

**COMPARATIVE EVALUATION OF THE HYPOTENSIVE EFFECTS OF THE SEED
AND LEAF EXTRACTS OF *MORINGA OLEIFERA LAM* (*Moringaceae*)
IN LABORATORY ANIMALS**

BY

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LABORATORY ANIMALS.**

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NIGERIA

JUNE, 2015

DECLARATION

I declare that the work in this thesis entitled “Comparative Evaluation of the Hypotensive Effects of the Seed and Leaf Extracts of *Moringa oleifera* Lam (*Moringaceae*) in Laboratory Animals” has been carried out by me in the Department of Pharmacology and Therapeutics under the supervision of Dr. J.I. Ejiofor, Prof. O.A. Salawu and Dr. A.U. Zezi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Timothy Ayowale OSAMEYAN

Name of Student

Signature

Date

CERTIFICATION

This thesis entitled “Comparative Evaluation of the Hypotensive Effects of the Seed and Leaf Extracts of *Moringa oleifera* Lam (*Moringaceae*) in Laboratory Animals” by Timothy Ayowale OSAMEYAN meets the regulations governing the award of the degree of M.Sc. Pharmacology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Lord God Almighty who has enabled me throughout my life. To Him be the glory and also to my late sister Mrs. Sarah Titilayo Solomon may her soul rest in peace. Amen.

ACKNOWLEDGEMENT

I wish to give glory to God Almighty who saw me through the challenges of life and brought me thus far.

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ABSTRACT

Moringa oleifera is locally used in Nigeria for the management of respiratory disorders, hypertension and poor blood circulation among other ailments. However, all parts of the plant are blindly used because the exact part of this plant and / or extractive solvent that provides the best of its cardiovascular or optimum hypotensive activity has not been reported. The impetus of this research work therefore is to compare the crude extracts of the leaf and seed of *Moringa oleifera* in their two solvent forms respectively, aqueous and methanol for the leaf; and methanol and pet-ether for the seed, since the seed is very oily for aqueous extraction. The phytochemical constituents of the leaf and seed extracts of *Moringa oleifera* were determined. The effect of *Moringa oleifera* leaf and seed extracts on cat blood pressure and guinea pig heart atria were investigated. Intravenous injection of drugs and test solutions injected through the femoral vein into a cannulated artery was used to investigate effects of drugs on arterial blood pressure in male cats. The effects of *Moringa oleifera* leaf and seed extracts on isolated guinea pig heart atria and on isoprenaline and Calcium chloride induced contraction, as well as interactions on specific receptor mediation (Cholinergic, Histaminergic and Adrenergic) as to elucidate the probable underlying receptor mechanism(s) involved in the actions of the extracts were also investigated. The aqueous and methanol leaf extracts of *M. oleifera* contained alkaloids, tannins, saponins, cardiac glycosides, carbohydrates, flavonoids, steroids and triterpenes, while anthraquinone was absent. The methanol seed extract of *M. oleifera* contained carbohydrates, saponins, alkaloids, tannins and cardiac glycosides with no flavonoids, steroids and triterpenes, but its pet-ether seed extract had only carbohydrates and alkaloids. The results revealed that the standard depressor agent, acetylcholine causes

a fall on blood pressure and both the leaf and seed extracts of *Moringa oleifera* lowered the blood pressure of cats in a dose dependent manner, but in varying degree. Both the aqueous and the methanol leaf extracts demonstrated a higher hypotensive property than the seed extracts which showed milder hypotensive effect, with its methanol seed extract causing slightly more relaxation of the arterial system.

The aqueous and methanol extracts of the leaves as well as the methanol seed extract inhibited adrenaline induced contraction of the cat blood pressure and caused blockade that was not reversed with higher concentration of adrenaline. Propranolol enhanced the relaxing effect of all the extracts in a manner that is synergistic including that of the pet-ether seed extract which was not able to block adrenaline contraction. Mepyramine (antihistamine,) potentiate the depressor effect of the aqueous and methanol leaf extracts. The cholinergic antagonist, (atropine), blocked only the aqueous leaf extract and showed no effect on the methanol leaf and both seed extracts. All the extracts exhibited a concentration-dependent reduction in both rate and force of the guinea pig atria spontaneous contraction, except the methanol seed extract which decreased only the rate of contraction. Similarly, all the extracts of the leaf and seed inhibited isoprenaline and calcium chloride induced guinea pig atria contraction. In conclusion, the aqueous and methanol leaf extracts of the plant were found to cause higher depressor effects than the seed extracts; and the extracts antagonistic actions on adrenaline contraction suggests adrenergic receptor mediation activity. The *in-vitro* blockade of calcium chloride-induced contraction also seemed to suggest the opening of calcium channels as part of the mode of activity of the extracts in their constrictor actions.

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ABBREVIATIONS

- ACE** – Angiotensin converting enzymes
- ACh** – Acetylcholine
- ACR** – Albumin creatinine ratio
- ADR** – Adrenaline
- ANP** – Arterial natriuretic peptides
- Aq Ex.** - Aqueous extract
- ARBs** – Angiotensin receptors blockers
- AT** – Atropine
- AV** – Atrioventricular
- BP** – Blood Pressure
- CaCl₂** – Calcium chlorides
- CCBs** – Calcium Channel Blockers
- CO** – Cardiac output
- CVDs** – Cardiovascular diseases
- DBP** – Diastolic blood pressure
- ECF** – Extracellular fluids
- ECG** – Electrocardiogram
- eGFR** – estimated glomerular filtration rate
- Ex.** - Extract
- FeCl₃** – Ferric chloride
- H₂SO₄** – Hydrogen tetraoxosulphate VI
- HCl** – Hydrogen chloride

HIS – Histamine

HLD – High density lipoprotein cholesterol

HTN- Hypertension

INH – Isoniazid

ISO – Isoprenaline

K⁺ - Potassium ions

KCl – Potassium chloride

KH₂PO₄ – Potassium hydrogen tetraoxophosphate V

LDL – Low density Lipoprotein cholesterol

MEP – Mepyramine

Met Ex. – Methanol extract

NaCl – Sodium chloride

NaHCO₃ – Sodium hydrogen trioxocarbonate IV

Pet- ether – Petroleum ether

PVR – Peripheral vascular resistance

PZA – Pyrazinamide

RAAS – Rennin angiotensin aldosteron system

RMP – Rifampicin

SA – Sinoatrial

SBP – Systolic blood pressure

TPR – Total peripheral resistance

WHO – World health organization.

CHAPTER ONE

1.0 INTRODUCTION

1.1 The Hypotensive Effect of *Moringa oleifera* Mediated through cardiovascular System

Many edible plants are reliable source of medicines in many different parts of the world and are often used both as food and drug to maintain health. *Moringa oleifera* is one of these numerous exceptionally nutritious vegetable plants that is widely cultivated and claimed to be useful for the management of various ailments (Fahey, 2005; Anwar *et al.*, 2007). The plant *Moringa oleifera* has been reported to have several biologically active constituents like minerals, fatty acids, carbohydrates, vitamins, proteins and amino acids alkaloids, flavonoids, anthocyanins, proanthocyanidins, cinnamates (Bhoomika *et al.*, 2007). *Moringa oleifera* plant has been reported to possess a wide range of pharmacological effects including hypotensive actions (Majambu, 2012). Various parts of this plant act as cardiac and circulatory stimulant and are highly used in the indigenous system of medicine for both high blood pressure and for poor blood circulation (Aliyu *et al.*, 2010). Usually, the constituents with pharmacological activity and / or their quantity vary with both the plant parts and choice of solvent extraction. This study was therefore carried out to compare the hypotensive effect of crude extracts of the leaf and seed respectively of *Moringa oleifera* plant on cat blood pressure and guinea pig heart atria.

Hypertension is a chronic medical condition and a term used to describe persistent high blood pressure. It is a condition that occurs as a result of increase in the arterial blood pressure or repeatedly elevated blood pressure exceeding 140 over 90 mmHg (Nahida and Feroz, 2011). Persistent high blood pressure (hypertension) requires the heart to work harder than normal in attempt to supply blood and this may cause damage to several body organs and / or results in other cardiovascular conditions such as stroke, heart attack, heart failure or even death (Oates and Brown, 2001; Cunha, 2011). Several other adaptive changes may also occur in blood vessels leading to endothelial dysfunction and / or increase in the extracellular matrix (Guyton and Hall, 2006).

Hypertension (HTN) as with other cardiovascular diseases affects mostly the heart and the blood vessels and it is noteworthy that cardiovascular diseases (CVDs) are one of the leading causes of death in the world and which accounts for approximately 17 million global deaths annually (Lim *et al.*, 2012). Hypertension alone is responsible for at least 45% of such deaths and then stroke which is responsible for about 51% (WHO, 2008), and which in most cases is predisposed by hypertension. Approximately 1 billion people were diagnosed of hypertension of which the low income countries including the African sub-region were found to be more susceptible (about 40% prevalence); and the developed countries like America also showed a prevalence of 35% (WHO, 2013). Thus, hypertension is of a public health importance in both developed and developing countries. It has been estimated that by 2025, the developing countries will have about 75% of the global incidence of hypertension (Kearney *et al.*, 2005).

1.2 Statement of Research Problem

Various parts of *Moringa oleifera* are used locally in their crude forms for self-care, disease prevention and treatment based on their ethnomedical claims. *Moringa oleifera* is locally recommended both for high blood pressure and for poor blood circulation (Anwar *et al.*, 2007). However, the exact part of this plant and or extractive solvent that provides the best of its cardiovascular activity had not been reported; and thus, the leaves, seeds or stem-bark are blindly used in various disease conditions.

High blood pressure is a chronic medical condition of elevated arterial blood pressure that results in inadequate blood circulation and / or adaptive changes in blood vessels that lead to endothelial dysfunctions (Guyton and Hall, 2006). Most of the cardiovascular disease (CVDs) related deaths have been attributed to hypertension and which has been reported to be responsible for about 45% of approximately 17 million global deaths annually (Lim *et al.*, 2012).

1.3 Justification of the Study

There is need to know the plant parts of *Moringa oleifera* and the extracting solvent that gives the optimum hypotensive effect and also the mechanism by which this effect occurs. Hypertension is a known common disease condition sometimes related to stress and lifestyle; and although it has been noted that the prevention of the prevalence of hypertension and other CVDs consists of the non-pharmacological measures that includes lifestyle modification (Michael, 2014), the need for available natural food plants that are eaten daily in diets or drunk as blood tonics is pertinent. This can be particularly useful in moderately elevated blood pressure conditions to control the system and reduce the dependence on orthodox medications.

1.4 Aim and Objectives of the Study

The aim of the study is to compare the crude extracts of *Moringa oleifera* leaf and seed for their hypotensive effects in cat blood pressure model and guinea pig heart atria.

The objectives of the study are to:

- Screen the phytochemical constituents of both the leaf and seed of *Moringa oleifera*
- Compare the crude extracts of *Moringa oleifera* leaf and seed for their hypotensive effects in both the cat blood pressure and guinea pig heart atria
- Assess the effect of specific receptor antagonists on the extracts' effects for the elucidation of probable underlying receptor-mechanism of the hypotensive action.

1.5 Hypothesis

Most of the hypotensive effects of *Moringa oleifera* are present in the methanol or petroleum ether seed extract and not in the aqueous or methanol leaf extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Description of *Moringa oleifera*

2.1.1 Taxonomy

Moringa oleifera is a plant tree belonging to the division *Magnoliophyta* and class *Magnoliopsida*.

Family	<i>Moringaceae</i>
Genus	<i>Moringa</i>
Species	<i>Moringa oleifera</i>

There are about fourteen (14) different species of moringa genus. However, only *Moringa oleifera* and *Moringa stenopetala* are most widely known (Quintin *et al.*, 2011). Other species of this genus include: *Moringa pelegra*, *Moringa aptera*, *Moringa concanesis*, *Moringa drouhardii*, *Moringa hildebrandti*, *Moringa longituba* and so on. (Fahey, 2005).

2.1.2 Origin

Moringa oleifera tree grows mainly in semi-arid tropical and sub-tropical areas. The plant originated from Asian countries including India, Sri Lanka, Bangladesh, Malaysia, Philippines, but is now widely cultivated and used in African countries (like Ethiopia, Zimbabwe, Nigeria); Latin America, Florida, Pacific Island and Mexico (Fahey, 2005; Durgash *et al.*, 2013). It is known and utilized by ancient Greeks, Romans, and Egyptians (Fahey, 2005).

2.1.3 Common names

The name *Moringa oleifera* pronounced as (moh-rin-guh oh-lee-if-er-uh) is derived from a Latin word meaning “oil bearing plant”.

Common names of the plant are:

- Drumstick tree (derived from the shape of the pod (Byrne, 2005))
- Benoil tree
- Horseradish tree
- Mother’s best friend

Other name(s) of *Moringa oleifera* (DictReIm 1994: Sheldon and Steve, 2010) include:

- Senjana/Saihjan — India (Hindu)
- Zakalanda — Zimbabwe (Binga district)
- Zogallan gandi (Zogale) — Northern Nigeria (Hausa)
- Okwe oyibo — Eastern Nigeria (Ibo)
- Ewe-igbale (Ewe ìle) or Idagba-monoye — Southern Nigeria (Yoruba)
- Malunggay — Philippines (Filipino)
- Moringa aileé — France (French)
- Moranga — Spain (Spanish)

2.1.4 Physical appearance

Moringa oleifera is a deciduous plant tree that sheds its leaves during the dry season to conserve water and it is both drought and parasite resistant. It is a perennial slender softwood plant tree of about 10 m tall with drooping branches, brittle stems and corky bark. It has feathery compound tripinnate leaves of many leaflets on the stalk and the

leaves are alternate, dark green in colour on the inside and pale green underneath. The flowers are white and hairy and have fragrant nice scented smell while the fruits are light-brown in colour and sprout around March – April with each fruit containing about 20 winged round seeds which are light and black in colour (Sheldon and Steve, 2010; Ganatra *et al.*, 2012).



