PHARMACOLOGICAL INTERACTION BETWEEN ETHANOL EXTRACT OF *MORINGA OLEIFERA* LEAVES AND METFORMIN IN ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS

By

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MSc/ Pharm-Sci /0463/2010-11

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
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AHMADU BELLO UNIVERSITY, ZARIA
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SEPTEMBER, 2014

Declaration

I declare that the work in the Thesis entitled 'Pharmacological Interaction between Ethanol Extract of *Moringa oleifera* Leaves and Metformin in Alloxan-induced Hyperglycaemic Wistar Rats' has been carried out by me in the Department of Pharmacology and Therapeutics. 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.

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Name of Student	Signature	Date

Certification

This thesis entitled "PHARMACOLOGICAL INTERACTION BETWEEN ETHANOL EXTRACT OF *MORINGA OLEIFERA* LEAVES AND METFORMIN IN ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS" by Precious Adejoh IDAKWOJI meets the regulations governing the award of the degree of Masters in Pharmacology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Acknowledgment

I wish to express my profound gratitude to God Almighty for blessing me with good health, knowledge, wisdom; grace, willpower and the resources that made this project a reality. I pledge my allegiance to you forever o Lord. Amen.

My profound gratitude to the Head of Department of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Dr. A. U. Zezi for providing a conducive and friendly environment for me to enhance my knowledge. I also wish to thank all the other academic and non-academic staff in the department of pharmacology and therapeutics.

I am forever indebted to Prof. (Mrs) A. O. Salawu, my supervisor for believing in me and allowing me to work under her guidance. Her tolerance, constant encouragement, constructive criticism and untiresome guidance helped in making this project a reality. I am proud to say that it has been fruitful and enjoyable working under her guidance. You will forever be remembered by God, Ma. I also wish to pay my humble regards to Dr. B. B. Maiha, my co- supervisor, for her encouragements and full effort in making sure that this study is a success.

I owe a very special thanks to Dr. A.Y Tijani for his valuable suggestions. His encouragements provided an additional source of energy and vitality for the success of this work. I also wish to warmly and gratefully acknowledge Pharm. Ifeoma Ezeonyi. Her tutorship, contributions, encouragement and her ready to help attitude helped in shaping this work. I also wish to thank the entire staff of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja

I remember and cherish with gratitude the timely help and support of the young ambitious crew, my lab mates at NIPRD, Kemi, Millicent, Kareema, Sarah, Stella, Success, Tamara, Emeka and Martins, may God bless you and cause you to do greater things than this. I sincerely appreciate my friends especially Esther Adama for all the support, encouragement and good times outside my lab environment. Thanks for keeping me connected to the network of life of which work is just a part.

This is the right moment to reveal my respect and love for my family. I am highly indebted to my parents, Dr E. A. Idakwoji and Mrs. L. Idakwoji for giving me the strength of will to pursue what I believed and for giving me strength of character to lead a moral life. Without them and all the sacrifices they made in training me, I wouldn't be the same person. The lord God almighty shall preserve your lives in good health and you shall live to enjoy the fruit of all your labours. I am eternally grateful to my sister Mrs Victoria Ocheja and her family for providing a befitting accommodation for me till the completion of this project. My other siblings, Mrs Helen Edicha, Mrs Peace Ogu, Mr Cyril Idakwoji and Mr Timothy Idakwoji, I am forever indebted to you for your moral support and everlasting love.

Abstract

Herbs with anti-diabetic activity could initiate interactions if used concurrently with orthodox drugs. *Moringa oleifera* is one of such herbs usually taken as an adjunct to orthodox oral hypoglycaemic agents. This study investigated the possible pharmacological interaction between the ethanol extract of *Moringa oleifera* leaves (MOE) and metformin co-administered to hyperglycaemic Wistar rats and the effect of MOE on normoglycaemic rats. Hyperglycaemia was induced by administration of a single dose (150 mg/kg *i.p*) of alloxan. Rats having fasting blood glucose (FBG) ≥ 200 mg/dl after 72 h were considered hyperglycaemic. A dose-response study for MOE was carried out with 8 groups of hyperglycaemic rats which were administered 100, 200, 400, 800, 1000 and 2000 mg/kg MOE respectively. Blood glucose level was monitored hourly and a plot of percentage glycaemic reduction at 4h versus log dose was used to estimate the median effective dose (ED_{50}), to be 750 mg/kg. Pharmacological interaction study was carried out for 28 days with 8 groups of hyperglycaemic rats. Group 1 served as control, groups 2, 3, and 4 received 375, 750 and 1500 mg/kg MOE corresponding to ½ ED_{50}, ED_{50} and $2ED_{50}$ respectively. Groups 5, 6 and 7 received 375, 750 and 1500 mg/kg MOE but co-administered with metformin (150 mg/kg) while group 8 received metformin (150 mg/kg). For normoglycaemic rats, group 1 served as control while groups 2, 3 and 4 were administered 375, 750 and 1500 mg/kg MOE respectively. Parameters such as FBS, feed/water intake, body weight changes, lipid/protein profiles, electrolytes, creatinine and urea levels were measured in both hyperglycaemic and normoglycaemic rats. Relative organ weight (ROW) and histopathological examination of the pancreas, heart, brain, kidney, liver, lungs, spleen and stomach were also carried out. Results of the hyperglycaemic study, showed that 375mg/kg MOE significantly ($p<0.05$) reduced FBS on day 28, 750 and 1500 mg/kg

MOE significantly ($p<0.01$) reduced FBS on days 21 and 28. The 375 and 750 mg/kg MOE co-administration with metformin produced significant ($p<0.01$), ($p<0.001$) reduction in FBS on days 14, 21 and 28 respectively while 1500 mg/kg MOE/metformin co-administration produced a significant ($p<0.001$) reduction in FBS on days 7, 14, 21 and 28 compared to hyperglycaemic control. A dose- dependent significant reduction in food and water intake was observed throughout the experiment with no significant change in body weight. Each of cholesterol, triglycerides and low density lipoprotein was dose-dependently reduced significantly ($p<0.001$) with a corresponding significant ($p<0.001$) increase in high density lipoprotein in all groups. Total protein and albumin were significantly ($p<0.001$) increased in all the groups except in the 750mg/kg MOE that showed significantly ($p<0.05$) increase. Serum aspartate transaminase, alanine transaminase, alkaline phosphatase and bilirubin levels reduced more significantly ($p<0.001$) in the extract/metformin co-administered groups. A significant ($p<0.001$) increase in serum Na^+, K^+ and HCO_3^+ levels with a corresponding decrease in Cl^- ions was observed at all doses when compared to the hyperglycaemic control. The MOE/metformin co-administered groups showed the most significant ($p<0.001$) reduction in creatinine and urea levels. Among all the organs examined, only the liver and spleen showed a dose-dependent increase in ROWs while only the pancreas showed remarkable changes in the form of beta-cell necrosis on histopathological examination. However, regeneration of beta-cells was observed in a dose-dependent fashion in all groups. The normoglycaemic study did not show any significant difference for all the parameters examined. When compared to the metformin treated group, MOE/metformin co-administration showed significant changes in all parameters monitored. In conclusion, MOE showed additive interaction with metformin. This could be important in maximizing diabetes management

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List of Abbreviations

ADA - American Diabetes Association

ATP - Adenosine tri-phosphate

BGC- Blood Glucose Concentration

Chol- Cholesterol

DDI- Drug-drug interaction

DIs- Drug interactions

DNA - Deoxyribose nucleic acid

DPP-IV - Dipeptidyl peptidase-IV

FBS- Fasting blood Sugar

GDM - Gestational diabetes mellitus

GIT- Gastrointestinal tract

H&E- Haematoxylin and eosin

HDL - High density lipoprotein

HFD - High fat diet

IAAs - Insulin autoantibodies

ICAs - Islet cell antibodies

IDDM - Insulin dependent diabetes mellitus

IGT - Impaired glucose tolerance

LDL - Low density lipoprotein

MOE - Ethanol extract of *Moringa oleifera* leaves

NIDDM - Non-insulin dependent diabetes mellitus

PD- Pharmacodynamics

PK- Pharmacokinetics

PPAR- Peroxisome proliferators activated receptor

SUR- Sulfonylurea receptors

T1DM - Type I diabetes

T2DM - Type 2 diabetes

TAG - Tri-acyI glycerol

TBIL- Total bilirubin

WHO- World Health Organisation

CHAPTER ONE

1.0 INRODUCTION

1.1 Statement of Research Problem

Increasing number of patients and consumers are using herbs and herbal products as complementary therapy in the treatment and management of chronic ailments such as tuberculosis, diabetes, hypertension, HIV/AIDS and cancer. Herbs are also employed in the treatment of endemic diseases especially malaria, as well as other social conditions like obesity (Obodozie *et al.,* 2004). This upsurge in the use of phytomedicines is a global phenomenon, with more than 80% of people in Africa and Asia using herbal medicines and an increasing number in the Western world (WHO, 1999). Herbs are widely acceptable; accessible, affordable and are also considered to be safe since they are 'natural'. As a result, the use of herbs and herbal products alongside conventional therapy for better therapeutic outcome continues to grow rapidly. Herbs and herbal products contain a multiple of both active and inactive principles. This increases the possibility of interaction when co-used with orthodox drugs. Herbal-drug interaction can occur either at pharmacodynamic (PD) or pharmacokinetic (PK) level (Mohammed and Mohammed, 2009).

Some diabetics use an antidiabetic herb concurrently with a conventional anti-diabetic drug for a synergistic glycaemic control (Adikwu *et al*., 2010). This could lead to drug-herb interaction. The clinical significance of these interactions varies; it can lead to an increase/decrease of therapeutic efficacy or even increase in the toxic effects of drug therapy (Mohammed and Mohammed, 2009). In some instances, the interaction may have a beneficial effect by increasing drug efficacy or diminishing potential side effects (Tende *et al*., 2011). The issue of drug-herb interactions should therefore be seriously

considered while a patient is combining a potent antidiabetic agent and herbal remedies (Adikwu *et al*., 2010).

Moringa oleifera belongs to the family of Moringaceae, a fast growing drought-resistant tree, native to Sub-Himalayan tracts of Northern India but also distributed world wide in the tropics and sub tropics (Fuglie, 1999). In Nigeria, though the Moringa tree is widespread throughout the states, it is usually found around farms and compounds as fence in Northern part of the country. In Nigeria, it is cultivated for its use as an alternative green vegetable source for human consumption and other medicinal uses. The pharmacological properties of *Moringa oleifera* have received a lot of attention in recent years due to its wide usage in the management and treatment of a number of ailments. Ethanol extract of the leaves has been shown to possess antifungal activity against a number of dermatophytes (Chaung *et al*., 2007) while, the methanol extract was shown to have a CNS depressant activity (Pal *et al*., 1996) and the aqueous extract also has demonstrated anti-fertility activity (Prakash, 1998). *Moringa oleifera* is used to manage diabetes mellitus and it has also been confirmed scientifically to possess hypoglycaemic properties (Jaiswal *et al*., 2009; Chinwe and Isitua, 2010; Tende *et al*., 2011).

The wide consumption of *Moringa oleifera* for its therapeutic and nutritional benefits poses a possibility of pharmacological interaction when co-used with a conventional first-line antidiabetic agent such as metformin. Metformin is a biguanide commonly used by type II diabetic patients because of its fewer side effects and less likelihood to cause weight gain or raise cholesterol levels. A scientific study is therefore important to ascertain the beneficial or toxicity potentials of such combinations.

1.2 Justification for the Study

The World Health Organization (WHO) estimates that about 80% of African and Asian populations rely on traditional medicine as primary method for their health care needs. The scenario in developed countries is very similar with 70 to 80% of the population using some form of alternative and complimentary medicine (WHO, 2008). Herbal medicines are often used under a false sense of security due to the perception that 'natural' ensures safety (Brantley *et al.,* 2013). This misconception is as a result of the lack of regulatory quality control and standardization enforcement for herbal products. It should be understood that herbal preparations contain active phytochemicals in varying proportions which have a tendency like any other active pharmacological substance to alter the enzymatic systems, transporters and/ or the physiologic process (Vidushi, 2013).

Herbal products have increasingly been incorporated into Western health care as various reports suggest a high contemporaneous prevalence of herb-drug use in developing and developed countries (Vidushi, 2013). Herb-drug interaction is the single most important clinical consequence of this practice (Fasinu *et al.,* 2012). Despite these consequences, a standard system for interaction predictions and evaluation is non-existent as most researchers concentrate on potential therapeutic effects and mechanism of action of medicinal plants and often ignoring their potential interactions with conventional drugs. Consequently, the mechanisms underlying herb-drug interaction remain an understudied area in pharmacology (Brantley *et al.,* 2013). The knowledge of the interactions of herbs with prescription and/or over-the-counter drugs is essential for minimizing clinical risks (Alissa, 2014).

The interaction of drugs with herbal medicines is a significant safety concern, especially for drugs with narrow therapeutic indices (e.g. warfarin and digoxin), drugs for chronic ailments and drugs used in the management of life threatening conditions due to the fact that an alteration in the pharmacokinetics and/or pharmacodynamics of the drug by herbal remedies could bring about altered efficacy and/or toxicity, adverse reactions that are sometimes life threatening or lethal (Elvin-Lewis 2011). Several clinically important drugs have been reported to interact with some herbs leading to an exaggerated pharmacological activity or toxicity. Typical examples are the bleeding observed when warfarin is combined with garlic (*Allium sativum*), mild serotonin syndrome in patients who mix St. John's Wort (*Hypericum perforatum*) with serotonin-reuptake inhibitors; decreased bioavailability of digoxin, theophylline and cyclosporine when these drugs are combined with St John's Wort; induction of mania in depressed patients who mix antidepressants and *Panax ginseng* and increased risk of hypertension when tricyclic antidepressants are combined with yohimbine (Fugh-Berman, 2000).

In the management of diabetes mellitus, a number of herbs have been observed to interact with oral hypoglycaemic drugs. These include enhanced anti-hyperglycemia observed with co-administration of aqueous leaf extract of *Vernonia amygdalina* and metformin (Adikwu *et al.,* 2010) and co-administration of the fruit juice of *Mormodica charantia* with glibenclamide (Lal *et al.,* 2011). Similar observation was made when *Mormodica charantia* was consumed with chlorpropamide (Aslam and Stockley, 1979). Clinical studies have also shown that dietary gums such as the gum from the guar plant (*Cyamopsis tetragonobulus*) reduce the absorption of metformin and glibenclamide, consequently reducing hypoglycaemic effect (Izzo, 2004).

Moringa oleifera is used traditionally to manage a number of conditions. Moringa is considered an effective treatment for anaemia, loss of appetite and lactation enhancer in women. It also combats gastric discomfort, stomach ulcers, diarrhoea, dysentery, colitis and can be used as a laxative, purgative and a diuretic; it is also used to fight colds, bronchial infections, fever and head pain, rheumatic discomfort, muscular cramp and bruises, skin infections, scabies, ringworm, insect bites and also stabilizes sugar in diabetes (Nadkarni, 2009). Tende *et al*. (2011) established the anti-hyperglycaemic activity of the leaves of this plant in diabetic rats. Due to its wide consumption, some diabetics take *Moringa oleifera* as an adjunct therapy to oral hypoglycaemic agents. One of such oral hypoglycaemic agent is metformin, a biguanide which is currently used as the first drug of choice in the treatment of uncomplicated type 2 diabetes mellitus. This concurrent use of *Moringa oleifera* and metformin could lead to a drug-herb interaction.

Identification of potential herb-drug interactions is of importance for effective therapy. This can be achieved by using appropriate in vitro and in vivo testing methods. Thus, this investigation of the possible pharmacological interaction between the ethanol extract of *Moringa oleifera* leaves and metformin is important towards maximizing therapy and minimizing toxicity in the management of diabetes. On establishing the type of interaction, there will now be scientific data to justify or discourage the co-use of *Moringa oleifera* extract and metformin by diabetics.

1.3 Theoretical Frame Work

1.3.1Acute toxicity studies

Acute toxicity studies in animals are conducted to determine the nature and extent of the adverse effects which might follow the administration of a single dose of a drug. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for chronic toxicity studies.

The index of acute toxicity testing is the median lethal dose (LD_{50}), which is the dose that will cause lethality in 50% of the test animals. The method described by Lorke adopted in this study postulates that acute toxicity should be tested in two phases using the smallest number of animals per group. In the initial phase, the range of doses producing the toxic effects is established and the nature and time of all adverse effects noted. In the second phase, further specific doses based on the results of the initial phase, are administered to calculate the $LD_{50.}$ An appropriate LD_{50} value is usually adequate to estimate the risk of acute intoxication. LD_{50} values usually guide the choice of doses used in efficacy and toxicity studies.

1.3.2 Dose-response study

The graphical representations of quantitative measurements of drug action are called dose- response curves (DRCs). Drugs exhibit a characteristic relationship between the dose and the pharmacologic response. Graphical representation of the dose and the magnitude of effect is the best way of clarifying the meaning of dose-response relationship. Knowledge of the relationships among dose, drug concentration in blood,

and clinical response is important for the safety and effective use of drugs in individual patients. This information can help identify an appropriate starting dose, the best way to adjust dosage to the needs of a particular patient, and a dose beyond which increases would be likely to provide added benefit or would produce adverse effects. A dose-response study is important to establish the safety and effectiveness of a drug therapy.

Often the dose-response curve is sigmosidal in appereance. Both ends of the curve are usually representative of doses of the drug which are either without effect, 0%, or least effect and the maximal effect, 100%. Subsequent doses beyond this latter level result in a plateuing of response. Sometimes, the plateau end of the curve may represent the toxic or even the lethal point. The smallest or the largest dose that produces 50% of the maximum response obtainable is known as the 'effective median dose' (ED_{50}). ED_{50} value is used to characterize the potency of an agent.

The graded doses of *Moringa oleifera* extract were selected based on the LD_{50} values and the logarithm of doses plotted against the pharmacological response (percentage glycaemic reduction) to each dose level. The plot of percentage glycaemic reduction versus log dose was used to estimate the ED_{50} which was in turn used for the interaction studies.

1.3.3 Alloxan-induced diabetes in rats

The alloxan model of experimental diabetes was first described by Dunn, *et al.*, (1943). Alloxan was originally prepared by the oxidation of uric acid using nitric acid. It is regarded as a strong oxidizing agent that forms a hemiacetal with its reduction product; dialuric acid. Daluric acid can be further reduced to form alloxantin. Alloxan has been

noted to exert its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously. Furthermore, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status (Federiuk *et al.,* 2004). Alloxan is non-toxic to the human beta-cells, even at very high doses; this might be attributed to the differing glucose uptake mechanisms in humans and rodents (Eizirik *et al.,* 1994).

Alloxan induces diabetes by selective necrosis of the insulin-producing pancreatic beta-islets. This is similar to the auto-immune destruction of beta cells of the pancreas of type 2 diabetics as lack of insulin secretion results in both cases. Induction by alloxan leads to a triphasic blood glucose response.The first phase which starts a few minutes after alloxan administration is characterized by a transient hypoglycemic phase that lasts maximally for 30 minutes. This occurs as a result of stimulation of insulin secretion (Kliber *et al*., 1996). The underlying mechanism of this transient hyperinsulinemia may be attributed to a temporary increase in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition. The second phase appears one hour after administration of alloxan. This stage is characterized by an increase in blood glucose concentration with a corresponding decrease in plasma insulin concentration. This marks the first hyperglycemic phase after the contact of the pancreatic beta cells with alloxan. These changes are as a result of inhibition of insulin secretion from the pancreatic beta cell due to the beta-cell toxicity of alloxan. The third phase is again a hypoglycemic phase that occurs 4-8 hours after alloxan injection. This severe transitional hypoglycemia phase usually lasts for several hours and it occurs as a result of alloxan-induced secretory granule and cell membrane rupture (Banerjee and

Bhattacharya, 1945). In addition, other subcellular organelles such as the rough endoplasmic reticulum and Golgi complex are also ruptured.

1.3.4 Pharmacological interaction study

Herb-drug interaction can occur either at pharmacokinetic or pharmacodynamic level. Pharmacokinetic interaction results when there is an alteration in the absorbtion, distribution, metabolism or elimination of a conventional drug by an herbal product or vice versa (Lal *et al.,* 2011). A pharmacodynamic drug-herb interaction results when both the herb and drug are directed at a similar receptor target or physiological system. The resulting effect of an herb-drug interaction could be synergetic, whereby the herbal medicine potentiates the action of synthetic drugs or antagonistic whereby, the herbal medicine reduces the efficacy of synthetic drugs (Izzo *et al.,* 2002).

1.4 Aim of the Study

The aim of the study is to investigate the pharmacological interaction between the ethanol extract of *Moringa oleifera* leaves and metformin when co-administered to alloxan-induced hyperglycaemic Wistar rats.

1.4.1 Specific objectives

The specific objectives of the study are as follows

(i) To estimate the median lethal dose (LD_{50}) of the ethanol extract of *Moringa oleifera* leaves.

(ii) To estimate the dose of the ethanol extract of *Moringa oleifera* leaves required for 50% glycaemic reduction in fasted hyperglycaemic rats (ED_{50}).

(iii) Using hyperglycaemic rats, to carry out long-term (28 days) efficacy and toxicological studies on graded doses of ethanol extract of *Moringa oleifera* leaves (MOE), metformin and MOE/metformin co-administration. Parameters monitored were:

a. Blood glucose level

b. Food and water intake

c. Body weight (BW) changes.

d. Serum biochemistry, lipid and protein profiles

e. Serum electrolyte, creatinine and urea levels

f. Relative organ weight and histopathology of the liver, kidney, heart, brain, lungs, spleen, stomach and pancreas.

(iv.) Using normoglycaemic rats, to carry out long-term (28 days) efficacy and toxicological studies on graded doses of ethanol extract of *Moringa oleifera* and monitor same parameters as above.

1.5 Statement of Research Hypothesis

There is no pharmacological interaction when ethanol extract of *Moringa oleifera* leaves and metformin are administered concurrently to hyperglycaemic rats and *Moringa oleifera* possess hypoglycaemic activity in normoglycaemic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Drug Interactions

A drug interaction is said to have occurred when the effectiveness or toxicity of one medication is altered by the administration of another medicine or a substance that is administered either as food or for therapeutic purposes. The effects can be positive and sometimes form the basis of very important drug combinations. Synergism, additive effects, potentiation and antagonism are all outcomes of drug interactions and the magnitude of the effect seen depends on the mechanism by which the interaction occurs.

