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HYPOGLYCERMIA AND SERUM BIOCHEMISTRY: ROOT AND LEAF COMBINATION OF *COMBRETUM HISPIDUM*. LAW EXTRACT ON ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT
Antidiabetic activity of *Combretum hispidum* and Biochemical indices was checked. Twenty-five rats were used for the research were grouped into five of five rats each. Groups 1 was the untreated diabetic group, 2, 3 and 4 were the treated groups which received equal doses of root and leaf extract at 100 +100, 200+200 and 400+400 mg/kg body weight of the *C. hispidum*, extracts. Group 5 was the positive control and was administered known antidiabetic drug Glibenclamide. Diabetes was induced in the rats with alloxan monohydrate. *C. hispidum*, extract was administered to rats for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis. Effect of *C. hispidum*, extract was checked on blood glucose level and serum biochemistry. All results in treatment groups were compared with the untreated diabetic group at statistical confidence of $p<0.05$. Result shows that *C. hispidum*, extract reduced blood glucose level in the test groups as dose of extract increased. *C. hispidum*, demonstrated hypoglycermic effect. Biochemical indices shows safety of liver, kidney and heart cells.

KEYWORD: Alloxan, Blood glucose, Diabetes, *Combretum hispidum*, Glucose oxidase, Root+Leaf.

INTRODUCTION
The disease diabetes mellitus, usually just 'diabetes' is a metabolic disease characterized by raised levels of blood glucose and elevated fat and protein metabolism. It is one of the most prevalent human metabolic defects ADS (2007), Guyton (2006). It is caused by failure of the β-cell, of islet of Langerhans of the pancreas to produce insulin. Lenzen, (2008), Ezeja et al (2015). It can be treated successfully by regulating the diet alone otherwise it is necessary to give daily injection of insulin or treat with sulphonylurea drugs. Dietary management is very important in the control of diabetes Bantle et al (2006). Diabetes is a risk factor for cardiovascular diseases such as hypertension, heart failure and nephropathy Bantle et al 2006. Neutritional therapy, counseling and the use of specialized nutritional supplement are recommended for diabetic cases Pastor et al (2002). Chronic complications of diabetes results from elevated blood glucose levels and associated with impairment of lipid and other metabolic pathways Sheetz and King (2003), Diabetic Control Trial (1993). Diabetic nephropathy is the leading cause of chronic kidney disease/failure, ADS (2007), US Renal Data System (1998). The term diabetic nephropathy is used to describe the combination of lesions that occur concurrently in the diabetic kidney, Sheetz and King (2000).

Diabetic retinopathy which is a leading cause of blindness is closely linked to elevation in blood glucose and hyperlipidemia seen in people with uncontrolled diabetes. Seen in cataracts and glaucoma ADS 2004.

Diabetic neuropathies which can affect the somatic and autonomic nervous systems, results from uncontrolled diabetes Boulten 2004, ACCE 2003.

Macrovascular disorder such as coronary heart disease, stroke and peripheral vascular disease reflect the combined effects of unregulated blood glucose levels, elevated blood pressure and hyperlipidemia due to metabolic disorder Martfin (2007), Grundy and Panel (2001).

Diabetic foot ulcers has been associated with diabetic patients, causing ulceration, infection and eventually the need for amputation, ADS (2004).

The chronic complications of diabetes are best prevented by measures aimed at tight control of glucose levels, maintenance of normal lipid levels and control of hypertension. ADS (2004). Hypoglycermia occur when an agent, drug or plant extract causes lowering of blood glucose level below normal reference range Masharani and Gitelman (2007), ADS Workgroup 2005.

Plants play a vital role in the treatment and prevention of diseases. They help in the prevention and reduction of the adverse side effects of conventional drugs Bachrach (2012). They are sources of biological and pharmacological important chemicals. It has been reported that plants are sources of successful drugs, and will continuously be in the front line for screening novel lead compounds Atanasov et al (2015). An essential part of organic chemistry and biochemistry of plant is the identification of the novel bioactive compounds present in plant leading to further biological and pharmacological studies Momin et al (2014), Farid et al (2015), Guo et al (2013).