Plate i- *Moringa oleifera* seed



Plate ii- *Moringa oleifera* plant

***Moringa oleifera* seed and leaves**

2.2 Chemical Properties

Moringa oleifera has been reported to contain many chemical constituents (Fahey, 2005; Ganatra *et al.*, 2012). They include,

- i. Minerals – Calcium, Phosphorus, Iron, Copper, iodine
- ii. Fatty acids – Palmitic, Stearic, Behemic, Oleic, Myristic, Lignoceric acids.
- iii. Vitamins A, B, C, and E
- iv. Proteins and Amino acids – Arginine, Histidine, Lysine, Tryptophan, Phenylalanine, Methionine, Threonine, Leucine, Isoleucine, and Valine
- v. Carbohydrates – Rhamnose, Glucosinolates and Isothiocyanates.
- vi. Estrogenic substances – Antitumor compound, β – sitosterol, pectinesterase
- vii. Alkaloids – moringine, moringinine, spirochine
- viii. Steroid – phytosterin
- ix. Flavonoids – zeatin, quercetin, β – sitosterol, Caffeoyl/quinicacid, kaempferol

2.3 Uses of *Moringa oleifera*

Moringa oleifera is regarded as a vegetable of high nutritive value in the developing world and is used both as human and animal forage (Fahey, 2005; Anwar *et al.*, 2007; Ganatra *et al.*, 2012). It has a widespread use as fertilizer and natural pesticide as well as for water purification (Ganatra *et al.*, 2012). *Moringa oleifera* is generally cultivated for food, natural medicine, cash earning and many other uses (Fahey, 2005; Sheldon and Steve, 2010) as described below:

2.3.1 Use as food

The growing tips, young leaves and shoots as well as pods are used soups and sauces as vegetables. These have been found to be low in fats and carbohydrates and rich in minerals, iron, calcium, phosphorus and vitamins (Anwar *et al.*, 2007; Sheldon and Steve, 2010). The seed has the appearance of green beans and flavour-taste of mushroom (asparagus) and often referred to as peas. The seed contains about 35% oil (known as benoil) which is not only used for cooking purposes, but also for lubrication, perfume and soap. The flowers are also edible when cooked and are said to taste like mushrooms. The several food nutrients contained in *Moringa oleifera* pods and leaves are responsible for its immune boosting defensive mechanism (Anwar *et al.*, 2007).

2.3.2 Use as animal fodder

The leaves and young shoots and branches are also used for livestock feed especially for cattle, sheep, pigs, and rabbits (Adegun *et al.*, 2011; Tete *et al.*, 2013).

2.3.3 Use as fertilizer and natural pesticide

The seed-cakes by-product of oil extraction contains about 58.9% crude proteins and serves as nutritive fertilizer for the soil and therefore used in agriculture. It also has bactericidal and fungicidal properties for soil protection (Emmanuel *et al.*, 2011; Prabhu *et al.*, 2011). The leaves of *Moringa oleifera* also make useful mulch to prevent seedling damping off (Council for Scientific and Industrial Research, 1962).

2.3.4 Use for water purification

The seed-kernel powder is used for cleaning dirty water and serves as a substitute for aluminium sulphate. The powder joins with the suspended solid silt particles, bacteria

and other microorganisms in the water and causes this dirt to stick together or coagulate and sink to the bottom of the container. Thus, removing about 90 – 99% of bacteria contained in the water (Aho and Lagasi, 2012; Mununi *et al.*, 2013).

2.3.5 Ornaments and other uses

Moringa oleifera is often planted as backyard vegetables and as border garden plant in urban areas to serve as a shade tree and as hedge plant (Maroyi, 2006). The wood provides a pulp used for newsprint, wrapping paper, printing and writing papers. The bark and gum from *Moringa oleifera* can be used in tanning hides (as shoe and bag industry). The moringa oil is of high quality and potentially has a high market value and as a source of income. The oil is of equal value both as cooking oil and as the main ingredient for soap manufacture (Council for Scientific and Industrial Research, 1962; Mehta *et al.*, 2011).

2.4 Pharmacological and Medicinal Properties of *Moringa oleifera*

Moringa oleifera is very important for its medicinal values and various parts of the plant (including leaves, roots, seeds, barks, fruits and flowers and immature pods) have medicinal properties. They are widely employed as natural medicine and are used in various forms as extracts, decoctions, creams, oils, powders e.t.c (Fahey, 2005; Biswas *et al.*, 2011). The medicinal uses of *Moringa oleifera* as herbal plant in traditional medicine consist in its claims to have medicinal properties, (Fahey, 2005; Ganatra *et al.*, 2012), which include:

- Cardiac and circulatory stimulation
- Antitumor agent
- Antimalarial and Antipyretic

- Anti-inflammatory
- Antiulcer
- Antispasmodic
- Antiepileptic
- Diuretic
- Antihypertensive
- Cholesterol lowering
- Antioxidant
- Antidiabetic
- Hepatoprotective
- Antibiotics
- Antifungal

2.4.1 Traditional medicinal uses

The various parts of *Moringa oleifera* used as traditional medicine include:

2.4.1.1 Leaf and pod (as tea)

This is used for gastric ulcer and diarrhoea, dysentery and colitis (colon inflammation) and It also treats intestinal parasites and constipation when used as a purgative and digestants and also cures gonorrhoea (Durgesh *et al.*, 2013). The seed pod tea is used as a de-wormer. And it also has some analgesic properties and used to treat joint pains (Rao *et al.*, 2008). The Leaves are also used as source of sulphur-containing amino acids like methionine and cystine and this high concentration of amino acids, minerals and vitamins' including β – carotene, high protein and fibre content makes *Moringa oleifera* an ideal nutritional supplement in preventing anaemia and most forms of malnutrition (Council of Scientific and Industrial

Research, 1962; Zongo *et al.*, 2013). The leaves of *Moringa oleifera* is therefore highly recommended for children and pregnant women who believe that drinking of the broth from cooking *Moringa oleifera* leaves as soon as uterine contraction pains are felt speeds up and facilitates delivery (Council of Scientific and Industrial Research, 1962; Maroyi, 2008). The leaf juice is believed to have a controlling effect on blood pressure and also used to control glucose level in diabetics (Council of Scientific and Industrial Research, 1962; Durgesh *et al.*, 2013).

2.4.1.2 *Seed (decoction and oil)*

The Seeds and oil of *M. oleifera* are used for their antibiotic and anti inflammatory properties in arthritis, rheumatism, gout, cramp, sexually transmitted diseases and boils (Fahey, 2005). They are also known as anticonvulsant for epilepsy (Anwar *et al.*, 2007). The seed oil is used in perfumes and hair cream and also in treating external skin diseases. The seeds are effective against skin infecting bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. An antibacterial and antifungal substance known as pterygospermin is found in *M. oleifera* seeds. (Council of Scientific and Industrial Research, 1962; Kumar *et al.*, 2010).

2.4.1.3 *Flower (juice)*

Moringa oleifera flower (juice) is used to improve the quality and flow of mothers' milk (galactagogue or lactation) in breastfeeding women also as diuretic and aphrodisiac properties and used as cold remedy (Durgesh *et al.*, 2013).

2.4.1.4 *Root and bark (decoction)*

Decoction of the boiled roots *M. oleifera* is bitter and often used as tonic and to wash sores and ulcers including sore throats or asthma and this expectorant property helps to eliminate secretions from the respiratory tracts (Mehta *et al.*, 2011). The decoction

also is used in paralytic afflictions, epilepsy, and hysteria as mild stimulant (Parankh, 2013). The root juice is applied externally as rubefacient or counter-irritant on inflammatory pains, otalgia (earaches) and ulcers (Parankh, 2013). The juice from the bark is used to treat inflammatory swellings and also used to calm excitement and restlessness (Parankh, 2013). However, the use of roots, barks and gums as medicines are restricted or used sparingly because they are highly concentrated with the alkaloid spirochine which is a potentially fatal nerve paralysing agent (Ecubantang, 2011).

2.4.2 Scientific research validation of some claimed medicinal uses of *Moringa oleifera*

The nutritional properties of *Moringa oleifera* is now well known in many parts of the world including Nigeria where is largely used as food. A large number of reports on the nutritional qualities of *Moringa oleifera* now exist in scientific literature to justify its indigenous use as food plant (Anwar *et al.*, 2007). Studies have shown that *Moringa oleifera* contain more vitamin A than carrots, more vitamin C than oranges, more potassium than bananas and a protein quality that can be compared to milk and eggs (Fahey, 2005; Anwar *et al.*, 2007; Sheldon and Steve, 2010). Although substantial reports are available on the nutritional benefits of *Moringa oleifera*, well controlled or documented clinical use studies are still fragmentary. Published *in-vitro* and *in-vivo* trials have provided practical support for the folkloric claims, but such scientific research reports in literature for *Moringa oleifera*, are very few. Some of the areas in which *Moringa oleifera* research had been reported are described below:

- The ethanolic extract of the leaves of *Moringa oleifera* when administered orally has been reported to have hepato-protective effect on the liver damage induced by antituberculosis drugs like isoniazid (INH), rifampicin (RMP) and

pyrazinamide in rats. The effects of *Moringa oleifera* was assessed on the levels of glutamic oxaloacetic transaminase (Aspartate amino transferase), glutamic pyruvic transaminase (alanine amino transferase), alkaline phosphatase and bilirubin in the serum and on the levels of lipid peroxidation in the liver (Ruckmani *et al.*, 1998; Pari and Kumar, 2002; Anwar *et al.*, 2007).

- The crude extract of *Moringa oleifera* leaves fed to wistar rat fed high fat diet have been shown to reduce high fat diet-induced increase of cholesterol levels in the serum, liver and kidney. The extract however showed no observable effect on the serum total protein (Chumark *et al.*, 2009; Jain *et al.*, 2010).
- *In-vitro* antifungal activities of the seeds and leaves of *Moringa oleifera* against dermatophytes such as *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum canis* has also been demonstrated (Chuang *et al.*, 1998/2007; Jinn Shieh *et al.*, 2007; Kekuda *et al.*, 2010)
- The protective effect of alcohol (methanol and ethanol) and acetone extracts of the leaves of *Moringa oleifera* on gastric and duodenal ulcers evaluated on acetic acid and indomethacin induced chronic gastric ulcers in pylorus-ligated rats had also been reported (Devaraj *et al.*, 2007; Das *et al.*, 2011). The study showed that the acetone and methanol extract produced gastric anti-secretory effects. Gastric cytoprotective effect was observed in the ethanol induced and indomethacin induced gastric ulcers in albino rat. The leaf extract also produced significant reduction of stress-induced gastric ulcers and cysteamine induced duodenal ulcers (Devaraj *et al.*, 2007; Das *et al.*, 2011).
- The intravenous (i.v) administration of either of the pure active principles (Thiosulphate glycosides, Niazinin A, Niazinin B, Niazimicin, Niazimin A +

B) of *Moringa oleifera* has been reported to possess hypotensive and bradycardiac effects in anaesthetized rats. The compounds also exhibited spasmolytic activity on isolated rat uterus and ileal tissues, justifying its traditional use in gastrointestinal motility disorders (Anwar *et al.*, 1993; Gilani *et al.*, 1999).

- Pterygospermin one of the constituents of carbohydrates in *Moringa oleifera* has been identified and found to have antimicrobial properties (Kekuda, *et al.*, 2010). The other carbohydrate constituents also found to have a wide range of bacterial and fungal activities are benzyl isothiocyanate and its glycosylated derivatives (Mikkon *et al.*, 2003; Anwar *et al.*, 2007). This therefore provides some justification in the use of the plant parts in a variety of dermal and internal infections. However, some of the traditional antibiotic claims for other constituents have not been validated. The antibiotic activity of (4 – (α – L – rhamnopyranosyloxy) benzyl isothiocyanate) and (benzyl isothiocyanate) of the carbohydrate constituent has also been demonstrated on *Helicobacter pylori* – the bacterium responsible to cause gastritis of gastric and duodenal ulcers and a major risk factor for gastric cancer having been classified as a carcinogen by (WHO, 1993) (Das *et al.*, 2011). Cultures of *Helicobacter pylori* were found to be susceptible to (4 – (α – L – rhamnopyranosyloxy) benzyl isothiocyanate) in an antibiotic concentration 1000 folds lower than the concentrations which had been used in earlier studies against a wide range of bacteria and fungi (Das *et al.*, 2011). Human studies carried out to authenticate this antibiotic activity finding using a prototype isothiocyanate against human *Helicobacter pylori* infection have also shown some efficacy (Das *et al.*, 2011)

- Compounds (4 – (4 – o – acetyl – α – L – rhamnopyranosyloxy) benzyl isothiocyanate and (4 – (α – L – rhamnopyranosyloxy) benzyl isothiocyanate) have been isolated from carbohydrate constituents of *Moringa oleifera* and have been shown to have anticancer potential (Fahey, 2005). A dramatic reduction in skin papillomas has also been demonstrated in mouse models with these compounds. Although the claimed traditional cancer prevention and therapy of *Moringa oleifera* was adequately demonstrated in animal models, it still has not been possible to authenticate this effect in human subjects owing to the inability of researchers to discover the biomarkers of the plants protection (Fahey, 2005).
- The methanol seed extract has been shown to possess local anaesthetic effects as well as prolong diazepam-induced sleeping time in mice, (Bandana *et al.*, 2003; Saroj *et al.*, 2006).
- The alcohol seed and aqueous leaf extracts also showed antinociceptive activity (Sulaiman *et al.*, 2008; Sutar *et al.*, 2008). Similarly, the wound healing and immunostimulatory effects have also been demonstrated with the aqueous extract of the leaf which also inhibited oedema in rats (Ndiaye *et al.*, 2002; Rathis *et al.*, 2006; Anwar *et al.*, 2007). Furthermore, the seed powder also reduced bronchial asthma and other respiratory tracts effects as demonstrated by Babita *et al.*, (2008). The anticonvulsant activity of the methanol leaf extract has also been demonstrated (Amrutia *et al.*, 2011).