2.2 Mechanism of Drug Interactions

They are two primary mechanisms through which drug interactions can occur: pharmacodynamic and pharmacokinetic. Pharmacokinetic interactions relate to the mechanism in which absorption, distribution, metabolism and/or excretion of the interacting drugs are altered resulting in an increase, decrease or delayed pharmacological actions. Pharmacodynamic interactions on the other hand are considered more with actions of drug at various sites and will usually not have effect on timing of drug action as much as pharmacokinetic interactions.

2.2.1 Pharmacokinetic drug interactions

These are interactions which can affect the processes by which drugs are absorbed, distributed, metabolized and excreted.

2.2.1.2 Absorption

The term absorption describes the process by which herbs or drug molecules pass across biological membranes. The majority of all absorption occurs in the intestines, where herbs or drugs must pass through the intestinal wall to enter the blood stream. A number of factors may interfere with the absorption of drugs through the intestines. Herbal medications can alter the absorption of concomitantly administered medicines through a number of mechanisms. Changes in the gastrointestinal pH and other biochemical factors can alter dissolution properties and the absorption of pH-dependent drugs such as ketoconazole and itraconazole. Chelation and formation of insoluble complexes could also be a possible way of herb-drug interaction (Fugh- Berman, 2000).

2.2.1.2 Distribution

Distribution refers to the process in which herbs or drugs are carried and released to different parts of the body. After absorption, herbs or drugs need to be presented to the affected area to exert their effect. Interaction at distribution level occurs mainly with drugs that are bound by the same protein. Herbs such as meadowsweet and black willow, which contain pain-reducing salicylates, are highly protein bound as such may displace relatively less protein bound drugs such as warfarin and carbamazepine thus increasing the adverse effects of these drugs. These products are usually not to be taken concurrently as there are chances of drug- herbal interactions (Khun *et al.,* 2001).

2.2.1.3 Metabolism

The liver, intestines, kidneys, and lungs are the sites of drug metabolism and pharmacokinetic interactions (Bressler and Bhal, 2003). However, the liver is the major

site of drug metabolism, with the intestines playing a secondary yet potentially important role. The cytochrome p450s (CYPs) are a group of enzymes generally involved in oxidative, peroxidative, and reductive biotransformation of xenobiotics and endogenous compounds (Nebert and Russell, 2002; Hiratsuka, 2011). Certain herbal products have been shown to be capable of inducing CYP (Zhou, 2008). Concomitant administration of enzyme-inducing herbal products and prescription drugs can therefore result in sub-therapeutic plasma levels of the latter with therapeutic failure as a possible clinical consequence. Apart from enzyme induction, herbal products can also inhibit enzyme activities. A clinically important consequence of enzyme inhibition is drug accumulation due to subdued hepatic clearance. These effects will be of particular concerns in drugs with narrow therapeutic window or steep dose–response profiles.

2.2.1.4. Elimination

A possible mechanism of herbal-drug interactions is the modification of renal clearance of a drug by an herb or vice-versa. After the metabolism of drugs and herbs by the liver, the process of elimination is completed by excretion. The kidneys are responsible for eliminating the substances and their metabolites from the body. Some herbs and herbal products are capable of interacting with renal functions, leading to altered renal elimination of drugs. Such interactions can result from the inhibition of tubular secretion, tubular re-absorption, or interference with glomerular filtration (Isnard *et al*., 2004).

2.2.2 Pharmacodynamic drug interactions

Concurrent use of herbs and drugs with similar therapeutic actions poses potential for pharmacodynamic herb-drug interactions. Herb-drug interaction can lead to

antagonistic effect where the administration of a herb and a drug with opposing pharmacodynamic effects reduce the response to one or both the drug and the herb. A positive outcome of drug-herb interaction is synergistic or additive effects. This happens when the herb or herbal products and the conventional medications have affinities for common receptor sites and when given together shows an exponential clinical impact (Ma *et al.,* 2009).

2.3 Types of Drug Interactions

There are different types of drug interactions which include drug-drug interactions, drug-disease interactions, drug–laboratory test interactions, drug–food interactions and drug- herb interactions.

2.3.1 Drug-drug interactions

A clinically relevant drug-drug interaction (DDI) occurs when the effectiveness or toxicity of one drug is altered by the administration of another drug (Dresser *et al.,* 2002). DDIs have many clinical implications which include diminished therapeutic effect or even toxicity. Based on the properties of a drug, its route of administration and some other patient-specific factors, the possibility of a DDI could be predicted (Dresser *et al*., 2002).

2.3.2 Drug- disease interactions

A drug-disease interaction is said to have occurred when a drug causes undesirable effect in patients with certain disease states. For instance, there is a high rate of co-morbidity in people with diabetes. Cardiovascular, renal, gastrointestinal, neurological, and thyroid diseases and ophthalmological complications are all disorders that are

associated with diabetes. These diseases increase the chance of having drug-disease interactions. Metformin and impaired renal function is a well-documented drug-disease interaction (Somogyi *et al.,* 1987).

2.3.3 Drug- laboratory test interactions

A drug-laboratory test interaction occurs when a drug administered prior to a diagnostic laboratory test causes alteration in its result. Herbal medicines can affect laboratory test results for example; liver toxicity and elevated ALT, AST and bilirubin concentrations may be observed in healthy individuals taking *Piper methysticum* (Kava-Kava).

2.3.4 Drug-food interactions

The interactions between foods, their nutrients and drugs are common in the health care and medical fields (Mohammed and Mohammed, 2009). A drug-food interaction occurs when the food affects the ingredients in a medicine which the patient is taking affecting the efficacy of medicine being administered. Certain foods and specific nutrients in foods, if ingested concurrently with some drugs, may affect the overall bioavailability, pharmacokinetics, pharmacodynamic and therapeutic efficacy of the medication.

2.3.5 Drug-herb interaction

Herbs and herbal products are considered to be safe as such people take it concurrently with orthodox medicines in order to maximise therapeutic effect. Worthy of note is that not all combinations of herbs and drugs are safe as it has been reported that some may be beneficial while others may be harmful (Amita *et al.,* 2012). When an orthodox drug is co-administered orally with a herbal product, there is a possibility of alteration in each others pharmacokinetic profile, that is, absorption, distribution, metabolism,

and/or excretion as both orthodox and herbal drugs share the same set of metabolizing enzymes and transporters (Miller, 1998).

2.4 Diabetes Mellitus

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Ugochukwu *et al.,* 2003), lipoprotein abnormalities (Scoppla *et al*., 2001), raised basal metabolic rate (Nawata *et al*., 2004; Okwu *et al.,* 2006), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Unwin *et al.,* 2001). The hyperglycaemia in diabetes mellitus can result from an absolute deficiency in insulin secretion (type 1 DM), insulin action (type 2 DM) or both (Ahmed and Goldstein, 2006; Andrade-Cetto *et al*., 2007). If not controlled, hyperglycaemia can lead to the development of microvascular disorders such as nephropathy, retinopathy and macrovascular complications including cardiomyopathy, neuropathy, atherosclerosis and myocardial infarction (Randle *et al*., 1963; WHO, 1999).

A number of pathogenic processes are involved in the development of diabetes. These range from an autoimmune destruction of the β cells of the pancreas, with consequent insulin deficiency, to abnormalities that result in resistance to insulin action (Xu *et al.,* 2008).

2.4.1 Prevalence of diabetes mellitus

At least 346 million people worldwide suffer from diabetes or about 5.6% of the population (WHO, 2011). Diabetes mellitus affects all races around the world, but is more prevalent in the more developed countries (Ramachandran *et al.,* 2001). Type 2

diabetes accounts for almost 90% of all cases of diabetes in adults worldwide. The mortality rate of diabetes varies sharply with the prosperity of the country (WHO, 2011). Approximately 1.2 adults die of a diabetes-associated illness per 1,000 cases in 2011 in low and middle income countries which are more than double the mortality rate of high income countries. Mortality rates are much lower in high income countries with the greater healthcare resources, but those tolls are still high: approximately 180,000 people died in the United States in 2011 (WHO, 2011).

Diabetes afflicts only about 4.5% of adults in Sub-Saharan Africa but prevalence is predicted to double over the next 20 years, the fastest rise of any region in the world. In Nigeria, it afflicts about 3 million people or about 3 percent of the population (WHO, 2011).

2.4.2 Classification of diabetes mellitus

Diabetes mellitus (DM), as already mentioned, is a complex and heterogeneous disorder with diverse etiologic mechanisms; therefore, any given classification is arbitrary but nevertheless useful.

Diabetes has been previously classified into five distinct types namely: Insulin Dependent Diabetes Mellitus (IDDM), Non-Insulin Dependent Diabetes Mellitus (NIDDM), Gestational Diabetes Mellitus (GDM), malnutrition related diabetes and other types (ADA, 2008). These types were characterised by either fasting hyperglycaemia or elevated levels of plasma glucose during an oral glucose tolerance test (OGTT).

The current classification puts into consideration both the etiology and clinical stages of the disease. It categorizes diabetes into four classes. The two main types are: type 1(T1D), which accounts for 5–10% of all cases, and type 2(T2D), which accounts for 90-95% of all individuals with diabetes. The third group consists of other less common types of diabetes that are caused or associated with certain specific conditions and/or syndromes. The last group includes diabetes diagnosed during pregnancy, called gestational diabetes mellitus (GDM) (ADA, 2008).

2.4.2.1 Type 1 diabetes mellitus (T1D)

The type 1 diabetes mellitus was previously referred to as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes. T1D results from a progressive cellular mediated autoimmune destruction of the pancreatic β-cells that leads to complete insulin deficiency (Knip and Siljander, 2008).

Idiopathic diabetes:

There are some forms of Type 1 diabetes which have no known etiology. Some of these patients have varying degree of β-cell dysfunction, permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (McCarty, 2005).

2.4.2.2 Type 2 diabetes mellitus (T2D)

The type 2 diabetes was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It is a term used for individuals who have relative rather than absolute insulin deficiency. Type 2 diabetes is associated with two main metabolic defects: insulin resistance, a decreased response of peripheral tissues to insulin and β-cell dysfunction that is manifested as inadequate insulin secretion or

relative insulin deficiency in the face of insulin resistance and hyperglycaemia (Defronzo *et al.*, 1991).

Type 2 diabetes is determined primarily by lifestyle factors and genes (Riserus *et al.*, 2009). Unlike type 1 diabetes, autoimmune destruction of β-cells does not occur. The two most important risk factors of this disorder are obesity and sedentary lifestyle.

2.4.2.3 Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus, GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. This does not include women with diabetes prior to becoming pregnant, who have diabetes and pregnancy rather than gestational diabetes. In early pregnancy both fasting and post-prandial plasma glucose levels are usually lower than normal so that any deviation at this stage could certainly be attributed to undetected type 2 diabetes (Riserus *et al.*, 2009).

2.4.2.4 Other specific types of diabetes

The frequency of occurrence of the other specific types of diabetes is low when compared to type 1, type 2 and gestational diabetes mellitus. The group includes the following; Genetic defects in β-cell function, Genetic defects in insulin action, Diseases of the exocrine pancreas and Endocrinopathies.

2.4.3 Microvascular and macrovascular complications in diabetes

The cardiovascular system is made up microvessels and macrovessels. The microvessels include arterioles, capillaries, and venules which collectively function to regulate blood pressure and to deliver nutrients to tissues. The macrovessels on the

other hand include the veins and arteries and they supply blood to organs. The chronic hyperglycemia associated with diabetes promotes long-term damage and dysfunction of both micro- and macrovessels, with much of the impact on the microvessels (Skyler *et al.,* 1996).

The narrowing and blockage of microvessels due to hyperglycaemia causes a lot of damages to some organ systems of the body particularly the eye, kidneys and the nervous system. Apart from the complications with retina, kidneys, and peripheral nervous system that are referred to as retinopathy, nephropathy and neuropathy respectively, macrovascular complications are a problem in type 1 (T1D) and type 2 diabetes mellitus (T2D). Just like the microvascular complications, the hallmark of these macrovascular complications is the atherosclerotic process initiated by hyperglycaemia. Atherosclerosis causes the gradual narrowing of vascular lumina of aorta and large and medium-sized arteries throughout the body, resulting in increased risk of myocardial infarction, stroke, and lower-extremity gangrene (Boyle *et al.,* 2007).

2.4.4 Diagnosis and monitoring of diabetes

A series of glucose tests are employed in the diagnosis and monitoring of diabetes. Impaired fasting glucose and impaired glucose tolerance are the diagnostic criteria for diabetes. The oral glucose tolerance test and fasting plasma glucose level are the common tests used for diagnosis since they are less complicated and cheap (Rohl, 2004). Acute infections, trauma and circulatory stress may induce hyperglycaemia, any reading taken under these conditions be should not in itself be regarded as diagnostic of diabetes. A single abnormal blood glucose value in an individual without symptoms

should not be confirmed as diabetes. For such an individual, at least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either fasting, from a random (casual) sample, or from the oral glucose tolerance test (WHO, 1999).

Apart from the two tests that have been mentioned earlier for the diagnosis of diabetes, other tests for blood glucose estimation include glycosylated haemoglobin, which reflects average glycaemia over a period of weeks, intravenous glucose tolerance test, glucose challenge test, lipid panel, two hour postprandial glucose and glucose clamping tests.

2.4.5 Prevention and treatment of diabetes

Diabetes mellitus is a chronic, progressive and degenerative disease, thus it can only be managed but not cured. A number of treatment options are available and effective in preventing complications of diabetes. Obesity has been linked with insulin resistance and hence type 2diabetes. It has been therefore suggested that moderate weight loss in obese individuals and avoidance of sedentary life style can reduce the risk of developing type 2 diabetes (Goodpaster *et al.,* 2003). Increasing physical activity, controlled diet or weight loss at times is not enough in managing diabetes, the use of oral hypoglycaemic agents and/or insulin injections are necessary to return blood glucose level to near normal.

2.4.5.1 Exercise, diet and weight management

Insulin sensitivity can be improved by the use of diet, exercise and weight management. If this leads to a reasonable control of blood glucose level, oral hypoglycaemic agents may not be needed or the dose reduced (Nelson *et al*., 2002).

The development of type 2 diabetes can be slowed by exercising regularly and a regular walking program was shown to reduce total mortality by 42% in patients with TID (Zarich, 2003).

Diet plays a role in the management of diabetes. Oxidative injury appears to be the fundamental mechanism underlying a number of human disorders (Atawodi, 2005), for instance, in diabetes, increased oxidative stress which co- exists with reduction in anti-oxidant status has been postulated (Sabu and Kuttan, 2002) .There is some controversy over the optimal diet for adults with T1D. More consumption of fruits and vegetables and decrease daily consumption of saturated fats have been suggested (ADA, 2002). Fruits and dark green vegetables contain antioxidants that prevent the development of cardiovascular complications of diabetes. Phenolic compounds that are found in fruits prevent oxidative damage while vitamin C prevents the oxidation of low density lipoprotein and cholesterol (Block, 1992).

2.4.5.2 Oral hypoglycaemic agents

Oral hypoglycaemic drugs are used for the management of type 2 diabetes. The effects of these drugs are usually limited to the pancreatic islets, liver and skeletal muscles since they are the main organs affected by diabetes (Dey *et al.*, 2002). Some of the drugs tend to improve insulin release; some inhibit hepatic glucose production while others improve the sensitivity of body tissues to insulin. There are seven distinct classes

of hypoglycaemic agents namely, sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, meglitinides, incretin mimetics and dipeptidyl- peptidase-4 (DPP-4) inhibitors.

Sulfonylureas:

Sulfonylureas are related to sulphonamides structurally and chemically. Sulfonylureas are divided into first and second generation. The first generation include chlorpropamide, tolazamide, tolbutamide and acetahexamide while the second generation include glyburide, glipizide, gliclazide, glimeperide etc.

Mechanism of action:

Sulfonylureas acts by stimulating insulin release from pancreatic ß-cells. The sulfonylureas bind to specific cell-surface receptors on the ß-cell plasma membrane called Sulfonylureas receptors (SUR). The binding of sulfonylureas to these receptors leads to the closure of adenosine triphosphate (ATP)-sensitive potassium channels, leading to depolarization of the cell membrane. Consequently, the voltage-gated channels open, allowing massive influx of calcium ions and subsequent release of preformed insulin granules.

The extra-pancreatic mechanisms of action of the sulfonylureas include reduction in serum glucagon levels which possibly contribes to its hypoglycemic effects. The precise mechanism by which this occurs remains unclear but may result from indirect (secondary) inhibition due to enhanced release of both somatostatin and insulin. Sulfonylureas may also potentiate insulin action at target tissues (drug-dependent characteristic).

Pharmacokinetics:

The rate of absorption of the drugs in this group differs markedly. However, all are effectively absorbed from the gastrointestinal tract. The presence of food in the GIT has been reported to affect the absorption of sulfonylureas. In general sulfonylureas with short half-lives may be more effective when given 30 min before eating due to the time required to reach optimal concentration in the plasma.

Adverse reactions:

The most common side effect of sulfonylurea is weight gain and hypoglycaemia, which though usually mild to moderate, can cause fatal complication (Seltzer, 1989).

Therapeutic uses:

Sulfonylureas have an important role in the management of type 2 DM patients who cannot achieve proper control with changes in diet alone. However, continued dietary restrictions are essential to maximize the efficacy of sulfonylureas.

Contraindications:

Contraindications to the use of these drugs include type 1 DM, pregnancy, lactation, and significant hepatic and renal insufficiency.

Drug interactions:

Several drugs interfere with the efficacy of sulfonylureas, particularly the first-generation agents, by influencing their pharmacokinetics and/or pharmacology. Drugs like sulphonamides, clofibrate, discumarol, salicylates, and phenylbutazone displace the sulfonylureas from their binding proteins, thereby increasing the free concentration transiently (Seltzer, 1989).

Several clinically important drug-herb interactions involving sulfonylureas have also been reported (Elvin-Lewis 2011). An example of such interaction is seen in the enhanced hypoglycemia observed when a meal containing garlic and *Mormodica charantia L*. (balsampear) *family: curcubitaceae*, a herb traditionally used in the treatment of type 2 diabetes was consumed with chlorpropamide (Aslam and Stockley., 1979).

Biguanides:

Metformin (Glucophage®) and phenformin were introduced in 1957 and buphormin was introduced in 1958. They were widely used in Europe for treating type 2 diabetes. The latter two were withdrawn in many countries in the 1970s because they produced fatal lactic acidosis (Schafer, 1983). Metformin has a very low rate of lactic acidosis compared with phenformin and has been widely used in Europe, Canada, Middle East and other countries. It became available in the United States in 1995.

Mechanism of action:

The mechanism of action of metformin is not fully understood. Metformin is antihyperglycaemic, not hypoglycaemic (Bailey, 1992). It does not cause insulin release from the pancreas and does not cause hypoglycaemia; even in large doses (Hermann, 1979). Metformin has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin. It has been shown to increase peripheral uptake of glucose (Hundal *et al.,* 1992) and to reduce hepatic glucose output by approximately 20-30% when given orally (pariello *et al.,* 1994) but not intravenously. Impaired absorption of glucose from the gut has also been suggested as a mechanism of action, but has not been shown to have clinical relevance. Metformin has also been

shown to decrease serum triglycerides and fatty acid concentration and slows the rate of lipid oxidation (pariello *et al.*, 1994). These actions indirectly inhibit gluconeogenesis. Metformin treatment is associated with statistically and clinically significant reduction in body weight in obese patients with type 2 diabetes. These are independent of its effect on glycaemic control (Defronzo *et al.*, 1991).

Pharmacokinetics:

Metformin is mainly absorbed from the small intestine. The drug is stable, does not bind to plasma proteins, and is excreted unchanged in urine. It has a half-life of 2 hours. The maximum daily dose of metformin in the United States is 2.5 g taken in three doses with meals.

Adverse effects:

Approximately one-third of patients on metformin will have transient nausea, anorexia or diarrhoea, abdominal discomfort, and metallic taste. These side effects can be minimized by starting with a low dose (500 mg daily) and gradually increasing the dose up to 800-850 mg thrice daily. Intestinal absorption of vitamin B12 and folate is often decreased during chronic metformin therapy. Other advers effects reported are headache, agitation, dizziness and tiredness.

Contraindications:

Metformin is contraindicated in patients with impaired renal, respiratory or hepatic function, cardiac failure, or a history of alcohol abuse.

Uses:

Metformin is the drug of first choice in overweight patients in whom strict dieting has failed to control diabetes. It may also be considered as an option in patients who are not overweight and when diabetes is inadequately controlled with sulfonylurea. (Rubin *et al.*, 2002).

Drug interactions:

Metformin is not metabolized in human tissues, hence metabolic-based drug interactions are not known to occur. Cationic drugs that are eliminated by tubular secretion may compete with metformin for elimination and this may result in clinically significant interactions.

Alpha glucosidase inhibitor:

α- Glucosidase inhibitors have been developed specifically to delay the digestion of complex carbohydrates and decrease the postprandial rise in plasma glucose, thus reproducing the effect of a low glycaemic index/high fiber diet (Bischoff, 1995). These actions significantly reduce postprandial glycaemic and insulinaemic increase whether they are used as monotherapy or combined in the treatment of type 1 and type 2diabetes. These drugs have an excellent safety profile.

Mechanism of action:

α- Glucosidase inhibitors competitively block small intestine brush border enzymes that are necessary to hydrolyze oligosaccharides and polysaccharides to monosaccharides (Bischoff, 1995). Inhibition of this enzyme slows the absorption of carbohydrates; the

postprandial rise in plasma glucose is blunted in both normal and diabetic subjects (Reabasa- Lhoret and Chiasson, 1998).

Pharmacokinetics:

Acarbose is poorly absorbed (less than 2%) on oral administration and 35% of an oral dose appears as metabolites in the urine (Lebovitz, 1998). (Bressler and Johnson., 1992). Miglitol is not metabolized and is excreted quantitatively by the kidney (Lebovitz, 1998).

Uses and adverse effects:

Alpha-glucosidase inhibitors may be used as monotherapy in elderly patients or in patients with predominately postprandial hyperglycaemia. α- glucosidase inhibitors typically are used in combination with other oral antidiabetic drugs and/or insulin.

Drug interactions:

Since acarbose and miglitol may potentiate the hypoglycemic effects of insulin and the sulfonylureas, the doses of these agents may require adjustment when used concurrently. When acarbose is used, hypoglycemia will not respond as efficiently to oral carbohydrates.

Thiazolidinediones:

Thiazolidinediones (TZDs) are chemically and functionally unrelated to the other classes of oral antidiabetic agents. A thiazolidine-2, 4-dione structure is common to all agents in this group. Rosiglitazone (Avandia) and pioglitazone (Actos) are the two

thiazolidinediones currently in use (Papoushek, 2003).The third, troglitazone, was withdrawn from use because of its severe hepatic toxicity (Papoushek, 2003).

