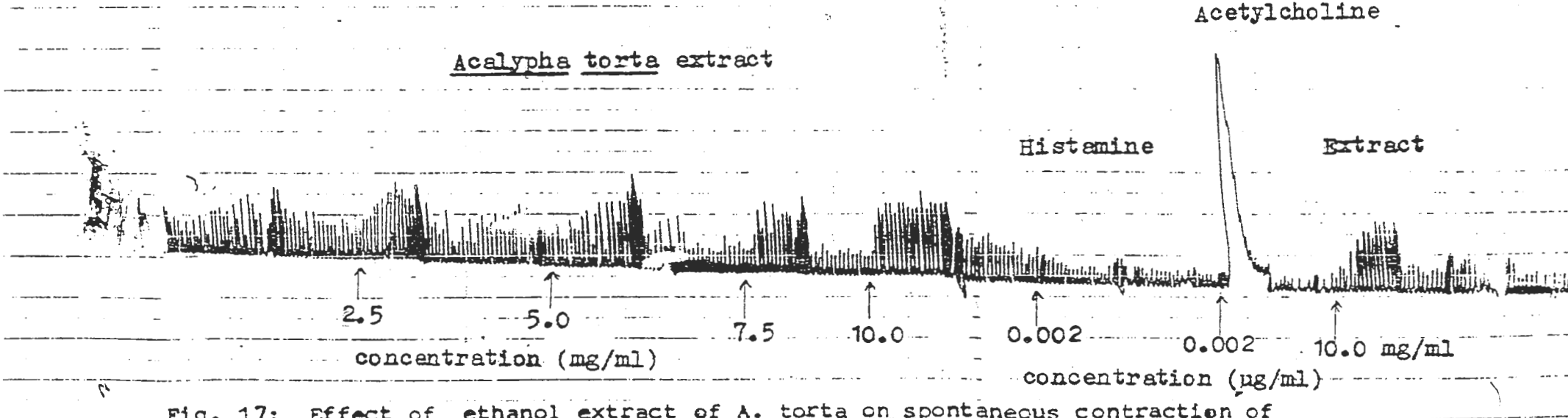

Fig. 17: Effect of ethanol extract of A. torta on spontaneous contraction of isolated rabbit gut.

3.5.2 Effect of Extract on $CaCl_2$ – Induced Platelet Aggregation

Fig. 18 shows the effect of the extract on $CaCl_2$- induced platelet aggregation. Like normal saline (control), *A. torta* extract *per se* did not have any effect on the aggregatory property of blood platelets in the absence of $CaCl_2$. Addition of $CaCl_2$ (4 mM) into the medium caused approximately 88.40% aggregatory effect. Incubation of the platelet – rich plasma with 5 mg/ml of *A. torta* extract decreased the observed aggregatory response by 78.55% (i.e from 88.20 ± 0.09 to 11.75 ± 0.43). This activity was dose-dependent as 15 mg/ml of the extract completely abolished the aggretory effect of $CaCl_2$.

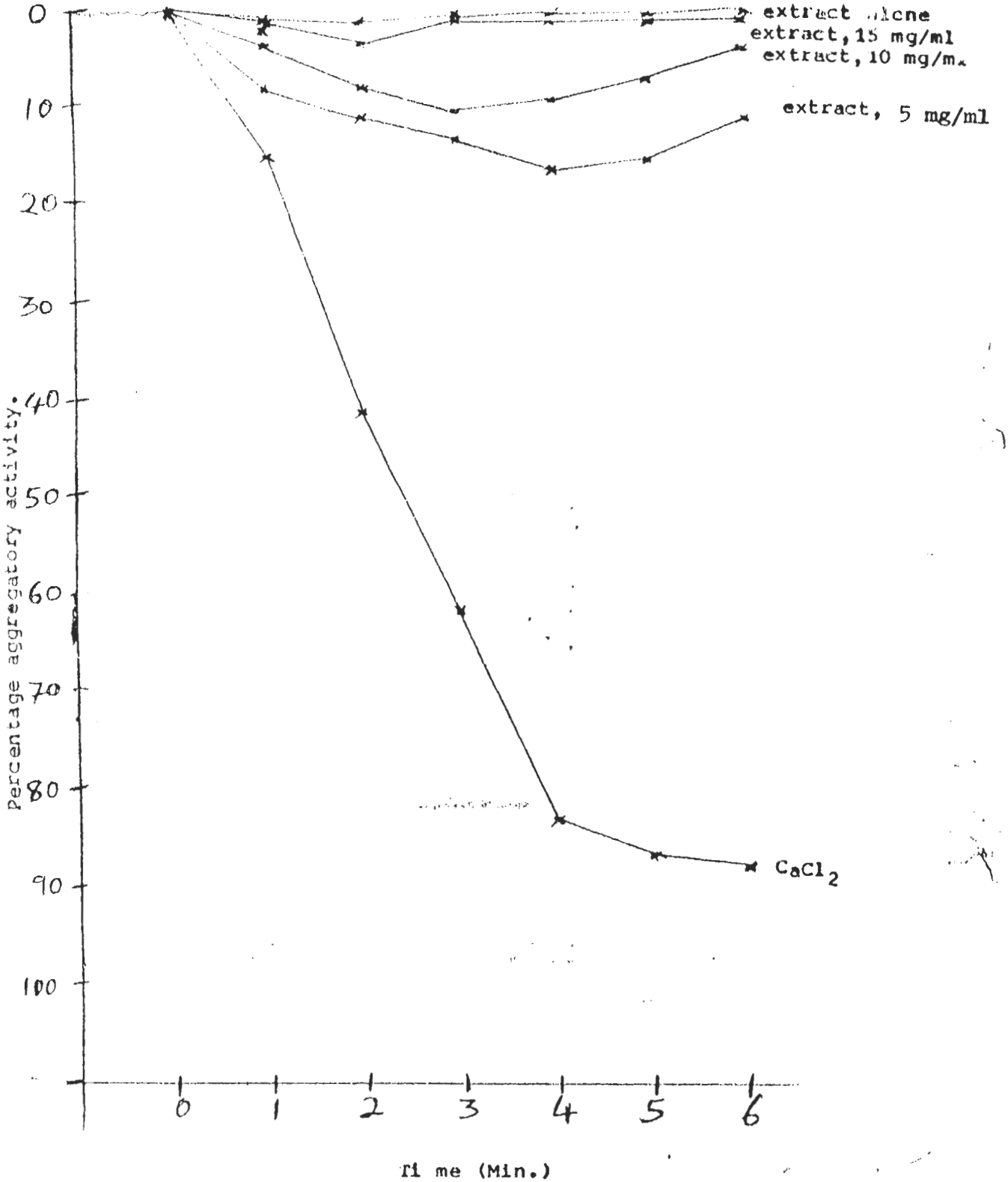

Fig. 18: Effect of ethanol extract of A. torta on calcium chloride - induced platelet aggregation.

3.5.3 Lipid Profile of Rabbits

3.5.3.1 Effect of Extract on Serum Triacylglycerol levels

Comparison of the changes triacylglycerol amount of treated groups of animals with that of the control showed that the extract caused a fall in the serum triacylglycerol levels by the 21st day of treatment – 23.50% and 35.20% decreases at 10.0 mg/kg and 20.0 mg/kg body weight respectively.

Increases in triacylglycerol levels of all the animals were noted on the 7th day of treatment. But steady and significant ($p<0.003$) increase in the triacylglycerol level was observed in the control group (see Fig .19).

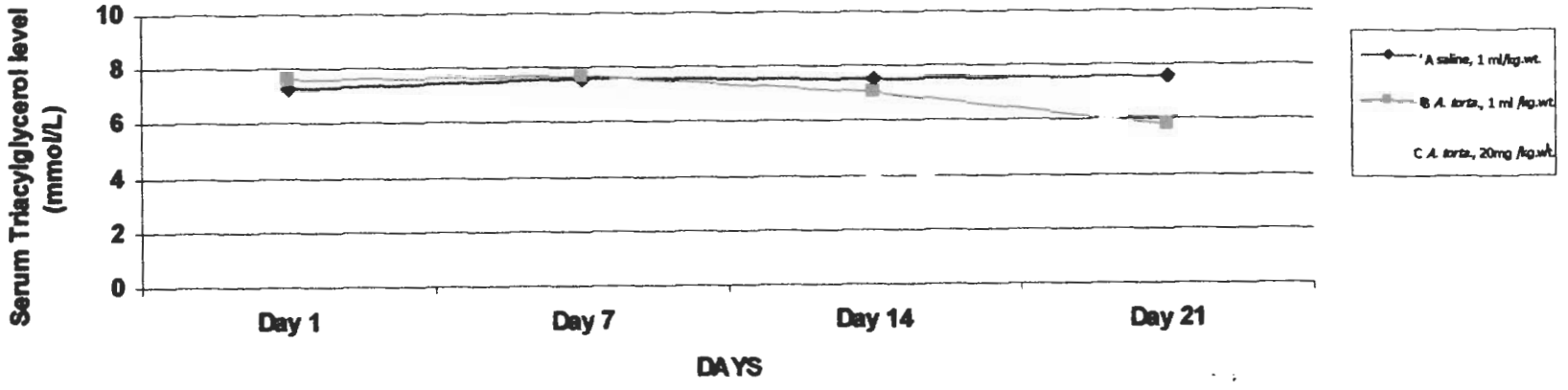

Fig 19:Effect of ethanol extract of *A. torta* on serum triacylglycerol levels in rabbit

3.5.3.2 Effect of Extract on Total Cholesterol Levels.

Fig.20 shows that the extract lowered the serum total cholesterol levels in all the treated groups at the end of the 21days of treatment. This effect was found to be dose-dependent i.e 20.50% and 53.31% reductions at 10.0 mg/kg and 20.0 mg/kg body weight respectively. Increases in total cholesterol levels of all the animals were recorded on the 7^{th} day while that of the control group consistently increased up to the 21^{st} day.