2.5 Blood Pressure

2.5.1 Definition and types

Blood pressure (measured in millimeters of mercury) is the force of blood on the walls of the circulatory vessels which normally distribute the blood that is pumped out of the heart; and this pressure is the product of cardiac output (CO) and peripheral vascular resistance (PVR) (Guyton and Hall, 2006). Cardiac output depends on the force (tensile) strength of the cardiac volume, arterial elasticity and the two-phase activity of the heart - contraction (systole) and relaxation (diastole). The maximum (arterial) contraction is of the peak or systolic blood pressure (SBP) during which a volume of blood (stroke volume) is pumped into the two ventricles at different pressures of 120 mmHg for the left and 25 mmHg for the right in human (Guyton and Hall, 2006). The minimum (ventricular) repolarisation or relaxation phase is of the diastolic blood pressure (DBP) during which the blood in the ventricles is discharged at a pressure of 70 mmHg in human (Guyton and Hall, 2006). The output from each ventricle per minute is the cardiac output and it varies with the heart rate (No. of systoles and diastoles in a minute = cardiac cycle which normally is 70 cycles / min) and stroke volume (volume pumped into the ventricles).

Arterial pressure is conventionally written as the ratio of the left ventricular systolic pressure (120) and the diastolic pressure (70) mmHg and the difference between this maximum and minimum pressures (= 50) is called pulse pressure or end systolic ventricular blood volume (Guyton and Hall, 2006). The mean arterial blood pressure is an approximate value of $1/3^{\text{rd}}$ of the pulse pressure. There is no universal upper or lower limits, but many cardiologists consider an upper limit of 160/100 mmHg

(systolic / diastolic) and a lower limit below 110 / 70 mmHg to be pathological (Cunha, 2011).

Usually, SBP < 90 mmHg and diastolic < 60 mmHg is often regarded as low bp (hypotension). Normal blood pressure is that at rest (systolic range of 100-140 mmHg and diastolic range of 60-90 mmHg) and thus, SBP ≤ 120 with DBP ≤ 80 is regarded normal. SBP of ≥ 120 - 139 with DBP ≥ 80 - 89 is of borderline HTN, while SBP of ≥ 140 with DBP ≥ 90 is typically regarded as high blood pressure (arterial hypertension - HTN) (Chobanian *et al.*, 2003). A high or elevated SBP with normal DBP or vice versa is often described as isolated systolic HTN and this is commonly seen in the elderly. The SBP also often tend to increase during exercise, but usually to not more than between 200 - 230 mmHg (Wallace, 2003). Resistant HTN is used to describe medication resilient types.

2.5.2 Blood pressure regulation

The anatomic sites responsible for maintaining and regulating blood pressure and intravascular volume are the heart, arterioles, capacitance vessels (veins), baroreceptors and the kidneys (Katzung, 2007).

2.5.2.1 The heart, arterioles and capacitance vessels

Agents that affect both the force and rate of myocardial contraction positively to stimulate it or negatively to depress it are generally referred to as inotropic agents, while those that increase only the heart rate are called chronotropic agents (Katzung, 2007). Each contraction and recovery events of the heart presents an electrical complex waves pattern such as P-wave, QRS complex and T-waves. Although it is Ca²⁺ (Ca²⁺ gated voltage channel), as with all muscle cells that triggers the electrical

activity or action potential of the heart and vascular smooth muscles to cause permeability of metabolic sodium and potassium ions that leads to contraction, potassium is usually the dominant ion conductive pathways that maintains the membrane potential (Guyton and Hall, 2006). Ca^{2+} is however, required for efficient contraction and many vasoconstrictor and vasodilatory agents exert their effects by increasing or reducing intracellular calcium concentrations (Guyton and Hall, 2006).

The arterioles are the major site of resistance to the outflow of blood and as the pressure exerted on the arterial walls of the arteries rises linearly, the radii diameter of the arterioles decreases in proportionate manner such that the ratio of the pressure that now distends the heart to that of the decreasing radius of the arterioles produces the outflow (in mL/s) (pressure/resistance) (Guyton and Hall, 2006). It is also the diastolic recoil of the arteries that moves the blood forward depending on the blood viscosity. Arterial receptors in the walls of the left and right atria are often regarded as low pressure sensitive receptors stimulated only by distention of the structures in which they are located (Guyton and Hall, 2006). Vasoconstriction and vasodilation are terms used to describe the caliber of resistance vessels. Constrictor tone reduces the radius of arterioles (arteriolar vasoconstriction) increasing PVR and bp as with vasoconstrictor agents (impulses from the adrenergic sympathetic nerves; vasopressin, angiotensin II, serotonin, drop in temp. as in cold etc); while dilator tones increases the radius of arterioles (arteriolar vasodilation) decreasing PVR and bp as with vasodilator agents (impulses from the cholinergic vagal nerves, low O_2 and pH, high CO_2 , temp and K^+ , vasoactive substances like kinins and ANP, histamine) decrease bp (Katzung, 2007).

The pressure mount on the veins occurs only when large volumes accumulates to a certain capacity and it does not rise linearly as with the arteries, thus, the veins are usually referred to as the capacitance vessels (Katzung, 2007). The metaarterioles (capillaries) serve for exchange of substances between interstitial fluid and bloodstream and also serve as link between the resistance vessel (arterioles) and the capacitance vessels (veins) (Guyton and Hall, 2006).

2.5.2.2 *Baroreceptors and chemoreceptors*

Baroreceptors are high pressure or stretch sensitive receptors located in the aortic arch and in the common carotid artery and which monitor the arterial circulation (Thresher, 2007). The baroreceptors in the carotid sinus are responsible for the regulation of the bp in the brain, while those in the aortic arch are responsible for regulation of systemic blood pressure. Chemoreceptors (carotid and aortic bodies) are of small structure located close to the baroreceptors and which detect and react to changes in the chemical compositions (O_2 and CO_2 levels or pH) of the blood. Changes in blood pressure often tend to stimulate the baroreceptors and while the vagus nerve of the parasympathetic nerve controls the decreases in blood pressure (vasodilation), the sympathetic nervous system controls the increases (vasoconstriction) associated with accelerated heart rate and contractility (Heusser *et al.*, 2005). The sympathetic motor fibres to arterioles regulates tissues blood flow *vis a vis* bp and are responsible for short term blood pressure fluctuations and they often respond to transient changes in blood pressure via baroreflex mechanisms. Thus, the barosensitive sympathetic nerve controls the release of noradrenaline from the adrenal chromaffin cells and tends to constrict the resistance arterioles of the heart and kidneys as to control the rennin secretion and renal tubular sodium reabsorption for neural control of blood pressure.

2.5.2.3 Kidneys

The kidney responds to changes in bp by increasing or decreasing excretion of water and electrolytes and its rapid increase in the excretion of sodium and water restores the arterial or systemic bp to normal (Crowley *et al.*, 2006). The kidney secretes some vasoactive polypeptides that are often involved in its regulatory mechanisms and these include:

- Kinins formed from body substrates (kininogens) and the three types are bradykinins (a nonapeptide from plasma), lysylbradykinin (a decapeptide from tissues) and methonyllysylbradykinin (in urine) (Katzung, 2007). Kinins are often converted to their active peptides by kininases (carboxypeptidase and converting enzyme dipeptidyl carboxypeptidase). lysylbradykinin (angiotensin I) is a fraction of the plasma glycoprotein molecules which rennin often split off from its α_2 -globulin protein (angiotensinogen) component fraction that it uses as its substrate.
- Renin is one of the three hormones (1,25-dihydroxycholecalciferol, erythropoietin) released into blood from the juxtaglomerular cortex of the kidney and which in high release causes rise in blood pressure (Katzung, 2007). The splitted off lysylbradykinin (the decapeptide kinin fraction or angiotensin I) now freely susceptible to angiotensin converting enzyme, is then converted to an active octapeptide form (angiotensin II, hypertension or angiotonin) which is a potent vasoconstrictor that causes the rise in bp. The activity of the plasma angiotensin II in the elevation of extracellular fluid volume and bp for which angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are used in HTN consists in:

- i. Increase in aldosterone release from the adrenal cortex (rennin-angiotensin aldosterone system - RAAS) to cause increase in the reabsorption of sodium chloride / K^+ and fluid in the distal nephron. Increases rennin and / or angiotensin II formation is part of a homeostatic mechanism that operates to maintain the ECF volume in response to sodium depletion, decreased renal perfusion, cirrhosis, nephrosis, dehydration and low bp. High rennin-angiotensin aldosterone activity may cause profound rise in bp and / or 2^o hyperaldosteronism (Crowley *et al.*, 2006).
 - ii. Increase in release of arginine vasopressin from the posterior pituitary gland to enhance fluid retention in the renal collecting duct for blood volume conservation. (Crowley *et al.*, 2006).
 - iii. It stimulates thirst and the subsequent increase in fluid ingestion expands blood volume and raises bp (Cervenka *et al.*, 2000).
 - iv. Angiotensin II receptors in the brain increases or facilitates sympathetic output of noradrenaline to both the heart (increasing cardiac output, TPR and bp) and vascular bed causing vasoconstriction of renal and systemic blood vessels (especially of small arteries and arterioles) (Crowley *et al.*, 2006).
- Natriuretic peptides: these include arterial natriuretic peptide (ANP) synthesized in the atrium of the kidney cortex or aorta, brain (or B-type) natriuretic peptide (BNP) synthesized in the ventricle, and CNS (C-type) natriuretic peptide (CNP) all of which play an important role in regulating bp and volume at varying intensities causing diuresis and natriuresis (urinary removal of sodium). The release of ANP and BNP from the heart has the most

immediate biologic effect of antagonizing the salt sparing role of the rennin-angiotensin aldosterone system by blocking the release of rennin-aldosterone and promoting renal-artery excretion of electrolyte, sodium and water (Bricca and Lantelme, 2011). The peptides also oppose various vasoconstrictor agents including catecholamines and modulate systemic vascular resistance.

2.6 Hypotension

The SBP < 90 mmHg and diastolic < 60 mmHg could be termed healthy in the absence of associated abnormal body functions or symptoms, but according to Klabunde (2014), abnormally low bp generally may often results from:

- reduced cardiac output (amount of blood pumped out of the heart): heart valve problems, severe congestive heart failure, bradycardia or low heart rate which often progress to cardiogenic shock and arrhythmias often results in hypotension.
- hypovolemia (reduction in the blood volume of the body): often seen in conditions of insufficient fluid intake as in starvation or excessive fluid and electrolyte losses from diarrhea or vomiting, haemorrhage, use of drugs like diuretics.
- blood volume redistribution: the amount of blood getting to a particular organ or tissue determines the amount of nutrients and oxygen supplied, thus, low blood volume redistribution can lead to reduced oxygen resulting in hypotension.
- reduced peripheral vascular resistance (PVR): this may lead to anaphylactic reactions, septic conditions, neurogenic or autonomic disfunctions etc.

- vascular obstruction as in pulmonary embolism: this may cause diminished venous return that reduces the amount of blood that gets to the heart and / or cardiac output
- reduction in the activities of peripheral and central sympathetic systems
- interference with rennin-mediated angiotensin production
-

2.7 Hypertension

2.7.1 Types of hypertension

Hypertension (HTN) affects mostly the heart and the blood vessels on which it exerts a higher than normal pressure work load. Hypertension is of two basic types (Katzung, 2007):

- i. Primary- 1^o (idiopathic or essential) HTN
- ii. Secondary- 2^o (symptomatic) HTN.

About 90 - 95% of hypertensive cases are of the 1^o type, having no obvious underlying medical causes and are often thought to be related to a complex interaction of genes and environmental factors (Chobanian *et al.*, 2003). This type of HTN often develops gradually with age and is mostly heritable (Carretero and Oparil, 2000). The other 5 - 10% cases of HTN are of the 2^o type which often results from underlying causes that requires treatment as with conditions of complex structural and functional changes such as:

- Inability of the kidneys to excrete sodium: resulting in secretion of atrial natriuretic factors that promote salt excretion vis a vis increased TPR.

- Over activity of rennin-angiotensin system that causes vasoconstriction and retention of sodium and water that leads to increase in blood volume and /or HTN
- Over activity of sympathetic nervous system that leads to increase in stress responses and /or HTN
- Over productions of adrenal glands cortisol, cushing's syndrome, hypo or hyper -thyroidism, kidney diseases, metabolic disorder, some drug medications, congenital defects (coarctation), pre-eclampsia during pregnancy (gestational) are all causes of 2° HTN (Crowley *et al.*, 2006; Takahashi, 2008; Dodt *et al.*, 2009).