Mechanism of action:

Thiazolidinediones are selective agonists for nuclear peroxisome proliferator-activated receptor-gamma (PPAR) (Lemberger *et al.,* 1996). The PPAR are a family of nuclear receptors consisting of three subtypes designated as PPARa, PPARb and PPARd or ß (Desvergne, 1999). PPAR receptors are found in key target tissues (adipose tissue, skeletal muscle and liver) for insulin action. Binding of TZDs to PPAR activates insulin-responsive genes that regulate carbohydrate and lipid metabolism. They require insulin to be present for their action. Thiazolidinediones exert their principal action by lowering insulin resistance in peripheral tissue, but has also been reported to decrease glucose production by the liver (Park *et al.,* 1998).

Pharmacokinetics and pharmacodynamics:

Rosiglitazone (Avandia®) and pioglitazone (Actos®) are taken once a day. Both are absorbed within 2 h, but the maximum clinical effect is not observed until after 6 to 12 weeks. Pioglitazone has a plasma half-life of 3-7 h, reaching peak concentration about 2 h after administration while rosiglitazone has a plasma half-life of 3-4 h, reaching peak plasma concentration after about an hour. Both are highly (>99%) plasma protein bound and undergo hepatic metabolism.

Contraindication:

Thiazolidinediones combined with insulin are contraindicated in patients with a history of cardiac failure (New York Heart Association classes I-IV), severe renal insufficiency (rosiglitazone) or on dialysis (pioglitazone), pregnancy, and breast- feeding.

Clinical use and adverse effects:

Rosiglitazone, when used in combination with a sulfonylurea, is given at a dose of 4 mg/day, which can be increased to 8 mg/day when used in combination with metformin. It is given once or twice a day, with or without food. The dose of pioglitazone is 15-30 mg/day, with or without food.

Drug interactions:

Pioglitazone and rosiglitazone do not inhibit any of the major cytochrome P450 isoenzymes: troglitazone, the parent member of this series was a potent CYP3A4 isoenzyme inducer. Studies are required to elucidate the potential interaction of inducers and inhibitors of the CYP3A4 isozyme with pioglitazone which is metabolized, in part, by this enzyme. The steadystate pharmacokinetics of glipizide and metformin are not altered by pioglitazone coadministration (DeRuiter, 2003).

Meglitinides:

The meglitinides are a new class of drugs developed to improve early-phase insulin secretion, which is one of the earliest pathophysiologal manifestations of type 2 DM (Hu *et al.,* 2000). These are derived from the meglitinide portion of sulfonylureas and they include repaglinide and nateglinide.

Mechanism of action:

Meglitinides have the same mechanism of action as the sulfonylureas. They bind to the sulfonylurea receptor subunit on the ß-cell receptors to stimulate insulin release by closing the K+ ATP channel (Hu *et al.,* 2000). Closure of the potassium channel leads to depolarization of ß-cell plasma membrane, which promotes influx of calcium ions through voltage-gated calcium channels, resulting in exocytosis of insulin granules.

Pharmacokinetics:

Repaglinide is rapidly absorbed, and peak plasma levels are reached within 30-60 min. Oral bioavailability is 65% and the plasma half-life is approximately 60 min in healthy volunteers and in patients with type 2 diabetes (Wolffenbuttel and Graal., 1996). Nateglinide is rapidly absorbed after oral administration and peak plasma levels are reached within 45 min (Gribble *et al.,* 2001). Nateglinide is metabolized by the liver.

Uses and adverse effects:

Residual ß-cell function is necessary for meglitinide analogues to be effective. Repaglinide is licensed as monotherapy in the treatment of patients with type 2 diabetes and may be used in combination with metformin when metformin alone is inadequate. (Moses *et al.,* 1999). It may also be used in combination with thiazolidinediones.

Drug interactions:

Currently no clinically significant drug interactions have been reported with repaglinide. However, repaglinide metabolism may be decreased by inhibitors of CYP 3A4 such as the azole antifungal (ketoconazole, miconazole, etc.) and some antibiotics including erythromycin, resulting in increased serum concentrations.

Incretin mimetics and Incretin enhancer drugs:

Incretins (glucagon like peptide-1 (GLP-1) and glucose dependent insulinotrophic polypeptide (GDIP)) are enteroendocrine hormones released into bloodstream from L and K cells throughout the gastrointestinal tract (Chee *et al.,* 2009). In presence of nutrients, GLP- 1 is released. In type 2 diabetics, GLP- 1 is secreted in low quantities.

Mechanism of action:

The mechanism of action of GLP- 1 is the stimulation of glucose-dependent insulin release from the pancreatic islets, slowing gastric empting with consequent reduction in glucagon release (Rocca *et al.,* 1999).

Clinical uses:

GLP-1 is thought to have beneficial effects on myocardial function by improving myocardial contractility, enhancing glucose uptake in normal and post ischemic rat hearts and induced an endothelial dependent reduction in vascular tone of rat lungs.

2.4.5.3 The use of herbs for the management of diabetes

Medicinal plants are increasingly being used in most parts of the world as hypolipidemic (Xu *et al.,* 2008), antihypertensive (Nworgu *et al.,* 2008) and hypoglycaemic agents (Farswan *et al.,* 2009; Lee *et al.,* 2009). Several plants and isolated compounds have been reported to have anti-diabetic potentials (Li *et al.,* 2004; Grover *et al.,* 2000; Rahman and Zaman, 1989). Many of the conventional drugs in use today were derived from phytochemicals. The development of metformin, an effective glucose lowering agent was based on the use of *Galega officinalis* to treat diabetes (Bailey and Day, 1989).

There has been increasing demand for the use of plant products with anti-diabetic activity. The high cost, availability, uncertainly of use during pregnancy and undesirable side effects of synthetic drugs have been some of the factors leading to a strong preference for hypoglycemic drugs of plants origin, which are believed to be suitable for chronic treatments (Okigbo and Mmeka, 2006). Till date, numerous medicinal plants have been reported to be effective in the management of diabetes, yet plenty of research is still needed to be done (Vasim *et al.,* 2012).

2.5 *Moringa oleifera*

2.5.1 Description

Moringa oleifera belongs to the family of Moringaceae, a fast growing drought-resistance tree, native to sub Humalayan tracts of northern India but has been introduced and become naturalized in other parts of India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian peninsula, east and west Africa, southern Florida, throughout the West Indies, and from Mexico to Peru, Paraguay, and Brazil (Fuglie, 1999). There are about 13 species of the genus Moringa; these are *Moringa oleifera, M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. ovalifolia, M. peregrine, M. pygmaea, M. rivae, M. ruspoliana, and M. stenopetala.*

Moringa oleifera is known by names such as clarifier tree, horseradish tree, drum stick, Mother's Best Friend, and Miracle Tree (Duncan, 2009). As a result of the multiple importance of the plant, it is referred to in Hausa as *Zogale Mataimaki*, meaning *Zogale* the helper.



PLATE 1: *Moringa oleifera* tree in its Natural Habitat



PLATE II: Leaves of *Moringa oleifera*

2.5.2 Traditional uses of *Moringa oleifera*

Moringa has found use in traditional medicine for centuries in many cultures around the world, for skin infections, anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera conjunctivitis, cough, diarrhoea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation ,diabetes and pregnancy. Moringa oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner (Johnson, 2005; Manzoor *et al.,* 2007).

The most common species of Moringa, *Moringa oleifera* is a very impressive and amazing plant due to its tested, trusted and potential benefits from nutritional and therapeutical points of view (Khawaja *et al.,* 2010).

2.5.3 Nutritional benefits of *Moringa oleifera*

Nutritionally, *Moringa oleifera* is the most nutrient-rich plant yet discovered. This plant has been used in many societies for thousands of years, and it has been the center of nutritional research for decades now. Moringa provides a rich and rare combination of nutrients like vitamins, amino acids, antioxidants, antiaging and anti-inflammatory properties used for nutrition and healing (Khawaja *et al.,* 2010). The nutritional importance of Moringa is recognized by the World Health Organization (WHO) and in 1999 WHO recommended the use of Moringa as an alternative to imported food supplies to treat malnutrition (Manzoor *et al.,* 2007; Screelatha and Padma, 2009). Moringa contains vitamin C which helps in fighting a host of illnesses including colds and flu. It contains vitamin A, which protects the eyes against disease, skin disease,

heart ailments, diarrhoea, and many other diseases. It also contains calcium which strengthens the bones and teeth and helps prevent osteoporosis. Potassium which is essential for the functioning of the brain and nerves is also contained in Moringa, and Proteins, the basic building blocks of all our body cells (Babu, 2000; Fuglie, 1999).

Moringa leaves also contain all of the essential amino acids in the right proportions for the body, which are the building blocks of proteins. Most of the vegetables do not contain as many amino acids as *Moringa oleifera*. The leaves could be of an advantage to people who do not get protein from meat (Lockett and Calvert, 2000). The amino acids contained in Moringa include arginine and histidine which are very important for the development of infants. Research shows that about 30% of children in sub-Saharan Africa are protein deficient. Moringa therefore could be of importance in providing these essential nutrients to the people (Manzoor, 2007).

2.5.4 Researched properties of *Moringa oleifera*

2.5.4.1 Antibacterial and antifungal efficacy

Kekuda *et al.,* (2010) reported that the steam distillate of *M. oleifera* caused a reduction in the growth of *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, B. subtilis* and also a fungus. This they attributed to the essential oil fraction of the plant.

2.5.4.2 Anti-oxidant effect

Screelatha and Padma, (2010) showed that the aqueous extract of mature and tender leaves of *Moringa oleifera* exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid per

oxidation, as such prevent oxidative damage to major bio-molecules and afford significant protection against oxidative damage.

2.5.4.3 Gastric ulcer protective activity

Das *et al*., (2011) studied the possible anti-ulcer effects of water extracts of *M. oleifera* in pyloric ligation and ibuprofen induced gastric ulcer models and reported that the anti-ulcer effect of *M. oleifera* was comparable with that of the standard drug, famotidine.

2.5.4.4 Analgesic activity and local anaesthetic activity

Sutar *et al.,* (2008), reported that the alcoholic extract of *M. oleifera* seeds showed potent analgesic activity which is comparable to that of aspirin at the dose of 25 mg/kg of body weight.

2.5.4.5 Anti-inflammatory and antinociceptive activity

The anti-inflammatory action of an aqueous extract of *M. oleifera* root was investigated in rats by Ndiaye *et al.,* (2008) and reported that the extract reduced the carrageenan induced oedema to a similar extent as the potent anti-inflammatory drug indomethacin.

2.5.4.6 Cardioprotective activity

Nandave *et al*., (2009), evaluated cardio-protective effect of lyophilized hydro-alcoholic extract of *M. oleifera* in the isoproterenol (ISP)-induced model of myocardial infarction. They reported that chronic treatment with *M. oleifera* demonstrated mitigating effects on ISP-induced hemodynamic perturbations and favorable

modulation of the biochemical enzymes (superoxide dismutase, catalase, glutathione peroxidase, lactate dehydrogenase, and creatine kinase-MB).

2.5.4.7 Wound healing activity

Rathi *et al.,* (2006) investigated the aqueous extract of leaves of *M. oleifera* for its wound healing activity using resutured incision, excision, and dead space wound models in rats and concluded that the extract has significant wound healing property.

2.5.4.8 Hypotensive and spasmolytic activities

Faizi *et al*., (1998) investigated the hypotensive activity of the ethanol and aqueous extracts of *M. oleifera* whole pods and their parts, namely, coat, pulp, and seed. It was found that the ethyl acetate phase of the ethanol extract of pods was found to posses more hypotensive and spasmolytic activity which they attributed to the presence of methyl p-hydroxybenzoate, beta-sitosterol and p-hydroxybenzaldehyde in it.

2.5.4.9 Hypolipidaemic and anti-atherosclerotic activities

Chumark *et al*., (2008) investigated the hypolipidaemic and anti-atherosclerotic activities of *M. oleifera* leaf extract. They found that in hypercholesterol-fed rabbits, at 12 weeks of treatment, the water extract of the plant significantly lowered the cholesterol levels and reduced the atherosclerotic plaque formation to about 50% and 86%, respectively and these effects were at degrees comparable to those of simvastatin.

2.5.4.10 Antiurolithiatic activity

The effect of oral administration of aqueous and alcoholic extract of *M. oleifera* root-wood on calcium oxalate urolithiasis was studied in male Wistar albino rats by Karadi

et al., (2006). They reported that supplementation with aqueous and alcoholic extract of *M. oleifera* root-wood significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis.

2.5.4.11 Antiviral activity

Other Activities of the *Moringa* powder tested so far include Anti-HIV. This activity could be due to its immunostimulatory effect. Sudha *et al.*, (2010) reported that both low dose (25 mg/kg, p.o.) as well as high dose (750 mg/kg, p.o.) of *M. oleifera* stimulated immune system by acting through cellular and humoral immunity in experimental models of immunity in animals.

2.5.4.12 Interactions involving Moringa oleifera

A study by Taesotikul *et al.*, (2010) revealed that ethanol and aqueous extracts of *M oleifera* inhibited CYPIA2, CYP2D6, CYP2El and CYP3A4 activities in a dose-dependent manner. This suggests the possibility of potential interactions of *M .oleifera* with drugs that are metabolized by these enzymes.

2.6. Animal Models for Experimental Diabetes

Using animal models for diabetes studies presents many advantages. Many aspects of diabetes such as the etiology and its pathogenesis can be easily understood using animal models. The use of animal models is also important in the screening and development of new anti- diabetic agents. There are however, a number of limitations in the use of animals for diabetes research which include but not limited to expensiveness, practical difficulties and ethical considerations.

Experimental diabetes can be induced in animals chemically, by surgical removal of part of the pancreas, viral induction and genetic manipulation (Gehard *et al*., 2001).

2.6.1 Diabetogenic chemicals

Streptozotocin and alloxan are the most commonly used diabetogenic chemicals. In inducing experimental diabetes, the species, strain, sex and diet of the animals must be put into consideration as there exists variations in susceptibility.. The type of diabetes induced is a function of the amount of the chemical used.

2.6.1.1 Alloxan

Alloxan is also known as mesoxalyuria, mesoxalylcarbamide and pyrimdinetertrone. It was discovered by Dunn *et al.,* in 1943 to possess diabetogenic activity and was confirmed by Goldner and Gomori in the same year (Grollman *et al.,* 1970).

Alloxan exists in a tetrahydrate form but can easily be converted to a monohydrate by drying over sulphuric acid. The monohydrate form is mostly used in diabetic studies. Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cell in the pancreas when administered to rodents and diabetes mellitus (called "Alloxan diabetes"). In those animals, with characteristics similar to type 1 diabetes in humans, Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT 2 glucose transporter, Alloxan in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cells toxic action of Alloxan is initiated by free radicals formed in this redox reaction (Bowmann *et al.,* 1980).

Apart from guinea pigs, all other experimental animals are susceptible to alloxan when administered orally, intravenously, intramuscularly and subcutaneously. Intraperitoneal and intrapulmonary routes are also effective. Depending on the dose and the species injected, the induced diabetes is either reversible or irreversible (Ghosh *et al.*, 2005).

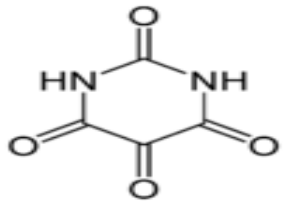


Figure 2.1: Structural Formula of Alloxan

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Fresh leaves of *Moringa oleifera* were collected in Samaru, Sabon Gari Local Government Area of Kaduna State, Nigeria in April, 2013. The botanical identity was confirmed by Mallam Mohammed Musa at the Herbarium Section of the Department of Biological Science, Ahmadu Bello University, Zaria where a voucher specimen (Voucher No 571) has been deposited for future reference.

3.1.2 Animals

Healthy Wistar rats of either sex weighing 100-150 g, purchased from the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, of the National Institute for Pharmaceutical Research and Development, NIPRD, Idu, Abuja, Nigeria were used for the study. They were maintained in stainless steel cages and maintained on clean water and pelleted rat feed. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

3.1.3 Chemicals and drugs

The following drugs and chemicals were used in the experiment

(i) Metformin 500mg tabs (Glucophage) manufactured by Merke Sante s.a.s France, Batch No 102682, Manufacturing date: 10/2011, Expiry date: 10/2016 were purchased from Newlife Pharmacy, Wuse Zone 2, Abuja

(ii) Alloxan monohydrate (powder) and absolute ethanol (99.5% $^{V/}_{V}$) (Sigma Aldrich, St. Loius USA).

Sundry consumables and other materials

(i) Animal cages and animal feeds (Ladokun feeds Limited Ibadan)

(ii) Measuring cylinders

(iii) Scissors, cotton wool, gloves and antiseptics

(iv) Normal saline and distilled water

(v) Disposable syringes and needles (1ml, 2mls, 5mls, and 10mls).

3.2 Methods

3.2.1 Preparation of extract

The extraction was carried out in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Reasearch and Development (NIPRD), Abuja. Fresh leaves of *Moringa oleifera* were cleaned with distilled water to remove adulterant, shade-dried to a constant weight and pulverized with a mortar and pestle. Eight hundred grammes (800 g) of the pulverized leaves was macerated with 99.5% ethanol for 24 hours and filtered with Whatman filter paper (size No 1) to obtain the extract. The extract was evaporated to dryness over a water bath maintained at 40^{o}c and weighed. The percentage yield of the extract was calculated as follows:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of pulverized leaves (g)}} \times 100$$

The extract was then stored in a refrigerator until required for use. The extract will henceforth be refered to as MOE.

3.2.2 Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method described by Lorke (1983). The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given MOE at doses of 10, 100, and 1000 mg/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included but were not limited to paw-licking, stretching, respiratory distress and mortality for the 24h. Based on the results of the initial phase, three (3) other rats were administered 1600, 2900 and 5000 mg/kg MOE respectively. The rats were also monitored closely for the 24 h after treatment for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the $LD_{50.}$ The LD_{50} was calculated as the geometric means of the maximum dose producing 0 % mortality (*a*) and the minimum dose that produced 100 % mortality (*b*) and mathematically expressed as:

$$LD_{50} = \sqrt{ab}$$

3.2.3 Induction of hyperglycaemia

The method described by Dunn and McLetchie (1943) was adopted in both the dose-response and pharmacodynamic interaction studies. Experimental rats were fasted overnight; freshly prepared alloxan (150 mg/kg body weight) was administered intraperitoneally. The animals were thereafter allowed food and water *ad libitum*. The day which alloxan was administered was considered as day 1. After 4 days (day 5), the blood glucose levels in the rats were determined following an overnight fast, using a Fine Test® digital glucometer and the corresponding test strip. Rats having fasting blood glucose (FBS) level $\geq$ 200mg/ dl were considered hyperglycaemic.

3.2.4 Dose-response study and estimation of ED_{50}

Fourty-eight (48) rats having FBS level ≥ 200 mg/dl, were randomized into 8 groups of 6 rats each. The rats in group 1 served as diabetic control group and were administed distilled water orally, groups 2, 3,4,5,6 and 7 were administered 100, 200, 400, 800, 1000 and 2000 mg extract/kg body weight respectively while group 8 rats received metformin at a dose of 150 mg/kg body weight. The dose selection was based on the LD_{50} value of the extract.

The post-treatment blood glucose concentration (BGC) was measured hourly for 4h and percentage glycaemic reduction (PGR) at each hour by each dose level was calculated as follows:

$$\text{PGR (\%)} = \frac{\text{Mean FBS of control} - \text{Mean BGC of treated group}}{\text{Mean FBS of control}} \times 100$$

A plot of percentage glyceamic reduction at 4h versus log dose was used to estimate the ED_{50} (the dose required for 50% glycaemic reduction in diabetic rats) of the extract.

3.2.5 Pharmacological interaction studies

3.2.5.1 Experimental design

The interaction study was carried out in hyperglycaemic and normoglycaemic groups of rats. In the hyperglycaemic group, hyperglycaemia was induced in 80 rats on day 1 using the method described by Dunn and McLetchie, (1943). While for the normoglycaemic group, rats having FBS of 50-90 mg/dl were considered normoglycaemic and used for the experiment.

3.2.5.2 Treatment

Hyperglycaemic group:

Fourty- eight (48) hyperglycaemic rats were randomized into 8 groups of 6 rats each. Group 1 served as the diabetic control group and received 5ml distilled water/kg body weight. Rats in groups 2, 3 and 4 were treated with 375, 750 and 1500 mg/kg MOE respectively corresponding to ½ ED_{50}, ED_{50} and $2ED_{50}$ of the extract. Rats in groups 5, 6 and 7 were treated with these different doses (375, 750 and 1500 mg/kg respectively) in combination with a fixed dose (150 mg/kg body weight) of metformin. Rats in group 8 were treated with 150 mg metformin/ kg body weight only.

Normoglycaemic group:

Twenty- four normoglycaemic rats were randomized into 4 groups of 6 animals each. Group 1 served as control and received 5ml distilled water/kg body weight daily while rats in groups 2, 3 and 4 were treated with 375, 750 and 1500 mg extract/kg body weight.

Metformin Hydrochloride solution was usually prepared fresh for each day's experiment to ensure stability. Treatment was carried out daily for 28 days and both the extract and metformin hydrochloride solutions were freshly prepared on each day of the experiment to ensure stability.

Route of administration:

The extract of *Moringa oleifera* leaves and metformin were administered orally throughout the study.

3.2.5.3 Weekly fasting blood sugar (FBS) estimation

FBS was estimated on day 0 (i.e day 4 post induction for the hyperlycaemic sub-groups) and subsequently on days 7, 14, 21 and 28. Blood samples were collected from the tail vein with a sterile scissors and tested using the digital glucometer and its test strips.

3.2.5.4 Weekly food and water intake

The quantity of food and water consumed by rats in each group was measured daily as the difference between the quantity of feed and water supplied and the quantity remaining after 24 hours respectivey and a weekly average determined.

3.2.5.5 Weekly body weight change

Rats in all groups were weighed weekly during the period of treatment and on the day of sacrifice for changes in body weight.

3.2.5.6 Serum biochemical analysis

On the 28th day of the experiment, all the rats were euthanized by chloroform inhalation and blood samples were collected by cardiac puncture. The blood was collected into plain serum tubes, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4 ^{o}C and used for evaluation of biochemical parameters, (aspartate transaminase (AST), alanine transaminase (ALT) levels, alkaline phosphatase (ALP) levels, total protein, albumin, total cholesterol, HDL-Cholesterol, LDL-Cholesterol, triglyceride, total bilirubin (TBIL), serum electrolytes (Na, K, HCO_3 and Cl^-), serum urea and creatinine levels.

