ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF THE LEAF EXTRACT AND FRACTION OF *Alchornea cordifolia (*Schumach & Thonn.) Müll. Arg ON STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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MARCH, 2014

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B.Sc (BUK) 2007

M.Sc/SCIEN/05044/09-10

A Thesis Submitted to the Postgraduate School, Ahmadu Bello University, Zaria.

In Partial fulfillment of the requirement for the Award of Master of Science Degree in Biochemistry

Department of Biochemistry, Faculty of Science

MARCH, 2014

DECLARATION

The research works in this Thesis entitled ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF THE LEAF EXTRACT AND FRACTION OF *Alchornea cordifolia (*Schumach & Thonn.) Müll.Arg. ON STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS was carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma at this or any other Institution.

Mohammed-Rabiu Karima		09/03/2014
Name of Student	Signature	Date

CERTIFICATION

This Thesis entitled ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF THE LEAF EXTRACT AND FRACTION OF *Alchornea cordifolia (*Schumach & Thonn.) Müll.Arg. ON STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS, meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria, and is approved for its scientific contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENTS

Immense praise and gratitude to Almighty Allah for all that he has blessed me with. Special gratitude goes to Chairman, Supervisory Committee, Dr. S. Ibrahim for his untiring support, unsurpassed encouragement, and motivation, a true source of inspiration. His contribution to the sound outcome of this work is immeasurable. My deepest gratitude goes to member, Supervisory Committee Prof. S.E. Atawodi for his enthusiastic guidance. His resourcefulness helped in the academic enrichment of this research work. My profound appreciation goes to Head of Department, Prof. I, A Umar for making available the facilities and enabling learning environment

My sincere appreciation goes to Eze E. Daniel of Physiology Department, A.B.U, Zaria, especially for his assistance in animal handling. Many thanks go to Dr. I. A. Umar, Dr. B. Sallau, Dr. D. B. James, and all Laboratory Technologists of Biochemistry Department, A.B.U, Zaria. I also appreciate Hajiya L. Nasiru formally of Biochemistry Department, A.B.U, Zaria I wish to acknowledge the assistance of Dr. M. K. Atiku of Department of Biochemistry, Bayero University, Kano

Utmost appreciation goes to my family members for their all-round support, love and care, without which I may not be able to keep afloat and swim to the riverbank; My parents, Alhaji Mohammed Rabiu and Hajiya Fatima Salis, you two beam with positive energy and hope for the future and that will always be a great source of inspiration for the family. Much gratitude goes to my siblings, Auwal, Aisha, Abubakar, Alhassan and Fatima for their comfort at all times. Warm appreciation goes to my friends, class colleagues; Maimuna Zubairu, Fatima Hassan, Abdulmalik Salman, Aliyu Sani, Oche, Ogechi, Mal. Ibrahim, Kingsley, Haroun, Amaya, Victoria, Samuel Atabo, Mfon, Chichi, Mayowa, Sarah, Chijioke, Chidi, Ewa and Jerry.

ABSTRACT

Aqueous concoction made from the crushed leaves of *Alchornea cordifolia* (Christmas bush) is used in Nigeria and other parts of Africa as a remedy for the treatment of diabetes mellitus and other ailments. The aim of the study was to evaluate the hypoglycemic and hypolipidemic effect of *Alchornea cordifolia* leaf extract and fraction in Streptozotocin-induced diabetic rats. Liver and heamatological biochemical parameters were also investigated. In the experiment, a total of forty five (45) rats were used. They were randomly divided into 9 groups of 5 rats each. Normal rats were assigned group 1, untreated diabetic rats were assigned group 2. Groups 3, 4 and 5 received varying doses (200, 400, 800 mg/kg bw/day) of n-butanol fraction of *Alchornea cordifolia* leaf ethanolic extract either extract/fraction or standard anti-diabetic drug daily for four (4) weeks, orally. Groups 6, 7 and 8 received varying doses (200, 400, 800 mg/kg bw/day) of ethanolic extract, while rats in group 9 were treated with the standard drug, glibenclamide (10mg/kg bw/day). After the 14th, 21st and 28th day treatment of diabetic rats with all doses of the plant extract and glibenclamide, there was a significant increase in the body weight of diabetic rats. The study showed that there was a significant reduction in the fasting blood sugar (FBG) levels in the entire streptozotocin-induced diabetic rats treated with ethanolic extract and its fraction, with a maximum reduction of 13.6% and 16.2% in the FBG level at 400 and 800mg/kg bw doses of n-butanol fraction respectively. There was significant decrease in the serum levels of total cholesterol, triglyceride, LDL-C and increased HDL-C in streptozotocin-induced diabetic treated groups, and percent reduction of 12.4% , 18.7% , 19.5% of TC, TAG, LDL-C respectively were observed in the groups treated with 800mg/kg n-butanol fraction when compared with the group of diabetic rats (85.3, 117.2, 91.9 %), and percent increase in the concentration of HDL-C, (10.8 and 12.9 %) was recorded in the group treated with 800mg/kg N-

butanol fraction and 800mg/kg ethanol extract respectively. Maximum reduction in the concentration of ALT, 2.7% was recorded in the group treated with 800mg/kg ethanol extract. A marked reduction in the concentration of AST, (2.51 and 2.42%) was observed in the groups treated with 400 and 800mg/kg N-butanol fraction respectively. A high decrease in the level of ALP, 5.4% was recorded in the group administered with 400mg/kg n-butanol fraction. The levels of TB and DCB were reduced significantly (0.4 and 0.6%) respectively for groups treated with 200mg/kg ethanol extract. There was a significant increase in packed cell volume, red cell count, haemoglobin concentration and total protein in the extract and glibenclamide treated diabetic. Total white blood cell count and lymphocyte revealed a significantly increased levels after treatment with the n-butanol fraction of *Alchornea cordifolia* leaf ethanol extract after 28th days. No death was recorded after three (3) weeks of sub-acute toxicity study. The histopathological studies of the pancreas of diabetic animals revealed the degeneration of pancreatic Islet cells, but with the restoration of pancreatic Islet cells in the pancreas of diabetic group treated with various doses of the plant extract. The implications of the results obtained in the present study provide the scientific rationale for the use of *Alchornea cordifolia* as antidiabetic agent in the management of diabetes and dyslipidemia usually associated with the disease. In addition, the plant had erythropoietic effect on the experimental animals.

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ABBREVIATIONS

ADA: American diabetic association

AHA: American Heart Association

ALP: Alkaline Phosphatase

ALT: Alanine Amino Transferase

AST: Aspartate Amino Transferase

BASO: Basophil

CETP: Cholesterol ester transfer protein

CHD: Coronary heart disease

DCB: Direct Conjugated Bilirubin

EOSINO: Eosinophil

FPG: Fasting plasma glucose

GLP: Glucagon-like peptide

Hb: Haemoglobin

HDLC: High-density lipoprotein cholesterol

HMG CoA: 3-hydroxyl-3-methyl glutaryl Coenzyme A

IDL: Intermediate density lipoprotein

LDLC: Low –density lipoprotein cholesterol

LYMP: Lymphocytes

MONO: Monocytes

N-BUT: N-butanol

NCEP: National Cholesterol Education Programme

NEUTR: Neutrophil

NIDDM : Non-insulin dependent diabetes mellitus

NIHCD: National Institute of health Consensus Development

PCV: Packed cell volume

PPARs: Peroxisome proliferator-activated receptors

RBC: Red blood cell

STZ: Streptozotocin

TB: Total Bilirubin

TC: Total cholesterol

Total Pro: Total Protein

TRIG: Triglyceride

WBC: White blood cells

W H O: World Health Organization

CHAPTER 1

INTRODUCTION

Diabetes is a metabolic disease characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism (Jensen and Clark, 1988). Diabetes has become one of the devastating diseases afflicting health of many people, in recent times, and has accounted for a high proportion of health problems worldwide (Sushruta *et al*., 2006). The worldwide prevalence of diabetes is about 2%, and the prevalence in Nigeria as at 1997 is 2.2%, which means that about 2.6 million Nigerians are diabetic (The Expert Committee on Non-communicable Disease, 1997.) It is now recognized as one of the leading causes of death in the developing countries where the high prevalence of the disease is attributed to increase in sedentary lifestyle coupled with a gross lack of modern facilities for the early diagnosis of the disease.

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating one of the following: Fasting blood glucose level at or above 126mg/dl or 7.0mmol/L, blood glucose at or above 200mg/dl or 11.1mmol/L two hours after a 75g oral glucose load in a glucose tolerance test, random plasma glucose at or above 200mg/dl or 11.1mmol/L, glycosylated hemoglobin (HbA1c) at or above 6.5, two fasting glucose measurements above 126mg/dl or 7.0mmol/L or random blood sugar level greater than 20.0mg/dl on two occasions is considered diagnostic for diabetes mellitus, patients with fasting sugars between 1l0mg/dl and l25mg/dl (6.1 and 7.0rnmol/L) are considered to have impaired glucose tolerance (Shankar and Sundarka, 2001). Two main types of diabetes are identified based on their clinical manifestations: Type 1 diabetes known as juvenile onset or insulin

sensitive diabetes and Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM). The latter is the most prevalent. Type 2 diabetes may have as its underlying metabolic causes, the combined effects of impairment in the insulin mediated glucose disposal and defective secretion of insulin by the cells of the pancreas (Grundy *et al*., 1999). Oral hypoglycacmic agents, especially the sulphonylureas and biguanides have been commonly employed in the management of type 2 diabetes. Sulphonylureas are the most widely used oral hypoglycacmic agents but may have some adverse effects such as exacerbating hyperinsulinaemia, thereby causing weight gain in patients (Rastigo, 1977; Egwim, 2005). Biguanides are only weak hypoglycaemics and have limited clinical use (Rastigo, 1977). For these cogent reasons, therefore, there is a great need for a search for an acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agents that would be effective in the management of diabetes and devoid of serious side effects of the currently used oral hypoglycaemic agents. Herbs and marine sources have been considered the best option. The use of herbs and natural product drugs from various plant sources is now of great interest in the management of diabetes mellitus. Several herbs have been reported in folk medicine to be successfully employed in the management of diabetes and have shown effectiveness in non-insulin dependent diabetes (Rastigo, 1977).

Medicinal plants are plants that provide health promoting characteristics, provide temporary relief of symptoms or have curative properties (Calixto, 2000). The use of plant herbs and other natural products for the restoration of health dates back to ancient times. The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. Industrialized nations are developing interest in the use of herbs. In the United States, increasing public dissatisfaction with conventional medicine and its increased cost,

combined with an interest in returning to natural or organic remedies with the mistaken impression that natural products are safe, has led to an increase in the use of herbal medicines (Mathews *et al*., 1999). Herbal medical practice has been the main form of treatment especially among indigenous populations around the world particularly in developing countries, and the World Health Organization has also estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare (WHO, 2002). In Germany it is estimated that roughly 600 to 700 plant-based medicines are available and are prescribed by approximately 70% of German physicians. In Germany, herbal medicines are actually prescribed more often than conventional medicines. These plant based medicines have been studied and the claims of a number of compounds in them shown to be valid while others have been ineffective or more toxic than prescription alternatives (Blumenthal *et al*., 1998).

Scientific investigation into some folk remedies has provided leads to bioactive compounds and many of these compounds have been developed into drugs. Examples of these folk remedies are as follows: willow (*Salix purpurea*), the plant from which the famous analgesic, aspirin was first obtained (Hedner and Everts, 1998), quinine (an antimalarial) from *Cinchona succirubra*, artemisinin (qinghaosu) which is the antimalarial from a Chinese medicinal herb (*Artemisia annua*) (Klayman, 1985), Forskolin, which is the antihypertensive agent from *Coleus forskohlii* Briq. (Kinghorn, 1987) and the ginkgo tree (*Ginkgo biloba*), mentioned, in Chinese medicinal books used in anti-asthmatic and antitussive preparations (Hamburger *et al*., 1991). Folklore medicine seems to have a stock of valuable lead compounds which when scientifically investigated can provide very useful drugs especially in the management of infectious conditions.

1.1 STATEMENT OF RESEARCH PROBLEM

The worldwide survey reported that Diabetes Mellitus (DM) is affecting nearly 10% of the world's population (Siddharth, 2001). Wild *et al*, (2004) estimated that the number of people with diabetes will increase to over 366 million by 2030 and that large increases will occur in developing countries, especially in people aged between 45 and 64 years. The prevalence of diabetes for all age groups worldwide is on the increase, and has been projected as the World's main disabler and killer in the next 25 years (Edwin *et al*., 2008). DM is a major cause of disability and hospitalization and it results in significant financial burden (Vats *et al.,* 2002). It is now apparent that most of the currently used synthetic drugs for the treatment of diabetes mellitus are not affordable to low income earners and not easily accessed by the users. This is also coupled with serious side effects posed by these drugs to the potential users (Rang *et al*., 1991). Amongst the numerous herbal plants used for treatment of DM, some have been validated by scientific criteria (Frode and Medeiros, 2008).

1.2 JUSTIFICATION

The ever growing interest in the use of herbal medicine demands information on the efficacy, toxicity and also risk assessment on various plant concoctions used in management of diseases. The use of herbal medicines is coupled with the characteristic profile of serious side effects to the potential users. Therefore, there is a need in evaluating the toxic effects on various tissues and organs of the body system (Chan, 1997). There is a need for scientific backing for the use of *A. cordifolia* in treating diabetes and the possible active principle(s). The use of medicinal plants in modern medicine suffers from the fact that though, hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most

cases. However, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles (Singh *et al*, 2000).

Throughout the world many traditional plants treatments for diabetes exist. However, few have received scientific or medical scrutiny and the World Health Organization (WHO) has recommended that traditional plants treatments for diabetes warrant further eva1uation (WHO, 1980). And with the increasing incidence of this disease in the rural population throughout the world there is clear need for development of indigenous, inexpensive botanical sources for antidiabetic crude or purified drugs (Venkatesh *et al*, 2003). Often the toxicity of herbal extracts is ignored but they may be important in designing treatment regimes. In this study, the antidiabetic effect and toxicity of *A. cordifolia* was investigated in induced diabetic wistar rats.

1.3 AIM OF THE STUDY

The aim of this study is to evaluate the hypoglycemic effect of *Alchornea cordifolia* leaf extract in streptozotocin induced diabetic rats.

1.4 OBJESTIVES OF THE STUDY

i. To assess and compare the efficacy of aqueous extract, ethanol extract and ethanol fractions of *Alchornea cordifolia* leaf as hypoglycemic agents in streptozotocin induced diabetic rats and determine the most active fraction from the most active extract.

ii. To evaluate (qualitative and quantitative) the phytochemical composition of all extracts and fractions.

iii. To assess the effect of the most active extract and the most active fraction on other parameters associated with metabolic syndrome of diabetes.

iv. To carry out sub-acute toxicity evaluation of the aqueous extract (the form in which the plant is used locally) on the histopathology of the liver and kidney of experimental rats.

CHAPTER 2

LITERATURE REVIEW

2.1 THE PLANT: *Alchornea cordfolia*

Alchornea cordifolia (Schumach & Thonn) Müll.Arg belongs to the Family Euphorbiaceae. It has several local names across African countries, such as Bondji or dibonbunji in Cameroon, Tsehufu or Tsehifu in Togo, Agyarna, Ogyama, and Ayraba in Ghana, Me.Njekoi, Susu bolonta or bolontoi in Sierra Leone, Casamance bugong and bulora in Senegal, Mand.Hira in Gambia (Mavar-Manga *et al*., 2004). In Nigeria, *Alchornea cordifolia* is called Ewa Ipa, Ewe epa, Isin amongst the Yorubas, Ububa amongst the Ibos, and Bambami amongst Hausas. Other local names include Upia in Igede, Uwonwen in Benin, Mbom in Efik, Ukpaoromi in Ijaw and Asheshen amongst the Bekwarras. Common name is Dove wood or Christmas Bush.

The plant is an evergreen dioecious shrub or small tree up to 8m tall (Plate 2.1). Leaves alternate, simple; stipules triangular, 1.5mm long, acute, soon falling; petiole 5-15cm long; blade ovate to elliptical-ovate, 10-25cm x 7-15 cm, base cordate, with basal lobes slightly auriculate and overlapping, apex acute to acuminate, margins toothed, shortly hairy when young, later almost glabrous, 3-5 veined at the base with 4 glandular patches in the angles of the veins. Male inflorescence an axillary panicle up to 30-45 cm long, sparingly hairy, bracts minute; female Inflorescence an axillary spike or lax panicle up to 30-45 cm long, several together, bracts broadly triangular ovate, 1mm long, acuminate. Flowers unisexual, sessile; male flowers with 2 cup-shaped sepals, petals absent, stamens 8, the united filaments forming a basal plate; female flowers with 2-4 lobed calyx, lobes obtuse, hairy, petals absent, ovary superior, conical, 2mm x 2mm, smooth, densely silky hairy, styles 2-3, 1-2cm long. Free or fused at base, dark red. Fruit a

2-lobed capsule 1 .5cm x 1 .5cm lobe somewhat compressed, smooth, shortly hairy, green to red, 2-seeded. Seeds are ovoid ellipsoid, 6 mm long, smooth, bright red (Agbor *et al*., 2004). The plant is geographically distributed in secondary forests' usually near water, moist or marshy places (Dalziel, 1956) this shrub comprises of 70 tropical species.

Plate 2.1: Leaves of *Alchornea cordifolia* in its natural habitat

2.1.1 Medicinal Properties of *A. cordfolia*

Alchornea cordifolia is commonly used as a medicinal plant throughout its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits enter in local medicine. *Alchornea cordifolia* has been used widely in the treatment of rheumatism, arthritis, colds, muscle pains, cough, infertility, impotence, diabetes and diarrhoea. A decoction of fresh leaves of *A.cordifolia*, *Erythrina senegalensis*, *Abrus precatorius* and ripe fruit of *Musa spp*, when drunk is sometimes used for diarrhoea (Dalziel, 1956). The infusion of the dried leaf of *A.cordfolia* was used for diarrhoea in Zaire (Kambu *et al*, 1990; Muanza *et al*, 1994). The fresh chewed leaf juice of the plant was used for diarrhea in Sierra Leone (Macfoy and Sama, 1990).

Crushed fresh leaves or powdered dry leaves are applied externally as a cicatrisant. Tona *et al*, (1999) reported that the decoction of *A.cordifolia* was active against *Salmonella enteriditis* and *Shigella flexneri*. These bacteria species have been reported in diarrhoea alongside with *Escherichia coli* (Brooks *et al*., 1998). Extracts from leaves of *A.cordifolia* have been reported to inhibit the growth of bacteria such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *E.coli*, *Pseudomonas aeruginosa*, *S.albus* and *Escherichia coli* (Ogunlana and Rarnastad, 1975; Gatsing *et al*., 2010; Adeshina *et al*., 2011). The leaves are also taken as a blood purifier, as a tonic and to treat anaemia (Kambu *et al*., 1990) and epilepsy. In Senegal a leaf decoction is taken to treat tachycardia. Young stem pith is biller and astringent and is chewed for the same use. The leaves are eaten in Guinea-Bissau as an emmenagogue (Kambu *et al*., 1990) and to facilitate delivery, and in Gabon as an abortifacient. A cold infusion of the dried and crushed leaves acts as a diuretic (Kio *et al*., 1984) Leaf and root decoctions are widely used as mouth wash to treat ulcers

of the mouth, toothache and caries, and twigs are chewed for the same purposes. A decoction or paste of leafy twigs is applied as a wash to treat fever, malaria (Banzouzi *et al*., 2002), rheumatic pains (Daiziel 1956, Gbile and Adeshina, 1986 and Ogungbamila and Samuelsson, 1990) enlarged spleen and as a lotion or poultice to sore feet (Daiziel, 1956) vapour baths can also be taken for toothache in Gabon (Akendengue and Louis, 1994). The root pith is chewed for thrush and buccal ulceration. lt is also given along with young leaves, peppers and white clay in form of an enema to check abortion (Dalziel,1956).The water root-extract of the plant was used as an analgesic (applied externally) and as an antiseptic (inhalation) in Equatorial Guinea (Akendengue,1992). In Cote d'Ivoire and Ghana the leaves are applied as a haemostatic to stop prolonged menstruation and a decoction of roots or leaves is applied in the vagina to stop post-partum haemorrhage and to treat vaginitis. In Sierra Leone and Congo young leaves or pounded bark are made into a suppository to treat hemorrhoids. In DR Congo bruised leaves are applied as an enema to treat impotency.