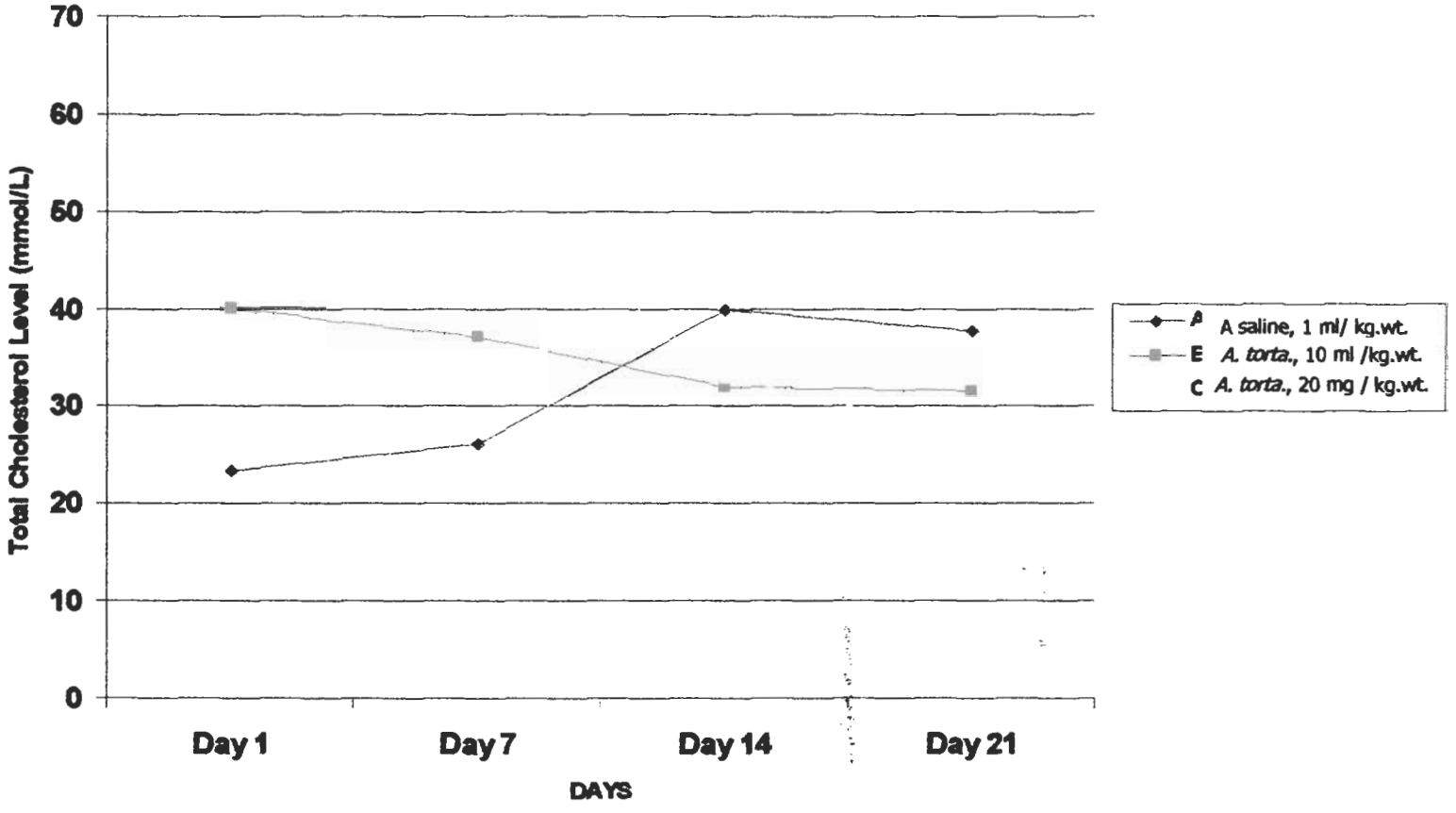

Fig 20: Effect of ethanol extract *A. torta* on serum total cholesterol level (mmol/L) in rabbit

3.5.3.3 Effect of Extract on Serum LDL Cholesterol Levels

Results presented in Fig.21 show that a steady increase in LDL – Cholesterol level was observed in the control group of rabbits. On the other hand, *A. torta* extract, at 10.0 mg /kg. body weight, reduced the LDL-cholesterol concentration by 30.76% on the 14th day. A higher dose of the extract (20.0 mg/kg. body weight) produced greater reduction (66.16%) in the LDL-cholesterol level by the 14th day. Surprisingly, increases in the LDL-cholesterol levels of the treated animals were observed after 14th day of treatment.

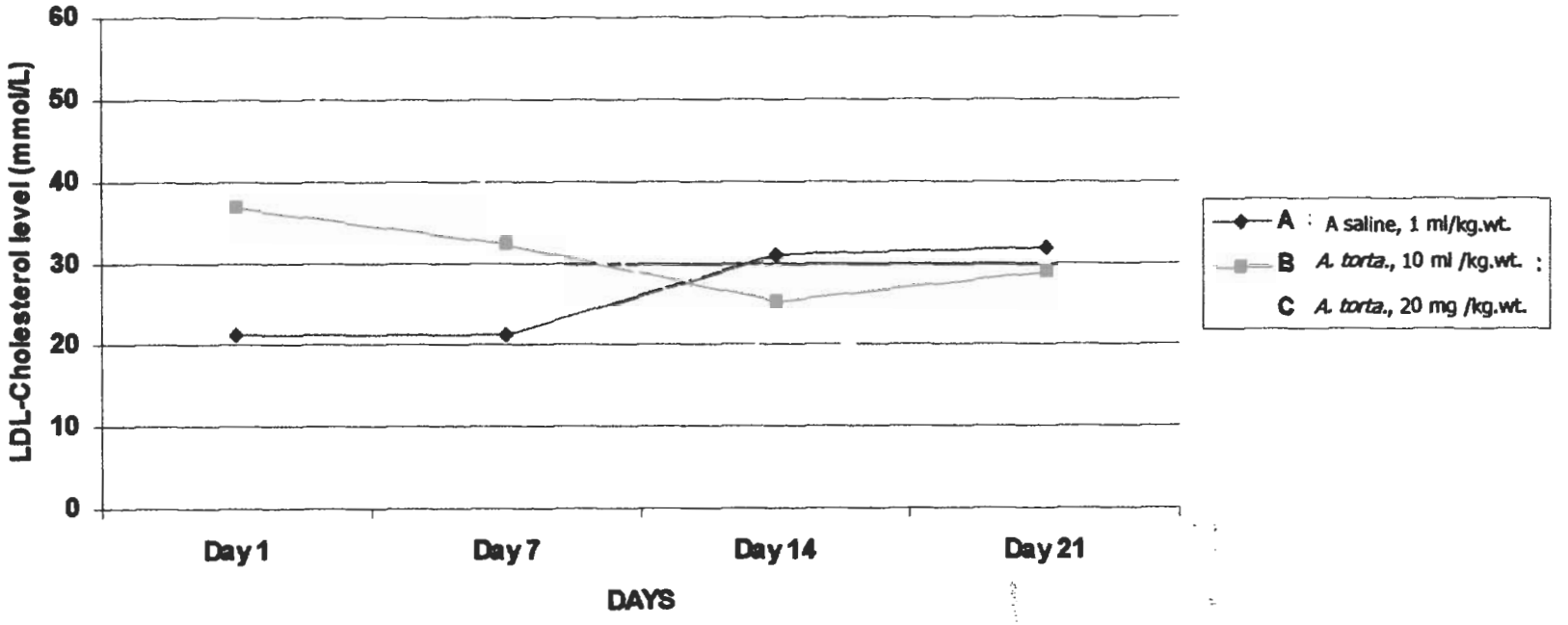

Fig 21: Effect of ethanol extract of *A.torta* on serum LDL- Cholesterol levels (mmol/L) in rabbits

3.5.3.4 Effect of Extract on Serum HDL-Cholesterol Levels

Continuous increase in HDL-cholesterol level was also recorded in the control animals (Fig.22). Treatment of animals with daily doses, 10.0 mg/kg and 20.0 mg/kg.body weight of the extract increased the HDL-cholesterol levels of the animals by 64.20% and 156.93% respectively by the 14th day. But by the 21st day of treatment the HDL-cholesterol levels had decreased by 61.93% and 70.30% respectively of their initial values.