3.2.5.7 Relative organ weight ratio

Major organs such as the liver, kidneys, heart, brain, lungs, spleen and stomach were removed, rinsed in normal saline, blotted dry and weighed. The relative organ-body weight ratio (ROW) for each rat was calculated as follows

$$ROW = \frac{\text{Absolute Organ Weight}}{\text{Body Weight of Rat on Sacrifice day (g)}} \times 100$$

3.2.5.8 Histopathological studies

The excised organs were fixed in 10% formal saline for about 48hours. Tissue slices of 3 to 4 mm thick were cut and kept in the autonomic tissue processor where they were fixed in 10% formal-saline saline solutions for 2 hours. They were then dehydrated for 2 hours. They were then dehydrated for 2hours in each of the following ascending grades of alcohol: 85%, 90%, and 100% $^{V}/_{V}$. the dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections were dehydrated in xylene and rehydrated in the following grades of alcohol 100%, 90%, and 70% $^{V}/_{V}$. They were then stained in haemotoxylin for about 5 minutes, differentiated in 1% acid alcohol, blotted in scotch's tap water and stained in eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100%; finally they were cleared in xylene and mounted in a pox. The slides were then examined microscopically for pathological lesions. The lesions observed were assessed for the following: mucosal atrophy, the presence of inflammatory cells in the wall, eosinopl, lymphocytes and plasma cells. Photomicrographs of representative lesions were taken at various magnifications.

3.2.5.9 Statistical analysis

All data were expressed as Mean ± SEM and statistical differences between means were determined by one- way ANOVA followed by Dunnett's *post –hoc* test for multiple comparison tests using GraphPad version 5.0. *P-values* less than 0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of the Extract

The weight of extract = 96.67 g

The weight of pulverised plant = 800 g

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of pulverized leaves (g)}} \times 100$$

$$= \frac{96.67\ \text{g}}{800\ \text{g}} \times 100$$

$$= 12.07\%$$

The yield of the ethanol extract was calculated to be 12.07%$^{w}/_{w.}$

4.2 Acute Toxicity Study

In both phases of the experiment, the ethanol extract of *Moringa oleifera* leaves did not produce any sign of toxicity or mortality at the doses administered orally (Table 4.1). The oral median lethal dose (LD_{50}) of the extract was therefore estimated to be greater than 5000 mg/kg body weight in rats (according to Lorke's method).

Table 4.1: Observed Effects of Ethanol Extract of *Moringa oleifera* Leaves on Rats in Acute Toxity Study

Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
1	MOE 10	0/3	-
2	MOE 100	0/3	-
3	MOE 1000	0/3	-
4	MOE 1600	0/1	-
5	MOE2900	0/1	-
6	MOE 5000	0/1	-

MOE: *M. oleifera* ethanol leave extract, D/T: number of deaths/number of rats treated, - : Absence

4.3 Dose -Response Study

Treatment of hyperglycaemic Wistar rats with graded doses of MOE, dose and time-dependently reduced fasting blood glucose (FBS) when compared to the control (Table 4.2). At 400 mg/kg, MOE showed a statistically significant ($p<0.05$) reduction in FBS at 3h (29.71%) and 4h (41.88%). At 800 mg/kg, MOE showed significant ($p< 0.001$) decrease in FBS at 3h (52.63%) and 4h (55.13%). Statistically significant ($P<0.01$, 0.001) decreases in FBS was observed for the 1000 mg/kg extract group at 2h (37.76 %), 3h (60.53 %) and 4h (61.25 %). A significant ($p< 0.01$,0.001), decrease in FBS was also observed for 2000 mg/kg extract at 2h (42.34%) 3h (66.38%) and 4h (65.63%). Metformin at a dose of 150mg/kg showed a significant ($p< 0.001$) decrease in FBS at 3h (55.66%) and 4h (53.84%) when compared to the control.

From the plot of percentage glycemic reduction at 4h versus log dose (Fig.4.1), the ED_{50} value of MOE was estimated to be approximately 750 mg/kg.

Table 4.2: Effect of MOE and Metformin on Fasting Blood Sugar of Alloxan-induced Hyperglycaemic Rats at Different time intervals

	Post Treatment Time in hours (h)				
Treatment (mg/kg)	**0**	**1**	**2**	**3**	**4**
Control	529.3±48.02	403.5±39.62	393.0±29.90	456.0±48.41	431.5±36.49
MOE 100	501.8±54.20	499.8±24.74 (-23.87%)	471.0±23.67 (-19.84%)	415.8±15.20 (8.82%)	349.7±42.62 (18.96%)
MOE 200	454.2±63.44	428.3±28.72 (-6.14%)	387.2±27.56 (1.48%)	361.3±18.44 (20.77%)	297.3±3.97* (31.56%)
MOE 400	474.2±55.54	497.04±45.09 (-23.18%)	393.3±46.53 (-0.08%)	320.5±19.14* (29.71%)	250.8±57.97** (41.88%)
MOE 800	486.5±38.08	391.7±35.20 (2.92%)	242.2±25.28**^{c} (38.37%)	216.0±21.59***^{c} (52.63%)	193.6±15.92***^{c} (55.13%)
MOE 1000	469.2±46.88	335.0±24.98^{a} (16.98%)	209.7±14.89**^{c} (46.64%)	108.0±16.79***^{c} (60.53%)	167.2±18.10***^{c} (61.25%)
MOE 2000	454.7±33.70	335.8±43.02^{a} (16.78%)	215.0±16.76**^{c} (45.29%)	153.3±8.54***^{c} (66.38%)	148.3±8.79***^{c} (65.63%)
Metformin 150	457.2±49.87	364.5±33.67 (9.67%)	231.7±47.44**^{b} (41.04%)	202.2±38.69***^{c} (55.66%)	199.2±34.00***^{c} (53.84%)

Data represented as mean ± S.E.M (n=6), analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons.

*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, statistically significant reduction in FBS compared with the control and $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, statistically significant reduction in FBS compared with the baseline values respectively.

Values in paranthesis represent percentage glycaemic reduction at the various hours

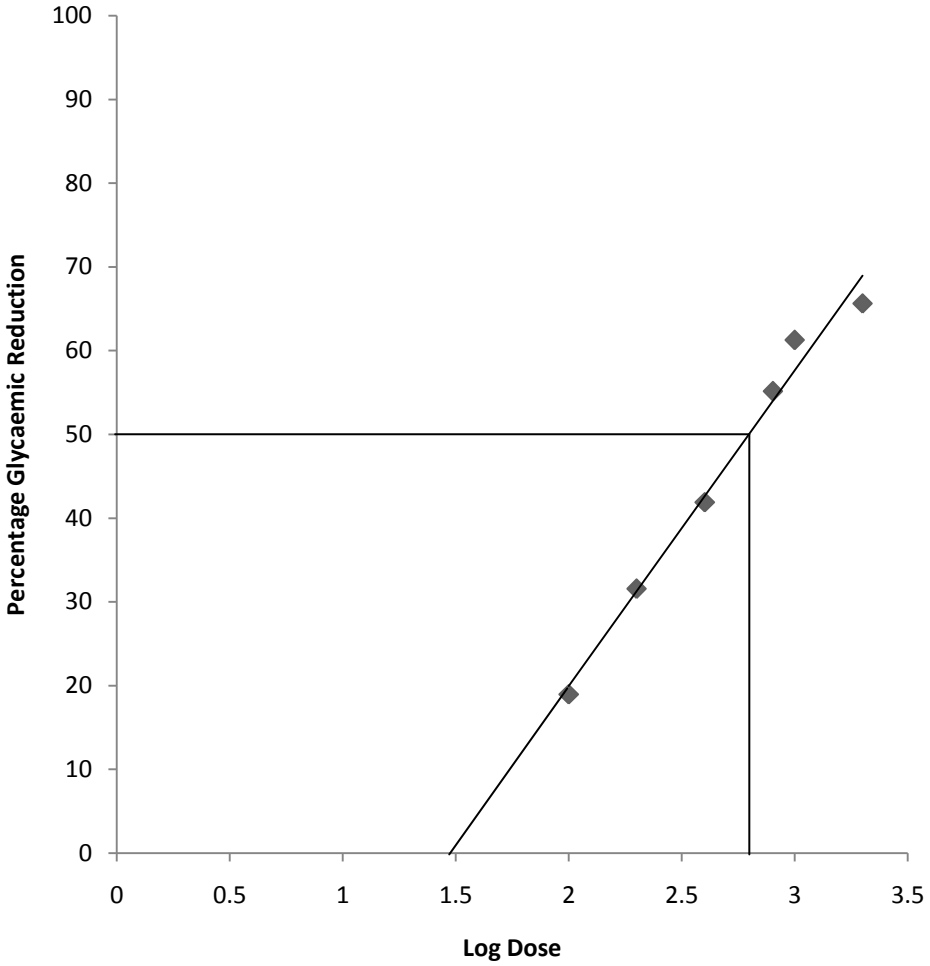


Figure 4.1: 4 h post-treatment dose-response curve of ethanol extract of *Moringa oleifera* leaves

50% glycaemic reduction (ED_{50}) corresponds to 2.8 on the log-dose axis,

Thus, ED_{50}= Anti-log of 2.8= 750

ED_{50} of Ethanol Extract of *Moringa oleifera* Leaves is 750 mg/kg.

4.4: Effect of MOE, Metformin and MOE/Metformin Co-administration on FBS of Rats

The FBS of the various hyperglycaemic groups are presented in Table 4.3. The MOE (375 mg/kg) treated group showed a significant ($p< 0.05$) reduction in FBS only on day 28 when compared to the control group. On days 21 and 28, the MOE (750 mg/kg) treated group showed a statistically significant ($p<0.01$) reduction in FBS when compared to the control group. The MOE (1500 mg/kg) treated group showed a statistically significant reduction in FBS on day 7 ($p<0.05$) days 14, 21 and 28 ($p<0.01$) respectively when compared to the control group.

The co-administration of the extract MOE and Metformin (375 mg/kg MOE/Metformin and 750 mg/kg MOE/Metformin) showed significant reduction in FBS on day 7 ($p<0.05$), on days 14 and 21($p< 0.01$) respectively and on day 28 ($p< 0.001$) when compared to the control group. The 1500 mg/kg MOE/Metformin treated group however showed a significant ($p< 0.001$) reduction on days 7, 14, 21 and 28.

Metformin (150 mg/kg) showed significant ($p<0.05$), ($p< 0.01$) reduction on day 14 and days 21 and 28 when compared to the control respectively.

When compared to metformin, the 375 mg/kg MOE/metformin treated group showed significant ($p<0.001$) reduction in FBS on day 28. On days 14 and 21, the 750 mg/kg MOE/metformin treated group showed a significant ($p<0.05$) in FBS and on day 28, a significant ($p<0.001$) reduction. The 1500 mg/kg MOE/metformin showed significant ($p<0.05$) reduction in FBS on days 7 and a significant ($p< 0.01$), ($p< 0.001$) reduction on days 14, 21 and 28 respectively.

The normoglycaemic study did not show any statistically significant reduction in FBS when compared to either the control or to the baseline FBS of the respective groups (Table 4.4).

Table 4.3: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Fasting Blood Sugar (FBS) (mg/kg) of Hyperglycaemic Rats

	Post- Treatment Time in days (d)				
Treatment Mg/kg	**0**	**7**	**14**	**21**	**28**
Control	454.4±67.21	517.6±43.91	539.6±13.07	517.6±25.28	487.4±27.112
MOE 375	448.0±55.49	445.5±57.19	417.5±56.00	368.5±47.98	316.2±60.14*****
MOE 750	415.0±56.17	356.5±49.78	360.0±56.00	281.2±50.23**	265.0±51.61******
MOE 1500	433.8±61.75	307.0±47.57*	288.8±51.25**	284.5±45.31**	244.3±50.94******
MOE 375+ Met	430.2±60.56	303.0±53.55*	288.7±59.35**	251.7±53.36***	132.3±19.01*****[b]**
MOE 750+Met	435.7±59.45	283.0±45.53*	268.8±47.21**[a]	200.6±37.98***[a]	112.8±13.74*****[b]**
MOE 1500+Met	423.2±65.87	198.0±14.59***[a]	156.7±12.45***[b]	119.5±8.84***[c]	96.33±5.57*****[c]**
Met 150	416.3±57.81	373.5±53.17	308.7±54.79*	291.2±47.13**	257.0±47.81******

Data represented as mean ± S.E.M (n=6), data was analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons.

*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, statistically significant reduction in FBS compared with the control.

[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, statistically significant change in FBS by MOE/metformin co-administered groups compared with metformin

Table 4.4: Effect of MOE administration on Fasting Blood Sugar (FBS) (mg/kg) of Normoglycaemic Rats

	Post-Treatment Time in Days (d)				
Treatment (mg/kg)	**0**	**7**	**14**	**21**	**28**
Control	84.80±5.60	72.40±4.42	76.40±3.41	79.20±2.67	73.80±2.44
MOE 375	74.38±4.98	78.20±7.59	74.80±6.40	81.20±2.69	78.80±2.71
MOE 750	83.80±6.27.	76.80±2.85	75.60±5.56	79.00±0.95	76.60±2.29
MOE 1500	76.80±4.95	72.20±4.28	79.60±4.86	76.80±3.61	81.40±3.11

Values represented as Mean±SEM of FBS in (mg/dl), n= 6

4.5. Effect of MOE, Metforminand MOE/Metformin Co-administration on Weekly Food consumption of Rats

There was a reduction in the average food consumption of the rats in both the hyperglycaemic and normoglycaemic groups when compared to the control.

For the hyperglycaemic groups, in weeks 3 and 4, the MOE (375 mg/kg) treated group showed a statistically significant ($p<0.01$) reduction in food consumption when compared to the control group. The MOE (750 mg/kg) treated group showed significant ($p< 0.05$) reduction in food consumption in week 2 and significant ($p< 0.001$) reductions in weeks 3 and 4 when compared to the control group. The MOE (1500 mg/kg) treated group showed significant ($p< 0.001$) reductions in weeks 2, 3 and 4 when compared to the control.

The 375 mg/kg MOE/Metformin treated group showed significant ($p< 0.001$) reduction in weeks 2, 3 and 4. The 750 mg/kg MOE/Metformin, 1500 mg/kg MOE/Metformin and metformin-treated groups showed significant reduction in food consumption in week 1 ($p<0.05$), weeks 2, 3 and 4 ($p<0.001$) when compared to the control.

When compared to metformin, the 750 and 1500 mg/kg MOE/metformin treated groups showed significant ($p<0.001$) reduction in food consumption in week 2.

There was no significant difference in the mean food consumption by the normoglycaemic rats administered MOE when compared to the control (Table 4.6).

Table 4.5: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Weekly Food Consumption (g) in Alloxan-induced Hyperglycaemic Rats

	Post- treatment time in weeks (wk)			
Treatment (mg/kg)	**1**	**2**	**3**	**4**
Control	160.3±09.12	192.5±04.61	200.7±04.79	215.7±03.38
MOE 375	163.0±05.62	186.8±04.29	169.3±09.84**	149.0±06.60***
MOE 750	165.0±02.52	177.8±02.86*	163.3±06.00***	160.3±16.67***
MOE 1500	138.2±04.47	164.0±03.86***	152.8±03.07***	132.5±03.97***
MOE 375 + Met	153.2±16.08	154.5±04.54***	146.0±03.94***	136.7±05.31***
MOE 750 +Met	126.7±04.76*	125.0±01.59*** [c]	137.5±05.79***	123.2±02.01***
MOE 1500 +Met	123.8±10.57*	115.8±02.10*** [c]	119.2±05.21***	120.3±02.32***
Met 150	121.3±06.35*	157.8±02.04***	128.8±05.79***	131.3±04.07***

Data represented as mean ± S.E.M of quantity of feed in (g), analysed by one-way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons. (n=6)

Statistically significant *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the control

[a]$p< 0.05$, [b]$p <0.01$, [c]$p< 0.001$, statistically significant change in weekly food consumption by MOE/metformin co-administered groups compared with metformin

Table 4.6: Effect of MOE administration on Weekly Food Consumption (g) in Normoglycaemic Rats

	Post- treatment time in weeks (wk)			
Treatment (mg/kg)	**1**	**2**	**3**	**4**
Control	131.5±13.63	115.3±02.88	119.5±02.75	118.7±02.22
MOE 375	122.8±07.33	120.0±02.68	114.5±01.80	120.8±03.95
MOE 750	122.5±05.78.	118.0±04.36	125.5±03.92	119.7±03.64
MOE 1500	130.7±10.68	124.2±04.79	122.0±04.27	120.0±01.79

Values represented as Mean±SEM, n= 6

4.6: Effect of MOE, Metformin and MOE/Metformin Co-administration on Weekly Water intake of Rats

The mean water intake of hyperglycaemic groups is presented in Table 4.7. There was a reduction in the water intake in all the groups. At the three doses used, the MOE treated groups showed significant ($p< 0.001$) reduction in water intake in weeks 2, 3 and 4 when compared to the control group.

The 375 mg/kg MOE/Metformin and 750 mg/mg/kg MOE/Metformin treatment showed significant ($p<0.01$) reduction in water intake in week 1 and significant ($p< 0.001$) reduction in weeks 2, 3 and 4. The 1500 mg/kg MOE/Metformin and the metformin- treated group showed significant ($p<0.001$) reduction in water intake in week 1 through week 4.

When compared to metformin, the 750 mg/kg MOE/metformin treated group showed significant ($p<0.05$) reduction in water intake in week 2. In week 2 and 4, the 1500 mg/kg MOE/metformin treated group showed a significant ($p<0.01$) in water intake.

There was no significant difference in the water intake by the normoglycaemic groups administered MOE at graded doses when compared to the control (Table 4.8).

Table 4.7: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Weekly Water Intake in Alloxan-induced Hyperglycaemic Rats

	Post- treatment time in weeks (wk)			
Treatment (mg/kg)	**1**	**2**	**3**	**4**
Control	328.3±28.22	394.3±09.67	424.3±06.85	404.3±03.69
MOE 375	256.7±15.63	318.6±05.95***	265.7±10.20***	238.6±04.59***
MOE 750	255.0±15.44	281.4±09.11***	238.6±09.11***	222.9±05.65***
MOE 1500	271.7±29.60	255.7±05.28***	225.7±08.41***	200.0±04.88***
MOE 375 + Met	225.0±16.07**	242.9±07.14***	217.1±04.74***	174.3±09.48***
MOE 750 + Met	218.3±12.76**	214.3±07.19***[a]	201.4±05.53***	172.9±05.22***
MOE 1500 + Met	198.3±14.47***	184.3±02.02*** [b]	175.7±05.71***	140.0±03.78*** **[b]**
Met 150	203.0±22.61***	245.7±09.48***	192.9±05.65***	180.0±05.35***

Data represented as mean ± S.E.M of volume of water in (ml). Data was analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons. (n=6)

*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant reduction compared with the control

[a]p< 0.05, [b]p <0.01, [c]p< 0.001, statistically significant change in weekly water intake by MOE/metformin co-administered groups compared with metformin

Table 4.8: Effect of MOE administration on Weekly Water Intake in Normoglycaemic Rats

	Post-treatment time in weeks (wk)			
Treatment (mg/kg)	**1**	**2**	**3**	**4**
Control	168.3±10.14	165.7±06.12	164.3±05.71	158.6±05.95
MOE 375	170.0±06.33	152.9±06.80	158.6±09.62	151.4±04.59
MOE 750	170.0±10.95	164.3±05.28	155.7±08.69	145.7±02.97
MOE 1500	171.7±10.14	162.9±07.78	167.10±04.74	168.6±03.40

Values represented as Mean±SEM, n= 6

4.7: Effect of MOE, Metformin and MOE/Metformin Co-administration on Body Weight of Rats

There was no statistically significant difference in the mean body weight of rats in the hyperglycaemic (Table 4.9) and normoglycaemic groups, when compared with the controls (Table 4.10).

Table 4.9: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Average Body Weight (g) of Hyperglycaemic Rats

	Post-treatment time in days (d)				
Treatment (mg/kg)	**0**	**7**	**14**	**21**	**28**
Control	121.2±13.75	110.0±13.50	108.4±12.74	108.0±12.17	103.4±09.70
MOE 375	137.3±14.24	132.2±13.76	134.2±14.10	138.2±13.11	147.5±12.13
MOE 750	125.5±12.91	116.8±13.45	120.3±13.49	124.8±14.45	131.2±13.13
MOE 1500	115.8±13.46	111.7±12.47	117.5±13.42	120.0±13.84	121.5±13.37
MOE 375 + Met	139.0±15.27	128.3±10.91	133.3±10.82	137.5±10.25	145.0±10.75
MOE 750 +Met	125.8±12.67	114.8±14.75	120.0±16.10	121.2±16.15	126.8±15.92
MOE 1500 +Met	138.7±12.99	127.3±14.11	132.7±13.14	136.5±13.56	144.8±13.18
Met 150	130.0±57.81	119.0±5.62	121.3±5.03	126.5±4.92	131.8±5.45

No statistically significant difference between treated groups and control

Table 4.10: Effect of MOE administration on Average Body Weight (g) of Normoglycaemic Rats

	Post-treatment time in days (d)				
Treatment (mg/kg)	**0**	**7**	**14**	**21**	**28**
Control	133.5±14.26	149.0±17.18	151.2±15.55	152.6±02.67	155.4±16.91
MOE 375	104.4±04.07	108.4±04.23	118.6±05.30	127.2±08.17	133.0±07.93
MOE 750	113.6±12.09.	114.4±12.11	127.8±13.89	137.4±11.65	143.2±10.54
MOE 1500	113.4±15.56	114.6±18.75	121.20±19.07	132.4±16.97	140.8±17.62

Values represented as Mean±SEM, n= 6

4.8: Effect of MOE, Metformin and MOE /Metformin Co-administration on the Lipid and Protein Profiles of Rats

The MOE (375 mg/kg) treated group showed a statistically significant ($p<0.05$) reduction only in LDL-C compared to the control. The MOE (750 mg/kg) treated group showed significant ($p< 0.05$) reduction in total serum protein and albumin levels when compared to the control. This dose also showed significant ($p< 0.01$) reduction in total cholesterol (144.3 mg/dl) compared to the control (157.4 mg/dl) while the MOE (1500 mg/kg) treated group showed significant ($p<0.001$) reduction in total protein, albumin, total cholesterol, HDL-C, LDL-C and TAG levels as compared to the control.

The 375 mg/kg MOE/Metformin, 750 mg/kg/Metformin and 1500 mg/kg MOE/Metformin and Metformin- treated group showed significant ($p<0.001$) reduction in total protein, albumin, total cholesterol, LDL-C and TAG levels with a corresponding increase in HDL-C levels (Table 4.11).