The juice of the leaf also when taken orally was employed as an abortive in Gabon (Raponda and Sillans, 1961). The leaves or leafy stems, as an infusion or chewed fresh, are taken for their sedative and antispasmodic activities to treat a variety of respiratory problems including sore throat, cough and bronchitis (Le Grand and Wondergen, 1987; LeGrand, 1989). Dried leaves or roots, alone or with tobacco, are smoked to cure cough. The leaves and root bark are externally applied to treat leprosy and as an antidote to snake venom as eye drops to cure conjunctivitis. In Nigeria a decoction of bruised fruit is taken to prevent miscarriage. The sap of the fruit is applied to cure eye and skin diseases (Neuwinger, 2000). The hepatoprotective activity of the ethanol extract of *A. cordifolia* leaves against paracetamol-induced toxicity has also been reported

(Olaleye *et al*., 2006). Other uses include; in veterinary medicine a leaf or root infusion is given to livestock to treat trypanosomiasis (Adewunmi *et al*., 2001). In Nigeria the stem bark is thrown in dammed streams as a fish poison (Iwu, 1993).

2.1.2 Economic Importance of *A. cordfolia*

Alchornea cordifolia is used for alley cropping for in-situ mulch production in banana or maize plantations in West and East Africa. In Burkina Faso the plant is used as a windbreak around crops. In West Africa the leaves are used as forage for small ruminants and poultry. Chicken produce egg yolks with a deeper yellow colour when fed regularly with the leaves (Kanmegne *et al*., 1999). In West Africa the leaves are used for packing cola nuts and 'okpeye', a Nigerian condiment produced by fermenting seeds of *Prosopis africana*. Taub Pipe stems are made from the branches with the pith removed. The Iwo people of Nigeria chew the leaves as an appetizer. Dried leaves are a tea substitute. In the same region, mats and cloth are cooked with the fruits and natron to colour them black; the fruits are often combined with fermented *Parkia* pods or the bark of *Bridelia ferruginea Benth*. This dye is also used on pottery, calabashes and leather. The leaves are often added to indigo to darken its colour. In Nigeria fishermen use leaves and fruit for dyeing and preserving fishing nets; dried leaves give a darker colour than fresh ones (Kang *et al*., 1999). In Gabon, bark and leaves are used to blacken cloth and pottery. The wood ash serves as a mordant. The wood ash is light, soft and perishable and is used for house construction, stakes and kitchen utensils, and also benches when large stems are available. The wood is also used as fuel. In DR Congo the split sterns are used to line baskets (Kang *et al*., 1999). In Cameroon and Gabon the acidulous fruits are considered edible and are also used as bait to trap birds. The inflorescences are used in decorations (Kang *et al*., 1999). *Alchornea cordifolia* extract has been

patented for various other applications: antifouling adjuvant in paints, coatings and polymers, and alchorneic acid was proposed as a raw material for semi-synthesis of plastic (Kanmegne *et al*., 1999).

2.1.3 Phytochemical Constituents of *A. cordifolia*

Phytochemical screening revealed the presence of alkaloids, saponins, tannins (naturally occurring polyphenol), flavonoids, terpenes and glycosides with steroidal rings. (Osadebe and Okoye, 2003). Ellagic acids, coumarin, flavonoid and quercetin, (Ogungbamila and Samuelsson 1990). Abdullahi (2003) also' reported the presence of inulin, tannins, alchornine and alkaloid in the plant leaf. Alkaloids such as alchornine, tannins and polyphenols have been found in the leaf of *A.cordifolia* in Ghana (GHP, 1992). Glycosides, phenols and saponins were also, found in the leaves of *A. cordfolia* (Adeshina *et al*., 2007).

2.1.4 Research in Herbal Medicine

There is renewed research interest in herbal medicine for the following reasons (among others): The toxicity of many synthetic drugs has become obvious and by relying exclusively on these preparations, patients pay heavy price in adverse reactions. The overuse of antibiotics has also contributed to a profoundly dangerous medical threat to modern healthcare and this has made it necessary for major prescription to be reviewed and approved by personnel attuned to the problems of antibiotic abuse and aware of the alternative treatment. Active ingredients occur in small quantities in plants and are very much diluted when a decoction is prepared (Sofowora, 1982) and this perhaps makes the need for accurate dosage unnecessary. Finally in the light of our present economic predicament that is plaguing all sections especially healthcare; continued

efforts are needed to search for and identify those plants that could be useful medicinally to the society at large. Due to the problems of drug resistance and the ever increasing cost of modern drugs, scientists especially in Africa and developing countries are conducting researches into local plants which are used in traditional medicine. The use of plants for curing diseases has been known and practiced in Nigeria for many centuries and it is a well-known fact that in Nigeria about 65%of the population when sick seeks for herbal remedies from local herbalists. Research into herbs in Nigeria is undermined by several problems such as underfunding, lack of co-operation between the traditional herbalists and researchers in modem medicine, indiscriminate bush burning and attitude of some modem/orthodox medical practitioners and their desire for medical monopoly.

2.2 BLOOD GLUCOSE HOMEOSTASIS AND METABOLIC SYNDROME OF DIABETES

2.2.1 Blood Glucose Homeostasis

Beta cells in the islets of Langerhans release insulin in two phases. The first phase insulin release is rapidly triggered in response to increased blood glucose levels as follows: Glucose enters the beta cells through the glucose transporter GLUT2. Glucose goes into glycolysis and the respiratory cycle where multiple high- energy ATP molecules are produced by oxidation. Dependent on ATP levels, and hence blood glucose levels, the ATP-controlled potassium channels (K^+) close and the cell membrane depolarizes. On depolarization, voltage controlled calcium channels (Ca^{2+}) open and calcium flows into the cells. An increased calcium level causes activation of phospholipase C, which cleaves the membrane phospholipid phosphatidyl

inositol 4, 5-biphosphate into inositol 1, 4, 5- and diacylglycerol. Inositol 1, 4, 5-triphosphate (1P3) binds to receptor proteins in the membrane of endoplasmic reticulum (ER). This allows the release of Ca^{2+} from the ER via 1P3 gated channels, and further raises the cell concentration of calcium. Significantly increased amounts of calcium in the cells causes release of previously synthesized insulin, which has been stored in secretory vesicles.

The second phase is a sustained, slow release of newly formed vesicles that are triggered independently of sugar (Kumar and Stephen, 2005). In addition, some insulin release takes place generally with intake of food, not just glucose or carbohydrate intake, and beta cells are also somewhat influenced by the autonomic nervous system. The signaling mechanisms controlling these linkages arc not fully understood (Ivanova *et al*., 2009).When the glucose level comes down to the usual physiologic value, insulin release from beta cells slows or stops. If blood glucose levels drops lower than this, especially to dangerously low levels, release of hyperglycemic hormones, most prominently glucagon from Islet of Langerhans' alpha cells forces release of glucose into blood from cellular stores of glycogen (Reaven and Ami 1999).

2.2.2 Metabolic Syndrome of Diabetes

Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. It affects one in five people, and prevalence increases with age. Some studies estimate the prevalence in the USA to be up to 25% of the population. (NCEP, 2001). Metabolic syndrome is also known as metabolic syndrome X, or insulin resistance syndrome (Ford *et al*., 2002). The International Diabetes Federation consensus worldwide defines metabolic syndrome as central obesity (defined as waist circumference with ethnicity

specific values) and any two of the following: Raised triglycerides greater than 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality. Reduced HDL cholesterol less than 40 mg/dL (1.03 mmol/L) in males less than 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality. Raised systolic blood pressure greater than 130 or diastolic blood pressure greater than 85 mmHg, or treatment of previously diagnosed hypertension. Raised fasting plasma glucose (FPG) > greater than 100 mg/dL (5.6 mrnol/L), or previously diagnosed type 2 diabetes (khan *et al*., 2003)

2.3 COMPLICATIONS OF DIABETES MELLITUS

Diabetes is sometimes complicated by acidosis and coma, and in long standing diabetes there are additional complications. These include microvascular, macrovascular, and neuropahic disease. The microvascular abnormalities are proliferative scarring of the retina (diabetic retinopathy), leading to blindness; and renal disease (diabetic nephropathy), and leading to renal failure. The macrovascular abnormalities are due to accelerated atherosclerosis, which is secondary to increased plasma low-density lipoprotein cholesterol. The result is an increased incidence of stroke and myocardial infarction. The neuropathathic abnormalities (diabetic neuropathy) involve the autonomic nervous system and peripheral nerves (Arlan and Silverstein, 2003). The neuropathy plus the atherosclerotic circulation insufficiency in the extremities and reduced resistance to infection can lead to chronic ulceration and gangrene, particularly in the feet. The ultimate cause of the microvascular and neuropathy complications is chronic hyperglycemia, and tight control of diabetes reduces their incidence (Arlan and Silverstein, 2003).

2.4 INSULIN MANAGEMENT OF DIABETES MELLITUS

Insulin is a polypeptide hormone consisting of two peptide chains that are connected by disulfide bonds. It is synthesized as a precursor a (pro-insulin) that undergoes proteolytic cleavage to form insulin and C peptide, both of which are secreted by the beta cells of the pancreas (Richards and Pamela, 2009). Insulin is a hormone that is central to regulating the energy and glucose metabolism in the body. Insulin causes cells in the liver, muscle and fat tissue to take up glucose from blood, storing it as glycogen in the liver and muscle. When control of insulin level fails, diabetes mellitus result. Patients with type 1 diabetes mellitus depend on external insulin (most commonly injected subcutaneously) for survival because the hormone is no longer produced internally (William *et al*., 1998).

2.4.1 Mechanism of Insulin Action

Insulin receptors are found in different cells of the body, including cells in which insulin does not increase glucose uptake. The insulin receptor, which has a molecular weight of approximately 340,000, is a tetramer made up of two α and β glycoprotein subunits. The α subunit bind insulin and is extracellular, whereas the β subunits span the membrane. The intracellular portions of the β subunits have tyrosine kinase activity. The α and β subunits are both glycosylated, with sugar residues extending into the interstitial fluid. Binding of insulin triggers tyrosine kinase activity of the β subunits, producing autophosphorylation of the β subunit on the tyrosine residues. The autophosphorylation which is necessary for insulin to exert its biologic effects, triggers phosphorylation of some cytoplasmic proteins and dephosphorylation of others, mostly on serine and theorine residues (Williams *et al*.,1998). Four

related insulin receptor substrate (IRS) proteins in cells have been described: IRS-1, IRS-2, IRS-3 and IRS-4. IRS-1 has received the most attention (Williams *et al*., 1998). When insulin binds to its receptor, they aggregate in patches and are taken into the cell by receptor mediated endocytosis. Eventually, the insulin-receptor complexes enter lysosomes, where the receptors are broken down or recycled (Williams *et al*., 1998). The number or the affinity, or both, of insulin receptors is affected by insulin and other hormones, exercise, food, and other factors. Exposure to increased amounts of insulin receptor concentration (down regulation), and exposure to decreased insulin levels increases the number of receptors (Hellman *et al*., 2007).

2.5 BLOOD LIPID PROFILE

Plasma lipids consist mostly of lipoproteins-spherical macromolecular complexes of lipids and specific proteins (apolipoproteins). The clinically important lipoproteins, listed in decreasing order of atherogenicity, are: Low-density lipoprotein (LDL), Very-low-density lipoprotein (VLDL) and Chylomicrons, and High-density lipoprotein (HDL) (Richard and Pamela, 2009). A lipid profile is a direct measurement of three blood components: Cholesterol, triglycerides and high-density lipoproteins (HDL). Cholesterol and triglyceride are transported in the blood by combination of lipids and proteins called lipoproteins (Olson, 1998). Factors such as age, sex, and genetics can influence lipid profile, including diet, level of physical activity; level of diabetes control, and smoking status (USDA, 2008).

2.5.1 Plasma Cholesterol

Cholesterol is an important component for the manufacture of bile acids, steroid hormones, and several fat-soluble vitamins (Smith, 1991). Since cholesterol is essential for all animal life, it is

primarily synthesized from simpler substances within the body (Emma, 2009).However, high levels in blood circulation, depending on how it is transported within lipoproteins, are strongly associated with progression of atherosclerosis. The body therefore compensate for cholesterol intake by reducing the amount synthesized. Cholesterol is recycled. It is excreted by the liver via bile into digestive tract. Typically about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the blood stream (Olson, 1998).

The occurrence of coronary heart disease (CHD) is positively associated with high cholesterol, and more strongly with elevated LDL cholesterol in the blood (Richard and Pamela, 2009). Total cholesterol is the sum of HDL, LDL, and VLDL (Wang *et al*., 2009).The NCEP suggest that the total (fasting) blood cholesterol level should be: < 200mg/dl normal blood cholesterol, '200-239mg/dl borderline, and >240mg/dl high cholesterol (NCEP, 1988).

2.5.2 Plasma Triacyglycerols

Triglyceride is an ester composed of a glycerol bound to three fatty acids (Hemat, 2003). It is the main constituent of vegetable oil and animal fats. Triacylglycerols are formed from a single molecule of glycerol, combined with three molecules of fatty acid. The glycerol molecule has three hydroxyl (OH-) groups. Each fatty acid has a carboxyl group (COOH-). The enzyme pancreatic lipase acts at the ester bond, hydrolyzing the bond and releasing the fatty acid. (Daley *et al*., 2004). In triacylglycerol form, lipids cannot be absorbed by the duodenum. Fatty acids, monoglycerides (one glycerol, one fatty acid) and some diglycerides are absorbed by the duodenum, once the triacylglycerols have been broken down (Balch, 2006). Most natural fats contain a complex mixture of individual triacylglycerols (AHA, 2009). Usually, triacylglycerols

cannot pass through cell membrane freely. Special enzymes on the walls of blood vessels called lipoprotein lipases must break down triglycerides into free fatty acids and glycerol. Fatty acids can then be taken up by cells via the fatty acid transporters (Balch,2006).

In the human body, high levels of triacylglycerols in the blood stream (hypertriglyceridemia) have been linked to atherosclerosis, and by extension, the risk of heart disease and stroke. However, the relative negative impact of raised levels of triglycerides compared to that of LDL: HDL ratios is as yet unknown. This risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level (Balch, 2006). Triglyceride measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction.

2.5.3 Low- Density Lipoprotein Cholesterol (LDL-C)

Low-density lipoprotein (LDL) is one of the five major groups of lipoproteins (Chylomicrons, VLDL, IDL, HDL, LDL) that enable lipids like cholesterol and triglycerides to be transported within the water-based blood stream. Medically, estimates of cholesterol content carried by LDL particles are used as part of a cholesterol blood test. Since higher levels of LDL particles can promote medical problems like cardiovascular disease, they are often called bad cholesterol particles. Each native LDL particle contain a single apolipoprotein B-100 molecule (Apo B-100) which circulates the fatty acids, keeping them soluble in the aqueous environment (Warnick *et al.*,1990). Studies have shown that LDL particle vary in size and density, and that a pattern that has more small dense LDL particles, called pattern B, equates to a higher risk factor for coronary heart disease than does a pattern with more of the larger and less LDL particles (pattern A). This

is because the smaller particles are more easily able to penetrate the endothelium (Segrest *et al*., 2001; Superko *et al*., 2002).

2.5.4 High - Density Lipoprotein Cholesterol

High-density lipoproteins (HDL) are one of the major classes of plasma lipoproteins (chylornicrons, VLDL, IDL, LDL), that enable lipids like cholesterol and triacylglycerols to be transported within the water-based blood stream. In healthy individuals, about 30% of blood cholesterol is carried by HDL-C (AHA, 2009). It is hypothesized that HDL can remove atheroma within arteries and peripheral cells and transport it back to the liver for excretion or re-utilization, which is the main reason why HDL-bound cholesterol is sometimes called "good cholesterol" or. A high level of HDL-C seems to protect against cardiovascular disease, and low MDL cholesterol levels (less than 40mg/dl or about 1mm/L) increase the risk for heart disease (NIHCD, 1992).

2.6 HYPERLIPIDEMIA AND ANTIHYPERLIPIDEMIC DRUGS

2.6.1 Hyperlipidemias

Hyperlipidemias result from elevated levels of low-density lipoprotein (LDL) cholesterol and triglycerides and with low levels of high-density lipoprotein (HDL) cholesterol. It is a metabolic complication of both clinical and experimental diabetes. It is correlated with the incidence of coronary heart disease (CHD). Other risk factors for CHD include: hypertension, obesity, cigarette smoking and diabetes. Hyperlipidemias can also results from a single inherited gene

defect in lipoprotein metabolism or, more commonly, from a combination of genetic and life style factors (Richard and Pamela, 2009).

2.6.2 Antihyperlipidemic Drugs

Antihyperlipidemic drugs target problem of elevated serum lipids within complementary strategies. Some of these agents decrease production of the lipoprotein carriers of cholesterol and triglycerides, whereas others increase the degeneration of lipoprotein. Still others decrease cholesterol absorption or directly increase cholesterol removal from the body (Richard and Pamela, 2009).

2.6.2.1 HMG CoA Reductase Inhibitors

3- Hydroxy-3-methylglutaryl (HMG) coenzyme CoA reductase inhibitors (commonly known as statins) lower elevated LDL cholesterol levels, resulting in a substantial reduction in coronary events and deaths from CHD. This group of antihyperlipidemic agents inhibits the first committed enzymatic step of cholesterol synthesis in the cell, and they are the first- line and more effective treatment for patients with elevated LDL-C. To compensate for the decreased cholesterol availability, synthesis of hepatic LDL-C receptors is increased, resulting in an increased clearance of LDL-C from blood (Richard and Pamela, 2009). Rosuvastatin and atorvastatin are the most potent LDL cholesterol lowering statin drugs.

2.6.2.2 Niacin (Nicotinic Acid)

Niacin can reduce LDL (the “bad” cholesterol carrier) levels by 10-20 % and is the most effective agent for increasing HDL (the “good” cholesterol carrier) levels. At gram doses, niacin

strongly inhibits lipolysis in adipose tissue-the primary producer of circulating free fatty acids (Richard and Pamela, 2009). The liver normally utilizes these circulating fatty acids as a major precursor for triglyceride synthesis. Thus, niacin causes a decrease in the liver triglyceride synthesis, which is required for VLDL production. LDL (the cholesterol- rich lipoprotein) is derived from VLDL in the plasma. Therefore, a reduction in the VLDL concentration, thus, both plasma triglyceride (in VLDL) and cholesterol (in VLDL and LDL) are lowered (Richard and Pamela, 2009).

2.6.2.3 The Fibrates (Fenofibrate and Gemfibrozil)

Fenofibrate and gemfibrozil are derivatives of fibric acid that lower serum triglycerides and increase HDL levels. Both have the same mechanism of action (Richard and Pamela, 2009). The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor supergene family that regulate lipid metabolism. PPARs functions as a ligandactivated transcription factor. Upon binding to its natural ligand (fatty acids), PPARs are activated. They bind to peroxisome proliferator response elements, which are localized in numerous gene promoters. In particular, PPARs regulates the expression of genes encoding for proteins involved in lipoprotein structure and function. Fibrate-mediated gene expression ultimately leads to decreased triglyceride concentrations by increasing the expression of lipoprotein lipase and decreasing ApoC-II concentration. Fibrate also increase the level of HDL cholesterol by increasing the expression of ApoA-I and Apo A-II (Richard and Pamela, 2009).

2.6.2.4 Bile Acid-binding Resins

Bile acid sequestrants (resins) have significant LDL cholesterol-lowering effects, although the benefits are less than those observed with statins (Richard and Pamela. 2009). Cholestyramine, colestipol and colesevelam are anion-exchange resins that bind negatively charged bile acids salts in the small intestine. The resin-bile acid complex is excreted in the Feces, thus preventing the bile acids from returning to the liver by the enterohepatic circulation. Lowering the bile acid concentration causes hepatocytes to increase conversion of cholesterol to bile acids, resulting in a replenished supply of these compounds, which are essential components of the bile. Consequently, the intracellular cholesterol concentration decreases, which activate an increased hepatic uptake of cholesterol-containing LDL particles, leading to a fall in plasma LDL. In some patients a modest rise in plasma HDL levels is also observed. The final outcome of this sequence of events is a decreased total plasma cholesterol concentration (Richard and Pamela, 2009).