Combretum hispidum (Laws) (Combretaceae) is a common climbing weed of exist in the forest and savanna region. It regrows rapidly after forest and grass fires. It has trailing branches. It produces from seeds and vegetatively from basal stumps. The leaves are opposite, oblong, elliptic, 10 – 25 cm long and 5 – 11 cm wide. It has a cylindrical woody stem that is covered with short bristly hairs.The outer part of the root is fleshy which covers the inner wooden centre Figure 1b. The pharmacological use of plants of the family Combretaceae is widely reported in the scientific literature Atindehou et al (2004), Muthu et al (2006), Gansane et al (2010). Combretaceae families exist predominately in tropical and subtropical areas, for example, in Africa and Brazil. Pictorial view of the root is shown in Figure 1a.

Phytochemical analysis on the genus *Combretum* have revealed the presence of triterpenes, flavonoids and non-protein amino acids, Pietrovsky et al (2006). In the past few decades, numerous unusual phytocompounds have been isolated from *Combretum* species. It has been reported that 9,10-dihydrophenanthrenes and a substituted bibenzyl was isolated and characterized from *C. molle*, Rogers and Verotta (1996). Isolation of eleven triterpenes and their glycosides from *C. laxum* were reported by Bisoli and co-workers, Bosoli et al (2008). Cycloartane dienone lactone and alkaloids (combretine and betonicine) were isolated from *C. quadrangulare*, Banskota *et al* (2000), and *C. micranthum*, Ogan, (1972). Flavonoids such as rhamnoctrin, quercetin-5,3'-dimetylether, ramnazin and kaempferol were isolated from *C. erythrophyllum*, Martini *et al* (2004).

Analysis of bioactive phytochemicals present in the leaves of *C. hispidum* was carried out by Ikpeazu *et al* (2020) and revealed presence of antidiabetic compounds. Bioactive compounds in plants have been identified by many researchers using Gass Chromatography-Mass Spectrometry analysis (Igwe *et al* 2016, Otuokere *et al* 2016, Ikpeazu *et al* 2017) There are no published literatures that determine the hypoglycermic potentials of ethanol extracts of *C. hispidum* root hence the research.

This study is aimed to investigate the hypoglycermic potentials and biochemical indices of root of *C. hispidum.* and to disprove or otherwise the natives claim that *C. hispidum* root extract can be used to reduce the blood glucose level of diabetic patients.

MATERIALS AND METHODS

(1a) *Combretum hispidum* leaf

(1 b) *Combretum hispidum* root section.

(1c) *Combretum hispidum* root

Figures 1: a,b,c shows pictures of *Combretum hispidum* root and leaf.

Plant Materials
Fresh leaves of *Combretum hispidum* were collected from the Obi Ngwa, in Abia State, Nigeria and was identified by Prof. M. C. Dike and Mr Ibe at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract
The identified leaves of *C. hispidum* was shade dried for 10 days and pulverized to a coarse powder using manual grinder [Corona-Landers YC 1A SA]. The plant leaves extract was prepared using Soxhlet method described by **Jensen (2007).** Thirty five grams (45g) of coarse powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at 70^0C throughout the extraction period of 48 hours. At the end of the extraction period the extract was concentrated using oven at 30^0C to obtain dried extract which was weighed and kept in a well labelled sterile specimen bottle.

Different doses of 200, 400 and 800 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively. These doses were calculated from a stock solution dissolved in distilled water.

Chemicals
Alloxan was used in this study and was obtained from Sigma and Alderich USA. Other reagents/chemicals used were obtained within Nigeria and were of analytical grade.

Experimental Animals
Adult albino rats (148 to 253 g) were purchased from University Farm. Approval was obtained from College of Vet Medicine, Michael Okpara University of Agriculture Umudike, Nigeria, in line with the guidelines for the care and use of laboratory animals as given by the National Research Council (NRC, 1985). The rats were acclimatize and fed *ad libitum.*

Experimental Design
Twenty-five rats were used for the research were grouped into five of five rats each. Groups 1 was the untreated diabetic group, 2, 3 and 4 were the treatment groups which received equal doses of root and leaf extract at 100 +100, 200+200 and 400+400 mg/kg body weight of the *C. hispidum*, extracts. Group 5 was the positive control and was administered known antidiabetic drug Glibenclamide. Diabetes was induced in the rats with alloxan monohydrate. *C. hispidum*, extract was administered to rats for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis.