When compared to metformin, the 375 mg/kg MOE/metformin treated group showed significant ($p<0.001$) reduction in LDL-C. The 750 mg/kg MOE/metformin treated group showed a significant ($p<0.05$) increase in total protein and HDL-C concentration and a significant ($p<0.001$) reduction in LDL-C level. The 1500 mg/kg MOE/metformin showed showed significant ($p<0.05$), ($p<0.01$) increase in HDL-C, total protein and albumin level respectively and a significant ($p<0.001$) reduction in LDL-C and a significant ($p< 0.05$) reduction in TAG level.

There was no statistically significant difference in the lipid and protein profiles of the extract- treated rats in the normoglycaemic groups when compared to the control (Table 4.12).

Table 4.11: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Lipid and Protein Profiles of Alloxan-induced Hyperglycaemic Rats

	Lipid and Protein Levels					
Group/Treatment (mg/kg)	**TP (g/dl)**	**ALB (g/dl)**	**CHOL (mg/dl)**	**HDLC (mg/dl)**	**LDLC (mg/dl)**	**TAG (mg/dl)**
Control	54.80±1.36	20.40±0.81	157.4±4.26	30.80±1.36	117.6±3.20	181.6±2.36
MOE 375	57.17±0.87	26.00±0.82	147.7±3.53	31.67±0.71	108.3±3.74*	168.7±10.61
MOE 750	62.00±2.79*	27.83±0.65*	144.3±3.33**	32.17±0.87	104.6±1.21**	157.0±2.36*
MOE 1500	72.83±1.35***	42.67±2.59***	83.00±2.03***	43.50±2.25***	14.0±1.27***	109.2±4.48***
MOE 375 + Met	70.17±1.35***	44.33±1.67***	83.83±1.15***	45.50±0.99***	15.40±0.87***[c]	83.00±2.32***[c]
MOE 750 +Met	78.40±1.94***[a]	40.60±1.66***	84.00±0.89***	51.60±2.06***[a]	14.0±1.58***[c]	68.20±1.66***[c]
MOE 1500 +Met	80.83±1.05***[a]	46.00±1.24***[b]	81.00±2.70***	51.33±2.40***[a]	10.80±1.16***[c]	34.67±1.61***[a]
Met 150	69.00±1.93***	41.67±1.52***	84.67±0.95***	46.83±1.42***	30.20±3.31***	56.00±1.03***

Data represented as mean ± S.E.M. Data was analysed by one-way ANOVA followed by Dunnett's *post– hoc* test. (n=6)
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically significant change in parameter compared with the control
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$), statistically significant change by MOE/metformin co-administered groups compared with metformin.

Table 4.12: Effect of MOE administration on Lipid and Protein Profiles of Normoglycaemic Rats

	Lipid and protein profiles					
Treatment (mg/kg)	**TP (g/dl)**	**ALB (g/dl)**	**CHOL (mg/dl)**	**HDLC (mg/dl)**	**LDLC (mg/dl)**	**TAG (mg/dl)**
Control	71.00±1.82	40.40±1.54	84.20±1.32	52.60±1.12	12.00±1.05	35.40±2.93
MOE 375	71.00±1.14	42.60±2.29	86.80±1.50	45.00±1.82	13.25±0.63	36.60±1.57
MOE 750	71.80±0.49	42.20±1.56	84.60±1.60	48.80±2.56	13.23±1.32	35.20±2.65
MOE 1500	72.80±1.53	42.00±2.21	84.20±1.50	45.20±2.62	12.80±1.07	31.20±1.46

Values represented as Mean±SEM, n= 6

4.9: Effect of MOE, Metformin and MOE/Metformin Co-administration on some Serum Electrolytes of Rats

The MOE (375 mg/kg) treated group showed a significant ($p<0.001$) reduction in chloride ions and an ($p<0.001$) increase in sodium, potassium compared to the control. The MOE (750 mg/kg) treated group showed a significant increase in bicarbonate ion ($p< 0.01$, in sodium and potassium ion level ($p< 0.001$) respectively when compared to the control. However, a significant ($p< 0.001$) decrease in chloride ion was observed. The MOE (1500 mg/kg) treated group showed significant ($p<0.001$) reduction in chloride ions and a significant ($p< 0.001$) increase in sodium, potassium and bicarbonate ions.

The 375 mg/kg MOE/Metformin, 750 mg/kgMOE/Metformin, 1500 mg/kg MOE/ Metformin) and metformin- treated group showed significant ($p<0.001$) reduction in chloride ions and a significant ($p< 0.001$) increase in sodium, potassium and bicarbonate ions (Table 4.13).

When compared to metformin, the MOE/metformin treated group showed no significant changes in serum electrolytes.

There was no significant difference in the serum electrolytes of the rats in the treated normoglycaemic groups when compared to the controls (Table 4.14).

Table 4.13: Effect MOE, Metformin (Met) and MOE/Metformin Co-administration on some Serum Electrolytes of hyperglycaemic Rats

	Serum Electrolytes (mmol/L).			
Treatment (mg/kg)	**Na^+**	**K^+**	**Cl^-**	**HCO_3^-**
Control	130.6±0.81	3.06±0.18	109.2±2.50	17.80±0.37
MOE 375	145.0±1.39***	5.13±0.22***	97.0±1.16***	19.67±0.49
MOE 750	144.7±0.80***	4.97±0.18***	99.3±1.26***	21.17±0.70**
MOE 1500	145.2±1.42***	5.40±0.19***	99.0±1.18***	22.83±0.79***
MOE 375 + Met	146.5±0.85***	5.01±0.08***	98.8±1.14***	23.00±0.68***
MOE 750 +Met	144.8±1.24***	5.41±0.24***	99.4±1.23***	23.40±0.68***
MOE 1500 +Met	145.7±0.99***	5.13±0.35***	98.2±0.60***	23.33±0.56***
Met 150	143.3±1.52***	5.26±0.10***	97.5±0.81***	22.00±0.68***

Data represented as mean ± S.E.M of concentration of serum electrolytes. Data was analysed by one- way ANOVA followed by Dunnett's *post–hoc* test for multiple comparisons. (n=6)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically significant compared with the control.

No statistically significant difference between MOE/Metformin co-administered groups and metformin group

Table 4.14: Effect of MOE administration on some Serum Electrolytes of Normoglycaemic Rats

	Serum Electrolytes (mmol/L).			
Treatment (mg/kg)	**Na^+**	**K^+**	**Cl^-**	**HCO_3^-**
Control	145.4±1.83	5.42±0.29	97.0±1.58	21.6±1.07
MOE 375	145.2±0.58	5.20±0.29	97.8±0.73	21.4±1.12
MOE 750	146.6±0.68	5.44±0.19	99.8±0.86	21.4±0.60
MOE 1500	145.6±1.29	5.36±0.09	96.8±1.11	20.8±0.49

Values represented as Mean±SEM, n= 6

4.10: Effect of MOE, Metformin and MOE/Metformin Co-administration on Serum Creatinine and Urea levels of Rats

The MOE (375 mg/kg) treated group showed a significant ($p<0.01$) reduction in creatinine level when compared to the control. The MOE (750 mg/kg) treated group showed significant ($p< 0.05$) reduction in urea level (3.97mmol/L) when compared to the control (4.86 mmol/L) and a significant ($p<0.01$) reduction in serum creatinine level. The MOE (1500 mg/kg) treated group, showed a significant ($p<0.001$) reduction in urea level (2.76 mmol/L) and a significant ($p<0.01$) reduction in creatinine level when compared to the control.

The 375 mg/kg MOE/ Metformin, 750 mg/kg MOE/Metformin and 1500 mg/kg MOE /Metformin showed a significant ($p<0.001$) reduction in both creatinine and urea levels. The metformin- treated group showed a significant ($p< 0.01$) reduction in urea level (3.75 mmol/L) and a significant ($p<0.05$) reduction in serum creatinine level when compared to the control (Table 4.15).

When compared to metformin, the 375 and 1500 mg/kg MOE/metformin treated groups showed significant ($p<0.01$) reduction in serum creatinine and urea levels and the 750 mg/kg MOE/metformin treated group showed a significant ($p<0.05$) in serum creatinine and urea levels.

There was no significant difference in the treated normoglycaemic groups when compared to the control group (Table 4.16).

Table 4.15: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Serum Creatinine and Urea levels of Alloxan-induced Hyperglycaemic Rats

	Creatinine and Urea Levels	
Treatment (mg/kg)	**Creatinine (µmol/L)**	**Urea(mmol/L)**
Control	76.20±3.83	4.86±0.46
MOE 375	60.00±2.27**	4.37±0.18
MOE 750	58.00±4.41**	3.97±0.27*
MOE 1500	57.83±3.40**	2.76±0.08***
MOE 375+ Met	47.67±3.18***[b]	2.52±0.13***[b]
MOE 750+Met	53.40±3.54***[a]	2.74±0.16***[a]
MOE 1500+Met	49.67±2.97***[b]	2.60±0.18***[b]
Met 150	61.17±1.85*	3.75±0.21**

Data represented as mean ± S.E.M of concentration of creatinine and urea, (n=6).

Data was analysed by one-way ANOVA followed by Dunnett's *post-hoc* test for multiple comparisons.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically significant change in serum creatinine and urea compared with the control

[a]$p< 0.05$, [b]$p <0.01$, [c]$p< 0.001$, statistically significant change in serum creatinine and urea levels by MOE/metformin co-administered groups compared with metformin

Table 4.16: Effect of MOE, administration on Serum Creatinine and Urea levels of Normoglycaemic Rats

	Creatinine and Urea Levels	
Treatment (mg/kg)	**Creatinine (µmol/L)**	**Urea (mmol/L)**
Control	55.60±1.03	3.36±0.42
MOE 375	54.00±1.48	3.52±0.10
MOE 750	54.20±1.16	3.12±0.18
MOE 1500	51.60±0.60	3.36±0.15

Values represented as Mean±SEM of concentration of serum creatinine) and urea, n= 6

4.11: Effect of MOE, Metformin and MOE/Metformin co-administration on some Serum Biochemical Parameters of Rats

The MOE (750 mg/kg) treated group showed significant ($p< 0.05$) decrease in serum AST and ($p< 0.01$) decrease in ALT and total bilirubin when compared to the control. The MOE (1500 mg/kg) treated group showed a significant ($p<0.01$) reduction in AST and ALP and also a significant ($p<0.001$) decrease in ALT and Total bilirubin.

When compared to the control, the 375mg/kg MOE/Metformin, 750 mg/kg MOE/ Metformin and 1500 mg/kg MOE/Metformin treated groups showed significant ($p<0.001$) reduction in serum AST, ALT, ALP and Total bilirubin levels. The metformin treated group showed a significant ($p< 0.01$) decrease in serum AST, ALT, ALP and Total bilirubin levels (Table 4.16).

When compared to metformin, the MOE/metformin treated groups showed significant ($p<0.001$) reduction in serum AST, ALT, ALP and Total bilurubin levels.

There was no statistically significant difference in the serum AST, ALT, ALP and Total bilirubin levels of the rats in the treated normoglycaemic groups when compared to the control (Table 4.17).

Table 4.17: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on some Serum Biochemical Parameters of hyperglycaemic Rats

	Serum Biochemical Parameters			
Treatment (mg/kg)	**AST (µmol/L)**	**ALT (µmol/L)**	**ALP (µmol/L)**	**TBIL (mmol/L)**
Control	371.0±19.87	277.8±17.66	278.2±30.55	3.00±0.07
MOE 375	371.7±18.33	273.0±12.59	275.5±11.32	2.80±0.06
MOE 750	323.0±08.81*	229.3±10.22**	225.2±16.69	2.57±0.12**
MOE 1500	310.7±08.61**	157.7±07.29***	208.0±10.98**	1.70±0.11***
MOE 375+ Met	93.33±1.67***^{c}	88.0±03.68***^{c}	124.5±13.38***^{c}	1.17±0.03***^{c}
MOE 750 + Met	81.00±02.05***^{c}	62.0±04.80***^{c}	107.8±07.72***^{c}	1.08±0.08***^{c}
MOE 1500 +Met	35.85±1.58***^{c}	40.67±01.60***^{c}	98.67±02.35***^{c}	0.88±0.03***^{c}
Met 150	315.5±6.96**	222.2±10.83**	205.2±06.93**	2.62±0.06**

Data represented as mean ± S.E.M of concentration of serum AST, ALT, ALP and TBIL. Data was analysed by one- way ANOVA followed by Dunnett's *post– hoc* test for multiple comparisons. (n=6)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically significant compared with the control

$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, statistically significant change in serum biochemical parameters by MOE/metformin co-administered groups compared with metformin

Table 4.18: Effect of MOE administration on some Serum Biochemical Parameters of Normoglycaemic Rats

	Serum Biochemical Parameters			
Treatment (mg/kg)	**AST(µmol/L)**	**ALT(µmol/L)**	**ALP (µmol/L)**	**TBIL(mmol/L)**
Control	33.20±1.96	37.80±1.93	117.0±4.43	2.64±0.13
MOE 375	33.80±2.67	39.20±1.50	116.2±3.71	2.66±0.11
MOE 750	32.40±1.69	37.80±0.97	113.0±2.90	2.78±0.06
MOE 1500	32.00±1.92	36.20±1.74	114.4±4.43	2.76±0.07

Values represented as Mean±SEM, n= 6

4.12: Effect of MOE, Metformin and MOE/Metformin Co-administration on Relative Organ Weights (ROW) of Rats

The mean relative weights of organs of the hyperglycaemic groups are presented in Table 4.18. MOE (375 mg/kg and 750 mg/kg) treated groups showed a significant ($p< 0.05$) increase in the weights of liver and spleen when compared to the control. MOE (1500 mg/kg) treated group showed a significant) increase in the weights of liver ($p< 0.001$) and spleen ($p< 0.05$) respectively when compared to the control.

The 375 mg/kg MOE/Metformin, 750 mg/kg MOE/Metformin and 1500 mg/Metformin treated groups showed significant ($p< 0.001$), ($p<0.05$) increase in the weights of liver and spleen respectively when compared to the control while the 150 mg/kg Metformin treated group showed a statistically significant ($p< 0.05$) increase in liver weight and a significant ($p<0.01$) increase in the weight of spleen. The kidney, heart, brain, stomach and lungs of all the hyperglycaemic treated groups showed no statistically significant difference when compared to the control.

When compared to metformin, the 375, 750 and 1500 mg/kg MOE/metformin treated group showed significant ($p<0.05$) increase in the relative weight of spleen.

There was no significant difference in the mean relative organ weights of the normoglycaemic groups when compared to the control group (Table 4.19)

Table 4.19: Effect of MOE, Metformin (Met) and MOE/Metformin Co-administration on Relative Organ Weight (ROW) of Hyperglycaemic Rats

	Organ weights (g)						
Treatment (mg/kg)	**Liver**	**Kidney**	**Spleen**	**Heart**	**Brain**	**Stomach**	**Lungs**
Control	2.98±0.28	0.97±0.08	0.25±0.05	0.38±0.04	1.07±0.06	1.17±0.06	0.72±0.06
MOE 375	4.07±0.14*	0.87±0.03	0.43±0.05*	0.40±0.02	1.07±0.10	1.15±0.05	0.80±0.08
MOE 750	4.06±0.22*	0.94±0.06	0.45±0.04*	0.37±0.02	1.06±0.09	1.24±0.08	0.80±0.08
MOE 1500	4.39±0.37**	1.00±0.09	0.44±0.07*	0.38±0.02	0.96±0.09	1.26±0.06	0.85±0.05
MOE 375 + Met	4.65±0.22***	0.83±0.05	0.48±0.02*[a]	0.37±0.04	0.92±0.07	1.10±0.09	0.85±0.06
MOE 750 +Met	4.63±0.49***	1.04±0.14	0.49±0.05*[a]	0.49±0.09	0.91±0.12	1.29±0.14	0.86±0.09
MOE 1500 +Met	4.61±0.15***	0.93±0.07	0.48±0.07*[a]	0.39±0.01	0.92±13.56	1.24±0.10	0.77±0.04
Met 150	4.02±0.24*	0.91±0.09	0.51±0.06**	0.38±0.06	0.95±0.07	1.28±0.09	0.77±0.03

Data represented as mean ± S.E.M and analysed by one- way ANOVA followed by Dunnett's *post –hoc* test for multiple comparisons, n=6.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Statistically significant change compared with the control.
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$), statistically significant change in ROW by MOE/metformin co-administered groups compared with metformin.

Table 4.20: Effect of MOE on Relative Organ Weight (ROW) of Normoglycaemic Rats

	Organ weights (g)						
Treatment	**Liver**	**Kidney**	**Spleen**	**Heart**	**Brain**	**Stomach**	**Lungs**
Control	4.72±0.24	0.98±0.15	0.40±0.04	0.38±0.06	0.91±0.09	1.14±0.14	0.96±0.20
MOE 375mg/kg	4.57±0.30	1.19±0.06	0.38±0.40	0.44±0.03	1.06±0.10	1.04±0.06	0.92±0.13
MOE 750mg/kg	4.07±0.32	1.19±0.03	0.39±0.03	0.38±0.03	0.94±0.08	1.01±0.08	0.75±0.04
MOE 1500mg/kg	3.90±0.15	1.04±0.03	0.35±0.03	0.39±0.02	1.07±0.10	1.10±0.05	0.80±0.04

Values represented as Mean ± SEM, n=6.

4.13. Histopathology of organs of hyperglycaemic and normoglycaemic rats after 28 days of MOE, Metformin and MOE/ Metformin oral administration

Following 28 days of drug administration, the effect of the MOE, metformin and MOE/metformin co-administration on the histological appearance of the selected organs was microscopically evaluated following Haematoxylin and Eosin stain.

The hyperglycaemic control rats showed necrosis of pancreatic acini and beta-cell degeneration after alloxan treatment. However, the rats in all the treated hyperglycaemic groups showed significant regeneration of beta-cells. For all the other organs studied, brain, heart, liver, lungs, kidney, spleen and stomach, no remarkable changes was seen in the hyperglycaemic control group and all the hyperglycaemic treated rats.

For all the normoglycaemic groups, there were no remarkable changes in all the organs of the rats at all the doses of the extract.

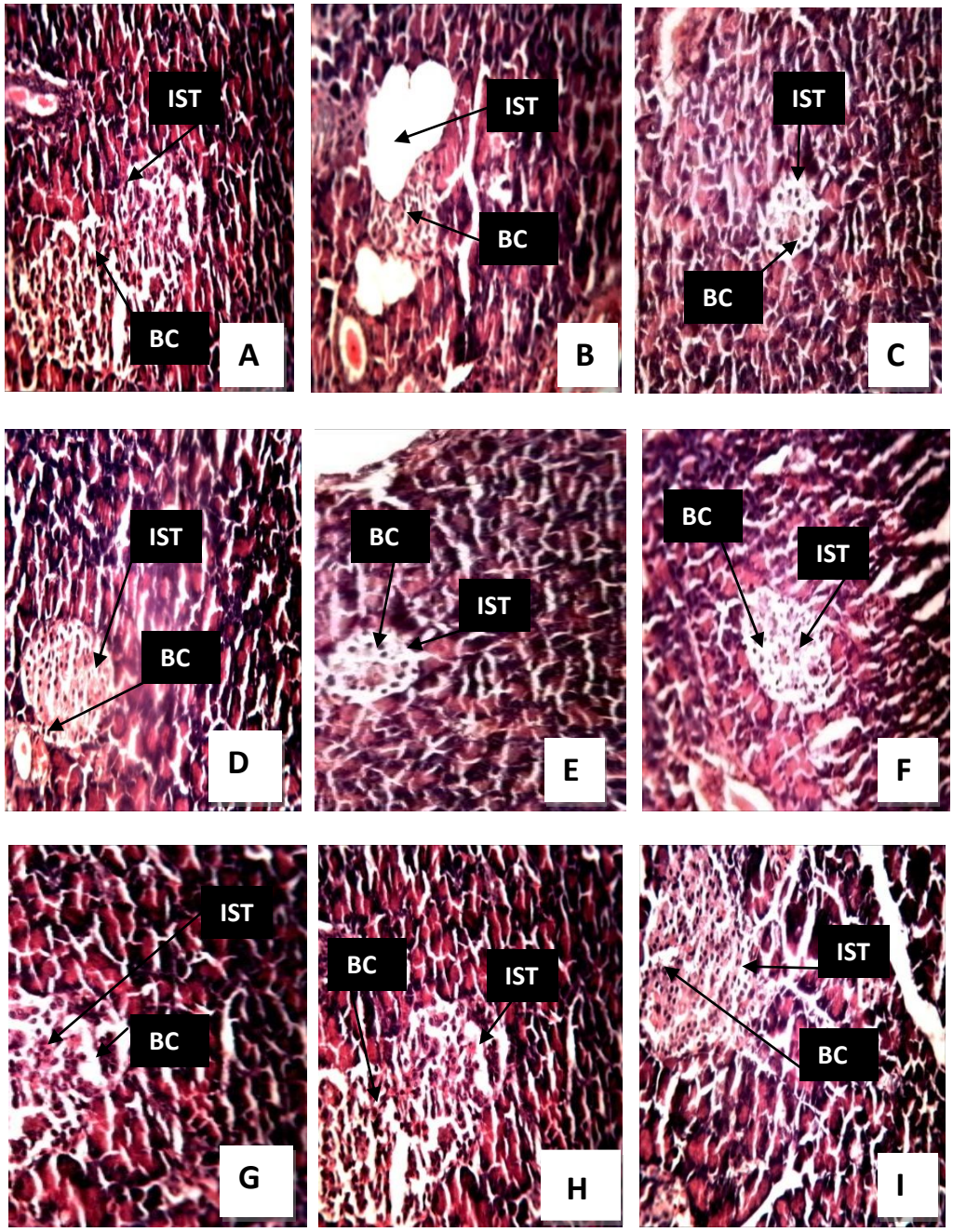


Plate III: Photomicrograph of a section of the pancreas of a (A) normoglycaemic control rat showing normal islets and beta-cell population (B) hyperglycaemic control rat showing extensive damage to the islets and reduced dimension of islets (C) metformin-treated rat showing scanty beta-cell regeneration (D), (E) and (F) a 375, 750 and 1500 mg/kg MOE treated-rat respectively showing gradual restoration of size of islets and beta-cells (G) (H) and (I) a 375, 750 and 1500 mg/kg MOE/metformin co-administered rat respectively showing better beta-cell restoration and islet size at 28 day post treatment (HE x250)
IST=Islet, BC=Beta-cells.