2.6.2.5 Cholesterol Absorption Inhibitors

Ezetimibe selectively inhibits intestinal absorption of dietary and biliary cholesterol in the small intestine, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood. Ezetimibe lowers LDL cholesterol by 17 % and triglycerides by 6 %, and it increases HDL cholesterol by 1.3 % (Richard and Pamela, 2009).

2.7 LIVER FUNCTION TESTS AND LIVER ENZYMES

The liver, like the salivary gland and pancreas, is an outgrowth of the digestive tract, a very large and versatile organ endowed with metabolically active cells which independently coordinate

virtually all biochemical transformations. Accordingly, the regenerating power of these cells is tremendous and involved metabolic, secretary, excretory, storage, detoxification, protective and synthetic functions, among others. Many plasma proteins are synthesized in the liver and their plasma levels therefore depend on the balance between synthesis and catabolism and/or loss from the body (Chatterjea, M. N. and R. Shinde, 2002.). These liver enzymes and proteins generally are very important biomarkers in the body utilized in the diagnosis and assessment of normal function of otherwise of the tissues, organs and the body as a whole. Major or minor changes in the integrity of cellular membranes in tissues or organs have culminated in changes in enzyme activities. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total protein levels, as well as the tissue histological assay are known to be useful in assessing the functional integrity of the liver.

2.7.1 Alanine Amino Transferase (ALT)

Alanine amino transferase (ALT) catalyzes the transfer of amino group of alanine α-ketogluterate resulting in the formation of pyruvate and glutamate. Alanine amino transferase is found in the liver, kidney, heart, skeletal muscle and pancreas in descending order of concentration (Wooton and Freeman, 1972). Normal concentration of ALT in plasma is 5-35 unit/ml (Amdur *et al*., 1998). Serum elevations of ALT activity are observed rarely in conditions other than parenchymal liver disease. Moreover elevations of ALT activity persist longer than those of AST activity (Ellis *et al*., 1978).

2.7.2 Aspartate Amino Transferase (AST)

Aspartate amino transferase (AST) catalyzes the transfer of amino group of aspertate to α-ketogluterate resulting in the formation of oxaloacetate and glutamate. Extreme rise of plasma AST more than 100 times the upper limit of normal is viewed in acute hepatic necrosis and viral or toxic hepatitis. This sign may occur before clinical signs or symptoms manifest. It is thus a sensitive index of active damage of hepatic parenchymal cells, being useful in detecting the onset of early hepatitis or latent liver cell damage (Schmidt and Saudway, 1963). Moderate elevation of AST between 40-100 units/ml is seen in patients with intrahepatic cholestasis, post hepatic jaundice, etc (Kumar and Clark, 1998). Amino transferase activities may also be elevated in myorcardial infaction, muscle disorders, drug toxicity and infection (Kumar and Clark, 1998). The normal range of plasma AST is 5-40 units/ml (Amdur *et al*., 1998).

2.7.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is an enzyme that catalyzes the hydrolysis of organic phosphate to produce inorganic phosphate in an alkaline medium. In cholestatic jaundice, levels may be 4-6 times the normal upper limit. Raised levels may also occur in condition with infilteration of the liver (e.g. metastitis) and in cirrhosis (Kaplan, 1972). Slight or moderate increase in ALP one or two times the normal occurs in many patients with parenchymal liver disorders such as hepatitis and cirrhosis. Transient increase may occur in all types of liver diseases (Podolsky and Isselbacher, 1998). The response of the liver to any form of biliary tract obstruction is to induce the synthesis of ALP. The main site of new enzyme synthesis is the hepatocytes adjacent to the biliary canaliculi. Some of the newly formed enzymes enter the circulation to raise the enzyme

level in serum. The elevation tends to be more marked in eases of extrahepatic obstruction than in those of intrahepatic obstruction.

2.8 MECHANISM OF STREPTOZOTOCIN ACTION ON β-CELLS OF RAT PANCREASE

Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action on B cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of both diabetogenic agents is mediated by reactive oxygen species, however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells. Streptozotocin enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, B cells undergo the destruction by necrosis (Szkudelski, 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Reagents

Streptozotocin was purchased from (Sigma Chemical Company St. Louis U.S.A.). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline Phosphatase (ALP), bilirubin, total cholesterol, triacylglycerol and HDL-cholesterol assay kits were purchased from Randox laboratories Ltd (Northern Ireland, UK). All chemicals and reagents used in this study were of analytical grade.

3.1.2 Collection of Plant Material and Identification

Alchornea cordfolia leaves were picked from a field at old Karu village, Abuja, Nigeria in the Month of June 2011. The specimen was identified and authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria - Nigeria and a voucher specimen number, 401 was deposited.

3.1.3 Animals

Seventy two apparently healthy Wister albino rats of both sexes weighing between I50-200g were obtained from the Animal house, Department of Pharmacology, ABU, Zaria. Male and female rats were housed separately in standard cages to avoid complications of pregnancy. They were acclimatized for 2 weeks under laboratory conditions maintained at a temperature of 25°C and humidity of 50%. The animals were maintained on standard pellets, grower’s mash (Vital feed, Grand Cereal Plc, Bukuru, Jos, Plateau State) and and water *ad libitum.*

3.2 METHODS

3.2.1 Extract Preparation

The Leaves of *A. cordfolia* were air dried and then reduced to coarse powder using wooden mortar and pestle. Then 400grams of coarsely powdered leaves were extracted with 2000mls distilled water using cold maceration for 24hours. The extract was filtered through cheese cloth with fine pore, and filtrate evaporated to dryness on a hot water bath at 100°C. Again another 500grams of the coarsely powdered leaves were extracted with l500mls of 70% (v/v) ethanol by cold maceration for 24 hours. The extract was similarly filtered and evaporated to dryness. The extracts were kept in suitable amber coloured containers until needed (Aliyu *et. al*., 2008).

3.2.2 Extract Fractionation

Hundred and eighty (180g) gram of Ethanol extract was suspended in 1000mls of distilled water. The filtrate was partitioned in a separating funnel with 750mls of Ethyl acetate. The Ethyl acetate fraction was concentrated on a water bath. The aqueous portion was further partitioned with 750mls of n-butanol to get the n-butanol fraction after concentration (Aliyu *et. al*., 2008). The fractions were kept in suitable containers until needed.

3.2.3 Comparative Hypoglycemic Activity of the Extracts and Fractions

A total of thirty (30) Wister rats were used and divided into six (6) groups of five (5) rats each, group 1 contained normal rats which were given distilled water, group 2 contained Streptozotocin- induced diabetic rats while groups 3, 4, 5 and 6 were treated with 200mg/kg body weight doses of Aqueous extract, Ethanol extract, Ethyl acetate and n-Butanol fractions of Ethanol extract respectively for three (3) weeks, orally. The choice of dose was based on

estimates of the LD50 and the minimum calculated dose was used to determine and compare the glucose suppressing activity of the extracts and ethanolic extract fractions. Blood glucose level of rats was monitored by glucometer at seven (7) day interval after treatment.

3.2.4 Phytochemical Screening

3.2.4.1 Qualitative Phytochemical Screening

Preliminary phytochemical screening of the aqueous extract, ethanol extract and its n-butanol and ethyl acetate fractions of *Alchornea cordifolia* leaf was carried out using standard methods as described by Sofowora (2008) to screen for the presence of various chemical constituents.

Test for Carbohydrate

Molisch Test: Few drops of Molisch reagent were added to 2mls of the extracts and fractions and then a small quantity of concentrated sulphuric acid was added and allowed to form a lower layer. A violet ring indicated the presence of carbohydrate.

Test for Glycosides

Concentrated sulphuric acid (5 mls) was added to each extract and fraction and boiled for 15 minutes. This was then cooled and neutralized with 20 % Potassium hydroxide and was divided into two portions. Another part of the extract/fraction was dissolved in distilled water; this was then used as a control, no acid hydrolysis.

(i) Fehling's solution test: 1:1 Fehling's solution A and B was added to one portion and boiled for few minutes. The presence of glycosides was indicated by a red colour

(ii) Ferric Chloride test: 3 drops of Ferric chloride was added to the second portion. Red colour was noted.

Test for Anthraquinone Derivatives

Test for Free Anthraquinone (Borntrager's test)

2mls of the extract/fraction was shaken with 10mI of chloroform and filtered. 5m1 of 10% ammonia solution was added to the filtrate and stirred. A pink colour indicated the presence of anthraquinone derivatives.

Test for Cardiac Glycosides

Keller-Killani Test: Extracts/Fractions were dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degrees, 1ml of concentrated sulphuric acid was added down the side. A greenish ring at the interface indicated the presence of cardiac glycosides

Test for Saponin

Frothing Test: Each sample, 2 g was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

Shisnoda Test: Each extract and fraction (0.5g) was dissolved in 2mls of 50% methanol by boiling on water bath for 5 minutes. Metallic Magnesium and 5 drops of concentrated HCL was added. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Tannins

Sample, 0.5 g was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for Alkaloids

Wagner's Test: Three drops of the reagent were added to a small amount of each extract and fraction. A reddish brown precipitate confirmed the presence of alkaloids.

3.2.4.2 Quantitative Estimation of Phytochemicals

Flavonoid: Quantitative determination of flavonoid was done by the method of Boham and Kocipai- Abyazan (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Alkaloid: Harborne (1973) method was used. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the

original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin: Van-Burden and Robinson (1981) method was used**;** 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin: The method used was that of Obdoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

3.2.5 Acute Toxicity Studies of *A. cordifolia* Extracts and Fractions on Wistar rats

The median lethal dose (LD50) of the plant extracts and fractions was carried out in order to select a suitable dose for the evaluation of antidiabetic activity. This was done using the method described by Lorke (1983.) using 12 rats. In the initial phase, rats were divided into 3 groups of 3 rats each and were treated orally with 10mg, 100mg and 1000mg of the extract per kg body weight. The rats were observed for 48 hours for signs of toxicity including death. Based on the results of phase one, three fresh rats were divided into 3 groups of one rat each, and were treated with 1600, 2,900 and 5,000 mg per kg body weight of the extracts and fractions. The rats were also observed for 48 hours for signs of toxicity including death.

3.2.6 Experimental Induction of Diabetes Mellitus

Diabetes was induced by single intraperitoneal injection of Streptozotocin (Sigma St. Louis, M.S., U.S.A.) at a dose of 150mg/kg body weight dissolved in 0.9% cold normal saline solution into 16-18 hours fasted rats (Katsumata *et al*., 1999). Since Streptozotocin is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 hours. The rats were then kept for the next 24 hours on 5% glucose solution in their cages to prevent hypoglycemia. After a period of three days of Streptozotocin treatment, blood was collected from tail vein of the rats. Rats having fasting blood glucose level greater than 200 mg/dl were considered as diabetic (Burcelin *et al*., 1995).

3.2.7 Experimental Design

From the preliminary studies it was established that the ethanolic extract and its n-butanol fraction were more active than their counterparts and hence they were used in the main work.

In the experiment, a total of forty five (45) rats were used. They were randomly divided into 9 groups of 5 rats each which received varying doses of either extract/fraction or standard anti-diabetic drug daily for four (4) weeks, orally. The blood glucose level was monitored weekly.

Group 1: Normal rats that received distilled water orally (Normal Control).

Group 2: STZ induced Diabetic rats that received distilled water orally (Diabetic Control).

Group 3: STZ induced Diabetic rats that received 200mg/kg body weight of n-Butanol fraction of ethanolic extract orally.

Group 4: STZ induced Diabetic rats that received 400mg/kg body weight of n-Butanol fraction of ethanolic extract orally.

Group 5: STZ induced Diabetic rats that received 800mg/kg body weight of n-Butanol fraction of ethanolic extract orally.

Group 6: STZ induced Diabetic rats that received 200mg/kg body weight of ethanolic extract orally.

Group 7: STZ induced Diabetic rats that received 400mg/kg body weight of ethanolic extract orally.

Group 8: STZ induced Diabetic rats that received 800mg/kg body weight of ethanolic extract orally.

Group 9: STZ induced Diabetic rats that received 10mg/kg body weight of standard drug, glibenclamide orally.

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3.3 BIOCHEMICAL ASSAYS

3.3.1 Collection and Preparation of Sera Samples

The study period lasted four (4) weeks after which the rats were fasted for 12 hrs then rats were sacrificed by exposing them to an over dose of chloroform soaked in cotton wool placed in anesthetic box covered with lid. Blood samples were drawn from the heart of each sacrificed rat from all groups by puncture. Blood samples were collected in Eppendorf tubes and allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 2556 g for 10minutes. The sera collected were then subjected to various biochemical analyses as shown below.

3.3.2 Determination of Blood Glucose Level

Blood samples were collected from the tail vein of the rats at 7 day interval (weekly) for 28 days (4 weeks). Fasting blood glucose levels were determined by using glucose oxidase method (Beach and Turner, 1958) using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) and the results were expressed in the unit of mg/dl (Rheney and Kirk, 2000). Based on the principle; the glucose in the blood sample reacts with the glucose oxidase to form gluconic acid, which then reacts with ferricyanide to form ferrocyanide. The electrode oxidizes the ferrocyanide, which then generates a current directly proportional to the glucose concentration. The total charge passing through the electrode is proportional to the amount of glucose in the blood that has reacted with the enzyme.

3.3.3 Estimation of Serum Lipid Profile

Serum lipid profiles were determined spectrophotometrically, using enzymatic colornetric assay kits (Randox, Northern Ireland) as follows:

3.3.3.1 Determination of Serum Total Cholesterol

The serum (0.1ml) level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25°C after mixing and the absorbance of the sample (A sample) and standard' (A standard) was measured against the reagent blank within 30 minutes at 546nm. The value of TC present in serum was expressed in the unit of mg/dl. TC concentration = A sample/A standard x 196.86 mg/dl

3.3.3.2 Determination of Serum Triacylglycerol

The serum triacyglycerol level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). First l000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A sample) and standard (A standard) was measured against the blank within 30 minutes at 546nm.The value of triacylglycerol present in the serum was expressed in the unit of mg/dl.

TGL concentration = A sample/A standard x 194.0 mg/dI

3.3.3.3 Determination of Serum High-Density Lipoprotein Cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes

at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dl.

3.3.3.4 Determination of Serum Low-Density Lipoprotein Cholesterol

The serum level of (LDL-C) was measured according to protocol of Friedewald *et al*., (1972) using the equation below:

$$LDL\text{-}C = \{TC - (TGL/5) - HDL\text{-}C\}.$$

The value was expressed in the unit of mg/dl.

3.3.4 Estimation of Liver Enzymes

3.3.4.1 Determination ofAspartate aminotransferase (AST)

Activity of aspartate arninotransferase was assayed by the method described by Sini *et al*., (2006). First 0.2m1 of sample fraction and 0.5m1 of substrate solution; aspartate and 2-ketogluterate were incubated at 37°C for 60 min. After incubation, 0.5 ml of DNPH solution was added to each to stop the reaction and allowed to stand for 20min at room temperature. To this, lml of 0.4N NaOH was added and absorbance read at 5l0 nm. Activities were expressed as lU/L.

3.3.4.2 Determination of Alanine aminotransferase (ALT)

Activity of alanine aminotransferase (ALT) was assayed by the method described by Sini *et al*., (2006). 0.2m1 of sample fraction and 0.5m1 of substrate solution of alanine and 2-ketogluterate were incubated at 37°C for 30 min. After incubation, 0.5 ml of DNPH solution was added to

arrest the reaction, which was kept for 20min at room temperature. To this, lml of 0.4N NaOH was added and absorbance read at 5l0 nm. Activities were expressed as lU/L.

3.3.4.3 Determination of Alkaline phosphatase (ALP)

Activity of alkaline phosphatase (ALP) was determined using Randox assay kit from Randox laboratories Ltd. Ardmore, Co. Antrm UK. Substrate solution (3 ml) was incubated at 37°C for 15 minutes and then 0.5 ml of the samples was added to it. This was mixed with 9.5 ml of 0.085 N NaOH. This corresponded to zero time assay (blank). The remaining solution (substrate + enzyme) was incubated for 15 minutes at 37°C and then 0.5 ml was drawn and mixed with 9.5 ml of 0.085N NaOH. Absorbance was measured at 405nm against the reference blank. Specific activities were expressed as moles of p- nitrophenol formed.

3.3.4.4 Bilirubin Assays

Determined by Colorimetric method based on that described by Jendrassik and Grof (1938), using Randox assay kit from Randox laboratories Ltd. Ardmore, Co. Antrm UK. Total bilirubin was determined in the presence of caffeine which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

3.3.5 Haematological Analysis

An auto heamatological analyzer (XE-2100 by Sysmex Corporation) was used to determine the heamoglobin concentration (Hb), red blood cell count (RBC), total white blood cell count (WBC), lymphocytes, Monocytes, Eosinophill and Packed cell volume (PCV), using the method of Dacie and Lewis (1991).

3.3.6 Histopathological Studies

Sacrificed animals from each group were subjected to postmortem gross examinations. Then the pancreatic tissues, livers and kidneys were dissected out and washed on ice cold saline immediately. A portion of the tissues were fixed in 10% neutral formal saline fixative solution for histological studies. After fixation, tissues were embedded in paraffin, solid sections were cut at 5 mm and various sections were stained with haematoxylin and eosins as described by Strate *et al*., (2005). The slides were viewed at magnification of X 250 and photomicrographs taken.

3.4 SUB-ACUTE TOXICITY STUDIES

This was under taken to ascertain the safety and toxicity if any; the plant may have on the liver and kidney of the experimental animals. The aqueous extract of the plant is the usual form that is consumed orally as anti-diabetic agent in traditional medicine. To achieve this, fifteen (15) fresh adult albino wistar of both sexes weighing 150-200g were used for this study. They were randomly divided into three groups of five animals each as follows:

Group 1: Received 200mg/kg b w aqueous extract of *Alchornea cordifolia* orally

Group 2: Received 400 mg/kg b w aqueous extract of *Alchornea cordfolia* orally

Group 3: Received 800mg/kg b w aqueous extract of *Alchornea cordfolia* orally

The animals were subjected to oral administration of the aqueous plant extract for a period of 28 days. After the last day of administration three animals from each group were sacrificed as described in section 3.3.1. The liver and kidney of the rats were removed dissected and studied as in section 3.3.5 above.

3.5 STATISTICAL ANALYSIS

Data obtained was expressed as mean ± SD and statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests to compare the level of significance between the test groups. The values of $p<0.05$ were considered as significant.

CHAPTER 4

RESULTS

4.1 Comparative Hypoglycemic Studies

Table 4.1 reveals the degree of effectiveness of the extracts and fractions in reducing the level of fasting blood glucose in diabetic rats after three (3) week treatment with 200 mg/kg body weight of the aqueous extract, ethanol extract and its n-butanol and ethylacetate fractions in comparison with the positive control, untreated diabetic rats. On day 1, before the administration of STZ, the fasting blood glucose level did not differ significantly (P>0.05) between the six groups of experimental rats. Likewise, at the end of the first week, day 7, there was no statistically significant (P>0.05) difference between the groups treated with 200 mg/kg bw aqueous extract, ethanol extract and its ethyl acetate fraction. However, the blood glucose level of animals administered 200 mg/kg bw (284.5 ± 83.3 mg/dl) n-butanol fraction, significantly (P<0.05) decreased when compared to the untreated diabetic group (384.3 ± 41.0). At day 14, the average blood glucose levels of aqueous extract (280 ± 30.37 mg/dl), ethanol extract (243 ± 61.83 mg/dl), ethyl acetate fraction (292 ± 61.49 mg/dl) and n-butanol fraction (200.0 ± 60.1 mg/dl) decreased significantly (P<0.05) when compared to the positive control, untreated diabetic rats (372.5 ± 24.4 mg/dl). Also, at day 21, there was a significant (P<0.05) decrease in fasting blood glucose level of groups given 200 mg/kg bw (258 ± 38.68, 193.2 ± 64.2, 275 ± 48.39 mg/dl) aqueous extract, ethanol extract, ethyl acetate fraction, n-butanol fraction, while the group administered 200 mg/kg bw (152.2 ± 51.9 mg/dl) n-butanol fraction recorded a significantly (P<0.05) reduced blood glucose level when compared with the positive control, untreated diabetic rats (372.6 ± 26.1 mg/dl). Ethanol extract (193.2 ± 64.2 mg/dl) and its n-butanol fraction (152.2 ± 51.9 mg/dl) were most effective in reducing the blood glucose level of diabetic rats.

