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Research Paper

EVALUATION OF ANTIDIABETIC AND ASSOCIATED EFFECT OF METHANOL LEAF EXTRACT OF *Combretum micranthum* G. Don (COMBRETACEAE) ON NORMOGLYCEMIC AND ALLOXAN-INDUCED DIABETIC RATS

Odoh, Uchenna Estella[1], Osadebe, Patience Ogoamaka[2] and Osadebe, Onyinye Hilda[1]

[1]Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutica Sciences, University of Nigeria, Nsukka,
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences,University of Nigeria, Nsukka, Nigeria.

Abstract

Introduction: Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism. It results from defects in either or all the following: insulin secretion, insulin sensitivity and insulin action. *Combretum micranthum* is a plant known for its antidiabetic, antioxidant, anti-inflammatory and various other activities. **Method:** The methanol leaf extract of C. micranthum was screened for antidiabetic and associated effects on both normoglycemic and alloxan-induced diabetic animals. The phytochemical investigation of the extract and the acute toxicity study (Lorke's method, 1983) was also carried out. Diabetes mellitus was induced using 150 mg/kg of alloxan monohydrate intraperitoneally. **Results:** The phytochemical analysis revealed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, protein, tannins, saponin, fats and oils, steroids and terpenoids. The extract was found to possess a significant non dose-dependent ($p<0.05$) antidiabetic effect in rats at doses of 100 (67.34 %) and 200 mg/kg (60.56 %) which was comparable to 5 mg/kg glibenclamide (90.62 %). The plant extract also produced some effect on the haematological and serum biochemical parameters. The study shows that the antidiabetic activity of C. micranthum is not dose dependent and the plant extract is not nephrotoxic since it didn't increase urea and creatinine levels after 21 days treatment, and also had hemanitic, immune boasting and lipid lowering effects in addition to the antidiabetic effect. The methanol leaf extract was found to be non toxic after the acute toxicity test, since at 5000 mg/kg no death or behaviourial changes were observed.

Conclusion: From this study, the methanol leaf extract of *Combretum micranthum* was found to possess antidiabetic activity which justifies its use in traditional medicine for the management of Diabetes mellitus.
Key words: *Combretun micranthum*, diabetes mellitus, phytochemical investigation, biochemical analysis.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism. It results from defects in either or all the following: insulin secretion, insulin sensitivity and insulin action [1]. Diabetes mellitus is an important endocrine and metabolic disease causing considerable mortality and morbidity in human population. Diabetes mellitus is a hereditary, metabolic disease characterized by hyperglycemia and eventual glycosuria. It is caused by inability of tissues to carry out normal metabolism of carbohydrates, fats, and proteins due to absolute or relative lack of insulin [2]. According to International Diabetes Federation (IDF) report, elevated blood glucose is the third uppermost factor for premature mortality, following high blood pressure and tobacco use globally [3].

In 2015, according to IDF report, 415 million (8.8 %) adults (aged 20 – 79) worldwide were estimated to have diabetes; this number is expected to rise to 642 million (10.4 %) by 2040 or one adult in ten people [3]. An estimated 14.2 million adults aged 20 – 79 ad diabetes in the Africa Region that represents a regional prevalence of 3.2 5 (2.1 -6.7 %) in 2015, which can be projected to 3.7 % (2.6 – 7.3 %; 34.2 million) by 2040. South Africa (2.3 million), Democratic Republic of Congo (1.8 million, Nigeria (1.6 million) and Ethiopia (1.3 million) are among the highly populated African countries containing the highest number of people living with diabetes [3].