2.7.2 The risk factors of hypertension

The risk factors of HTN include:

- Sedentary lifestyle,
- Stress
- Obesity
- Potassium deficiency (hypokalemia)
- Salt sensitivity
- Alcohol intake
- Vitamin D deficiency
- Genetic mutations and family history (Michael, 2011).

2.7.3 Symptoms of hypertension

Hypertension may be asymptomatic at the initial stage, but some signs may occur such as

- headache

- confusion
- fatigue
- irregular heart beats
- vision changes and
- dizziness (Chobanian *et al.*, 2003).

2.7.3 Diagnosis of hypertension

To measure the blood pressure, an inflatable cuff with an attached meter (sphygmomanometer) and stethoscope for the ears is placed around the arm at the level of the heart and inflated to briefly interrupt arterial blood flow. It is then slowly deflated while listening to the pumping sounds from the heart (the 1st being for the systole and then the diastole) noting the metre readings corresponding to the sounds as the blood pressure measurement (Hodgkinson *et al.*, 2011).

Routine laboratory tests include:

2.7.3.1 Electrocardiogram -ECG

Obtaining a record of the electrical activity of the heart (electrocardiogram -ECG) that evaluates the heart rate and rhythm as to check for changes from the normal heart events that indicates damage or abnormality (Javon, 2011).

2.7.3.2 Urinalysis

This assesses the kidney functions. Urinalysis is a physico-chemical microscopic examination of the urine to quantitatively detect the cellular materials or compounds (proteins, albumin, globulin) normally passed out in urine in conditions of kidney disorders. Albumin excretion is often used to check the albumin – creatinine ratio (ACR). Creatinine or the corresponding estimated glomerular filtration rate (eGFR) is

also used to monitor kidney dysfunction. The presence of decreased GFR or albuminuria has prognostic implications in kidney disorders (Mann *et al.*, 2001; Beddhu *et al.*, 2002).

2.7.3.3 *Blood glucose*

High blood sugar above the normal range as occurs in diabetes can lead to heart or kidney disease complications (Hemmelgarn *et al.*, 2005).

2.7.3.4 *Haematocrit*

This parameter as with haemoglobin level is often used to monitor the proportion of red blood cells in blood. It evaluates the fluid level as to check dehydration or excess loss of water which often leads to electrolyte imbalance and changes in blood pressure (Hemmelgarn *et al.*, 2005).

2.7.3.5 *Serum potassium and other body electrolytes*

The opening of K^+ channels results in out-diffusion of K^+ (membrane hyperpolarisation and vasodilatation), while the opening of the Cl^- channels results in efflux of Cl^- and this being negatively charged, causes depolarization and vasoconstriction as with the closure of K^+ channels. Normal potassium level maintains fluid and electrolyte balance within and outside the cell because the sodium level also changes in reverse direction with changes in potassium level. Other factors that affect the serum potassium level include the adrenal gland hormone –aldosterone, renal condition, blood pH, ingested potassium, diuretics and emesis. Too high or too low level of potassium is often a serious condition that can cause changes in cardiac rhythm (Hemmelgarn *et al.*, 2005).

2.7.3.6 *Calcium level*

Increased serum calcium often seen in conditions of increased activity of the parathyroid has been found to be associated with HTN (Hemnelgarn *et al.*, 2005).

2.7.3.7 *fasting lipoprotein profile*

This parameter is also used to check for the presence of atherosclerosis as a risk factor of HTN and it evaluates the levels of the high and low density lipoprotein cholesterol (HDL & LDL) as well as the triglycerides. Cholesterol is a fatty substance essential in the maintenance of cell membranes and formation of many hormones and fat digestible bile acids, but excess cholesterol accumulates in the walls of arteries and increases the risk of heart diseases including HTN (Hemnelgarn *et al.*, 2005).

2.8 Treatment of Hypertension

The treatment goal of HTN is to lower the systolic blood pressure to less than 140 mmHg and the diastolic below 90 mmHg using both lifestyle modification and drug therapy (Michael, 2014). The lifestyle or non-pharmacological measures is often regarded as the foundation stone to prevent the prevalence of HTN and CVD. It is particularly useful in moderately elevated bp in which it is sometimes used alone to achieve treatment goal or used to reduce the frequency of medications or drug therapy.

2.8.1 Non-pharmacological treatment measure

- Lifestyle modification

This includes:

- Weight loss in overweight by reducing calorie intake and increasing physical activity

- Salt (NaCl) restriction
- Reduction in alcohol intake
- Consumption of diets of reduced saturated fat, fresh fruits and vegetables
- Some other lifestyle adjustments which have not been found to have significant antihypertensive effect include intake of calcium and magnesium supplements, reduction in caffeine intake etc

2.8.2 Drug therapy (Pharmacological measures) in the management of Hypertension

The common clinical drug therapy strategies to achieve a lowering of blood pressure include the use of diuretics, calcium channel blockers, beta adrenergic antagonists, angiotensin II type 1 receptor antagonists and angiotensin converting enzyme (ACE) inhibitors. Others include: centrally acting sympatholytic drugs, alpha blockers

2.8.2.1 Diuretics

Thiazides (chlorthalidone, chlorothiazide, hydrochlorothiazide); loop diuretics (furosemide, bumetanide, torsemide, ethacrynic acid) and potassium sparing diuretics (amiloride, triameterene, spiro lactone). The principal use of these three types of diuretics is to limit sodium in the body (Benowitz, 2007). Thiazides have an initial effective diuretic effect that reduces cardiac output and extracellular volume that results in reduction in systemic vascular resistance and bp and they also improve the action of other antihypertensive drugs (Benowitz, 2007).

2.8.2.2 Calcium channel blockers (CCB)

Phenylalkylamines (e.g. verapamine); dihydropyridines (eg. nifedipine, minodipine, amlodipine); benzothiazepines (eg. diltiazem): these block the entry of calcium through long lasting (L - type) channels to cause negative inotropic effect of reduced

excitability in nodal cells and peripheral vasodilatation. The phenylalkylamines act mainly on cardiac conducting tissues and are mostly valued as antiarrhythmic and antiangina agents as with benzothiazepines that act preferentially on coronary vessels, while the vasodilating dihydropyridines are mostly used as antihypertensive (Thompson, 2007).

2.8.2.3 *Beta adrenergic blockers*

Beta blockers are classified as first generation non-selectives - propranolol, sotalol; second generation cardio-selectives - metoprolol, bisoprolol, atenolol; third generation vasodilators - carvedilol, nebivolol: these generally cause hypotension via several mechanisms that include reduction in:

- Cardiac output, heart rate and contractility
- Central sympathetic nervous activity
- Plasma rennin concentrations and
- peripheral resistance

Their antihypertensive effects are mostly valued in conditions of ischemic heart disease, obstructive cardiomyopathy, congestive heart failure, arrhythmias, anxiety and thyrotoxicosis (Benowitz, 2007).

2.8.2.4 *Angiotensin converting enzyme inhibitors (ACEIs)*

Enalapril, lisinopril and angiotensin II type 1 receptor antagonists (AT1RB, - losartan): these inhibitors act on the rennin angiotensin pathway by inhibiting angiotensin converting enzyme to prevent the production of the potent vasoconstrictor angiotensin II (Thompson, 2007).

2.8.2.5 Centrally acting sympatholytic drugs

Clonidine, methyldopa, etc mainly stimulate central α_2 adrenergic receptors in the brain stem centres to reduce sympathetic nerve activity and neuronal release of noradrenaline. This class of drugs is often associated with side effects of drowsiness, fatigue and dry mouth (Vongkatanasin *et al.*, 2011).

2.8.2.6 Alpha blockers

The non-selectives - short acting phentolamine and long acting phenoxymethamine; the selectives for α_1 , prazosin and doxazosin: this group of drugs inhibits the action of catecholamine at peripheral α -adrenergic receptors. The short acting phentolamine is often used in hypertensive crises, while the long acting phenoxymethamine is used in the preoperative management of pheochromocytoma. Prazosin and doxazosin cause vasodilation and are used for benign prostatic hyperplasia, relaxation of urinary tract smooth muscle, in congestive heart failure and Raynaud's disease (Thompson, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Animals

Adult male cats (2.0-2.5 kg) and guinea pigs (300-400 g) obtained from Samaru market Zaria Kaduna state were used for the experiments.

3.1.2 Drugs

- Heparin sodium multiparin (5000 iu/ml) CP Pharmaceutical Ltd, Wrexham, U.K)
- Thiopental sodium (Rotex Medica Tritau Germany)
- Adrenaline ampoules (5ug/ml) (Sigma Chemical Company, Louis MO, USA)
- Acetylcholine (ACh) (Sigma Chemical Company, Louis MO, USA)
- Isoprenaline (Sigma Aldrich chemical company St. MO, Louis USA)
- Histamine (Sigma Aldrich Chemical Company St. Louis MO, USA)
- Propanolol (Actavis, Barnstaple, EX32 8NS, UK)
- Atropine (Halewood Chemicals Limited, Middlesex, England)
- Mepyramine (Sigma Aldrich chemical company St. MO, Louis USA)

3.1.3 Chemicals and reagents

NaCl, KCL (10%), CaCl₂ (molar), D-Glucose, NaHCO₃, KH₂PO₄ (10%), MgSO₄, 7H₂O (10%) were obtained from British Drug House (BDH), UK.

- Ringer Locke's physiological salt solutions
- Aerating gases (atmospheric air and 95% oxygen)

- De-ionised water and Normal saline (0.9% w/v)
- Methanol (British Drug House (BDH), UK)
- Petroleum ether (British Drug House (BDH), UK)
- Tween 80 (British Drug House (BDH), UK)

3.1.4 Equipment

- i. Avery balance with a sensitivity of 1 g – 3.5 kg (W. & T. Avery Limited, Birmingham, U.K) and the Top loading mettler balance (P162 Gallenkamp, U.K) were used for weighing the animals and drugs accordingly.
- ii. Thermostatically – controlled Organ Bath set-ups of 25 ml capacity were used to suspend the guinea pig heart atria. The Ugo Basile recording microdynamometer (Model No. 7050; Comerio-Varese, Italy) was used to record the isometric contractions of the preparation. This same recording machine was also used to record the effect of drugs on blood pressure of the cat.
- iii. Digital thermostatic water bath (HH-S6 Digital Water Bath Shanghai Sican Industries limited China) for drying the extracts.
- iv. Other materials include needles and syringes, petri dishes, dissecting kits, beakers, masking tapes, volumetric flasks, sample bottles, evaporating dish, round bottom flask, measuring cylinders, thread

3.2 Methods

3.2.1 Plant collection and identification

The fresh leaves and seeds of *Moringa oleifera* were collected around the month of June 2013 from Bida, Niger state. The plant was identified in the Herbarium, Department of Biological Sciences, Amadu Bello University, Zaria, by Mallam U.S

Gallah where a specimen voucher number of 571 had been assigned to it for reference purposes.

3.2.2 Extraction of plant material

The leaves and seeds of *Moringa oleifera* were air dried in the shade and individually made into powder form using mortar and pestle. About 525g for the leaf and 350g for the seed powder were obtained. The leaf powder was divided into 250 grams used for the aqueous extraction and 275 grams used for the methanol extraction. Cold maceration of the leaf powder were obtained with 2.0 litres of distilled water soaked for 24 hours (aqueous leaf extraction) and 2.5 litres of methanol soaked for 48 hours (methanol leaf extraction). The seed powder (350 grams) was soaked in 2.0 liters of pet-ether for 48 hours to extract the oil (pet-ether extraction). The resulting mixture was then air dried to evaporate the pet-ether solvent. The resulting seed powder residue (296 grams) was cold macerated for 48 hours with 1.5 liters of methanol (methanol seed extraction). The maceration process was subjected to occasional shaking. The resulting mixtures were filtered and the filtrates concentrated at 40°C in an evaporating dish and over digital thermostatic water bath. The yields for each of the extracts were then calculated as follows:

$$\text{Percent (\%) yield} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered sample}} \times 100$$

3.2.3 Preparation of physiological solution (Ringer-Locke's solution)

Ten litres (10 L) of Ringer-Locke's solution was prepared in de-ionised water by adding the chemical constituents as follows:

NaCl – 90 g

KCl (10%) - 42 milliliters

D-Glucose – 10 g

NaHCO₃ – 5 g

CaCl₂ (molar) – 10.8 milliliters

3.2.4 Preparation of drug solutions

Stock solutions of thiopental sodium (60 mg/ml), acetylcholine (10 µg/ml), adrenaline (10 µg/ml), histamine (10 µg/ml), propranolol (4mg/ml), atropine (25 mg/ml) and mepyramine (1 mg/ml) were prepared using de-ionised water to obtain stock solutions. Working concentrations of 100 mg/kg *Moringa oleifera* leaf and seed of the leaf and seed extracts of *Moringa oleifera* were also prepared by dilution with de-ionised water and were used for the study.

3.2.5 Phytochemical screening

The method described by Trease and Evans (1983) was used to screen the extracts for the presence of metabolites.

3.2.5.1 Carbohydrates (Molisch' reagent test)

Two or three drops of molisch reagent was added to 5 ml of the extract in a test tube and 1 ml of concentrated sulphuric acid (H₂SO₄) was added down the test tube side. Formation of a purple ring coloured layer interphase beneath the aqueous layer confirms the presence of carbohydrates.

