PATHOPHYSIOLOGICAL EFFECTS OF *IRVINGIA GABONENSIS/IRVINGIA WOMBOLU* SEED POWDERS COMBINATIONS ON ALLOXAN-INDUCED DIABETIC MALE RATS.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, FACULTY OF BIOLOGICAL SCIENCES,

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APPROVAL PAGE

Nwatu, Kate Uchechi, a post graduate student in the Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka with registration number PG/M.Sc./13/66940 has satisfactorily completed the course and research work requirements for the award of Master of Science degree (M.Sc.) in Zoology and Environmental Biology (Physiology). The work embodied in this project report is original and has not been submitted in part or in full for any other diploma or degree in this or any other university.

_____ _____

Prof. J. E. Eyo **Date**

(Project Supervisor)

_____ _____

Prof. B. O. Mgbenka **Date**

(Head of Department)

_____ _____

(External Examiner) **Date**

DEDICATION

This project work is dedicated to my beloved husband Lt. MO Nnamani and my awesome daughter for their support and understanding throughout the period of this study. God bless you both richly.

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I deeply thank the Almighty God for upholding and seeing me through in this academic endeavour. I also wish to tender my sincere appreciation to my supervisor and the Dean, Faculty of Biological Sciences, Prof. J. E. Eyo who by his fatherly attention and patience took his time and energy to advice and correct me in the course of this work. I also wish to express my profound gratitude to the Head of department, Prof. B. O. Mgbenka who desiring studentsô excellence advised me on how to make the best out of this work. I also pray that the Almighty God will bless and reward Dr. V. C. Ejere for his fatherly guidance to me throughout the period of this study. I also appreciate Mr. Gerald Attama for his analytical support.

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May Almighty God from who all good things come from bless you all.

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ABSTRACT

This study aimed at investigating the pathophysiological effects of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* combined in different proportions on alloxan-induced diabetic rats. Both species of *Irvingia* were analysed for phytochemical content. A total of 72 male albino rats were used for the experiment. A latin square design of six treatment groups replicated thrice, with each replicate having 4 albino rats each was used for the experiment. Diabetes was induced in 5 groups of the experimental animals by the intraperitoneal administration of alloxan in a dose of 120mg/kg body weight, while one group served as the positive control. Crude seeds of *Irvingia gabonensis* and *Irvingia wombolu* combined in 3 different proportions (80% *I.gabonensis*: 20% *I.wombolu* (IgIw1), 20% *I. gabonensis:* 80% *I. wombolu*(IgIw2) and 50% *I. gabonensis* : 50% *I.wombolu* (IgIw3)) were administered to 3 groups of the diabetic animals while the remaining 2 diabetic groups served as the diabetic and standard drug control. Body weight, blood glucose levels and serum levels of low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and malondialdehyde (MDA) were monitored at 7 days interval for 21 days. Pancreas, kidney and liver were harvested from a rat in each group at the end of the experiment for histological studies. Phytochemical analysis of both species of *Irvingia* reveals that *I. wombolu* has more flavonoids, alkaloids, glycosides, terpenoids and steroids than *I. gabonensis.* There was no significant difference ($P < 0.05$) in the body weight of the diabetic animals compared to the positive control. Administration of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* and the standard drug glibenclamide significantly ($P < 0.05$) lowered blood glucose levels of the diabetic rats. Serum TC, TG and LDL-C decreased significantly ($P < 0.05$) while HDL-C increased significantly ($P < 0.05$) in the treated animals compared to the diabetic control. There was also a significant decrease ($P < 0.05$) in serum levels of ALT, AST and ALP of the treated rats compared to the diabetic control. However, there was no significant difference ($P < 0.05$) between the serum levels of MDA of the treated animals and the diabetic control. The histopathological damage on the pancreas, kidney and liver of the diabetic rats was not entirely revised within the period of experiment. This study therefore suggests that *Irvingia gabonensis* and *Irvingia wombolu* combined has blood glucose lowering effect and may also protect against dislipidemia. Hence, *Irvingia gabonensis* and *Irvingia wombolu* may be used as ingredients in health and functional food to ameliorate diabetes.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Glucose is an indispensable fuel for the brain and other tissues (Whitney *et al.,* 2007). However, chronic amounts of circulating glucose cause toxic effects on the structure and function of organs, including pancreatic islets (Matsinkou *et al.,* 2012). Therefore, to regulate glucose in the body, the body produces insulin to drive glucose into cells for use or storage; and glucagon, epinephrine and other hormones to bring glucose out of storage again for use during extreme starvation. Chief among these hormones produced for glucose regulation is insulin; which is produced by the beta cells of the pancreatic islets of Langerhans. Insufficient production or resistance of body cells to insulin results in a disease known as diabetes mellitus; characterized mainly by hyperglycemia (chronic amounts of circulating glucose) and most times hyperlipidemia (chronic amounts of ôbadô fats in circulation) (Kavishankar *et al.,* 2011; Matsinkou *et al,.* 2012; Saravanamuttu and Sudarsanam, 2012).

Diabetes mellitus is a lifestyle disorder that is rapidly becoming a major threat to populations all over the globe. Over the past 30 years, the status of diabetes has changed from being considered as a mild lifestyle disorder of the elderly to one of the major causes of morbidity and mortality, affecting people of all ages (Saravanamuttu and Sudarsanam, 2012). Diabetes mellitus is one of the most common health problems worldwide (Shukla *et al.* 2012), and the prevalence of this disease is rapidly increasing, leading to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Umar *et al.,* 2010). The number of individuals with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Firdous, 2014).

Essentially, insulin deficiency results in faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by the tissues and this condition leads to hyperglycemia. Hyperglycemia in diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra *et al*., 2006). The fatty acids from adipose tissue are mobilized for energy purpose, as a result of inability of the cells to make use of insulin. Hence, excess fatty acids are accumulated in the liver, which are converted to triglyceride (Shih *et al*., 1997). Accumulation of triglyceride leads to increase in the formation of low density lipoproteins (LDL) and a reduction in high density lipoproteins (HDL).

Multiple biochemical pathways and mechanisms of action of glucose toxicity have been suggested. Reactive oxygen species (ROS) attack unsaturated fatty acids of the membrane phospholipids to initiate lipid peroxidation, causing severe damages to the membrane structure with sequential fluidity. Diabetes mellitus is usually accompanied by impaired antioxidant capacity (Matsinkou *et al.,* 2012). There is a considerable evidence that hyperglycemia results in the generation of ROS (Punitha *et al.,* 2006), ultimately leading to increased oxidative stress in a variety of tissues (Evans *et al*., 2002), variations in its fluidity and ability to function correctly (Shukla *et al.,* 2012). In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive intracellular signaling pathways (Evans *et al*., 2002). All of these pathways produce ROS in excess and which over time causes chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis (programmed cell death) of the beta cells (Robertson, 2004).

Free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and subsequent oxidative degradation of glycation proteins (Kangralkar

et al., 2010). These free radicals produced as a result of normal biochemical reactions in the body, are implicated in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, hair loss and neurodegenerative disorders such as Alzheimerôs disease and Parkinsonôs disease (Olutayo *et al.,* 2013).

Although the body possesses innate defence mechanisms to counter free radicals in the form of enzymes such as superoxides dismutate (SOD), catalase and glutathione peroxidase, this increased free radical generation along with declined antioxidant defense system may damage enzymes, cellular organelles, which leads to lipid peroxidation (Kangralkar *et al.,* 2010). All these activities can cause or exacerbate diabetes mellitus. This may happen as a result of decreased insulin production (Type I) or insufficient insulin utilization (Type II) (Marshal and Bangert, 2004; Matsinkou *et al,.* 2012). Insulin deficiency contributes to increased serum levels of transaminase enzymes due to easy availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes during diabetes inducing hyperglycemia and hyperlipidemia (Punitha *et al,.* 2006, Dzuefiet *et al*., 2009; Shukla *et al.,* 2012).

The hyperlipidemic condition certainly contributes to a major risk factor for atherosclerosis and cardio vascular diseases (Punitha *et al.,* 2006). The saturated fatty acids present in fat could increase the production of triglycerides and cholesterols by the liver and could decrease the catabolism of LDLs by the repression of their receptors (Shukla *et al.,* 2012). Actually, insulin deficiency inactivates the lipoprotein lipase, which promotes the conversion of free fatty acid into phospholipids and cholesterol in the liver, and they finally get discharged into the blood resulting into elevated serum phospholipids.

It is believed that any medicinal plant that can work as a potential antioxidant together with having anti-diabetic property could prevent or reduce diabetic complication more effectively

than the conventionally used anti-diabetic drug (Bhattaram *et al*., 2002). This is because most synthetic drugs have been associated with so many side effects. Plant secondary metabolites such as flavonoids and terpenoids etc. have been shown to play important role in the defence against free radicals (Devasagayan and Sainis, 2002; Govindarajan *et al.*, 2005). Hence, several plants have been found useful in ameliorating diabetic complication.

Currentlly available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidasae inhibitors, troglitazone, etc. But these are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea (Ngondi *et al*., 2006). In addition they are not suitable for use during pregnancy (Kangralkar *et al.* 2010). More so, although many anti-diabetic drugs are already available which have been commercially used by diabetic people, none of these have the dual properties of reducing blood glucose level and scavenging free radicals. Moreover the side effects and cost of the drugs are not affordable by all. Again, taking too much insulin can cause hyperglycemia (Whitney *et al.* 2007), by causing blood glucose level to fall so rapidly that it triggers the liver to release counter regulatory hormones (insulin antagonists) which may raise the blood glucose higher than the available insulin can handle.

Many traditional treatments for diabetes are used throughout the world (Ngondi *et al,.* 2006; Oduola *et al*., 2007). Some medicinal plants have been reported to be useful in diabetes treatment and have been used empirically as antihyperglycemic and antihyperlipidemic remedies (Bhattaram *et al,.* 2002). Most of these plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. that have been shown to have antidiabetic effects (Loew and Kaszkin, 2002; Haque *et al.,* 2012; Firdous *et al.,* 2014). The attributed antihyperglycemic effect of the plants is due to their ability to restore the function of the pancreatic tissues by causing an increase in insulin output or by inhibiting the intestinal

absorption of glucose or to increase the facilitation of metabolites in insulin dependent process (Ngondi *et al.,* 2006).

Irvingia gabonensis and *Irvingia wombolu* are highly valuable and extensively utilised tropical African trees. They are local fruit trees with wide distribution across West and Central Africa. *Irvingia* kernels are used in soup making as they form an important part of the West and Central African diet. The yellow fruit pulp of *Irvingia gabonensis* is edible having yellow pulp. However, the fruit pulp of *Irvingia. wombolu*, is bitter and has turpentine taste, so it is not edible. Fat extracted from the kernels can be used for food applications, such as in margarine or cooking oil, and is also suitable for soap, cosmetics and pharmaceuticals. Flour can be produced from the kernels. The seeds contain oil used in different culinary purposes. The wood is hardy and has resistance to termites. The bark is green in colour and is used as medicine for arthritis, rheumatism, dropsy, swellings, oedema, gout, eye treatment, fabrifuges, stomach trouble and venereal diseases (Singh, 2007). The only part of the *Irvingia* plant that doesnôt seem to have other medicinal application is its pulp.

Fractions of *Irvingia gabonensis* seeds were reported to have hypoglycemic effect (Ngondi *et al*., 2006; Dzuefiet *et al*., 2009; Omonkhua and Onoagbe, 2011).

Fruits of both *Irvingia* species possessed all five phytochemicals (alkaloids, flavonoids, saponins, tannins and glucosides). However, whilst both species had the same amounts of flavonoids and glycosides, *I. wombolu* possessed relatively higher amounts of alkaloids, saponins and tannins than *I. gabonensis. Irvingia wombolu* may be the preferred choice if domestication would be based on phytochemicals. In like manner, *I. gabonensis* may be the preferred choice for domestication if taste, weight and size of fruits are the parameters of interest (Etebu, 2013).

Several researches have been carried out to check for the hypolipidemic and hypoglycemic effects of both species of *Irvingia* (Ngondi *et al.,* 2005, 2006, 2009). The results of these researches showed that *Irvingia* species have antidiabetic effects. However, a comparison of the nutritive qualities of the kernels of both species conducted by Ndoye *et al*. (1997) indicated that *I. wombolu* is more energy-rich due to its higher percentage fat although both species are a good source of oil. The fat from *I. wombolu* kernel has lower iodine and saponification values. Therefore, there is a possibility that the differences in percentage of phytochemical composition of both species could lead to differences in their degree of biochemical activities in the body. Hence, the aim of this present research is to evaluate the pathophysiological effects of various fractions obtained from combinations of the seeds of *Irvingia gabonensis* and *Irvingia wombolu* harvested in Nigeria, on alloxan-induced diabetic rats.

1.1.2 Justification of study

This work investigated the hypoglycemic and hypolipidemic effect of the two species of *Irvingia* found in Nigeria. Two major factors that lead to diabetes includes; genetic factors and lifestyle. Oftentimes therefore, diabetes is usually managed to maintain blood glucose level at a healthy range and prevent further diabetic complications. The three basic management procedures for diabetes include diet, exercise and medication. Hence, there has been increase in the search for both medicinal and food plants that can be used to manage diabetes with little or no side effects. Food plants with antidiabetic effects are very useful in diabetes management, since they can easily be added in daily meal, eschewing the phobia of using only drugs and the side effects of injecting excessive insulin after a meal.

Irvingia gabonensis and *Irvingia wombolu* are highly valuable and extensively used tropical African trees. *Irvingia* seeds are used in soup making as they form an important part of West

and Central African diets. It has been reported from several studies that various parts of *Irvingia* species harvested from other West African countries like Cameroun have hypoglycemic and hypolipidemic effects. However, it has also been discovered that both species of *Irvingia* vary in both phytochemical and proximate content. Hence, there is need to evaluate the pathophysiological effects of various combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* harvested in Nigeria, and compare this with glibenclamide, a standard diabetic drug, so as to determine the best and as well help dieticians in planning adequate diet management for diabetics.

1.1.3 Objectives of study

The objectives of the study were to determine the effects of combinations of crude seed powders of *I. gabonensis* and *I. wombolu* combined on:

(i) fasting blood glucose level in alloxan induced diabetic rats,

(ii) body weight in alloxan induced diabetic rats,

(iii) serum lipid profile (total cholesterol, triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL)) in alloxan induced diabetic rats,

(iv) liver enzyme activity in alloxan induced diabetic rats,

(v) malondialdehyde (MDA) concentration of plasma, liver and kidney and

(vi) histopathology of the pancreas, liver and kidney in alloxan induced diabetic rats.

1.4 Literature Review

1.2.1 Medicinal plants

Medicinal plants are those plants (parts, extract etc) that are used in treating and preventing specific ailments and diseases that affect human beings (Nwachukwu *et al.* 2010). Hence the important role of medicinal plants in health care delivery (services) cannot be over emphasized. Demand for medicinal plants is increasing in both developing and developed countries. Hence, research on medicinal plants is one of the leading areas of research globally (Soetan *et al.* 2009; Omonkhua and Onoagbe, 2012; Olutayo *et al.* 2013).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Most plant parts (extract) identified e.g. (bark root, seeds, fruit, leaf) serve as major source of active ingredient and products of secondary metabolites like alkaloid, terpenoids etc used in curing diseases, production of drugs as well as in maintaining good health by both the traditional and orthodox medical practitioners, although it has been opined that plant leaves are about 51% more favorable for storing active ingredients, as compared to other parts of the medicinal plants (Mannan *et al.* 2014). Medicinal plants are either ñwild plant speciesò those growing spontaneously in self maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or the contrasting ñdomesticated plants speciesò those that have arisen through human actions such as selection or breeding and depend on management for their existence.

Medicinal plants contain physiologically active prhytochemicals that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebajo *et al*., 1983). The drugs contained in medicinal plants are known as active principles. Cowman (1999) and Banso and Olutimayin (2001) reported that plants contain a wide variety of active

principles. In the case of most drugs, herbs, ethnomedicines, essential oils and cosmetics are derived from the secondary products of plant metabolism such as the alkaloids, terpenoids and flavaonoids (Alaribe, 2008). These substances have evolved as responses of plants to stress, predation and competition constituting to what is regarded as the vast chemical library of biological systems. It is usually ñextractsò not the plants themselves or their parts such as fruits, seeds leaves etc; that are used for medicinal effects. However, medicinal plants possess what is referred to as pathological niche and they assume pathogenomic structure. This means that medicinal herbs can be used for different ailments with respect to its on human physiology.

Some examples of indigenous medicinal plants in Nigeria and their active parts, according to recent researches include; *Azadirachta indica* (neem/dogoyaro) leaves, roots and bark, *Aspilia africana* (haemorrhage plant) leaves, *Gongronema latifolia* (Utazi) leaves, stem, fruit and root, *Costus afar* (Ginger lily/Bush cane) leaves, *Psidium guajava* (Guava) leaves, stem, fruit and root, *Carica papaya* (Paw-paw) seeds (Eyo *et al.,* 2013a)root, bark, and leaves, *Vernonia amagdalina* (Bitter leaf) leaves (Eyo *et al.,* 2013b), *Ocimum gratissimum* (Scent leaf) leaves, (Nwachukwu *et al*., 2010 Eyo *et al.,* 2014), *Entandrophragma angolense Welw* (*Meliaceae*) stem-bark, *Khaya senegalensis* Desr. (*Meliaceae*), *Anogeissus leiocarpus* (*Combretaceae*), *Pavetta crassipes*, *K.Schum* (*Rubiaceae*) flowers and *Abrus precatorius Linn*.(*Leguminosaceae*) (Olutayo *et al.,* 2013), *Allium cepa* L. (onion): (*Liliaceae*), *Allium sativum* L. (garlic): (*Liliaceae*) (Kavishankar *et al*., 2011; Eyo *et al.,* 2011; Ozougwu *et al.,* 2014; Ozougwu and Eyo, 2014). These plants have been shown to contain active phytochemicals which are useful in curing many human diseases.

1.2.2 Food plants and their biochemical components

Plant foods have remained the ultimate source of nutrients for larger population of the world. They are simply described as irreplaceable food resources for humans, which exclude animal sources. These foods contain many chemical compounds needed for metabolic functions in varying proportions. Some of these chemical compounds are non-nutrients that are beneficial to man while some others provoke some adverse reactions (e.g oxalic acid found in *Amaranthus* species). However, the level of adverse effect produced by these non-nutrients depends on the levels of intake, interrelationships of nutrients and food habits. Plant foods are classified as cereals, roots and tubers, legumes, vegetables and fruits (Aremu and Ibrahim, 2014). According to the major nutrients they provide, plant foods are classified into three main categories namely; macronutrients (carbohydrate, protein, fat and water), micronutrients (minerals and vitamins) and non-nutrient components (dietary fibre, phytochemicals, anti-nutrients, food toxicants and additives) (Lutz and pryztulski, 2008).

The bioactive compounds or secondary metabolites are the non-nutrient components in plant foods. They have some nutritional effects and health benefits. They are those substances contained in foods which supply no nutrients. They could contain some compounds that are beneficial to health or toxic to humans and/or act as antagonists to nutrients in foods. These include tannins and other phenolic compounds (phenols, flavonoids, isoflavonoids), saponins, glucosinolates, alkaloids (Drewnowski and Gomez-Carneros, 2000), phytate and dietary fibre (Gibson, 2007). These chemical compounds are found in different classes and parts of plant foods in varying amounts. They are more concentrated in plant storage organs (leaves and seeds) than in other parts of the plants (Chan *et al*. 2012). These constituents have their individual health-promoting qualities that compel people to combine the different food sources to achieve healthy eating and maintain good health. Several authors have studied therapeutic potentials and metabolic effects of foods rich in dietary fibre and phytochemical constituents (Jenkins *et al*. 2003; McCarty, 2004; Soetan, 2008). These include lower risk of

colon cancer (Ricciardiello *et al.,* 2011), promotion of early satiety and normal laxation (Hossain *et al.,* 2012), moderation of post-prandial blood glucose responses and improved insulin sensitivity (Ngondi *et al.,* 2006; Hossain *et al.,* 2012), reduction in total and low density lipoprotein (LDL)-cholesterol (Ngondi *et al.* 2006; Baldeon *et al*. 2012) and regulation of appetite and enhancement of sodium and fluid balance (Camargo *et al.,* 2004). They are also used to treat constipation and prevent development of diverticulosis and diverticulitis (Wintola *et al.,* 2010). Diets adequate in dietary fibre are usually rich in micronutrients and phytochemicals, and frequently less calorically dense and lower in fat and added sugars. However, environmental factors, cultural food habits and insufficient nutritional information about health benefits of traditional plant foods still pose a problem to healthy food choices. Drewnowski and Gomez-Carneros (2000) reported that most of the bioactive compounds are bitter, acrid or astringent and aversive to the consumer and may be wholly incompatible with consumer acceptance. These factors have caused increasing epidemic of diet-related diseases across the regions. They suggested the need to take sensory properties and food preferences into account when advocating for increased consumption and diversification of rich sources of these secondary metabolites in plant foods. The challenge of achieving adequate supply of energy and nutrient intake as well as the health-promoting compounds from plant-based foods/diets without compromising the health of an individual forms the basis for current dietary recommendations aimed at promoting consumption of plant foods to reduce diet-related non-communicable diseases.

1.2.3 Biological activities of phytochemicals

The most commonly encountered secondary metabolites of plants (phytochemicals) are saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides, phlobatannins, resins, balsam and volatile oils (Soetan *et al.,* 2008; Olutayo *et al.,* 2013). The presence of these secondary metabolites in plants probably explains the

various uses of plants for traditional medicine, because most of them play important role in the defence against free radicals. The pharmacological and other beneficial effects of antinutritional factors in plants have been reviewed by Soetan (2008). Plants rich in chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes have been found to be protective to the liver (Bhavna and Sharma, 2012; Ozougwu and Eyo, 2014; Ozougwu *et al.,*2014). Hence the hepatoprotective potency of the plant could be attributed to its antioxidant property.

Saponins are glycosides of both triterpenes and steroids having hypotensive and cardiac depressant properties (Olaleye, 2007). Saponins bind to cholesterol to form insoluble complexes. Dietary saponins in the gut of monogastrics combine with endogenous cholesterol excreted via the bile. This prevents cholesterol reabsorption and results in a reduction of serum cholesterol. Saponins have been found to be potentially useful for the treatment of hypercholesterolaemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol.

Tannins are complex phenolic polymers which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Nwogu *et al*., 2008). Tannins from the bark, roots and other parts of many plants especially Euphorbiaceae are used to treat cells that have gone neoplastic. Tannins are also reported to have astringent properties on mucous membranes (Egunyomi *et al*., 2009).

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent anti-oxidant activity against the superoxide radical. Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of low density lipoproteins (LDL) oxidation known to have been attributed to the

dietary and supplemental intake of flavonoids and other micronutrients. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary artherosclerotic disease.

Alkaloids are basic natural products occurring primarily in many plants. They are generally found in the form of salts with organic acids and they are haemolytically active and are also toxic to microorganisms. Alkaloids, comprising a large group of nitrogenous compounds are widely used as therapeutic agents in the management of cancer. Alkaloids also interfere with cell division. An alkaloid isolated from *Hibiscus sabdariffa* demonstrated its ability to prevent mutagenesis.

Cardiac glycosides are cardioactive compounds belonging to triterpenoids class of compounds. Their inherent activity resides in the aglycone portions of their sugar attachment. Their clinical effects in cases of congestive heart failure are to increase the force of myocardiac contraction. They exert their hypotensive effect by inhibiting Na^+ -K^+ ATPase. They also act directly on the smooth muscle of the vascular system. They exert a number of effects on neural tissue and thus indirectly influence the mechanical and electrical activities of the heart and modify vascular resistance and capacitance (Olaleye, 2007).

