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EVALUATION OF BIOCHEMICAL PARAMETER AND HYPOGLYCERMIC POTENTIAL OF COMBRETUM HISPIDUM.LAW ROOT EXTRACT ON ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

Antidiabetic activity of *Combretum hispidum*, was checked. Twenty-five rats were used for the research and were grouped into five of five rats each. Groups 1 was the untreated diabetic group while groups 2, 3 and 4 were the treatment which received 200, 400 and 800 mg/kg body weight of the *C. hispidum* extract respectively. 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 for possible hypoglycermic potential and biochemical parameters. All results in treatment groups were compared with the untreated diabetic group at statistical confidence of $p<0.05$.This significant effect was recorded at Day 1, Day 3 and Day 7 of the treatment. 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 indicated safety of liver, kidney cardiac cells.

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

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).

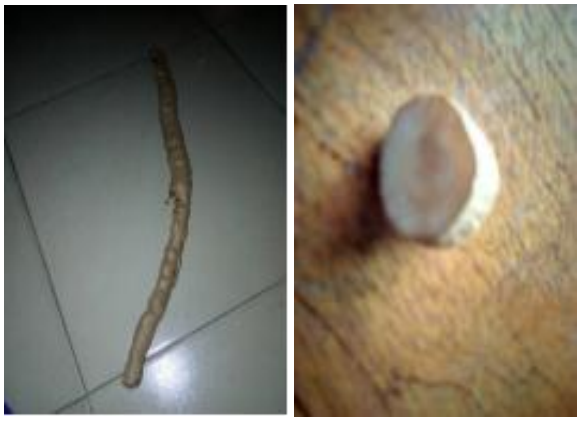
Combretum hispidum (Laws) (Combretaceae) is a common climbing weed of exist in the forest and savanna region. It regrows rapidly after forest and grass fires. It has trailing branches. It produces from seeds and vegetatively from basal stumps. The leaves are opposite, oblong, elliptic, 10 – 25 cm long 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



1(a) **1(b)**

Fig. 1: *C. hispidum* root.

Plant sample

Fresh roots of *C. hispidum* were collected from Obi-Ngwa, Abia State Nigeria on 24th May, 2020. Sample of plant roots was identified by a Botanist at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

Extraction of crude extracts

The identified root 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 root extract was prepared using Soxhlet method described by Jensen (2007). Forty-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.

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 root extract, the treated rats were still healthy and active. This observation shows that the root extract is safe at dose below 5000 mg/kg b.w. Based on this safety determination, 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 acclimatized 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 200, 400 and 800 mg/kg body weight of the *C. hispidum*, extract. 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.

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. 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 enzyme glucose oxidase reacts with glucose, water and oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide can then be used to oxidize a chromogen or the consuption of oxygen measured to estimate the amount of glucose present. Glucose oxidase is specific for B-D-glucose so cross reaction with other sugars is not a problem Aziz (1983), Bergman (1984), Howanitz and Howanitz (1984). The blood samples were collected by cutting the tip of the tail artery of the rats, and a drop allowed touching 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

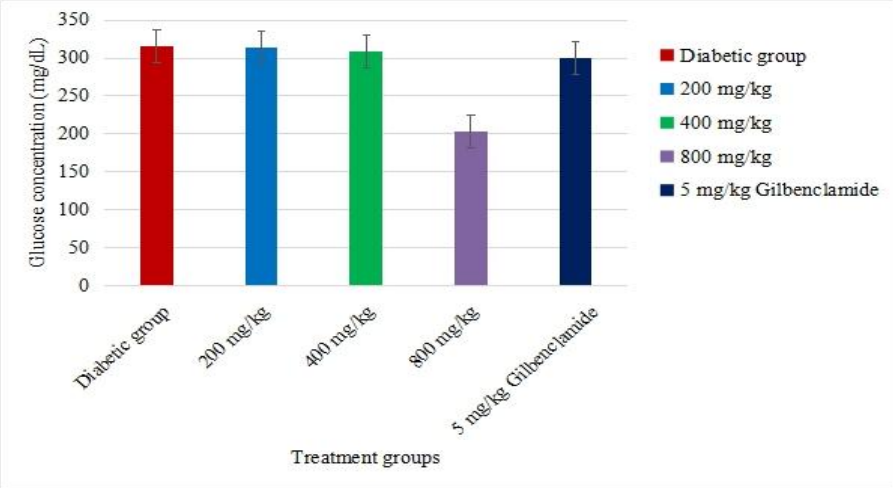


Figure 2: Represent glucose level at Day 1.

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 313.40 ± 19.43, 309.20.20 ±11.82 and 283.00 ± 13.08 when compared to the diabetic untreated group 316.00 ± 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.