LD_{50} and Dose selection
A preliminary acute toxicity test was done using rats to determine the LD_{50} (lethal dose that kills 50% of the rats) Acute toxicity (LD_{50}) was determined according to Lorke method, Lorke (1983). At ≤ 5000 mg/kg body weight of administered leaf extract, the treated rats were still healthy and active. This observation shows that the extract is safe at dose below ≤ 5000 mg/kg b.w. Based on this safety determination, different dose combinations of 200, 400 and 800 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively. These doses were calculated from a stock solution dissolved in distilled water.

Experimental Diabetes Induction
The method of Lenzen (2008) was adopted. The animals were fasted for 16–18 hours with free access to water before the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan Monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% v/v normal saline solution at a dose of 150 mg/kg body weight (Katsumat *et al.*, 1999). The diabetes was assessed in alloxan induced rats by determining the blood glucose concentration using one touch glucometer and Accu-check strips at day 1 and day 3 after injection of alloxan monohydrate. The rats that recorded elevated blood glucose level above 240 g/dL were considered diabetic and were selected for the study.

Blood Glucose Levels determination
The procedure of Aziz (1983), Bergman (1984)**)** based on the glucose oxidase principle was adopted in the determination of blood glucose level of the experimental rats. The blood samples were collected by cutting the tip of the tail artery of the rats, and a drop allowed to touch the sensor part of one touch glucometer strips. The values obtained were recorded in mg/dl. The blood glucose levels were sampled at intervals of day 1, day 3 and day 7 of treatment.

Statistical Analysis
All the data were expressed as mean ± SEM. The data was analysed using SPSS vision 20 Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests to separate the mean. The results were considered statistically significant at $p<0.05$.

RESULTS

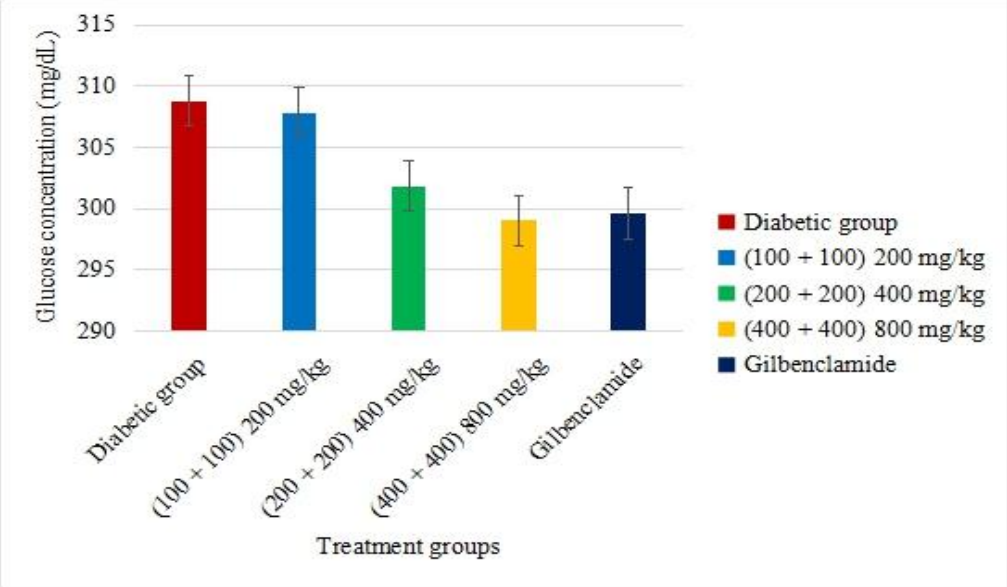

Figure 2: Synergistic effect of leaf and root extracts of *C. hispidum* on blood glucose concentration (mg/dl) of Wistar rats after day 1 of treatment.