1.2.4 Anti-diabetic phytochemicals

Plants can provide biologically active molecules and structural compounds for the development of modified derivatives with enhanced activity and reduced toxicity (Mannan *et al.,* 2014). Plants have chemical compounds which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect (Malviya *et al.*, 2010). *Galega officinalis* is a plant from which hypoglycemic drugs was obtained traditionally (oseph and Jini, 2011). Insulin, biguanides, sulfonylurease and thiazolinediones

are mordern pharmacotherapeutics, but still except glycemic control with insulin, there is need to look for new drugs for more efficacious agents with less side effects is needed.

Several studies have shown that tannins and saponins found in most plants are the components responsible for their hypoglycemic effect (Ngondi *et al*., 2005, 2006; Omonkhua and Onoagbe, 2010; Eyo *et al.,* 2011). The administration of different plant extracts in diabetic rats may act by a direct stimulation of insulin secretion in remaining ϐ- cells. This effect could be attributed to compounds like glycosides, alkaloids, flavonoids, anthocyanin, tannins (Matsinkou *et al*., 2012). Their action may involve insulin-like extrapancreatic mechanisms such as the stimulation of glucose utilisation and the reduction of hepatic gluconeogenesis (Hossain *et al.,* 2012).

Polyphenols have also been suggested to decrease the oxidative stress in human especially through inhibition of the LDL-cholesterol oxidation (Fuhrman and Aviram, 2001). Flavonoids found in the pulp extracts may inhibit the oxidative stress by: i) scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers; ii) chelating metal ions; iii) sparing other antioxidants (e.g. carotene, vitamin C and E); and iv) preserving HDL associated serum paraoxonase activity (Fuhrman and Aviram, 2001). Antioxidant properties of polyphenols are related to their chemical structure and depend on the number and arrangement of their phenolic hydroxyl groups. The amount of phenolics varies considerably in the different pulp extracts.

Some plant constituents appear to be disease specific. Plants with considerable hypoglycaemic property have beenreported. Drewnowski and Gomez-Carneros (2000) and Noor *et al* (2013) reported phenols and polyphenols, flavonoids, isoflavones, terpenes and glucosinolates in vegetables and fruits. Several studies have published similar effects with dietary fibre (non-starch polysaccharides (NSPs)) (Jekins *et al*. 2003). A new classification of

dietary fibre (water-soluble and insoluble dietary fibre) was based on their solubility characteristics (Gray, 2003). The soluble dietary fibre is highly viscous and has added viscosity as functional property in the evaluation food/diets. These NSPs lower blood glucose level by impeding glucose absorption from the gastrointestinal tract and reduce post-prandial hyperglycaemia (Hossain *et al.,* 2012). The water-insoluble NSP are mainly obtained from structural carbohydrates (cellulose and lignin of the cell walls) of starchy roots/tubers and cereals. The water-soluble NSP are obtained from storage carbohydrates (gum and hemicellulose) of legumes and as pectin from fruits and vegetables (Busch, 2015).

Ngondi *et al*. (2006) observed that high fiber diet has been shown to work better in controlling diabetes. On the basis roles of phytochemicals and antioxidant constituents of plant foods, it is believed that they hold good promise for diabetes.

1.2.5 Aetiology and pathophysiology of diabetes

Diabetes mellitus is a chronic life-long disease, which has been known to mankind for over 2000 years. It requires careful monitoring and control. Diabetes is a chronic metabolic disorder, characterized by high blood glucose (hyperglycemia), associated with impaired carbohydrate, fat and protein metabolism, resulting from either insufficient or no release of insulin by pancreas in the body (American Diabetes Association, 2012).

Evans *et al*. (2002) has reported that pathogenesis of diabetes is better appreciated and should be discussed in line with hyperglycaemia and elevated free fatty acid in the blood in relation to oxidative stress and production of free radicals; where oxidative stress refers is the imbalance between production and removal of reactive oxygen species (ROS) and free radicals.

Diabetes is usually accompanied by impaired antioxidant capacity (Matsinkou *et al.*, 2012). Several reports have shown that there are alterations in the endogenous antioxidant enzymes in diabetic condition (Preet *et al*., 2005), especially the anti-oxidative defense system, like superoxide dismutase and catalase, which are lowered in diabetic subjects. There is a considerable evidence that hyperglycemia results in the generation of reactive oxygen species (Punitha *et al*., 2006), ultimately leading to increased oxidative stress in a variety of tissues (Evans *et al*., 2002). ROS attack unsaturated fatty acids of the membrane phospholipids to initiate lipid peroxidation, causing severe damages to the membrane structure with sequential variations in its fluidity and ability to function correctly (Shukla *et al.,* 2012). In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive intracellular signaling pathways (Evans *et al*., 2002). Multiple biochemical pathways and mechanisms of action of glucose toxicity have been suggested. All of these pathways produce ROS in excess and which over time causes chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis (programmed cell death) (Robertson, 2004).

Again, free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and subsequent oxidative degradation of glycation proteins (Kangralkar *et al.,* 2010; Matsinkou *et al*., 2012). These free radicals, produced as a result of normal biochemical reactions in the body are implicated in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, hair loss and neurodegenerative disorders such as Alzheimerôs disease and Parkinsonôs disease (Olutayo *et al*., 2013). Although the body possesses innate defence mechanisms to counter free radicals in the form of enzymes such as superoxides dismutate, catalase and glutathione peroxidase, increased free radical generation along with declined antioxidant

defense system may damage enzymes, cellular organelles, cause lipid peroxidation and increase diabetic abnormalities (Kangralkar *et al.,* 2010). All these activities can cause or exacerbate diabetes. This happens as a result of decreased insulin production (Type I) or insufficient insulin utilization (Type II) (Marshal and Bangert, 2004; Matsinkou *et al*., 2012). Insulin deficiency contributes to increased serum levels of transaminase enzymes due to easy availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes during diabetes inducing hyperglycemia and hyperlipidemia (Punitha *et al*., 2006; Shukla *et al*., 2012).

The hyperlipidemic condition certainly contributes to a major risk factor for atherosclerosis and cardio vascular diseases (Punitha *et al*., 2006). The saturated fatty acids present in the fat could increase the production of triglycerides and cholesterols by the liver and could decrease the catabolism of LDCs by the repression of their receptors (Shukla *et al*., 2012). Also, insulin deficiency inactivates the lipoprotein lipase, promoting the conversion of free fatty acid into phospholipids and cholesterol in the liver and finally it gets discharged into the blood resulting into elevated serum phospholipids (Hossain *et al*., 2012).

1.2.5.1 Aetiological classification of diabetes

Diabetes mellitus includes type I diabetes (immune-mediated and idiopathic), type II diabetes, gestational diabetes and other specific types (American Diabetes Association, 2012). However, types I and II diabetes mellitus appeared to have gained much more popularity among researchers and have generally been considered as the two major categories.

Type I diabetes insulin dependent diabetes mellitus)

Type I diabetes is also known as insulin dependent diabetes mellitus (IDDM), juvenile onset diabetes. In this type of diabetes, the pancreas becomes completely unable to or insufficiently produces insulin, due to autoimmune destruction of the pancreatic beta cells (Whitney *et al*., 2007). This results in variation in blood glucose levels, which makes the patient prone to ketoacidosis; characterized by hyperglycemia and ketones, and hypoglycemia (Lutz and Przytulski, 2008).

In type 1 diabetes, glucose absorbed from the digestive tract remains in the blood, even though the cells are starved of it. Insulin is therefore injected regularly to assist the cells in taking up the needed glucose; hence, the descriptive term insulin dependent (Whitney *et al*., 2007).

Since no glucose enters the cells (in the case of untreated type I diabetes), excessive hunger and overeating (polphagia), the cells breakdown protein and fat in order to generate the needed energy fuels. Consequently, weight loss occurs and ketones are produced for energy resulting in ketonemia (ketones in the blood, which causes acidiosis) and ketones in the urine (ketonuria). Ketonuria is a sign that diabetes has gone out of hand.

Researchers have also found that people with diabetes type I have certain genes associated with immune response. However, not everyone with this gene expresses clinical diabetes.

Type I diabetes occurs in about five to ten percent of all the cases of diabetes and is frequently develop in childhood, although some cases arise in adulthood (Lutz and Przytulski, 2008).

Type II diabetes (non-insulin dependent diabetes)

Type II diabetes has also been called non-insulin dependent diabetes mellitus (NIDDM) and adult onset diabetes. In this case, the pancreas produces insulin but the cells are less sensitive

to it. Though the pancreas may respond by making more insulin, glucose uptake is still inadequate to meet the cells need. Over time, the pancreas produces less insulin; hence, the blood glucose rises.

Due to inadequate energy fuels for the cells, the cells hunger for energy which results in overeating and consequently more increase in blood glucose. The blood then converts excess glucose into fat; hence, overweight occurs. This is why obesity is commonly associated with type 2 diabetes. Overweight and obesity leads to adverse metabolic effects like high blood pressure and hyperlipidemia (WHO, 2003). Obesity or increased body fat (especially central or abdominal obesity) leads to increased insulin resistance, due to the secretion of a group of hormones that may possibly impair glucose tolerance; and also increase in the serum resistin level which in turn correlates to insulin resistance. It is also believed that fat tissues reduce insulin receptors thereby causing insulin insensitivity in the cells (Lutz and Przytulski, 2008).

Type II diabetes occurs in 90 to 95 percent of all the cases and it develops in people of over 40 years of age.

1.2.6 Genus *Irvingia*

Classification

Kingdom: Plantae

Phylum: Angiosperm

Class: Eudicots

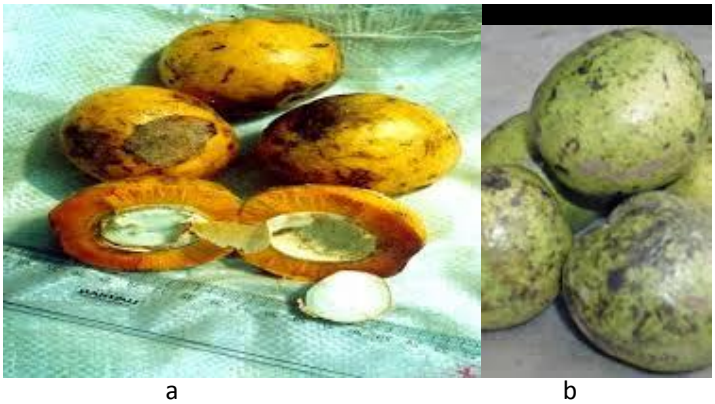
Order: Rutales

Family: Irvingiaceae

Genus: *Irvingia*

Species: *Irvingia gabonensis, Irvingia wombolu* (Kengni *et al.,* 2011)

The genus *irvingia* is commonly known as Bush mango, African mango, Dika nut, or wild mango. Two species of this genus are widely distributed in West and Central African namely, *I. gabonensis* and *I. wombolu.* They are found in their natural range in humid forest zones in Angola, Cameroun, Nigeria, Ghana, Senegal, etc. In Nigeria, two varieties of this species were identified in 1974, *I. gabonensis var. gabonensis* (with sweet edible fruit) and *I. gabonensis* var. *excelsa* (with bitter fruit). In a revision of the taxonomy of the Irvingiaceae family, Harris renamed the bitter variety *I. wombolu vermoesen* and the sweet variety *I. gabonensis*Aubry-Lecomte ex O'Rorke. Other species of the same genera are *I. excelsa, I. robur, I. smithii and I. grandifolia.* The kernels of these species also have various local names: in Nigeria, they are ó*gbono'* in Ibo and ó*apon'* in Yoruba, ó*goron*, or ó*biri'* in Hausa. Igbo people of Nigeria distinguish between kernels from I. gabonensis and I. wombolu, referring to the former as ó*ugiri'* and the latter ó*gbono'*.



a b

c d

e f

Figure 1: (a) fruits of *Irvingia gabonensis* (b) fruits of *Irvingia wombolu* (c) Seeds of *Irvingia gabonensis* (d) Seeds of *Irvingia gwombolu* (e) seed powder of *Irvingia gabonensis* (f) seed powder of *Irvingia gabonensis*

The fruits of *I. wombolu and I. gabonensis* are similar in appearance to that of cultivated mango (Mangifera indica) (Tchoundjeu *et al*. 2005) and their color varies from green to yellow when mature. *I. gabonensis* flowers in February-March and fruits of the rainy season (July- September) while *I. wombolu* flowers in October and fruits in the dry season (January-March).

The kernels of both species are used as a condiment in soups, increasing their viscosity and drawability (sliminess), but *I. wombolu* is preferred due to its better sliming qualities (Awono *et al*., 2009). They form an important diet providing carbohydrates, oils and proteins to enhance health and nutrition (Fajimi *et al*., 2007). Kengni (2003) reported the composition of seeds from Cameroon to be 68.5% fat, 6.1% total carbohydrate, 2.7% ash, 6.2% crude protein, 6.9% soluble fibre, 17.3% insoluble fibre, 0.1% phenolic compounds. The fruit of *Irvingia gabonensis* has a sweet mesocarp and it is eaten fresh while that of *Irvingia wombolu* is sour and is not consumed locally (Fajimi *et al*., 2007; Awono *et al*. 2009). A comparison of the nutritive qualities of the kernels of both species indicates that *I. wombolu* is more energy-rich due to its higher percentage fat although both species are a good source of oil. The fat from *I. wombolu* kernel has lower iodine and saponification values. However, the percentage of crude protein is low for the two species (near 7%).

1.2.6.2 Ecology and biology of *Irvingia Species*

Both bush mango species occur at altitudes between 200 - 500 m, with mean annual temperature of 25 - 32ºC. Sweet bush mango grows best in a dense moist forest on well-drained acidic soils, with mean annual rainfall of 1500 ï 3000 mm. Bitter bush mango can tolerate a wider range of soils, growing in swamps and seasonally flooded forest as well as dry land forest, where annual rainfall is1500 - 2500 mm (Kengni *et al*., 2011). Sweet bush

mango can reach 40 m in height under good conditions while bitter bush mango can reach 25 m in height.

1.2.6.3 Morphological traits and their variation

The two species are very similar in their morphological traits, except that sweet bush mango is taller and has a more elongated crown than bitter bush mango. The crown is dense and compact. The bark is grey and smooth or very slightly scaly. Leaves are green, simple and elliptic (Kengni *et al*., 2011). They are placed alternately along the twigs. Petiole length is 5 - 10 cm. Flowers are yellowish to greenish-white, and grow in slender, clustered racemes or small panicles above the leaves. Individual flower stalks are slender, about 6 mm long and petals bend right back. Fruit of *I. gbonensis* is yellow when ripe, broadly ellipsoid and varies in size from 5 to 20 cm long, and 4 to 11 cm wide. Fruit consists of yellow, fibrous pulp surrounding a large seed. Variation has been noted in flowering and fruiting phenology, crown shape, flower colour, fruit production, precocity, fruit characteristics (shape, quality, colour, size, pulp colour, sweetness, fibrousnesses) and seed hardness. Many of these traits are important to farmers. Depending on the degree to which the variation in these traits is inherited, there may be opportunities for substantial improvement in a breeding and domestication programme.

1.2.7 Bioactive constituents and health effects of *Irvingia* species

The bioactive compounds or secondary metabolites are the non-nutrient components in plant foods. They have some nutritional effects and health benefits. They are those substances contained in foods which supply no nutrients. They could contain some compounds that are beneficial to health or toxic to humans and/or act as antagonists to nutrients in foods (Soetan,

2008). They include tannins and other phenolic compounds (phenols, flavonoids, isoflavonoids), saponins, glucosinolates, alkaloids (Drewnowski and Gomez-Carneros, 2000), phytate and dietary fibre (Gibson, 2007).

Plant contains useful extractable substances in their storage organs (leaves and seeds/roots) in quantities sufficient to be economically useful as raw materials for various scientific technological and commercial applications.

Evidences from several researches have shown that *irvingia* spp contain many phytochemicals.

Singh (2007) summaried the physicochemical properties of *I. gabonensis* at 39 ºC - 40 ºC as follows; saponification value 212 - 220, smoking point 213 - 220, free fatty acid value 0.25 - 0.30 iodine value 1.99mg/gm, acid value 1.36, total lipid content. In another study, Nangue *et al*. (2011) observed that *I. gabonensis* contains two main saturated fatty acids namely; lauric acid (40.7%) and mystric acid (49.05%). These two fatty acids was claimed to be associated with capric acid (1.54%), palmitic acid (5.06%) and stearic acid (2.38%), making saturated fatty acids in I. gabonensis up to 98.86% of the whole fatty acid content. Oleic acid was found to be the only unsaturated acid in the sample. Therefore, I. gabonensis was found to increase the serum triglycerides in the treated rats except for HDL. It was claimed that lauric acid was found to modify the activities in hepatic lipid metabolism, leading to triglycerides accumulation. It was also said that the fat in *I. gabonenesis* may have also slowed down protein synthesis, either by interrupting the activities of liver enzymes or by the detoriation initiated by the fatty acids which may be in excess in the liver. Surprisingly, in the male rats, they observed that this effect may have been countered by the secretion of regulatory hormones. However, several studies (Ngondi *et al*., 2006; Omokhua and Onoagbe, 2011; Hossain *et al*., 2012), have suggested that intake of *I. gabonensis* reduced serum lipids but

increased HDL. It could be that the reduction of ôbadô serum lipids in these researches resulted from the body defense system of the model animals, which may have been ellicted by *the I. gabonenesis*. Moreover, the diets of the experimental rats used by Nangue *et al.* (2011) contain high concentrations of *I. gabonensis* fat. On the other hand, Nangue *et al.* (2011) suggested that the mystric acid found in *I. gabonensis* could be beneficial for of cellular metabolism, since the roles of mystric acid includes; participation in a óswitchô mechanism, permitting the protein to cycle in a regulated manner between membranes and cytosol and influencing protein conformation leading to protein stability or ligand binding).

A study carried out by Matsinkou *et al.* (2012), revealed that the pulp extract of *Irvingia wombolu* fruits is rich in polyphenols. This is in agreement with the result of Oviasogie *et al.* (2009). Polyphenols are usually known to act as antioxidants. They are thought to rid the body of harmful molecules known as free radicals, which can damage a cell's DNA and may trigger some forms of cancer and other diseases (American Cancer Society, 2013). Matsinkou *et al.* (2012) suggested that the polypenols decrease the oxidative stress in humans, especially through the inhibition of LDL cholesterol oxidation. They observed that the amount of phenolics varies considerably in the fruit pulp extract. Polyphenol content in the aqueous extract was found to be 1.94 times higher than that of hydroethanolic extract. This showed the influence of the type of solvent used on the polyphenol content.

Joyal (2012) indicated that *I. gabonensis* extracts has beneficial effects on a variety of metabolic targets involved in carbohydrate metabolism thus: inhibitory effect on glycerol-3-phosphate dehydrogenase, a key enzyme involved in conversion of glucose to stored fat, inhibitory effect on alpha-amylase, a key enzyme involved in the digestion of dietary complex carbohydrates into maltose and dextrin,

beneficial impact on PPAR-gamma, a key enzyme involved in both adipogenesis (new fat cells metabolism) as well as insulin sensitivity, up-regulation of adipopectin, a key protein hormone involved in enhancing insulin sensitivity and endothelial function, and enhancing leptin sensitivity and therefore decreasing leptin sensitivity.

Irvingia gabonensis seeds have been suggested to have delayed stomach emptying effect, thus leading to a more gradual absorption of dietary sugar (Dzeufiet *et al*., 2009; hossain *et al*., 2012). This effect led to reduced the elevation of blood sugar levels that is typical after a meal. Ngondi *et al*. (2006) attributed this hypoglycemic effect to the high dietary fibre content of the seed. They also observed that *Irvingia gabonensis* seeds protein have anti-amylase activity. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being digested by pancreatic amylase.

According to Bard (2010), consumption of *Irvingia* has been linked to increased` effective fat loss via multiple pathways:

i. Reducing glucose levels and insulin induced lipogenesis (A.K.A reducing insulin sensitivity)

ii. Reducing the absorption of sugar (inhibiting amylase activity)

iii. Inhibiting the conversion of glycerol to triglycerides (reducing fat cell triglycerides and glucose-3-phosphate dehydrogenase enzyme)

iv. Reducing leptin resistance (reducing CRP binding to leptin)

v. Lowering serum leptin levels (leptin unable to be used by cells)

vi. Increasing anti-inflamatory, anti-atherogenic and anti-diabetic effects (increasing adipopetin levels).

In a similar research, Ngondi *et al*. (2009) found that IGOB131, a relatively rich plant-derived protein, extracted from *I. gabonensis*, safely and significantly reduced body weight in overweight and /or obese subjects and had favourable impact on a variety of other metabolic parameters. IGOB131 administration was associated with increases in plasma adipopectin levels and decreases leptin and CRP level. Plasma leptin levels are closely related and correlated with the levels of adipose tissue. Hence, decrease in plasma leptin levels associated with the decrease of adipose tissue was a consequence of weight loss.

Irvingia gabonensis extract was also found to significantly reduce body weight of rabbits (Ngondi *et al*., 2005; Oben *et al*., 2008; Omonkhua and Onoagbe, 2011). It was suggested that the significant reduction in subcutaneous fat of the animals implies that weight reduction was as a result of loss of fat deposits and not muscle wasting (Oben *et al*., 2008). The weight lowering effect may as a result of the hypoglycemic effect of *I. gabonensis*, since obesity is a predisposing factor to diabetes and loss of weight has been shown to improve insulin sensitivity. Omonkhua and Onoagbe (2011) attributed this effect to presence of phytochemicals such as tannins, saponins and high fibre content. Dzeufiet *et al*. (2009) also observed that defatted seeds of *I. gabonensis* consumed by diabetic rats produced a more positive effect on body weight. This they said could probably be due to its fat free composition. Fatty diets as have been observed are usually less consumed due to its high energy content (Dzeufiet *et al*., 2009). Nangue *et al*., (2011) opined that the oil extracted from *I. gabonensis* seed is made of 90 percent saturated fatty acids. Saturated fatty acids are known to increase cholesterol concentration and expose us to the risk of cardiovascular diseases.

Bhavna and Sharma (2012) investigated the *in-vitro* hepatoprotective effect of methanol extract of *I. gabonensis* on CC14-induced liver cell damage as well as the possible antioxidant mechanism involved in this protective effect. Phytochemical analysis of the

extract showed the presence of seven compounds identified as: 3-friedelanone, betulinic acid, oleanolic acid, 3, 3ĝ, 4ĝ-tri-O-trimethylellagic acid, methyl gallate, hardwickiic acid and 3-ƃ-acetoxyursolic acid. It was found that compounds such as oleanolic acid, 3-ƃ-acetoxyursolic acid, methyl gallate and betulinic acid showed significant hepatoprotective activity as indicated by their ability to prevent liver cell death and LDH leakage during CCl4 intoxication compounds oleanolic acid, methyl gallate and 3-ƃ-acetoxyursolic acid showed significant antioxidant effects involving radical scavenging action, inhibition of microsomal lipid peroxidation, ƃ-CLAMS and FRAP assays.

Furthermore Hossain *et al*. (2012), reported that the oral consumption of food incorporated with *I. gabonensis* crude powder resulted in reduced serum glucose level. In addition to its high dietary fibre, *I. gabonensis* posses insulinomimetic or insulin sensitizing effect. Based on this study *I. gabonensis* was suggested not to have changed liver glycogen contents significantly. Hence, it seems that lowering of blood glucose by *I. gabonensis* seeds may not have been accomplished through glycogenesis.

