www.abiosci.com

RESEARCH ARTICLE

Annals of Biological Sciences
2013, 1 (1):14-22

ISSN: 2348-1927

Sub-chronic Toxicity and Hypoglycemic Studies of Ethanol Leaf Extract of *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg.in Streptozotocin-induced Diabetic wistar Rats

E.D. Eze[1], R.K. Mohammed[2], O. Onaadepo [3], A.Shaibu[4], D.M. Adams [5] and I.S. Malgwi[4]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Bingham University, Karu, Nasarawa State, Nigeria.
[2]Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.
[3] Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences University of Abuja, Abuja, Nigeria.
[4] Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.
[5] Department of Biochemistry, Faculty of Science and Technology, Bingham University, Karu, Nasarawa State, Nigeria.

ABSTRACT

Diabetes mellitus has remained one of the most common endocrine disorders in both developed and developing *countries and the disease is increasing rapidly in most parts of the world. The study investigated the sub-chronic toxicity and hypoglycemic effects of ethanol leaf extract of Alchornea cordifolia in streptozotocin-induced diabetic wistar rats. The animals were made diabetic by single intraperitoneally injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. Diabetic rats were randomly assigned into the following groups: Group 1: Normal control, Group 2: Diabetic untreated rats, while Group 3 to Group 4 received 200, 400 and 800mg/kg b w of A. cordifolia and glibenclamide 10mg/kg b w respectively orally for a period of 28 days. Blood glucose levels were assessed weekly. For the sub-chronic study, fifteen (15) fresh animals were divided into three groups of (n=5) and they treated with 200, 400 and 800mg/kg b w of A. cordifolia leaf extract for four weeks. At the end of treatment animals from each group were sacrificed and liver and kidney tissues exicised and subjected to routine histological investigation. The result revealed a significantly decreased ($p<0.05$) blood glucose levels in the groups administered with all doses of A. cordifolia, leaf extract when compared to diabetic control group. Prememinary phytochemical screening of the extract revealed the presence of flavonoids, saponin, tannins, cardiac glycosides, triterpenes and reducing sugars. The LD_{50} of the extract of A. cordifolia was found to be safe up to 5000mg/kg b w. The sub-chronic toxicity study of A. cordifolia on the liver and kidney tissues showed that the extract did not cause any alteration to the structure of the liver cells. There was no potential toxicity or damage to the cell structure of kidney of A. cordifolia treated groups. In conclusion, the results obtained from this study provide the scientific basis on use of this plant in the management of diabetes mellitus.*

Key words: *Alchornea cordifolia*, sub-chronic toxicity, Hypoglycemia, streptozotocin, blood glucose.

INTRODUCTION

Diabetes mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8% [1, 2]. The disease arises when insufficient insulin is produced, or when the available insulin does not function

properly. Thus diabetes is characterized by hyperglycaemia resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. The long-term manifestation of diabetes can result in the development of some complications, broadly classified as microvascular or macrovascular disease. Microvascular complications include neuropathy (nerve damage), nephropathy (renal disease) and vision disorders (retinopathy, glaucoma, cataract and corneal diseases), while macrovascular complications include heart disease, stroke and peripheral vascular disease, which can lead to ulcers, gangrene and amputation [3, 4]. Currently available therapies for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral anti-diabetic agents suffer from various adverse effects, thus, managing diabetes without any side effects is still a challenge to the workers, and hence the search for more effective and safer therapeutic agents in eradicating diabetic syndromes has continued to be an important area of investigation [3]. To cope with severe problems associated with using of synthetic anti-diabetic drugs, there is a need to look for more efficacious drugs with lesser side effects and also of low cost [3]. Plants have always been exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [3]. It is always believed that plant is safe, but so many plant materials are not safe for human being, that's why toxicity study of these plants should be investigated before consumption of these plant materials [5]. However,most medicinal plants are used indiscriminately without knowing their possible adverse effect. Over the past decades, several reports in both developed and developing countries have indicated adverse effects allegedly arising from the use of medicinal plants [6, 7].Some of these effects include abortion of pregnancy, dizziness, vomiting, diarrhea, abdominal pain, fast heart beat, death, ulcer and loss of appetite [8].These effects could be attributed to the presence of phytotoxic compounds in the plant extracts and lack of actual dosage necessary for the treatment of diseases [9]. *Alchornea cordifolia* is an erect and bushy perennial straggling, laxly branched,evergreen dioecious shrub or small tree up to 8 m tall reproducing from seeds. It is widespread in secondary forest and riverine forest, especially in marshy areas but sometimes in drier sites, from sea-level up to 1500 m altitude [10]. The leaves, roots and stem bark contain terpenoids, steroid glycosides, flavonoids (2–3%), tannins (about 10%), saponins, carbohydrates and the imidazopyrimidine alkaloids alchorneine, alchornidine and several guanidine alkaloids [11]. *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. 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, genital-urinary problems including venereal diseases and female sterility, and intestinal problems including gastric ulcers, diarrhoea, amoebic dysentery and worms. As a purgative, they are also taken as an enema; high doses taken orally are emetic. They are also taken as a blood purifier, as a tonic and to treat anaemia and epilepsy [12,13]. The present research work was designed to investigate the sub-chronic toxicity and hypoglycemic effect of ethanol leaf extract of *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg.in Streptozotocin-induced diabetic wistar rats.