Table 4.1: Comparative Hypoglycemic Potentials of Leaf Ethanolic and Aqueous Extracts of *Alchornea cordifolia* and of n-Butanol and Ethyl acetate Fractions of its Ethanolic Extract

Groups	Teatment	Fasting Blood Glucose Levels after Three (3) Week treatment (mg/dl)			
		Day 1	Day 7	Day 14	Day 21
1	Normal Rats + D/W	91.4 ± 13.1^{a}	88.6 ± 13.5^{a}	90.0 ± 12.2^{a}	86.10 ± 12.6^{a}
2	SIDR + D/W	386.0 ± 50.2^{b}	384.3 ± 41.0^{a}	372.5 ± 24.4^{a}	372.6 ± 26.1^{a}
3	SIDR + Aqueous Extract 200mg/kg bw	383.0 ± 87.8^{ns}	343 ± 91.8^{ns}	280 ± 30.3^{c}	258 ± 38.6^{c}
4	SIDR + Ethanol Extract 200mg/kg bw	375.8 ± 55.1^{ns}	297.4 ± 66.2^{ns}	243 ± 61.8^{c}	193.2 ± 64.2^{c}
5	SIDR + Ethyl actetate Fraction 200mg/kg bw	385.8 ± 70.0^{ns}	355 ± 34.5^{ns}	292 ± 61.4^{c}	275 ± 48.3^{c}
6	SIDR + n-Butanol Fraction 200mg/kg bw	380.0 ± 57.8^{ns}	284.5 ± 83.3^{a}	200.0 ± 60.1^{c}	152.2 ± 51.9^{a}

Values are presented as mean ± SD, after four (3) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

4.2 Yields of Extracts and Ethanolic Fractions of *Alchornea cordifolia* Leaf

The percentage yield of the leaf extracts and fractions is shown in Table 4.2. The Aqueous Extract, Ethanol Extract and Ethyl acetate fraction were green in color while the n-butanol fraction was Brown. The Aqueous Extract had the lowest yield of 38.05% followed by the Ethanol Extract which had a yield of 41.42%. Percentage yield of the two fractions, Ethyl acetate and n-butanol were close at about 50%.

4.3 Phytochemicals Present in Extracts and Ethanolic Fractions of *Alchornea cordifolia* Leaf

The phytochemical screening revealed the presence of several medicinally active chemical constituents in the various extracts and Ethanolic fractions (Table 4.3). All the extracts and fractions contained carbohydrates, glycosides, saponins, flavonoids, cardiac glycosides, alkaloids and tannins but Anthraquinones are only present in Ethanolic extract.

4.4 Quantitative Estimation of Some Phytochemicals Present in Ethanolic Extract and Fractions of *Alchornea cordifolia* Leaf

The five (5) major groups of phytochemical constituents in the extracts and ethanolic fractions of the plant are shown in Table 4.4; n-butanol fraction of ethanolic extract contained the highest crude yield of Flavonoids (78.76%), while aqueous extract contained the lowest yield of flavonoid (10.76%). Likewise, n-butanol fraction contained the highest yield of alkaloids (9.11%), and ethylacetate fraction contained the lowest crude yield of alkaloids (4.40%). The highest crude yield (25.23%) of tannin was found in n-butanol, while ethylacetate fraction contained the lowest crude yield of tannin (17.72%). Also, n-butanol fraction contained the

highest yield of saponin (12.20%), while ethylacetate fraction contained the lowest crude yield of saponin (4.90%).

4.5 LD_{50} of *A. cordifolia* Leaf Extracts and Fractions

There were no deaths recorded in the first phase, 48 hours after oral administration of 10, 100 and 1000 mg/kg body weight of the extracts and ethanolic fractions to the rats. However, after oral administration of 1000, 1600, 2900 and 5000 mg/kg body weight of the extracts and ethanolic fractions, there was 100% mortality in the groups administered 2900 and 5000 mg/kg body weight of the extracts and ethyl acetate fraction of the ethanolic extract in 48 hours, while signs of toxicity and suffocation were observed in the rats administered similar doses of n-butanol fraction of ethanol extract of *A.cordifolia* leaf in 72 hours. The LD50 was thus calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, 1600 x 2900 = 2154mg/kg.

Table 4.2: Percentage Yield of The Aqueous, Ethanol Extracts and Ethyl acetate and n-Butanol Fractions of Ethanolic Extract of Leaves of *Alchornea cordiforlia*

EXTRACTS/FRACTIONS	% yield
Aqueous Extract	38.05
Ethanol Extract	41.42
Ethyl acetate Fraction of Ethanolic Extract	50.46
n-Butanol Fraction of Ethanolic Extract	49.60

Table 4.3: Some Phytochemicals Present in Extracts and Ethanolic Fractions of the Leaf of *Alchornea cordifolia*

Phytochemicals	Ethanol Extract	Aqueous Extract	n-Butanol Fraction	Ethylacetate Fraction
1. Carbohydrate	+	+	+	+
2. Glycosides	+	+	+	+
3.Anthraquinones	+	-	-	-
4.Cardiac glycosides	+	+	+	+
5. Saponins	+	+	+	+
6. Flavonoids	+	+	+	+
7. Tannins	+	+	+	+
8. Alkaloids	+	+	+	+

(+) = Present
(-) = Absent

Table 4.4: Phytochemical Contents of *A.cordifolia* Leaf Ethanolic Extract and its n-Butanol Fraction (%)

Phytochemicals	Ethanol Extract	Aqueous Extract	Ethylacetate Fraction of Ethanolic Extract	n-Butanol Fraction of Ethanolic Extract
Flavonoid (%)	45.23 ± 22.92	10.76 ± 1.53	36.69 ± 27.27	78.76 ± 10.56
Alkaloids (%)	4.86 ± 1.62	6.46 ± 0.72	4.40 ± 2.09	9.11 ± 3.11
Tannin (%)	20.02 ± 2.46	31.56 ± 3.09	17.72 ± 2.09	25.23 ± 8.63
Saponin (%)	6.80 ± 1.31	5.13 ± 0.23	4.90 ± 1.65	12.20 ± 1.70
Glycosides (%)	13.05 ± 0.67	21.91 ± 3.19	11.07 ± 0.44	29.10 ± 2.68

Values are presented as mean ± SD of three determinations

4.6 Effect of Daily Oral Doses of *Alchornea cordifolia* Leaf Ethanolic Extract and its n-Butanol Fraction on the Body Weights of Experimental Animals

There was no significant change in the body weights of animals, as shown in Table 4.5, throughout the first week study period when compared to the positive control, untreated diabetic mice. In the second week, however, there was a statistically significant (P<0.05) change in the average body weight of groups treated with 200, 400 mg/kg body weight (161.4 ± 10.5, 161.2 ± 7.6 g) n-butanol fraction and 400, 800 mg/kg body weight (161.8 ± 4.8, 169.2 ± 4.3 g) ethanol extract when compared with the positive control, untreated rats (146.6 ± 5.7 g). There was a statistically significant (P<0.05) change in the body weight of animals treated with 800mg/kg body weight (165.0 ± 8.1 g) n-butanol fraction, while no significant change occurred in the group given 200mg/kg bodyweight (159.0 ± 4.5g) ethanol extract. In the third week, the average body weight of experimental animals given varying doses of the ethanol extract and its n-butanol fraction were statistically significant (P<0.05) when compared with the positive control, untreated diabetic rats. In the fourth and final week, a significant (P<0.05) dose dependent increase in body weight of experimental animals treated with 200, 400, 800 mg/kg bw (171.4 ± 6.4, 172.8 ± 6.6, 174.6 ± 8.2 g) n-butanol fraction when compared with the positive control, untreated diabetic animals (135.0 ± 4.5 g). Also the diabetic group administered 800mg/kg bw (174.6 ± 8.2 g) n-butanol fraction recorded a better increase in body weight when compared with diabetic groups that received 400 and 200mg/kg bw of the fraction. There was a decrease in body weight of animals in the test groups treated with the extract and its fraction when compared with the group treated with the standard drug, glibenclamide 10mg/kg bw (178.2 ± 7.6 g). Also, decrease in body weight was observed between the test groups and the negative control, non-diabetic (192.4 ± 3.9 g) with a much higher value of body weight.

At the end of the study period (week 4), there was a statistically significant (P<0.05) dose dependent increase in body weight of experimental animals treated with 200 400 800 mg/kg bw (169.6 ± 3.8, 173.4 ± 3.1, 174.6 ± 4.7 g) ethanol extract when compared with the body weight of positive control group, untreated diabetic animals (135.0 ± 4.5 g). For this, the diabetic group administered 800mg/kg bw (174.6 ± 4.7 g) of the ethanol extract recorded a better increase in body weight when compared with diabetic groups that received 400 and 200mg/kg bw (169.6 ± 3.8, 173.4 ± 3.1 g) of the ethanol extract. There was a decrease in body weight in groups treated with varying doses of ethanol extract and its n-butanol fraction when compared to the group treated with the standard drug, glibenclamide 10mg/kg bw (178.2 ± 7.6 g) while a dose dependent decrease in body weight was observed in the test groups when compared to the negative control, non-diabetic group (192.4 ± 3.9 g).

There was a dose dependent increase in the body weight of diabetic animals treated with 200, 400 mg/kg bw (171.4 ± 6.4, 172.8 ± 6.6 g) n-butanol fraction when compared with values of body weight (169.6 ± 3.8, 173.4 ± 3.1 g) obtained with similar concentrations of the ethanol extract. However, at concentration, 800 mg/kg bw (174.6 ± 4.7 g), the body weight of animals treated with the ethanol extract and the body weight (174.6 ± 8.2g) of animals treated with its n-butanol fraction had similar values.

Table 4.5: Effect of Daily Oral Doses of *Alchornea cordifolia* Leaf Ethanolic Extract and its n-Butanol Fraction on the Body Weights of Experimental Rats

Group	Treatment Given (n=5)	CHANGES IN BODY WEIGHT (g)				
		Day 1	Day 7	Day 14	Day 21	Day 28
1	Normal Rats + D/W	163.4 ± 8.0[a]	174.0 ± 5.9[c]	183.0 ± 4.6[c]	191.8 ± 3.6[a]	192.4 ± 3.9[c]
2	SIDR+ D/W	167.8 ± 11.6[a]	153.8 ± 5.0[a]	146.6 ± 5.7[a]	137.6 ± 5.5[b]	135.0 ± 4.5[b]
3	SIDR Rats + n-But 200 mg/kg bw	164.8 ± 10.8[ns]	159.2 ± 10.9[ns]	161.4 ± 10.5[a]	164.8 ± 9.5[c]	171.4 ± 6.4[c]
4	SIDR Rats + n-But 400 mg/kg bw	163.0 ± 10.8[ns]	155.8 ± 9.3[ns]	161.2 ± 7.6[a]	166.6 ± 7.6[c]	172.8 ± 6.6[c]
5	SIDR Rats + n-But 800 mg/kg bw	167.4 ± 12.5[ns]	156.4 ± 10.6[ns]	165.0 ± 8.1[b]	169.2 ± 9.2[c]	174.6 ± 8.2[c]
6	SIDR Rats + Eth 200 mg/kg b w	160.0 ± 8.6[ns]	153.0 ± 5.5[ns]	159.0 ± 4.5[ns]	162.8 ± 4.3[c]	169.6 ± 3.8[c]
7	SIDR Rats + Eth 400 mg/kg b w	161.6 ± 7.1[ns]	155.6 ± 6.7[ns]	161.8 ± 4.8[a]	169.0 ± 2.9[c]	173.4 ± 3.1[c]
8	SIDR Rats + Eth 800 mg/kg b w	160.0 ± 2.3[ns]	153.2 ± 3.0[ns]	169.2 ± 4.3[a]	172.0 ± 4.5[c]	174.6 ± 4.7[c]
9	SIDR Rats + Std 10mg/kg b w	166.4 ± 13.0[ns]	154.2 ± 9.2[ns]	165.2 ± 6.2[b]	177.0 ± 6.2[c]	178.2 ± 7.6[c]

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

4.7 Hypoglycemic Effect of *Alchornia cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction

A study of the effect of administration of A.cordifolia leaf ethanolic extract and its n-butanol fraction on blood glucose level of Streptozotocin-induced diabetic rats is shown in Table 4.6. There was a significant (P<0.05) dose dependent decrease in fasting blood glucose level of the diabetic groups treated with 200, 400, 800 mg/kg bw (114.0 ± 29.2, 102.2 ± 11.2, 104.8 ± 7.6 mg/dl) n-butanol fraction when compared with the positive control group, untreated diabetic animals (364.4.0 ± 39.6 mg/dl), at the end of the study period. Diabetic group treated with 400mg/kg bw (102.2 ± 11.2 mg/dl) of the n-butanol fraction had the lowest level of fasting blood glucose, slightly lower than the group treated with 800mg/kg bw (104.8 ± 7.6 mg/dl) n-butanol fraction. When compared with the diabetic group treated with 10mg/kg bw (95.8 ± 3.9 mg/dl) of the standard drug, glibenclamide, there was a dose dependent increase in fasting blood glucose level of the test groups given ethanol extract and its n-butanol fraction. Also, a dose dependent increase in fasting blood glucose level was observed in the test groups when compared with the negative control, non-diabetic (88.6 ± 4.5 mg/dl).

A statistically significant (P<0.05) dose dependent decrease in blood glucose level of diabetic animals treated with ethanol extract 200, 400 and 800 mg/kg bw (152.2 ± 32.6, 141.8 ± 36.9, 127.6 ± 23.3 mg/dl) when compared with the positive control group, untreated diabetic animals (364.4.0 ± 39.6 mg/dl). There was an increase in the level of fasting blood glucose of diabetic groups administered varying doses of the ethanol extract when compared with the diabetic group treated with 10mg/kg bw (95.8 ± 3.9 mg/dl) of the standard drug, glibenclamide. An increase in blood glucose level was recorded in the ethanol extract and n-butanol fraction treated diabetic

groups when compared with the negative control, normal rats (88.6 ± 4.5mg/dl). The diabetic group administered 400mg/kg bw (141.8 ± 36.9 mg/dl) of the ethanol extract had the lowest fasting blood glucose level at the end of the four (4) week study period.

There was a dose dependent decrease 200, 400, 800 mg/kg bw (114.0 ± 28.7, 102.2 ± 11.2, 104.8 ± 7.6 mg/dl) in fasting blood glucose levels of diabetic animals treated with n-butanol fraction of the ethanol extract when compared with fasting blood glucose levels (152.2 ± 32.6, 141.8 ± 36.9, 127.6 ± 23.3 mg/dl) of diabetic groups treated with the ethanol extract.

Table 4.6: Effect of Daily Oral Doses of *Alcornia cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction on Blood Glucose Levels of Normal, Streptozotocin induced Diabetic Rats.

Group	Treatment Given (n=5)	FASTING BLOOD GLUCOSE LEVELS (mg/dl)				
		Day 1	Day 7	Day 14	Day 21	Day 28
1	Normal Rats + D/W	91.4 ± 6.8[a]	88.6 ± 8.0[a]	90.0 ± 9.1[a]	87.8 ± 8.5[a]	88.6 ± 4.5[a]
2	SIDR Rats + D/W	381.0 ± 124.3[b]	383.4 ± 66.5[a]	384.0 ± 60.0[a]	363.8 ± 40.2[c]	364.4 ± 39.6[c]
3	SIDR Rats + n-But 200 mg/kg bw	382.4 ± 93.6[ns]	282.2 ± 64.7[a]	203.0 ± 34.6[c]	154.2 ± 41.1[a]	114.0 ± 28.7[c]
4	SIDR Rats + N-But 400 mg/kg bw	372.6 ± 45.2[ns]	246.6 ± 42.5[b]	185.8 ± 45.0[c]	140.0 ± 29.2[ns]	102.2 ± 11.2[c]
5	SIDR Rats + N-But 800 mg/kg bw	382.6 ± 72.6[ns]	260.8 ± 55.6[b]	175.8 ± 37.3[c]	120.8 ± 14.0[ns]	104.8 ± 7.6[c]
6	SIDR Rats + Eth 200 mg/kgbw	375.8 ± 51.8[ns]	297.4 ± 44.6[ns]	243.0 ± 41.8[c]	190.0 ± 19.5[c]	152.2 ± 32.6[c]
7	SIDR Rats + Eth 400mg/kgb w	384.6 ± 51.7[ns]	282.8 ± 50.9[a]	218.6 ± 38.6[c]	166.8 ± 41.4[b]	141.8 ± 36.9[c]
8	SIDR Rats + Eth 800mg/kgb w	378.4 ± 51.8[ns]	278.2 ± 24.1[a]	198.6 ± 34.9[c]	155.2 ± 42.4[a]	127.6 ± 23.3[c]
9	SIDR Rats + Std 10mg/kg b w	358.6 ± 30.8[ns]	187.0 ± 43.6[c]	129.0 ± 4.3[c]	103.0 ± 4.3[ns]	95.8 ± 3.9[c]

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

4.8 Anti-hyperlipidemic Effect of *Alchormea cordifolia* Ethanolic Leaf Extract and its Fraction

Data shown in Table 4.7 reveals the study of serum lipid profile levels of extract and treated groups as compared to the diabetic group. There was no significant change in the level of serum TC between diabetic groups treated with 200mg/kg bw (135.57 ± 11.15 mg/dl) n-butanol fraction and 200mg/kg bw (136.47 ± 13.91 mg/dl) ethanol extract with the positive control, untreated rats (164.63 ± 14.71 mg/dl). There was significant (P<0.05) dose dependent decrease in the concentration of TC at dose concentrations 400mg/kg bw (108.64 ± 15.83 mg/dl), 800mg/kg bw (91.73 ± 20.87 mg/dl) n-butanol fraction and 400mg/kg bw (109.02 ± 18.88 mg/dl), 800mg/kg bw (112.65 ± 18.46 mg/dl) ethanol extract when compared with the positive control, untreated diabetic group (164.63 ± 14.71 mg/dl). At dose concentration 800mg/kg bw (91.73 ± 20.87 mg/dl), n-butanol fraction showed a better TC lowering effect than the standard drug, glibenclamide 10mg/kg bw (105.42 ± 21.34 mg/dl), this renders it less effective than the standard drug. The level of serum total cholesterol in the negative control, non-diabetic group (79.29 ± 3.67 mg/dl) was significantly less than that in the test groups (treated with ethanol extract and its n-butanol fraction). There was significant (P<0.05) change in the level serum triacylglycerol for dose concentration 200mg/kg bw (159.33 ± 9.94 mg/dl) n-butanol fraction and highly significant (P<0.05) decrease at dose concentrations 400mg/kg bw (117.00 ± 14.27 mg/dl), 800mg/kg (107.79 ± 15.78 mg/dl) n-butanol fraction and 400mg/kg bw (121.96 ± 25.33 mg/dl), 800mg/kg bw (143.69 ± 15.61 mg/dl) ethanol extract when compared with the positive control, untreated diabetic group (206.23 ± 13.42 mg/dl). Similar value of serum triacyglycerol was recorded in the diabetic group treated with 800mg/kg bw (107.79 ± 15.78 mg/dl) n-butanol fraction as the diabetic group treated with 10mg/kg bw (107.41 ± 36.57 mg/dl) of the standard

drug, glibenclamide. No significant change was observed at 200mg/kg bw (172.00 ± 15.94 mg/dl) ethanol extract. Evidently, the concentration of serum triacyglycerol in the negative control, non-diabetic group (89.00 ± 18.24 mg/dl) was lesser than that in the test groups (treated with ethanol extract and its n-butanol fraction). For serum HDL, no statistically significant change was observed in diabetic groups administered 200mg/kg bw (22.92 ± 37.29 mg/dl) and 400mg/kg bw (29.79 ± 10.21 mg/dl) n-butanol fraction, also, 200mg/kg bw (21.35 ± 5.08 mg/dl), 400mg/kg bw (29.31 ± 12.33 mg/dl) and 800mg/kg bw (32.54 ± 12.91 mg/dl) ethanol extract when compared with the positive control, untreated diabetic group (15.39 ± 6.46 mg/dl). However, there was a statistically significant ($P<0.05$) change in the HDL level of diabetic animals administered 800mg/kg bw (34.61 ± 8.18 mg/dl) n-butanol fraction of the extract when compared with the negative control group, normal rats. There was a slight increase in the concentration of serum HDL of diabetic group treated with 800mg/kg bw (34.61 ± 8.18 mg/dl) n-butanol fraction in comparison to a lesser concentration of serum HDL in diabetic group treated with 10mg/kg bw (35.14 ± 9.20 mg/dl) glibenclamide. All treated diabetic groups maintained a lesser HDL level when compared with the negative control, non-diabetic group (45.48 ± 10.74 mg/dl). Serum LDL-C level was significantly ($P<0.05$) lowered at dose concentrations 400mg/kg bw (55.44 ± 14.42 mg/dl) and 800mg/kg bw (35.56 ± 16.43 mg/dl) n-butanol fraction, also 400mg/kg bw (55.31 ± 25.28 mg/dl) and 800mg/kg bw (52.44 ± 11.68 mg/dl) ethanol extract compared with the positive control, untreated diabetic group (107.98 ± 15.83 mg/dl). At dose concentrations of 200mg/kg bw (80.78 ± 12.42 mg/dl) n-butanol fraction and 200mg/kg bw (80.71 ± 12.77 mg/dl) ethanol extract, there was no significant change. At dose concentration 800mg/kg bw (35.56 ± 16.43 mg/dl) n-butanol fraction, showed a better serum LDL-C lowering effect than the standard drug, glibenclamide 10mg/kg bw (48.76 ± 13.75

mg/dl). There was a significant difference in the level of LDL-C between the treated diabetic groups and the negative control, normal rats (16.01 ± 7.98 mg/dl).