3.2.5.2 Alkaloids (Mayer,s reagent test)

Five millilitres of the extract was mixed with equal volume of 1% aqueous hydrochloric acid (HCl) stirred over water bath for it to warm and filtered; 1 ml of the filtrate was mixed with few drops of Mayer's reagent; and another 1 ml of the filtrate with Wagner's reagent; and yet another 1 ml with Drangendorff's reagent. The

mixtures were checked for a white precipitate, red precipitate and rose red precipitate respectively that indicate the presence of alkaloids.

3.2.5.3 *Cardiac glycosides (Kella-killiani test)*

About 0.5 g of the extracts was dissolved in 2 ml of glacial acetic acid, and 1 ml of ferric chloride solution was added. To the solution, 1 ml of conc.H₂SO₄ was also added at an angle of 45 degree

Kedde's Test: To 1ml of 2% 3, 5-dinitrobenzoic acid in 95% alcohol was added a little portion of extract and 5% sodium hydroxide and mixed. Both tests were checked for a purple ring interphase and a purple blue colour respectively that indicate presence of cardiac glycosides.

3.2.5.4 *Steroidal Glycosides (Liebermann-Burchard's reaction)*

Five grams of the extract was dissolved in 10 ml of distilled water in a test tube and was evaporated on a boiling water bath (55° C) to dryness. The residue was dissolved in 0.5 ml chloroform and Concentrated H₂SO₄ acid (2 ml) in a pipette was dropped at the bottom of the test tube for Liebermann-Burchard's reaction. Separation of the two liquids by a reddish brown ring colour indicates the presence of steroids and triterpenes.

3.2.5.5 *Anthraquinones (Borntrager's test)*

Three grams of the extract was dissolved with 10 ml of benzene and filtered to obtain a filtrate which was mixed with 5 ml of 10% ammonia solution and stirred. Another 3 g of the extract boiled with 5 ml of 10% hydrochloric acid for 3 minutes was filtered hot and allowed to cool. The filtrate was mixed with 5 ml of benzene and produced a benzene layer which was filtered off and half the volume of the residue was mixed

with 10% ammonia solution and shaken gently. A red colour in the ammonia (lower) phase for both the filtrate and residue indicates the presence of free and combined anthraquinones respectively

3.2.5.6 *Saponins (frothing test)*

Quantity of the extract was dissolved in 10 ml of distilled water and shaken gently for 30 seconds. The persistence of foams for 30 minutes indicate the presence of saponin.

3.2.5.7 *Flavonoids (Shinoda test)*

Two grams of the extract was dissolved in 2 ml of 50% methanol and warmed over water bath and filtered. Magnesium metals (4-5 pieces) and few drops of concentrated hydrochloric acid (HCl) were added to the filtrate and observed for the appearance of pink colouration which indicates the presence of flavonoids.

3.2.5.8 *Tannins (Lead sub-acetate test; FeCl₃ test)*

Three grams of the extract was boiled with 10 ml of water was cooled and filtered. Three drops of lead sub-acetate solution was added to 1ml of the filtrate. The formation of a white precipitate is indicative of tannins;

A drop of 1% FeCl₃ solution added to 1 ml of the filtrate was also checked for blue-black precipitate of tannins.

3.2.6 Effect of *M. oleifera* leaf and seed extracts on cat blood pressure

Intravenous infusion of drugs and test solutions injected through the femoral vein into a cannulated artery is/has been used to investigate the effect of substances on arterial blood pressure in cats. Male cats weighing 2.0 - 2.5 kg were anaesthetized with i.p. injections of thiopental sodium (50 mg/kg) and the anaesthetized cat was fixed with its leg fastened to an operating or dissecting table. Heparin, 0.2 ml of 5,000 units / ml

was injected into the femoral vein to avoid blood coagulation as it is being exposed and cannulated for drug injections. Anaesthesia was maintained with intermittent injections of small volumes of the prepared 60 mg/ml thiopental sodium. A longitudinal midline incision was made in the neck to expose the trachea (tracheotomy) and a tracheal cannula was inserted. The carotid arteries were located and carefully separated from the attached vagi and two ligatures were placed around the left artery: one around the upper end of the artery (cephalad ligature) and the second tied nearest to the heart (cardiac ligature). A bulldog clip was also placed at the end of the cardiac ligature to occlude this segment of the artery completely. A small incision was made on the artery around the cardiac ligature and the tip of arterial cannula is inserted into the incision and tied firmly with the thread of this cardiac ligature. Usually the arterial cannula is fixed on a three-way tap that also carries the blood pressure transducer on one tap and a pressure bottle or syringe containing heparinised saline on the other. This heparinised saline is used in flushing the carotid artery from time to time to prevent blood clotting in the cannula. The tap of the transducer is closed to flush out any bubbles in the cannula with the heparinised saline in the syringe. Another syringe full of heparinised saline is also fixed to an outlet on the transducer for adjusting the pressure of the system. The transducer was then connected to the recording machine and attached to the artery. The tracing baseline and sensitivity (mV) of the recorder to the transducer output were selected and the speed of the chart trace was also set at 96 mm / min. The setup was allowed to equilibrate for 15 minutes and control records of the cat's blood pressure were obtained as a reference for evaluating the changes in blood pressure by the various drug infusions. Each drug injection was followed up with 0.2 ml of normal saline

(0.9% NaCl) used to flush the vein after each drug injection. The various experiments carried out include:

- i. The effect of adrenaline and ACh as standard drugs
- ii. The hypotensive effects of the crude extracts of both the leaf and seed of *M. oleifera* plant and their interactions with atropine, propranolol and mepyramine.

3.5.6 Effect of *M. oleifera* leaf and seed extracts on isolated guinea pig heart atria

The method described by the Pharmacology Department Staff, (1970) of Endinburg University was used in assessing the effect of *M. oleifera* leaf and seed extracts on isolated guinea pig heart atria. A guinea pig was killed by a blow on the head and then autenzied and the chest was quickly opened. The heart was then carefully dissected out from the pericardium and adherent tissues and placed into a petri dish containing aerated Ringer Locke's solution. The ventricles were trimmed off and a thread was tied to both the right atrium and the left atrium. The right atrium containing the sinoatrial (SA) node (pacemaker or automatic cell) that usually initiates the basic myogenic rhythm of the heart was attached to the spring lever of the transducer, while the left atrium was attached to the tissue holder (a fixed pin) at the base of the bath. The preparation was mounted in oxygenated Ringer-Locke's solution at 37°C in a 25 ml organ bath, washed 2-3 times and then allowed for about 15 minutes to recover before addition of test substances. The transducer was then connected to the recording apparatus and the baseline and sensitivity (mV) of the recorder to the transducer output were selected. The speed of the chart trace was also set at 96 mm / min. After the 15 minutes of tissue equilibration, the normal rate and

amplitude of the heart was recorded (control) the drugs were added to the organ bath with 1 ml syringes and responses to Ach (10 µg/ml), and *Moringa oleifera* leaf and seed extracts (100 mg/ml) as well as the effect of the extracts on isoprenaline (0.5 µg/ml) and calcium chloride (25 mg/ml) – induced contractions were obtained.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of the Leaf and Seed Extracts of *Moringa oleifera*

Weight of powdered materials for the leaf extractions: aqueous =250g; methanol = 275g

Weight of the dry extracts = 90.64g; 91.96g respectively

Weight of powdered materials for the seed extractions: methanol =296g; pet-ether = 350g

Weight of the dry extracts = 48.30g; 88.41g respectively

The yield of the different extracts obtained from the leaf and seed of *M. oleifera* is presented on table 4.1

Table 4.1: Percentage yield of *Moringa oleifera* leaf and seed extracts

Extracts	Percentage yield
Aqueous leaf	33.57
Methanol leaf	35.44
Methanol seed	16.32
Pet-ether seed	25.26

4.2 Phytochemical Constituents of *M. oleifera* Leaf and Seed Extracts

Alkaloids, tannins, saponin, cardiac glycosides, carbohydrates, flavonoids, steroids and triterpenes were detected in the aqueous and methanol leaf extracts of *Moringa oleifera*. For the methanol seed extract, only carbohydrates, cardiac glycosides, saponin, tannins and alkaloids were observed, while the pet-ether extract contained only carbohydrates and alkaloids as shown in Table 4.2.

Table 4.2: Phytochemical constituents in *Moringa oleifera* Leaf and Seed Extracts

Constituents	Test	Aqueous Leaf	Methanol Leaf	Methanol Seed	Pet-ether Seed
Carbohydrates	Molisch'reagent	+	+	+	+
Anthraquinone	Borntrager	-	-	-	-
Saponins	Frothing	+	+	+	-
Steroids and Triterpene	Liebermann-Burchard's	+	+	-	-
Flavonoids	Shinoda	+	+	-	-
Alkaloids	Mayer,s reagent, Dragendorff Wagner	+	+	+	+
Tannins	FeCl ₃ tests	+	+	+	-
Cardiac glycoside	Kella-killiani	+	+	+	-

Key: (+) Present; (-) Absent

4.3 Effect of *Moringa oleifera* Leaf and Seed Extracts on Cat Blood Pressure

Acetylcholine (ACh) produced a decreased blood pressure effects. That effect of ACh on cat blood pressure has been well documented (Bako *et al.*, 2010). The extracts of *M. oleifera* showed similar effect by reducing cat blood pressure which in this study was compared to Ach.

All the extracts used in this study produced a dose dependent decrease in blood pressure with the aqueous and methanol leaf extracts showing more pronounced reduction that are almost equal to acetylcholine. The hypotensive effect of the seed extracts was milder; with the methanol seed extract having more effect than its pet-ether seed extract (Fig. 1).

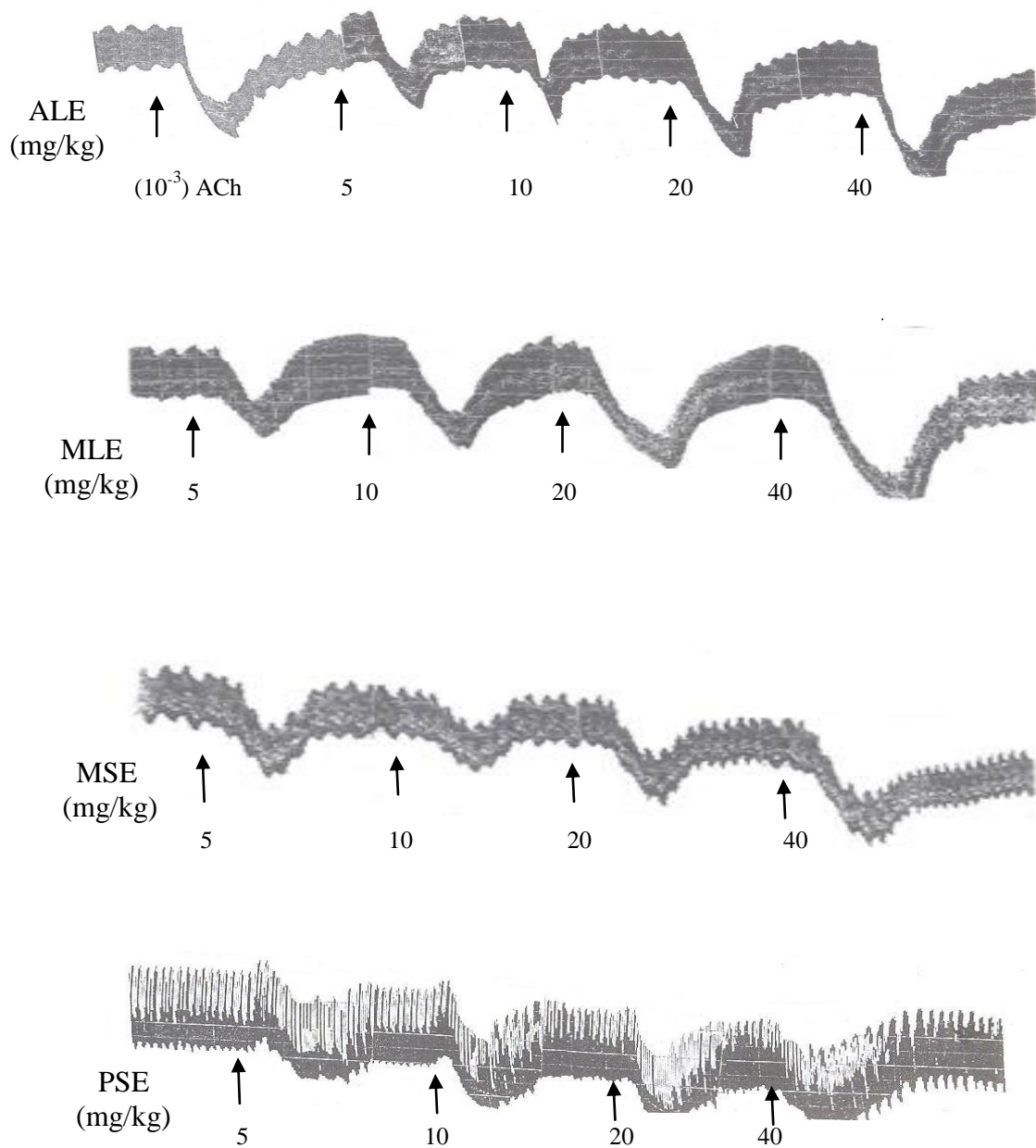


Figure 1: The Hypotensive Effect of the Extracts of Leaf and Seed of *Moringa oleifera* on Cat Blood Pressure

Keys = ACh- Acetylcholine, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

4.3.1 Interactions of *Moringa oleifera* leaf and seed extracts with some receptor specific antagonists

4.3.1.1 Adrenergic receptor mediation of the extracts of leaf and seed of Moringa oleifera

Adrenaline exhibited positive chronotropic and ionotropic effects, characteristic of cardiovascular control agents on sustained cardio contraction. However, both the aqueous and methanol leaf extracts inhibited the contractility action of adrenaline. On the other hand, it was only the methanol seed extract that inhibited adrenaline contraction, the pet-ether seed extract did not. Propranolol also abolished the adrenaline contraction as expected of a standard β -adrenergic antagonist, but the concurrent administration of propranolol with each of the solvent extracts of both the leaf and seed of *Moringa oleifera* enhanced the relaxation actions of the extracts (figure 3).