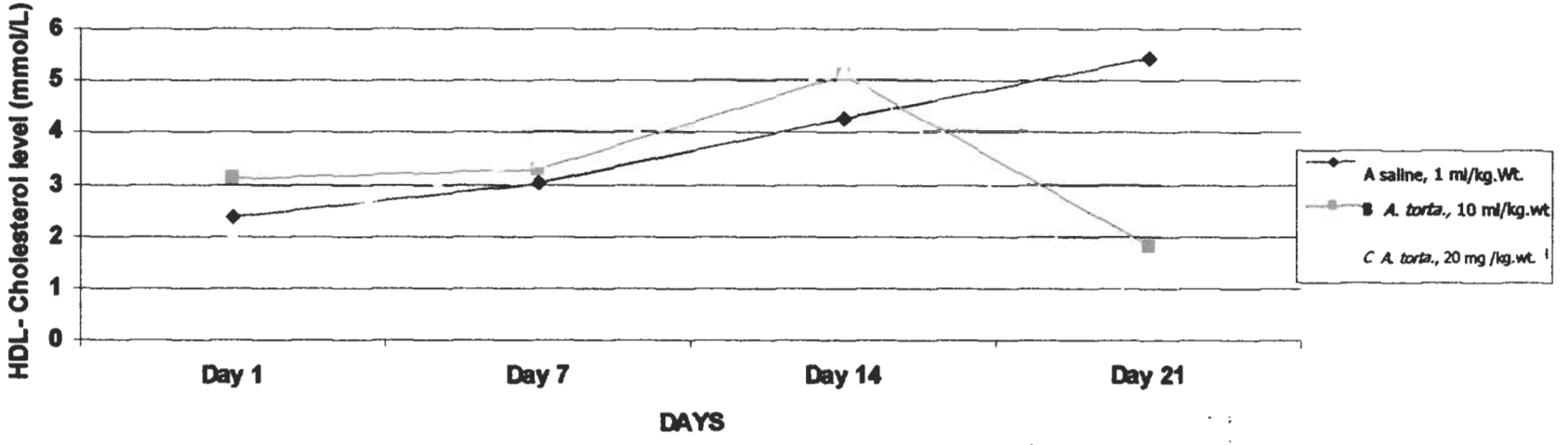

Fig 22: Effects of ethanol extract of *A.torta* on serum HDL-Cholesterol level (mmol/L) in rabbits

3.5.4 Serum Electrolyte Levels of Rabbits

3.5.4.1 Effect of *A. torta* Extract on Serum Cation Levels

Fig. 23 shows slight increase of 0.4% in serum Na^+ level in the control group by the 21st day, whereas 10.0 mg/kg and 20.0 mg/kg. body weight of extract reduced the serum Na^+ levels by 1.56% and 2.14% respectively. These changes were insignificant ($p>0.05$).

On the 21st day, K^+ level was decreased by 22.4% in the control group, while increases of 11.60% and 20.37% were elicited by 10.0 and 20.0 mg/kg body weight of *A. torta* extract respectively.

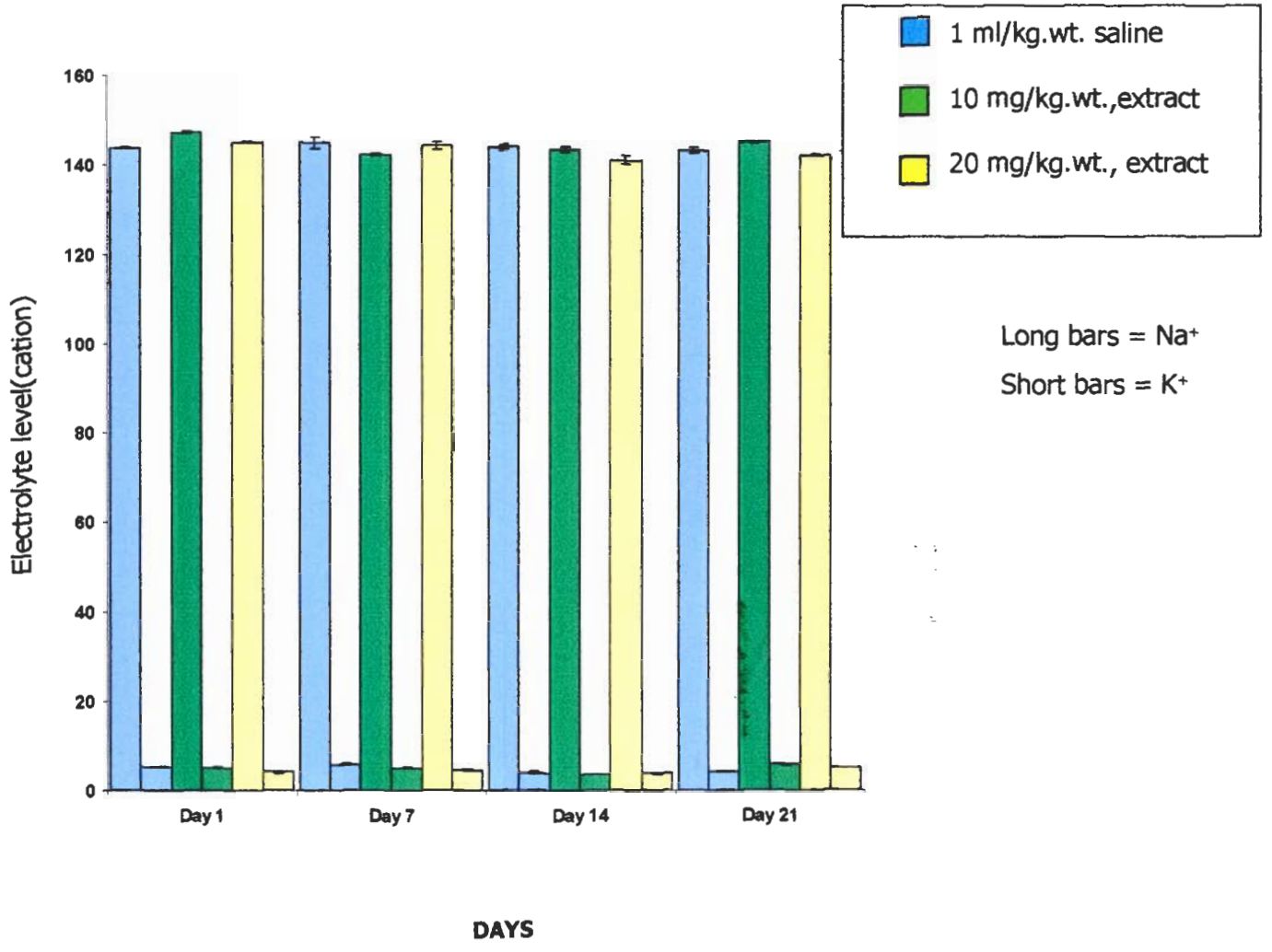

Fig. 23: Effect of ethanol extract of *A. torta* on serum cation levels in rabbits

3.5.4.2 Effect of *A. torta* Extract on Serum Anion Levels

Significant increases in Cl^- level were observed in all the groups of animals ($p<0.001$) at the end of the study (14.38%,15.46% and 5.54% in the control, 10.0 and 20.0 mg/kg body weight of extracts respectively).

For serum HCO^-_3 levels, significant decrease of 27.31% ($p<0.001$) was seen in the control group whereas insignificant decreases of 6.33% and 1.23% were recorded with 10.0 and 20.0 mg/kg body weight of extracts respectively, $p>0.05$. (see fig. 24)