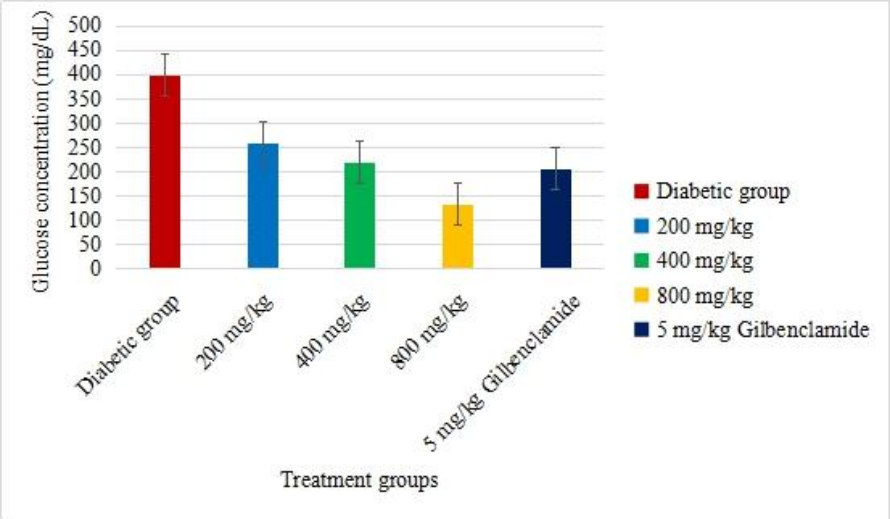


Figure 3: Represent glucose level at Day 3.

Graph in Fig 4 represent hypoglycermic result of root extract of *C. hispidum* on alloxan induced diabetic rats at Day 3 of treatment. Values are presented as Mean ± S.E.M.

There was more reduction of glucose level in the treatment groups 258.60 ± 22.26, 258.60 ± 22.26 and 133.60 ± 3.50 when compared to the diabetic untreated group 398.00 ± 25.05. The reference standard drug was 206.40 ± 14.12. The reduction was statistically significant at $p<0.05$ on Day 3 treatment.

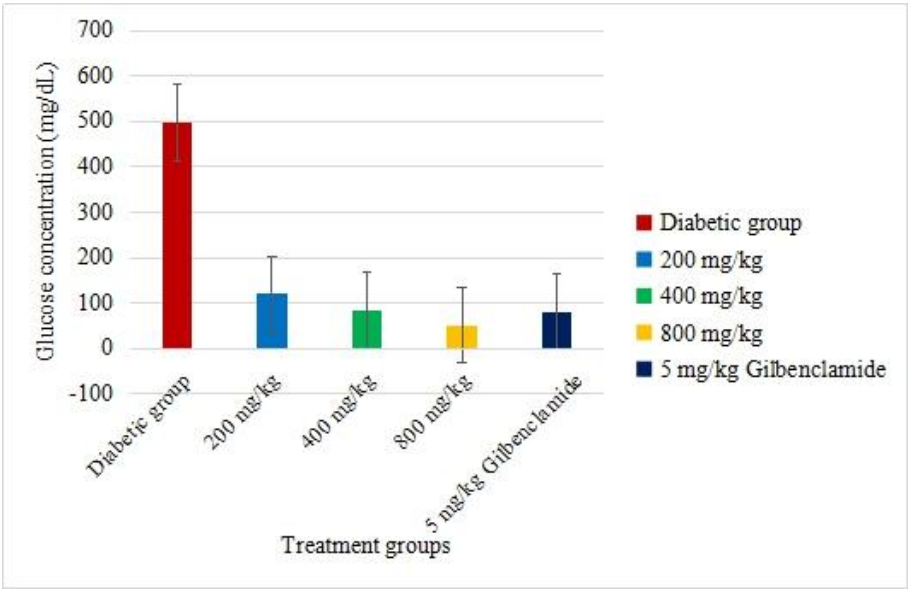


Figure 4: Represent glucose level at Day 7.

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 ± S.E.M.

There was a very noticeable reduction of glucose in the treatment groups 120.40 ± 3.07, 83.20 ± 2.26 and 51.20 ± 1.85 when compared to the diabetic untreated group 496.80 ± 4.74. The reference standard drug was 81.80 ± 1.52. The reduction was statistically significant at $p<0.05$ on Day 7 treatment.

RESULT

The result as presented in Table 1 showed the percentage protection of *C. hispidum* extract on blood glucose concentration (mg/dl) of alloxan induced diabetes in Wistar rats after seven (7) days of treatment.

Table 1: Percentage Reduction of root extract of *C. hispidum* on mean fasting blood glucose concentration (mg/dl) of alloxan induced diabetic wistar rats after seven days of treatment.