(a) Extracts' inhibition of adrenaline contraction

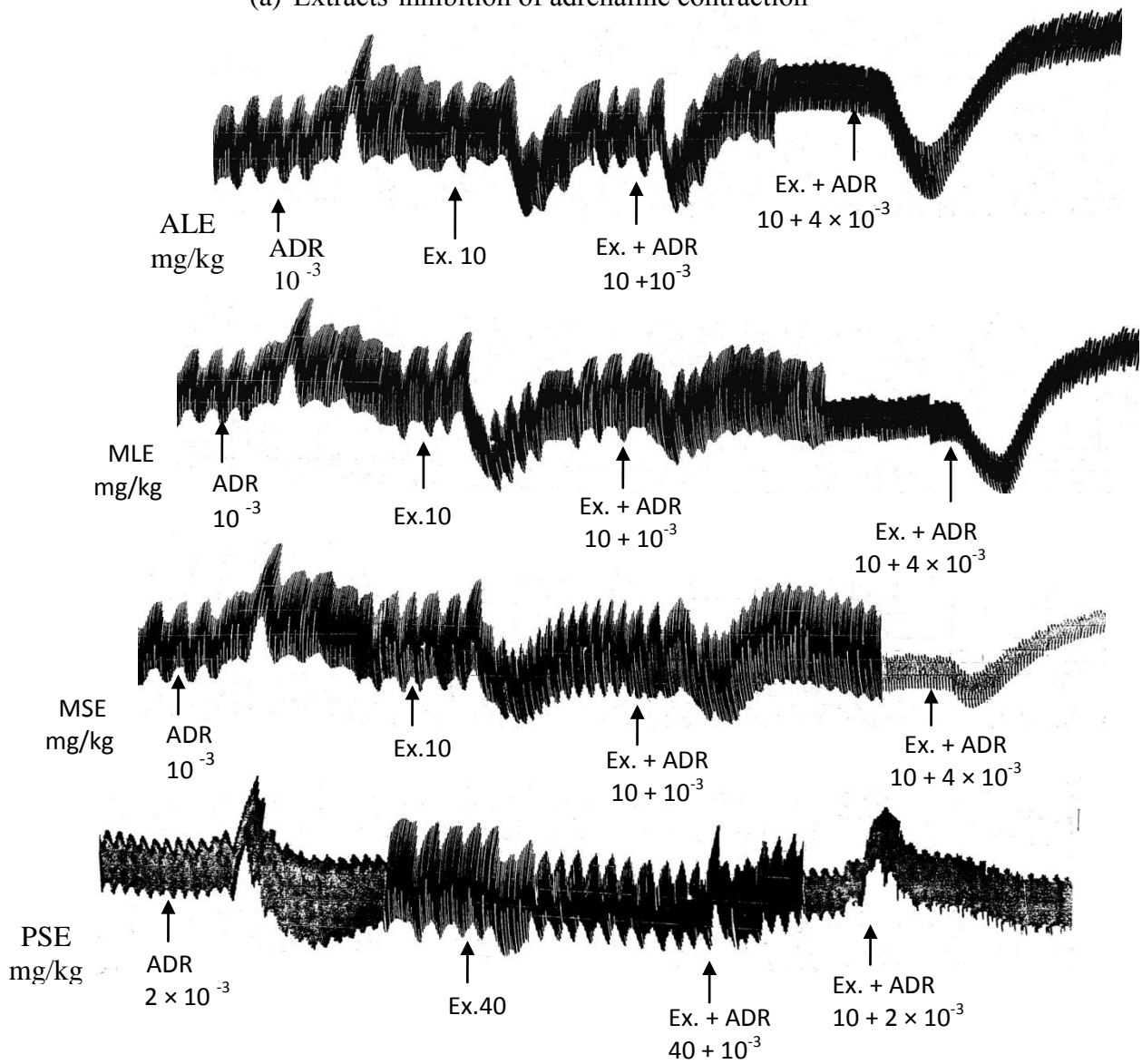
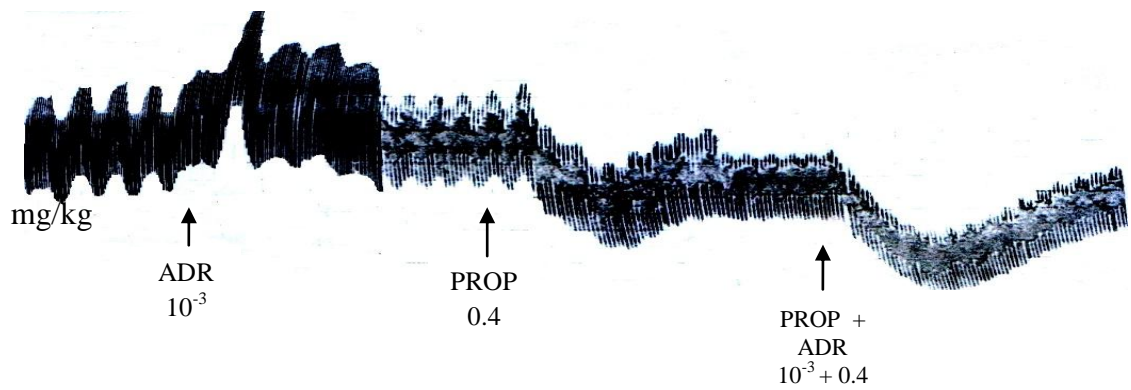


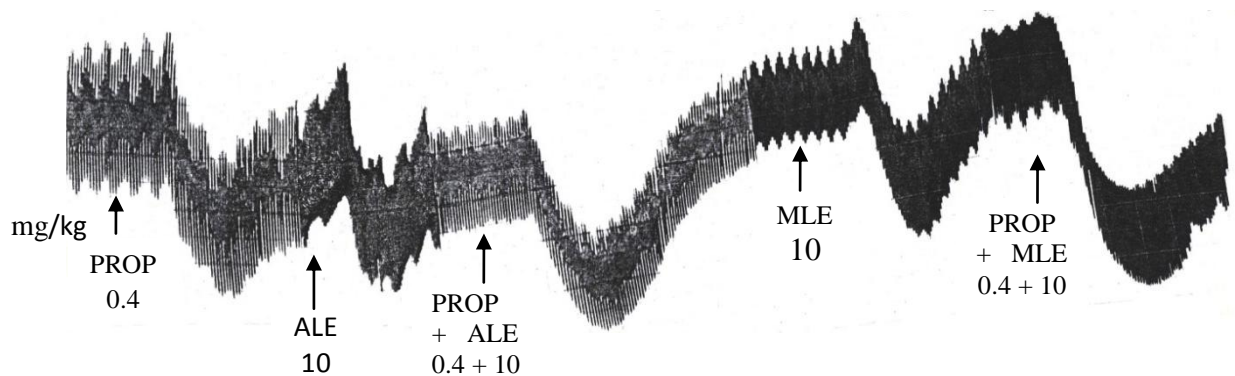
Figure 2: Effect of *M. oleifera* leaf and seed Extracts on Adrenaline Induced Increase of Cat Blood Pressure

Keys = ADR – Adrenaline, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

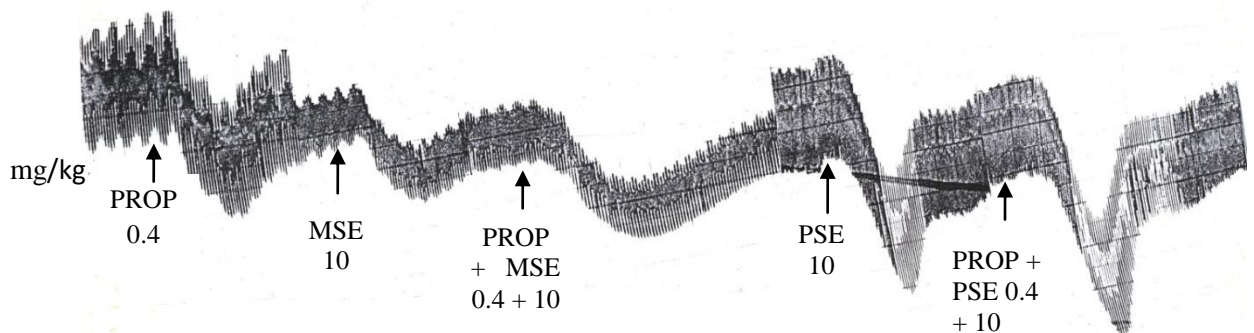
Propranolol Inhibition of adrenaline contraction



(c) Propranolol- enhanced Extracts' relaxing effect



i. Leaf Ex.



ii. Seed Ex.

Figure 3: The Inhibitory (a) and Enhanced (b) Hypotensive Effect of Propranolol on Adrenaline and *Moringa oleifera* Leaf and Seed Extracts on Cat Blood Pressure

Keys = ADR- Adrenaline, PROP- Propranolol, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

4.3.1.2 *Histaminergic receptor mediation of Moringa oleifera leaf and seed extracts*

Mepyramine blocked the effect of histamine, but was not able to block the effect of the extracts. It enhanced the effect of both aqueous and methanol leaf extracts, but did not show any effect on both seed extracts (fig. 4).

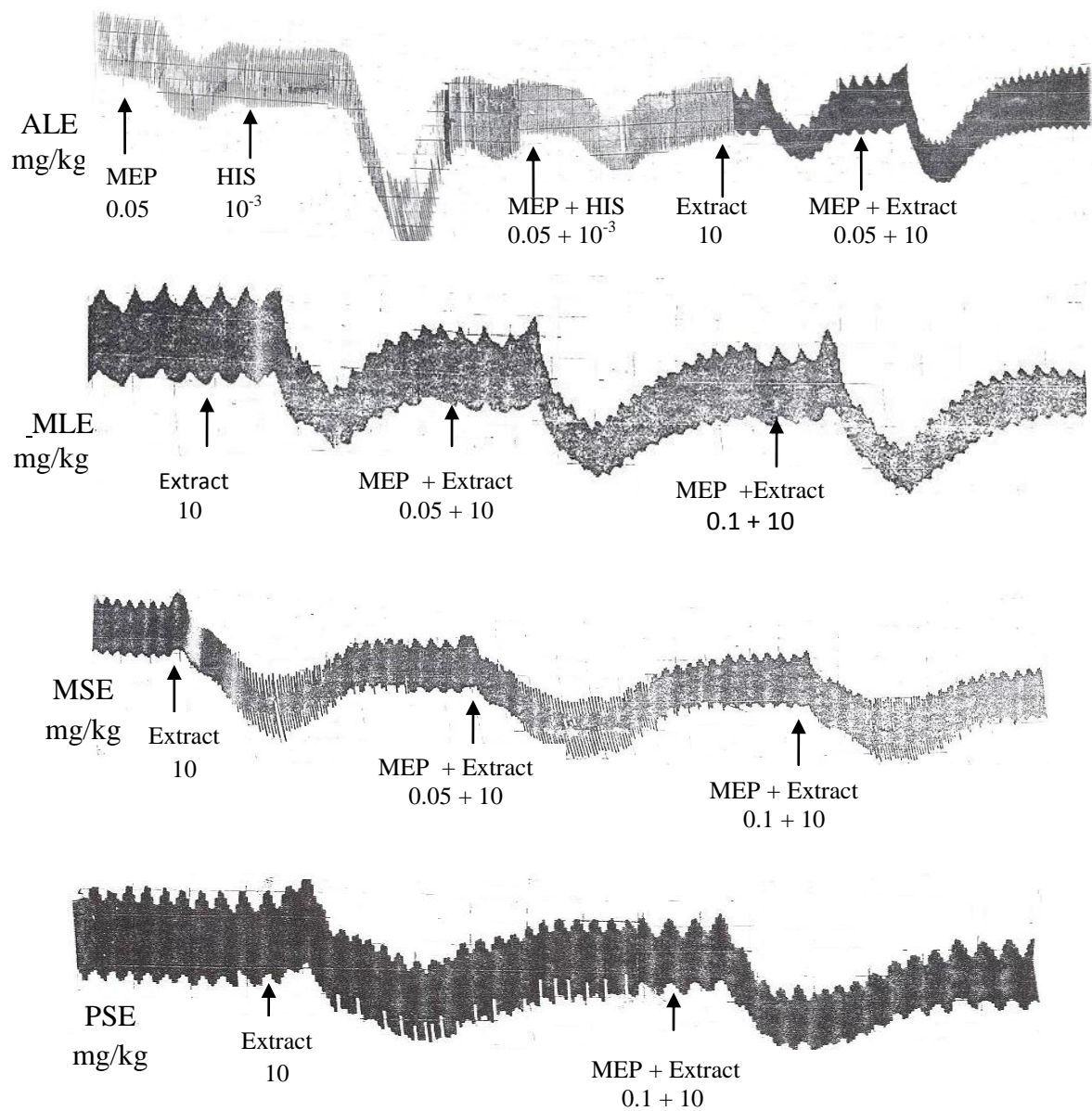


Figure 4: Effect of Mepyramine on Histamine and *Moringa oleifera* Leaf and Seed Extracts on Cat Blood Pressure

Keys = HIS – Histamine, MEP – Mepyramine, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

4.3.1.2 Cholinergic receptor mediation of Moringa oleifera leaf and seed extracts

Atropine also showed cholinergic antagonistic action by blocking the effect of acetylcholine as expected; and it also blocked the relaxing effect of only the aqueous leaf extract. It had no effect on the methanol leaf extract and on both extracts of the seed (fig. 5).