MATERIALS AND METHODS

Chemicals and drugs used

Streptozocin (STZ) was purchased from Sigma chemicals (St Louis U.S.A). Glibenclamide was obtained from pharmaceutical store in Zaria, Kaduna state, while Accu-chek Advantage, a digital glucometer was used for the determination of blood glucose levels.

Plant material collection

Fresh leaves of *Alchornea cordifolia* were collected from old Karu village, Karu Local government of Nasarawa State, Nigeria in the month January 2011.The plant was then taken to the herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria, Kaduna state, where the plant was identified by Mal. M. Musa and a voucher specimen (Number 401) deposited.

Plant extracts preparation

The fresh leaves *Alchornea cordifolia* collected were air dried under the shade and ground into fine powder. The powder (490g) was macerated in 70% of ethanol and 30% of distilled water at room temperature for 72 hours. This was then filtered using a filter paper (Whatmann size no. 1) and the filtrate were evaporated to dryness using (HH-S Digital thermostatic water bath) maintained at 30-35°C. A brownish residue weighing 34 g was obtained and kept in an air tight sample bottle, labeled and stored in a desiccator until it was reconstituted in appropriate volume of distilled water for administration.

Acute toxicity study of A. *cordifolia*
The median lethal doses (LD_{50}) of fresh leaves of ethanol leaf extract of *Alchornea cordifolia* was carried out by method of Lorke [14].

Phytochemical screening
The methods of analysis employed were those described by Trease and Evans [15]. The ethanol leaf extract of *Alchornea cordifolia* leaf extract obtained were subjected to preliminary phytochemical screening to identify the presence or absence of phytochemicals constituents.

Animal management
Strains of albino wistar rats of both sexes that weighed between 150 – 200 g were obtained from the Department of Human Physiology, Animal House, Ahmadu Bello University, Zaria. The animals were kept and maintained under laboratory condition of temperature, humidity and light. The animals allowed to acclimatize for two weeks, but were allowed free access to water, before commencement of the study.

Experimental Induction of Diabetes Mellitus
Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 which served as the vehicle into 18 hrs fasted rats. Three days after Streptozotocin injection, blood was taken from tail artery of the rats. Rats having blood glucose levels greater than 200mg/dl were considered diabetic and included in the study.

Experimental design
In the experiment, a total of thirty six rats were used, the animals were divided into six groups of six (6) animals each as follows:
Group 1: Non-diabetic untreated and received distilled water
Groups 2: Diabetic untreated administered with distilled water
Group 3: Diabetic and treated with 200mg/kg b w of *A. cordifolia* leaf extract
Group 4: Diabetic received 400mg/kg b w of *A. cordifolia* leaf extract
Group 5: Diabetic received 800mg/kg b w of *A. cordifolia* leaf extract
Group 6: Diabetic received Glibenclamide 10mg/kg b w

All extract and drug administration was given orally once daily for a period of twenty eight (28) days.

Sub-chronic toxicity studies of *A. cordifolia* leaf extract
Twenty fresh Wistar rats of both sexes weighing between 150 – 200 g were used for this study. The animals were divided separately into three groups of five (5) animals each. Group I served as the control and were given 1ml of distilled water, Group II were administered with 200 mg/kg b w of the extract, Group III were given 400 mg/kg b w of the extract, while Group IV also received the highest dose of 800 mg/kg b w of *A. cordifolia respectively* for a period of 28 days. The animals from all groups were observed for general signs of toxicity such as decreased locomotor activity and sensitivity to touch and pain, decreased water and feed intake, tachypnoea and prostration including death throughout the period of the plant extracts administration [16].

Histological preparation of liver and kidney tissues
At the end of twenty eight days of extract and drug treatment, all animals from each group were sacrificed and liver and kidney tissues dissected out and fixed immediately in 10% neutral formal-saline fixative solution for histological studies as well as the postmortem gross examination. After fixation, tissues were embedded in paraffin, solid sections were cut at 5µm and various sections were stained with haematoxylin and eosins as described by Galozhger and Kocloff [17].

Statistical analysis
Data obtained were expressed as mean ± SEM The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was evaluated using SPSS Version 17.0 software. The values of $p < 0.05$ were considered as significant [18].

RESULTS

Acute Toxicity Study

In the oral acute toxicity study, all the graded doses of ethanol leaf extract of *Alchornea cordifolia* leaf extract administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD_{50} of ethanol leaf extract *Alchornea cordifolia* was found to be ≥ 5000 mg/kg body weight.