Phytochemicals from other plants have also been found to have hypoglycemic effect. Cooked *Lupinus mutabilis* (a legume) and its purified extract were found to have hypoglycemic effects on subjects with type 2 diabetes (Baldeon *et al*. 2012). Shukla *et al*. (2012) also found that lepidine and semilepidine found in *Lepidium sativum* linn (garden grass) had anti-diabetic effect against alloxan-induced diabetic rats, probably through the reduction of oxidative damage and modulating antioxidant enzymes. They claimed that the possible mechanism *L. sativum* seed total alkaloid brought about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from the remaining islet beta cells.

1.2.8 Mechanism in alloxan action

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyura- cil) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution (Rohilla and Ali, 2012). Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 °C is about 1.5 minutes and is longer at lower temperatures.

Alloxan was first described by Brugnatelli in 1818. Wöhler and Liebig used the name ñalloxanò and described its synthesis by uric acid oxidation. The diabetogenic properties of this drug were reported many years later by Dunn *et al.* (1943), who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Since then, alloxan diabetes has been commonly utilized as an animal model of insulin-dependent diabetes mellitus (IDDM). The name Alloxan emerged from the merging of two words, i.e., allantoin and oxaluric acid. Allantoin is a product of uric acid excreted by the foetus in the allantois and oxaluric acid has been derived from oxalic acid and urea that is found in urine. Alloxan was originally prepared by the oxidation of uric acid by nitric acid. It has been regarded as a strong oxidizing agent that forms a hemiacetal with its reduced reaction product; dialuric acid, in which a carbonyl group is reduced to a hydroxyl group, that is called alloxantin (Rohilla and Ali, 2012).

Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose in alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse. The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg body weight. When alloxan is given intraperitonealy or subcutaneously its effective dose must be 2 - 3 times higher. The intraperitoneal dose below 150 mg/kg b.w. may be insufficient for

inducing diabetes in the rat. Fasted animals are more susceptible to alloxan (Szkudelski *et al*. 2001), whereas increased blood glucose provides partial protection (Szkudelski *et al*., 2001).

Alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment (Lachin and Reza, 2012). This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used. Further, the alloxan action in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups. Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result in alloxan reduction, dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species and superoxide radicals (Das *et al*., 2012). The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions. In addition, superoxide radicals undergo dismutation to yield hydrogen peroxide (H_2O_2) in the presence of superoxide dismutase. As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of ferrous and H2O2. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. The fragmentation of DNA takes place in the beta cells exposed to alloxan that causes DNA damage, which stimulates poly ADP-ribosylation, a process participating in DNA repair (Rohilla and Ali, 2012). Antioxidants like superoxide dismutase, catalase and the non- enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity (Ebelt *et al*., 2000). In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an important step in the

diabetogenic action in alloxan. It has been noted that alloxan elevates cytosolic free Ca2+ concentration in the beta cells of pancreatic islets. Calcium influx is resulted from the ability in alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca2+ ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets (Szkudelski, 2001; Etuk, 2010).

Induction of diabetes in the laboratory animals by alloxan injection is the result of selective uptake in alloxan via GLUT2 into pancreatic beta cell (Elsner *et al*. 2000). This is because alloxan is an unstable chemical which also have a similar shape with glucose.

The chemical induction of diabetes appears to be the most popularly used procedure in inducing diabetes mellitus in experimental animals. The foremost drug-induced diabetic model is the alloxan diabetes that is capable of inducing type I diabetes mellitus in experimental animals. The surgical and genetic methods of diabetes induction are associated with a high percentage of animal morbidity and mortality. Hence, Rohilla and Ali (2012) are of the opinion that alloxan induced diabetes model appears to be the most reliable and easily reproducible method of inducing diabetes mellitus in experimental animals.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Procurement of *Irvingia* (*Wombolu* and *Gabonensis*) Seeds

The fruits of *Irvingia gabonensis and Irvingia wombolu* were collected from Ibeagwa-aka in Nsukka Local Government Area, Enugu state Nigeria. They were duely identified at the Taxonomy unit, Department of Plant Science and Biotechnology, University of Nigeria Nsukka, where voucher specimens were deposited at the herbarium. The seeds were removed from the nuts of the fruit, dried in an oven at 40 ºC for 24 hours. The dried seeds were powdered with electrical grinders, and stored in laboratory.

2.2 Procurement of Experimental Drug

Glibenclamide (5 mg) was procured from Juhel Nigeria Limited, Nkwubor road Emene Enugu, Enugu State, Nigeria.

2.3 Procurement and Management of Experimental Animals

A total of 72 adult albino rats were used for the experiment. The animals were procured from the Genetics and Animal Breeding Unit, Zoology and Environmental Biology Department, University of Nigeria Nsukka. The animals were acclimatized in clean rat cages at the Animal Breeding Unit of the Department of Zoology and Environmental Biology, University of Nigeria Nsukka for 14 days. They were feed ad-libitum with standard diet (Top Feed growers mesh) and allowed free access to water as well for the period of acclimatization.

2.4 Preparation of *Irvingia* Seeds

Dried *I. gabonensis* and *I .wombolu* seeds were pulverized using electrical grinders. The seed powders of both species were combined in three different proportions namely; 20% *I.*

gabonensis : 80% *I .wombolu* (IGIW1), 80% *I. wombolu* : 20% *I. gabonensis* (IGIW2) and 50% *I. gabonensis* : 50% *I. wombolu* (IGIW3). IGIW1, IGIW2 and IGIW3 were then oven dried at 40 ºC for 24 hours and in the laboratory.

2.5 Phytochemical Analysis

The alkaloid, flavonoids, tannin, saponins, and total phenol contents of the seeds of *I. gabonensis* and *I.wombolu* were determined as detailed below:

2.5.1 Alkaloid determination

This was done according to Okwu and Omodamiro (2005). Five gram each of powdered samples of *I. gabonensis* and *I. wombolu* mixture were weighed into a 250 ml beaker and 200 ml of 10% acetic acid and ethanol was added, covered and allowed to stand for 4 hours. These were filtered and the filtrate evaporated in a Water bath at 60 ºC to about ¼ of the original volume. Concentrated ammonium hydroxide was added drop-wise until precipitation was completed. The solution was then allowed to settle and precipitate collected and washed with dilute ammonium hydroxide solution, and then filtered. The residues were weighed and recorded as the total alkaloid.

2.5.2 Determination of tannin content

This was carried out by the method of Van-Burden and Robinson (1981). Five grams each of the powdered seeds was weighed into a 100 ml plastic bottle and 50 ml of distilled water added and shaken for 2 hours in a volumetric flask and made up to the mark. Then 5 ml of each filtrate was pipetted into a tube and mixed with 3 ml of 0.1M $FeCl_2$ in 0.1 N HCI and 0.008M potassium ferricyanide. The absorbance of the colour developed was then read at 120 nm within 10 minutes. A standard was prepared by using tannin acid.

2.5.3 Saponin determination

Total saponin content was determined according to Obadoni and Ochuko (2001). Two grams each of the seed powder sample was added to 200 ml of 20% ethanol. The suspensions formed were heated for 4 hours with stirring at 55^{o}C. The mixtures were then filtered and the residues re-extracted with another 200 ml of 20% ethanol. The resulting extracts were evaporated to about 20 ml in a water bath at 90^{o}C. The concentrates were transferred to a 250 ml separating funnel and 10 ml of diethylether added. The aqueous layer of each was recovered while the ether layer was discarded. The purification process was repeated by adding 30 ml of n-butanol. The combined n-butanol extracts were washed two times with 5 ml of 5% aqueous sodium chloride. The remaining solutions were then evaporated in a water bath. The samples were dried in an oven to a constant weight and the weight recorded in grams.

2.5.4 Flavonoid determination

Ten ml of ethylacetate was added to about 0.2g each of the samples and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for the following test.

a. **Ammonium test:** Four ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicated the presence of flavonoids.
b. **1% Aluminum chloride solution test:** Four ml portion of the filtrate was shaken with 1 ml of 1% aluminum chloride solution. The layers were allowed to separate. A yellow colour in the aluminum chloride layer indicated the presence of flavonoids.

2.5.5 Determination of anthraquinones

Two milliliters of 10% hydrochloric acid were added to the crude seeds in a test tube and boiled for about two minutes. Equal amount of chloroform was added to the test tube and vortexes twice, the chloroform layer was pipette out and then equal volume of 10% ammonia was added. The mixture was allowed to stand for 30 minutes after which absorbance was read at 550 nm (Trease and Evans, 1983).

2.5.7 Determination of total steroid

This was done according to Harbone (1973). One gram each of the samples was weighed into a mortar and macerated with 20 ml of ethanol and filtered. Two ml of colour reagent was added and allowed to stand for 30 minutes. Absorbance was read at 550 nm.

2.6 Toxicity Test

The lethal dose (LD_{50}) of each of *I. gabonensis* and *I. wombolu* was determined according to the method of Lorke (1983). A preliminary test was done using three graded doses (10, 100 and 1000 mg/kg bw) of each sample.

Each dose served as a group with three mice (mean weight 26.3 ± 4.84) each. No deaths were recorded after 24 hours, then, three higher doses (1600, 2900 and 5000 mg/kg of body weight) was used as groups with two mice each. The mice were closely observed for 24 hours for any mortality and next three days for any delayed toxic effects. Their food consumption, behaviour and weight were also examined once daily up to three days.

2.7 Induction of Diabetes in the Rats

Alloxan monohydrate (Sigma Aldrich, St, Louis, Missouri, USA; stored at 4 ^{o}c) was dissolved in 9% normal saline at room temperature. Diabetes was evoked in the animals by intraperitonal (IP) injection in alloxan in a dose of 120 mg/kg body weight after an overnight fast.

A day after alloxan injection, fasting blood glucose level was assessed to confirm the diabetic state. Rats with fasting blood glucose values of at least 150 mg/dl were used for the experiment.

2.8 Experimental Design

A total of 72 adult male albino rats were used for the experiment. A latin square design of six treatment groups replicated thrice, with each replicate having 4 male albino rats each (figure 2) was used for the experiment. Group 1 served as the positive control receiving standard diet and distilled water, group 2 served as diabetic control receiving distilled water and standard feed, group 3 on the other hand served as the standard drug control and will receive 5mg/kg body weight of Glibenclamide by oral medication, while, groups 4, 5 and 6 received 800mg/kg body weight each of IGIW1, IGIW2 and IGIW3 combinations of crude seed powder of *I. gabonensis* and *I. wombolu* respectively. The IGIW1, IGIW2 and IGIW3 of the crude seed powder were macerated in aqueous solution and given to the rats by oral route. The animals were fed once a day while their water was changed anytime in the day when the need arises. The quantity of feed and water consumed by each group of animal was measured at the end of every day. One animal each was taken from all replicates of each group at 7 days interval for 21 days for blood sample collection and further analyses.

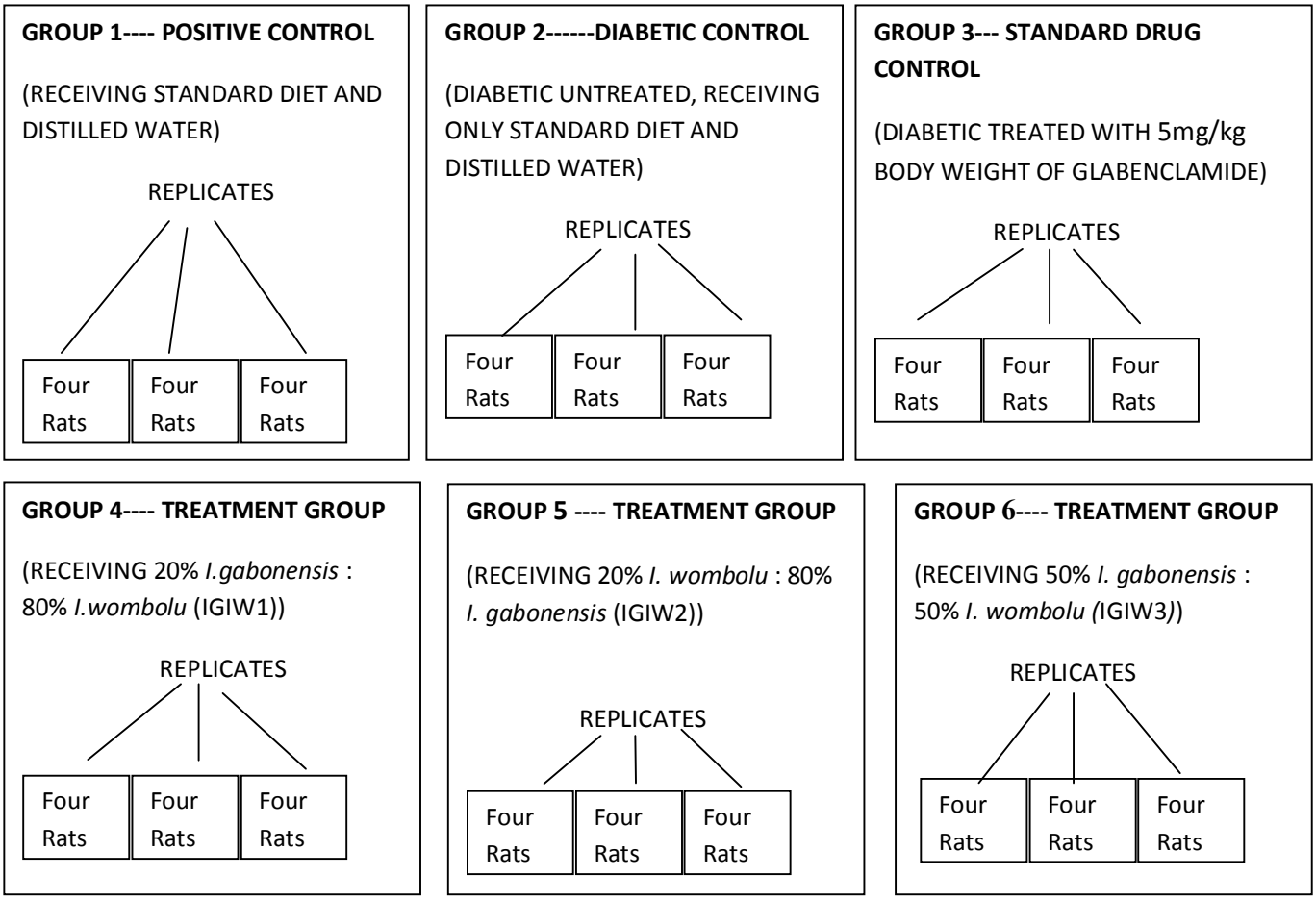


Figure 2: Experimental design to study the pathophysiological effect of combinations of *Irvingia gabonensis* and *Irvingia wombolu* on alloxan-induce diabetic rats.

2.9 Blood Sample Collection

Blood samples were collected from the orbital sinus into ethylenediaminetetracetic (EDTA) tubes for analyses. Blood samples were centrifuged for serum collection and analyses. Analyses were carried out at Shalom Laboratories in Nsukka Local Government Area of Enugu State.

2.10 Determination of Blood Glucose Level

After overnight fasting, fasting blood glucose levels were determined by slightly cutting the tip of the ratôs tail with a scissors and dropping the blood on the electronic glucometer known as Accu-Check. The fasting blood glucose level was read off in mg/dl.

2.11 Serum Lipid Profile

2.11.1 Total cholesterol (TC)

The determinations of serum total cholesterol (TCL) levels were done using the CHOD-PAP method (Trinder, 1969). 1.0 ml of total cholesterol working reagent was pipette into each of three clean dry test tubes labeled standard, blank and test. 0.01 ml of distilled water was added into the blank, while 0.01 ml each of standard cholesterol and serum was added into the standard and the test respectively. The contents of the test tubes were mixed thoroughly and allowed to incubate at 37^{o}C for 5 minutes. The absorbance of the standard (Ab. S) and the test sample (Ab. T) were measured against the blank at 500 nm within 6 minutes. Total cholesterol was calculated using the formular:

TC (mg/dL) = Ab.T/Ab. S x 200.

High density lipoprotein cholesterol (HDL-C)

High density lipoprotein cholesterol (HDL-C) was first of all be precipitated in the presence

of phosphotungistic acid and magnesium chloride (Trinder, 1969). 500 µl of plasma sample was added into marked Eppendorf tubes. Then, 50 µl of precipitating reagent was pipetted into the same tube. After mixing, the tube was centrifuged at 10,000 rpm for 15 minutes. The clear supernatant was pipetted into a tube and HDL determined. The colour that developed was read at 550 nm.

2.11.3 Total triglyceride (TG)

Total triglyceride was determined by the enzymatic method described by Buccolo and David (1973) using commercially available kit.

Triglycerides were first hydrolyzed to glycerol and free fatty acids. Glycerol was then phosphorylated by adenosine-5'-triphosphate (ATP) to form glycerol-1-phosphate (G-l-P) and adenosine-5'-diphosphate (ADP) in the reaction catalyzed by glycerol kinase. The triglyceride working reagent was prepared according to the manufacturer's instructions, while the spectrophotometer was set at 550 nm. After warming the working reagent to assay temperature, a series of cuvets labeled blank, standard and sample were set up. 1 ml of triglyceride working reagent was pipetted into each cuvet. 10 µl of water, glycerol standard, and sample were added into corresponding cuvet. The mixture was homogenized by inversion. The mixture was incubated for 5 minutes at 37^{o}C. The optical density or absorbency (A) of blank, standard, and sample was read at 550 nm.

2.11.4 Low density lipoprotein cholesterol (LDL-C)

Low density lipoprotein concentration was determined using the method of kit product of Randox, UK. 0.4 mL of sample was pipetted into a centrifuge test tube. Then, 0.2 mL of LDL-C precipitating reagent was added to the sample in the test and mixed thoroughly. The mixture was allowed to stand for 15 minutes at room temperature; then centrifuged at 4000 rpm for 15 minutes, after which the supernatant was carefully collected

into another test tube. The Absorbance was then read at 500 nm against a blank.

2.12 Liver Enzyme Assay

The determination of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities were done using kit product of Randox UK (Bergmayer *et al,* 1986).

Aspartate aminotransferase and alanine aminotransferase (AST and ALT) assay

0.5 ml of reagent 1 (buffer) was added into each of two test tubes containing 0.1 ml of serum (sample test) and 0.1 ml of distilled water (sample test) respectively. The contents of the test tubes were mixed thoroughly and incubated at 37°C for exactly 30 minutes. 0.5 ml of reagent 2 (2, 4 - dinitrophenylhydrazine) was then added into each of the sample blank and the sample test. The contents were mixed thoroughly and allowed to incubate at 25°C for exactly 20 minutes, after which 5 ml of NaOH was added into each of the test tubes. The contents were mixed and the absorbance of the sample was read against the reagent blank at 546 after 5 minutes.

Alkaline phosphatase assay (ALP)

3.0 ml, 1.0 ml and 0. 5 ml of the reagent was pipetted into cuvettes, labeled macro, semi-macro and micro, containing 0.05 ml, 0.02 ml and 0.01ml of serum respectively. The contents of the cuvettes were mixed thoroughly and the initial absorbance was read, after which absorbance was read repeatedly after 1, 2 and 3 minutes. ALP was calculate using the formulae:

U/L = 3300 x 405 nm/minute Macro

U/L = 2760 x 405 nm/minute Semi-micro

U/L = 2760 x 405 nm/minute Micro

2.13 Assessment of Malondialdehyde (MDA) Concentration in the Plasma

Plasma homogenate (0.1 ml) was added to 0.4 ml of thiobarbituric acid, and allowed to boil at 100°C for 15 minutes; tubes were uncovered and refrigerated in cold water for 15 minutes. The tubes were later centrifuged at 3000 rpm for 5 minutes and the absorbance of the supernatant was read at 532 nm. MDA concentration was estimated using molar extinction coefficient (Ů=1.53, 105M-1cm-1).

2.14 Histopathological Studies

Tissue specimen of liver, kidney and pancreas were collected from each group (1 - 6) after clinical and gross examination of the stunned albinorats and immediately fixed in 10% neutral buffered formalin. Dehydration was done using ascending grades of ethanol (70, 80, and 100%) for 1hour each. The specimens were then cleared in two changes of xylene. After blocking using soft paraffin, serial sections of 4µm thickness were done. The sections were stained using routine haematoxylin and eosin stain and examined using binocular microscope at magnifications of X100 and X400 and photomicrograph taken using motic camera.

2.15 Statistical Analysis

Data obtained were recorded as mean ± SEM. One way Analysis of Variance (ANOVA) was used to determine significant difference between treatments means. Duncan's Multiple Range Test (DMRT) was used to separate means of groups. Level of significance was set at $P<0.05$. The probit value was determined from the probit model developed by Finney (1971).

CHAPTER THREE

RESULTS

3.1 Qualitative and Quantitative Phytochemical Composition of Crude Seed Powder of *Irvingia gabonensis*

Qualitative and quantitative phytochemical screening of crude seed powder of *Irvingia gabonensis* indicated that reducing sugar, tannin and glycosides are abundantly present in the crude seed powder of *Irvingia gabonensis,* while alkaloid and steroid are moderately present. However saponins, flavonoids, terpenoids and anthocyanins are present in small quantities (Table 1). Steroids (69.983 ± 0.78) had the highest concentration, followed by alkaloids (66.98 ± 0.25), reducing sugar (22.80 ± 2.91), terpenoid (4.83 ± 0.10), flavonoid (3.02 ± 0.11), glycoside (1.77 ± 0.04), tannin (1.37 ± 0.01), anthocyanin (1.04 ± 0.13), the least in concentration being saponin (0.64 ± 0.03) (Table 1).

3.2 Qualitative and Quantitative Phytochemical Composition of Crude Seed Powder of *Irvingia wombolu*

Qualitative and quantitative phytochemical screening of crude seed of *Irvingia wombolu* showed that reducing sugar and tannin are abundantly present in the crude seed powder of *Irvingia wombolu*, while alkaloid, glycosides, flavonoids, terpenoids and steroid are moderately present. However saponins, and anthocyanins are present in small quantities (Table 2). Alkaloid (80.47 ± 0.11) had the highest concentration, followed by steroid (78.86 ± 0.78), reducing sugar (9.19 ± 1.49), terpenoid (3.80 ± 0.05), flavonoid (3.77 ± 0.06), glycoside (1.94 ± 0.01), tannin (1.63 ± 0.07), saponin (0.79 ± 1.12), the least in concentration being anthocyanin (0. 68 ± 0.07) (Table 2).

Table 1: Qualitative and quantitative phytochemical compositions of crude seed of *Irvingia gabonensis*

Parameter	Quality	Quantity (mg/100g)
Flavonoid	+	3.02 ± 0.11
Alkaloid	++	66.98 ± 0.25
Saponin	+	0.64 ± 0.03
Tannin	+++	1.37 ± 0.01
Terpenoid	+	4.83 ± 0.10
Reducing sugar	+++	22.80 ± 2.91
Glycoside	+++	1.77 ± 0.04
Steroid	++	69.33 ± 0.78
Anthocyanins	+	1.04 ± 0.13

+++ = Abundantly present

++ = Moderately present

+ = Present in small amount

Table 2: Qualitative and quantitative phytochemical compositions of crude seed of *Irvingia wombolu*

Parameter	Quality	Quantity (mg/100g)
Flavonoid	++	3.77 ± 0.06
Alkaloid	++	80.47 ± 1.11
Saponin	+	0.79 ± 0.04
Tannin	+++	1.63 ± 0.07
Terpenoid	++	3.80 ± 0.05
Reducing sugar	+++	9.19 ± 1.49
Glycoside	++	1.94 ± 0.08
Steroid	++	78.86 ± 0.78
Anthocyanins	+	0.68 ± 0.07

+++ = Abundantly present

++ = Moderately present

+ = Present in small amount

3.3 Acute Toxicity Test of Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu*

At the expiration of the 24 hours period of observation for both death and behavioural manifestations, it was observed that the animals maintained stable emotion and level of activity after the administration of the different doses (10 mg/kg, 100 mg/kg, 200 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of body weight) of crude seed powders of both *Irvingia gabonensis and Irvingia wombolu,* although the animals were seen scratching the mouth region. This implies that *Irvingia gabonensis and Irvingia wombolu* have wide range effective dose and utility. Hence, usage within and above these doses used is safe for consumption.