Table 4.7: Effect of Daily Oral Doses of *Alchornia cordifolia* Ethanol Leaf Extract and its n-Butanol Fraction on Serum Lipid Profile Level of STZ-Induced Diabetic Rats (mg/dl)

Group (n=5)	Treatment Given	Serum total cholesterol	Serum Triglyceride	SerumHDL	SerumLDL
1	Normal Rats + D/W	79.29 ± 3.67[c]	89.00 ± 18.24[c]	45.48 ± 10.74[c]	16.01 ± 7.98[b]
2	SIDR Rats + D/W	164.63 ± 14.71[a]	206.23 ± 13.42[c]	15.39 ± 6.46[a]	107.98 ± 15.83[c]
3	SIDR Rats + N-But 200 mg/kg bw	135.57 ± 11.15[ns]	159.33 ± 9.94[b]	22.92 ± 37.29[ns]	80.78 ± 12.42[ns]
4	SIDR Rats + N-But 400 mg/kg bw	108.64 ± 15.83[c]	117.00 ± 14.27[c]	29.79 ± 10.21[ns]	55.44 ± 14.42[c]
5	SIDR Rats + N-But 800 mg/kg bw	91.73 ± 20.87[c]	107.79 ± 15.78[c]	34.61 ± 8.18[a]	35.56 ± 16.43[c]
6	SIDR Rats + Eth 200 mg/kg bw	136.47 ± 13.91[ns]	172.00 ± 15.94[ns]	21.35 ± 5.08[ns]	80.71 ± 12.77[ns]
7	SIDR Rats + Eth 400 mg/kg bw	109.02 ± 18.88[c]	121.96 ± 25.33[c]	29.31 ± 12.33[ns]	55.31 ± 25.28[c]
8	SIDR Rats + Ethanolic 800 mg/kg bw	112.65 ± 18.46[c]	143.69 ± 15.61[c]	32.54 ± 12.91[ns]	52.44 ± 11.68[c]
9	SIDR Rats + Std 10mg/kg bw	105.42 ± 21.34[c]	107.41 ± 36.57[c]	35.14 ± 9.20[a]	48.76 ± 13.75[c]

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

Table 4.8: Effect of Daily Oral Dose of *Alchornia Cordifolia* Ethanol Leaf Extract and its n-Butanol Fraction on Serum HDL and LDL Levels of STZ-Induced Diabetic Rats (mg/dl).

Group (n=5)	Treatment Given	Serum HDL	Serum LDL	HDL/LDL RATIO
	Normal Rats + D/W	45.48 ± 10.74	16.01 ± 7.98	2.84
	SIDR + D/W	15.39 ± 6.46	107.98 ± 15.83	0.14
	SIDR + N-But 200 mg/kg b w	22.92 ± 37.29^{ns}	80.78 ± 12.42^{ns}	0.28
	SIDR + N-But 400 mg/kg b w	29.79 ± 10.21^{ns}	55.44 ± 14.42^{c}	0.53
	SIDR + N-But 800 mg/kg b w	34.61 ± 8.18^{a}	35.56 ± 16.43^{c}	0.97
	SIDR + Eth 200 mg/kg b w	21.35 ± 5.08^{ns}	80.71 ± 12.77^{ns}	0.26
	SIDR + Eth 400 mg/kg b w	29.31 ± 12.33^{ns}	55.31 ± 25.28^{c}	0.53
	SIDR + Eth 800 mg/kg b w	32.54 ± 12.91^{ns}	52.44 ± 11.68^{c}	0.62
	SIDR + Std 10mg/kg bw	35.14 ± 9.20^{a}	48.79 ± 13.75^{c}	0.72

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$). SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide). HDL/LDL Ratio-Preferred (0.3 and above) Ideal (0.4 and above)

4.9 Effect of *Alchornea cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction on Liver Enzymes

Data presented in Table 4.9 shows the study of serum liver enzymes levels of normoglycemic and diabetic treated groups as compared to diabetic group. There was a statistically significant (P<0.05) decrease in ALT activity in n-butanol treated diabetic group at dose concentration of 200 mg/kg bw (64.66 ± 12.44 IU/L) and a highly significant (P<0.05) decrease of both n-butanol fraction at dose concentrations 400 mg/kg bw (55.68 ± 14.67 IU/L), 800mg/kg bw (44.50 ± 4.86 IU/L) and ethanol extract administered diabetic groups 200 mg/kg bw (56.44 ± 16.81 IU/L), 400 mg/kg bw (51.65 ± 9.41 IU/L), 800 mg/kg bw (37.66 ± 20.11 IU/L) when compared to the positive control, untreated diabetic group (99.00 ± 7.67IU/L). The activity of ALT in group given 800 mg/kg bw (37.66 ± 20.11 IU/L) ethanol extract was lower than that in the group treated with 10 mg/kg bw (46.80 ± 14.36IU/L) standard drug, glibenclamide. In comparison with the ethanol extract and its n-butanol fraction treated groups, ALT activity was lower in the negative control, non-diabetic group. There was also a statistically significant (P<0.05) dose dependent decrease in the activity of AST in n-butanol treated groups 200 mg/kg bw (166.10± 14.76 IU/L), 400 mg/kg bw (143.41 ± 8.42 IU/L), 800 mg/kg bw (143.50 ± 25.77 IU/L) and in the ethanol extract treated groups 200 mg/kg bw (160.62 ± 11.32 IU/L), 400 mg/kg bw (154.02± 20.44 IU/L), 800 mg/kg bw (149.66 ± 9.07 IU/L) when compared with the positive control, untreated diabetic group (220.60 ± 22.38IU/L). There was lower activity of AST in diabetic group given glibenclamide 10 mg/kg bw (133.64 ± 13.51 IU/L) than diabetic groups administered varying doses of ethanol extract and its n-butanol fraction. However, there was less difference of AST activity in groups given 200 mg/kg bw (143.41 ± 8.42 IU/L) and 800 mg/kg bw (143.50 ± 25.77 IU/L) n-butanol fraction when compared to the negative control, non-diabetic group (145.92 ± 5.35 IU/L). A significant (P<0.05) change was observed in the activity

of ALP for the group treated with n-butanol 200 mg/kg bw (56.73 ± 13.49 IU/L) and a statistically significant (P<0.05) decrease of ALP activity in groups administered 400 mg/kg bw (31.97 ± 7.42 IU/L), 800 mg/kg bw (58.16 ± 8.20 IU/L) n-butanol fraction and groups administered 200, 400, 800 mg/kg bw (33.91 ± 20.69, 39.95 ± 20.89, 41.95 ± 13.53 IU/L) ethanol extract. The test group administered 400 mg/kg (31.97 ± 7.42 IU/L) n-butanol fraction recorded a lesser ALP activity when compared to the test group given standard drug, glibenclamide 10 mg/kg bw (39.37 ± 17.84IU/L) and the negative control, non-diabetic group (37.37 ± 6.11 IU/L). No significant (P>0.05) change in total bilirubin level of the diabetic group treated with n-butanol 200mg/kg bw (2.66 ± 0.75 IU/L) when compared to the positive control, untreated diabetic group (4.31 ± 0.64 IU/L) but a recorded significant (P<0.05) decrease of 400, 800 mg/kg bw (1.51 ± 0.91, 1.38 ± 0.62 IU/L) n-butanol fraction and 200 mg/kg bw (1.05 ± 0.73 IU/L) ethanol extract treated diabetic groups. While a statistically significant (P<0.05) decrease was observed at dose concentrations 400, 800 mg/kg bw (2.05 ± 0.66, 2.10 ± 0.95 IU/L) of the ethanol extract. Except in diabetic groups administered 200 mg/kg bw (2.66 ± 0.75 IU/L) n-butanol fraction, all diabetic groups administered varying doses of ethanol extract and its n-butanol fraction recorded low levels of total bilirubin compared with the diabetic group given 10mg/kg bw (2.44 ± 0.91 IU/L) of the standard drug, glibenclamide. Also the diabetic group treated with 200 mg/kg bw (1.05 ± 0.73 IU/L) of the ethanol extract had a slightly lower level of bilirubin compared to the negative control, non-diabetic group (1.45 ± 0.78 IU/L). There was no significant (P>0.05) change in the level of direct conjugated bilirubin in the group treated with 200 mg/kg bw (2.42 ± 0.64 IU/L) n-butanol and group treated with 400 mg/kg bw (2.17 ± 0.80 IU/L) ethanol extract as compared to the positive control, untreated diabetic group, but there was statistically significant (P<0.05) decrease for groups treated with 400 mg/kg bw (1.11 ± 0.55

IU/L) n-butanol fraction and groups given 200 mg/kg bw (062 ± 0.49IU/L) ethanol extract. Also a significant ($P<0.05$) decrease was observed at dose concentrations of both 800 mg/kg bw (1.38 ± 0.62 IU/L) n-butanol and 800mg/kg bw (1.51 ± 0.89 IU/L) ethanol extract. Diabetic group that received 200 mg/kg bw (062 ± 0.49 IU/L) ethanol extract, recorded lower level of direct conjugated bilirubin when compared with the group administered 10 mg/kg bw (1.49 ± 0.69 IU/L) of the standard drug, glibenclamide, and the negative control, non-diabetic group (1.23 ± 0.53 IU/L).

Table 4.9: Effect of Daily Oral Doses of Ethanolic Leaf Extract of *Alchornia cordifolia* and its n-Butanol Fraction on Serum Liver Enzymes Level of STZ-Induced Diabetic Rats.

Group (n=5)	Treatment Given	ALT (IU/L)	AST (IU/L)	ALP (IUL)	Total Bilirubin (mg/dl)	Direct Conjugated bilirubin (mg/dl)
1	Normal Rats + D/W	40.40 ± 4.99^{c}	145.92 ± 5.35^{c}	37.37 ± 6.11^{a}	1.45 ± 0.78^{c}	1.23 ± 0.53^{c}
2	SIDR + D/W	99.00 ± 7.67^{a}	220.60 ± 22.38^{b}	96.23 ± 9.49^{a}	4.31 ± 0.64^{a}	3.30 ± 0.57^{b}
3	SIDR Rats + N-But 200mg/kg bw	64.66 ± 12.44^{b}	166.10 ± 14.76^{c}	56.73 ± 13.49^{b}	2.66 ± 0.75^{ns}	2.42 ± 0.64^{ns}
4	SIDR Rats + N-But 400mg/kg bw	55.68 ± 14.67^{c}	143.41 ± 8.42^{c}	31.97 ± 7.42^{c}	1.51 ± 0.91^{c}	1.11 ± 0.55^{c}
5	SIDR Rats + N-But 800mg/kg bw	44.50 ± 4.86^{c}	143.50 ± 25.77^{c}	58.16 ± 8.20^{c}	1.38 ± 0.62^{c}	1.38 ± 0.71^{b}
6	SIDR Rats + Eth 200mg/kg bw	56.44 ± 16.81^{c}	160.62 ± 11.32^{c}	33.91 ± 20.69^{c}	1.05 ± 0.73^{c}	0.62 ± 0.49^{c}
7	SIDR Rats + Eth 400mg/kg bw	51.65 ± 9.41^{c}	154.02 ± 20.44^{c}	39.95 ± 20.89^{c}	2.05 ± 0.66^{b}	2.17 ± 0.80^{ns}
8	SIDR Rats + Eth 800 mg/kg bw	37.66 ± 20.11^{c}	149.66 ± 9.07^{c}	41.95 ± 13.53^{c}	2.10 ± 0.95^{b}	1.51 ± 0.89^{b}
9	SIDR Rats + Std 10mg/kg	46.80 ± 14.36^{c}	133.64 ± 13.51^{c}	39.37 ± 17.84^{c}	2.44 ± 0.91^{c}	1.49 ± 0.69^{b}

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

4.10 Effect of *Alchornea cordfo1ia* Leaf Ethanolic Extract and its Fraction on Haematological Parameters

Presented in Table 4.10 is the effect of *Alchornea cordfo1ia* Leaf ethanolic extract and its fraction on haematological parameters. There was a significant (p<0.05) increase in the PCV count of the n-butanol fraction 200, 800 mg/kg bw (52.20 ± 7.62, 51.73 ± 20.87 %) treated groups of diabetic rats. A statistically significant (P<0.05) increase in same was also noted in both n-butanol fraction 400 mg/kg bw (48.40 ± 4.30 %) and ethanol extract 400, 800 mg/kg bw (48.00 ± 9.25, 49.20 ± 5.37 %) treated diabetic groups but no significant (P>0.05) change was recorded in diabetic group treated with ethanol extract 200 mg/kg bw (44.20 ± 6.71 %) when compared to the positive control, untreated diabetic group. The table reveals a higher percentage of PCV in the group treated with n-butanol fraction, 800 mg/kg bw (51.73 ± 20.87 %) when compared with the group treated with glibenclamide, 10 mg/kg bw (50.40 ± 2.94 %) and the negative control, non-diabetic group (49.60 ± 4.95 %).

Statistically significant (P<0.05) increase was observed in Hb level at dose concentrations of n-butanol 200, 400, 800 mg/dl bw (17.34 ± 2.54, 16.72 ± 1.29, 16.82 ± 0.95 g/dl) and ethanol extract 400, 800 mg/kg bw (16.62 ± 3.16, 16.60 ±1.80 g/dl). However, there was a statistically significant P<0.05) increase in diabetic group treated with ethanol extract 200 mg/kg bw (16.18 ± 4.05 g/dl) when compared with the positive control, untreated diabetic group (10.48 ± 1.51 g/dl). The Hb level of the group administered n-butanol fraction, 800 mg/dl bw (16.82 ± 0.95 g/dl) was slightly higher than that of group treated with 10 mg/kg bw (16.54 ± 1.89 g/dl) glibenclamide and of the negative control group, non-diabetic group (16.32 ± 1.96g/dl). There was a statistically significant (P<0.05) increase in the total protein in diabetic groups treated with varying doses of n-butanol fraction and ethanolic extract when compared to the negative control

(3.78 ± 0.75 g/dl). However the diabetic group treated with n-butanol 200 mg/kg bw (7.76 ± 1.29 g/dl) presented with a lower level of total protein when compared with the group treated with 10 mg/kg bw (7.92 ± 0.93 g/dl) glibenclamide and the negative control, non-diabetic group (7.52 ± 0.89 g/dl). RBC count in diabetic animals treated with n-butanol 200 mg/kg bw (5.76 ± 0.86 10^{12}L) was satistically significant (P<0.05) and, a significant (P<0.05) increase in the RBC count in diabetic groups treated with n-butanol 400, 800 mg/kg bw (5.54 ± 0.42, 5.56 ± 0.28 10^{12}L) and the group given ethanol extract 200, 400 mg/kg bw (5.36 ± 1.33, 5.50 ± 1.04 10^{12}L) compared with the positive control, untreated rats (3.46 ± 0.49 10^{12}L). Also there was a significant (P<0.05) increase in the diabetic group treated with ethanol 800 mg/kg bw (5.07±0.52 10^{12}L) when compared to diabetic control. The group administered n-butanol 200 mg/kg bw (5.76 ± 0.86 10^{12}L) recorded a slightly higher concentration of RBC when compared to the group that received glibenclamide 10 mg/kg bw (5.48 ± 0.24 10^{12}L) and the negative control, normal rats (5.40 ± 0.66 10^{12}L).

Data in Table 4.11 shows that there was no significant change (P>0.05) in the neutrophils, monocytes and eosinophils, count at varying dose concentrations of the ethanol extract and its n-butanol fraction in the test groups when compared with the negative control, normal rats.. However, the neutrophil count of group given 800 mg/kg bw (12.40 ± 5.90 %) n-butanol and group given 200 mg/kg bw (12.40 ± 5.10 %) ethanol extract are lower than the values obtained from the 10 mg/kg bw (14.00 ± 6.42 %) glibenclamide treated group, and values from the negative control, normal rats (2.20 ± 6.15 %). Likewise, values obtained for eosinophil count in groups administered 800 mg/kg bw (1.40 ± 1.65 %) n-butanol fraction and 200 mg/kg bw (1.40 ± 2.58 %) ethanol extract were similar to eosinophil count of the negative control (1.40 ± 0.67 %).

All values of monocytes count obtained from groups treated with varying doses of ethanol extract and its n-butanol fraction were lower compared to the negative control, normal rats (3.00 ± 0.69 %) and 10 mg/kg bw (2.60 ± 1.94 %) glibenclamide treated group. There was no significant change in the WBC count of groups treated with 400 mg/kg bw (6.60 ± 1.65 $x10^9$/L) n-butanol fraction and 200 mg/kg bw (5.60 ± 2.05 $x10^9$/L) ethanol extract when compared with the positive control, untreated diabetic rats (4.00 ± 2.54 $x10^9$/L). There was a significant ($P<0.05$) change in WBC count of groups treated with 200 mg/kg bw (8.60 ± 1.11 $x10^9$/L) n-butanol fraction and 400 mg/kg bw (8.60 ± 2.49 $x10^9$/L) ethanol extract. Also, there was a statistically significant ($P<0.05$) change in WBC count of group administered 800 mg/kg bw (11.20 ± 2.85 $x10^9$/L) n-butanol fraction, this value was higher than that obtained from the group treated with 10 mg/kg bw (9.20 ± 1.29 $x10^9$/L) glibenclamide and only slightly lower than the value from the negative control, normal rats (11.40 ± 2.05 $x10^9$/L).

The value obtained from WBC count of the group administered 800 mg/kg bw (10.80 ± 2.76 $x10^9$/L) ethanol extract was also higher than that of the glibenclamide treated group. There was a statistically significant ($P<0.05$) change of lymphocytes count in groups given 200, 400, 800 mg/kg bw (79.40 ± 6.55, 80.20 ± 3.32, 81.00 ± 6.37 %) n-butanol fraction and groups given 400, 800 mg/kg bw (80.00 ± 13.24, 82.00 ± 6.42%) ethanol extract when compared to the positive control, untreated rats (59.60 ± 8.80 %). However, there was no significant change in the group administered 200mg/kg bw (73.00 ± 9.18%) ethanol extract. At 200mg/kg bw, the ethanol extract exhibited similar effect with 10 mg/kg bw (82.80 ± 7.76 %) glibenclamide, and slightly less effective when compared to the negative control, non-diabetic group (84.40 ± 2.94%)

Table 4.10: Effect of Daily Oral Doses of *Alchornia cordifolia ethanolic* Leaf Extract and its n-Butanol Fraction on Haematological Parameters of STZ-Induced Diabetic Rats.