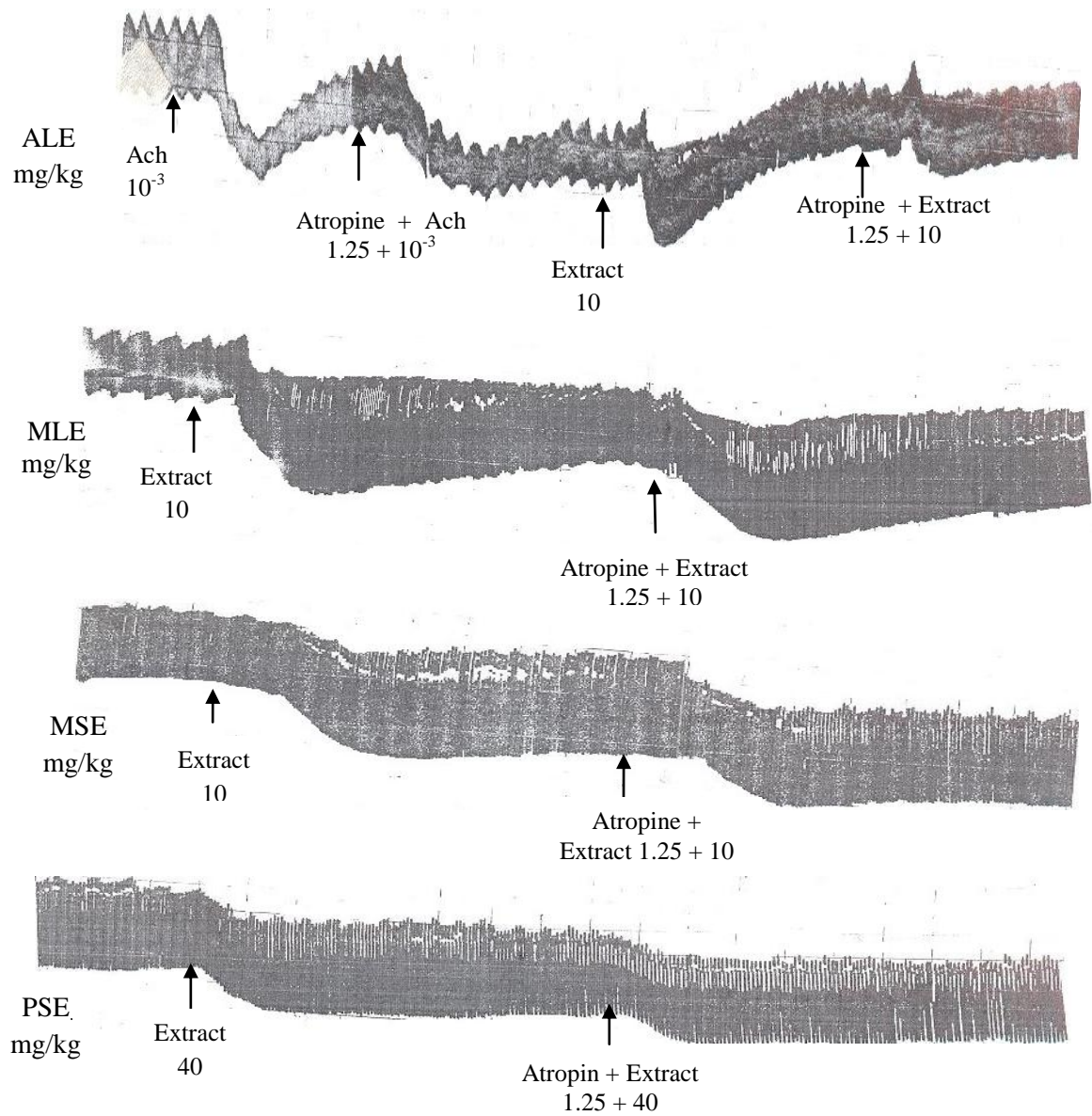


Figure 5: Effect of Atropine on Acetylcholine and *Moringa oleifera* Leaf and Seed Extracts on Cat Blood Pressure.

Keys = ACh- Acetylcholine, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

4.4 The Direct Effect of *Moringa oleifera* Leaf and Seed Extracts on the Spontaneous and Induced Contractions of the Isolated Guinea Pig Heart

Atria

In the *in-vitro* study of the direct effect of the extracts on the contractility of the heart using isolated guinea pig atria, both the aqueous and methanol leaf extracts decreased the spontaneous contraction of the atria similarly and in concentration dependent manner. The methanol seed extract reduced the rate of contraction as can be seen with the reduced frequency or speed in the tracing, but not the force of contraction which remained unaltered. However, this effect also occurred in a concentration dependent manner. The petether seed extract reduced both force and rate of the heart beats as with the leaf extracts and concentration dependently (Fig. 6). All the extracts for both the leaf and seed abolished the isoprenaline-induced and calcium chloride-induced contractions of the guinea pig atria (Figs 7 and 8).

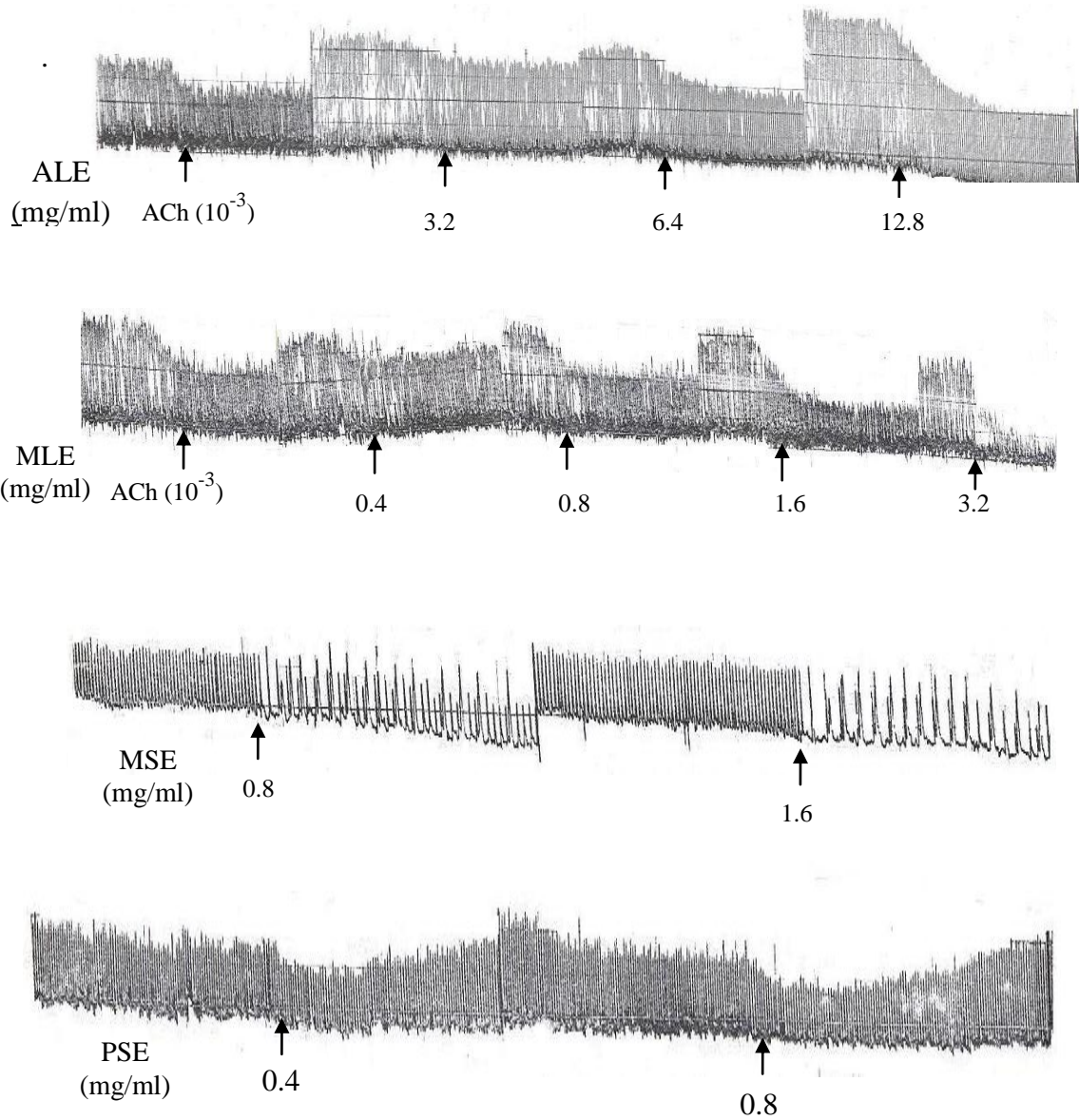


Figure 6: Concentration-Dependent Effect of the Extracts of Leaf and Seed of *M. oleifera* on the Guinea Pig Heart Atria.

Keys = ACh- Acetylcholine, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract.

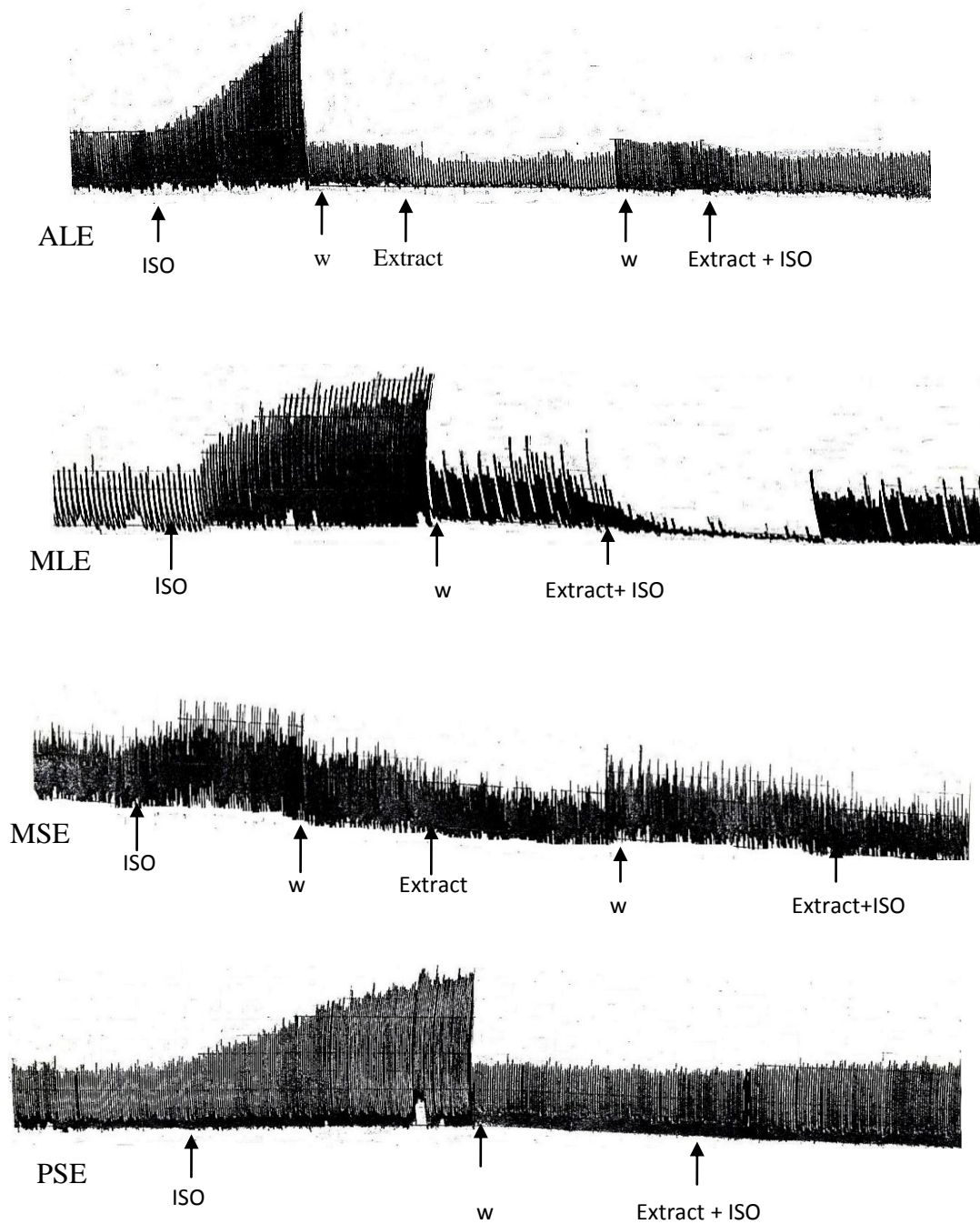


Figure 7: Inhibitory Effect of *Moringa oleifera* Extracts on Isoprenaline-induced Guinea Pig Heart Atria Contraction.