Phytochemical Screening of *A. cordifolia*

Preliminary phytochemical screening of the plant extract showed the presence of cyanogenetic glycosides, saponins, carbohydrate, flavonoids, tannins, cardiac glycosides and steroids and Triterpenoids.

Effect of ethanol leaf *A.cordifolia* on fasting blood glucose level in streptozotocin-induced diabetic Wistar rats

There was no statistically significantly change ($p > 0.05$) on blood glucose level after day 1 and when compared to the diabetic untreated group. Day one (1) indicates the day before commencement of treatment. However, there was a significantly ($p < 0.05$) decreased blood glucose level in the group administered with 400 and 800 mg/kg b w of the extract, with a non significant reduction ($p > 0.05$) observed in the group treated with 200mg/kg b w of extract after day 7 when compared to diabetic control group. There was also a significant reduction ($p < 0.05$) on blood glucose levels in all the groups administered with all doses of *A. cordifolia* after 14, 21 and 28th day when compared to diabetic control group .The effect of the extract in decreasing the blood glucose was comparable to the standard drug (glibenclamide 10mg/kg b w) used in this study (figure).

Microscopic findings on the histology of liver tissues of rats administered with *A. Cordifolia.*

The histological features of liver of control and the groups treated with *A. cordifolia* extract in the sub-chronic toxicity study are shown in plate 1-4. The histological findings showed that the plant extract did not adversely affect the morphology of liver tissues in the group treated with 200, 400 and 800 mg/kg b w of the extract. The liver tissues showed cords of hepatocytes that are 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 2-4) when compared to control group (plate 1).

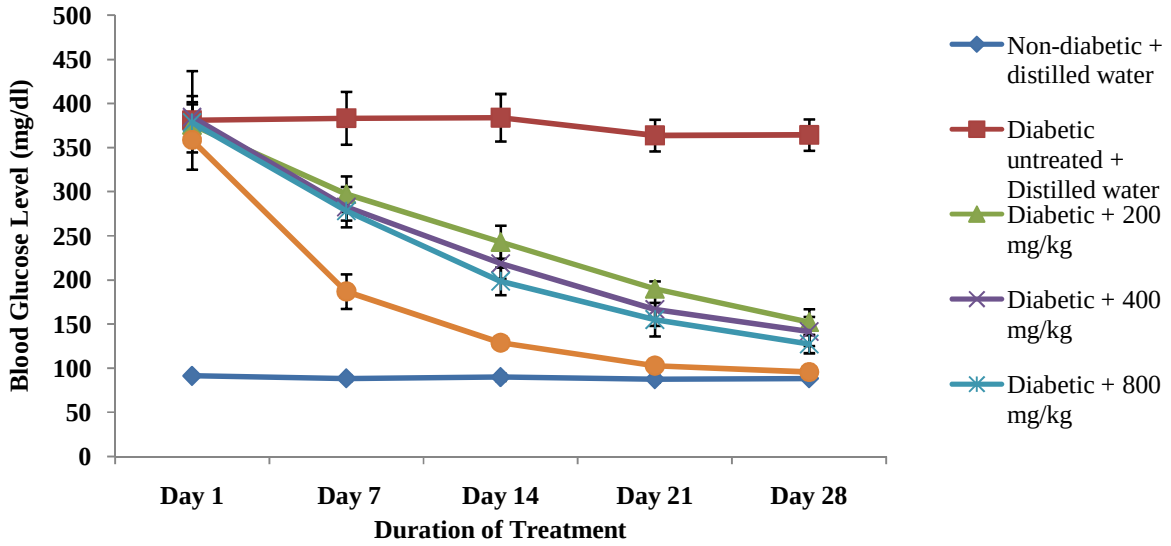

Figure 1: Effect of ethanol leaf extract of *Alchornea cordifolia* on (± mean) fasting blood glucose levels in streptozotocin-induced diabetic wistar rats.

Microscopic findings on the histology of kidney tissues of rats administered with *A. Cordifolia.*

In this study, microscopic evaluation of the kidney tissues of rats administered with 200, 400 and 800 mg/kg b w of *A. cordifolia* in the sub-chronic toxicity study revealed that the extract did not cause any alteration to the glomerular

and renal tubular epithelium, with a well preserved, intact and essentially normal renal tubular cells (plate 6 –8) when compared to the control group (plate 5).