Group (n=5)	Treatment	PCV (%)	HB (g/dl)	PROTEIN (g/dl)	RBC (10^{12}/L)
1	Normal Rats + D/W	49.60 ± 4.95[c]	16.32 ± 1.96[c]	7.52 ± 0.89[a]	5.40 ± 0.66[c]
2	SIDR+ D/W	33.00 ± 2.89[a]	10.48 ± 1.51[a]	3.78 ± 0.75[a]	3.46 ± 0.49[a]
3	SIDR Rats + N-But 200 mg/kg bw	52.20 ± 7.62[c]	17.34 ± 2.54[c]	7.88 ± 0.84[c]	5.76 ± 0.86[c]
4	SIDR Rats + N-But 400 mg/kg b w	48.40 ± 4.30[b]	16.72 ± 1.29[c]	8.24 ± 0.93[c]	5.54 ± 0.42[b]
5	SIDR Rats + N-But 800 mg/kg bw	51.73 ± 20.87[c]	16.82 ± 0.95[c]	7.76 ± 1.29[c]	5.56 ± 0.28[b]
6	SIDR Rats + Eth 200 mg/kg bw	44.20 ± 6.71[ns]	16.18 ± 4.05[b]	9.04 ± 2.11[c]	5.36 ± 1.33[b]
7	SIDR Rats + Eth 400 mg/kg bw	48.00 ± 9.25[b]	16.62 ± 3.16[c]	8.22 ± 0.78[c]	5.50 ± 1.04[b]
8	SIDR Rats + Eth 800 mg/kg bw	49.20 ± 5.37[b]	16.60 ± 1.80[c]	8.42 ± 0.57[c]	5.07 ± 1.15[a]
9	SIDR Rats + Std 10mg/kg b w	50.40 ± 2.94[b]	16.54 ± 1.89[c]	7.92 ± 0.93[c]	5.48 ± 0.24[b]

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)
SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

Table 4.11: Effect of Daily Oral Doses of n-Butanol and Ethanol leaf extract of *Alchornia cordifolia* on Erythrocytes Indices and Total Protein of STZ-Induced Diabetic Rats (%).

Group	Treatment Given	WBC × 10^9/L	NEUTROPHILS	LYMPHOCYTES	MONOCYTES	EOSINOPHILS	BASOPHILS
1	Normal Rats + D/W	11.40 ± 2.05^{a}	2.20 ± 6.15^{c}	84.40 ± 2.94^{c}	3.00 ± 0.69^{a}	1.40 ± 1.49^{c}	0.00 ± 0.00
2	SIDR + D/W	4.00 ± 2.54^{a}	12.60 ± 3.41^{b}	59.60 ± 8.80^{a}	1.40 ± 0.53^{b}	0.40 ± 0.53^{b}	0.00 ± 0.00
3	SIDR + N-But 200mg/kg b w	8.60 ± 1.11^{a}	18.80 ± 1.62^{ns}	79.40 ± 6.55^{b}	0.40 ± 0.53^{ns}	0.20 ± 0.44^{ns}	0.00 ± 0.00
4	SIDR + N-But 400mg/kg b w	6.60 ± 1.65^{ns}	15.80 ± 3.36^{ns}	80.20 ± 3.32^{b}	1.20 ± 1.29^{ns}	0.40 ± 0.53^{ns}	0.00 ± 0.00
5	SIDR + N-But 800mg/kg b w	11.20 ± 2.85^{c}	12.40 ± 5.90^{ns}	81.00 ± 6.37^{b}	2.00 ± 1.56^{ns}	1.40 ± 1.65^{ns}	0.00 ± 0.00
6	SIDR + Eth 200mg/kg b w	5.60 ± 2.05^{ns}	12.40 ± 5.10^{ns}	73.00 ± 9.18^{ns}	1.00 ± 0.98^{ns}	1.40 ± 2.58^{ns}	0.00 ± 0.00
7	SIDR + Eth 400mg/kg b w	8.60 ± 2.49^{a}	17.00 ± 2.72^{ns}	80.00 ± 13.24^{b}	1.80 ± 1.78^{ns}	0.60 ± 0.89^{ns}	0.00 ± 0.00
8	SIDR + Eth 800mg/kg b w	10.80 ± 2.76^{b}	18.60 ± 5.48^{ns}	82.00 ± 6.42^{b}	1.00 ± 0.98^{ns}	1.00 ± 0.69^{ns}	0.00 ± 0.00
9	SIDR + Std 10mg/kg b w	9.20 ± 1.29^{a}	14.00 ± 6.42^{ns}	82.80 ± 7.76^{b}	2.60 ± 1.94^{ns}	0.60 ± 0.89^{ns}	0.00 ± 0.00

Values are presented as mean ± SD, after four (4) week treatment. n=5. Values with different superscript along the row are significantly different ($P<0.05$)

SIDR = Streptozotocin Induced Diabetic Rats. DW= Distilled Water. N-But= n-Butanol Fraction. nEth = Ethanolic Extract. Std = Standard Drug (Glibencalmide).

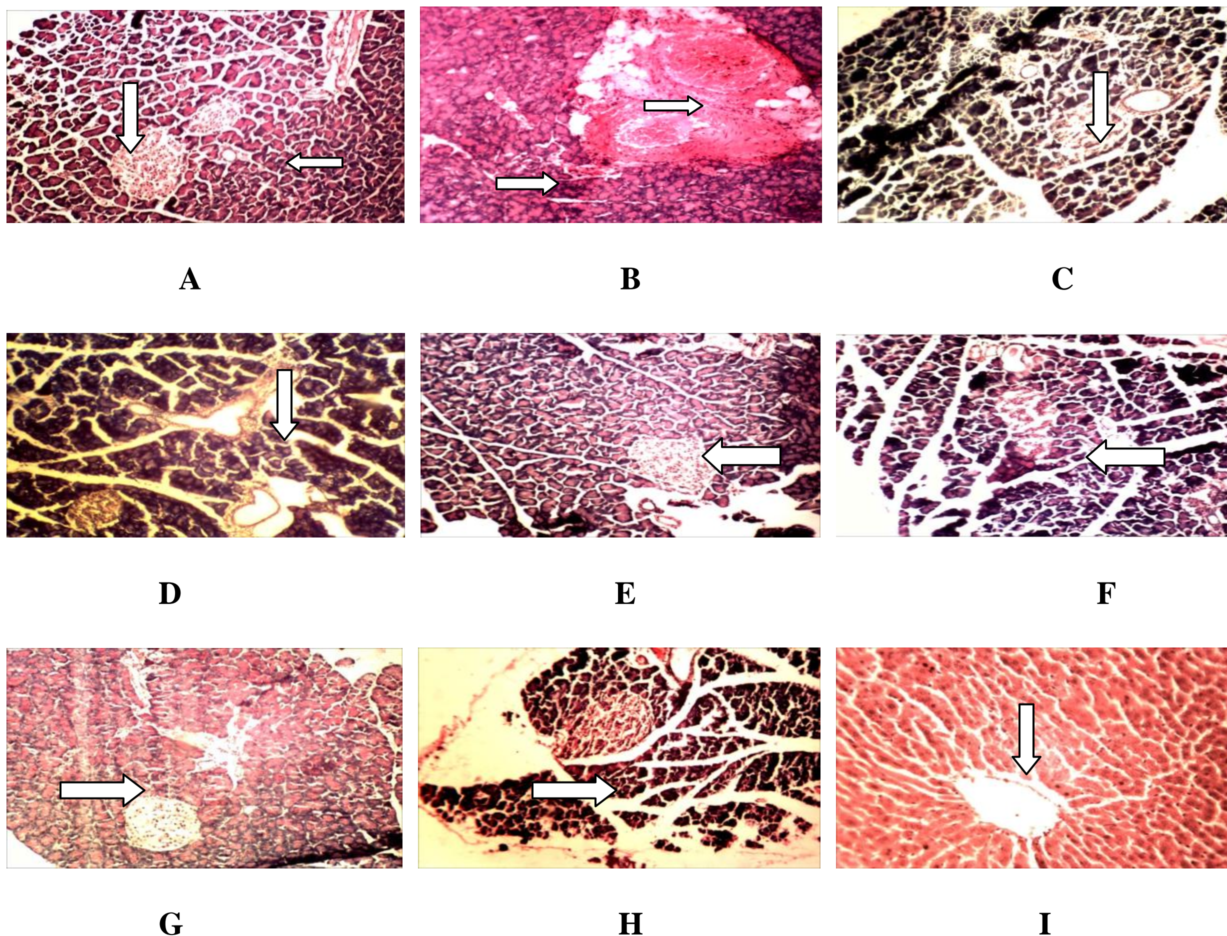
4.11 Histopathological Studies of the Pancreas Liver and Kidney of Experimental Rats

Plate 4.1 is a photomicrograph of a section of pancreas. Plate 4.1 (A) is a section from normal wistar rats showing intact Pancreatic Islet Cells. Plate 4.1 (B) is from the pancreas of STZ-induced diabetic wistar rats showing areas necrosis or atrophy of pancreatic islet cells. Plate 4.1 (C) reveals a section of pancreas of STZ-induced diabetic wistar rats administered n-Butanol fraction of *Alchornea cordifolia* ethanol leaf extract 200mg/kg body weight. It shows areas of slight regeneration of pancreatic islet cells. Plate 4.1 (D) shows a section of pancreas of STZ-induced diabetic wistar rats administered n-Butanol fraction of *Alchornea cordifolia* ethanol leaf extract 400mg/kg body weight showing good areas of restoration of pancreatic islet. Plate 4.1 (E) shows a section of pancreas of STZ-induced diabetic wistar rats administered n-Butanol fraction of *Alchornea cordifolia* ethanol leaf extract 800mg/kg Body. There was regeneration of pancreatic islet. Plate 4.1 (F) is another photomicrograph section of pancreas of STZ-induced Diabetic wistar rats administered ethanolic extract of *Alchornea cordifolia* 200mg/kg body weight Shows areas of slightly regenerated pancreatic islet cells. Plate 4.1 (G), photomicrograph of a Section of pancreas of STZ-induced diabetic wistar rats administered ethanolic extract of *Alchornea cordifolia* 400mg/kg Body Weight reveals areas of good restoration of pancreatic Islet. Plate 4.1 (H) is a section of pancreas of STZ-induced diabetic wistar rats administered ethanolic extract of *Alchornea cordifolia* 800mg/kg body weight shows areas of better regeneration of pancreatic islet. While Plate 4.1 (I) is a photomicrograph of a section of the liver from normal wistar rats administered with distilled Water 1mg/kg body weight showing normal architecture of the central vein (CV), intact hepatocytes (H) and sinusoids arranged in fairly radial position in relation to the central vein.

Plate 4.2 is a photomicrograph of liver of rats treated with *Alchornia cordifolia* ethanolic leaf extract and its n-butanol fraction. Plate 4.1 (A), a section of liver from STZ-induecd diabetic wistar rats administered with distilled water 1mg/kg body weight showing multifocal necrotic areas which revealed destructed hepatic cells with nuclear changes in the form of condensation of chromatin mass and fragmented nuclei, intense leucocytes infiltration and also kupffer cells hyperplasia. Plate 4.2 (B) is another section of liver from STZ-induced diabetic wistar rats administered n-butanol fraction of *Alchornea cordifolia* ethanol leaf extract 200mg/kg Body Weight showing slightly degenerated changes of hepatic cells single cell necrosis. Plate 4.2 (C) a photomicrograph of section of liver from STZ-induced diabetic wistar rats administered n-butanol fraction of *Alchornea cordifolia* ethanol leaf extract 400mg/kg body weight reveals evidence of slight regeneration of hepatic cells single cell Necrosis. Plate 4.2 (D) shows a section of the liver from STZ-induced diabetic wistar rats administered n-butanol fraction of *Alchornea cordifolia* ethanol leaf extract 800mg/kg body weight Shows better regeneration of hepatic cells which is manifested by increased number of binucleated cells, slight cytomegally of hepatocytes with cloudy cytoplasm, hyperchromatic nuclei with haphazardly arranged hepatocytes. Plate 4.2 (E) represents a section of the liver from STZ-induced diabetic wistar rats administered *Alchornea cordifolia* ethanol leaf extract 200mg/kg body weight showing slight degenerated changes of hepatic cells and single cell necrosis. Plate 4.2 (F) is a section of the liver from rats administered *Alchornea cordifolia* ethanol leaf extract 400mg/kg body weight. It reveals mild degenerative changes of hepatic cells and sinusoid cell lining. Plate 4.2 (G), a photomicrograph of a section of the liver from STZ-induced diabetic wistar rats administered *Alchornea cordifolia* ethanol leaf extract 800mg/kg body weight revealing evidence of good regeneration of hepatic cells accompanied by increased number of binucleated cells with cloudy cytoplasm,

hyperchromatic nuclei. Plate 4.2 (H) is a photomicrograph of a section of kidney from normal wistar rats administered 1ml/kg body weight of distilled water orally showing intact and normal architecture of glomerulus and renal tubular epithelium. Plate 4.2 (I), photomicrograph of a section of kidney from STZ-induced diabetic wistar rats administered 1ml/kg body weight of distilled water shows intense glomerular and renal tubular epithelium necrosis with many collapsed tubules.

Plate 4.3 presents a photomicrograph section of the kidney of rats administered Alchornea cordifolia leaf ethanolic extract and it's n-butanol fraction. Plate 4.3 (A) shows a photomicrograph of a section of kidney from STZ-induced diabetic wistar rats administered n-butanol fraction of *Alchornea cordifolia* ethanol leaf extract 200mg/kg body weight shows mild necrosis of glomerular and renal epithelium and vascular congestion. Plate 4.3 (B) shows good regeneration of glomerular and renal epithelial cells with mild vascular congestion after administering n-butanol fraction 400mg/kg body weight. While Plate 4.3 (C) shows better regeneration of glomerular and renal tubular epithelial cells at dose concentration of 800mg/kg body weight, n-butanol fraction. Plate 4.3 (D) reveals a section of kidney from STZ-induced diabetic wistar rats administered ethanol leaf extract of *Alchornea cordifolia* 200mg/kg body weight orally reveals slight tubular congestion, glomerular and renal tubular epithelial cells necrosis. Plate 4.3 (E) shows slight regeneration of glomerular and renal tubular epithelial cells at dose 400mg/kg body weight ethanol extract. Plate 4.3 (F) shows a section of kidney from STZ-induced diabetic wistar rats administered ethanol leaf extract of *Alchornea cordifolia* 800mg/kg body weight orally showing good regeneration of glomerular and renal tubular epithelial cells lining.



Plat 4.1: Photomicrograph of Pancreas of Rats treated with *Alchornia cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction. H & E Stain (X 250)

A: Section of pancreas of normal rat administered distilled water 1ml/kg bw
B: Section of pancreas of STZ-induced diabetic rat administered distilled water 1ml/kg bw
C: Section of pancreas of STZ-induced diabetic rat administered n-butanol fraction 200mg/kg bw
D: Section of pancreas of STZ-induced diabetic rat administered n-butanol fraction 400mg/kgbw
E: Section of pancreas of STZ-induced diabetic rat administered n-butanol fraction 800mg/kg bw
F: Section of pancreas of STZ-induced diabetic rat administered ethanolic extract 200mg/kg bw
G: Section of pancreas of STZ-induced diabetic rat administered ethanolic extract 400mg/kg bw
H: Section of pancreas of STZ-induced diabetic rat administered ethanolic extract 800mg/kg bw
I : Section of liver of normal rat administered distilled water 1ml/kg bw

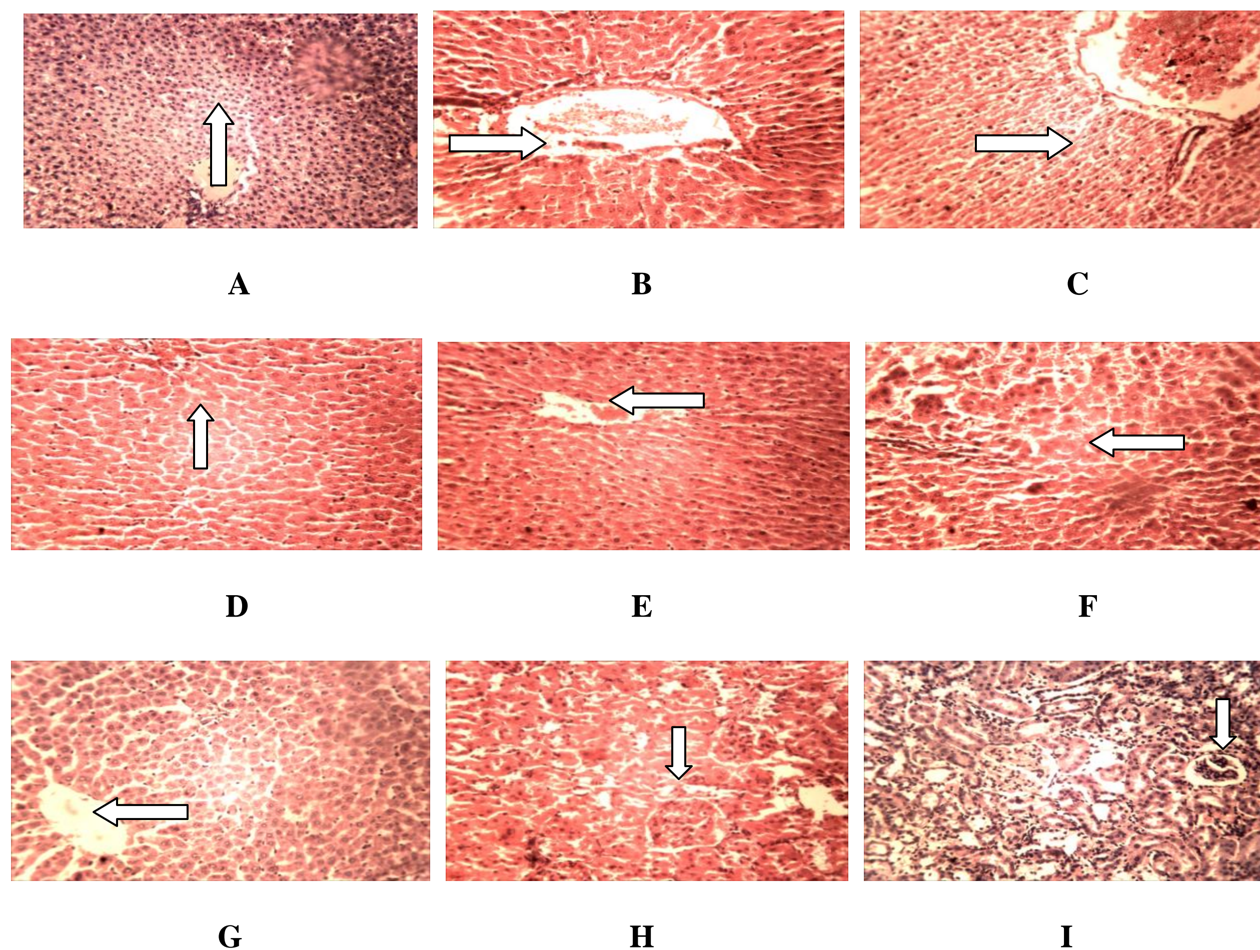


Plate 4.2: Photomicrograph of Liver of Rats treated with *Alchornia cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction. H & E Stain (X 250)

A: Section of liver of STZ-induced diabetic rat administered distilled water 1ml/kg bw
B: Section of liver of STZ-induced diabetic rat administered n-butanol fraction 200mg/kg bw
C: Section of liver of STZ-induced diabetic rat administered n-butanol fraction 400mg/kg bw
D: Section of liver of STZ-induced diabetic rat administered n-butanol fraction 800mg/kg bw
E: Section of liver of STZ-induced diabetic rat administered ethanolic extract 200mg/kg bw
F: Section of liver of STZ-induced diabetic rat administered ethanolic extract 400mg/kg bw
G: Section of liver of STZ-induced diabetic rat administered ethanolic extract 800mg/kg bw
H: Section from kidney of normal rats administered distilled water 1ml/kg bw
I : Section of kidney of STZ-induced diabetic rat administered distilled water 1ml/kg bw