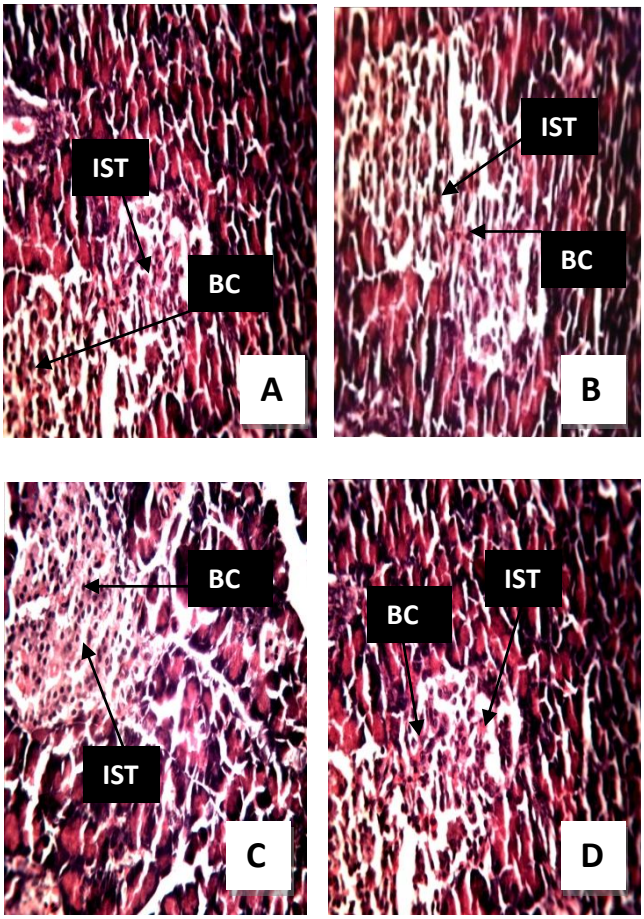


Plate IV: Photomicrograph of a section of the pancreas of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal islets and beta-cells at 28 day post treatment (HE x250)
IST=Islet, BC=Beta-cells

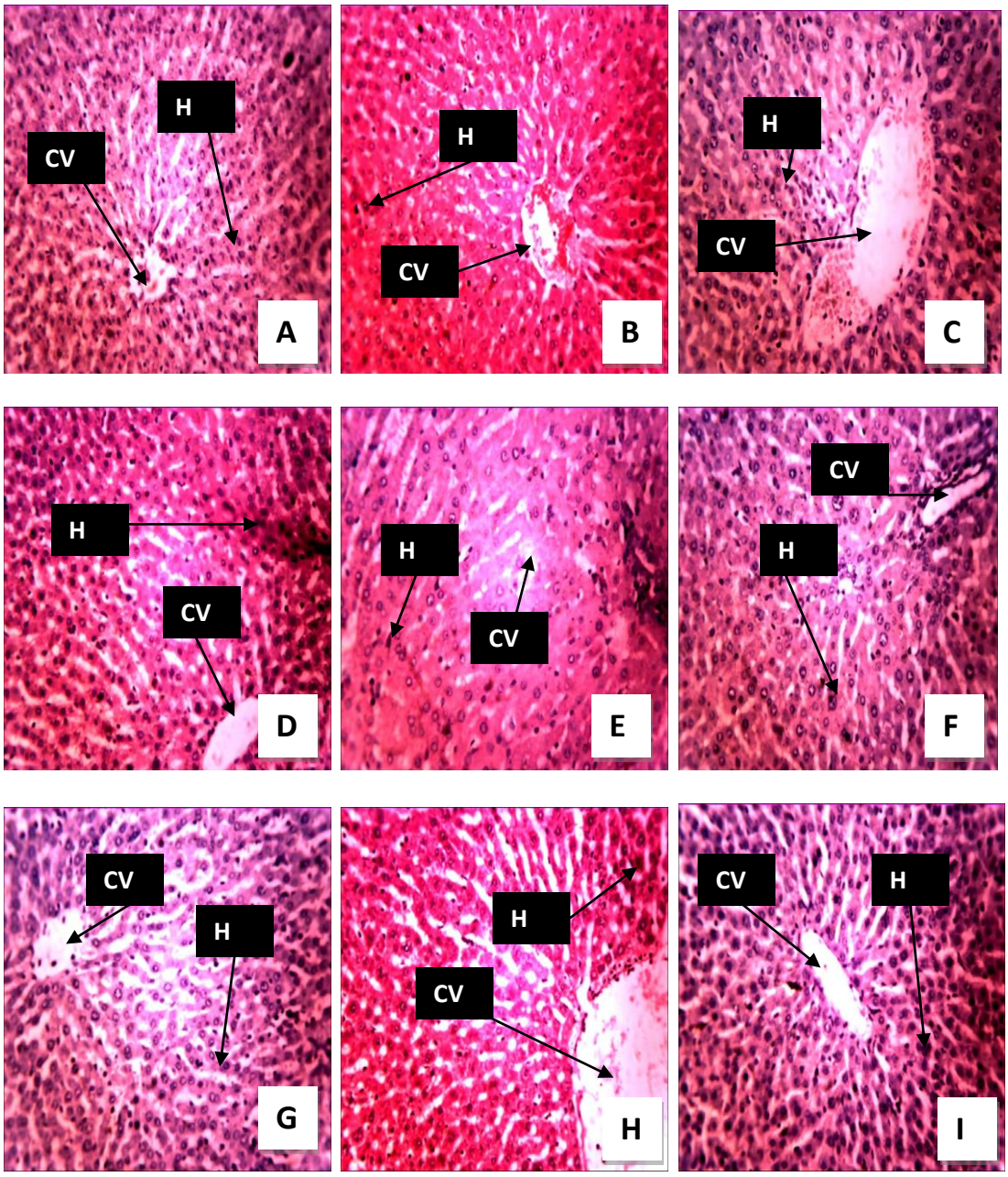


Plate V: Photomicrograph of a section of the liver of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered-hyperglycaemic rat respectively all showing normal lobular architecture at 28 day post treatment (HE x250)
CV= Central vein, H= Hepatocytes

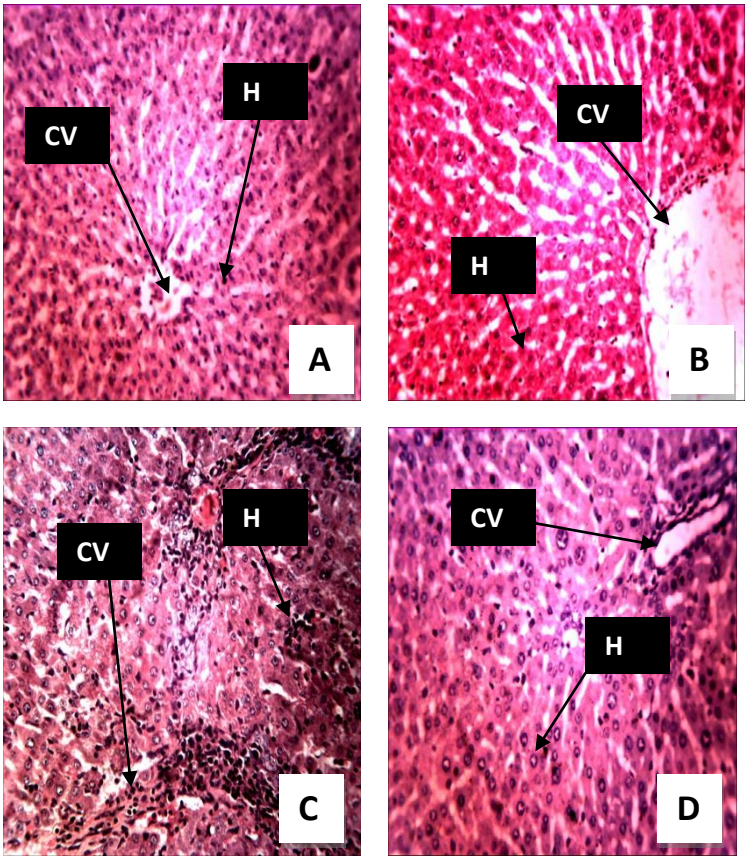


Plate VI: Photomicrograph of a section of the liver of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal lobular architecture at 28 day post treatment (HE x250)
CV= Central vein, H= Hepatocytes

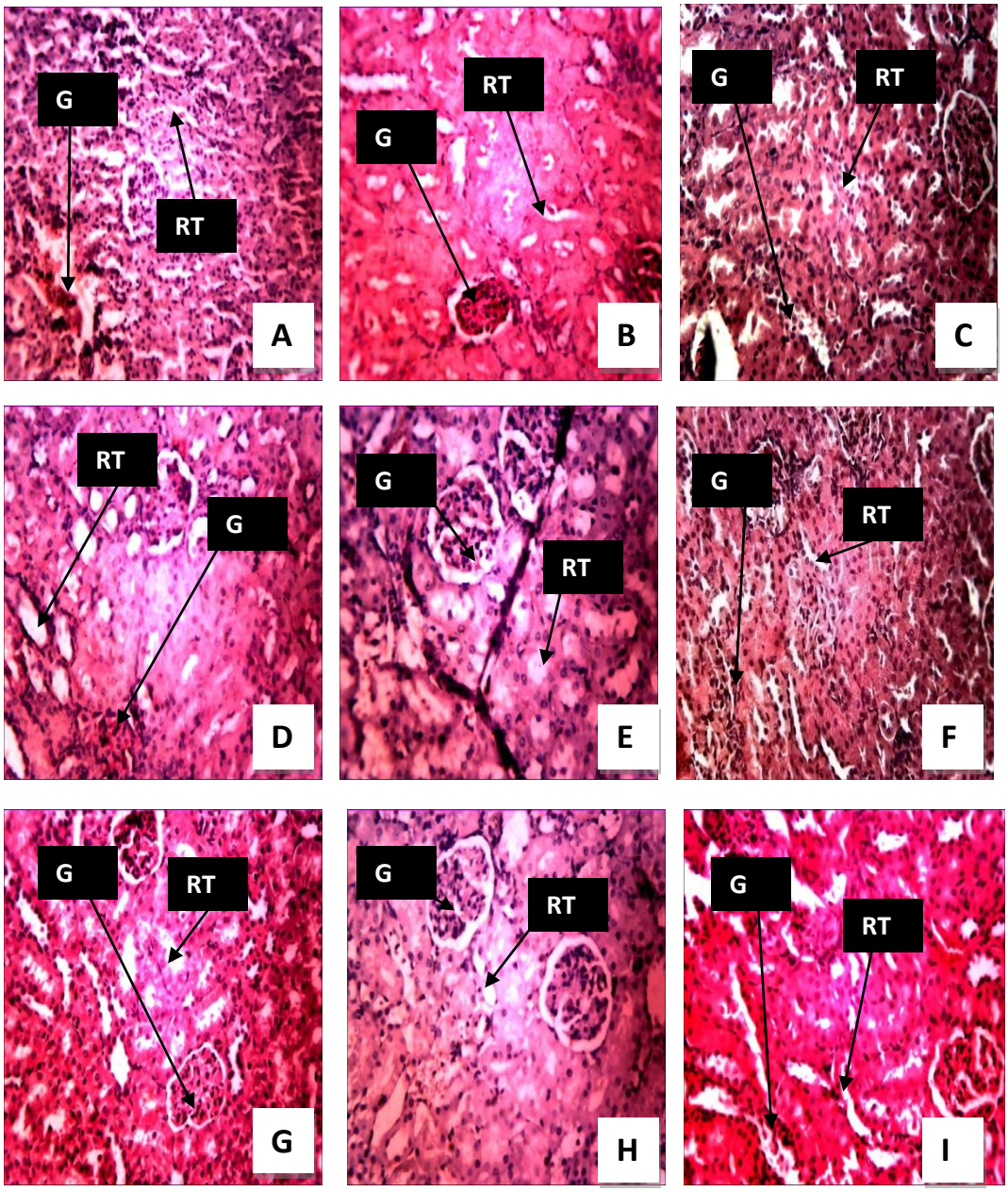


Plate VII: Photomicrograph of a section of the kidney of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered hyperglycaemic rat respectively all showing no remarkable changes at 28 day post-treatment (HE x250)
RT= Renal tubule. G= Glomerulus

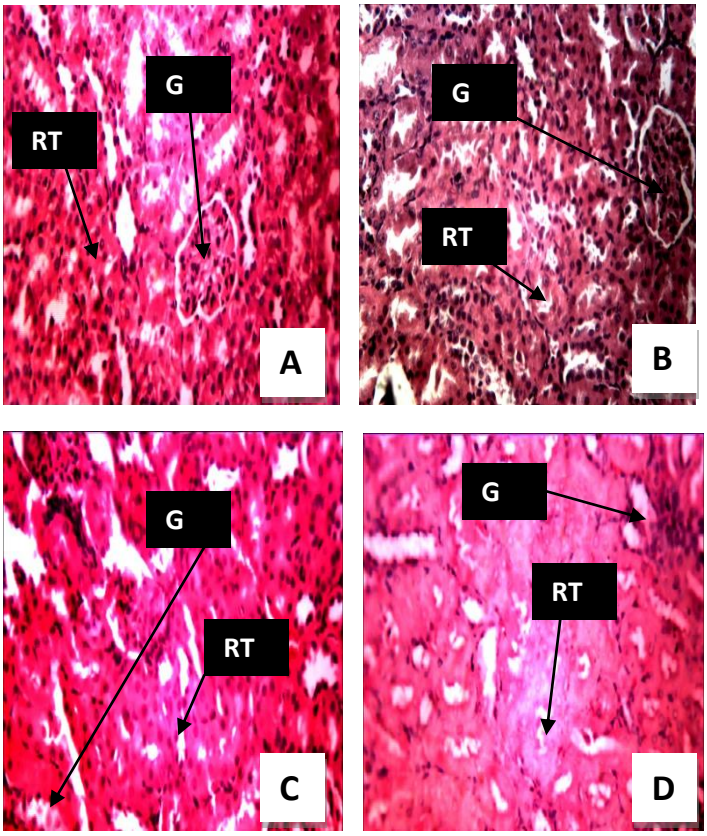


Plate VIII: Photomicrograph of a section of the kidney of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat all showing normal architecture at 28 day post treatment (HE x250)
RT= Renal tubule. G= Glomerulus

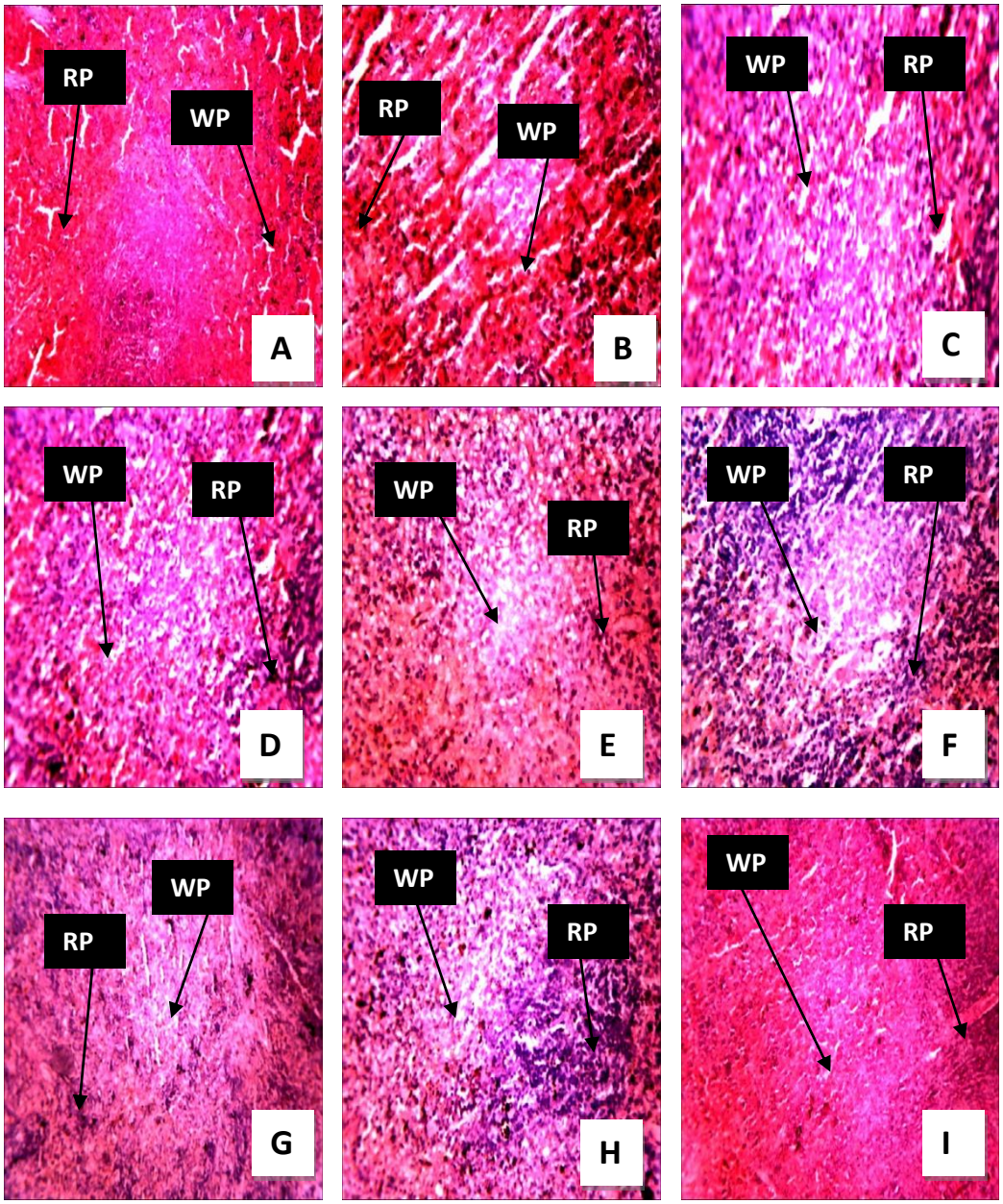


Plate IX: Photomicrograph of a section of the spleen of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat, (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered hyperglycaemic rat respectively all showing no remarkable changes at 28 day post-treatment (HE x250)
WP= White pulp, RP= Red pulp

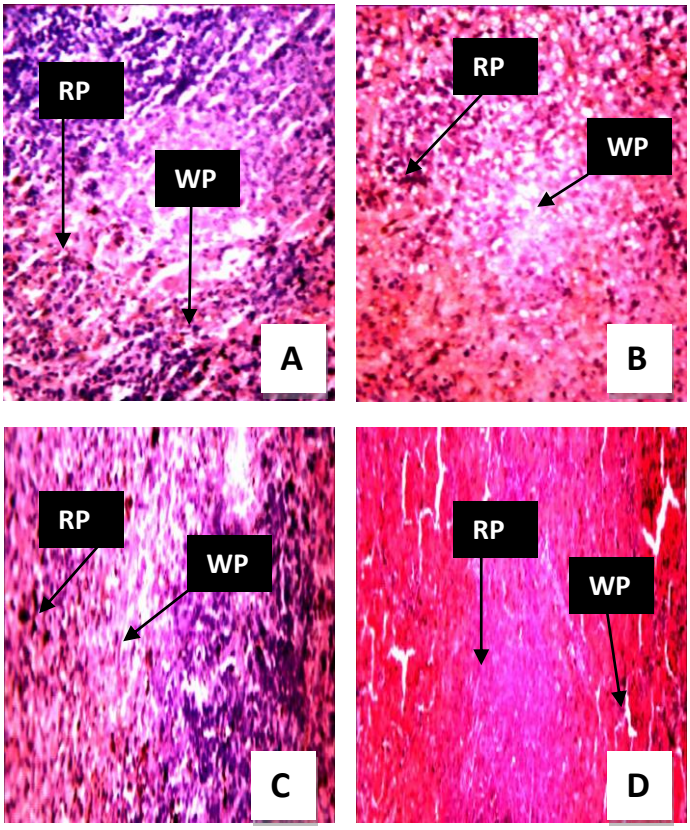


Plate X: Photomicrograph of a section of the spleen of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat all showing normal architecture at 28 day post treatment (HE x250)
WP= White pulp, RP= Red pulp

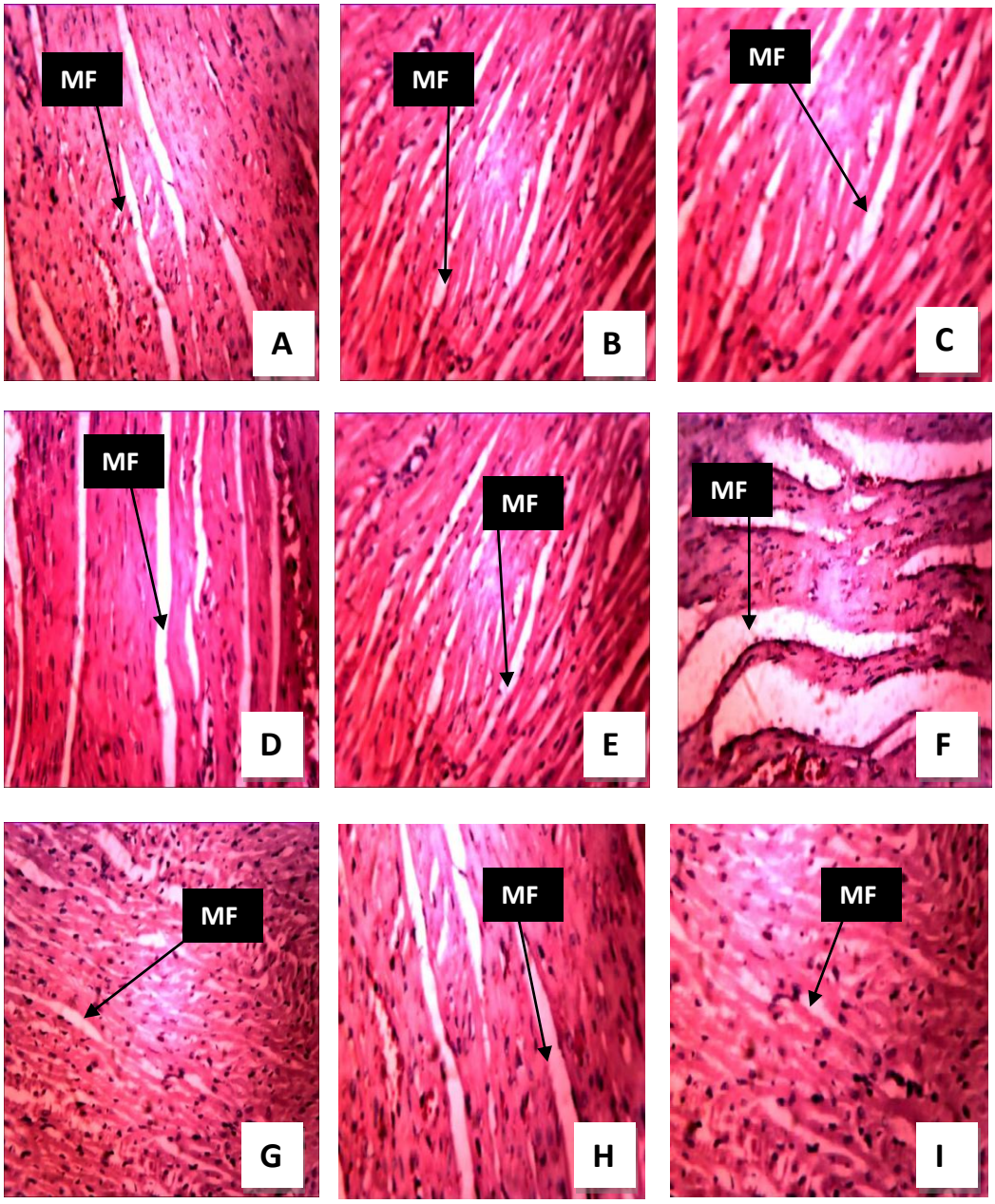


Plate XI: Photomicrograph of a section of the heart of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered hyperglycaemic rat respectively all showing no remarkable changes at 28 day post-treatment (HE x250)
MF= Muscle fibres