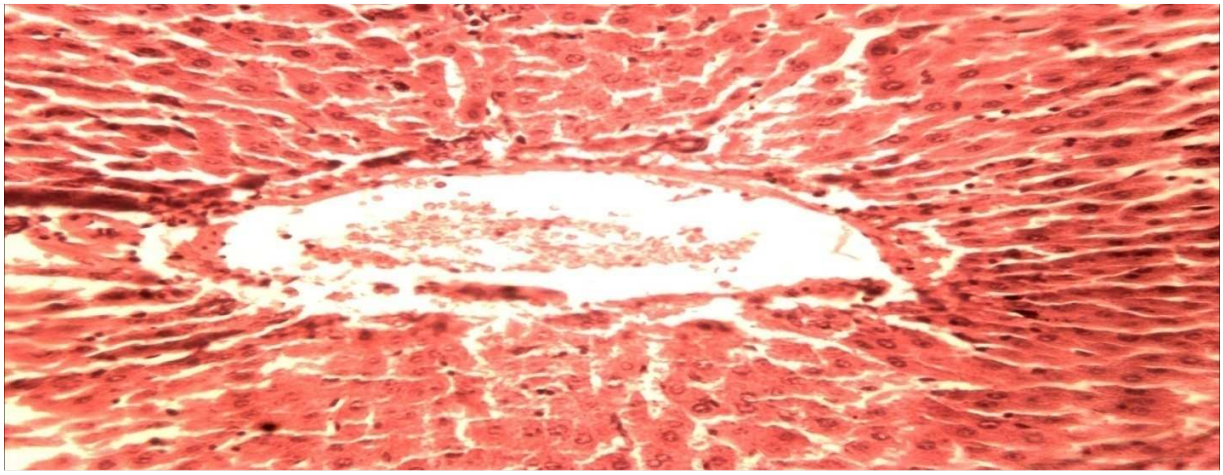

Plate 1: Photomicrograph of a section of liver of rat that received distilled water orally. H &E Stained X 250.

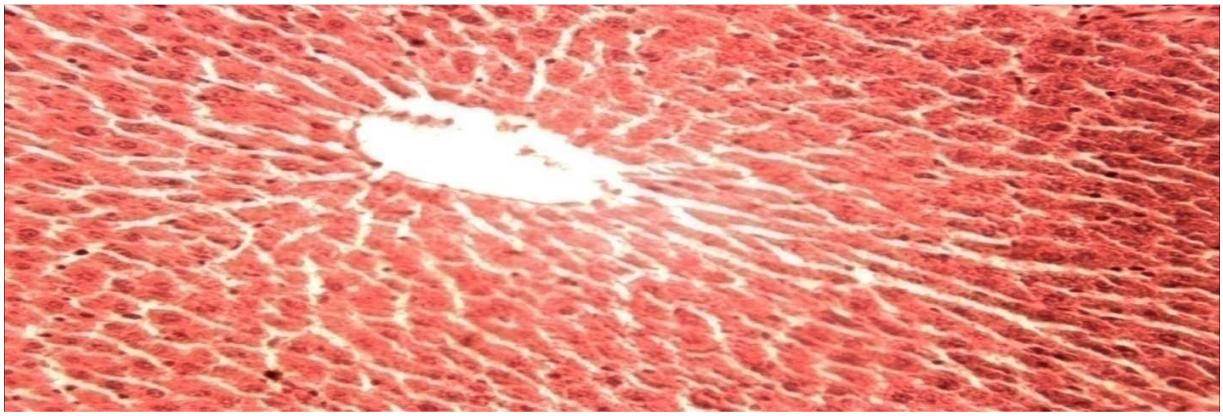

Plate 2: Photomicrograph of a section of liver rat administered with ethanol leaf extract of *A. cordifolia* 200 mg/kg body weight orally. H&E Stained X 250

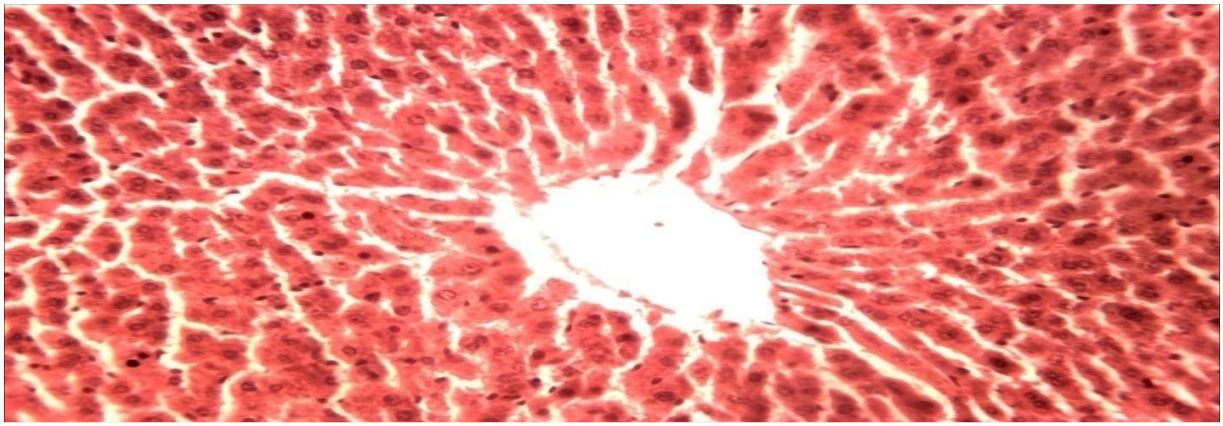

Plate 3: Photomicrograph of a section of liver of rats administered with ethanol leaf extract of *A. cordifolia* 400 mg/kg body weight orally. H&E Stained X 250.