Keys = ISO – Isoprenaline, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet-ether seed extract, W- wash.

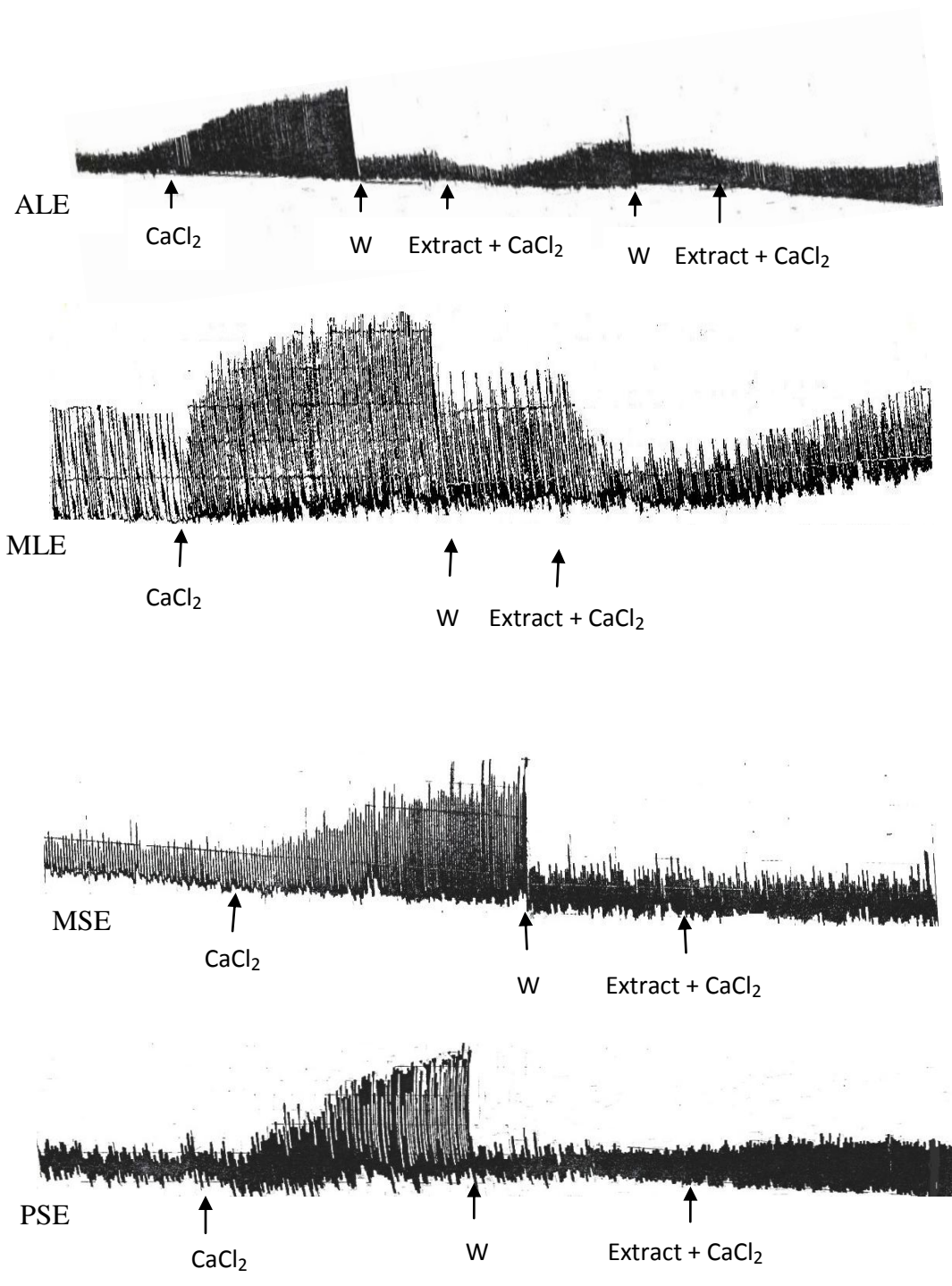


Figure 8: Inhibitory effect of *Moringa oleifera* Extracts on Calcium chloride - induced Guinea Pig Heart Atria Contraction.

Key = CaCl₂ = calcium chloride, ALE – Aqueous leaf extract, MLE – Methanol leaf extract, MSE – Methanol seed extract, PSE – Pet ether seed extract, W-wash.

CHAPTER FIVE

5.0 DISCUSSION

The phytochemical screening of *Moringa oleifera* revealed the presence of alkaloids, tannins, saponin, cardiac glycosides, anthraquinones, carbohydrates, steroids and triterpenes in the aqueous and methanol leaf extracts, this is in agreement with the work reported by Sunkar *et al.* (2012). In his study, flavonoids were not found in the leaf extracts. However, flavonoids were reported present in a different work by Roopalatha and Vijay (2013). All these constituents were found present in this study with the exception of anthraquinones, which was reported to be present by Roopalatha and Vijay (2013). For the seed extracts, only carbohydrates, cardiac glycosides, saponin and alkaloids were found present, while flavonoids, steroids, triterpenes and anthraquinones were absent and this agrees with the report of Ogunjimi and Taiwo (2012). The petroleum ether seed extract showed presence of alkaloids and carbohydrates, which have also been reported by Usman and Barhate (2012). The absence of flavonoids by Sunkar *et al.* (2012); and anthraquinones in this present study could be as a result of the time of collection, the nature of the soil, climatic condition and geographical location to which the plant is exposed, as this has been reported to affect the type and quantity of secondary metabolites in plants (Coulidiati *et al.*, 2009). The different plant parts and choice of solvent for extraction can also affect the plant constituents. In this study, more of the constituents were found present in the leaf extracts than the seed and even of the same solvent (methanol). Some of the chemical constituents found present in plants are responsible for their pharmacological activities including the side effects associated with their use (Aliyu *et al.*, 2010), and this probably might be the reason why the leaf, which had more of the phytoconstituents, showed more hypotensive effect than the seed extracts.

The response of the buffer nerves of the arterial system to the standard drugs (Adr and ACh) were as expected. Adrenaline stimulated the noradrenergic sympathetic nerves (carotid or pressor nerves) and increased the cardiac rate and force of contraction by increasing the systolic blood pressure, while ACh depressed the cholinergic vagal fiber (aortic arch or depressor fibers) to decrease the heart rate and force and which is an activity on the diastolic blood pressure. Usually vasoconstrictor agents like adrenaline stimulate the adrenergic receptor and increase the pulse pressure, and agents that stimulate the muscarinic receptors like ACh depress blood pressure and are referred to as negative inotropic agents. Generally, drugs acting on the cardiovascular system are traditionally characterized by their effects on blood pressure and are often classified as pressor or depressor agents.

In this study, all the extracts caused relaxation of the arterial system in a dose dependent manner indicating that the hypotensive effect of *Moringa oleifera* plant is actually on the diastolic pressure as with that of acetylcholine. The extracts reduced the cardiac rate almost in the same manner as ACh and with sustained effects that may even overstretch the elasticity of the heart with increasing dose. Thus, *Moringa oleifera* leaf extracts of either the aqueous or methanol has a strong hypotensive property. It is obvious in this study that the leaf extract of *M. oleifera* has a potential of causing a serious or extreme hypotension and / or heart failure if used beyond a certain dosage level. The result of this experiment therefore seemed to suggest that the extract must be used within a specific dosage range that reduces an elevated blood pressure to a near normal level. The hypotensive effect of the seed extracts (methanol and pet-ether) was milder. The seed is very oily and usually, oily substances can not be extracted in aqueous solvent and this was the reason for the choice of pet-ether as the solvent of its extraction. Methanol seed extract caused hypotensive effect that is

more than that of the pet-ether seed extract because methanol extracted more of the phytochemical constituents as was observed in the phytochemical screening test.

Study with the pharmacological antagonists was to ascertain the possible mechanisms of the hypotensive effect of *M. oleifera* leaf and seed extracts under investigation.. The effect of the extracts on adrenaline was investigated prior to propranolol antagonistic activity. Both the aqueous and methanol leaf extracts inhibited the contractility action of adrenaline – an effect that seemed like a pharmacological antagonism. The antagonistic effect of the extracts on adrenaline contraction on cat blood pressure was not reversed with higher concentration of adrenaline and thus, seemed as though the affinity of adrenaline for the β -adrenergic receptor and its potency was altered. This blocking of adrenaline contraction resembles a complete receptor-response inhibition often described as non-competitive or non-equilibrium blockade. The methanol seed extract also completely inhibited the contraction caused by adrenaline, but the pet-ether seed extract caused little or no reduction of the adrenaline induced contraction of cat blood pressure. The observed inhibitory effect seemed to suggest activity of the extracts on the same receptor with adrenaline.

Propranolol caused hypotensive effect when administered alone, by acting on the β -adrenergic receptor. Propranolol also abolished the hypotensive effect of adrenaline typical of its standard antagonistic activity, and it enhanced the relaxation activity of all the extracts including that of pet-ether in a manner that is seemingly synergistic. Propranolol enhanced the hypotensive effect of pet-ether seed extract even though the extract did not inhibit adrenaline induced contraction as to conclude β -adrenergic receptor mediation.

Mepyramine did not attenuate the hypotensive effects of *M. oleifera* extracts, but blocked the effect of histamine. Mepyramine enhanced the relaxing effect of the leaf extracts with no effect on either the methanol or pet-ether seed extracts. Again this observation suggests lack of histaminergic receptor mediation of any of the extracts for either the leaf or seed; but similar other sites' synergistic activity of mepyramine with the extracts of the leaf.

Atropine is a typical cholinergic antagonist that blocks the effect of acetylcholine and in this study it seemed to also have produced a blockade effect on aqueous leaf extract indicating that only the aqueous extract of the leaf had cholinergic receptor mediated activity. It showed no effect on neither the methanol leaf extract or both solvent extracts of the seed.

Hypotensive drugs act on peripheral and sympathetic systems, to lower the peripheral vascular resistance and / or to interfere with rennin angiotensin production. For intact preparations or whole animals as with the anaesthetized cannulated cat artery, it is possible that many concurrent actions of a drug on different sites that synergizes with the receptor do occur to yield an enhanced effect.

The antagonistic actions of the aqueous leaf and methanol leaf extracts as well as the methanol seed extract on the contraction of adrenaline, suggested adrenergic receptor mediated activity. The pet-ether seed extract activity may be via a different mechanism other than adrenergic receptor. The blockade of atropine by aqueous leaf extract is an indication of cholinergic receptor mediated activity. Thus, observation from this study showed that different receptor mechanisms are involved in the activity of the various extracts even those of the same plant part.

The direct effect of the extracts on the contractility of the heart was investigated using isolated guinea pig atria. The extract blocked isoprenaline-induced contraction of isolated guinea pig atria indicated the involvement of β -adrenergic mediation. This is in agreement with the antagonistic effect of the extracts on adrenaline contraction seen with the *in-vivo* study. Isoprenaline is an adrenaline derivative that also dilates the heart in similar manner, thus, its blockade further strengthens the possibility of adrenergic receptor mediated activity. The blockade of calcium chloride-induced contraction, suggests interruption of receptor operated calcium channels or influx of intracellular calcium. Usually, it is Ca^{2+} that maintains the integrity of cell excitations and it is known that many vasoconstrictor agents exerts their effects by increasing intracellular calcium concentrations, while vasodilator agents exert their effects by its reduction (Guyton and Hall, 2006).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

In conclusion, this experiment confirmed the claim that *Moringa oleifera* leaf and seed extracts lowers high blood pressure and enhances blood circulation. The study further revealed that the activities of the extracts are dose related and may be harmful if used beyond a certain dosage level and thus calls for caution in the use of the crude extracts. The aqueous and methanol leaf extracts of the plant were found to cause depressor effects, that were more profound than those of the seed extracts. The hypotensive effect of the seed extracts was milder with the methanol extract producing more of the effect. When used as a tonic to enhance blood circulation, a milder concentration is required of the leaf extracts; but for antihypertensive activity a relatively higher concentration may be used.

The aqueous leaf and methanol leaf extracts as well as the methanol seed extract may be said to have adrenergic receptor mediation activity because of their antagonistic actions on adrenaline contraction. The activity of the pet-ether seed extract may be via a different mechanism other than adrenergic, histaminergic or cholinergic receptor activation. This inhibition of adrenaline induced contraction calls for caution in the concurrent use of the extracts and drugs that act via this mechanism. Atropine blocked the effect of the aqueous leaf extract, this is an indication of cholinergic receptor mediation.

From the *in-vitro* study of the direct effect of the extracts on the contractility of the heart, all the extracts decreased the spontaneous contraction of the atria in concentration dependent manner, but the methanol seed extract decreased only the

rate and not the force of contraction. The blocking of isoprenaline-induced contraction indicated the involvement of β -adrenergic mechanism. The *in-vitro* blockade of calcium chloride-induced contraction on the other hand, seemed to suggest the opening of calcium channels as part of mode of activity of the extracts in their constrictor actions.

6.2 Recommendation for further work

1. Work should be carried out to establish the level of safety of the crude extracts of either the leaf or seed to be taken, especially as daily tonic for enhancing blood circulation.
2. Further studies should be carried out to check interactions of these plant extracts with conventional antihypertensive drugs in case of concurrent use of both crude extracts and orthodox hypotensive drugs.

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