Treatment groups	Value before induction	Day 1	Day 3	Day 7	Percentage protection after Day 7 (%)
5 mg/kg Gilbenclamide	60.60±2.24	290.02±3.27	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
200 mg/kg *C. hispidum* root extract	61.20±0.58	303.06±5.04	258.60±22.26^{b}	120.40±6.07^{b}	61.1
400 mg/kg *C. hispidum* root extract	61.60±0.92	311.13±2.41	253.40±14.52^{b}	83.20±2.26^{c}	73.1
800 mg/kg *C. hispidum* root extract	64.40±0.93	302.16±3.65	197.60±41.04^{b}	59.40±6.31^{d}	80.6

Values are presented as mean ± S.E.M. Different superscripts represent significant differences at p<0.05.

Table 2: Effect of *C. hispidium* root 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^{c}	0.72±0.18^{b}	3.96±0.47^{c}	1.81±0.05^{a}	0.11±0.03^{c}
Untreated group	8.34±0.45^{b}	1.58±0.33^{a}	6.01±0.58^{b}	1.47±0.10^{b}	0.27±0.06^{b}
200 mg/kg)	4.51±0.20^{d}	0.35±0.01^{b}	3.92±0.03^{c}	1.84±0.01^{a}	0.04±0.02^{c}
400 mg/kg	6.78±0.16^{c}	0.54±0.01^{b}	5.22±0.34^{bc}	1.32±0.01^{c}	0.11±0.02^{c}
800 mg/kg	7.46±0.17^{a}	1.48±0.15^{a}	6.36±0.35^{a}	1.16±0.01^{d}	0.48±0.02^{a}
Glibenclimide (5 mg/kg)	8.15±0.42^{b}	1.34±0.19^{a}	6.02±0.55^{b}	1.51±0.01^{b}	0.25±0.05^{b}

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* Root 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^{d}	3.80±0.31^{a}	0.65±0.02^{d}
Untreated group	156.70±8.53^{b}	236.52±14.60^{a}	78.72±4.39^{a}	7.00±0.21^{b}	3.61±0.22^{b}	3.38±0.08^{abc}	0.75±0.03^{c}
200 mg/kg)	186.00±3.79^{a}	166.60±1.77^{bc}	56.40±0.92^{c}	5.56±0.14^{d}	2.60±0.19^{d}	2.96±0.16^{c}	0.48±0.01^{e}
400 mg/kg	156.98±1.90^{b}	151.40±0.60^{cd}	42.00±1.18^{d}	6.23±0.15^{c}	3.08±0.16^{cd}	3.15±0.12^{bc}	0.66±0.01^{d}
800 mg/kg	131.10±0.71^{c}	142.40±1.46^{d}	36.90±2.45^{d}	7.73±0.08^{a}	4.28±0.06^{a}	3.39±0.05^{abc}	0.95±0.01^{a}
Glibenclamide (5 mg/kg)	181.54±11.06^{a}	235.42±2.86^{a}	79.38±2.00^{a}	7.08±0.09^{b}	3.44±0.11^{bc}	3.63±0.05^{ab}	0.85±0.01^{b}

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* root 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^{c}	0.60±0.02	137.54±5.44	95.12±3.09^{b}	4.57±0.29^{c}	16.42±0.81^{bc}
Untreated group	41.32±1.78^{b}	0.65±0.03	135.24±1.93	101.40±2.76^{b}	5.21±0.37^{bc}	18.58±1.09^{bc}
200 mg/kg)	29.02±0.48^{c}	0.44±0.01	111.00±1.84	86.20±1.68^{c}	3.30±0.15^{d}	14.42±0.14^{c}
400 mg/kg	38.22±2.13^{b}	0.55±0.02	127.14±0.78	96.50±0.63^{b}	5.15±0.28^{bc}	24.90±2.24^{a}
800 mg/kg	48.36±0.86^{a}	0.81±0.02	119.86±20.27	110.68±2.41^{a}	7.87±0.09^{a}	25.72±2.07^{a}
Glibenclamide (5 mg/kg)	38.90±0.53^{b}	1.86±1.19	134.28±0.65	101.66±0.73^{b}	5.54±0.37^{b}	19.26±0.31^{b}

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). 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 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 extract of *C. hispidum* can compete favourably with known antidiabetic drugs, hence a good alternative for diabetic cases.

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 elevation in total cholesterol which was not significant. Triglyceride, LDL, HDL and VLDL showed reduction when compared with untreated diabetic rats. Though these reduction was not significant at $p<0.05$ (Table 2). This reduction 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 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 suggest hepatoprotective effect. 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.

CONCLUSION

Root extract of *C. hispidum* has a 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 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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