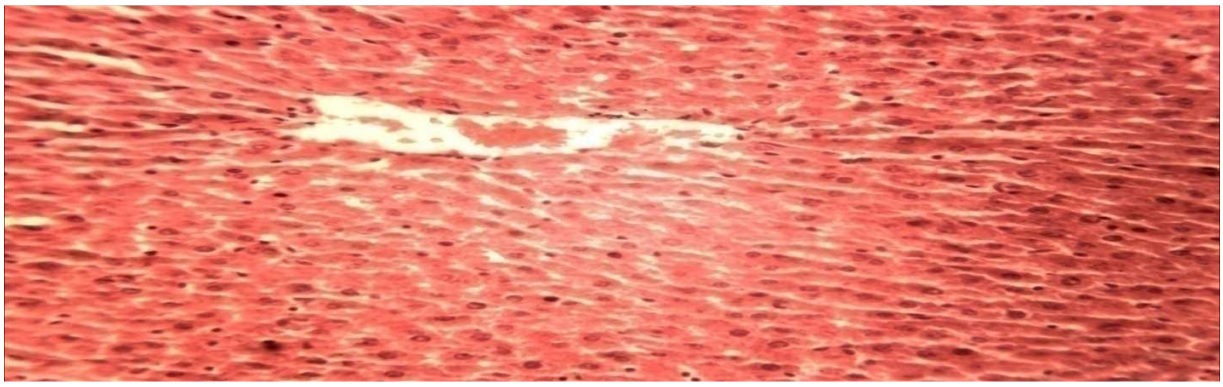

Plate 4: Photomicrograph of a section of liver of rats administered with ethanol leaf extract of *A. cordifolia* 800 mg/kg body weight orally. H&E Stained X 250.

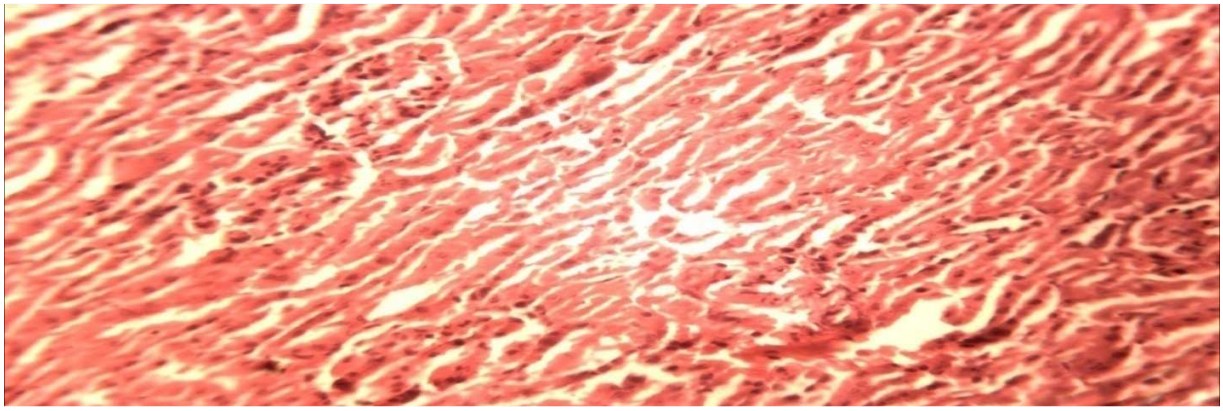

Plate 5: Photomicrograph of a section of kidney of rats that recieved distilled water orally. H&E Stained X 250.