Diabetes mellitus can be managed by diet, physical excercise, and modern drugs (insulin and /or oral hypoglycemic agents such as sulfonylureas and biguanides [4]. Different extracts from medicinal plants have also been used traditionally to manage diabetes globally, and these are considered as relatively inexpensive, less toxic and with relatively little or no side effects [5]. The treatment might include the whole plant, sin fusions like tea and the extraction and purification of single ingredients of plants. Many plants of different families have been reported to exhibited antidiabetic properties such as *Ceiba pentandra* [6], *Acalypha wilkesiana* [7], *Acacia Arabica* [8], *Allium cepa* [9], *Andrographis*

paniculata [10], *Aegle marmelose* [11], *Azadirachata indica* [12], *Brassica juncea* [13], *Helicteres isora* [14], *Catharanthus roseus* [15], *Chamaemelum nobile* [16], *Citrullus colocynthis* [17], *Embelia madagascariensis* [18], *Dioscorea dumetorum* [19], *Tetminalia bellerica* and *Emblica officinalis* [20], *Ficus racemosa* [21], *Eugenia jambolana* and *Ficus bengalensis* [22], *Magnifera indica* [23], *Moringa oleifera* [24], *Momordica charantia* [25], *Phyllanthus emblica* [26], *Tinospora cordifolia* [27] etc.

C. micranthum is commonly found in the tropical and sub-tropical countries including those of Africa like Senegal, Gabon, Gambia, Mali, Nigeria, Niger and Ghana where they are widely used in traditional medicine [28]. The flowers are usually bisexual and are borne as auxiliary cluster on scaly stalks. The fruits are small and scaly, while the leaves are dorsiventral or more rarely centric with short stalk; the leaves turn from green to orange over time and are very useful in herbal medicine [29]. Common names according to Burkill [30] are: Hausa: Farar Geezaa; Yoruba: Okan; Igbo: Nza Otego; Fulanis in Sokoto: Gumumi. A root decoction is vermifugal and is said to have been used for sore washes as antiseptic for washing open wounds and also drunk for treatment of guinea worm infestations [31]. The dried powdered root and fruit when mixed with palm oil is used in the treatment of sprain, bruises, suppurating swellings and abscesses whether of syphilic or other origin [30]. The leaf of C. micranthum is used in West Africa as herbal infusion in the treatment of biliary fever, kidney infections, naso-pharyngeal infection, colic and vomiting and has been found to possess antimalaria, diuretics, anti-inflammatory and antimicrobial properties against both gram positive and negative organisms [31]. The aqueous ethanol root extract had been reported to possess dose-dependent Anticonvulsant activity in both electroshock and chemically induced convulsions [32]. The aqueous ethanol leaf and stem-bark extracts were also reported to have shown analgesic, anti-inflammatory and antidiarrhoeal properties [28]; while the aqueous leaf extract showed antipyretic, analgesic and anti-inflammatory activity [33] as well as significant antidiabetic property for Type II diabetes mellitus [34]. The aqueous extract from the fresh leaves also showed antibacterial activity [35] and the methanol leaf extract showed inhibitory effect on herpes virus Types I and II [36].

The reported chemical constituents in the aqueous ethanolic root extract of *C.micranthum* include flavonoids, saponins, carbohydrates, anthraquinones, tannins and cardiac glycosides , while from the aqueous leaf extract, alkaloids, sterols and

terpenes were found in addition to the above [28; 37]. The chemical compositions from the ethyl-acetate and n-butanol fractions of the aqueous-ethanolic leaf extract as reported by Welch [37] include many polyphenolic compounds such as catechins, glycosylflavones, flavans and galloylated -C- glycosylflavone and epicatechin as well as gallic acid, mallic acid, betaine, choline, combretine, vitexin and isovitexin (C-glycosylflavone), m-inositol, sorbitol, myricetin-3- O -glucoside and myricetin-3-O-rutinosi.

With this alarming increase in the rate and incidence of diabetes, there is need for additional therapy which is well accepted and tolerated by the masses [38]. The aim of the study is to ascertain the anti diabetic effect of the crude methanol leaf extract of C. micranthum on both normal and alloxan induced diabetic rats and assess the effect of the extract on serum biochemistry and some haematological parameters.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant material (*Combretum micranthum*) was collected in July from Enugu state and was authenticated by Mr. A.O Ozioko of International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka. The voucher specimen was deposited at the Herbarium of the Department of Pharmacy and Environmental Medicines, University of Nigeria, Nsukka.