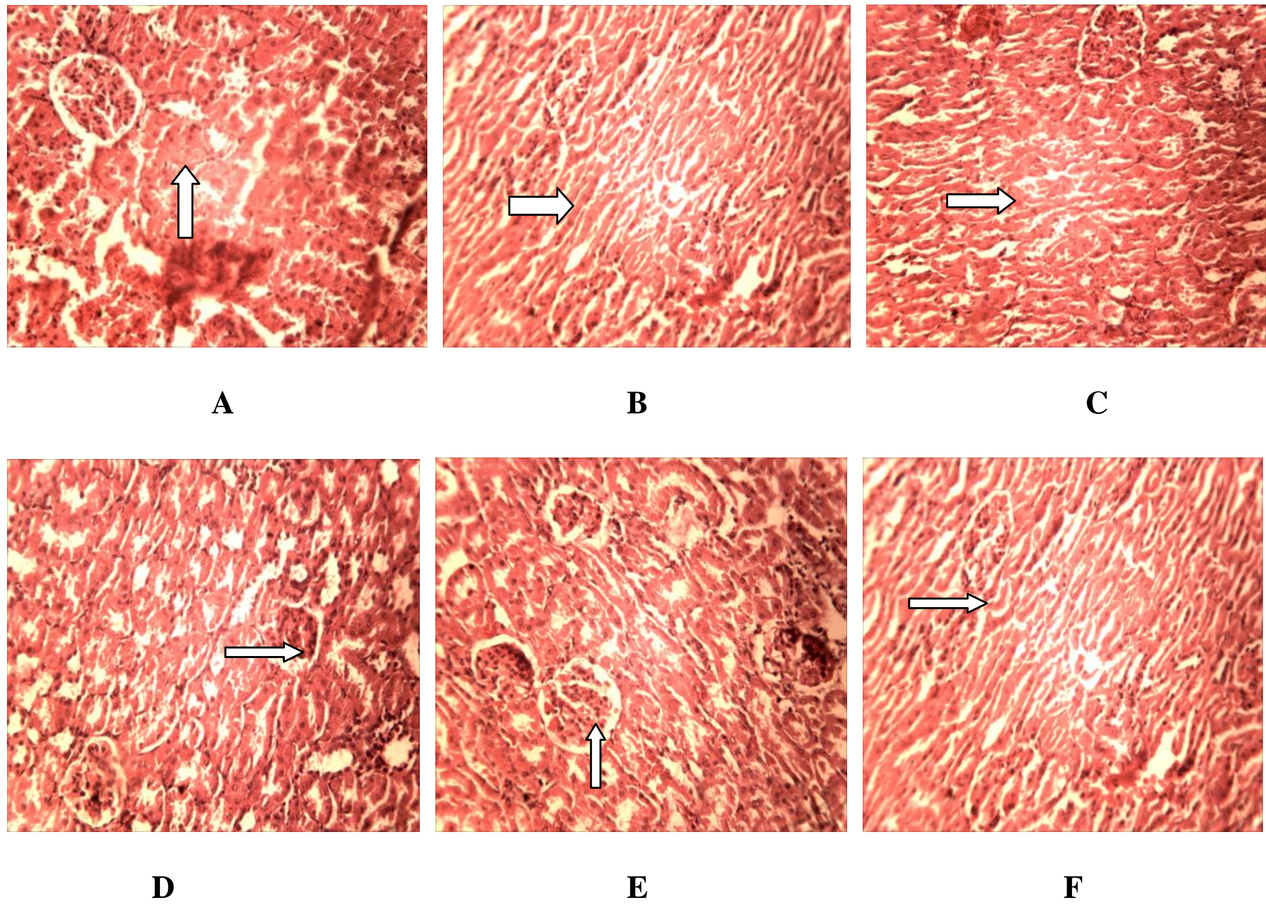


Plate 4.3: Photomicrograph of Kidney of Rats treated with *Alchornia cordifolia* Ethanolic Leaf Extract and its n-Butanol Fraction. H & E Stain (X 250)

A: Section of kidney of STZ-induced diabetic rat administered n-butanol fraction 200mg/kg bw
B: Section of kidney of STZ-induced diabetic rat administered n-butanol fraction 400mg/kg bw
C: Section of kidney of STZ-induced diabetic rat administered n-butanol fraction 800mg/kg bw
D: Section of kidney of STZ-induced diabetic rat administered ethanolic extract 200mg/kg bw
E: Section of kidney of STZ-induced diabetic rat administered ethanolic extract 400mg/kg bw
F: Section of kidney of STZ-induced diabetic rat administered ethanolic extract 800mg/kg bw

4.12 Sub-Acute Toxicity Studies

During this study, no sign(s) of stress was observed on the animals. Death was not recorded at the end. Histological examination of vital organs did not show alterations in their normal morphology. The histopathological studies carried out indicated that both leaf extract and its fraction caused some restoration of pancreatic islet cells in tissue damaged animals. The findings did not show any damage to the kidney of rats following oral administration of doses of *A. cordfolia* water extract after an extended period of 28 days. However, there was a slight change in the morphology of the liver at dose 800mg/kg Furthermore, no mortality was observed following oral administration.

Plate 4.4 (A) is a section of normal rat liver with intact cells. Plate 4.4 (B) shows a section of liver of rats administered with crude leaf extract of *Alchornea cordifolia* 200mg/kg body weight. It shows cords of hepatocytes well preserved and essentially normal and arranged in fairly radial position in relation to the central vein, cytoplasm not vacuolated, sinusoids well demarcated, no area of necrosis, no fatty degeneration and change.

Plate 4.4 (C) is a section of liver administered with aqueous leaf extract of *Alchornea cordifolia* 400mg/kg body weight. Shows normal architecture of central vein (CV), intact hepatocytes (H) and sinusoids arranged in fairly radial position in relation to the central vein. Plate 4.4 (D) reveals a section of liver from normal wistar rats administered with aqueous leaf extract of *Alchornea cordifolia* 800mg/kg body weight. The cords of hepatocytes are distinct and essentially normal, no fatty change and sinusoids arranged in fairly radial position in relation to the central vein.

Plate 4.4 (E) is a photomicrograph of a section of kidney from normal wistar rats. Plate 4.4 (F) reveals the photomicrograph of a section of kidney from normal wistar rats administered with

crude leaf extract of *Alchornea cordifolia* 200mg/kg body weight. It shows intact and normal architecture of glomerulus and renal tubular epithelium. Plate 4.4 (G) shows glomerular and renal tubular epithelium well preserved and essentially normal. Plate 4.4 (H) photomicrograph of a section of kidney from normal wistar rats administered with aqueous leaf extract of *Alchornea cordifolia* 800mg/kg body weight also shows intact and normal architecture of glomerulus and renal tubular epithelium.

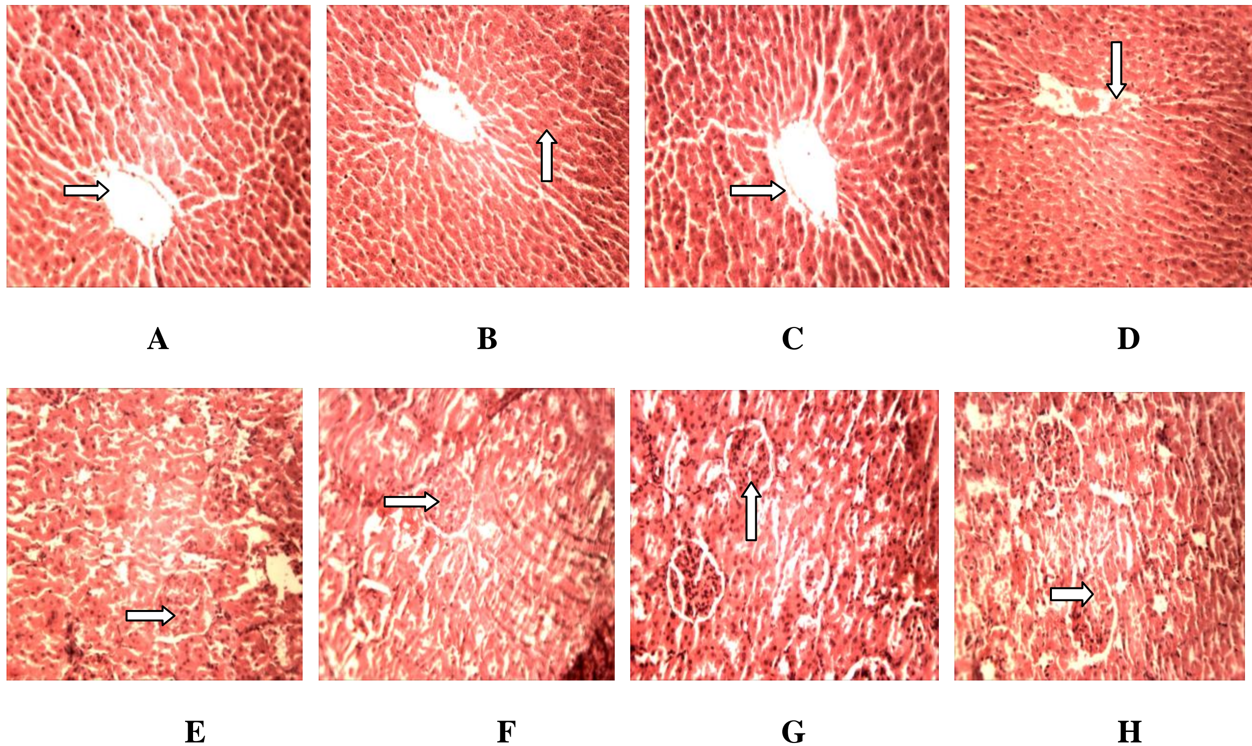


Plate 4.4: Photomicrograph of Liver and Kidney of Rats treated with *Alchornia cordifolia* Crude Leaf Extract. H & E Stain (X 250)

Plate A: Section from the liver of normal rats administered distilled water
Plate B: Section of rat liver administered crude extract of *A.cordifolia* leaf 200mg/kg bw orally.
Plate C: Section of rat liver administered crude extract of *A.cordifolia* leaf 400mg/kg bw orally
Plate D: Section of rat liver administered crude extract of *A.cordifolia* leaf 200mg/kg bw orally

Plate E: Section from the liver of normal rats administered distilled water
Plate F: Section of rat kidney administered crude extract of *A.cordifolia* leaf 200mg/kg bw orally.
Plate G: Section of rat kidney administered crude extract of *A.cordifolia* leaf 400mg/kg bw orally
Plate H: Section of rat kidney administered crude extract of *A.cordifolia* leaf 200mg/kg bw orally

CHAPTER 5

5.0 DISCUSSION

Medicinal plants are widely used in the management of diseases all over the world (Adewunmi and Ojewole, 2004). In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments (Iweala and Oludare, 2011). Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Kesari *et al*., 2007) of which *Alchornia cordifoIia* is one of them. The use of medicinal plants in most developing countries like Nigeria, as normative basis, maintenance of good health and treatment of many ailments has been widely observed (Edwin *et al*., 2008).

Management of diabetes without side effects is still a challenge to the medical system and has led search for plants with hypoglycemic properties and their employment in the management of diabetes (WHO, 2002 ; Iweala and Oludare, 2011;). However, several species of medicinal plants used in traditional treatment and management of diabetes have been evaluated; some of the plants have received scientific scrutiny (Brai *et al*., 2007). The World Health Organization has recommended and encouraged the use of alternative therapy especially in developing countries where access to the conventional treatment of diabetes is not adequate (Claudia *et al*., 2006). Streptozotocin Induced diabetes in experimental animals by destroying the beta cells of the Islet of Langerhans in the pancreas leading to reduction in the synthesis and release of insulin thereby inducing hyperglycemia leaving residual or less active Beta cells (Szkudelski, 2001). Streptozotocin has been shown to induce free radical generation and cause tissue injury. The pancreas is especially susceptible to action of streptozotocin induced free radical damage. The resultant effect which is insulin deficiency, leads to various metabolic alterations in the animals

such as, increase in blood glucose, total cholesterol and triacylglycerol levels (Vivek *et al*., 2010). Therefore, streptozoticin induced diabetes is one of the frequently used models for the study of IDDM in experimental animals (Abdel-Barry *et al*., 1997).

Preliminary phytochernical screening of *Alchornia cordifolia* ethanolic leaf extract and it' s n-butanol fraction revealed the presence of flavonoids, tannins, saponins, alkaloids, cardiac glycosides, carbohydrate, glycosides, anthraquinones and triterpenes. The effect of aqueous and ethanolic leaf extract and its n-butanol fraction on blood glucose levels, lipid profile, liver enzymes and heamatological parameters of streptozotocin induced diabetic Wistar rats was assessed. The results of this study indicate that the extract caused a decrease on the blood glucose levels. The mechanism by which the extract exert the hypoglycemic effect appear to be related to the presence of flavonoids among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituent in a group or as an individual responsible for the hypoglycemic activity of the plant extract (Marles and Farnsworth,1995).

Flavonoids have been shown to exert their antioxidant activity by scavenging or quenching free radicals or by inhibiting enzymatic systems responsible for free radical generation (Blaha *et al*.,2004;Dias *et al*.,2005;Lukacinova *et al*.,2008). Apart from being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 (SVCT I) and glucose transporter Isoform 2 (Glut 2), the intestinal transporters for vitamin C and glucose, leading to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration (Song *et al*., 2002). Several researchers have also demonstrated that flavonoids act

as reducer of hyperglycemia by causing inhibition of renal glucose reabsorption through inhibition of the sodium—glucose symporters located in the proximal renal convulated tubule (Hungo et al., 1998; Maghrani et al., 2005; Lukacinova *et al* 2008). Glibenclamide used as a standard drug in this study, exert its action mainly by inhibiting ATP-sensitive potassium channels in pancreatic beta cells (Serrano *et al*., 2006). This inhibition causes cell depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release. It also slows intestinal absorption of glucose and improves peripheral glucose uptake and utilization (Richard and Pamela, 2009). The Comparable effect of the plant extract with glibenclamide in this study may suggest similar mechanism of action, since streptozotocin causes destruction of pancreatic beta cells and the extract and fraction at all doses lowered blood glucose levels in alloxan induced diabetic rats to significant levels indicating that the extract and fraction may also possess extra pancreatic effect.

Hyperglycemia and dyslipidemia as well as oxidative stress generally coexist in diabetes subjects. Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes (Sobngwi *et al*., 2001; Beckman *et al*., 2002; Rotimi *et al*., 2011). High cholesterol levels and hyperlipidemia are associated consequences of diabetes (Mironova *et al*., 2000; Odetola *et al*., 2006; Iweala and Oludare, 2011). Diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to underutilization of glucose (Krishnakumar *et al*., 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter- regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al*., 2006;Rotimi *et al*.,

2011). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Suryawanshi *et al*., 2006). The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Claudia *et al*., 2006). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia *et al*., 2006).

In this study, administration of all doses of both ethanolic leaf extract and n-butanol fraction of *Alchornia cordfolia* significantly reduced serum levels of total cholesterol, triacylglycerol, low-density lipoprotein and increased serum levels of high-density lipoprotein in the streptozotocin induced diabetic Wistar rats. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia (Nirnenibo-uadia, 2003; Rotimi *et al*., 2011). Preliminary phytochemical screening of the extract revealed the presence of saponin among other polyphenolic compounds. This may be responsible for the lipid-lowering effect of *Alchornia cordifolia* on plasma lipid. Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion (Nimenibo-uadia, 2003; James *et al*.,2010; Rotimi *et al*., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi *et al*., 2011). Hence, saponins have been reported to have hypocholesterolic effect (James *et al*., 2010). Kumarappan *et al*., (2007) reported that administration of polyphenolic compounds to alloxan-induced diabetic rats reduced hyperlipidemia, and attributed this to a reduction in the activity of hepatic

HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This lowers elevated LDL cholesterol, levels, resulting in a substantial reduction in coronary events and deaths from CHD that occurs in diabetics (Richard and Pamela, 2009). Thus, the observed hypolipidemic effect of *Alchornia cordfolia* can be therefore, linked to the synergistic actions of phytochemicals like saponins contained in the plant extract. It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia (Brown and Goldstein, 1983; Austin and Hokanson, 1994).

In this study, all doses of the plant extract and fraction used produced a significant beneficial effect on serum lipid profile in streptozotocin induced diabetic rats. This beneficial effect on the lipid profile may be secondary to glycemic control .The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000; James *et al*.,20l0).

Therefore, the observed increase in the serum HDL-C level on administration of various doses of the extract and it's fraction in streptozotocin-induced diabetic rats, indicates that the extract and fraction have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract and fraction may be attributed to glucose utilization and hence depressed mobilization of fat (Momo *et al*., 2006; Iweala and Oludare, 2011). This implies that the plant extract may be useful in reducing the complications of hyperlipidemia and

hypercholesterolemia which often coexist in diabetics (Sharma *et al*., 2003). The study also revealed that administration of the extract and fraction at various doses significantly lowered the serum LDL-C in the streptozotocin-induced diabetic Wistar rats. Studies have shown that chronic insulin deficiency as observed in streptozotocin induced diabetes in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum level of LDL-C (Suryawanshi *et al*., 2006). Glibenclamide produces a beneficial effect on lipid profile mainly by correcting abnormal glucose metabolism (Defronzo and goodman, 1995). It also produces a moderate reduction in serum triacylglycerol as a result of decreased hepatic synthesis of very low-density lipoprotein (Chehade, 2000).

A similar effect was observed for glibenclamide in this present study. Hepatic cells contain higher concentrations of AST and ALT in the cytoplasm and AST in particular exists in the mitochondria (Nwaehujor *et al*., 2011). Damage of hepatic cells induces leakage of plasma to cause an increased level of hepato-specific enzymes in serum (Tolman and Rej, 1999). The measurement of serum AST, ALT and ALP levels serve as means for indirect assessment of liver function. *Alchornia cordifolia* leaf extract and its fraction reduced the serum levels of AST, ALT and ALP also preserved the functional ability of the liver. A similar observation was made by (Samudram *et al*., 2008). The mechanism of the hepatoprotective activity of *Alchornea cordifolia* leaf extract and fraction in streptozotocin induced diabetic Wister rats may be derived from some anti-inflammatory and antioxidant principles. This study also showed a marked reduction in both serum total bilirubin and serum direct conjugated bilirubin.

The histopathological studies of the endocrine region of pancreas of the diabetic rats revealed degenerated pancreatic islet cells in the diabetic rats. But the administration of various doses of plant extract and fraction showed restoration of pancreatic islet cells in all diabetic extract treated groups suggesting the antidiabetic potential of the plant.

Reactive oxygen species has been implicated in the mechanism of red cells damage (Rao *et al*., 2003). During diabetes the excess glucose present in blood reacts with heamoglobin to form glycosylated haemoglobin. So the total heamoglobin level is decreased in alloxan diabetic rats (Sheela and Augusti, 1992). This study revealed there was increase in the haemoglobin, red cell count,PCV and serum total protein. These results are consistent with the findings of some researchers on the effects of plant extracts on red cell indices of experimental animals (Maphosa *et al*., 2009).

The increase in total white blood cells, neutrophiles, lymphocytes and eosinophils by both ethanolic extract and its n-butanol fraction of *Alchornea cordifolia* may indicate an anti-inflammatory effect, but not a boost in the immune system as exhibited by some other plant extracts reported earlier by some researchers (Yakubu *et al*., 2007). Similarly, the results of this study are consistent with the results of some researchers who reported that triterpenoids such as ganodermanontriol from *G. lucidum* had a potent anticompliment activity against the classical pathway of compliment system (Min *et al*., 2001).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study assessed the comparative antiglycemic effect of both aqueous and Ethanol extracts of *A.cordifolia* and showed the latter is more effective. It also showed that the n-butanol fraction of ethanolic extract was more active. Phytochernical constituents and quantity in *Alchornea cordifolia* ethanol leaf extract and its n-Butanol fraction were determined. The study then investigated the effects of the extract and it's fraction on glucose level, lipid profile, liver enzymes and heamatological parameters and evaluated the sub-acute toxicity level of both extract and fraction. The following were the major findings: Qualitative and quantitative analysis of Alchornea cordifolia ethanol leaf extract and its n-Butanol fraction revealed:

i The presence of flavonoids, alkaloids, tannins, sponin, carbohydrate, glycosides and anthraquinones.

ii Phytochemicals were found to be higher in the n-Butanol fraction, with flavonoids having largest proportion.

iii Antidiabetic and antihyperlipidemic analysis of *Alchornea cordfolia* ethanol extract and its n-butanol fraction revealed:

a Significant reduction in levels of fasting blood sugar and significant increase in body weight with treatment.

b Significant reduction in levels of TC, TG and LDL and significant increase in level of HDL.

iv Liver function test and haematological analysis conducted also showed that:

a Levels of serum AST, ALT, ALP, serum total bilirubin and serum direct conjugated bilirubin were reduced significantly.

b Likewise, protein, haemoglobin, PCV, RBC, WBC levels were significantly increased.

v Subacute toxicity studies:

a No mortality was observed following oral administration of *Alchornea cordifolia* water extract for four (4) weeks.

b The findings did not show any damage to the kidney of rats.

c However, there was a slight change in the morphology of the liver at dose 800mg/kg.