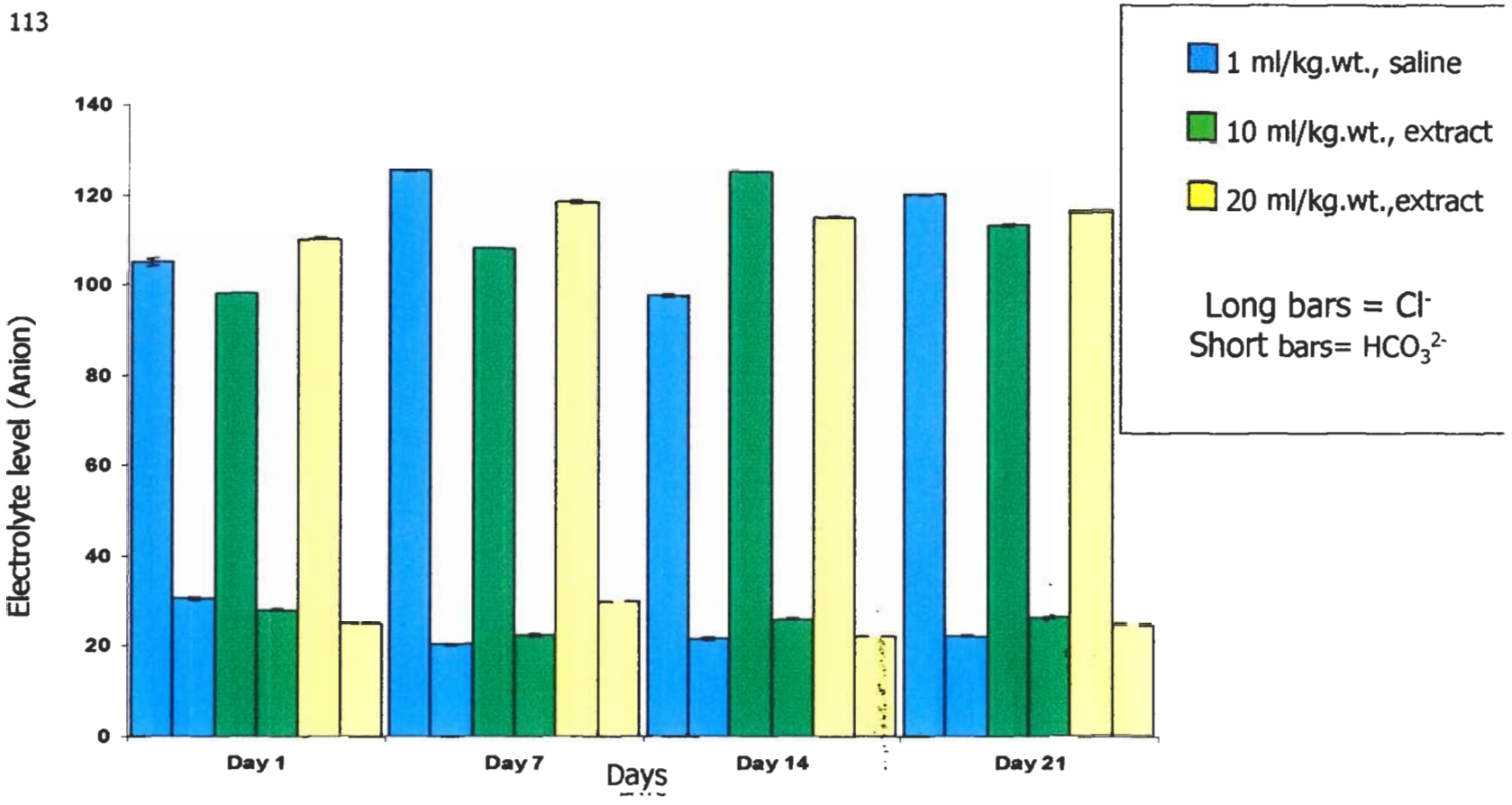

Fig. 24: Effect of ethanol extract of *A. torta* on **serum anion levels in rabbits**

3.6.0 RESULTS OF PHYOCHEMICAL AND PROXIMATE ANALYSES

3.6.1 Phytochemical Analysis of Whole *A. torta* leaves

Results in Table 1 show that flavonoids, glycosides, tannins, sterols and alkaloids were present in whole *Acalypha torta* leaves.

Table 1: Results of Phytochemical Analysis of Whole (Dried) *Acalypha torta* leaves

Test	Observation	Inference
ALKALOIDS		
a. Mayer's Test	Whitish precipitate formed	Alkaloids indicated
b. Wagner's Test	Reddish-brown precipitate formed	(trace)
		Alkaloids present (trace)
SAPONINS		
a. Frothing Test	No frothing appeared	Saponins absent
b. Emulsion Test	No transparency	Saponins absent
FLAVONOIDS		
a. Aluminium–chloride Test	A yellow colouration formed	flavonoids (+) indicated
b. Dilute ammonia Test	A yellow colouration observed	Present (++)
TANNINS		
a. Ferric chloride Test	Blue-black colouration	Present (++)
STEROLS AND TRITERPENES		
a. Moleschott Test	Light green and brown interface	Present (++)
b.Salkowski's Test	Blackish – green colouration	Present (++)
GLYCOSIDES		
a. Hydrolysis Test	A brick red colouration on standing	Present (++)
REDUCING SUGAR		
a. Fehling's Test	A brick red precipate observed	Present (+)

3.6.2. Proximate Analysis of Whole *A. torta* leaves

Table 2 shows high content of moisture and crude fat in *A. torta* leaves. But low percentages of protein and carbohydrates were detected.

Table 2: Proximate composition of whole *A. torta* leaves and the ethanol extract of *A. torta* leaves.

Percentage composition		
	Whole *A. torta* Leaves	**Ethanol extract of *A. torta***
Moisture	42.0 ± 0.06	69.7 ± 0.05
Crude fibre	11.01 ± 0.01	4.62 ± 0.02
Crude protein	7.88 ± 0.02	2.90 ± 0.01
Crude fat	20.31 ±0.01	13.55 ± 0.01
Total carbohydrate	28.82 ± 0.06	3.07 ± 0.03

3.6.3 Phytochemical Analysis of the Ethanolic Extract of *A. torta* Leaves

Table 3 shows the results of the phytochemial analyses. Results indicated obvious presence of flavonoids, steroids, tannins, alkaloids and glycosides, precisely cardiac glycosides.

Table 3: Result of Phytochemical Tests on Ethanol Extract of *Acalypha torta*

Test	Observation	Interference
ALKALOIDS		
a Dragendoff's Test b. Mayer's Test c. Wagner's Test	Red precipitate formed No precipitate formed A reddish brown precipitate was formed	Present (trace) indicated Absence supported Present (trace)
FLAVONOIDS		
a. Aluminium choleride Test b. Dilute Ammonia Test	A yellow colouration formed A yellow colouration was observed	Present (+) Present (++)
CARDIAC GLYCOSIDE		
Ferric chloride Test	Reddish brown ring formed	Present (++)
TANNINS		
Ferric chloride Test	Blue-black colouration	Present (++)
ANTHRACENE GLYCOSIDE		
Dilute ammonia Test	A pink colouration not formed	Absent
SAPONINS		
a. Emulsion Test b. Frothing Test	No emulsion formed No frothing formed	Absent Absent
TANNINS		
a. Lead subacetate Test b. Bromine water test c. Ferric Chloride Test	A gelatinous precipitate formed Pale brown precipitate formed Black colouration formed	Present (trace) Present (trace) Present (trace)

3.6.4. Phytochemical Analysis of Fraction L

Table 4 shows the results of the phytochemical analyses of fraction L that demonstrated the ability to lower blood pressure of anaesthetized normotensive cat. This fraction tested positive to flavonoids, steroid and cardiac glycosides. Traces of alkaloids and tannins were observed.

Table 4: Result of Phytochemical Analysis of Fraction L

Test	Observation	Interference
ALKALOIDS		
a. Mayer's Test	No precipitate formed	Absent
b. Wagner's Test	Reddish-brown precipitate formed	Present (trace)
FLAVONOIDS		
a. Aluminium–Chloride Test	A yellow colouration observed	Present (+)
b. Dilute ammonia Test	A yellow colouration observed	Present (++)
TANNINS		
a. Lead subacetate Test	A gelatinous precipitate formed	Present (+)
b. Ferric chloride Test	A blue – black colour observed	Present (++)
c. Bromine water Test	No precipitate was formed	Absent
CARDIAC GLYCOSIDE		
a. Ferric chloride Test	Reddish brown ring was formed	Present (++)

3.7.0 MINERAL CONTENTS OF THE ETHANOL EXTRACT OF *A.TORTA*

High levels of K^{+}, Ca^{2+}, Na^{+} and Mg^{2+} were detected in the ethanol extract of *A. torta* leaves (table 5).

Table 5: Mineral Contents of Ethanol Extract of *A. torta*

Mineral	Concentration (mg/100 g)
Na	61.5± 0.96
K	354.00 ± 0.86
Ca	79.25 ±1.29
Mg	68.80 ± 0.04
Cl^{-}	2.40 ± 0.02
HCO_3^{-}	12.00 ± 0.04
CO_3^{-}	2.30 ± 0.05
SO_4^{2-}	0.21 ± 0.01

3.8.0 ISOLATION OF THE HYPOTENSIVE FRACTION

3.8.1 Column Chromatography

Column chromatography over silica gel G-60-200 using chloroform – butanol- xylene (2:2:3) as the eluent produced a total of sixty-seven fractions (1 to 67) whereas elution with distilled water gave fractions 68 to 89.

3.8.2 Thin-Layer Chromatography

The fractions were concentrated down by using thin-layer chromatography over precoated silica gel G-60 plates and chroroform-butanol-xylene, as eluent thirteen fractions, A to M, were obtained by pooling together fractions with similar Rf values. Table 6- shows the fractions and their Rf values.

Table 6: Isolated Fractions and their Rf Values

Fraction Number	Designation	R, Values
1-2	A	0.95 ± 0.007; 0.10 ± 0.002
3 -12	B	0.23 ± 0.040; 0.03 ± 0.003
13 -19	C	0.01 ± 0.002
20 – 38	D	0.19 ± 0.023
39 – 43	E	0.02 ± 0.002
44 – 52	F	0.07 ± 0.003
53 - 59	G	0.03 ± 0.008
60 – 62	H	0.00
63 – 67	I	0.03 ± 0.003
68 – 72	J	0.01 ± 0.003
73 – 76	K	0.03 ± 0.001
77 – 80	L	0.02 ± 0.002
81 – 89	M	0.00

3.9.0 EFFECT OF FRACTIONS A TO M ON THE BLOOD PRESSURE OF A NORMOTENSIVE CAT.

Tracings in Fig. 25 show that fraction E – K, J and M caused slight increases in cat arterial blood pressure, whereas fraction L was the only fraction that reduced the blood pressure of cat. All other fractions had no effect on blood pressure.