Preparation of Plant Materials

The leaves of the plant (Combretum micranthum) were carefully plucked from their stalk and placed on a clean mat. The leaves were dried under shade for two weeks to obtain a dry flaky leaf. The dried leaves were pulverized using an End Runner Mill to obtain a coarse leaf powder.

Chemicals, Reagents, Drugs and Equipment

Chemicals and Reagents and Drugs

All chemicals, solvents and reagents were of analytical grade.

Chloral hydrate, mountant (glycerin), 50% gelatin, sodium hypochlorite, hydrochloric acid, phloroglucinol in hydrochloric acid and iodine, methanol (BDH), n-hexane (BDH), ethlyacetate (BDH) and n-butanol (BDH). Solution of crystalline $CuSO_4$ in sulphuric acid (Fehling's solution1), solution of Rochelle salt and potassium hydroxide (Fehling's solution 2), potassium bismuth iodide solution (Dragendorff's reagent), solution of iodine in potassium iodide (Wagner's reagent), potassium mercuric iodide solution (Mayer's reagent), saturated solution of picric acid (Hager's reagent), Million's reagent,

naphthol solution in ethanol (Molisch's reagent), α-naphthol, sulphuric acid (H_2SO_4), ammonium hydroxide (NH_4OH), Chloroform, sodium hydroxide (NaOH), carbon tetrachloride, ferric chloride, ethanol (70%,90%), lead subacetate, glacial acetic acid, ethyl acetate, aluminium chloride, olive oil, Glibenclamide (Hovid, USA), Alloxan monohydrate (Qualikems, India), Tween 80 (Nigeria) and Normal saline (Nigeria).

Equipment and other apparatus used

Electronic weighing balance (G & G, USA), Desiccator (Hindustan, India), Thermostatic water bath (Hindustan, India), separating funnel, Rotary evaporator (Stuart, UK), Acuu-Chek glucometer (Roche, Germany).

Animals

Forty-four (44) albino rats weighing between 100-210g were obtained from Department of Pharmacology, University of Nigeria, Nsukka. The animals were kept in metal cages and fed with Vital Feed (grower) and water with natural cycle of light. They were allowed to acclimatize to their new environment for two weeks. Ethical approval obtained from the University Ethics Committee.

Extraction

A 500 g weight of the pulverized plant leaf was extracted using the Soxhlet extractor and 2.5 litres of methanol as the menstrum (extracting solvent). The solvent was evaporated off using rotary evaporator at the temperature of 40 ^{0}C to obtain the dry leaf extract.

Phytochemical Tests

The extract was subjected to preliminary phytochemical analysis to test for the presence of alkaloids, saponins, glycosides, resins, proteins, carbohydrates, flavonoids, steroids, terpenoids, fats and oils using standard methods [39; 40].

Pharmacological Evaluation

Acute Toxicity Test

Lorke's method [41] was used for the acute toxicity test. Three groups consisting of 3 per group were set up for per-oral (p.o.) acute toxicity tests in rats. In the initial phase of the experiment, the extract at doses of 10, 100, 1000 mg/kg body weight was administered and observed for 24 hours for signs of changes in the behavioral pattern and/or death. The second phase was carried out based on the results of the first phase and the extract at doses of 1600, 2900, 5000 mg/kg body weight was administered through the same route to three groups consisting of one rat in each group .The median lethal dose was

calculated as the geometric mean of the highest non-lethal and the lowest lethal dose of the extract..

Induction of Diabetes in Rats

Twenty-four (24) rats were fasted overnight for about 8-12 hours prior to induction. Diabetes was induced by a single administration 150 mg/kg of Alloxan monohydrate intra-peritoneally (i.p) to the fasted rats [42]. Subsequently, the rats were fed with glucose solution (5 %w/v) for 24 hours to counter the initial hypoglycaemic surge caused by Alloxan (due to a sudden surge in the release of insulin). After three days blood was drawn from each rat by tail snipping, and the fasting blood glucose level of each rat was checked to confirm diabetes. The rats with a fasting blood sugar level of ≥250mg/dl were considered diabetic..