Graph in Fig 2 represent hypoglycermic result of root extract of *C. hispidum* on alloxan induced diabetic rats at Day 1 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was mild reduction of glucose in the treatment groups 307.80±11.68, 301.80±12.71 and 290.00±10.25 when compared to the diabetic untreated group 316.75 ± 20.31. The reference standard drug was 299.60 ± 16.85. There was no significant reduction of glucose level at $p<0.05$ on Day 1 treatment.

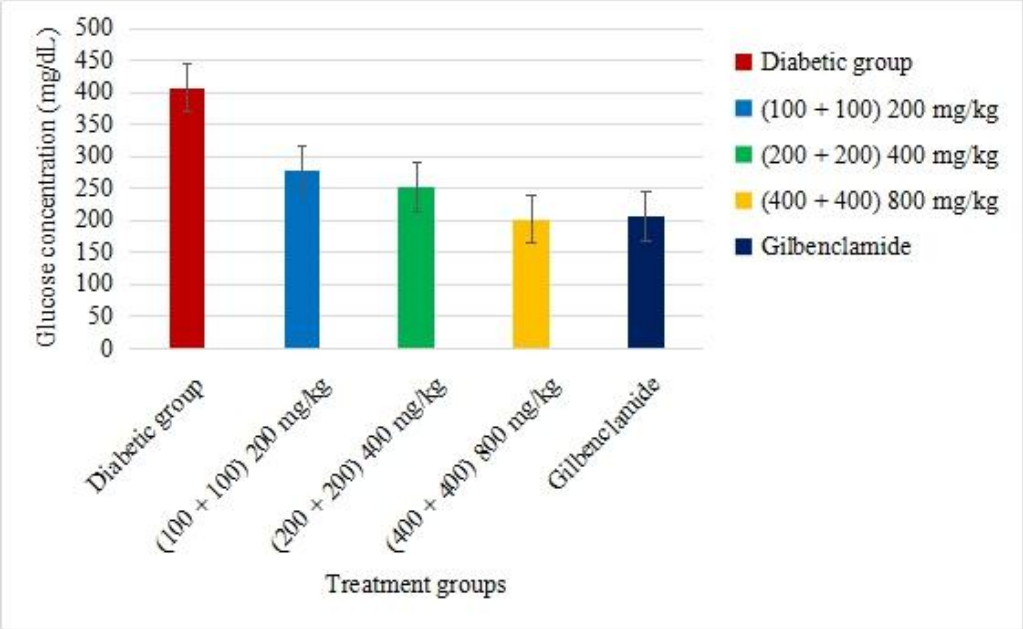

Fig. 3: Synergistic effect of leaf and root extracts of *C. hispidum* on blood glucose concentration (mg/dl) of Wistar rats after day 3 of treatment.

Graph in Fig 3 represent hypoglycermic result of root extract of *C. hispidum* on alloxan induced diabetic rats at Day 3 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was marked reduction of glucose in the treatment groups 279.00±5.07, 252.00±17.07 and 201.00±21.98 when compared to the diabetic untreated group 406.25±19.79. The reference standard drug was 206.40±14.47. There was marked significant reduction of glucose level at $p<0.05$ on Day 3 treatment.