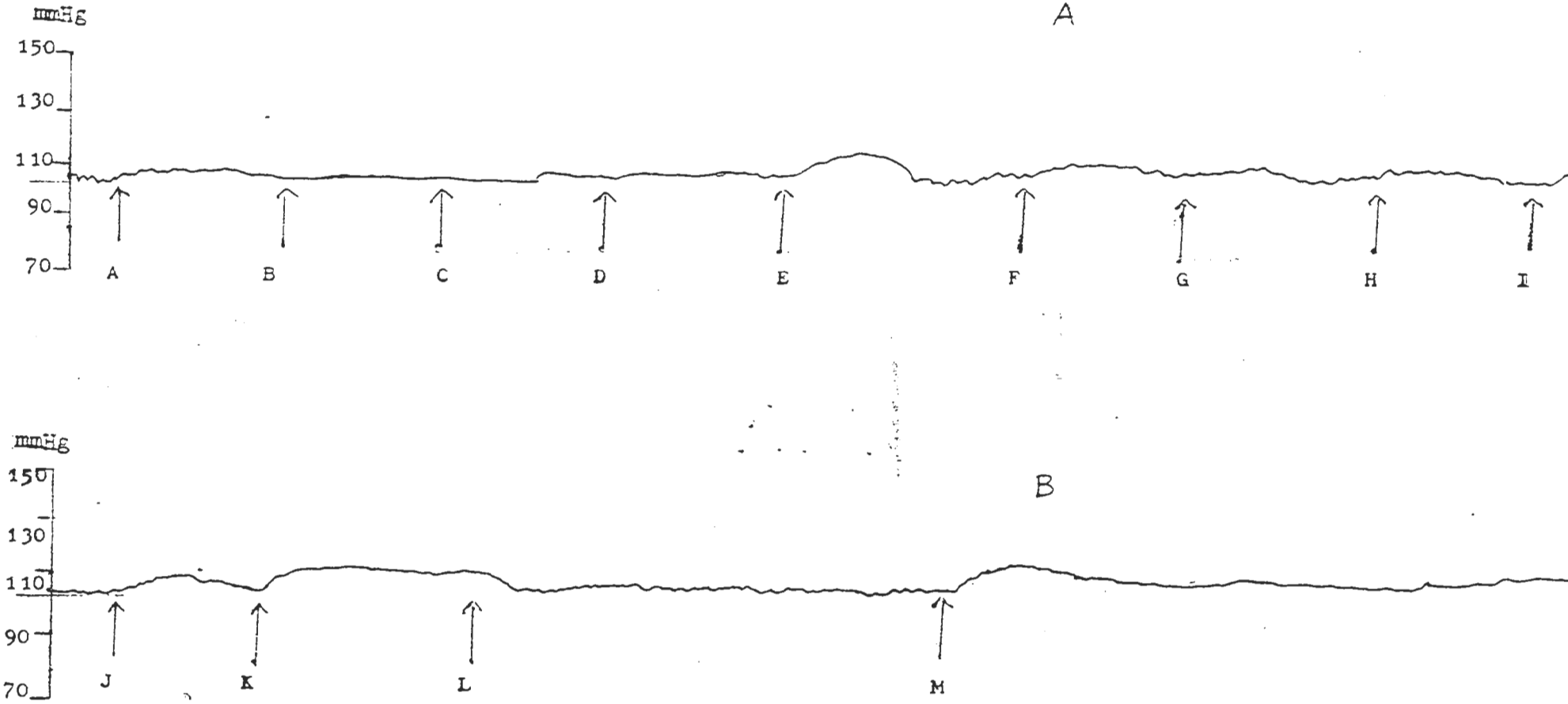

Fig. 25: Effect of isolated fractions, A to M, on the blood pressure of anaesthetized normotensive cat.

(B is a continuation of A).

3.10 DETERMINATION OF ACUTE TOXICITY OF FRACTION L

Seven groups of mice were given different doses of fraction L, ranging from 100-8000 mg/kg body weight and were observed for 24-hr period, the control mice received normal saline (10 ml/kg body weight), the median lethal dose (LD_{50}) of the fraction was extrapolated to be 1000 mg/kg body weight). (Fig. 26).

In all the treated groups, after administration of the extract, there was no agitation and the animals clustered together with signs of drowsiness. Normal feeding habit was also observed in all the groups. In groups 4-8 that received 500-8000 mg/kg body weight, peaceful deaths of 2,3,5,6 and 6 animals respectively were recorded. (Appendix 8).

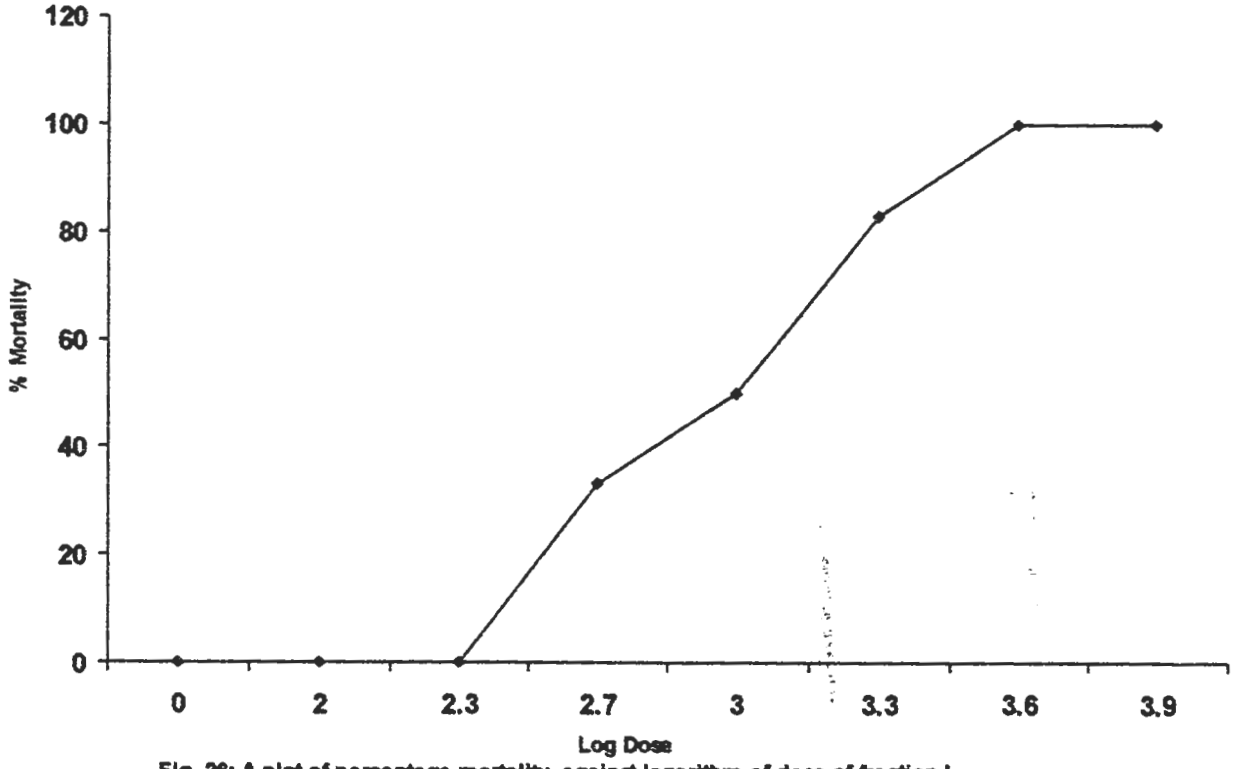

Fig. 26: A plot of percentage mortality against logarithm of dose of fraction L, obtained from the ethanol extract of *A. torta*, after a 24hr-actute toxicity study

CHAPTER FOUR

4.0 DISCUSSION

The leaves were collected, dried, pulverized and subjected to differential extraction in three solvent systems- chloroform: methanol (2:1), ethanol and water. When the effects of the three extracts (upper layer, ethanol, and water extracts) obtained on the blood pressure of normotensive cats were investigated, results (Fig. 4) showed that the upper layer (methanol) extract has no effect on blood pressure of normotensive cats. Water extract demonstrated vasopressor action in some cats and depressor effect in other cats. This inconsistency may be due to the influence of individual variation on the action of this extract. The ethanol extract, at the small doses of 12 .5 mg /kg body weight of cat, consistently lowered the arterial blood pressure of normotensive cats. The evidence that the alcohol extract of *A. torta* produced dose-dependent fall in the blood pressure of anaethetized normotensive cats supports the assertion that *A. torta* leaves possess a strong blood pressure lowering property which has also been found to be dose-dependent. The effect of the lower layer (chloroform) extract was not investigated because of its insolubility in all the solvent vehicles tested except chloroform. The upper layer, lower layer and water extracts were then discarded since the active blood pressure lowering principle was suspected to be embedded in the ethanol extract. Smith (1961) in his analysis of effects of drugs on the cardiovascular system suggested that any blood pressure lowering drug which produces a fall in blood pressure that lasts for less than 2 minutes was likely to be producing the fall either through direct vascular smooth muscle relaxation, or direct depressant action on the myocardium, but certainly not through the autonomic nervous system. Since the action of the ethanol extract of *A. torta* was seen to be immediate and unsustained, always returning to few mmHg pressure below the initial value within 2 minutes. This suggests that the ethanol extract may be exerting its effect on the vascular or cardiac smooth muscles. The activity of the ethanol extract at 12.5 mg/kg body weight was comparable to that of 100 µg/kg body weight of histamine.