3.4 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on the Body Weights (BW) in Alloxan-Induced Diabetic Rats

The effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the body weight in alloxan-induced diabetic rats indicated that there was no over dose-dependent significant diference ($P<0.05$) observed in the mean daily body weight of the treatment groups, when compared with the positive and the diabetic control (Table 3). At day 7, the mean BW of rats in all the treatment groups increase, although only the animal in the treatment group receiving IGIW2 increased significantly compared to the positive and diabetic control. By day 14, BW of animals in treatment groups IGIW1, IGIW2 and the standard control group increased significantly ($P<0.05$) when compared with the control. The standard control group and IGIW3 increased significantly ($P<0.05$) when compared with the positive control, while treatment groups IGIW1 and IGIW2 decreased though insignificantly ($P<0.05$) when compared with the other treatment groups and the positive control at day 21.

The time dependent analysis showed that the standard control group increased ($P<0.05$) with time, having the highest BW by day 21. The body weight of animals in other groups did not vary significantly from day 0 (Table 3).

3. 5 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Fasting Blood Glucose Levels (FBGL) in Alloxan-Induced Diabetic Rats

The effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the fasting blood glucose level in alloxan-induced diabetic rats showed that the FBGL of diabetic rats was significantly ($P<0.05$) higher compared with the positive control. However FBGL of the standard control and the treatment groups decreased significantly during the treatment days compared to day 0, the least values being recorded at day 21. It was rather observed that the variation in FBGL of diabetic control was insignificant throughout the period of treatment (Table 4).

3. 6 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Total Cholesterol (TC) Levels in Alloxan-Induced Diabetic Rats

The time dependent effects of combinations of crude Seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on total cholesterol levels in alloxan-induced diabetic rats showed that the TC levels in all the treatment groups, standard and diabetic control group varied significantly ($P<0.05$) with time. TC levels in diabetic control increased significantly ($P<0.05$) in all the treatment days compared with day 0. It was also observed that the TC values in all the treatment groups and the standard control group decreased significantly ($P<0.05$) compared to day 0, having the least values by day 21 (Table 5).

The dose dependent analysis showed that TC levels in the treatment groups and the standard control varied significantly ($P<0.05$) when compared with positive and diabetic control. It

was observed that TC levels in all the treatment groups and standard control decreased constantly, although the values in IGIW1and IGIW2 were statistically similar ($P<0.05$) at day 7 and 21. The values recorded in IGIW3 group were statistically similar ($P<0.05$) to the positive control. However, TC values increased significantly ($P<0.05$) in the diabetic control compared to the positive control (Table 5).

3. 7 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on High Density Lipoprotein Cholesterol (HDL-C) Levels in Alloxan-Induced Diabetic Rats

The time dependent effects of combinations of crude seeds of *Irvingia gabonensis* and *Irvingia wombolu* on high density lipoprotein-cholesterol (HDL-C) levels in alloxanï induced diabetic rats revealed that the diabetic and standard control, as well as the treatment groups varied significantly ($P<0.05$) with time compared to day 0, while there was no significant difference ($P<0.05$) in HDL-C levels of animals in the positive control group compared with time. HDL-Cvalues of diabetic control group decreased significantly with time compared to day 0. It was rather observed that the HDL-C levels of the standard control group and treatment groups increased significantly ($P<0.05$) compared to day 0 (Table 6).

It was revealed from the dose-dependent analysis that the HDL-C levels of the diabetic control group was significantly ($P<0.05$) lower compared to the values of the positive control. However, the standard control and the treatment groups increased significantly ($P<0.05$) compared with the positive control especially at by days 14 and 21 (Table 6).

Table 3: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the body weight (gm) of diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	115.80 ± 7.67^{a1}	130.90 ± 5.33^{b1}	119.87 ± 7.24^{b1}	128.57 ± 7.44^{b1}
Diabetic Control	130.27 ± 13.75^{ab1}	129.57 ± 15.03^{b1}	138.73 ± 14.26^{ab1}	146.00 ± 15.87^{ab1}
Standard Control	147.63 ± 1.89^{a1}	145.73 ± 2.78^{ab1}	158.48 ± 1.65^{a2}	167.47 ± 2.43^{a3}
IGIW1	126.83 ± 12.28^{ab1}	138.53 ± 10.66^{b1}	150.80 ± 10.90^{a1}	146.47 ± 16.56^{ab1}
IGIW2	145.77 ± 0.33^{a12}	167.17 ± 6.81^{a2}	168.13 ± 7.31^{a2}	139.57 ± 11.51^{ab1}
IGIW3	139.46 ± 5.29^{ab1}	153.83 ± 5.20^{ab12}	149.77 ± 5.63^{a12}	159.17 ± 6.21^{ab2}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).

Mean values with different numbers as superscript in a row are significantly different ($P<0.05$)

Table 4: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the fasting blood glucose level (mg/dL) of diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	70.00 ± 7.15^{d23}	53.67 ± 0.67^{c1}	63.00 ± 4.58^{d12}	75.33 ± 4.70^{c3}
Diabetic Control	218.00 ± 2.08^{b1}	201.67 ± 8.11^{b1}	211.67 ± 19.04^{ab1}	206.00 ± 23.12^{b1}
Standard Control	418.33 ± 16.24^{a3}	283.67 ± 43.05^{a2}	152.33 ± 37.17^{c1}	227.33 ± 60.14^{a2}
IGIW1	291.00 ± 124.00^{c3}	289.67 ± 96.34^{a3}	271.33 ± 90.79^{ab2}	174.00 ± 78.31^{b1}
IGIW2	341.67 ± 126.61^{c3}	294.67 ± 101.24^{a2}	269.00 ± 95.13^{ab12}	243.63 ± 75.74^{a1}
IGIW3	391.33 ± 116.70^{a3}	278.00 ± 113.70^{a2}	244.33 ± 103.63^{ab2}	153.67 ± 58.71^{b1}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

Table 5: Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on SerumTotal Cholesterol levels (mg/dL) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	60.33 ± 0.88^{b1}	60.33 ± 1.86^{d1}	64.33 ± 2.33^{cd1}	67.67 ± 2.96^{b1}
Diabetic Control	113.00 ± 4.73^{a1}	151.67 ± 3.18^{a2}	154.33 ± 3.84^{a2}	157.33 ± 3.71^{a2}
Standard Control	116.67 ± 16.70^{a2}	92.67 ± 1.86^{bc12}	67.00 ± 1.53^{cd1}	63.67 ± 4.18^{a1}
IGIW1	100.67 ± 5.46^{a2}	92.67 ± 1.76^{bc2}	71.00 ± 1.53^{c1}	66.00 ± 3.06^{b1}
IGIW2	101.33 ± 5.46^{a2}	94.33 ± 3.18^{b2}	81.33 ± 1.86^{b1}	74.00 ± 1.54^{b1}
IGIW3	102.33 ± 2.85^{a3}	85.33 ± 2.91^{c2}	63.00 ± 0.58^{d1}	62.00 ± 5.57^{b1}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$)

Table 6: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on high density lipoprotein cholesterol levels (mg/dL) alloxan-induced of diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	55.33 ± 4.37^{a1}	55.33 ± 1.86^{a1}	61.00 ± 1.53^{a1}	56.00 ± 1.53^{a1}
Diabetic Control	34.67 ± 2.40^{b3}	17.00 ± 1.53^{d1}	18.00 ± 1.15^{d1}	26.00 ± 1.15^{d2}
Standard Control	38.33 ± 2.18^{b1}	51.00 ± 0.58^{ab2}	44.33 ± 2.85^{c12}	39.33 ± 2.03^{c1}
IGIW1	39.67 ± 1.86^{b1}	52.33 ± 2.03^{ab23}	53.67 ± 2.19^{b3}	49.00 ± 1.73^{b12}
IGIW2	36.33 ± 2.19^{b1}	49.33 ± 0.88^{b2}	46.67 ± 1.20^{c2}	45.67 ± 2.85^{bc2}
IGIW3	35.00 ± 2.08^{b1}	43.33 ± 2.84^{c2}	45.33 ± 2.03^{c2}	43.67 ± 2.19^{bc2}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

Table 7 Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on total triglyceride (TG) levels (mg/dL) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	120.33 ± 3.93^{a12}	126.33 ± 1.43^{b12}	115.67 ± 3.76^{c1}	128.67 ± 2.91^{b2}
Diabetic Control	126.33 ± 6.44^{a1}	139.00 ± 2.52^{a2}	148.00 ± 1.53^{a23}	157.66 ± 1.45^{a3}
Standard Control	117.00 ± 4.34^{a2}	112.33 ± 3.28^{c12}	104.67 ± 2.19^{d1}	108.67 ± 3.52^{d12}
IGIW1	117.67 ± 5.93^{a12}	111.00 ± 1.53^{c1}	126.00 ± 3.46^{b2}	117.33 ± 4.37^{cd12}
IGIW2	114.33 ± 1.00^{a1}	113.67 ± 2.60^{c1}	125.33 ± 2.91^{b2}	118.67 ± 4.67^{cd12}
IGIW3	116.00 ± 4.73^{a1}	114.00 ± 5.51^{c1}	120.67 ± 1.76^{bc1}	109.33 ± 4.67^{d1}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3. 8 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Total Triglyceride (TG) Levels in Alloxan-Induced Diabetic Rats

The effects of combinations of crude seeds of *Irvingia gabonensis* and *Irvingia wombolu* on total triglyceride (TG) levels in alloxan-induced diabetic rats showed significant differences ($P<0.05$) in the mean daily TG levels of the treated rats compared with the diabetic and the positive control (Table 7). It was observed that the mean daily TG levels of the diabetic control increased significantly ($P<0.05$) compared with other control and the treatment groups, while it decreased significantly ($P<0.05$) in the treatment groups and the standard control groups compared to the positive control. More so, the TG levels in of the treatment groups were statistically similar ($P<0.05$) to the standard control especially on days 7 and 21 (Table 7).

The time dependent analysis of the mean daily TG levels showed that the standard control group and treatment groups IGIW1 and IGIW2 varied significantly ($P<0.05$) at days 14 and 21, compared to day 0. It was observed that the TG levels of the diabetic control increased insignificantly ($P<0.05$) with time, while the TG levels of the treated rats significantly reduced during the treatment days compared to day 0 (Table 7).

3. 9 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Low Density Lipoprotein-Cholesterol (LDL-C) Levels in Alloxan-Induced Diabetic Rats

The dose dependent effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on low density lipoprotein cholesterol (LDL-C) levels in alloxan-induced diabetic rats showed that there were some significant differences ($P<0.05$) in the LDL-C values of the treated rats and other control groups compared with the positive control (Table 8). The LDL-C values of the diabetic control was significantly higher ($P<0.05$) compared with the treated rats and other control groups, while it decreased significantly ($P<0.05$) in the standard control and the treated rats. However, the mean daily LDL-C levels of the standard control and the treatment groups were statistically similar ($P<0.05$), although IGIW2 varied significantly ($P<0.05$) from the others by day 7 (Table 8).

The time-dependent analysis also revealed that the LDL-C values of the standard control and the treatment groups decreased significantly ($P<0.05$) during the treatment days compared to day 0, although the values were statistically similar ($P<0.05$) in some groups. However, there was no significant difference ($P<0.05$) in the LDL-C values of the positive and diabetic

control (Table 8).

3. 10 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Alanine Aminotransferase (ALT) Levels in Alloxan-Induced Diabetic Rats

The dose dependent effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on alanine aminotransferase (ALT) levels in alloxan-induced diabetic rats showed that the mean daily ALT values of the treated rats and other control groups varied significantly ($P<0.05$) compared with the positive control (Table 9). The ALT levels of the standard control and the treatment groups decreased constantly during the treatment days. The ALT levels of the standard control was statistically similar ($P<0.05$) to that of IGIW3 at days 7 and 14, but differed significantly ($P<0.05$) from IGIW1 and IGIW2 at days 7 and 21 (Table 9).

The time-dependent analysis showed that there were some significant difference ($P<0.05$) in the mean daily ALT levels of the groups during the treatment days compared to day 0 (Table 9).

Table 8: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on low density lipoprotein-cholesterol levels (mg/dL) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	23.33 ± 2.91^{c2}	16.33 ± 0.88^{d1}	20.67 ± 1.76^{c12}	24.67 ± 1.76^{b2}
Diabetic Control	88.00 ± 7.02^{a1}	96.00 ± 1.15^{a1}	90.00 ± 1.15^{a1}	96.67 ± 1.33^{a1}
Standard Control	63.33 ± 2.40^{b3}	33.33 ± 2.90^{b2}	34.33 ± 3.28^{b2}	22.33 ± 2.03^{b1}
IGIW1	65.00 ± 2.65^{b3}	34.67 ± 2.40^{b2}	33.33 ± 2.40^{b12}	25.67 ± 2.03^{b1}
IGIW2	64.67 ± 1.76^{b3}	27.67 ± 0.88^{c2}	30.33 ± 0.88^{b2}	33.67 ± 0.88^{b1}
IGIW3	60.67 ± 1.45^{b4}	39.00 ± 1.53^{b3}	32.67 ± 1.76^{b2}	21.67 ± 2.73^{b1}

Mean values with different alphabets as superscript in a column are significantly different (P<0.05).
Mean values with different numbers as superscript in a row are significantly different (P<0.05)

Table 9: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on alanine aminotransferase (ALT) levels (U/L) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	39.67 ± 0.88^{c1}	43.33 ± 26.06^{d1}	44.00 ± 2.30^{c1}	45.33 ± 3.17^{d1}
Diabetic Control	92.33 ± 3.05^{a12}	163.67 ± 2.33^{a3}	82.67 ± 7.05^{a1}	97.00 ± 0.58^{a2}
Standard Control	84.33 ± 2.33^{b3}	71.00 ± 1.53^{c2}	67.33 ± 1.45^{b2}	54.33 ± 0.89^{c1}
IGIW1	91.67 ± 2.02^{a4}	83.33 ± 1.76^{b3}	69.33 ± 2.85^{b2}	66.33 ± 1.45^{b1}
IGIW2	93.33 ± 1.85^{a3}	90.00 ± 2.30^{b23}	84.67 ± 0.88^{a2}	67.00 ± 2.52^{b1}
IGIW3	$97.33 \pm 1.2.402^{a}$	69.33 ± 2.60^{c1}	66.67 ± 2.40^{b1}	62.00 ± 1.53^{b1}

Mean values with different alphabets as superscript in a column are significantly different (P<0.05).
Mean values with different numbers as superscript in a row are significantly different (P<0.05).

3. 11 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Aspartate Aminotransferase (AST) Levels in Alloxan-Induced Diabetic Rats

The effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on aspartate aminotransferase (AST) levels in alloxan-induced diabetic rat revealed that the mean daily AST levels of the treatment groups and other control groups varied significantly from the positive control (Table 10). The AST levels of the diabetic control were increasing constantly, though the differences were insignificant ($P<0.05$), while it decreased in the standard control and the treatment groups, with a significant variation ($P<0.05$) from the positive control (Table 10).

The time-dependent analysis showed that the AST levels in all the treatment groups and the standard control varied significantly ($P<0.05$) from day 0. However, there were no significant differences in the AST levels among the treatment days especially for IGIW3 (Table 10).

3. 12 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Alkaline Phosphatase (ALP) levels in Alloxan-Induced Diabetic Rats

The dosed dependent effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on alkaline phosphatase (ALP) levels in alloxan-induced diabetic rats showed that all the treatment groups and other control groups varied significantly from the positive control (Table 11). The mean daily ALP levels of the diabetic control group increased in all the treatment days, although the variation was insignificant ($P<0.05$). However, it was observed that the ALP levels in the treated rats and the standard control decreased significantly ($P<0.05$) from the values as at day 0. It was rather observed that the variation in the ALP levels IGIW3 group was insignificant, except on day 21 (Table 11)

Table 10: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on aspartate aminotransferase (AST) levels (U/L) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	51.33 ± 1.86^{b1}	59.00 ± 0.57^{d2}	59.00 ± 0.58^{d2}	65.33 ± 1.76^{c3}
Diabetic Control	101.67 ± 3.84^{a1}	147.00 ± 2.51^{a2}	158.00 ± 3.06^{a3}	160.66 ± 1.20^{a3}
Standard Control	101.33 ± 4.05^{a2}	94.00 ± 1.15^{b2}	58.67 ± 2.03^{d1}	56.33 ± 1.45^{d1}
IGIW1	103.67 ± 3.17^{a4}	83.33 ± 1.76^{c3}	66.00 ± 1.53^{c2}	55.33 ± 2.90^{d1}
IGIW2	106.67 ± 4.58^{a3}	93.67 ± 2.91^{b2}	85.33 ± 2.33^{b2}	63.00 ± 31.51^{cd1}
IGIW3	107.67 ± 1.85^{a4}	88.00 ± 2.08^{c3}	70.67 ± 1.76^{c2}	58.67 ± 2.48^{cd1}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$)

Table 11: Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on alkaline phosphatase (ALP) levels (U/L) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	66.53 ± 2.73^{c1}	67.00 ± 1.00^{d1}	62.33 ± 1.86^{d1}	61.00 ± 1.53^{c1}
Diabetic Control	96.00 ± 1.15^{a1}	128.33 ± 2.85^{a2}	124.00 ± 5.03^{a2}	141.67 ± 5.49^{a3}
Standard Control	95.33 ± 3.18^{ab4}	82.67 ± 1.76^{b3}	72.67 ± 1.76^{bc2}	62.00 ± 1.15^{c1}
IGIW1	93.67 ± 1.86^{ab3}	74.67 ± 2.33^{c2}	64.33 ± 2.40^{cd1}	$65. 67 \pm 2.85^{c1}$
IGIW2	95.33 ± 3.18^{a3}	87.00 ± 3.61^{b2}	82.33 ± 1.45^{b12}	75.67 ± 2.91^{b1}
IGIW3	88.33 ± 2.19^{b3}	85.33 ± 1.76^{b3}	76.00 ± 3.79^{b2}	66.33 ± 1.45^{c1}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3. 13 Effects of Combinations Crude Seed Powders of *Irvingia gabonensis* and *Irvingia wombolu* on Malondialdehyde (MDA) Levels in Alloxan-Induced Diabetic Rats

The effects of combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on malondialdehyde (MDA) levels in alloxan-induced diabetic rats showed that there was a significant difference ($P<0.05$) between the mean daily MDA levels of the treatment groups and the standard control when compared with the positive control (Table 12). The MDA levels of the diabetic animals were significantly ($P<0.05$) higher in the diabetic animals. The MDA levels of treatment group IGIW1 increased constantly in all the treatment days, though the variation was not significant ($P<0.05$) when compared to day 0. However, groups IGIW2 and IGIW3 decreased insignificantly ($P<0.05$) by days 14 and 21. The MDA levels of the animals in the standard control group also increased significantly compare with the control (Table 12).

Table 12 Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on malondialdehyde level (mg/dL) in alloxan-induced diabetic rats

Groups	Day 0	Day 7	Day 14	Day 21
Positive Control	1.26 ± 0.21^{b1}	1.61 ± 0.17^{c1}	1.49 ± 0.08^{cd1}	1.46 ± 0.01^{d1}
Diabetic Control	2.28 ± 0.02^{a1}	2.12 ± 0.05^{b1}	2.16 ± 0.08^{b1}	2.91 ± 0.34^{a1}
Standard Control	2.19 ± 0.03^{a1}	2.11 ± 0.16^{bc1}	2.27 ± 0.17^{ab1}	2.34 ± 0.14^{bc1}
IGIW1	2.57 ± 0.86^{ab1}	2.10 ± 0.46^{bc12}	2.40 ± 0.17^{a12}	2.68 ± 0.03^{b2}
IGIW2	2.17 ± 0.12^{a1}	2.42 ± 0.21^{a2}	1.82 ± 0.26^{bc12}	2.14 ± 0.08^{c12}
IGIW3	2.53 ± 0.18^{ab1}	2.55 ± 0.18^{a2}	2.32 ± 0.19^{ab2}	2.17 ± 0.24^{c12}

Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).
Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.14 Histopathological Features of the Pancreases, Kidney and Liver in Alloxan-induced Diabetic and Control Rats

3.14.1. Histopathological features of the pancreas of rat in alloxan-induced diabetic and control rats

3.14.1.1 Histopathological features of the pancreas of rat from the positive control group

The photomicrograph of the histopathological section of the pancreas of rat from the positive control group showed normal pancreatic micro-architecture. Intact intercalated ducts and islets of Langerhans were observed (Plate 1). Visible interlobular glands and connective tissues were also observed (Plate 1).

3.14.1.2 Histopathological features of the pancreas of rat from the diabetic control group

The photomicrograph of the histopathological section of the pancreas of rat from the diabetic control group showed marked changes in the normal pancreatic micro-architecture. The islet cells were relatively inconspicuous and generally decreased in number (Plate 2) depicting severe alteration in the endocrine portion of the pancreas. More so, the acinar cells were slightly disintegrated with scanty inflammatory infilterates; although the intercalated ducts were severely enlarged (Plate 2).