Effects of the Methanol Leaf Extract on the Blood Glucose Level of Normoglycaemic Rats

Twenty normal (non diabetic) rats were divided into four (4) groups randomly to receive; glibenclamide (5 mg/kg), 100 mg/kg of the extract, 200 mg/kg of the extract, and the vehicle respectively. The rats were treated once daily for twenty-one days and their fasting blood sugar level checked at day 0, 7, 14 and 21 [42].

Effects of the Methanol Leaf Extract on the Blood Glucose Level of Diabetic Rats

Twenty rats were affirmed diabetic (ie FBS ≥ 250mg/dl) after four days. The twenty diabetic rats were divided into four (4) groups (n=5) randomly; to receive Glibenclamide (5 mg/kg), Extract: 100 and 200 mg/kg and the vehicle respectively. The rats were fasted overnight prior to the experiment and the fasting blood glucose level of each animal was measured prior to treatment (0 hr), at 1/2, 1, 2, and 4h after the extract was administered [42].

Determination of Hematology Parameters

A 1 ml blood sample was collected from each normoglycemic rat and transferred into a sample bottle containing EDTA at day 0 i.e prior to treatment and day 21 after treatment. The blood samples were taken to the laboratory to determine the following blood parameters; Total White Blood Cell (T.WBC), Packed Cell Volume (PCV) and White blood cell Differential of each rat [43].

Determination of Biochemical Parameters

A 5ml blood sample was collected from each normoglycemic rat and transferred into a sample bottle; the blood samples were centrifuged at 100rpm in order to separate the serum from the blood cells at day 0 i.e prior to treatment and day 21 after treatment. The resulting serum for each rat was taken to the laboratory to determine the following biochemical parameters; total cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), triglyceride, urea and creatinine [44].

Statistical Analysis of Results

Data obtained was analysed using students's t-test. Difference between means were accepted significant at $p<0.05$. Results were presented as Mean ± SEM.

RESULTS AND DISCUSSION

The percentage yield of the extract (80 %) was high considering that methanol used for the extraction is a polar solvent and soxhlet extractor used must have extracted most of the polar constituents available to methanol as against a 12.92 % yield of the aqueous ethanolic leaf extract extracted by maceration reported Handa *et al*., [45]. The choice of extracting solvent and method of extraction particularly plays a role in the constituents found in an extract because the ability to extract sufficient/abundant secondary metabolites depends on the polarity of the solvent and the effectiveness of the extraction method.

The phytochemical screening carried out in this study shows the presence of the following secondary metabolites, alkaloids, carbohydrates, fats and oils, flavonoids, glycosides, proteins, reducing sugars, resins, saponins, steroids, tannins and terpenoids in the methanol leaf extract (Table 1). These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. Terpenes and tannins [46], steroids [47] have been implicated in the antidiabetic activities of plants. Sulphonyureas cause hypoglycemia by stimulating insulin secretion from the pancreas and these compounds are potent in mild alloxan-induced diabetes and inactive in intense alloxan induced diabetes whereby nearly all β – cells have been destroyed [48].

Welch [37] and Abdullahi [28] reported the presence of constituents such as alkaloids, carbohydrates, flavonoids, saponins, sterols anthraquinones, tannins and terpenoids which were found in the aqueous ethanol leaf extract collected in Kastina state of

Northern Nigeria in September 2011. However Osonwa et al., [50] and Uduma *et al.*, [35] reported absence of alkaloids and anthraquinones from the ethanolic leaf extract collected in August 2012 from Akwa-ibom State, Southern Nigeria. Generally, it has been reported that the type and quantity of secondary metabolites in plants depend on the nature of the soil, climate condition and geographical location to which the plant is exposed [49], thus the location and time of collection will affect the plant constituents as well as the different plant parts, choice of solvent and method of extraction.