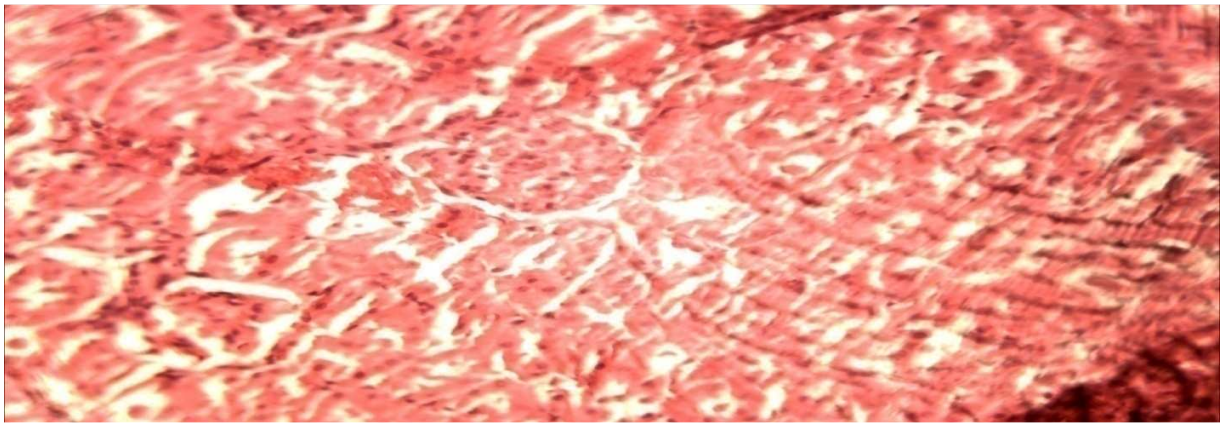

Plate 6: Photomicrograph of a section of kidney of rats administered with ethanol leaf extract of *A. cordifolia* 200 mg/kg body weight orally. H&E Stained X 250.

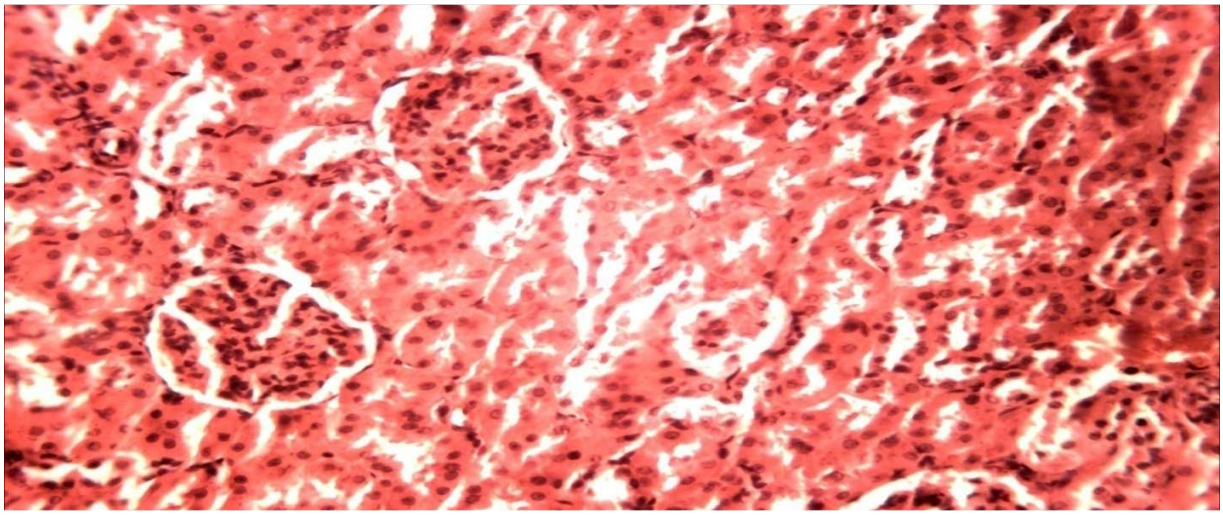

Plate 7: Photomicrograph of a section of kidney of rats administered with administered with ethanol leaf extract of *A. cordifolia* 400 mg/kg body weight orally. H&E Stained X 250.

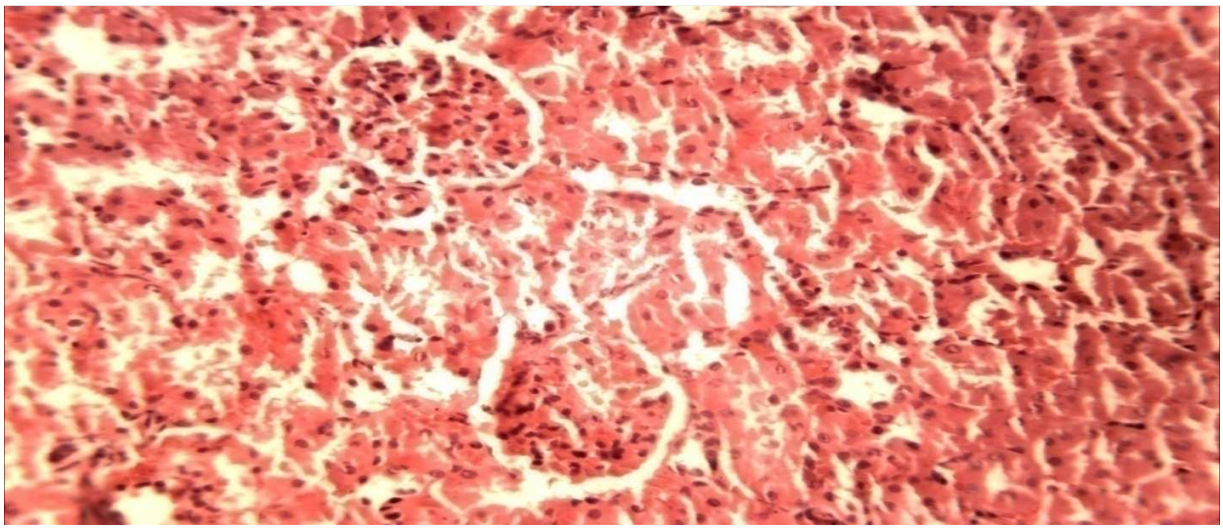

Plate 8: Photomicrograph of a section of kidney of rats administered with administered with ethanol leaf extract of *A. cordifolia* 800 mg/kg body weight orally. H&E Stained X 250.