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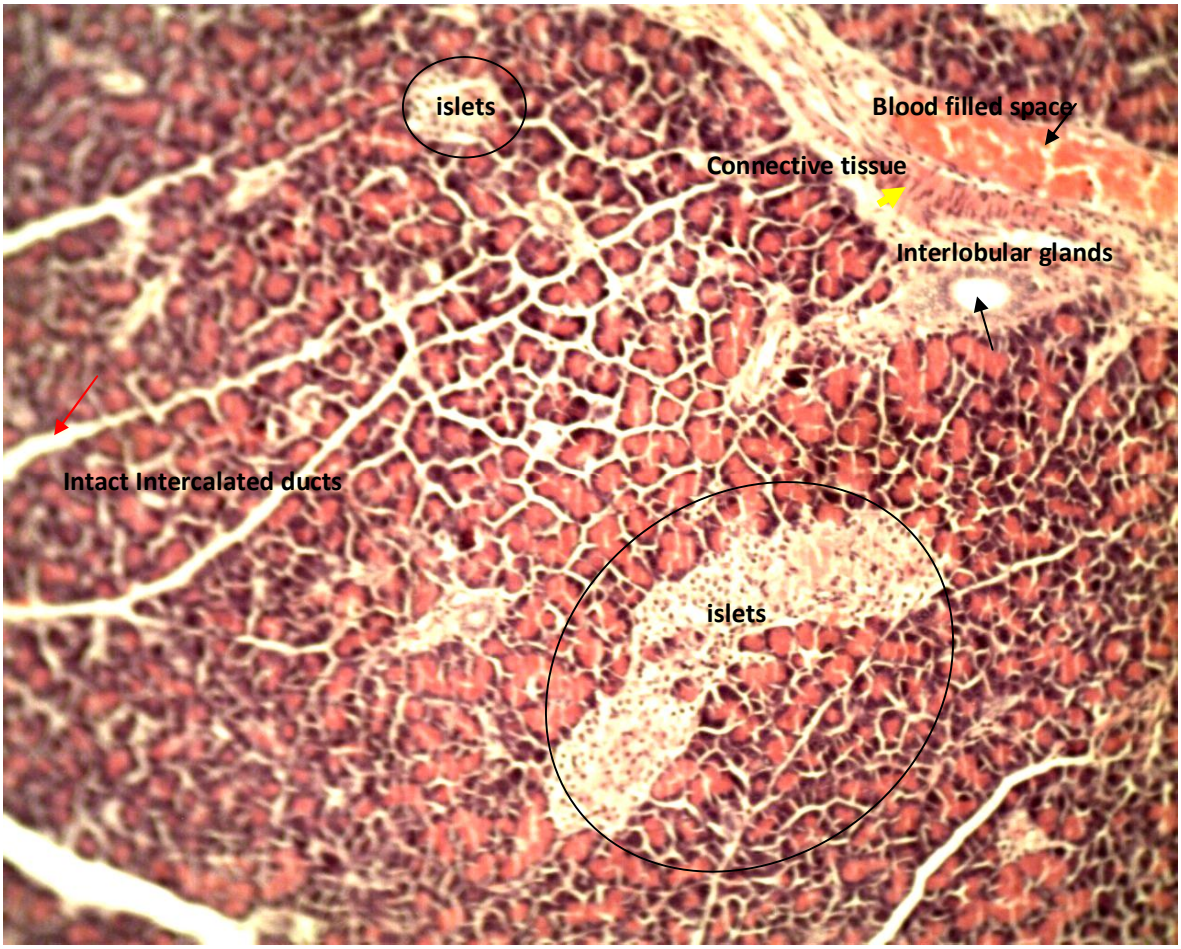

Plate 1: Photomicrograph of the pancreatic tissue of rat from the positive control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Islet cells good proportion of islet cell and intact intercalated duct.

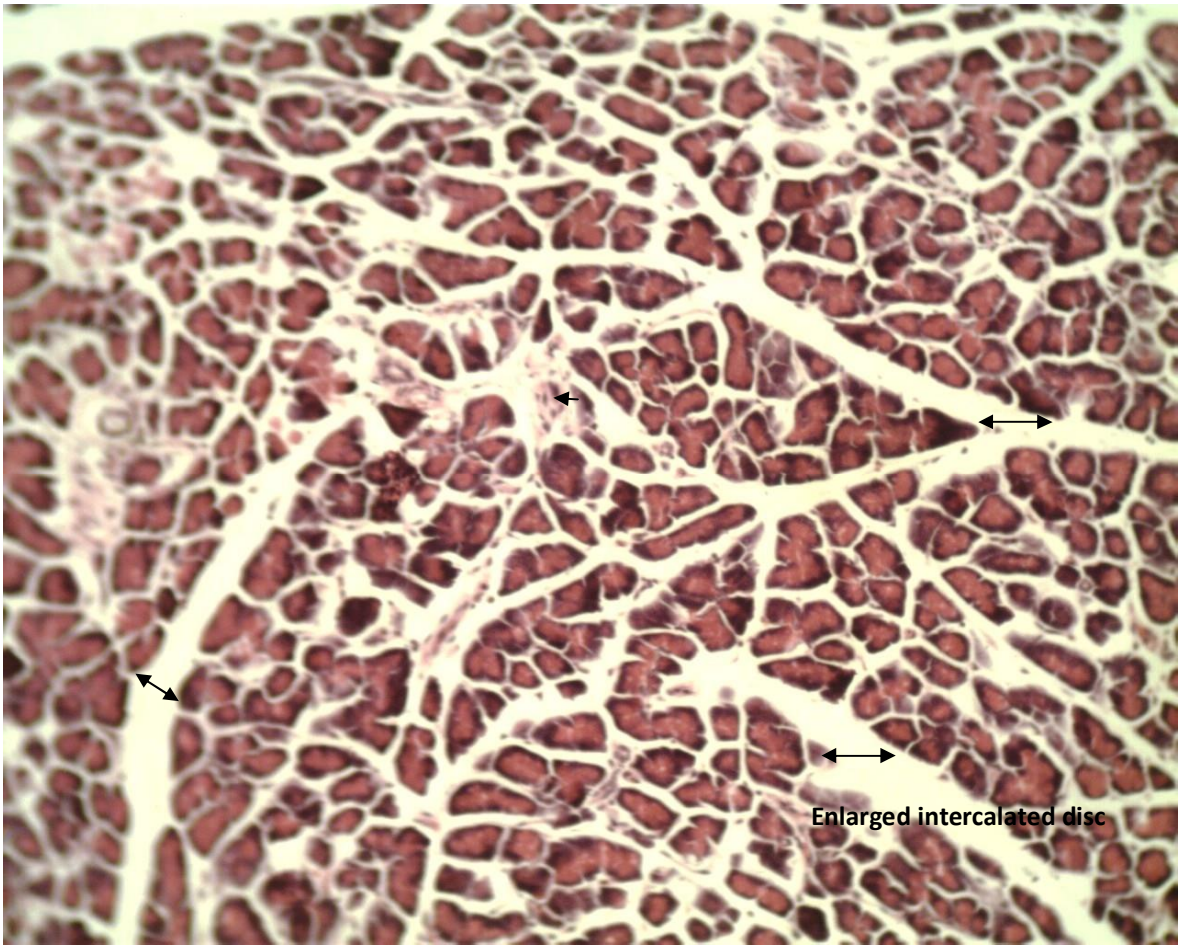

Plate 2: Photomicrograph of the pancreatic tissue of rat from the diabetic control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Severely enlarged intercalated ducts with presence of inflammatory infilterates
- Disintegrated acinar cells which appear not clearly arranged in a lobular form.

3.14.1.3 Histopathological features of the pancreas of rat from the standard control group

The photomicrograph of the histopathological section of the pancreas of rat from the standard control group revealed alterations in the normal pancreatic micro-architecture. Scanty inflammatory cell infilterations were observed and the intercalated ducts were also enlarged (Plate 3). However, good proportions of the islet cells were seen embedded in the acinar cells (Plate 3).

3.14.1.4 Histopathological features of the pancreas of rat from IGIW1 group

The photomicrograph of the histopathological section of the pancreas of rat from the IGIW1 group revealed a marked change from the normal pancreatic micro-architecture. The surrounding capsule of the islet cells appears to be lost; hence the islet cells were inconspicuous (Plate 4). More so, the intercalated ducts were severely dilated and the acini appear disintegrated (Plate 4).

3.14.1.5 Histopathological features of the pancreas of a rat from IGIW2 group

The photomicrograph of the histopathological section of the pancreas of a rat from the IGIW2 group revealed some changes from the normal pancreatic micro-architecture. Large proportions of islet cells were surrounded by normal proportion of acinar cells, which were rather filled with heavy inflammatory infilterates (Plate 5).

3.14.1.6 Histopathological features of the pancreas of a rat from IGIW3 group

The photomicrograph of the histopathological section of the pancreas of a rat from the IGIW3 group revealed alterations in the normal pancreatic micro-architecture. The lobular arrangement of the acinar cells was disintegrated; hence, islet cells were greatly reduced (Plate 6). Intercalated ducts were also enlarged and filled with mixed infilterates (Plate 6).

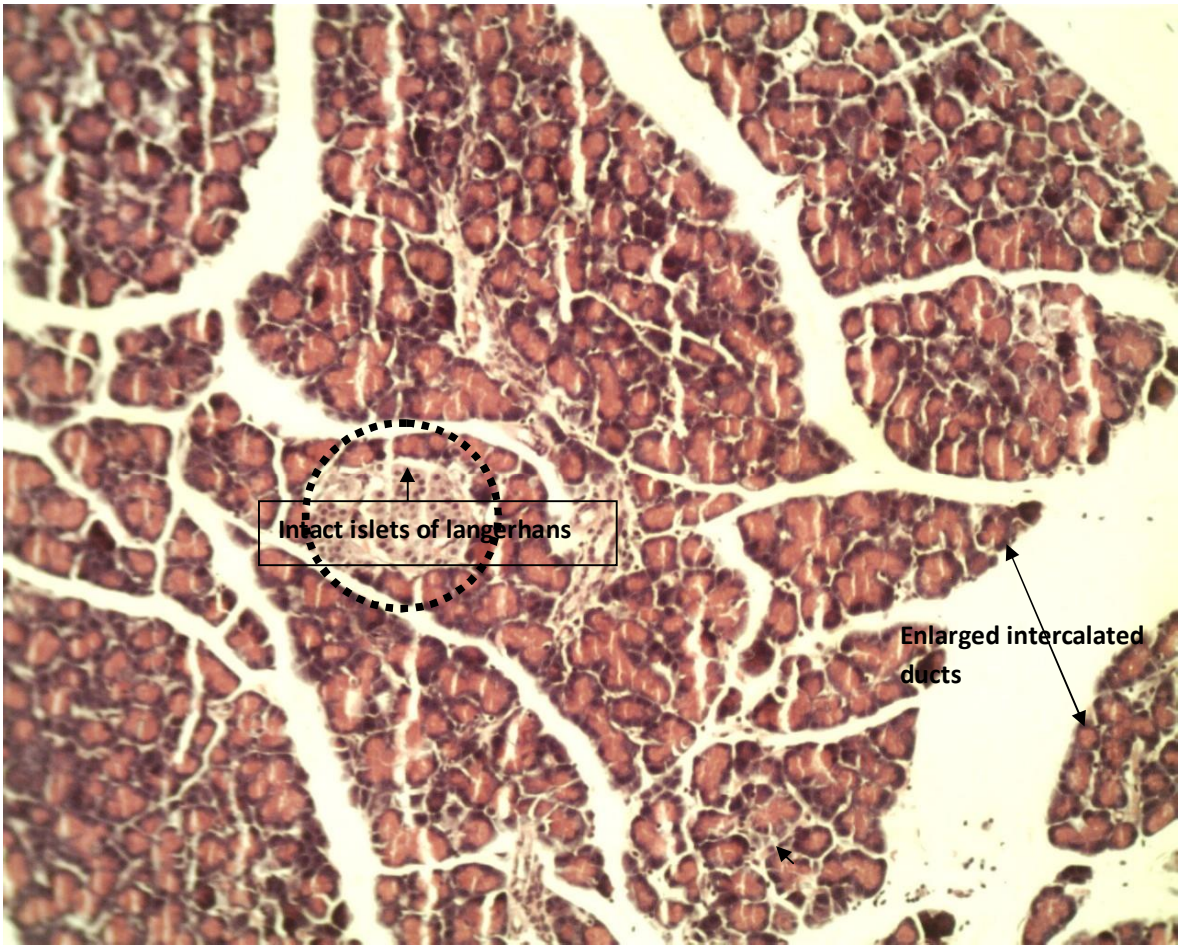

Plate 3: Photomicrograph of the pancreatic tissue of rat from the standard control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Severely enlarged intercalated ducts
- Intact islet cells

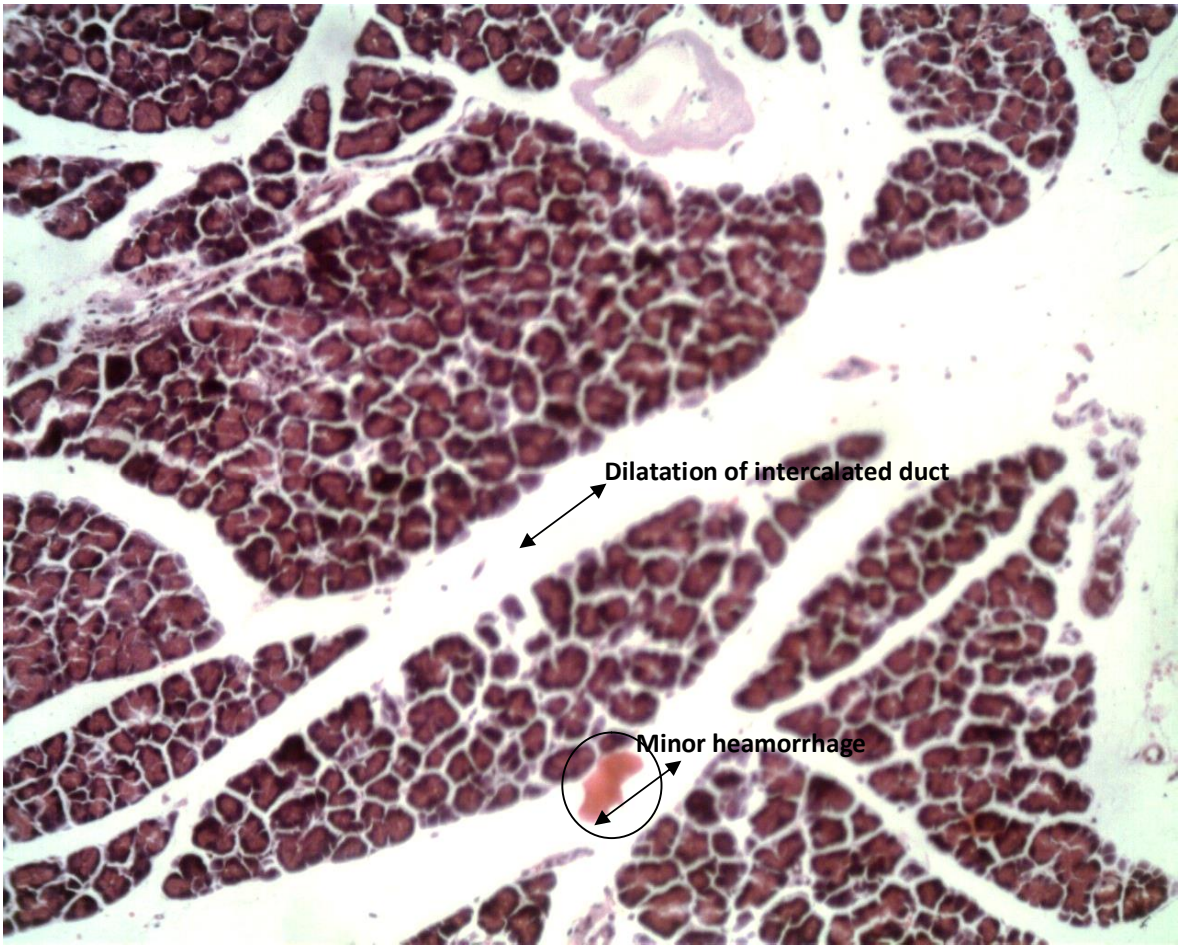

Plate 4: Photomicrograph of the pancreatic tissue of rat from the IGIW1 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Severely dilated intercalated ducts
- inconspicuous islet cells
- Disintegrated acini

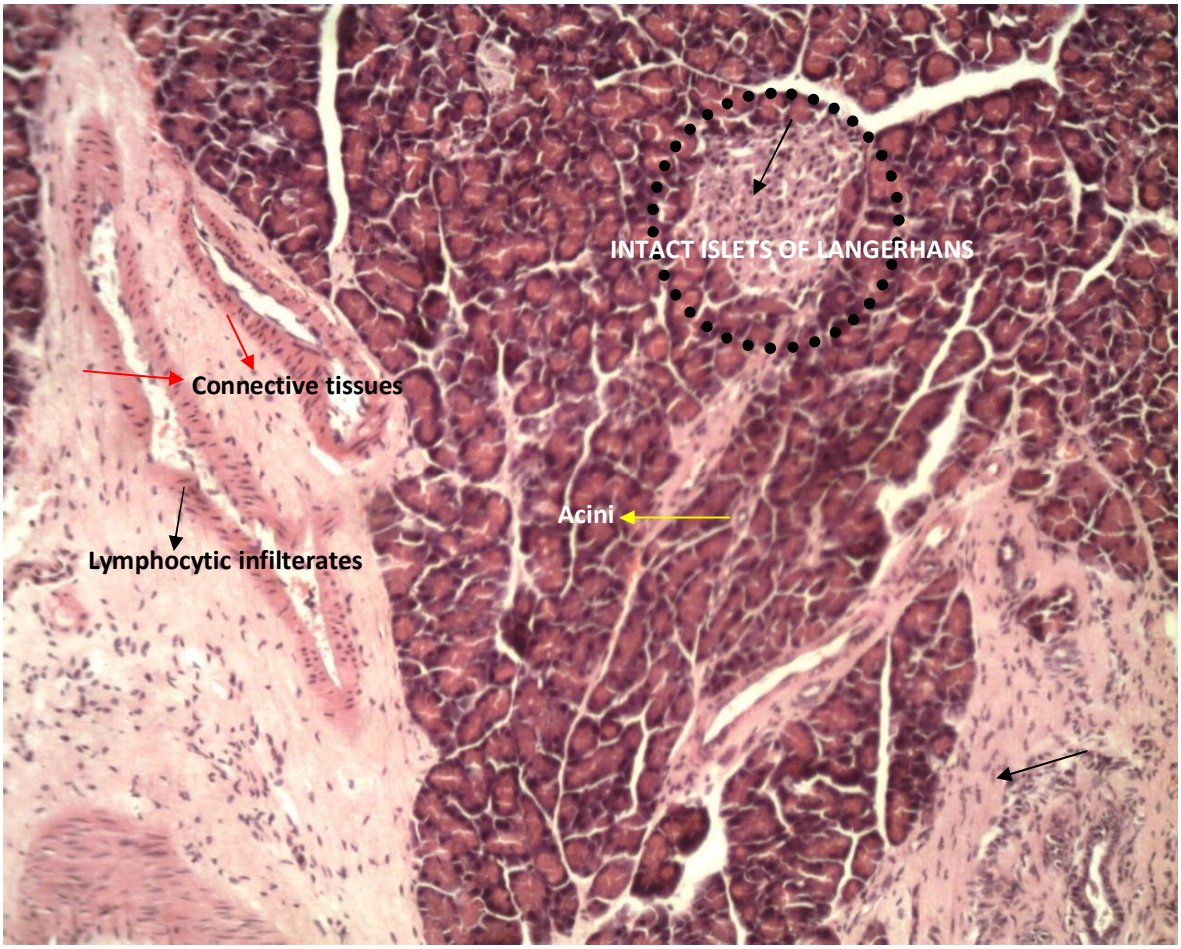

Plate 5: Photomicrograph of the pancreatic tissue of rat from the IGIW2 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Large proportion of islet cell surrounded by acinar cells
- Heavy lympocytic infilteration
- Heavy appearance of connective tissue

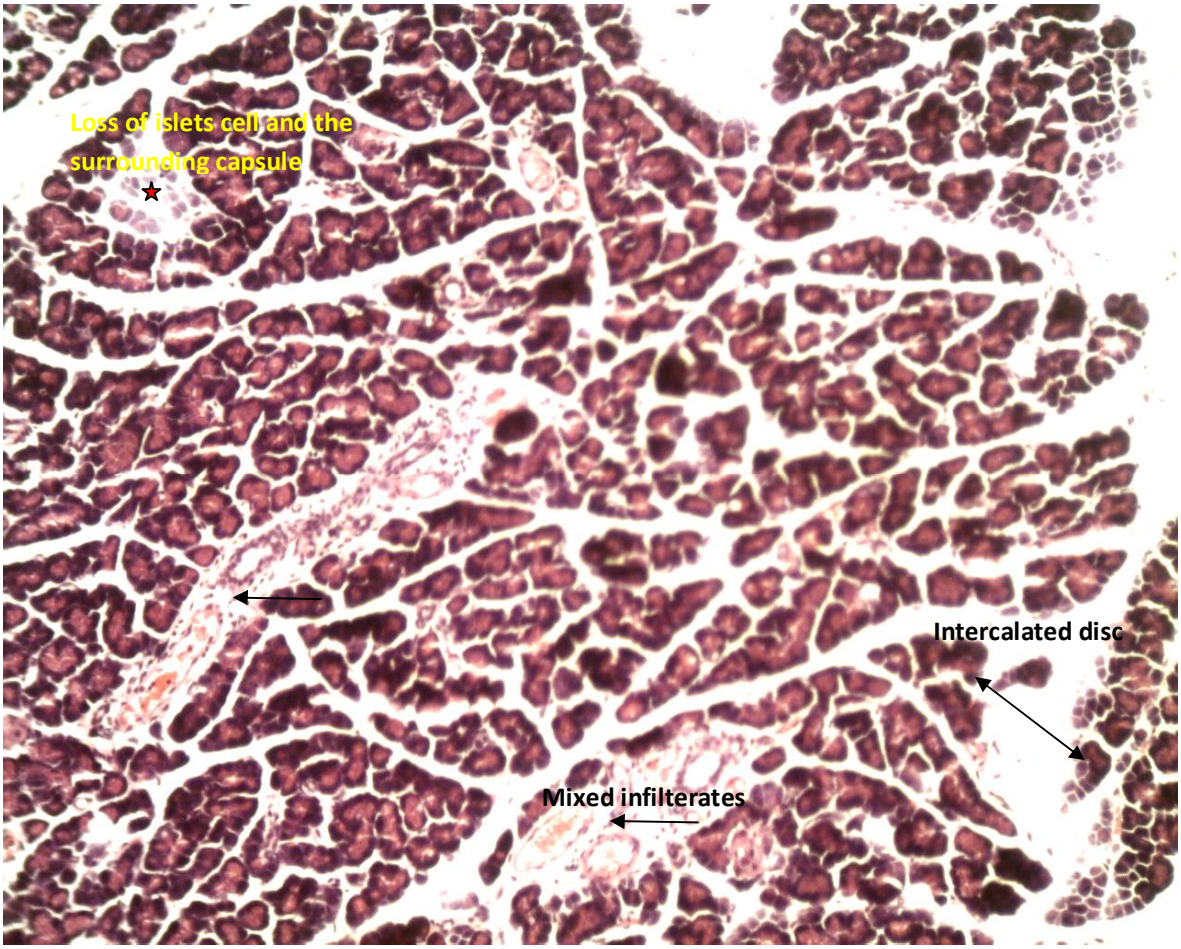

Plate 6: Photomicrograph of the pancreatic tissue of rat from the IGIW3 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Loss of islet cells and surrounding capsule
- Dilated intercalated ducts with mixed infilterates

3.14.2 Histopathological features of the kidney in alloxan-induced diabetic and control rats

3.14.2.1 Histopathological features of the kidney of a rat from positive control group

The photomicrograph of the histopathological section of the kidney of a rat from the positive control group revealed normal kidney micro-architecture. The renal corpuscles (glomerulus and bowmans space) and the distal and convulated tubules were intact (Plate 7).

3.14.2.2 Histopathological features of the kidney of a rat from the diabetic control group

The photomicrograph of the histopathological section of the kidney of a rat from the diabetic control group revealed alterations in normal kidney micro-architecture. Enlarged urinary spaces due to shrinkage of glomeruli were observed (plate 8). More so, there were presence of cytic tubules depicting a degenerative change and inflammatory infilterations owing to interstitial nephrities (Plate 8).

3.14.2.3 Histopathological features of the kidney of a rat from standard control group

The photomicrograph of the histopathological section of the kidney of a rat from the standard control group revealed alterations in normal kidney micro-architecture. Atrophy of the glomeruli and vacuolar degeneration was observed (plate 9). A congestion in the central zone of the cortex was also seen (Plate 9).

3.14.2.4 Histopathological features of the kidney of a rat from IGIW1 group

The photomicrograph of the histopathological section of the kidney of a rat from the IGIW1 group revealed shrinkage and contraction of glomerular tufts, with corresponding enlargement of urinary spaces, and presence of cystic spaces (Plate 10); all of which depict alterations in normal kidney micro-architecture.