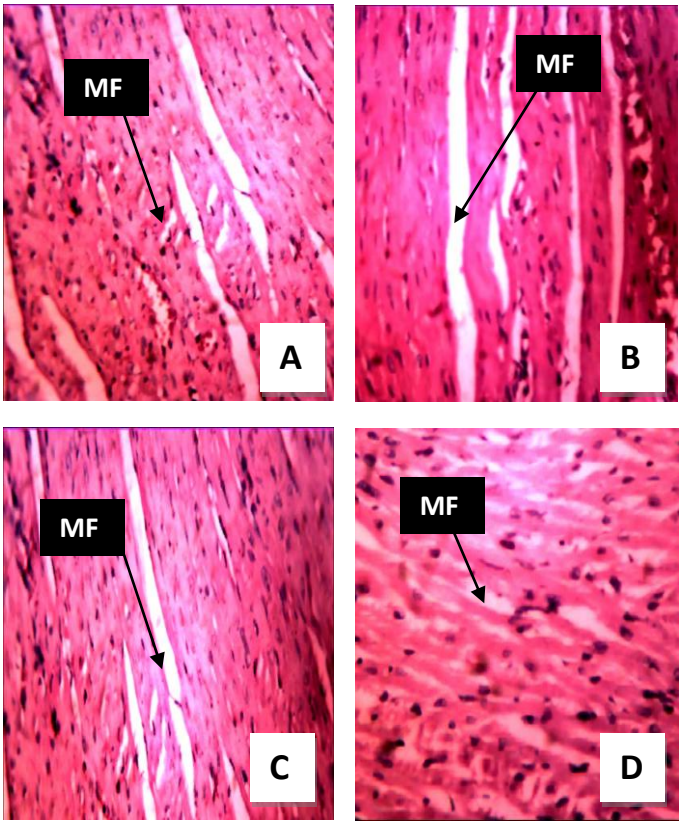


Plate XII: Photomicrograph of a section of the heart of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal architecture at 28 day post treatment (HE x250)
MF= Muscle fibres

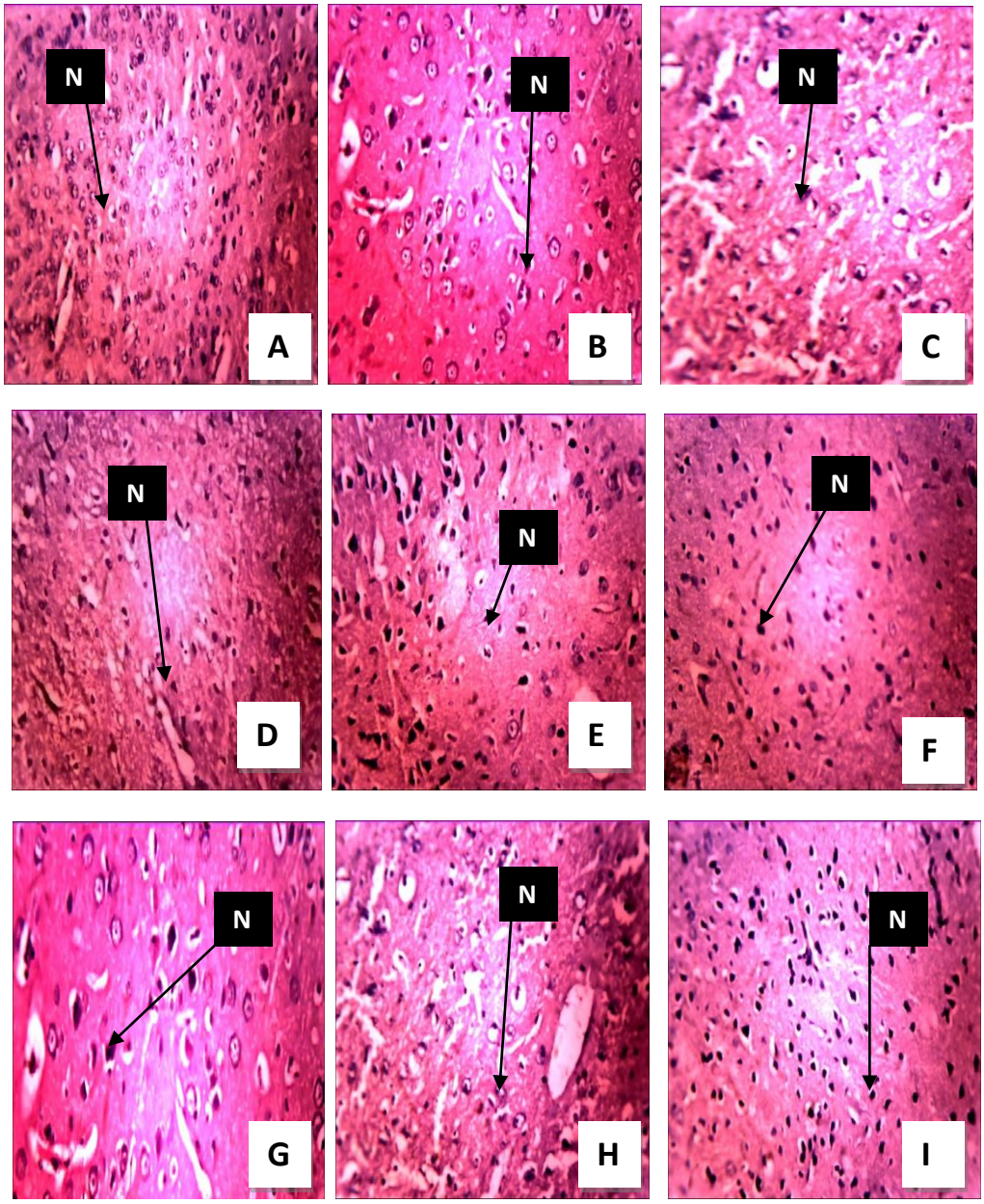


Plate XIII: Photomicrograph of a section of the brain of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered hyperglycaemic rat all showing no remarkable changes at 28 day post-treatment (HE x250)
N= Neurones

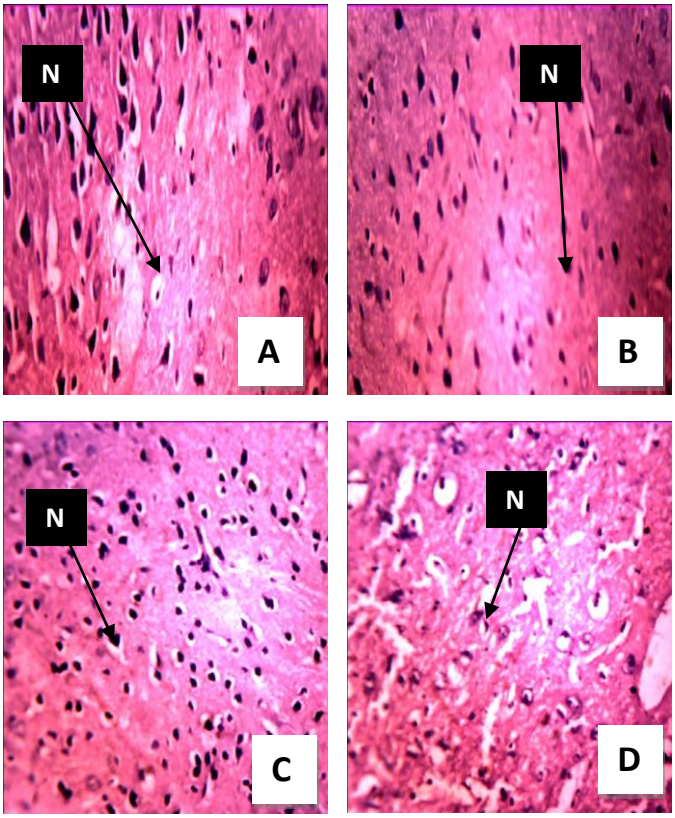


Plate XIV: Photomicrograph of a section of the brain of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal architecture at 28 day post treatment (HE x250)
N= Neurones

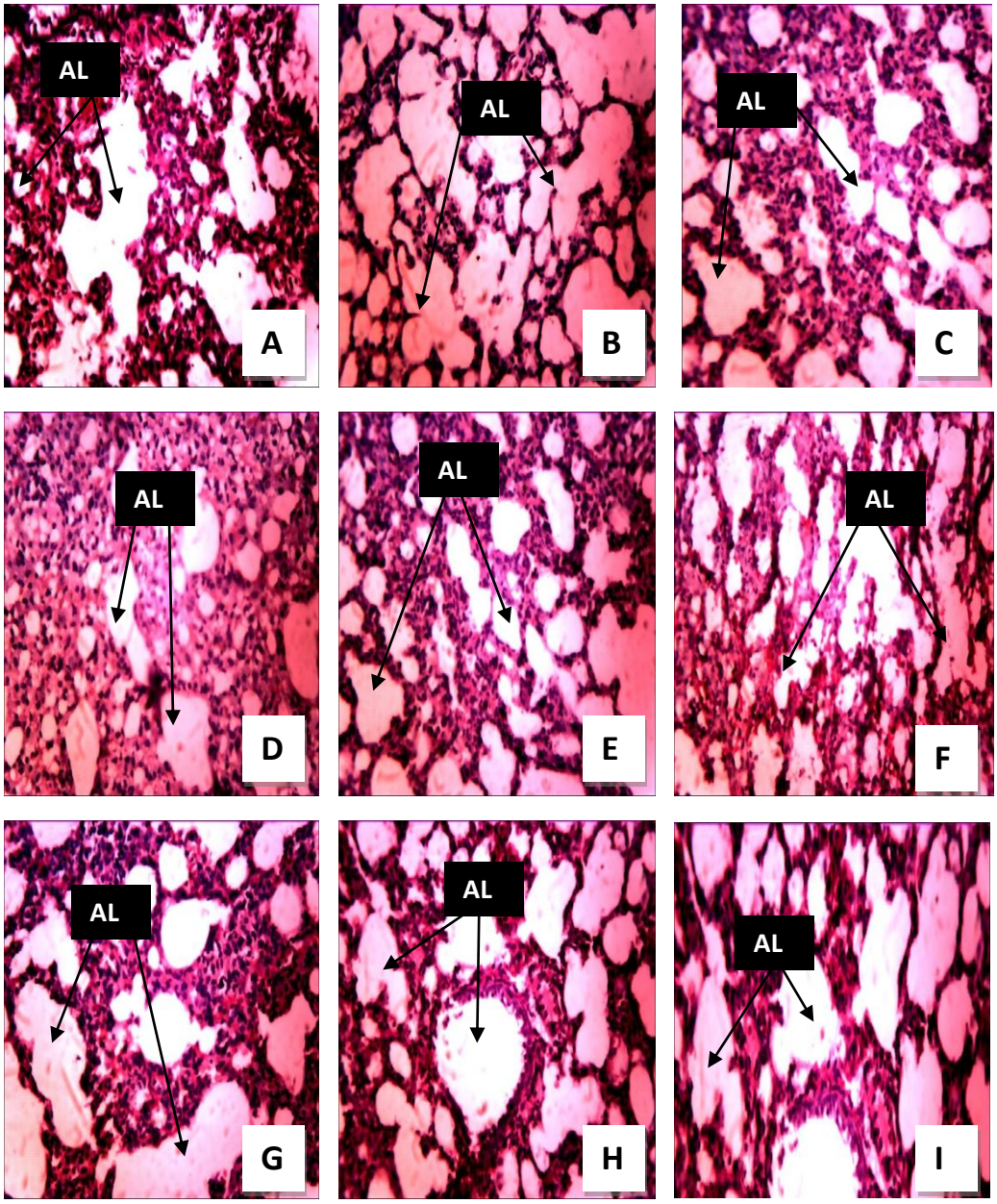


Plate XV: Photomicrograph of a section of the lung of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered rat respectively all showing no remarkable changes at 28 day post-treatment (HE x250)
AL= Alveoli

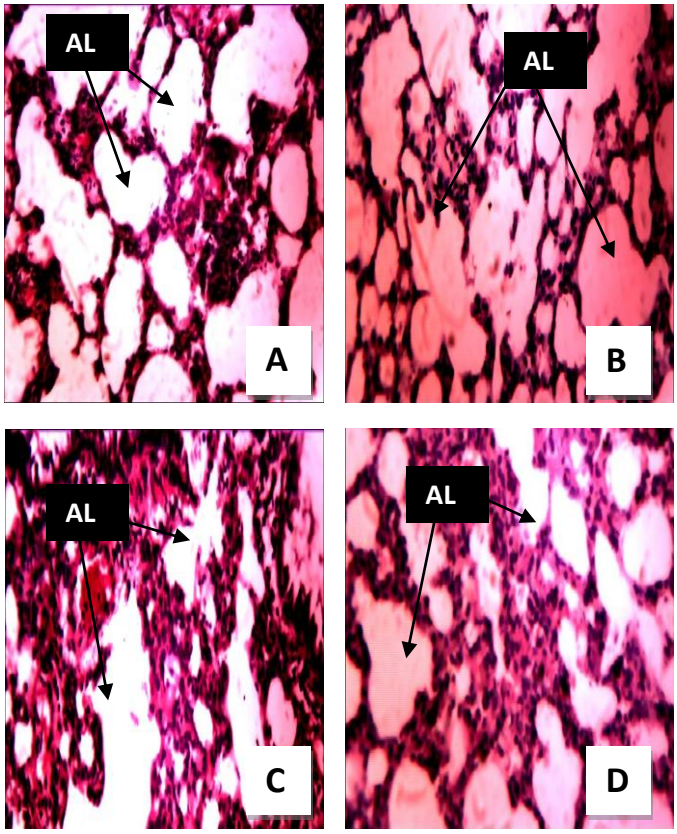


Plate XVI: Photomicrograph of a section of the lung of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal architecture at 28 day post treatment (HE x250)
AL= Alveoli

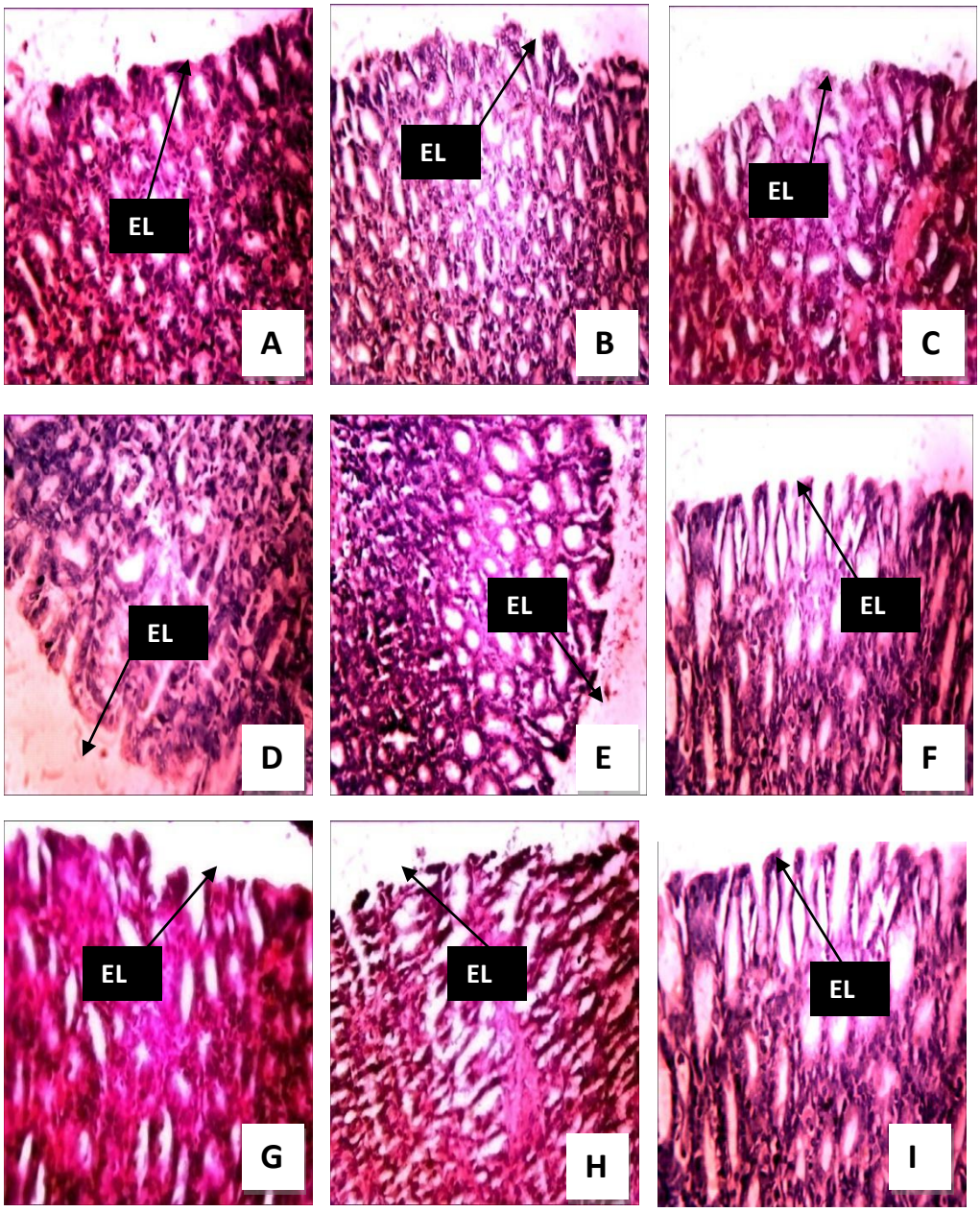


Plate XVII: Photomicrograph of a section of the stomach of a (A) normoglycaemic control rat (B) hyperglycaemic control rat (C) metformin-treated hyperglycaemic rat (D), (E) and (F) 375, 750 and 1500 mg/kg MOE treated-hyperglycaemic rat respectively (G), (H) and (I) 375, 750 and 1500 mg/kg MOE/metformin co-administered hyperglycaemic rat respectively) all showing no remarkable changes at 28 day post-treatment (HE x250).
EL= Epithelium linning

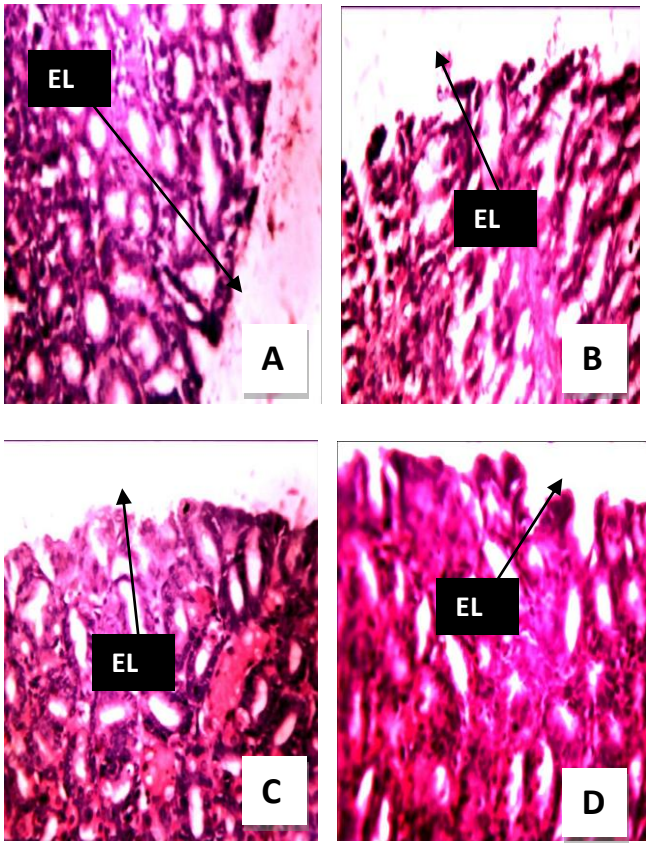


Plate XVIII: Photomicrograph of a section of the stomach of a (A) normoglycaemic control rat (B), (C) and (D) 375, 750 and 1500 mg/kg MOE treated-normoglycaemic rat respectively all showing normal architecture at 28 day post treatment (HE x250)
EL= Epithelium linning

CHAPTER FIVE

5.0 DISCUSSION

The primary aim of diabetes management is to reduce blood glucose levels to within the normal range. Various hypoglycemic medications are available for this purpose but most of them are not without severe adverse effects. Consequently, some diabetics often resort to medicinal plants and foods with antidiabetic activity but with fewer or no adverse effects while some co-use anti-diabetic herbs with conventional anti-diabetic agents for better glycaemic control. This study investigated the antidiabetic activity of various doses of the ethanol extract of *Moringa oleifera* leaves and also the possible pharmacological interaction when co-administered with metformin.

In this study alloxan was used to induce experimental diabetes. Alloxan induces diabetes by damaging the insulin secreting pancreatic beta-cells of the islets of Langerhans resulting in reduced synthesis and release of endogenous insulin (Ghosh, 2005). The cytotoxic action of alloxan is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration leading to a rapid total destruction of beta-cells producing a type 1-like diabetes mellitus (Szkudelski, 2001).

The acute toxicity study of the extract revealed that the oral LD_{50} value is greater than 5000 mg/kg. This is in agreement with the study of Bakre *et al.,* (2013) who reported that the oral LD_{50} of the ethanol extract of *M. oleifera* leaves was greater than 6400mg/kg. The extract is therefore relatively safe for oral consumption. The ED_{50} of the extract was also estimated to be 750 mg extract/kg. This implies that the extract possesses a wide safety margin as the LD_{50} value is far greater than the estimated ED_{50}.