Herbal medicines, however natural, can cause serious illnesses, ranging from allergy to cancer, to malfunction of vital organs such as liver, kidney, heart etc and even death (Aschwanden, 2001), thus they usually produced a wide range of side effects. The toxicological effects of the ethanol extract were then investigated. The 24hr-acute toxicity study revealed that the median lethal dose (LD_{50}) of the extract was 562.30 -

mg/kg body weight. This observation indicates that the extract may not be necessarily toxic since any chemical agent of LD_{50} 500 mg/kg body weight and above is usually considered non-toxic. For instance, Jacob (1996) reported LD_{50} values of castanospermine to be much greater than 500 mg/kg body weight and considered it to be relatively non-toxic. Certain behavioural changes noted such as drowsiness, reduced appetite and increased respiration shows that the extract actually has some other pharmacological effects. The reduced responses of the animals to pains recorded at large doses of the extract (> 4000 mg/kg body weight) indicate analgesic effect as one of the side actions of this extract.

The effects of intraperitoneal (i.p) administration of daily doses of the extract in adult rats of both sexes for 28 days were investigated at levels equal or below 50% of the LD_{50}, but in excess of the expected quantities that would appear in a single dose of 3-4 leaves usually taken by the patients. The i.p route was chosen because it provides a better spectrum of toxicological effects. Rats were observed for both toxicological and pharmacological manifestations. The fact that all the animals were agitated at the onset of the experiment, with greater agitation recorded in the animals that were treated with the extract, depicts destabilization of the animals equilibrium state by the presence of the extract. This effect was found to normalize by the third day in the groups that received the lowest dose-50 mg/kg body weight/day, single dose treatment with 100 mg/kg.body weight of extract and control groups.

Contrary to the gradual increase in body weight observed in the control rats throughout the 28-day sub-acute toxicity study, the ethanol extract of *A. torta* (200 mg/kg body weight) persistently caused significant reductions ($P< 0.0001$) in the body weights of rats. The decrease in body weight may be among the pharmacological effects of the extract but it also corresponded to the decline in feeding observed in all the treated groups within the first week of the experiment. Thus the extract, like anphatamine (Silverstone, 1986) may be inhibiting the hypothalamic centers of the brain responsible for controlling appetite. This effect was most prominent in the group that received the highest dose of the extract (200 mg/kg. body weight/day) and the animals died before the end of investigation. Activities of serum enzymes were measured since they are useful indicators for the assessment of liver function in the toxicity of chemicals and environmental pollutants (Torgny, 1984: Nsirim, 1999).

Elevation of serum ALT and AST is important in the diagnosis of liver and heart damage or disease caused by exposure to toxic drugs and chemicals (Nsirim, 1999: Nelson and Cox, 2000). Alanine transaminase ALT is raised in liver, myocardial infarction or skeletomuscular dysfunction. Results showed that the extract caused no significant change ($p > 0.05$) in serum ALT levels of the treated animals when compared with the control, whereas significant elevations ($p < 0.000\ 1$) in AST activity were recorded in all the groups (treated and untreated) on the 3rd and 7th days of the study followed by a decline in activity for days 14 and 28.

This may probably be the result of initial agitation observed in the animals coupled with the trauma caused by daily injections given to these animals. Normal activity was restored in the control group, and in groups that received low doses of the extract by the 28th day. High levels of alkaline phosphatase (ALP) imply liver, bone, kidney or, intestinal disease (Nsirim, 1999: Torgny, 1984). Although there were fluctuations, normal ALP activity was maintained in all the groups throughout the 28 days indicating that the extract did not have any significant effect on the above mentioned organs.

Histopathological studies were undertaken because the morphological evidence of pathological process is the most consistent of the changes that can be identified as the result of a toxic process and some forms of the injury are reversible while others are permanent (Hewitt *et al.*, 1991). The liver section of rats dosed 50 and 100 mg/kg body weight of ethanol extract of *A. torta* showed mononuclear cell infiltration and prominent necrosis. However, mononuclear cell infiltration (lymphocytes and plasma cells) could be indicating evidence of hepatitis.

The extract also caused mild haemorrhage, and vascular congestion in some areas of the spleen with infiltration of inflammatory cells. The degrees and distributions of these pathologic changes suggest mild tissue injury due to inflammatory reactions was statistically insignificant. Evidence of mild fibrosis and nuclear eosinophilia displayed in parts of the brain indicate the ability of the extract to cross the blood-brain barrier, and eosinophilia could be indicative of neutrotoxicity following prolonged use of the extract.

The proximal tubules are often the primary target of nephrotoxicants. Toxic products derived from reactive oxygen species have been implicated as mediators of renal injury (Hewitt *et al.*, 1991). The extract also caused mild tubular necrosis and glomerular

degeneration in the kidney sections. Histopathological changes noted in the lungs were mainly vascular congestion and rupture as well as bronchial dilatation. Lymphocytes were also noted indicating inflammatory lesions. These changes also seem to be dose-related.

Having seen that ethanol extract of *A. torta* possessed blood pressure lowering activity, and may probably be non-toxic to the body, preliminary investigation into the mode of action of the extract were then carried out. Hypertension is a haemodynamic disorder in which increased arterial pressure may be associated with an increased cardiac output or increased total peripheral resistance or both. But in most patients, increased total peripheral resistance is the ultimate cause of elevated arterial pressure (Alper, 2002). The target of many antihypertensive agents is therefore to reduce cardiac output and, or total peripheral resistance. The cardiac output is a function of the force and rate of contraction of the heart, and the elasticity and distensibility of the conducting arteries whereas the total peripheral resistance depends on the area available for blood flow (Oates and Brown, 2001). Results showed that the extract had no effect on the rate of contraction of perfused rabbit heart but the force of contraction was dose-dependently reduced. The extract (5.0 mg) caused up to 71% reduction of the height of contraction. This could result from the relaxation of the cardiac muscle (myocardium) due to the inhibitory action of the extract on the β_1- adrenoceptors which are predominantly found in the myocardium and mediate increase in the strength of contraction (Paul, 2001).

Intravenous administration of a pharamacological dose of adrenaline evokes a rapid rise in blood pressure. The mechanism of the rise in blood pressure may be due to direct myocardial stimulation that increases the force of contraction and an increased heart rate or vascular contraction in many vascular beds (Hoffman, 2001). In this work, the effect of the extract on isolated rabbit arterial strips was investigated. Results revealed that the contraction of rabbit aortic strip induced by 60µg of adrenaline was attenuated (33.3% decrease) by 20.0 mg of the extract after 2 minutes incubation.

The action of the extract was also found to be dose-dependent and adrenaline effect was completely abolished after 20 minutes of incubation with the extract (20 mg). These findings also suggest relaxation effect of the extract on vascular smooth muscle possibly via β_1- receptor blocking action. Unfortunately all efforts made to obtain β- and α-adrenoceptor antagonists such as propranolol and phentolamine respectively were futile.

Results also showed that the rate of flow of physiological fluid through the vessels of the rat hind-quarters preparation was increased by the extract (13.30-39.30 mg/ml) indicating dilatation of the peripheral blood vessels in the rat hind-quarters preparation. Vascular dilatation implies increase in the area available for blood flow; hence decrease in total peripheral resistance. Higher doses of the extract (up to 53.2 mg/ml) did not improve efficacy probably due to alterations in bioavailability of relevant mediators.

The fall in blood pressure produced by histamine in anaesthetized cat has been shown to be a consequence of vasodilation and increased permeability of the fine vessels (Douglas, 1985) and these actions are mediated through the histamine receptors principally H_1- receptors. The fall in blood pressure produced by the extract in anaesthetized normotensive cat was blocked by the classical antihistamine, promethazine, suggesting histamine receptor mediated activity. But whether histamine or histamine – like substance is the bioactive principle present in the extract is still unknown. This fact also lends support to suspicion that the extract may be causing vasodilatation of the peripheral vessels in the anaesthetized cat by releasing histamine from the mast cells (Langunoff *et al.*, 1983).

Atropine blocks all the muscarinic responses to injected acetylcholine (ACh) and related cholinomimetic drugs whether they are excitatory, as in the intestine, or inhibitory as in the heart (Ghasi, 1991). In this work, a dose of atropine (20 µg/kg body weight) which completely blocked the fall in blood pressure induced by acetylcholine also completely abolished the blood pressure lowering activity of the extract. Thus, this crude extract may also be acting through the muscarinic receptors.

Calcium ion, Ca^{2+} is involved in several physiological and biochemical processes in the body such as excitation- contraction coupling, excitation- secretion process, blood coagulation and platelet aggregation. Calcium antagonists lower blood pressure by relaxing smooth muscles of the arteries (dilatation) and cardiac muscles. They are particularly useful for people with angina (chest pain), certain types of rapid heart rates, or migraine headache (Robert, *et al.*, 1999). Findings in this research work showed that the ethanol extract of *A. torta* interferes with calcium utilizing processes as evidenced from the fact that calcium chloride- induced perturbation of perfused rabbit heart which caused abnormally rapid increase in the force and rate of contraction was drastically

antagonized by the extract. This observation supports the earlier report of Insel and Motulsky (1984) that reduced availability of intracellular Ca^{2+} causes the cells to be more resistant to contractile stimuli; and a change in the affinity and responses of cell surface receptors to vasoconstrictor hormones. This activity was completely abolished by 5.0 mg of the extract indicating the usefulness of this plant extract not only in lowering blood pressure but also in the management of patients with angina pectoris and palpitation. Similarly, $CaCl_2$ – induced blood platelet aggregatory activity was dose-dependently inhibited by the extract. Since platelets participate in pathological thromboses leading to myocardial infarction, stroke and peripheral vascular thromboses (Berkow *et al.*, 1999), inhibition of platelet aggregation by the extract is indicative of its possible role as an antithrombotic agent.