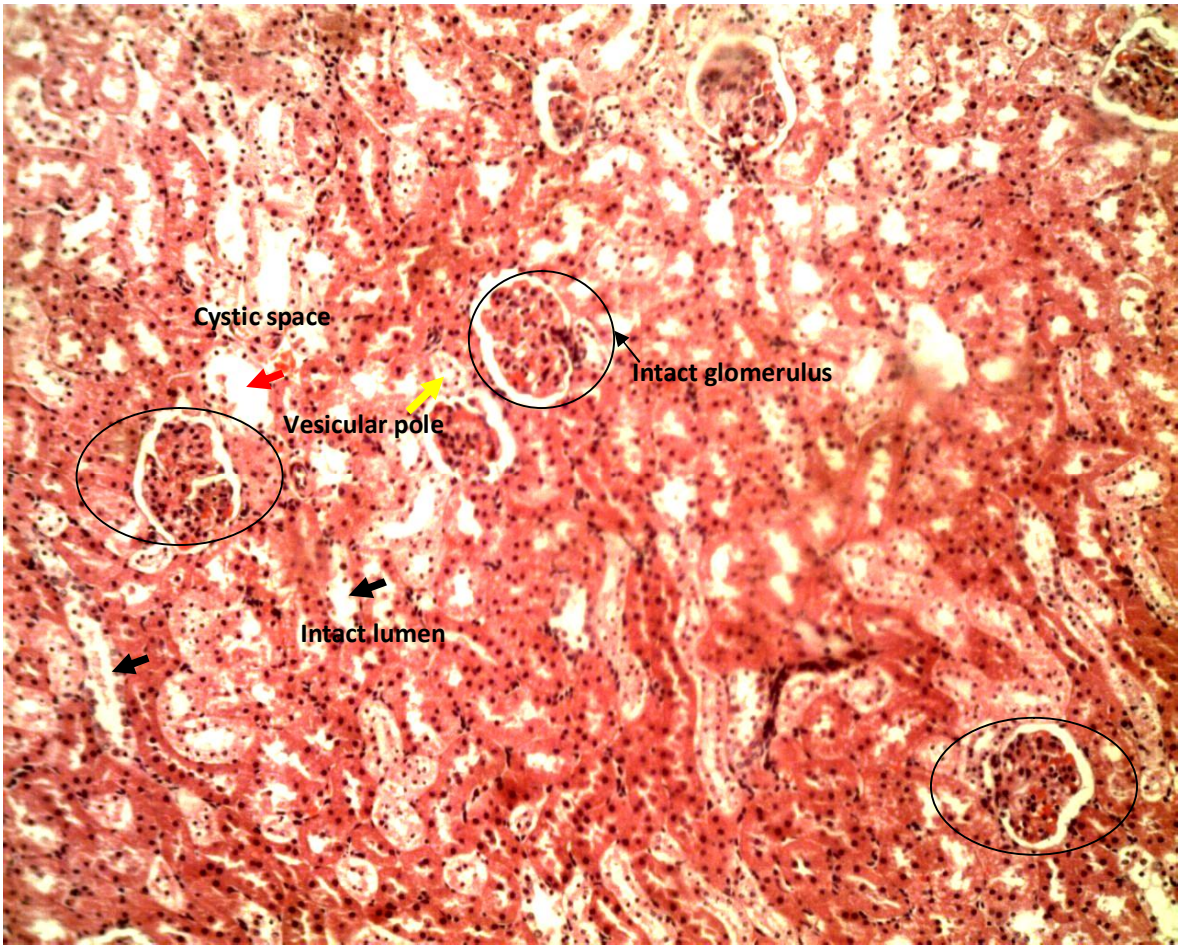

Plate 7: Photomicrograph of the kidney tissue of rat from the positive control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Intact renal capsules and tubules

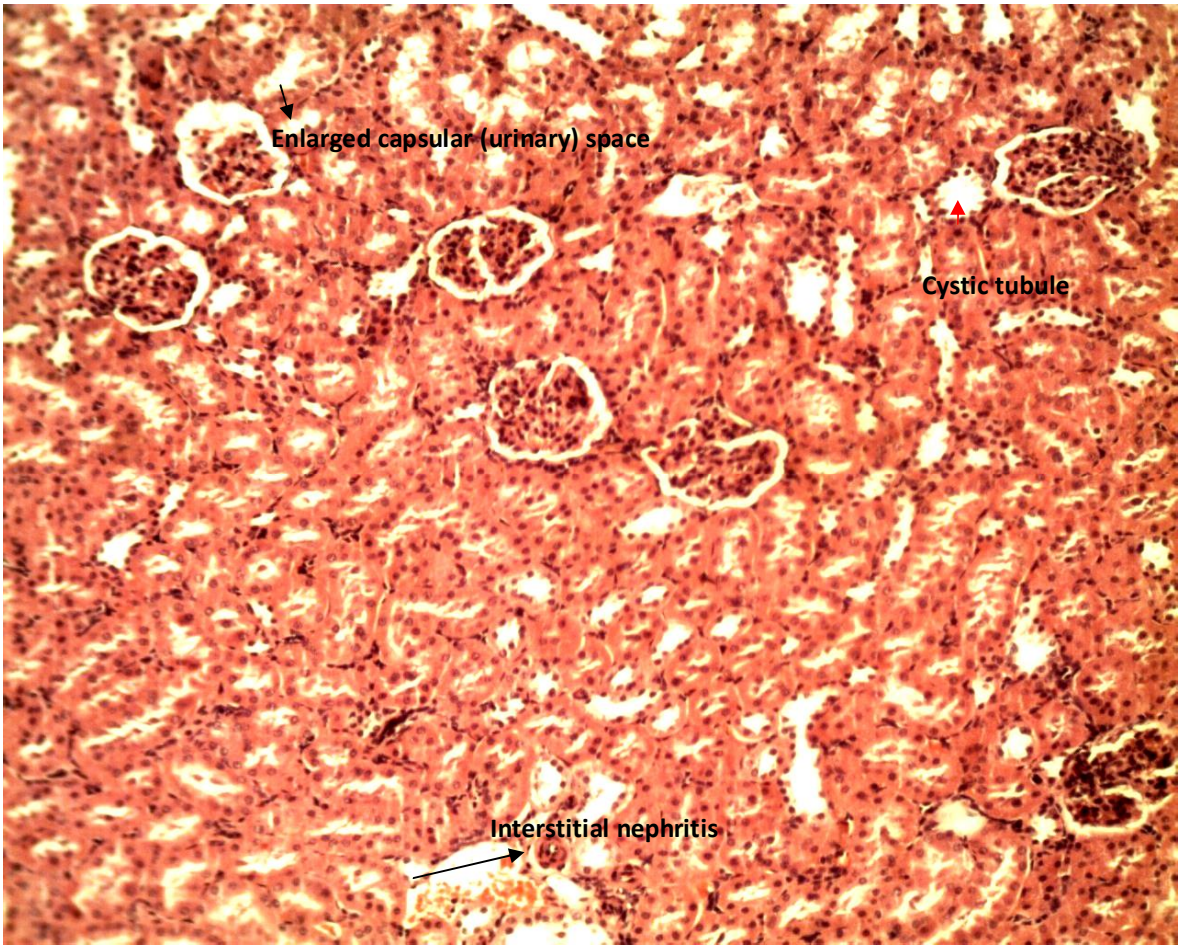

Plate 8: Photomicrograph of the kidney tissue of rat from the diabetic control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Enlarged capsular (urinary) space
- Presence of cystic tubule

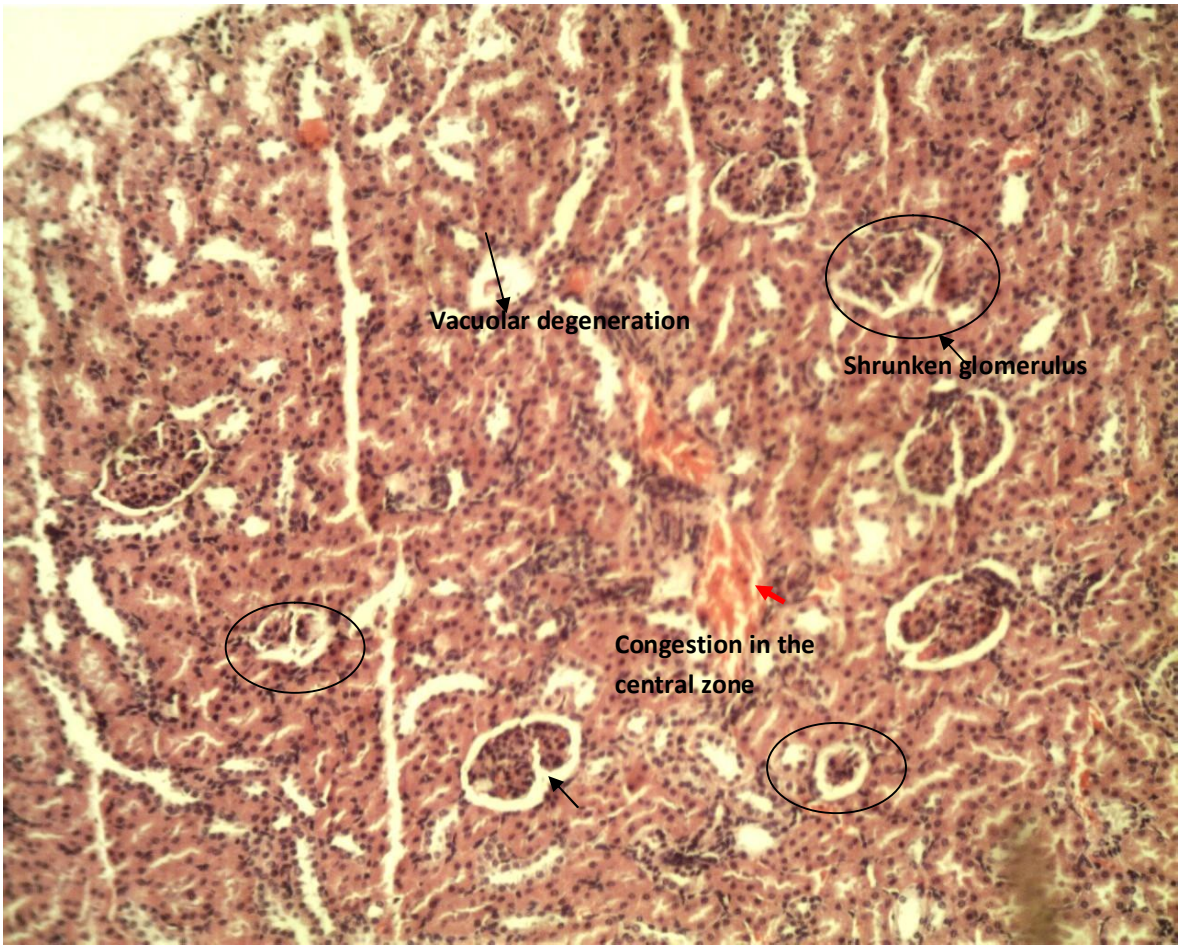

Plate 9: Photomicrograph of the kidney tissue of rat from the standard control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Shrunken glomeruli
- Vacuolar degeneration of the renal tubular epithelium

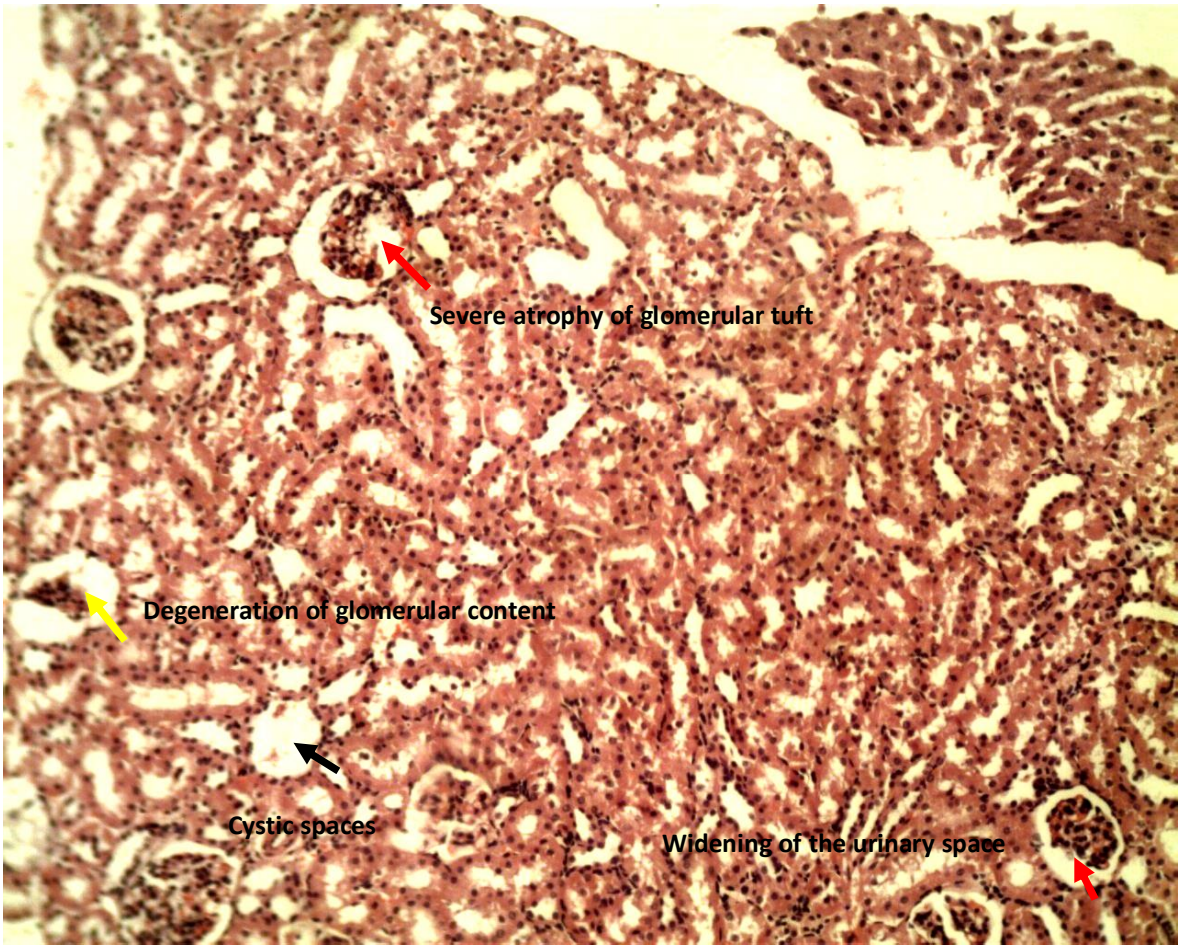

Plate 10: Photomicrograph of the kidney tissue of rat from the IGIW1 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Severe atrophy of glomerular tufts and widening of urinary space
- Presence of cystic space

3.14.2.5 Histopathological features of the kidney of a rat from IGIW2 group

The photomicrograph of the histopathological section of the kidney of a rat from the IGIW2 group revealed alterations in normal kidney micro-architecture. There were severe glomerular and tubular atrophy due to contraction and shrinkage of glomerular tufts and tubular structure (Plate 11).

3.14.2.6 Histopathological features of the kidney of a rat from IGIW3 group

The photomicrograph of the histopathological section of the kidney of a rat from the IGIW3 group revealed alterations in normal kidney micro-architecture. Intact renal corpuscles and tubules were observed, although one of the glomeruli was shrunken and reduced in size (Plate 12).

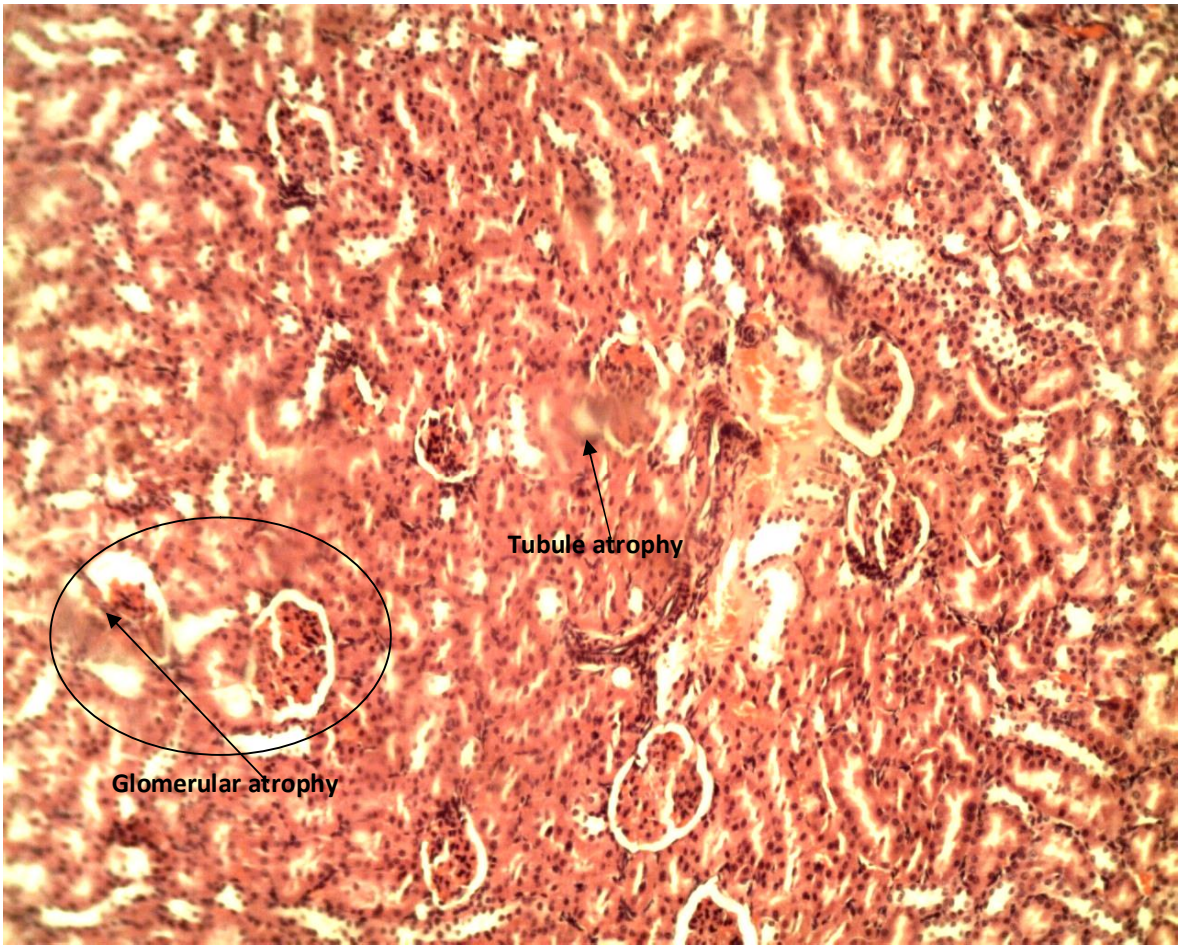

Plate 11: Photomicrograph of the kidney tissue of rat from the IGIW2 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Severe glomerular and tubular atrophy

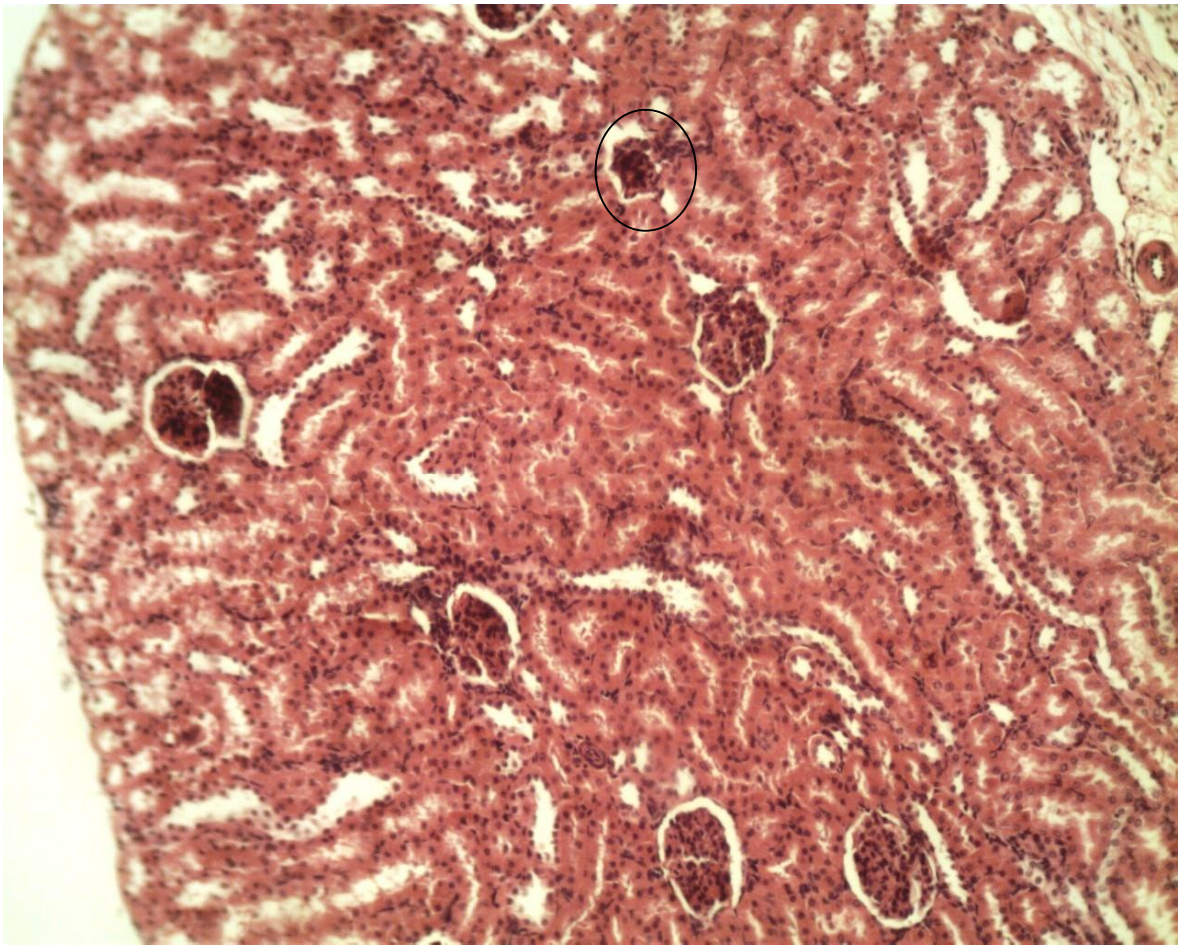

Plate 12: Photomicrograph of the kidney tissue of rat from the IGIW3 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Intact renal corpuscles and tubules

3.14.3 Histopathological features of the liver in alloxan-induced diabetic and control rats

3.14.3.1 Histopathological features of the liver of a rat from positive control group

The photomicrograph of the histopathological section of the liver of a rat from the positive control group revealed normal hepatic micro-architecture. Intact hepatocytes were seen embedded in lobules (Plate 13). Also, sinusoids with normal kupffer cells were observed (Plate 13).

3.14.3.2 Histopathological features of the liver of a rat from diabetic control group

The photomicrograph of the histopathological section of the liver of a rat from the diabetic control group revealed some deviations from normal hepatic micro-architecture. There was presence of minor distorted hepatic chords, neutrophilic infilterations and hyperplasia of kupffer cell, although the hepatocytes were relatively intact (Plate 14).