DISCUSSION

There is a growing interest in herbal remedies due to the side-effects associated with the existing therapeutic hypoglycaemic agents [19]. Streptozotocin-induced experimental diabetes is one of valuable models used for induction of type-I diabetes [20]. Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells leading to decreased synthesis and release of insulin and consequently hyperglycemia occurs [21]. In the present study there was a significantly increased ($p<0.05$) fasting blood glucose level in the diabetic untreated animals when compared to normal control group. The decreased serum insulin levels in the streptozotocin treated animals as result of the destruction of pancreatic beta cells which was not determined in this present study might be responsible for the increased blood glucose levels observed in the diabetic untreated animals. Our findings in this study showed that the administration of the graded doses 200, 400 and 800 mg/kg b w of *A. cordifolia* produced significantly ($p<0.05$) decreased fasting blood glucose level in the Streptozotocin induced diabetic animals when compared to the diabetic untreated group. The observed reduction in the level of fasting blood glucose was in dose dependent fashion. Streptozotocin have been reported to effectively destroy pancreatic cells and causes persistent

hyperglycemia, therefore the mechanism of anti-diabetic action of *A. cordifolia* leaf extract might therefore involve actions other than pancreatic cells insulin release/secretion (insulinotropic effect), i.e. possibly through other extra-pancreatic actions in these streptozotocin-induced diabetic rats [22]. The extra-pancreatic actions perhaps include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis [23]. Preliminary phytochemical screening revealed the presence of saponins, flavonoids and tannins among other host of other secondary metabolites. In addition, the mechanism of action of *A. cordifolia* leaf extract may be related to the presence of phyto-chemicals especially flavonoids found in the plant. Flavonoids have been reported to inhibit sodium-dependent glucose transporter Isoform 2 (Glut 2), the intestinal transporters for glucose, therefore it is also likely that it might reduce blood glucose level by inhibiting the glucose absorption from the intestine with consequent reduction in the blood glucose concentration as was observed in this present work [24]. Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells [16].Toxicity screening models provide important preliminary data to help select natural remedies with potential health beneficial properties for future work [25]. Therefore, acute oral toxicity study is vitally needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation. It is also a useful parameter to investigating the therapeutic index of drugs and xenobiotics [26]. In this study, after administration of various doses of *A. cordifolia* leaf extract by standard method of Lorke's, the rats were monitored for forty eight hours. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups [27].This present study showed that there were no observable signs and symptoms of toxicity distress, decrease food intake and water consumption. The physical appearance features such as skin, fur and eyes were found to be normal in the extract treated animals during the oral acute toxicity study. Studies have shown that determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested [28]. Hence, oral acute toxicity study revealed that the ethanolic extract of *A. cordifolia* did not cause any death and was considered safe at least up to 5000 mg/kg b w suggesting non-toxic nature of the plant extract. In general *in vivo* toxicity study is the toxicological analysis of many medicinal plants and its potency to evaluate qualitatively and quantitatively by histopathology, and to achieve this, long-term toxicity tests is done to find out which organs are affected at the end of the treatment period. In this study, histological evaluations of the liver and kidney tissues of rats administered with *A. cordifolia* for 28 days in the sub-chronic toxicity study was done to further confirm the alteration in cell structure of these organs. The liver is the main target organ of toxicity when it is exposed to foreign substances. When these substances are absorbed in intestines, they are metabolized to other compounds which may or may not be hepatotoxic to the rats [29]. In this study, the liver histology revealed evidence of normal hepatocytes. The study showed that the extract did not cause any alteration to the structure of the liver cells. Also there was no necrosis, inflammatory reaction, fibrosis or local fatty degeneration in the liver cells in the control group (plate 1) when compared to *A. cordifolia* treated groups (plate 2-4). The kidney micrograph displayed in (plates 5-8) shows that there was no potential toxicity or damage to the cell structure of kidney in the control group (plate 5) and the *A. cordifolia* treated groups (plate 6-8). These suggest that the *A. cordifolia* leaf extract does not have deleterious effects on the liver and kidney tissues respectively.