All β-adrenergic receptors stimulate adenylyl cyclase via interaction with Gs-protein leading to accumulation of cyclic AMP, and subsequent activation of cAMP-dependent protein kinase and altered function of numerous cellular proteins due to their phosphorylation (Taussig and Gilman, 1995). But it has been discovered that Gs can as well enhance directly the activation of Ca^{2+}- channels in the plasma membrane of skeletal and cardiac muscle, thereby providing an additional means of regulating the functions of these tissues (Taussig and Gilman, 1995). The suspected extracts inhibition of Ca^{2+} - utilizing processes may perhaps be related to its antagonism of β_1 – adrenergic receptor.

Results of investigation into other possible pharmacological effects of the extract showed that, contrary to its relaxatory effect on vascular and cardiac muscles, there was enhancement of intestinal smooth muscle contraction. This indicates that if the extract is administered per oral, intestinal colic should be expected as an adverse effect. In the same study, histamine (0.002μg) abolished spontaneous contraction of isolated rabbit gut whereas acetylcholine (0.002μg) induced a magnificient and prolonged contraction of the gut indicating that the extract is similar in action to neither histamine nor acetylcholin.

The interaction of hypertension and atherosclerosis has long been recognized (Mogenson *et al.*, 1983). Numerous population studies have linked elevated concentration of total cholesterol or LDL-cholesterol in plasma with increased incidence of atherosclerotic events (Goldstein *et al.*, 1973; Keys, 1975) and for whatever reason higher levels of

HDL-cholesterol are associated with a lower incidence of coronary heart disease (Castelli and Anderson, 1986).

The effect of the extract on the lipid profile of rabbit after three-weeks treatment was therefore investigated. Results showed that the extract caused significant ($P<0.0001$) and dose-dependent decreases in serum triacylglycerol and cholesterol levels by the end of 21st day. A significant fall ($P<0.0001$) in the level of LDL-cholesterol was also recorded but this effect was more on the 14^{th} day. On the other hand, HDL-level was increased. But the extract was more efficacious on the 14^{th} day than the 21^{st} day. Thus the extract may play a role in reducing the incidence of athrerosclerosis usually seen in severe hypertension but prolonged use of this plant extract may not be beneficial and is therefore discouraged. Steady and significant ($p<0.003$) increases in the levels of triacylglycerol, total cholesterol, and LDL –cholesterol noted in the control animals may be indicating high fat content of the animal feed used in this experiment.

Low potassium diet is associated with elevation in blood pressure and disturbances in several electrolytes in normotensive and borderline hypertensive patients (Lawton *et al.*, 1990) have been reported. Also, high plasma sodium ion and low potassium ion levels (i.e increased sodium/potassium ratio) have been considered a factor in the pathogenesis of hypertension (Khan and Barett-Connor, 1990). Since the extract did not cause any remarkable alteration in the plasma levels of the electrolytes (Na^+, K^+, Cl^- and HCO^-_3) it shows that the extract does not interfere with the Na^+/K^+ ATPase activity involved in the maintenance of Na^+ - K^+ ratio and therefore, may not be acting through this mechanism.

A comparison of results of the proximate analyses of whole *A. torta* leaves and the ethanol extract (Table. 2) showed that differential extraction in chloroform: methanol (2:1) and ethanol reduces the amount of fibre, proteins, fat and carbohydrates present.

The pharmacological or medicinal properties of many plants are attributed to their chemical constituents such as alkaloids, flavonoids, glycosides, saponins, tannins, sterols, triterpenes and coumarins (Harbone, 1984: Obidoa and Obasi, 1991 and Siems *et al.*, 1996). Blood pressure lowering activities of berberine, aspidospermine, aspidosamine and yarrow alkaloids have been reported (Preininger, 1975; Lyon *et al.*, 1973; Kudrzyeka-Bieloszabska and Glowniak, 1966).

Similarly, flavonoids from passion flower (*Passiflora incarnata*) have also demonstrated their ability to increase the rate of respiration and reduce arterial blood pressure. Since the results of phytochemical analyses of both *A.torta* leaves and the ethanol extract of *A. torta* leaves indicated the presence of flavonoids and trace amount of alkaloids, either of these bioactive principles may be responsible for the blood pressure lowering effect of the extract.

When the effect of each fraction, A to M, on blood pressure of anaesthetized cat was tested, fraction L (containing fractions 77-80) was the only fraction that lowered the blood pressure. This indicated that the blood pressure lowering principle of *A. torta* extract is present in fraction L and since this fraction was eluted with distilled water indicating hydrophilic nature of the substance one may suspect any of the phenolic substances. Result of phytochemical screening of this fraction clearly shows that it is rich in flavonoids, though trace of alkaloid was also observed. Fraction L was also found to be rich in cardiac glycosides and may also be useful in the treatment of heart failure. From the results of the 24-hr acute toxicity study, LD_{50} of fraction L was found to be 1000 mg/kg. body weight indicating non-toxicity of this fraction and that the fraction may be more and well-tolerated than the ethanol extract. Normal feeding habit was observed in all the animals that were fed unlike the ethanol extract, which caused loss of appetite in the experimental animals.

Summary

In the process of investigating the alleged use of *Acalypha torta* leaves in the treatment of hypertension more facts have been unraveled. The ethanol extract of *A. torta* leaves actually possesses blood pressure lowering potential (vasodepressor effect), but the extract should be used with extreme care since high dose of it may induce hypotension. This pharmacological effect of the extract may perhaps be mediated by blocking the β_1 – adrenergic receptors, especially on the heart, thus causing reduction of the force of contraction of the heart. Blood pressure lowering effect of the extract is antagonised by muscarinic receptor blocker (atropine) and histamine blocker (promethazine) but the active principle is neither acetylcholine nor histamine. The extract also antagonizes calcium-utilizing processes but has no effect on Na^+/K^+ ATPase activity.

The blood pressure lowering principle is only soluble in water and is therefore suspected to be a phenolic substance or an alkaloid, not yet identified.

Investigation of other pharmacological effects of the ethanol extract of *A.torta* revealed that the extract also possesses antithrombotic, hypolipidaemic and hypocholesterolemic effect, although long-term treatment with the extract should not be recommended. The ethanol extract may be regarded as non-toxic but further purification may reduce the chance of adverse reactions. Abundance of cardiac glycoside in fraction L may also confer therapeutic effect in the treatment of cardiac failure.

Indications for Further Research

The ethanol extract of *Acalpha torta* has promising pharmacological profile, as revealed by the findings of this research work. Even though it has proved to be highly efficacious in the management of hypertension in traditional medicine, the active ingredient responsible for this activity has not been successfully isolated and identified in order to open new possibilities for discovery of new antihypertensive agents. Further investigation will therefore focus on

(1) Isolation, complete characterization and structural elucidation of the active blood pressure lowering principle.

(2) A study of the structure- activity relationship (SAR) of the agent with the view to eliminating certain adverse reactions.

(3) Elucidation of the mechanism of action of the agent especially its effect on the renin-angiotensin-aldosterone system as well as the catecholamine metabolism.

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Appendices

Appendix 1

Effect of *A. torta* on serum total cholesterol level (mmol/L)

Serum Cholesterol level (mmol/L)				
TREATMENT	**DAY 1**	**DAY 7**	**DAY 14**	**DAY 21**
Saline, 1ml/kg wt.	23.4± 0.023	26.08±0.012	39.91±0.007	37.85±0.029
A. torta, 10mg/kg wt.	39.9 ± 0.003	37.19± 0.017	32.06 ±-0.006	31.74 ±0.012
A. torta, 10mg/kg wt.	58.32± 0.006	52.87 ±0.003	33.04 ±0.009	27.23 ±0.01

Appendix 2

Effect of *A. torta* on LDL-Cholesterol Level (mmol/l)

Serum LDL Cholesterol level (mmol/L)				
TREATMENT	**DAY 1**	**DAY 7**	**DAY 14**	**DAY 21**
Saline, 1ml/kg wt.	21.23± 0.017	22.33 ±0.03	33.02± 0.015	31.89±0.009
A. torta, 10mg/kg wt.	36.71 ± 0.01	32.5± 0.007	25.42 ± 0.003	29.05± 0.003
A. torta, 20mg/kg wt.	55.03 ± 0.028	38.43 ± 0.351	18.62 ± 0.025	29.71± 0.112

Appendix 3

Fig 24 Effect of *A. torta* on HDL-cholesterol level (mmol/L)

Serum LDL Cholesterol level (mmol/L)				
TREATMENT	**DAY 1**	**DAY 7**	**DAY 14**	**DAY 21**
Saline, 1ml/kg wt.	2.38 ± 0.017	3.017 ± 0.017	4.29 ±0.084	5.44 ± 0.031
A. torta, 10mg/kg wt.	3.1 ± 0.076	3.29 ± 0.015	5.09 ± 0.053	1.18 ± 0.009
A. torta, 20mg/kg wt.	2.017 ± 0.003	3.46 ± 0.006	5.19 ± 0.009	0.6 ± 0.0259