The LD_{50} obtained for the methanol leaf extract of *Combretum micranthum* in rat was found to be greater than 5000 mg/kg per oral. No death or observable behavioural changes were seen. Acute toxicity study is often used to describe the harmful effects including death which appear promptly or within 24 hours of exposure to a single or more doses of chemical substances and it is an important and initial step taken to access the safety of drugs in biological systems [50]. The acute effect is usually of the general observation in behavioural changes, while the amount of the drug that kills 50 % of the test animals is described as the median lethal dose (LD_{50}) and which is often taken as the end effect to acute studies. Agaie et al., [51] reported that substance with an i.p LD_{50} > 1000 mg/kg as nontoxic and substance with p.o LD_{50} >5000 mg/kg are regarded as practically non toxic. Thus, the LD_{50} of *C. micranthum* leaf extract was found to be relatively non toxic as the value obtained in the rats was >5000 mg/kg (p.o).

The methanol leaf extract of *C. micranthum* exhibited an antidiabetic and hypoglycaemic activity on alloxan- induced diabetic rats and normoglycemic rats as illustrated in Tables 2 and 3 respectively. In the antidiabetic study, a single dose of alloxan (150 mg/kg) induced diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cells resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by tissues and this leads to various metabolic aberrations in the animals such as increased blood glucose [52; 42]. Treatment of the alloxan-induced diabetic animals with the different doses of the extract produced a decline in the FBG which was generally sustained throughout the four (4) hours of observation. The 100 mg/kg of the methanol leaf extract of *C.micranthum* produced a significant effect ($p < 0.05$) after 30 mins, which persisted up to the fourth hour. The 200 mg/kg of the extract also produced a significant effect ($p < 0.05, 0.001$) after 30 mins which also persisted till the fourth hour. Maximum

reduction of 67.34 and 60.56 % in blood glucose was produced by 100 mg/kg and 200 mg/kg of the methanol extract respectively after 4h treatment. These effects were not comparable with that of 5 mg/kg of glibenclamide which produced maximum reduction of 90.62 % after 4 hr but was significant.

The normoglycemic animals were treated for 21 days to know the effect of prolonged use of the leaf of *C. micranthum* on a normal person taking the leaf extract for other health purposes. The significant lowering of the Fasting Glucose Concentration in both normal and alloxan-induced diabetic experimental animals by the extract shows the extract produces a non-dose dependant anti-diabetic activity, since the 100 mg/kg had a more pronounced effect than the 200 mg/kg. Comparison between the effect on the normoglycemic and diabetic experimental animals suggests that the extract has more activity in the presence of elevated blood sugar levels (hyperglycemia).

The effect of the extract on normoglycemic animals suggest that the leaf of *C. micranthum* has hypoglycemic effect which was sustained over the 21 day period. This effect though not comparable with that of the glibenclamide an insulin secretagogue which also lowers blood glucose in normal animals. Maximum reduction of 65.02 and 47.87 % were observed for both 100 and 200 mg/kg respectively after 21 days. Kahn and Shecter [53] have suggested that a 25 % reduction in blood glucose levels is considered a significant hypoglycemic effect. Some plant extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin [54] while others act through extra pancreatic mechanisms by inhibition of hepatic glucose production [16] or corrections of insulin resistance [55]. The results have shown the plant extract possesses both antidiabetic and hypoglycemic effect. Therefore, the plant extract may possibly act by potentiating the insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake. The extract could be acting similar to glibenclamide which secretes insulin from beta cells in Type 2. Type 2diabetes is characterized by a reduced pancreatic secretion of insulin (relative lack of insulin) or insulin resistance or both. Since glibenclamide as well as the test plant extract was found effective in lowering the blood glucose level in the alloxan-induced diabetic rats, it is possible that alloxan did not

totally destroy the pancreatic cells; this suggests a model of Type 2 for the present study.