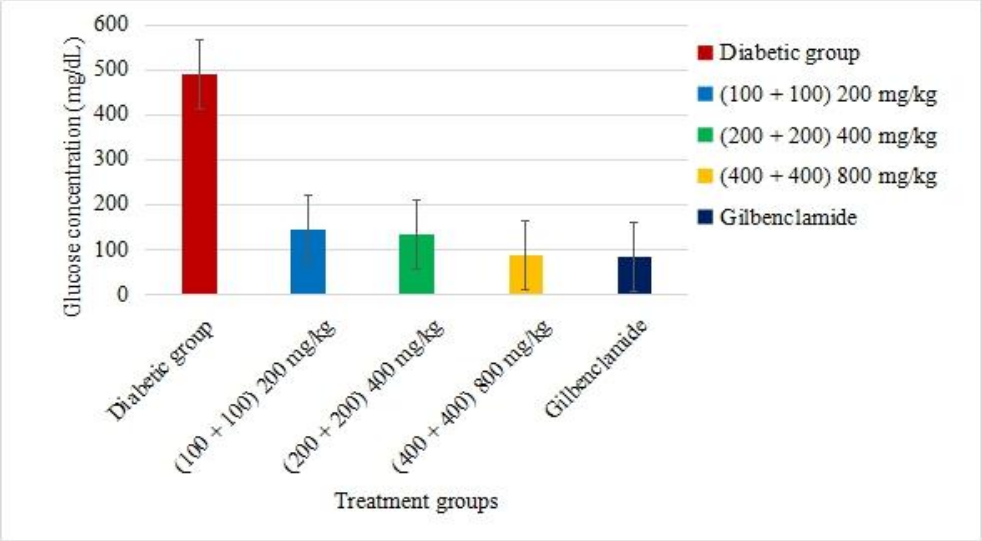

Figure 4: Synergistic effect of leaf and root extracts of *C. hispidum* on blood glucose concentration (mg/dl) of wistar rats after day 7 of treatment.

Graph in Fig 4 represent hypoglycermic result of root extract of *C. hispidum* on alloxan induced diabetic rats at Day 7 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was significantly marked reduction of glucose in the treatment groups 145.00±11.33, 132.40±3.18 and 86.00±3.88 when compared to the diabetic untreated group 499.00±3.21. The reference standard drug was 81.80±1.52. There was significantly marked reduction of glucose level at $p<0.05$ on Day 7 treatment.

Table 1: Comparing the effect of combination of leaf and root extracts of *C. hispidum* on blood glucose concentration (mg/dl) of Wistar rats after Day 7 of treatment.

Treatment groups	Value before induction	Day 1	Day 3	Day 7	Percentage reduction after Day 7 (%)
5 mg/kg Gilbenclamide	60.60±2.24	289.60±16.85	235.20±5.47^{b}	81.80±1.52^{c}	72.8
Diabetic group	60.80±1.39	315.17±3.16	398.00±25.05^{a}	496.80±4.74^{a}	0.0
100 mg/kg root + 100 mg/kg leaf extract of *C. hispidum*	64.20±1.11	288.18±9.08	252.00±17.07^{b}	145.00±11.33^{b}	53.6
200 mg/kg root + 200 mg/kg leaf extract of *C. hispidum*	62.60±1.02	280.11±10.20	219.00±5.07^{b}	122.40±3.18^{b}	55.7
400 mg/kg root + 400 mg/kg leaf extract of *C. hispidum*	62.00±0.57	298.08±10.42	209.00±15.03^{b}	93.00±7.68^{c}	70.3

Values are presented as mean ± S.E.M. Different superscripts represent significant differences at $p<0.05$.
At Day 7, combination of root and leaf extract of *C. hispidum* showed percentage reduction of Glibenclamide (72.8 %). Diabetic group treated with distilled water showed no reduction (0.0 %).Treatment groups 100 +100 mg/kg (53.6 %), 200 +200 mg/kg (55.7 %) and 400 + 400 mg/kg (70.3 %), Table 1.

Table 2: Effect of *C. hispidium* leaf and root extracts on lipid profile of alloxan-induced diabetic rats.

Treatment	Total cholesterol (mg/dl)	Triglycerides	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Normal control	6.54±0.37^{b}	0.72±0.18^{cd}	3.96±0.47^{c}	1.81±0.05^{ab}	0.11±0.03^{ab}
Untreated group	8.34±0.45^{a}	1.58±0.33^{a}	6.01±0.58^{a}	1.47±0.10^{d}	0.27±0.06^{ab}
200 mg/kg)	3.98±0.32^{c}	0.33±0.01^{d}	4.68±0.21^{bc}	1.85±0.05^{a}	0.01±0.00^{b}
400 mg/kg	4.84±0.41^{c}	0.59±0.02^{cd}	5.19±0.22^{abc}	1.87±0.04^{a}	0.31±0.20^{a}
800 mg/kg	7.45±0.22^{ab}	1.01±0.01^{bc}	5.29±0.15^{ab}	1.66±0.04^{bc}	0.21±0.00^{ab}
Glibenclamide (5mg/kg)	8.15±0.42^{a}	1.34±0.19^{ab}	6.02±0.55^{a}	1.51±0.01^{cd}	0.25±0.05^{ab}

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$.

Table 3: Effect of *C. hispidium* leaf and extracts on the liver markers of alloxan-induced diabetic rats.

Treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Total protein (g/dL)	Albumin (mg/dL)	Globulin (mg/dL)	Total Bilirubin (mg/dL)
Normal group	156.34±6.21^{b}	186.64±7.60^{b}	66.08±3.78^{b}	6.12±0.19^{c}	2.69±0.13^{b}	3.80±0.31^{a}	0.65±0.02^{c}
Untreated group	156.70±8.53^{b}	236.52±14.60^{a}	78.72±4.39^{a}	7.00±0.21^{a}	3.61±0.22^{a}	3.38±0.08^{ab}	0.75±0.03^{b}
200 mg/kg)	133.60±1.28^{c}	150.40±0.74^{c}	49.10±0.63^{c}	4.66±0.16^{e}	2.32±0.09^{b}	2.34±0.23^{c}	0.49±0.00^{d}
400 mg/kg	124.80±1.62^{cd}	125.00±2.68^{d}	43.22±1.10^{c}	5.64±0.09^{d}	2.73±0.13^{b}	2.91±0.20^{b}	0.67±0.01^{c}
800 mg/kg	117.00±0.44^{d}	111.20±3.61^{d}	35.40±1.07^{d}	6.56±0.08^{b}	3.18±0.11^{a}	3.37±0.13^{ab}	0.76±0.01^{b}
Glibenclamide (5 mg/kg)	192.44±1.98^{a}	235.42±2.86^{a}	79.38±2.00^{a}	7.08±0.09^{a}	3.44±0.12^{a}	3.63±0.05^{a}	0.85±0.02^{a}

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$. AST: Aspartate Transaminase, ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase.

Table 4: Effect of *C. hispidium* leaf and root extracts on the kidney markers of alloxan-induced diabetic rats.

Treatment	Urea (mMol/L)	Creatinine (µMol/L)	Na^{+} (mMol/L)	Cl^{-} (mMol/L)	K^{+} (mMol/L)	HCO_3^{-} (mMol/L)
Normal group	32.20±1.85^{b}	0.60±0.02	137.54±5.44^{a}	95.12±3.09^{b}	4.57±0.29^{b}	16.42±0.81^{cd}
Untreated group	41.32±1.78^{a}	0.65±0.03	135.24±1.93^{a}	101.40±2.76^{ab}	5.21±0.37^{ab}	18.58±1.09^{ab}
200 mg/kg)	14.40±0.97^{d}	0.60±0.06	114.60±1.72^{c}	86.04±1.18^{c}	3.05±0.06^{c}	14.70±0.28^{d}
400 mg/kg	22.74±1.88^{c}	0.63±0.05	124.92±1.31^{b}	96.08±1.16^{b}	3.74±0.06^{c}	16.48±0.19^{cd}
800 mg/kg	29.60±2.31^{b}	0.64±0.08	135.38±1.22^{a}	104.08±4.02^{a}	4.80±0.10^{ab}	17.12±0.46^{bc}
Glibenclimide (5 mg/kg)	38.90±0.53^{a}	0.86±1.19	134.28±0.65^{a}	101.66±0.73^{ab}	5.54±0.37^{a}	19.26±0.31^{a}

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$.

DISCUSSION

In this study diabetes was induced in rats by a single intra-peritoneal injection of alloxan monhydrate at 150 mg/kg body weight. Alloxan is a cytotoxic agent known to induce diabetes in a wide variety of animal species by damaging insulin secreting β-cell, resulting in decrease of insulin release. This results in decrease utilization of glucose by the tissues leading to hyperglycermia (Lenzen, 2008).

Diabetes mellitus is among the most common disorder in developed and developing countries (Makund *et al.*, 2008). The disease is increasing rapidly in most parts of the world (Kumar *et al.*, 2008). Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels (Jenson and Stender, 1998). Despite the presence of known anti diabetic medicines in the pharmaceutical market, screening for new anti-diabetic sources from plants is a good alternative because they contain substances which are safer for use on diabetes mellitus (Alli Smith, and Adanlawo, 2012). Other plant extracts have been known to show antidiabetic effects (Uhuegbu and Ogbuehi (2002, Nwanjo and Nwokoro (2004).