Appendix 4

SERUM ELECTROLYTE LEVELS AFTER TREATMENT WITH *A. TORTA* EXTRACT								
	Serum cation levels (mmol/l)							
	DAY 1		**DAY 7**		**DAY 14**		**DAY 21**	
Treatment	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
Saline, 1ml/kg wt.	143.8 ± 0.17	5. 40 ± 0.009	145.0 ± 1.16	6.02 ± 0.044	144.0 ± 0.58	4.05 ± 0.029	143.2± 0.60	4.19 ± 0.007
A. torta, 10mg/kg. Wt.	147.3 ± 0.35	5.17 ± 0.17	142.3 ± 0.27	5.18 ± 0.096	143.5 ±0.50	3.59 ± 0.064	145.0 ± 0.15	5.77 ± 0.15
A. torta, 20mg/kg. Wt.	145.2 ± 0.17	4.17 ± 0.165	144.3 ± 0.73	4.61 ± 0.049	141.0 ± 1.00	3.79 ± 0.124	142.1 ± 0.09	5.02 ± 0.023

Appendix 5

Effect of Ethanol Extract of *A. torta* on Serum Alkaline Phosphatase levels (mmol/l)					
GROUP	**DAY 1**	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**
A	78.1 ± 2.12	82.83 ± 4.56	50.9 ± 2.39	41.35 ± 6.70	59.8 ± 1.98
B	52.9 ± 0.98	54.3 ± 0.39	55.2 ± 3.96	50.6 ± 2.54	61.6 ± 1.36
C	54.8 ± 0.29	53.83 ± 0.71	54.4 ± 0.58	53.83 ± 0.67	Dead
D	54.3 ± 0.47	46.28 ± 3.95	55.05 ± 0.22	54.53 ± 0.32	36.35 ± 0.26
E	45.1 ± 0.47	43.85 ± 1.01	44.85 ± 0.47	44.88 ± 0.65	54.43 ± 0.60

Appendix 6

Effect of Ethanol Extract of *A. torta* on Serum Aspartate Transaminase					
GROUP	**DAY 1**	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**
A	14.75 ± 0.32	18.75 ±1.96	22.5 ± 0.29	17.3 ± 0.09	18.58 ± 0.72
B	17.3 ± 0.08	18.0 ± 2.20	22.0 ± 1.29	21.0 ± 0.91	23.75 ± 0.14
C	18.25 ± 0.10	18.75 ± 0.10	20.0 ± 0.41	24.5 ± 0.29	Dead
D	18.0 ± 0.35	21.5 ± 0.29	19.6 ± 0.40	21.25 ± 0.14	16.50 ± 0.24
E	17.5 ± 0.65	20.0 ± 0.41	23 25 ± 0.10	19.25 ± 0.78	13.5 ± 0.29

Appendix 7

Effect of Ethanol Extract of *A. torta* on Serum Alanine Transaminase					
GROUP	**DAY 1**	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**
A	9.05 ± 0.32	12.7 ± 1.33	9.75 ± 0.72	8.65 ± 0.38	11.13 ± 0.03
B	12.8 ± 0.69	15.0 ± 0.58	11.3 ± 0.17	10.4 ± 0.46	11.4 ± 0.12
C	13.0 ± 0.58	14.3 ± 0.40	11.3 ± 0.17	13.4 ± 0.06	Dead
D	15.4 ± 0.23	15.1± 0.06	13.5 ± 0.29	9.45 ± 0.55	9.6 ± 0.92
E	9.85 ± 0.14	8.8 ± 0.46	12.6 ± 1.73	9.4 ± 0.35	8.4 ± 0.23

Appendix 8

Determination of the Lethal Dose (LD_{50}) of Fraction L

Treatment	No of Deaths	Log Dose	% Mortality
Saline, 1ml/kg.wt.	0	0	0
A. torta, 100mg/kg.wt.	0	2.00	0
A. torta, 200mg/kg. wt.	0	2.30	0
A. torta, 500mg/kg. wt.	2	2.70	33.30
A. torta, 1000mg/kg. wt.	3	3.00	50.00
A. torta, 2000mg/kg. wt.	5	3.30	83.00
A. torta, 4000mg/kg. wt.	6	3.60	100.00
A. torta, 8000mg/kg. wt.	6	3.90	100.00

Appendix 9

Effect of Ethanol Extract of *A.* torta on average body weight (grams) of Rats					
GROUP	**DAY 1**	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**
A	125.8± 0.246	116.5 ± 1.137	109.8 ± 0.895	101.7 ± 0.806	84.15 ± 0.184
B	154.5± 0.572	141.1 ± 0.414	126.3 ± 0.471	106.5 ± 0.757	102.5 ± 1.035
C	165.5 ± 0.238	165.3 ± 1512	147.7 ± 0.900	98.8 ± 0.255	Dead
D	124.0 ± 0.618	121.3 ± 0.576	118.5 ± 0.216	132.4 ± 0.638	135.8 ± 0.049
E	143.2 ± 0.618	143.6 ± 0.245	146.1 ± 1.716	149.6 ± 0.163	157.8 ± 0.123

Appendix 10

Effect of Extract on Perfused Rat Hind Quarters Preparation

Concentration of Extract (mg/ml)	Flow rate at Equilibration (ml/min)	Flow Rate at peak Activity (ml/min)	Volume Change (ml/min)	% Volume change	Average % Change ± SEM
13.30	7.80 6.70 4.60 3.80	8.10 6.90 4.80 4.00	+ 0.30 + 0.20 + 0.20 + 0.20	+ 3.90 + 3.00 + 4.40 + 5.30	+ 4.15 ± 0.48
26.60	7.10 6.60 4.40 3.80	7.80 7.20 4.90 4.60	+0.70 + 0.60 + 0.50 + 0.80	+ 10.00 + 9.10 + 11.40 + 21.10	+ 12.9 ± 2.77
39.90	6.20 7.00 4.30 4.00	7.80 8.80 5.80 5.80	+1.60 + 1.80 + 1.50 + 1.50	+ 25.80 +25.70 + 34.90 + 37.50	+ 30.98 ± 3.06
53.20	7.00 7.00	7.80 7.80	+ 0.80 + 0.80	+ 11.40 + 11.40	+ 11.40 ± 0.00

Appendix 11

Proximate composition of whole *A. torta* leaves and the ethanol extract of *A. torta* leaves

Whole *A. torta* leaves

Moisture Content

= 42.0% Moisture

Crude Fibre Content:

Wt of crucible + dried residue = 94.2g

Wt of crucible + ashed residue = 93.98g

Wt of sample = 2.0g

$$\therefore \text{Crude fibre (\%)} = \frac{94.2 - 93.98}{2} \times \frac{100}{1}$$

= 11.0% Crude fibre

Crude fat content:

(Wt of conical flask) + (20ml solvent) + (wt of sample) = 123.4g

Wt of (conical flask + dry mass) = 122.99g

Wt of sample = 2.0g

∴ Wt of fat = (123.4 – 122.99) g = 0.41g

$$\text{\% Mass of fat} = \frac{0.41}{2} \times \frac{100}{1}$$

= 20.3%

Crude Protein:

Wt of fresh sample = 1.0g

Normadity of acid = 0.05N

Titre value = 18.0ml

$$\therefore \text{\% Nitrogen} = \frac{18.0 \times 0.05 \times 0.014 \times 100}{1}$$

= 1.26% Nitrogen

Crude protein (%) = % Nitrogen x 6.25 = 1.26 x 6.25

= 7.88% Crude protein.

Total carbohydrate :

Carbohydrate = 100 – (% moisture + % crude fibre + % Crude fat + % protein)

= 100 – (42.00 + 11.00 + 20.30 + 7.88)

= 28.82% Carbohydrates.

Ethanol Extract of *A. torta*

Moisture content.

Wt of fresh sample = 0.37g

Wt of fresh sample + dish = 20.45g

Wt of dry sample + dish = 20.19g

$$\% \text{ Moisture} = \frac{20.45-20.19}{0.37} \times \frac{100}{1}$$

= 69.7%

Crude Protein Content:

Titre value = 12.30ml

Normality of Acid = 0.01N

Weight of sample = 0.37g

$$\therefore \% \text{ Nitrogen} = \frac{12.30 \times 0.01 \times 0.014 \times 100}{0.37}$$

= 0.465

But crude protein (%) = % Nitrogen x 6.25

= 0.465 x 6.25

= 2.90%

Ash content:

Wt of crucible = 18.24g ---------- (W_1)

Wt of crucible + Sample = 18.61g---------- (W_2)

Wt of Crucible + Ash = 18.27g---------- (W_3)

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} = \frac{18.27 - 18.24}{18.61 - 18.24} \times \frac{100}{1}$$

$$= \frac{0.023}{0.37} \quad \frac{100}{1} = 6.22\% \text{ ash}$$

Crude fat content:

Wt of oil = 0.05g

Wt of sample = 0.37g

$$\% \text{ Fat} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$\text{i.e \% Fat} = \frac{0.05}{0.37} \times \frac{100}{1} = 13.51\%$$

Crude Fibre Content:

Wt of fibre = 0.017g

Wt of sample = 0.037g

$$\therefore \% \text{ Fibre} = \frac{\text{Wt of fibre}}{\text{Wt of sample}} \times \frac{100}{1} = \frac{0.017}{0.37} \times \frac{100}{1}$$

Carbohydrate Content:

% Carbohydrate = 100 – (69.7 + 2.90 + 6.22 + 13.51 + 4.60)

= 100 – 96.93

= 3.07%.