The results of the biochemical (Tables 4 and 5) and hematological (Tables 6 and 7) examination show that the methanol leaf extract of *C. micranthum* has effect on both hematological and biochemical parameters of the normal rats. The extract had notable effect on the PCV and the T.WBC. There was a dose dependent increase in PCV and T.WBC with 100 mg/kg producing a percentage change of 18.54 and 45.15 % for PCV and T.WBC respectively while the 200 mg/kg produced a percentage change of 28.93 and 51.26 % in PCV and T.WBC respectively. These changes were significant when compared to the negative control but it shows that the plant has a hematinic-like and immune boasting effect. *C. micranthum* produced a non dose - dependent effect on the serum biochemical parameters of the normoglycemic rats after 21 days of treatment. The doses of the plant extract were able to reduce the important biochemical parameters such as cholesterol, urea and low density lipoprotein. The dose of 100 mg/kg was the most effective with a percentage change of 33.0, 48.0 and 29.0 % in cholesterol ($p<0.05$), low density lipoprotein ($p<0.02$) and urea ($p<0.01$) respectively. The 200 mg/kg body weight of extract produced 23.0, 32.0 and 21.0 % change in cholesterol, low density lipoprotein ($p<0.02$) and urea ($p<0.001$). These changes shows that apart from the antidiabetic property of the leaf extract of *C. micranthum,* it can also be taken for other health benefits. The effect of the methanol leaf extract on urea and creatinine (no increase) shows that the plant extract has no deleterious effect on the kidney (no nephrotoxic effect), hence it's safe to be taken for a long period of time.

Table 1: Results of Preliminary Phytochemical Analysis

Test	**Observation**
Alkaloids	**+**
Carbohydrates	**+**
Fats and oil	**+**
Flavonoids	**+**
Glycosides	**+**
Proteins	**+**
Reducing sugars	**+**
Resins	**+**
Saponins	**+**
Steroids	**+**
Tannins	**+**
Terpenoids	**+**

Key: **+** present, - absent.

Table 2: Effect of extract on fasting blood glucose concentration of alloxan-induced diabetic rats

Treatment group	Dose (mg / kg)	Blood Glucose Concentration (mg/dL)				
		0	½ h	1h	2h	4h
Extract	100	305.60±1.86	237.60±1.91* (22.25)	184.20±2.08*** (39.73)	140.80±1.39* (54.18)	99.80±0.86* (67.34)
	200	308.80±2.99	254.40±1.81**** (17.62)	201.20±1.07*** (34.84)	160.40±1.63* (48.06)	121.8±1.16** (60.56)
Glibenclamide	5	336.80±1.93	229.40±1.63*** (31.89)	115.00±1.84*** (65.86)	85.80±1.46** (74.76)	31.60±1.08*** (90.62)
3% tween 80 (5ml/kg)	_	404.20±1.91	407.00±1.05 (0.70)	403.20±1.72 (0.25)	398.60±1.12 (1.39)	403.00±2.00 (0.30)

Values are Mean±SEM, n=5. Values in parenthesis are percentage reduction. Level of significance was calculated against the negative control at corresponding time,* significant at p<0.05 , *** significant at p<0.01,**significant at p<0.02 ,**** significant at p<0.002, ***** significant at p<0.00

Table 3: Effect of extract on fasting blood glucose concentration (upper value) and percentage reduction in blood glucose concentration of normoglycemic rats.

Treatment group	Dose (mg/kg)	Blood glucose concentration (mg/dL)			
		Day 0	Day 7	Day 14	Day 21
Extract	100	125.2± 2.131	64.2±1.53***** (48.72)	50.4±0.93** (59.74)	43.80±1.068**** (65.02)
	200	112.8±3.813	71±0.32***** (37.06)	64.4±0.68***** (42.91)	58.8±1.24**** (47.87)
Glibenclamide	5	122.60±1.806	36±0.949*** (70.64)	27.2±1.463*** (78.07)	21±0.63***** (82.87)
3% tween 80 (5ml/kg)		109±2.581	100±0.63 (8.59)	101.4±1.97 (7.31)	100.4±0.75 (8.23)