The findings indicate that administration of *C. hispidum* root extract at the graded dosage on alloxan-induced diabetic rats caused a significant ($p < 0.05$) reduction of the elevated glucose level. The extract from the root of *C. hispidum* caused a significant decrease of blood glucose in a dose dependent manner and hypoglycermic effect was highly pronounced at day 7, treatment (Fig 4). We suspect that the anti-hyperglycemic activity of this root extract may be partly due to insulin release from the existing cells of the pancreas, stimulation of insulin secretion and release, regeneration of β-cell of Langerhans islets or activation of enzymes responsible for glucose utilization (Spasov *et al.*, 2008). This findings is not in isolation as it is in agreement with other studies reported by various researchers, Ezeja *et al* (2015) who demonstrated that antidiabetic activities of plant extract may be due to its multiple effects involving both pancreatic and extra-pancreatic mechanisms. Figure 2 showed that reduction of blood glucose was not significant at Day 1 treatment. Figures 3 and 4 shows significant reduction of the root + leaf extract of *C. hispidum* at Day 3 and Day 7 treatment respectively. The hypoglycermic response was in a dose dependent manner when compared to the diabetic untreated group. The extract competed favourably with the reference drug Gilbenclamide. This suggest that the at higher doses the root+leaf extract of *C. hispidum* can compete favourably with known antidiabetic drugs, hence a good alternative for diabetic cases.

Biochemical parameters

Acute toxicity test shows that ≤ 5000 mg/kg of *C. hispidum* root was safe and was used for the study. The hypoglycermic potential of *C. hispidum* was evaluated by checking its ability to reduce the fasting blood sugar (FBS) of rats induced with alloxan monohydrate (150 mg/kg). Elevated serum lipids observed in diabetes mellitus are suspected to cause coronary heart disease in diabetic cases, Murugan et al (2009).There was mild reduction in total cholesterol, triglyceride, LDL, HDL and VLDL when compared with untreated diabetic rats. These reduction was not significant at $p<0.05$ (Table 2) and could be beneficial in preventing diabetic complications. We suspect that the reduction is due to a control in lipid metabolism (Cho et al, 2002). Therefore *C. hispidum* root+ leaf could be useful in preventing cardiac diseases associated with diabetes.Induction of diabetes with alloxan monohydrate can lead to leakage and elevated levels of liver enzymes ALT, AST, ALP into the blood, Edet et al (2011) as seen in untreated diabetic group (Table 3). The significant dose-dependant reduction in the elevated serum ALT, AST and ALP after administration of *C. hispidum* root + leaf suggest hepatoprotective effect but were in line with the reference drug, glibenclamide. This could be due to membrane stabilization and repair of tissue damage, Argawal et al (2012). There was mild reduction in total protein of treated rats when compared with the untreated diabetic rats. This effect was not significant and bilirubin level was not affected (Table 3). There was mild reduction in urea and creatinine levels which is an indication of kidney protective effect of *C. hispidum* leaf extract. Na+, Cl-, K+ and HCO3- were within normal reference range (Table 4). Diabetes mellitus is among the most common disorder in developed and developing countries (Makund *et al.*, 2008). The disease is increasing rapidly in most parts of the world (Kumar *et al.*, 2008). Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels (Jenson and Stender, 1998). Despite the presence of known anti diabetic medicines in the pharmaceutical market, screening for new anti-diabetic sources from plants is a good alternative because they contain substances which are safer for use on diabetes mellitus (Alli Smith, and Adanlawo, 2012). Other plant extracts have been known to show antidiabetic effects (Uhuegbu and Ogbuehi (2002, Nwanjo and Nwokoro (2004)

There is plan for further studies to find the bioactive compounds responsible for this antidiabetic activity in *C. hispidum* root and leaves.

CONCLUSION

Combination of root and leaf extract of *C. hispidum* has a more potent antidiabetic activity which is comparable with the known drug, Gilbenclamide in alloxan - induced diabetic rats and hence maybe a good alternative in the treatment of diabetes. Further studies are hereby recommended to isolate and characterize the active ingredients responsible for the hypoglycermic effect in *C. hispidum* root + leaf extract. Biochemical indices indicated liver, kidney and cardiac protective effect.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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