6.2 CONCLUSION

The result showed that oral administration of the plant extracts at all doses resulted in a significant decrease in the levels of blood glucose as well as decreased hyperlipidemia associated with diabetes. The n-butanol fraction of ethanol extract has the best hypoglycemic effect among the extracts and fractions tested.

The histopathological studies carried out indicated that both leaf extract and its fraction caused some restoration of pancreatic islet cells. This supports its usage in the management of diabetes.

No mortality was observed in sub chronic administration of *A.cordifolia* aqueous extract. However, there was a slight change in the morphology of the liver at dose 800mg/kg. Hence it was shown that aqueous extract that is normally orally consumed is relatively safe.

Extracts of *Alchornea cordfolia*, particularly the n-butanol fraction possess useful antidiabetic and antihyperlipidemic activities that can be used in the management of diabetes mellitus. Hence, this study is a justification of the traditional use of *A.cordifolia.*

6.3 RECOMMENDATIONS

i In further research, estimation of insulin level should be carried out in the experimental animals, as this may give more insight into the mechanism of antidiabetic activity exhibited by the plant extract.

ii Chronic toxicity studies of this plant should be done so as to ascertain the safety of usage of this plant extract on various body organs.

iii Further investigations are needed in other higher primate so as to validate the traditional used of the plant in the management of diabetes

iv Further phytochemical characterization of the plant extract should be done in order to identify the specific compound(s) involved in the observed anti-diabetic and anti-hyper lipidemic activities.

v It is necessary to isolate active principles of the extract in order to identify their specific activities and mechanism of action and subsequent formulation into appropriate dosage for safer use.

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APPENDIX I

Comparative Hypoglycemic Potentials of the Extracts and Fractions (mg/dl)

Group 1: Normal Rats + Distilled water

S/no	Day 1	Day 7	Day 14	Day 21
1	102	98	101	100
2	97	97	95	98
3	103	100	97	82
4	74	71	70	70
5	81	77	87	81

Group 2: Diabetic Rats + Distilled water

Day 1	Day 7	Day 14	Day 21
443	441	405	410
395	376	374	374
387	389	355	351
400	389	385	383
305	326	343	345

Group 3: Diabetic Rats + Aqueous Extract 200mg/kgBw

S/no	Day 1	Day 7	Day 14	Day 21
1	309	258	249	300
2	300	271	258	253
3	358	316	271	200
4	500	478	300	251
5	448	392	322	286

Group 4: Diabetic Rats + Ethanol Extract 200mg/kgBw

Day 1	Day 7	Day 14	Day 21
372	245	219	110
438	385	351	280
300	215	209	161
350	300	200	190
419	342	236	225

Group3: Diabetic group + Ethylacetate fraction 200mg/kg bw

S/no	Day 1	Day 7	Day 14	Day 21
1	422	358	315	297
2	477	391	375	328
3	335	303	293	290
4	395	380	270	260
5	300	343	207	200

Group4: Diabetic group + N-butanol Fraction 200mg/kg bw

Day 1	Day 7	Day 14	Day 21
388	272	251	153
441	354	253	216
425	386	193	190
346	210	198	105
300	201	105	97

APPENDIX II

Phytochemical Quantification

FLAVONOIDS % TAGE					
	I	II	III	TOTAL	MEAN
A	11.80	11.50	9.00	32.30	10.76
B	75.80	90.50	70.00	222.90	78.76
C	71.60	34.10	30.00	135.70	45.23
D	68.19	21.00	20.90	110.09	36.69
ALKALOIDS (% TAGE)					
A	7.30	6.10	6.00	19.40	6.46
B	10.20	11.55	5.60	27.35	9.11
C	6.00	5.60	3.00	14.60	4.86
D	5.80	5.41	2.00	13.21	4.40
SAPONINS (% TAGE)					
A	5.40	5.00	5.00	15.4	5.13
B	14.00	12.00	10.60	36.6	12.20
C	8.00	7.00	5.40	20.4	6.80
D	6.45	5.10	3.15	14.7	4.90
GLYCOSIDES(% TAGE)					
A	25.60	20.13	20.00	65.73	21.91
B	30.80	26.00	30.50	87.30	29.10
C	12.85	12.50	13.80	39.15	13.05
D	10.73	10.92	11.57	33.22	11.07
TANNINS (% TAGE)					
A	33.6	33.1	28	94.7	31.56
B	20.5	20	35.2	75.7	25.23
C	19.6	17.8	22.68	60.1	20.02
D	15.88	17.30	20.00	53.18	17.72

A-AQUEOUS EXTRACT

B- N-BUTANOL FRACTION

C- ETHANOL EXTRACT

D- ETHYLACETATE FRACTION

APPENDIX III

Percentage Mortality of Rats Administered Different Doses of Extracts and Fractions

Aqueous Extract

First phase	Dose (mg/kg)	Number of Animals	Number of death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group 1	1600	1	0	0
Group 2	2900	1	1	100
Group 3	5000	1	1	100

Ethanol Extract

First phase	Dose (mg/kg)	Number of Animals	Number of death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group 1	1600	1	0	0
Group 2	2900	1	1	100
Group 3	5000	1	1	100

Ethylacetate Fraction

First phase	Dose (mg/kg)	Number of Animals	Number of death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group 1	1600	1	0	0
Group 2	2900	1	1	100
Group 3	5000	1	1	100

N-Butanol Fraction

First phase	Dose (mg/kg)	Number of Animals	Number of death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0
Second phase				
Group 1	1600	1	0	0
Group 2	2900	1	1	100
Group 3	5000	1	1	100

APPENDIX IV

Changes in body weight (g)

Group 1: Normal Control given distilled water

S/no	Day 1	Day 7	Day 14	Day 21	Day 28
1	159	172	186	196	198
2	168	177	183	193	189
3	154	169	178	187	189
4	175	183	189	194	195
5	161	169	179	189	191

Group 2: Diabetic control given distilled water

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1		181	150	149	142	140
2		167	153	148	133	135
3		159	149	139	134	131
4		178	160	154	145	139
5		154	150	143	139	130

Group3: Diabetic group treated with N-butanol fraction

200mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1		182	176	178	179	183
2		154	150	153	158	164
3		168	163	166	169	176
4		159	149	154	155	169
5		161	148	156	160	168

Group4: Diabetic group treated with N-butanol fraction

400mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1		169	159	164	172	179
2		178	170	173	177	181
3		151	145	158	159	169
4		155	151	153	164	168
5		162	154	158	161	167

Group 5: Diabetic group treated with B-butanol fraction

800mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1		159	146	158	169	170
2		169	151	166	168	175
3		154	146	158	157	166
4		187	171	178	183	188
5		168	158	165	169	174

Group 6: Diabetic group treated with Ethanol extract

(200mg/kg bw)

S/no	Day 1	Day 7	Day 14	Day 21	Day 28
1	151	149	157	159	167
2	171	160	163	168	173
3	167	158	164	166	174
4	153	148	153	158	165
5	158	150	158	163	169

Group7: Diabetic group treated with Ethanol extract

(400mg/kg bw)

S/no	Day 1	Day 7	Day 14	Day 21	Day 28
1	158	161	164	171	176
2	162	151	158	167	171
3	165	154	161	166	169
4	152	148	157	168	176
5	171	164	169	173	175

Group8: Diabetic group treated with Ethanol extract

(800mg/kg bw)

S/no	Day 1	Day 7	Day 14	Day 21	Day 28
1	162	150	169	178	183
2	158	151	160	167	174
3	162	158	164	173	172
4	158	154	159	168	171
5	162	153	159	174	173

Group9: Diabetic group treated with

Glibenclamide (10mg/kg bw)

S/no	Day 1	Day 7	Day 14	Day 21	Day 28
1	152	140	159	174	177
2	177	162	169	179	181
3	168	158	168	170	176
4	154	150	158	164	168
5	181	161	172	186	189

APPENDIX V

Blood Glucose Levels

Group 1: Normal Control given distilled water

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	159	88	98	96	74	81
2	168	99	95	98	96	93
3	154	98	80	79	88	89
4	175	83	89	81	94	93
5	161	89	81	96	87	89

Group 2: Diabetic group given distilled water

100mg/kgBw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	181	354	369	358	388	394
2	167	594	498	472	423	416
3	159	364	359	381	341	351
4	178	347	368	401	339	343
5	154	264	323	308	328	318

Group3: Diabetic group treated with N-butanol fraction

200mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	182	307	246	182	145	112
2	154	527	384	224	201	164
3	168	355	231	188	124	99
4	159	421	310	253	193	102
5	161	302	240	168	108	93

Group4: Diabetic group treated with N-butanol fraction 400mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	169	334	228	180	164	121
2	178	362	238	118	126	99
3	151	335	256	186	110	98
4	155	445	313	243	122	91
5	162	389	198	202	178	102

Group 5: Diabetic group treated with B-butanol fraction

800mg/kg bw

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	159	367	217	131	109	102
2	169	435	323	224	138	113
3	154	311	281	178	131	108
4	187	479	294	198	105	94
5	168	321	321	148	121	109

Group 6: Diabetic group treated with ethanol extract

(200mg/kg bw)

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	151	300	271	181	172	128
2	171	365	301	253	198	183
3	167	445	367	289	210	188
4	153	387	300	268	168	114
5	158	382	248	224	203	148

Group7: Diabetic group treated with Ethanol extract

(400mg/kg bw)

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	158	434	364	283	207	188
2	162	441	298	221	187	160
3	165	381	236	181	98	91
4	152	349	248	200	164	148
5	171	318	268	208	178	122

Group8: Diabetic group treated with Ethanol extract

(800mg/kg bw)

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	162	353	258	158	122	122
2	158	442	301	251	192	159
3	162	408	302	208	161	128
4	158	383	281	198	102	99
5	162	306	249	178	199	140

Group9: Diabetic group treated with

Glibenclamide (10mg/kg bw)

S/no	Wt	Day 1	Day 7	Day 14	Day 21	Day 28
1	177	336	199	133	99	93
2	152	385	160	118	98	92
3	168	319	180	140	106	96
4	154	362	255	131	108	102
5	181	391	141	123	104	96

APPENDIX VI

Lipid Profile

Group 1: Normal Rats treated with distilled water

S/No	TC	TRIG	HDL-C	LDL-C
1	83.71	86.73	39.14	27.22
2	82.24	91.15	43.02	20.99
3	79.15	89.28	48.13	13.16
4	76.06	87.61	51.41	7.123
5	75.29	909.27	45.71	11.53

Group 2: Diabetic Rats treated with distilled water

S/No	TC	TRIG	HDL-C	LDL-C
1	167.42	199.34	23.36	104.19
2	182.13	203.21	11.52	129.97
3	158.44	218.19	19.28	95.52
4	172.06	189.03	16.08	118.14
5	143.11	221.41	6.72	92.108

Group 3: N-But 200mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	150.12	166.13	29.04	87.85
2	121.31	172.01	18.27	68.64
3	138.04	159.24	24.18	82.01
4	128.13	148.16	30.21	68.29
5	140.28	151.13	12.91	97.14

Group 4: N-But 400mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	111.24	133.21	22.18	62.42
2	118.18	108.62	39.1	57.36
3	128.21	129.73	28.68	73.58
4	96.14	99.13	41.21	35.1
5	89.43	114.31	17.81	48.76

Group 5: N-But 800mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	86.04	131.14	29.01	30.8
2	103.11	89.3	31.51	53.73
3	76.21	98.41	38.04	18.49
4	122.16	113.1	47.35	52.19
5	71.14	107.01	27.14	22.6

Group 6: Ethanolic 200mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	155.21	179.61	30.18	89.11
2	118.42	189.63	18.31	62.18
3	142.16	162.71	17.46	92.16
4	138.28	149.18	21	87.44
5	128.31	178.91	19.83	72.7

Group 7: Ethanolic 400mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	138.18	98.41	19.19	99.31
2	89.71	123.82	24.18	40.77
3	102.31	100.13	38.31	43.97
4	98.42	126.41	18.61	54.53
5	116.51	161.05	46.3	38

Group 8: Ethanolic 800mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	122.12	162.14	22.114	67.55
2	118.41	158.41	33.11	53.62
3	108.73	138.68	37	43.99
4	131.21	130.61	51.51	58.98
5	82.81	128.64	19.01	38.07

Group 9: Gilbenclamide 10mg/kg

S/No	TC	TRIG	HDL-C	LDL-C
1	138.01	167.13	34.13	70.45
2	101.14	118.16	26.27	51.24
3	91.16	89.12	39.11	34.23
4	83.41	79.48	27.41	40.1
5	113.41	83.18	48.81	47.96

APPENDIX VII

Liver Enzymes Assay

Group 1: Normal Rats treated with distilled water

S/No	ALT	AST	ALP	TB	DCB
1	41	138.31	34.04	1.51	0.63
2	34	149.3	29.04	0.87	1.78
3	48	152		0.65	1.82
4	39	143	40.5	2.66	0.94
5	40	147	38.21	1.58	0.99

Group 2: Diabetic Rats treated with distilled water

S/No	ALT	AST	ALP	TB	DCB
1	96	201	102.32	4.46	2.41
2	109	198	2999.21	3.3	3.69
3	84	217	79.92	4.44	3.83
4	89	238	83.16	5.12	3.56
5	104	249	106.56	4.25	3.02

Group 3: N-But 200mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	79.13	161.13	35.05	2.77	2.66
2	59.12	184	59.12	3.75	3.17
3	51.21	149.13	72.03	2.84	2.01
4	72	157.24	61.41	1.66	1.54
5	69	179.01	56.04	2.32	2.77

Group 4: N-But 400mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	54	141.21	40.48	2.51	1.63
2	67	183	28.04	1.03	0.56
3	44	153	38.04	0.88	0.63
4	74	151.24	22.08	2.52	1.79
5	39.41	138.31	31.24	0.62	0.98

Group 5: N-But 8000mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	52	114.21	61.64	1.35	3.1
2	46.12	144	49.62	3.11	1.44
3	42.03	183	69.92	3.32	3.04
4	56.41	168	51.23	0.77	1.12
5	61.23	198	58.41	1.95	0.31

Group 6: Ethanolic 200mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	65	166	26.68	1.29	0.71
2	34	156	26.68	1.18	0.22
3	72	201	35.12	0.46	0.66
4	43	151	12.88	0.24	0.16
5	68.21	178	68.21	2.11	1.39

Group 7: Ethanolic 400mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	58	120	69.92	2.07	3.56
2	56	153	36.56	1.71	1.94
3	41	169	50.5	2.89	2.23
4	61.21	171.1	16.56	2.5	1.46
5	42.04	157	26.21	1.12	1.7

Group 8: Ethanolic 800mg/kg

S/No	ALT	AST	ALP	TB	DCB
1	21	158.1	45.6	2.56	3.69
2	13	143.2	36.8	3.1	2.41
3	68	161	24.84	2.39	1.58
4	62	184	40.48	0.53	0.34
5	48.2	179	62.03	1.93	0.91

Group 9: Gilbenclamide (10mg/kg)

S/No	ALT	AST	ALP	TB	DCB
1	26	141	67.16	3.54	2.14
2	64	114	47.6	1.99	0.69
3	40	148	27.84	1.1	2.18
4	51	139.2	24.84	2.68	1.64
5.	53	149	29.44	2.93	2.21

APPENDIX VIII

Haematological Parameters

ERYTHROCYTES INDICES AND TOTAL PROTEIN

Group 1: Normal Rats treated with distilled water

S/No	PCV	Hb	Total Pro.
1	49	16.3	7
2	42	14	6.8
3	42	14	6.8
4	49	16.3	8.4
5	58	19.3	8.6

Group 2: Diabetic Rats treated with distilled water

S/No	PCV	Hb	Total Pro.
1	34	13.6	5.4
2	36	11.4	6
3	32	12.5	4.2
4	35	8.6	84.8
5	31	10.3	3.9

Group 3: N-But 200mg/kg

S/No	PCV	Hb	Total Pro.
1	50	16.6	6.8
2	63	21	7.4
3	55	18.3	7.8
4	51	17	9
5	42	16.8	8.4

Group 4: N-But 400mg/kg

S/No	PCV	Hb	Total Pro.
1	52	17.3	9.2
2	46	15.3	9
3	52	18.6	8.2
4	50	16.6	8
5	42	15.8	6.81

Group 5: N-But 800mg/kg

S/No	PCV	Hb	Total Pro.
1	66	21	6
2	52	17.3	8.2
3	49	16.3	6.8
4	48	16.6	9
5	44	15.7	8.8

Group 6: Ethanolic 200mg/kg

S/No	PCV	Hb	Total Pro.
1	48	16.6	10
2	46	15.6	10
3	44	18.1	9.1
4	40	13.3	8.2
5	43	17.3	7.9

Group 7: Ethanolic 200mg/kg 400mg/kg

S/No	PCV	Hb	Total Pro.
1	34	11.3	7.4
2	53	17.6	7.6
3	59	19.6	8
4	46	16.5	8.9
5	48	18.1	92

Group 8: Ethanolic 200mg/kg 800mg/kg

S/No	PCV	Hb	Total Pro.
1	53	17.6	8
2	55	18.3	8.8
3	49	16.3	8
4	41	13.6	8
5	48	17.2	9.3

Group 9: Glibenclamide (10mg/kg)

S/No	PCV	Hb	Total Pro.
1	46	15.3	8.2
2	51	17	9
3	49	16.3	8
4	53	17.6	6.4
5	53	17.6	8

Leucocytes Indices of Diabetic Treated Rats

Group 1: Normal Rats treated with distilled water

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	10	26	85	3	0	0
2	11	28	80	3	3	0
3	13	23	87	4	3	0
4	9	12	87	2	1	0
5	14	22	83	3	0	0

Group 2: Diabetic Control treated with distilled water

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	3	15	74	2	0	0
2	2	14	58	1	1	0
3	5	8	52	2	1	0
4	8	10	61	1	0	0
5	2	16	53	1	0	0

Group 2: N-But 200mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	9	20	80	0	0	0
2	8	18	87	1	0	0
3	10	21	79	0	1	0
4	9	18	82	1	0	0
5	7	17	69	0	0	0

Group 4: N-But 400mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	8	21	78	0	1	0
2	6	25	76	2	0	0
3	8	16	84	0	0	0
4	4	20	80	1	1	0
5	7	18	83	3	0	0

Group 5: N-But 800mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	8	16	84	0	0	0
2	15	6	84	3	4	0
3	9	22	70	4	2	0
4	11	18	86	2	1	0
5	13	17	81	1	0	0

Group 6: N-But 200mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	4	5	78	2	6	0
2	6	18	80	2	1	0
3	7	16	81	1	0	0
4	3	13	62	0	0	0
5	8	10	64	0	0	0

Group 7: Ethanolic Extract 400mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	8	13	97	0	0	0
2	7	20	78	2	0	0
3	13	19	90	3	1	0
4	7	17	68	0	2	0
5	8	16	67	4	0	0

Group 8: Ethanolic Extract 800mg/kg

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	10	25	75	0	1	0
2	8	13	86	0	0	0
3	9	24	90	1	1	0
4	12	15	76	2	1	0
5	15	16	83	2	2	0

Group 9: Glibenclamide (10mg/kg)

S/No	WBC	NEUTR	LYMP	MONO	EOSINO	BASO
1	8	5	88	2	0	0
2	10	12	93	0	0	0
3	11	13	77	5	2	0
4	9	22	74	4	0	0
5	8	18	82	2	1	0