3.14.3.3 Histopathological features of the liver of a rat from standard control group

The photomicrograph of the histopathological section of the liver of a rat from the standard control group showed a marked change from normal hepatic micro-architecture. Portal triads and periportal inflammation were observed (Plate 15). Amyloid deposits were also observed in the central canal (Plate 15)

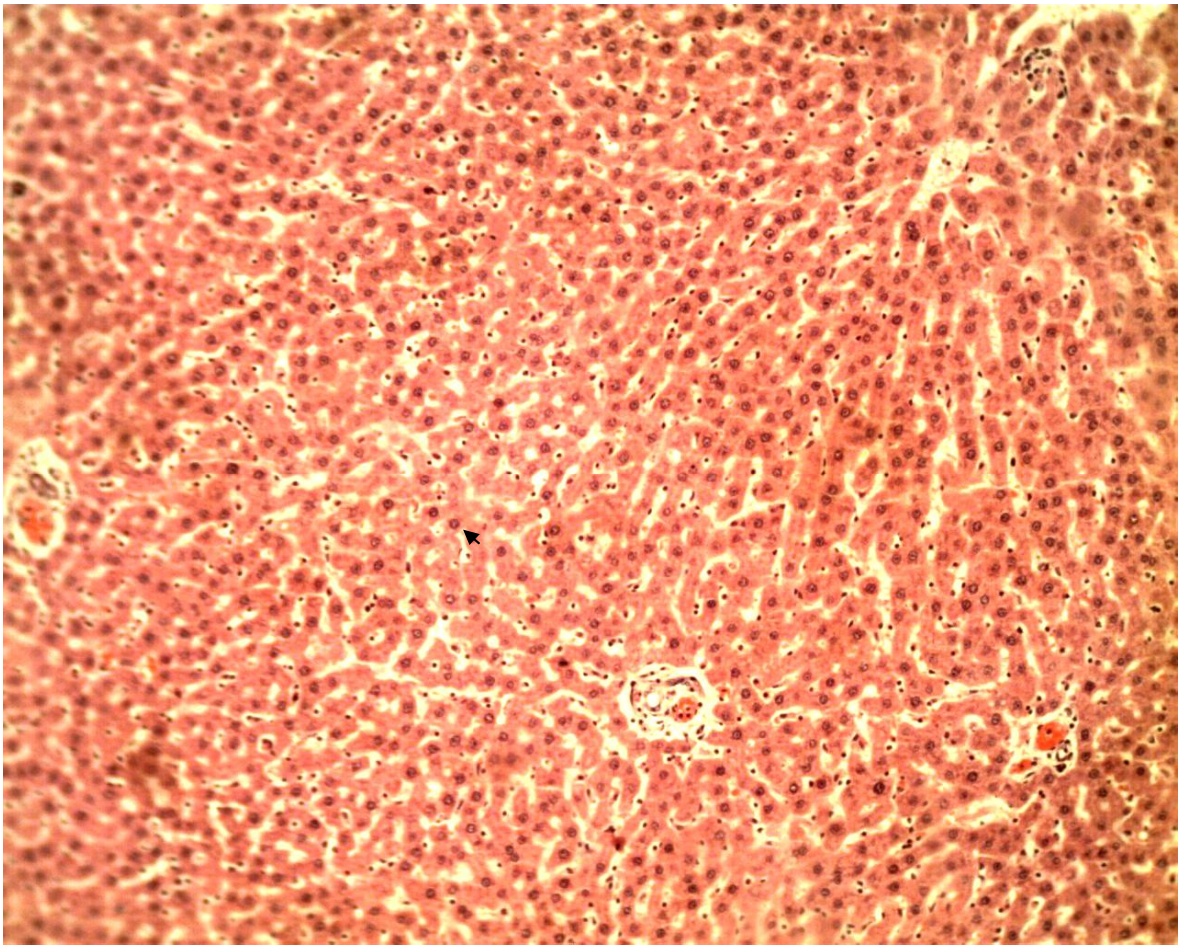

Plate 13: Photomicrograph of the liver tissue of rat from the positive control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Intact hepatocytes embedded in lobules
- Sinusoids with normal kupffer cells

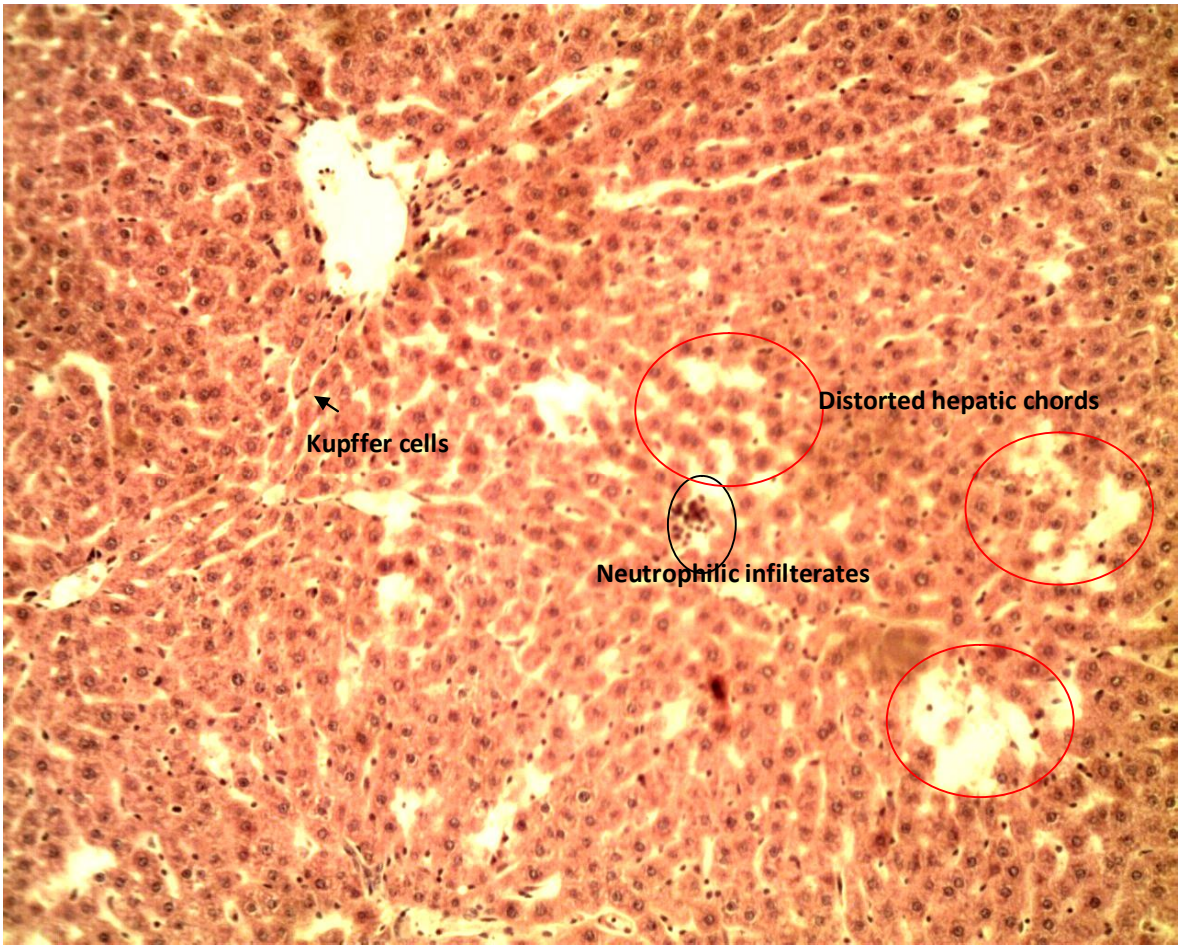

Plate 14: Photomicrograph of the liver tissue of rat from the diabetic control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Distorted hepatic chord
- Hyperplasia of kupffer cells

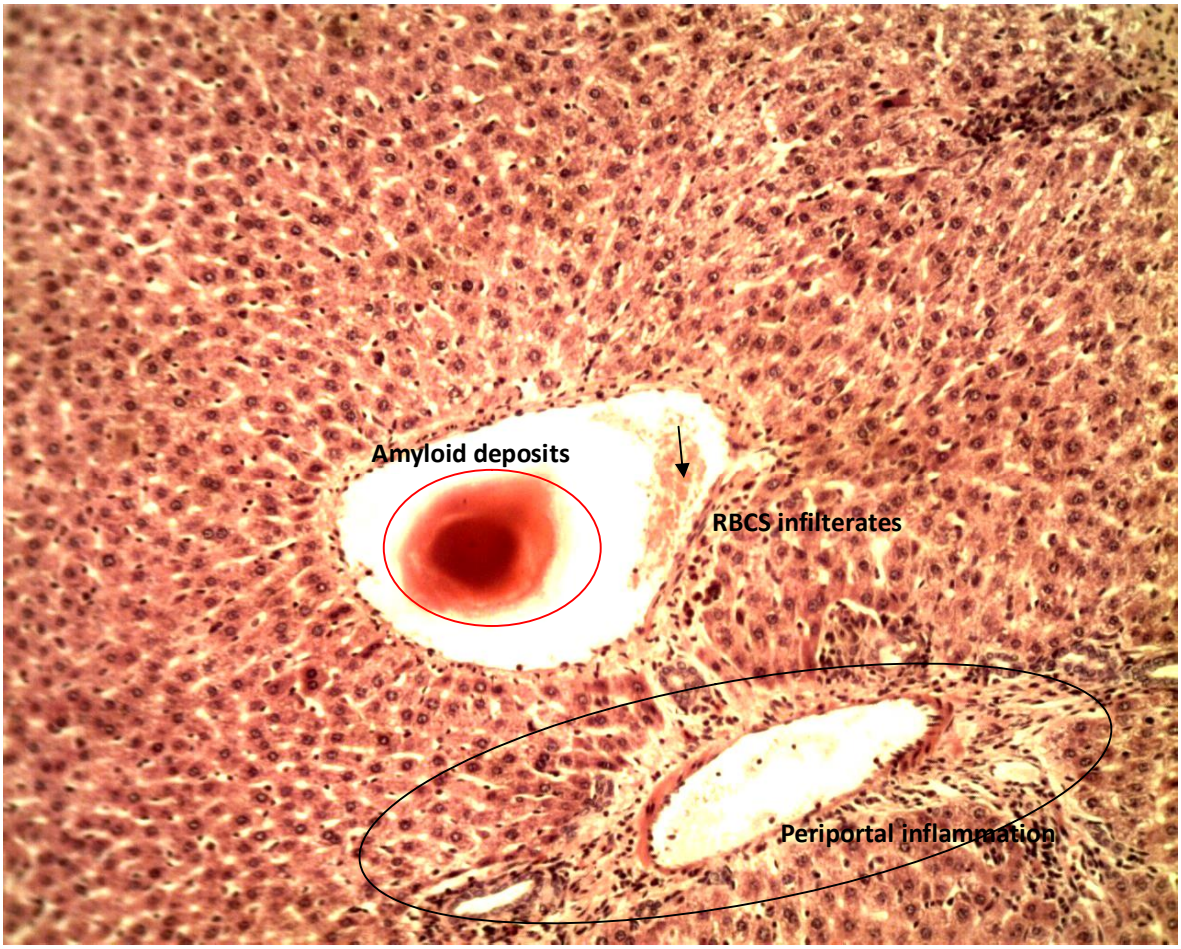

Plate 15: Photomicrograph of the liver tissue of rat from the standard control group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Periportal inflammation
- Amyloid deposit in the central canal

3.14.3.4 Histopathological features of the liver of a rat from IGIW1 group

The photomicrograph of the histopathological section of the liver of a rat from the IGIW1 group showed macrovesicular fatty change and enlarged sinusoids with inflammatory cells infiltration (Plate 16); all of which depicts a marked change from normal hepatic micro-architecture.

3.14.3.5 Histopathological features of the liver of a rat from IGIW2 group

The photomicrograph of the histopathological section of the liver of a rat from the IGIW2 group showed a marked change from normal hepatic micro-architecture. Loss of portal triad connective tissue and periportal inflammatory infilteration was observed (Plate 17). Prominient central vein as a result of inflammatory infilteration was also observed (Plate 17). There was multifocal necrosis as well.

3.14.3.6 Histopathological features of the liver of a rat from IGIW3 group

The photomicrograph of the histopathological section of the liver of a rat from the IGIW3 group showed a marked change from normal hepatic micro-architecture. There was presence of connective tissues above normal rates seen in the portal areas (Plate 18). Also, densely proliferated inflammatory cells were observed (Plate 18).

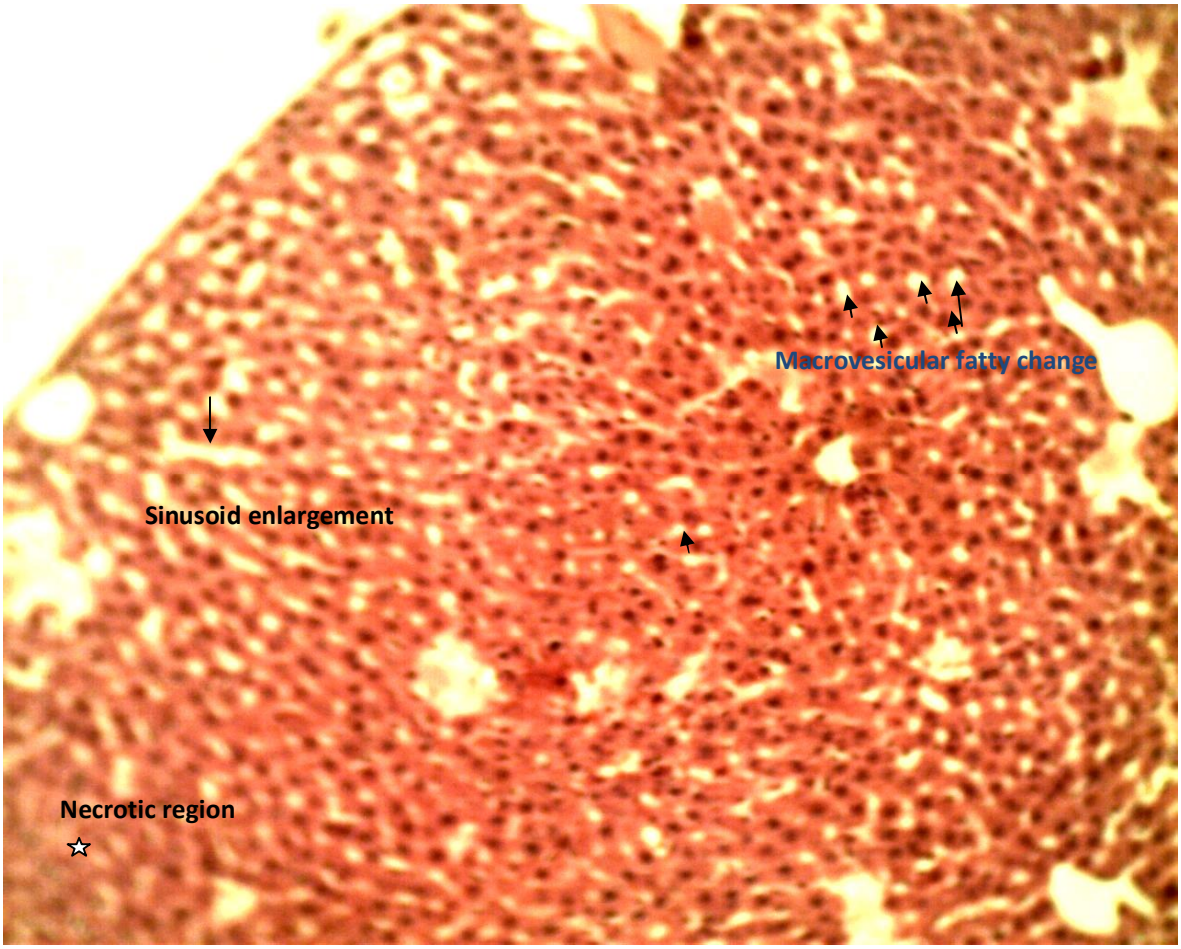

Plate 16: Photomicrograph of the liver tissue of rat from the IGIW1 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Macrovesicular fatty change
- Sinusoid enlargement

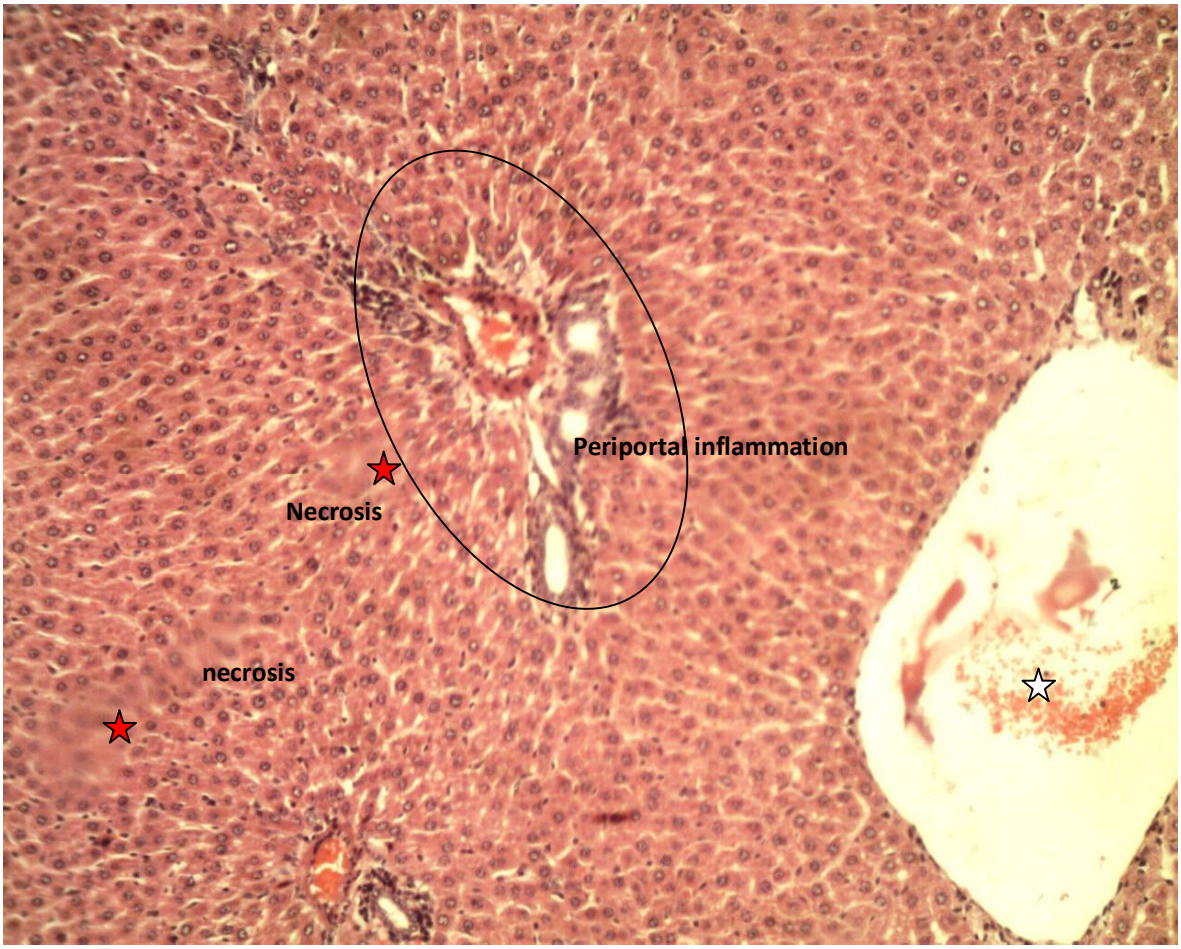

Plate 17: Photomicrograph of the liver tissue of rat from the IGIW2 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Multifocal necrosis
- Periportal inflammation
- Prominient central vein as a result of inflammatory infiltration

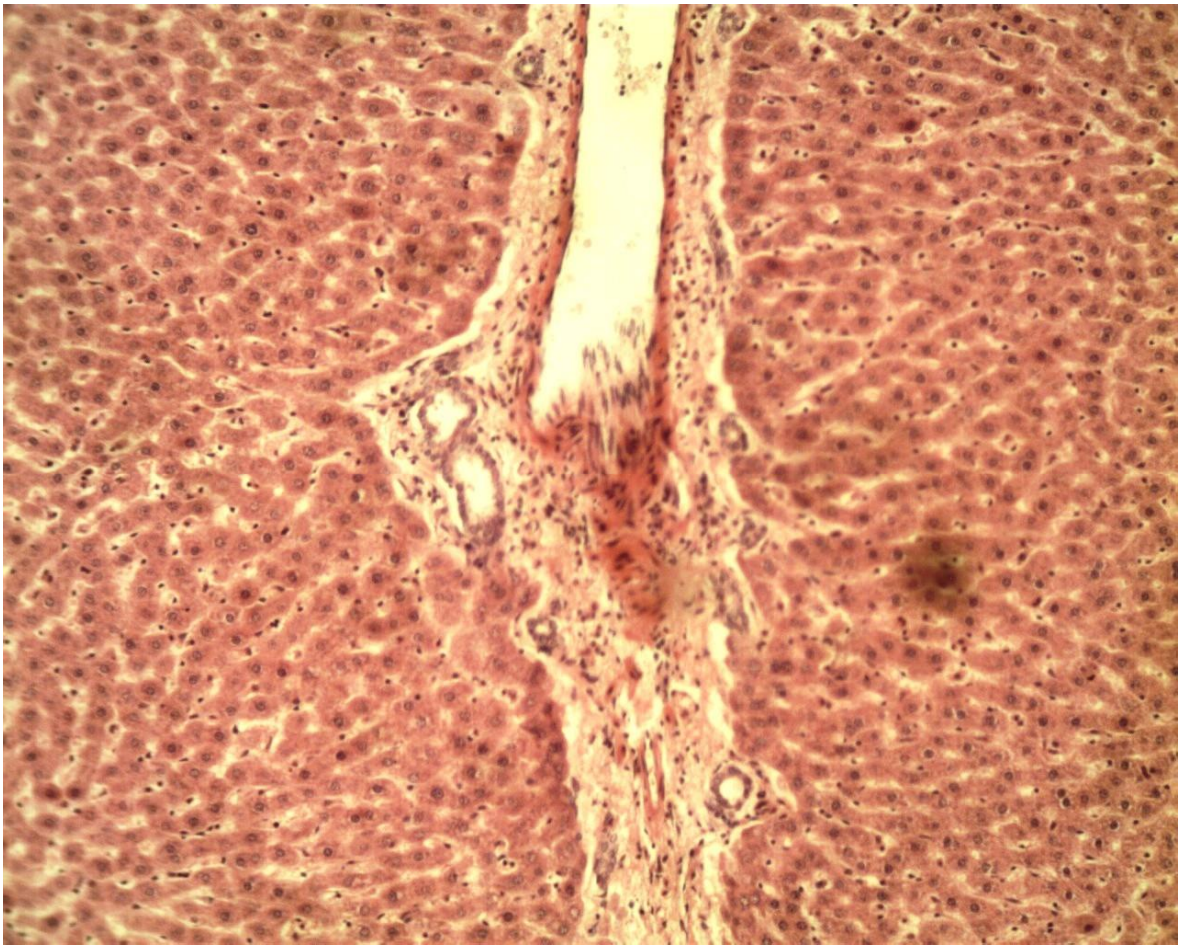

Plate 18: Photomicrograph of the liver tissue of rat from the IGIW3 group, stained with Haematoxylin and Eosin (H & E) and viewed at × 100 magnification (Mg)

- Densely proliferated inflammatory cell
- Presence of connective tissue above normal rate

CHAPTER FOUR

DISCUSSION

4.1 Discussion

Diabetes is a lifestyle disorder characterized mainly by hyperglycemia and hyperlipidemia, resulting from insulin deficiency which causes faulty glucose utilization and mobilization of fatty acids from the adipose tissue. Diabetes mellitus is usually associated with impaired antioxidant capacity (Matsinkou *et al.,* 2012). Reactive oxygen species (ROS) are usually generated in diabetes mellitus condition due to oxidation of excess glucose circulating in the blood (Punitha *et al.,* 2006). Hence, oxidative stress is increased, leading to damage of the structure and alteration of the functions of many organs in the body. As a result, microvascular (retinopathy, neuropathy and nephropathy) and macrovasculature (cardiovascular and peripheral vascular diseases) complications are usually associated with diabetes mellitus (Umar *et al.,* 2010).