Results obtained showed that the ethanol extract of *Moringa oleifera* leaves produced significant reduction in blood glucose level in alloxan-induced hyperglycaemic rats in a dose and time dependent manner. This is in agreement with those of other researchers, who systematically demonstrated that extracts of various parts of the plant (leaves, flowers and seed) possess antidiabetic properties (Jaiswal *et al*., 2009, Chinwe and Isitua, 2010). In a controlled study with untreated T2DM patients, William *et al.* (1993) reported the anti-hyperglycaemic activity of the *Moringa oleifera* leaves. Kumari (2010) also examined the hypoglycaemic effect of *M. oleifera* leaf dietary consumption in T2DM patients, and reported that it reduced glucose level significantly after a 40-day period.

The therapeutic actions of *Moringa oleifera* medication has been attributed to the relatively high antioxidant activity of its leaves, flowers, and seeds (Chumark *et al.,* 2008; Sreelatha and Padma, 2009; Verma *et al.,* 2009; Atawodi *et al.,* 2010). *Moringa oleifera* leaves contain many powerful antioxidant phytochemicals, especially quercetin and kaemferol (Fuglie, 1999). Hypoglycaemic activity of kaemferol derivatives from many medicinal plants has been reported by Desokky and Youssef (1997). These antioxidants in the extract might have played a role in inhibiting and scavenging the free radicals generated by alloxan consequently, the regeneration of the beta-cells which led to the realease of insulin and reduction in glycaemia. The extract might have also produced anti-hyperglycaemic activity through the release of insulin by inhibiting the ATP-sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue (Gerich, 2000).

In this study, the anti-hyperglycaemic activity of the extract/metformin co-administeration compared to the hyperglycaemic control was greater than the anti-hyperglycaemic activity of the extract at all the doses administered. Also, the anti-hyperglycaemic activity of the extract/metformin co-administeration was greater when compared to that of metformin. This suggests that the extract and metformin showed additive anti-hyperglycaemic activities. The implication of this is that, a diabetic patient could be placed on a reduced dose of metformin (which also implies lower adverse effect) while being encouraged to take more of *M. oleifera*. Therefore, *M. oleifera* can play a significant role in the management of diabetes.

The observation that the extract at the doses administered did not significantly change the glucose level of the normoglycemic rats is in agreement with the study of Tende *et al.* (2011) who reported that *M. oleifera* produces hypoglycaemia in streptozotocin-induced diabetic rats alone but not in normoglycaemic rats. This implies that, the extract is safe and does not cause hypoglycemia in a normal subject taking it either as food or for other therapeutic purposes.

Following injection with alloxan, the wistar rats displayed other symptoms of diabetes mellitus aside from hyperglycaemia, i.e. decrease of body mass gain and increase in food and water intake (polyphagia and polydipsia respectively) (Robert, 2001). The observed polydipsia could be as a result of excessive urination and dehydration brought about by plasma glucose levels exceeding 180mg/dl therefore producing an osmotic gradient where more liquid is drawn into the renal tubule while polyphagia could have resulted from the cells' inability to utilize glucose in the blood therefore sending signals to the hypothalamous to stimulate hunger (Bukonla *et al.*, 2012). The administration of

M. oleifera extract and metformin to different groups of hyperglycaemic rats caused statistically significant reduction in food and water intakes. However, the extract/metformin co-administration caused more reduction in food and water intake possibly due to the additive glycaemic control. With adequate glycaemic control, less water will be delivered to the renal tubes producing less urination, less dehydration and ultimately reduced water intake by rats. Also with more glucose uptake by the cells, there was a reduced stimulation of hunger by the nervous system leading to the reduced food intake observed in all the hyperglycaemic treated groups.

The body weights of the rats in all the hyperglycaemic groups showed a decrease in the first week. This is in agreement with the symptoms of diabetes as stated by American Diabetic Association (ADA) (2000) to include unexplained weight loss. This observation could be attributed to the increased conversion of storage fat and proteins to glucose (gluconeogenesis) (Champe *et al.,* 2005). However, after the first week, probably with improvement in glucose uptake by cells and subsequent reversal of gluconeogenesis, the body weights of the treated hyperglycaemic groups showed a steady increase, though with no significant difference throughout the course of the experiment. This study did not reveal any significant difference in the weights of the rats in the three groups that received graded doses of extract co-administered with metformin suggesting that the use of metformin and *M. oleifera* extract by diabetics is not likely to bring about an increase in weight. This observation is good for diabetes management as a number of oral hypoglycaemic drugs in use such as the sulphonylureas are associated with weight gain (Campbell and Howlett, 1995).

Diabetes is associated with increased dyslipidemia (Daniel *et al*., 2003). Dyslipidemia is a condition that is characterized by abnormal levels and composition of plasma

lipids. According to Adult Treatment Panel III (2001), plasma levels ≥200mg/dl for TC, ≥130mg/dl for LDL-C, <40mg/dl for HDL-C, and ≥150mg/dl for TG is termed dyslipidemia (Libby, 2011). Factors that could lead to dyslipidemia include inborn defects of lipoprotein metabolism, an unhealthy lifestyle such as excessive cigarette smoking or alcohol consumption, and other disease conditions such as obesity, diabetes, infection and obstructive liver disease (Libby, 2011). Drugs like β blockers and steroids could also predispose an individual to clogging of arteries by fatty substance which is the hall mark of atherosclerosis (Libby, 2011).

Dyslipidemia was also associated with alloxan induced hyperglycaemia as seen in the diabetic control group of this study where there was an elevation in LDL-C, TAG and total cholesterol level but with a corresponding decrease in HDL-C. However, treatment with the extract brought about a reversal of the abnormalities in the plasma lipids. Treatment with the extract produced a dose-dependent decrease in the serum total cholesterol. The extract/metformin co-administeration produced a reduction in serum total cholesterol which was comparable to the reduction produced by metformin alone. The observed reduction in serum cholesterol may be attributed to the levels of polyphenolic (flavonoids, tannin and saponins) compounds present in the extract (Chumark *et al.,* 2008). Other studies have shown that soluble dietary fibres (SDF) in plants are known to bind to dietary cholesterol and prevent or reduce its absorption by the small intestine (Oloyede, 2005: Gorinstein *et al*., 2006).

The extract produced a dose-dependent decrease in serum triacylglycerol level. The extract/metformin co-administration also produced a decrease in serum triacylglycerol level which was more than that of the extract at all the doses administered. However,

the dercrease produced by the extract/metformin co-administeration was not comparable to that produced by metformin administered alone except, the highest dose (1500mg) of the extract co-administered with metformin. Metformin has been shown to decrease serum triglycerides and fatty acid concentrations (Wu *et al.*, 1990) and the extract might have acted through an alteration in the level of interleukin-6 (IL-6) which mediates energy mobilization in the muscles and fat tissues (Oyewo and Akanji, 2010). An inverse proportionality exists between serum triacylglycerol level and IL-6 and TNF-α (Tracey and Cerami, 1994). In addition, reduction in the serum cholesterol level in this study does not seem to be accompanied by lipolysis, as seen in the reduction in the serum TAG levels. From this study, it could be inferred that the extract will only produce additive serum triacylglyceride lowering activity with metformin at a high dose.

The dose-dependent and additive increase in the serum HDL-C level by the extract and extract/metformin co-administration respectively could possibly be due to an increase in the biosynthesis of HDL-C in the liver due to the presence of flavonoids in the extract (Renaud *et al.*, 1999). With increase in HDL- C serum level, there will be an enhanced cholesterol excretion as more of it would be transported from the peripheral tissues to the liver for excretion. The observed dose dependent decrease in the serum LDL-C levels may explain the serum cholesterol-lowering capability of *M. oleifera* extract. This effect could possibly be due to enhanced reverse cholesterol transport and bile acid excretion through inhibition of apo B production, needed for LDL-C production, transport and binding (Turner *et al.*, 2004). The extract co-administered with metformin reduced serum level of LDL-C more than either metformin or the extract administered alone, this suggests an additive interaction. The observed additive

increase and decrease in the serum HDL-C and LDL-C respectively, is beneficial in diabetes as it reduces the risk of developing atherosclerosis, a vascular disease affecting blood circulation in the coronary, central, and peripheral arteries (Libby, 2011).

Serum total protein concentration was significantly decreased in alloxan-treated rats when compared with the normal non-diabetic ones. A number of factors that might be responsible for this reduction include increased rate of amino acids conversion to glucose (Chu *et al.,* 2007), decreased amino acid uptake (Garber *et al.,* 1980), and increased conversion rate of glucogenic amino acids to CO_2 and H_2O (Ahmed,2005). Other studies have revealed that total protein decrease might be as a result of a decrease in the amount and availability of mRNA, a loss of transitional factor, reduction of ribosomal protein synthesis as a result of insulin deficiency (Ahmed *et al.,* 2006) and decreased defensive mechanism (Mahboob *et al.,* 2005). The results of this study showed that, the treatment of diabetic rats with *M. oleifera,* metformin and *M. oleifera* extract/metformin co-administeration brought about marked increase in serum total protein contents. The extract/metformin co-administeration showed additive effect as the total protein and albumin in these groups increased more than the groups administered the extract or metformin alone. This increament could be as a result of increase hepatic uptake of amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzes amino acids transamination. A good correlation between protein synthesis and insulin level has also been recorded by Nahla *et al.,* (2006) in a study conducted in rats.

In this study, increase in serum levels of AST and ALT was observed in alloxan-induced diabetic animals. This is in agreement with the study by Derosa *et al.,* (2007)

where there was an elevation in serum AST and ALT levels after the administration of alloxan to Wistar rats. Another diabetogenic agent, streptozotocin also increased serum AST and ALT levels in rats as demonstrated by Cennet and Ebubekir, (2010). It has also been found by Larcan *et al.* (1979) that liver was necrotized in diabetic patients. The observation that the activity of serum AST appeared to be more elevated than ALT, is also in agreement with the findings of Tanaka *et al.,* (1988) and Nanbara *et al.,* (1990) that AST had more activity than did ALT in the serum of diabetic mice. The elevation of both enzymes may be a reflection of damage to the hepatic cells hence the leakage of these enzymes from the liver cytosol into the blood stream. Kim *et al.,* (2006) concluded that the elevation in AST and ALT levels may be due to the destructive changes in the hepatic cells. On the other hand, other investigators have postulated that diabetes could induce defects in sarcolemmal enzymatic activities (Micheal *et al.,* 1985) which leads finally to such effects. This study revealed that treatment of alloxan-diabetic rats with *M. oleifera* extract, metformin and various doses of extract/metformin co-administrations brought about decrease of the transaminases activity. The rate of decrease occurred in a dose- dependent fashion with the 1500mg/kg extract-treated group showing the most reduction among the extract-alone treated groups. An additive effect was observed when the extract was co- administered with metformin, as they produced the highest reduction compared to the extract-alone treated and metformin-alone groups. The decrease in AST and ALT activities could be atributted to the significant hepatoprotective and antioxidant activity of *M. oleifera* since antioxidants are known to reduce the development of chemically induced liver damage (Hui- Yin *et al.*, 2007).

There was also an increase in ALP activity after induction of diabetes in rats with alloxan. The data recorded in the present investigation showed a marked decrease of serum ALP activity after four weeks of treatment with the extract, metformin as well as extract/metformin co-administeration. This observation further indicates the protective effect of *M.oleifera* on the liver and this is in agreement with several studies that have demonstrated the hepatoprotective effect of the extract against chemically induced damage. Pari and Kumar, (2002) demonstrated the protective effect of *M. oleifera* leave extract on anti-tubular drug-induced liver damage in rats. Fakurazi *et al.*, (2008) reported that *M. oleifera* extract prevents acetaminophen induced liver injury through restoration of glutathione level. Buraimoh, (2011) also reported the hepatoprotective effect of *M. oleifera* extract on the histology of paracetamol-induced liver damage.

Furthermore, the reversal of the alloxan-induced liver damage by *M. oleifera* extract, metformin and their various co-administerations could be confirmed by the increase in level of plasma bilirubin produced when compared to non-diabetic control. Treatment of alloxan-induced diabetic rats with graded doses of *M. oleifera* extract produced significant decrease in serum bilirubin compared to the untreated hyperglycaemic rats. Metformin-alone treated group also showed a significant reduction in serum total bilirubin. The various doses of extract co-administered with metformin, however, through additive interaction brought about a greater decrease in serum bilirubin level. According to Rana *et al.* (1996), increase in plasma bilirubin (hyper-bilirubenimia) in alloxan-treated rats may be as a result of a decrease in liver uptake, conjugation or increased bilirubin production from hemolysis. Also, the elevation in plasma bilirubin indicates alteration in liver function as confirmed by the changes in the activities of plasma enzymes. The reversal of this increase in serum bilirubin levels that was

observed in this study suggests the hepato-protective potentials of the extract and extract- metformin co-administration.

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, and blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure, dehydration, fever and vomiting has also been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (Rao, 1992).The association between blood glucose and serum electrolytes is a complex one and is related to a number of other factors such as age and associated conditions (Shah *et al.*, 2011). Serum electrolyte imbalance in diabetes is primarily a result of elevated blood glucose. With hyperglycemia, the body tries to rid itself of the excess blood glucose by increasing urinary output. Increased urination produces water and electrolyte loss, thus giving rise to derangements in homeostatic balance with respect to electrolytes (Item, 2009; Bukonla *et al.*, 2012).

Significant decrease in the levels of serum sodium, pottasium and bicarbonate ions and an increase in chloride ions were recorded in the rats following treatment with alloxan. All the treated hyperglycaemic groups equally showed significant improvement in serum electrolyte levels. The sodium, pottasium, bicarbonate and chloride levels returned to the normal ranges in all the groups. This could be attributed to improvement in glucose homeostasis. Reduction in fluid and electrolyte loss could be responsible for the improvement seen in the electrolyte levels. It is important that drug combinations used in the management of diabetes mellitus should not bring about significant alterations in the levels of these ions as this could affect many body functions such as

nerve conduction (Item, 2009). No significant alteration in electrolyte levels was observed in the normoglycaemic rats treated with *M. oleifera*. This has implications for people taking *M. oleifera* for nutritional and other medical purposes, which suggests that they can take it without the fear of electrolyte imbalance.

Plasma creatinine and urea levels are established markers of glomerular filteration rate (GFR) (White *et al.,* 1973). They are therefore useful clinical tools in assessing renal function. Creatine is a non-protein nitrogen compound synthesized in the kidney, liver and pancreas from arginine, glycine and methionine (White *et al.,* 1973). After phosphorylation it provides energy for muscular contraction and it is converted into the waste product creatinine (White *et al.,* 1973), which is excreted in urine. Urea is a nitrogenous product of protein metabolism. It is usually excreted from the body via the urine. Creatinine and urea blood concentrations increase in case of any abnormality in kidney function. As their values exceed the normal limit, impairment in kidney function occurs (White *et al.,* 1973). Hence, creatinine and urea clearance is the best measure to check the glomerular filtration and its level in the blood reflects the functional state of the kidney (Munir *et al.,* 2003). Higher creatinine and urea levels may be detected in patients with history of diabetes or heart attack (Salive *et al.,* 1995).

In this study, alloxan produced increase in serum creatinine and urea levels. This might be as a result of a fall in the filtering capacity of the kidney thus leading to accumulation of these waste products within the system (Idonije *et al.,* 2011). Treatment with graded doses of *M. oleifera* extract however, significantly reduced both creatinine and urea levels. The extract at graded doses co-administered with metformin produced more reduction and this could be attributed to the additive interaction

between the two as metformin-alone treated group also showed marked reduction in urea and creatinine levels. Metformin is substantially excreted by the kidneys and risk of accumulation increases with the degree of renal impairment. Monitoring of kidney function is therefore important when used alone or in combination with other drugs.

There was no significant change in the creatinine and urea levels of the normoglycaemic rats treated with the *M. oleifera* extract. This implies that the extract has no toxic effect on the functioning capacity of a healthy kidney. However, this is in contrast to the findings of Oyagbemi *et al.,* (2013) that chronic administration of methanol extract of *M. oleifera* leaves raises serum creatinine and urea levels of normoglycaemic rats.

The relative weights of the heart, lungs, brain, stomach and kidney of both the *M. oleifera* treated hyperglycaemic and normoglycaemic groups showed no significant change difference in weights when compared to the respective controls. This may imply that *M. oleifera* and its combination with metformin had no adverse effect on these organs. However, this observation may not necessarily predict an absence of pathological conditions or functional alterations. Alloxan administration brought about a reduction in spleen weight as seen in the hyperglycaemic control rats when compared to the normoglycaemic control. This observation is in contrast with that of Indradevi *et al.,* (2012), who reported that alloxan administration produced an increase in the spleen weight of rats. The significant increase in spleen weight observed at all doses of extract and extract/metformin co-administered is a reversal of the reduction in speen weight and this could be attributed to the protective effect of the extract on the spleen due to its antioxidant properties.

The result of this study indicates a decrease in the liver weight of hyperglycaemic control rats when compared to the normoglycaemic control. This observation is in agreement with the study of Dodamani *et al.,* (2012) who reported a decrease in the liver weight of mice after alloxan administration. However, the findings of this study is in contrast with that of Indradevi *et al.,* (2012), who reported that alloxan administration produced an increase in the liver weight of rats. The liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases including diabetes (Stadler *et al.,* 2003). In experimental diabetes, diabetogenic agents exert their effects on liver and other organs in addition to the pancreas (Kume *et al.,* 2004). The insulin insufficiency and hyperglycaemia that result from beta-cell necrosis further augument liver damage through reactive free radical-mediated lipid peroxidation of hepatocellular membrane (Kume *et al.,* 2004). This pathophysiological sequence sets the scene for considering antioxidants important in the defence of diabetic liver. Thus the antioxidant properties of *M. oleifera* might have contributed to the significant restoration of the liver weight of the rats treated with graded doses of the extract and extract/metformin co-administration.

The histopathological studies of the pancreas of the hyperglycaemic rats revealed degenerated pancreatic islet. This observation is in agreement with Ragavan and Krishnakumari (2006) and Dunn *et al* (1943), where the ultra structure of alloxan induced diabetic rats showed considerable reduction in the pancreatic islets. However, the administration of various doses of the plant extract, extract/metformin co administration and metformin showed restoration of pancreatic islet cells in all

hyperglycaemic treated groups but more significant restoration with the extract/metformin co-administration. The cytotoxic action of alloxan is said to be through generation of free radicals. Antioxidants play an important role in inhibiting and scavenging free radicals and therapeutic actions of *Moringa oleifera* medication has been attributed to the relatively high antioxidant activity of its leaves, flowers, and seeds (Chumark *et al.,* 2008). As such the antioxidants of the extract might have scavenged the free radicals generated by alloxan hence, the regeneration of the beta cells. The normoglycaemic control rats and the treated normoglycaemic rats showed intact architecture of pancreatic islet cells with no observable microscopic lesions. The implication of this is that the extract has no effect on intact beta-cells.

Histopathological results of the liver and kidney sections of the hyperglycaemic control rats showed no remarkable changes. This is a deviation from the earlier observations of Ragavan and Krishnakumari (2006) that alloxan brought about marked tubular and glomerular damage in the kidney and necrosis of hepatocytes of rats. This observation could mean that alloxan only affected the functional integrity of these tissues as seen in the elevation in serum transaminases but did not cause any structural changes. There were also no remarkable changes in the histology of the heart, brain, spleen, lungs and stomach of the alloxan-induced hyperglycaemic control rats. MOE, metformin and MOE/metformin co-administration had no remarkable effect on all the organs studied apart from the pancrea. This implies that sub-chronic administration of *M.oleifera* extract or its co-administration with metformin may have no remarkable toxic effect on the organs of rats.

From this study it can be concluded that *M.oleifera* and metformin co-administration ameliorates the abnormalities associated with diabetes and its complications via additive mechanism. This could be important in reducing the dose of metformin to achieve enhanced therapeutic effect with minimum adverse effect.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

The ethanol extract of *M.oleifera* leaves and its co-administration with metformin at all doses used in this study, exhibited significant anti-hyperglycaemic activity in alloxan-induced diabetic rats after repeated administration for 28 days. This is evident in the dose-dependent and additive decrease in weekly FBS observed throughout the duration of the study. Graded doses of MOE and MOE/metformin co-administration gradually reduced the food consumption and water intake of the treated rats throughout the duration of the study when compared with the hyperglycaemic control. Repeated treatments with MOE and MOE/metformin co-administration, also brought about a slight increase in the body weights of the experimental rats. At all the doses used, MOE and MOE/metformin co-administration exhibited dose-dependent and additive hypolipidemic activity respectively as the serum levels of LDL-C, TAG, and total cholesterol were lowered. Conversely, an elevation of HDL-C was observed. An increase in serum total protein and albumin concentration was also observed. The liver function markers-serum total bilirubin, AST, ALT and ALP levels were also lowered after 28 days of repeated treatment with MOE and its co-administration with metformin. There was also a significant decrease in urea and creatinine levels at all the doses of MOE and MOE/metformin co-administration. Metformin administered alone showed similar effects. For the normoglycaemic experimental rats, there were no significant alterations in the body weight and biochemical parameters. Treatment with MOE, metformin and MOE/metformin cause significant increase in the weights of liver and spleen but no increament was observed in the weights of the other organ studied. The repeated administration of graded doses o MOE and its co-administration with metformin brought about the reversal of the degeneration of pancreatic beta-cells

caused by alloxan treatment. However, no remarkable changes occurred in the histology of the liver, kidney, spleen, heart, brain and stomach of the rats.

The anti-hyperglycaemic activity of the MOE and its co-administration with metformin occurred in a dose dependent manner. The interaction between MOE and metformin can be said to be additive as the various doses of MOE co-administered with metformin produced greater anti-diabetic effects than that of the extract or metformin administered alone.

In conclusion, ethanol extract of *M. oleifera* leaves and metformin ameliorated the hyperglycaemia and its attending disorders in diabetic rats through additive interaction. This investigation has demonstrated that the co-use of ethanol extract of the leaves of *M. oleifera* and metformin is not only efficacious but safe for the management of diabetes mellitus. This investigation also demonstrated the ability of *M. oleifera*/metformin to regenerate the lost beta-cells in diabetes as revealed in the histopathology of the pancreas.

It is recommended however, that

1. Chronic toxicity study of *M.oleifera* and metformin co-administration is carried out since diabetes requires life-long treatment.
2. Efficacy and toxicity study should also be carried out for the co-use of *M.oleifera* and other oral hypoglycaemic agents such as sulfonylureas.

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