CONCLUSION

The present results show that ethanol leaf extract of *A. cordifolia* at all doses caused a significant decrease on blood glucose level in STZ-induced diabetic animals. The study also revealed that the extract did not cause any apparent in vivo toxicity to animals. In the oral acute toxicity study, no death or signs of toxicity were observed in rats treated with the plant extract up to the dose of 5000 mg/kg b w. The histology examination in the sub-chronic toxicity study revealed no changes in the architecture of liver and kidney tissues at all tested doses of *A. cordifolia*, thus establishing its traditional safe usage in the management of diabetes mellitus.

Acknowledgement
The authors of this research work wish to acknowledge the technical assistance of Mr. A. Bamidele of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.

REFERENCES

[1] H Haller, M Drab, and F C Luft,, *Clinica Nephrology*, **1996,** 46, 246–255.

[2] D M Nathan, J Meigs, and D E Singer, *Lancet*, **1997,** 350, 1, 14–19.
[3] B. Goutam, A critical survey. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry*, **2011,** 187-212.
[4] J Mayfield, *American Family Physician*, **1998,** 58, 1355-1362.
[5] D K Patel, S K Prasad, R Kumar, and S Hemalatha, *Asian Pacific Journal of Tropical Biomedicine*, **2012,** 2, 4, 320-330.
[6] M Elvin-Lewis, *Journal of Ethnopharmacology*, **2001,** 75, 141-6.
[7] SO Oyedemi, G Bradley, and A J Afolayan, *Journal of Natural Pharmaceuticals*, **2010,** 1, 33-9.
[8] M C Gessler, D E Msuya, M H Nkunya, L B Mwasunbi, A Schar, and M Heinrich, *Journal of Ethnopharmacol*ogy, **1995,** 48, 131-44.
[9] H Azaizeh, S Fulder, K Khalil, and O Said, *Fitoterapia*, **2003,** .74, 98-108.
[10] A Lamikanra, A O Ogundain, and F O Ogungbamila, *Phytotherapy Reearch*, **1990,** 4, 198-200.
[11] HD Neuwinger, Medpharm Scientific, Stuttgart, Germany, **2000,** 589.
[12] PO Osadebe, and F BC Okoye, *Journal of Ethnopharmacology*, **2003,** 89, 19–24.
[13] DN Muanza, KL Euler, L Williams, and D J Newman, *International Journal of Pharmacognosy*, **1995,** 33 98–106.
[14] D Lorke, *Archives of Toxicology*, 1983, 275-287.
[15] G E Trease, and M C Evans,"Text book of pharmacognosy,13th edition, Bailler Tindal, London, **1983,** 247-762.
[16] LJ Subramanion, Z Zuraini, C Yeng, LL Yee, L Lachimanan, S Sreenivasan, *Molecules*, **2011,** 16, 5268-5282.
[17] A E Galozhger, and E N Kocloff, "Essential Practical Micro technique," 2nd Edn.,Lee and Febizer, Philadelphia, **!971,** 77.
[18] R C Duncan, RG Knapp, and M C Miller, In: Introductory Biostatistics for the health sciences," John Wiley and Sons Inc. NY, **1977,** 71 96.
[19] R Kameswara, B Giri, M M Kesavulu, and C Apparao, *Manphar Vaidhya Patrika*, **1997,** 1, 33-5.
[20] M Chhanda, C Kausik, G Mehuli, and G Debidas, *African.Journal of Traditional,Complementary and Alternative Medicine*, **2007,** 4, 3, 362 – 371.
[21] K Ikebukuro, Y Adachi, Y Yamada, S Fujimoto, Y Seino, and H Oyaizu, *Transplantation*, **2002,** 73, 512-518.
[22] H Jouad, M Eddouks, MA Dubois, and B Lyoussi, *Journal of Ethnopharmacology*, **2000,** 71, 169–177.
[23] A Saxena, and N K Vikram, *The Journal of Alternative and Complementary Medicine*, **2004,** 10, 369- 378.
[24] J Song, O Kwon, S Chen, R Daruwala, P Eck, and J B Park, *Journal of Biological Chemistry*, **2002,** 277, 5252-60.
[25] M P Badakhshan, YL Lachimanan, and S Sreenivasan, *Molecules*, 2011, 16, 3663-3674.
[26] H P Rang, M Dale, J Ritter, "*Pharmacology,"* 4th ed.; Churchill Livingstone: New York, NY, USA, **2001,** 13, 23.
[27] D L Eaton, and C D Klaassen, In *Casarett and Doull's Toxicology: The Basic Science of Poisons*," 5th ed.; Klaassen, C.D., Ed.; McGraw-Hill: New York, NY, USA, **1996,**13.
[28] K R Steven, and L Mylecrdfaine, In *Principles and Methods of Toxicology* 3rd ed.; Hayes, A.W., Ed.; Ravan Press: New York, NY, USA, **1994,** 673.
[29] H Rhiouania, J El-Hilalya, Z H.Israili, and B Lyoussia, *Journal of Ethno-pharmacology*, **2008,** 118, 378-386,