Mobilization of fatty acids from the adipose tissue usually results in accumulation of fatty acids in the liver, which are converted into triglycerides. Triglycerides also accumulate and lead to increased formation of LDL-C and reduction in HDL-C (Shih *et al.,* 1997).

Insulin deficiency also contributes to increased serum levels of transaminase enzymes due to easy availability of amino acids, which leads to enhanced occurrence of gluconeogenesis and ketogenesis during diabetes mellitus, inducing hyperglycemia and hyperlipidaemia (Punitha *et al.,* 2006; Dzuefiet *et al.,* 2009; Shukla *et al.,* 2012).

There has been an increase in the search for both medicinal and food plants that can be used to manage diabetes mellitus with little or no side effects. However, it is believed that any plant that can work as a potential antioxidant together with having anti-diabetic properties

could prevent or reduce diabetic complications more effectively than the conventionally used anti-diabetic drugs (Bhattaram *et al.,* 2002). Plant secondary metabolites have been shown to play important roles in the defence against free radicals (Devasagayan and Sainis, 2002; Park and Pezzutto, 2002).

Phytochemical analysis of both species of *Irvingia* reveals that *I. wombolu* has more flavonoids, alkaloids, glycosides, terpenoids and steroids than *I. gabonensis.*

Flavonoids have been reported to inhibit oxidative stress by scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers, chelating metal ions, sparing other antioxidants (e.g. carotene, vitamin C and E) and preserving HDL associated serum paraoxonase activity (Fuhram and Aviran, 2001).

Tannins are complex polymers which can bind proteins and carbohydrates resulting in reduction in digestibility of these macromolecules (Nwogu *et al.,* 2008). Hence, presence of tannins slowed down the digestion of carbohydrates, thereby reducing the impact of food materials on the blood glucose level, as such helping to control blood glucose level. Tannins have also been reported to have astringent properties on mucous membrane (Egunyomi *et al.,* 2009).

Alkaloids have also been reported to have glucose lowering effect, as such has potential beneficial effects in the treatment of diabetes and obesity (Zhang *et al.,* 2008). This was noted when an extract was used in the treatment of diarrhea. According to Shukla *et al.* (2012), the possible mechanism by which alkaloids bring about antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from the remaining islet beta cells. Saponins are glycosides of both triterpenes and steroids, having hypotensive and cardiac depressant properties (Olaleye, 2007). They have been found useful for the treatment of hypercholesterolaemia, which suggests that saponins might act by interfering with intestinal

absorption of cholesterol. Firdous *et al.* (2009) reported that charantins, a mixture of steroidal saponins from *Mormordica cymbalaria* plant had hypoglycemic effect and may have acted by increasing insulin secrection, probably by the regeneration of pancreatic beta cells.

Anthocyanins were reported to have significantly suppressed malondialdehyde levels and restored superoxide dismutase and catalase activities in diabetic rats (Nizamuldinova *et al.,* 2009). They reported that anthocyaninss taken from black soya bean seed coat had anti-diabetic effects that may be due, in part, to the regulation of glucose transporter 4 and prevention of insulin resistance and pancreatic apoptosis.

Induction of diabetes in laboratory animals by alloxan injection appears to be the most reliable and easily reproducible method of inducing diabetes mellitus in experimental animals (Rohilla and Ali, 2012). This is because alloxan is an unstable chemical which also has similar shape with glucose and is therefore selectively taken up via GLUT2 into pancreatic beta cells (Elsner *et al.,* 2000), where it is reduced, producing ROS which destroys the islet cells. Hence, suppression of islet cells results to high glucose concentration in the blood, leading to diabetes mellitus.

Due to insufficiency of insulin in diabetes mellitus disease condition, glucose absorbed from the digestive tract remains in the blood, though the cells are starved of it. Hence, organs and tissue such as the muscles, resorts to the breaking down of fats cell and tissue proteins in order to generate the needed energy fuels. Consequently, weight loss occurs. Our findings on the body weight in alloxan-induced diabetic rats in this study showed that *I. gabonensis* and *I. wombolu* combinations had no significant effect on the body weight of the experimental animals, as the animals maintained relatively body weights throughout the period of treatment (though there were some percentage of increase and decrease in body weight in some groups). This result is contrary to that of Ngondi *et al.* (2005), that reported that I.

gabonensis significantly reduced the body weight of obese subjects, but in agreement with the report of Hossain *et al.* (2012) where *I. gabonensis* had no significant effect on body weight of type II diabetic rats. In this study, our diabetic rats were not obese, may be that was why the samples had no effect on the treated animals.

High fasting blood glucose level is a major diagnostic feature of diabetes mellitus. This is because of insufficient amount of insulin to drive the glucose absorbed from the intestines into the cells where they are required, thereby leaving excess glucose in the blood circulation. It was observed that the FBGL of the diabetic rats in this study was significantly higher than that of the positive control. However, *I. gabonensis* and *I. wombolu* combinations significantly lowered the FBGL of the diabetic animal, where IGIW3 had the highest activity compared to IGIW1 and IGIW2. This effect could be attributed to the presence of alkaloid and tannins which have been said to posses blood glucose lowering effect (Nwogu *et al.,* 2008; Shukla *et al.,* 2012). The hypoglycemic action of the *Irvingia* species may be by slowing down the digestion of carbohydrates or by potentiation of pancreatic secretion of insulin from the remaining islet beta cells. Our findings are in agreement with the reports of Dzeufiet *et al.* (2009), where hexane extract of *Irvingia gabonensis* seeds significantly lowered the FBGL of diabetic rats. It is also in tandem with the findings of Jaiswal *et al.* (2009), where aqueous extract of *Moringa oleifera* leaves significantly reduced FBGL in streptozocin-induced diabetic rats.

Dyslipidaemic condition contributes to a major risk factor for atherosclerosis and cardiovascular diseases (Shukla *et al.,* 2012). Hyperlipidaemia is usually characterized by high levels of LDL-C, triglycerides, and total cholesterol and low levels of HDL-C. Insulin deficiency inactivates lipoproprotein lipase, promoting the conversion of free fatty acids into phospholipids and cholesterol in the liver, which finally gets discharged into the blood, resulting in elevated serum phospholipid levels (Pushparaj *et al.,* 2007). Hyperglycemia also

results in decreased insulin sensitivity, due to increase in serum resistin level. This leads to increase in lipogenesis and as a result, increase in circulating fatty acids. mobilization of fats from the adipose tissue to satisfy the body cells demand for energy in diabetes condition as a result of insulin deficiency also leads to increased levels of circulating fatty acids. Excess circulating fatty acids are assembled into triglycerides and then into LDL. Oxidized LDL particles accumulate in the endothelial walls of the arteries, leading to the formation of arterial plaque, which results in the narrowing of the arteries ï a condition known as atherosclerosis. Blockage of the coronary arteries due atherosclerosis causes poor oxygen supply to the heart, resulting in the death of heart cell (NIH, 2014). HDL-C is called the good cholesterol, because it carries away cholesterol from the tissues to the liver, lowering blood cholesterol levels. High HDL-C levels are associated with low risk of cardiovascular diseases. HDL-C levels are higher with exercise, higher estrogen levels and weight loss (Liji, 2015).

In this study, serum levels of TG, TC, and LDL-C were markedly increased while HDL-C was decreased in diabetic rats. However, all proportions of crude seeds of *Irvingia gabonensis* and *Irvingia wombolu* and the standard drug significantly reduced the serum TG, TC and LDL-C but at the same time raised the serum HDL-C levels compared to the diabetic control. This result is in agreement with the study of Dzeufiet *et al,* (2009) where n-hexane extract of *Irvingia gabonensis* seeds reduced serum levels of LDL-C, TG, and TC but raised HDL-C of diabetic rats. We found that IGIW2 (i.e. 20% *I. gabonensis*: 80% *I. wombolu*) had more activity on HDL-C and LDL-C. This may be because *I. wombolu* has more flavonoids and tannins than *I. gabonensis.* As such, more of *I. wombolu* will give more of these phytochemicals which have been shown to antioxidant effect. Hence, oxidation of LDL-C is reduced while HDL-C is increased.

In medicine, the presence of elevated serum transaminases (AST, ALP, and ALT) may be an indicator of liver damage or hepatotoxicity. The liver uses transaminases to synthesize and breakdown aminoacids and to convert them to energy storage molecules. The concentration of these enzymes in the liver is normally low. However, if the liver is damaged, the liver cells (hepatocytes) membrane becomes more permeable and some of the enzymes leak into blood circulation (Adebayo *et al.,* 2015).

In this study, elevated serum levels of ALT, ALP, and AST were recorded in diabetic rats compared to the positive control. Potential explanation to elevated serum levels of these transaminases of diabetic rats in this study may be the oxidative stress from reactive lipid peroxidation. In diabetes disease condition, oxidative stress arises as a result of oxidation of excess glucose the blood circulation, non-enzymatic glycation of protein and oxidation of protein glycation products, which leads to the generation of reactive oxygen species (ROS). Reactive oxygen species are known to attack lipid membrane of cells, e.g hepatocytes, leading to lipid peroxidation; as such, loss of functionality and intergrity of cell membrane. Hence, transaminase enzymes like ALT, ALP, and AST may increasingly leak into blood circulation. However, administration of the different combinations of the *Irvingia* species and the standard drug, glibenclamide, significantly reduced the serum levels of these enzymes, especially on the 21^{th} day of treatment. The action of IGIW1 on ALP and AST was similar to that of the standard drug while IGIW3 had the highest effect on serum ALT level. It can therefore be deduced that *I.gabonenesis* and *I. wombolu* have hepatoprotective effect on diabetic rats. This may be due to the presence of phytochemicals such as flavonoid and tannins, which have been said to posses antioxidant properties (Aljadi and Kamaruddin, 2004; Adebayo *et al.,* 2015). Our result is agreement with the work of Shukla *et al.* (2012), where *Lepidium sativum* linn seed total alkaloids (LSTA) showed hepatoprotective effect on alloxan-induced diabetic rats.

Malondialdehyde is one of the most frequently used indicators of oxidative stress (Adebayo *et al.,* 2015). Free radicals are formed disproportionately in diabetes mellitusby glucose degradation, non-enzymatic glycation of proteins and subsequent oxidative degradation, which may play an important role in the development of complications of diabetic patients (Matsinkou *et al.,* 2012). The generation of free radicals may lead to lipid peroxidation and several damages in diabetes mellitus condition. In this study, the degree of lipid peroxidation was measured in terms of serum MDA concentration. It was observed that serum MDA levels were elevated in diabetic animals compared to the control. This result clearly shows that the animals were exposed to increased oxidative stress through lipid peroxidation. Administration of crude seeds of *Irvingia* species reduced the serum levels of MDA, though insignificantly, probably because of the presence of flavonoids. The highest activity was found in IGIW2 treatment group. The standard drug seems not to produce any effect on the diabetic animals. This is result in agreement with the findings of Ereguwa *et al.* (2010), where glibenclamide and metaformin combination did not produce any significant effect on MDA levels of the pancreas in streptozotocin-induced diabetic rats

Hyperglycemia and hyperlipidaemia usually affect the histopathological features of the three major organs involved in the regulation of blood sugar level namely; pancreas, liver and kidney. The pancreas is an accessory organ of the digestive system which has both endocrine and exocrine functions. The endocrine portion of the pancreas consists of clusters of cells known as the islets of Langerhans, which produce insulin and glucagon- the two hormones that regulate the level of sugar in the blood. Reactive oxygen species (ROS) produced as a result of hyperglycemia usually causes necrosis of the islet cells, further reducing the level of insulin; as such resulting in more diabetic complications. In this present study, both the acinar cells and the islet cells were immensely damaged due to the action of the alloxan. However, intact cell were found in diabetic groups treated with IGIW1 and the standard drug.

The liver is an organ which is responsible for the deamination of proteins and detoxification of toxic substances from the body system. It is also involved in the regulation of the blood lipid profile. The liver cell (hepatocytes) are usually damage as a result of the oxidative stress generated due hyperglycemia, resulting in the reduce ability of the liver to regulate blood lipid levels giving rise to increased levels of circulating lipids, heavy fatty deposition in the liver and high levelsof transaminase enzymes in the blood following the loss of intergrity of hepatocytes cell membrane. Our findings in this study showed various levels of alterations from normal hepatic micro-architecture. However, hepatic tissues from some of the groups (standard control and IGIW3) were relatively restored.

The micro architecture of the kidney is usually distorted in diabetic conditions, resulting from non-enzymatic glycation of proteins lining the glomeruli and the tubules walls as well as oxidative stress. In our study, there were marked changes in the normal kidney micro architecture, ranging from atrophy of the glomeruli and corresponding widening of the urinary spaces, to the damage of the nephric tubules. However, intact renal corpsules were found in the IGIW3 group properly as a result of the protective effect of the treatment on the kidney.

Our findings on the histopathological studies of the pancreas, kidney and liver of alloxan-induced diabetic rats in the study are in agreement with the report of Ezejiofor *et al.* (2013) where aqueous extract of *Persa Americana* showed tissue protective effects on alloxan-induced diabetic rats.

Conclusion: The results obtained from this study suggest that combinations of crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* have hypoglycaemic and hypolipidaemic effects. Moreso, these combinations of *Irvingia* had relative tissue protective

and antioxidant effects, which could be related to their hypoglycemic and hypolipidaemic effects .

Recommendations:

1. Meals prepared with *Irvingia gabonenesis* and *Irvingia wombolu* seed powders should be included in the diet plan of diabetics.
2. The generally public, especially diabetic paitents should be enlightened on the antidiabetic effects of *Irvingia gabonenesis* and *Irvingia wombolu* seed powders.
3. Further research should be done to compare the pathophysiological effects of combinations of *Irvingia gabonenesis* and *Irvingia wombolu* with each of the species singly.
4. Further research should be done to determine the antidiabetic effects of combinations of *Irvingia* species and *Citrullus* species (ôEgwusiõ).
5. Further research should also be done to determine the antioxidant effect of the individual species of *Irvingia*.

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Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the body weight (gm) of diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	130.3	104.2	112.9
Diabetic Control	105.7	138.0	104.1
Standard Control	144.2	105.7	148.0
IGIW1	134. 5	102.8	143.2
IGIW2	145.7	145.8	145.9
IGIW3	133.4	150.0	135.0

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	141.4	124.1	127.2
Diabetic Control	152. 6	134.8	101.3
Standard Control	140. 5	146.7	150.0
IGIW1	139.1	119.8	156.7
IGIW2	162.9	180.5	158.1
IGIW3	149.1	162.2	150.2

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	134.0	110.0	115. 6
Diabetic Control	157.7	147.7	110.8
Standard Control	159.7	155.2	160. 5
IGIW1	157.0	129. 6	165.8
IGIW2	179.7	154. 6	170.1
IGIW3	142.8	160.9	145. 6

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	143.0	118.2	124. 5
Diabetic Control	167.8	155.0	115.2
Standard Control	168.3	162.9	171.2
IGIW1	165.7	113.5	160.2
IGIW2	138.1	120.4	160.2
IGIW3	169.2	147.8	160. 5

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on the fasting blood glucose level (mg/dL) of diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	72	70	68
Diabetic Control	217	222	215
Standard Control	187	554	514
IGIW1	166	168	539
IGIW2	265	171	589
IGIW3	185	589	400

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	55	53	53
Diabetic Control	187	215	203
Standard Control	272	214	362
IGIW1	239	154	476
IGIW2	220	169	495
IGIW3	83	419	362

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	72	60	57
Diabetic Control	193	223	219
Standard Control	78	189	190
IGIW1	96	161	395
IGIW2	138	215	454
IGIW3	48	400	285

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	79	81	66
Diabetic Control	160	233	225
Standard Control	346	151	185
IGIW1	108	84	330
IGIW2	140.2	199.5	391.2
IGIW3	91	271	99

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on low density lipoprotein-cholesterol levels (mg/dL) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	41	40	38
Diabetic Control	89	95	93
Standard Control	80	85	88
IGIW1	95	92	88
IGIW2	92	91	97
IGIW3	94	102	96

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	48	39	43
Diabetic Control	168	163	160
Standard Control	74	70	69
IGIW1	84	86	80
IGIW2	90	86	94
IGIW3	69	74	65

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	44	48	40
Diabetic Control	72	80	96
Standard Control	64	70	65
IGIW1	66	67	75
IGIW2	85	83	86
IGIW3	68	62	70

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	39	48	49
Diabetic Control	94	96	98
Standard Control	54	56	53
IGIW1	60	65	62
IGIW2	62	69	70
IGIW3	60	61	65

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on aspartate aminotransferase (AST) levels (U/L) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	50	49	55
Diabetic Control	109	100	96
Standard Control	102	94	108
IGIW1	104	109	98
IGIW2	115	103	100
IGIW3	109	104	110

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	58	60	59
Diabetic Control	142	150	149
Standard Control	94	92	96
IGIW1	84	80	86
IGIW2	91	92	98
IGIW3	94	91	89

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	58	60	59
Diabetic Control	152	162	160
Standard Control	62	55	59
IGIW1	69	65	64
IGIW2	81	89	86
IGIW3	68	70	74

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	62	68	66
Diabetic Control	163	160	159
Standard Control	59	54	56
IGIW1	50	56	60
IGIW2	59	60	70
IGIW3	60	54	62

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on alkaline phosphatase (ALP) levels (U/L) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	61	68	70
Diabetic Control	98	96	94
Standard Control	98	99	89
IGIW1	90	96	95
IGIW2	94	94	98
IGIW3	90	91	84

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	69	66	66
Diabetic Control	125	134	126
Standard Control	82	80	86
IGIW1	71	74	79
IGIW2	80	92	89
IGIW3	88	86	82

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	61	60	66
Diabetic Control	128	130	114
Standard Control	72	70	76
IGIW1	69	61	63
IGIW2	80	82	85
IGIW3	70	75	83

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	64	59	60
Diabetic Control	132	151	142
Standard Control	64	60	62
IGIW1	68	60	69
IGIW2	74	73	80
IGIW3	66	69	64

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on serum total cholesterol levels (mg/dL) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	62	60	59
Diabetic Control	104	120	115
Standard Control	98	100	102
IGIW1	90	108	104
IGIW2	98	94	112
IGIW3	108	100	99

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	59	58	64
Diabetic Control	158	149	148
Standard Control	94	95	89
IGIW1	92	90	96
IGIW2	88	97	98
IGIW3	90	86	80

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	68	60	65
Diabetic Control	162	150	151
Standard Control	65	66	70
IGIW1	69	70	74
IGIW2	80	85	79
IGIW3	64	62	63

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	62	69	72
Diabetic Control	162	160	150
Standard Control	72	60	59
IGIW1	68	60	70
IGIW2	72	76	74
IGIW3	69	66	51

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on total triglyceride (TG) levels (mg/dL) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	115	128	118
Diabetic Control	118	139	122
Standard Control	124	118	109
IGIW1	109	115	129
IGIW2	116	112	115
IGIW3	114	125	109

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	129	124	126
Diabetic Control	134	141	142
Standard Control	106	114	117
IGIW1	109	110	114
IGIW2	118	114	109
IGIW3	125	109	108

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	122	109	116
Diabetic Control	149	150	145
Standard Control	109	102	103
IGIW1	132	120	126
IGIW2	130	126	120
IGIW3	124	120	118

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	134	128	124
Diabetic Control	158	160	122
Standard Control	114	102	110
IGIW1	114	126	112
IGIW2	126	120	110
IGIW3	108	118	102

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on low density lipoprotein-cholesterol levels (mg/dL) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	18	24	28
Diabetic Control	74	96	94
Standard Control	62	68	60
IGIW1	60	66	69
IGIW2	62	68	64
IGIW3	61	58	63

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	18	16	15
Diabetic Control	98	96	94
Standard Control	28	34	38
IGIW1	38	36	30
IGIW2	29	28	26
IGIW3	40	41	36

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	18	20	24
Diabetic Control	88	90	92
Standard Control	39	28	36
IGIW1	38	32	30
IGIW2	29	30	32
IGIW3	36	32	30

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	24	22	28
Diabetic Control	94	98	98
Standard Control	19	22	26
IGIW1	29	22	26
IGIW2	24	25	22
IGIW3	20	18	21

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on high density lipoprotein cholesterol levels (mg/dL) alloxan-induced of diabetic 6 5

Day 0

Groups	**Replicates**		
	1	**2**	**3**
Positive Control	64	52	50
Diabetic Control	38	30	36
Standard Control	34	40	41
IGIW1	36	41	42
IGIW2	30	39	32
IGIW3	39	34	32

Day 7

Groups	**Replicates**		
	1	**2**	**3**
Positive Control	53	54	59
Diabetic Control	14	18	19
Standard Control	50	5 2	51
IGIW1	56	5 2	49
IGIW2	49	51	48
IGIW3	49	40	41

Day 14

Groups	**Replicates**		
	1	**2**	**3**
Positive Control	60	64	59
Diabetic Control	18	20	16
Standard Control	41	42	50
IGIW1	51	5 2	58
IGIW2	46	49	45
IGIW3	42	49	45

Day 21

Groups	**Replicates**		
	1	**2**	**3**
Positive Control	5 4	59	55
Diabetic Control	24	28	26
Standard Control	36	39	43
IGIW1	46	49	43
IGIW2	40	49	48
IGIW3	42	48	41

Effects of combinations crude seed powders of *Irvingia gabonensis* and *Irvingia wombolu* on malondialdehyde level (mg/dL) in alloxan-induced diabetic rats

Day 0

Groups	Replicates		
	1	**2**	**3**
Positive Control	1.70	1.07	1.04
Diabetic Control	1.29	1.25	1.30
Standard Control	1.93	1.87	1.95
IGIW1	1.40	1.67	1. 64
IGIW2	1.95	1.64	1. 54
IGIW3	1.26	1.87	1.47

Day 7

Groups	Replicates		
	1	**2**	**3**
Positive Control	1.95	1.42	1.44
Diabetic Control	1.09	1.21	1.09
Standard Control	2.42	1.97	1.92
IGIW1	2.94	1.36	1.98
IGIW2	2. 67	2. 60	2.00
IGIW3	2. 64	2.20	2.80

Day 14

Groups	Replicates		
	1	**2**	**3**
Positive Control	1.64	1.39	1.43
Diabetic Control	1.07	1.10	1.33
Standard Control	2.61	2.07	2.12
IGIW1	2.41	2.70	2.10
IGIW2	2.05	2.11	1.30
IGIW3	2. 66	2.30	2.00

Day 21

Groups	Replicates		
	1	**2**	**3**
Positive Control	1.44	1.44	1.50
Diabetic Control	1.03	1.11	1.17
Standard Control	2.48	2.46	2.07
IGIW1	2.73	2. 67	2.64
IGIW2	2.30	2.02	2.12
IGIW3	1.94	2.64	1.93