Values are Mean ± SEM, n=5. Values in parenthesis are percentage reductions. Level 0f significance was calculated against the negative control at corresponding time.* significant at p<0.05, **** significant at p<0.01, **significant at p<0.02 , ***** significant at p<0.002, *** significant at p<0.0001

Table 4: Effect of methanol extract of C. micranthum on haematological parameters of normoglycemic rats prior to treatment; day 0 and after treatment; day 21

Treatment Group	Dose (mg/kg)	PCV (%)		T.WBC (µL)		Neutrophils (%)		Lymphocytes (%)	
		Day 0	Day21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Extract	100	41.00±1.55	48.60±4.04	4740.00±358.61	6880.00±342.64	56.00±1.38	52.00±4.57	43.40±1.66	47.40±4.96
	200	39.40±2.50	50.80±1.80	6320.00±566.92	9560.00±646.22	56.00±5.79	50.20±4.57	41.00±5.31	49.20±4.96
Glibenclamide	5	36.40±2.50	50.80±1.80	6320.00±566.92	9560.00±646.22	57.00±5.65	53.00±6.92	42.20±3.54	46.60±7.00
3% Tween 80 (5ml/kg)	-	43.00±1.11	47.00±1.14	5960.00±974.99	5280.00±1161.64	56.60±5.65	57.00±4.63	43.20±5.60	42.00±4.47

PCV- packed cell volume, T.WBC- total white blood cell Values are Mean±SEM, n=5. Level of significance ($p<0.05$) was calculated against the negative control at corresponding time.

Table 6: Effect of methanol extract of C. micranthum on biochemical parameter of normoglycemic rats prior to treatment (day 0) and after treatment (day 21)

Treatment Group	Dose (mg/kg)	Cholesterol (mg/dL)		Triglycerides (mg/dL)		HDL (mg/dL)		LDL (mg/dL)		Urea (mg/dL)		Creatinine (mg/dL)	
		Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Extract	100	181.20±20	121.40±6.40*	104.80±9.27	96.60±14.83*	51.20±7.42	70.60±2.60**	78.00±5.25	40.00±2.78**	66.20±3.07	47.00±4.15*	2.12±0.11	1.96±0.25*
	200	181.20±11.38	138.80±7.17**	93.60±21.38	92.80±14.41**	54.40±8.82	70.80±2.25*	75.40±7.99	51.20±3.38***	58.40±4.49	45.80±1.93****	1.88±0.12	1.68±0.11*
Glibenclamide	5	221.00±8.67	181.00±1.00	121.40±8.27	118.80±5.43	87.00±14.36	85.00±1.84	72.60±8.10	73.00±1.58	72.60±8.11	66.60±1.12	1.84±0.21	1.74±0.23
3% Tween 80 (5ml/kg)	-	185.80±24.36	183.00±1.14	85.80±17.79	85.00±2.56	65.40±10.74	66.00±2.28	76.60±7.99	79.00±1.50	70.00±3.42	69.00±0.32	2.14±0.18	2.04±0.06

PCV- packed cell volume, T.WBC- total white blood cell values are Mean ± SEM, n=5Level of significance was calculated against the negative control at corresponding time, * significant at $p<0.05$, **** significant at $p<0.01$, **significant at $p<0.02$, ***** significant at $p<0.002$, *** significant at $p<0.001$

Fig. 1: A photograph of Stachytarpheta jamaicensis.

Taxonomic Classification

Kingdom: Plantae

Division: Angiospermae

Sub division: Dicotyledon

Order: Verbenales

Family: Verbenaceae

Genus: Stachytarpheta

Species: S jamaicensis

Geographical source: Eastern Nigeria (Udenu L.G.A) Enugu State, Nigeria.

Common names: blue vervain, bastard vervain, blue snake weed, Brazilian tea, Aaron's rod, rat tail e.t.c.

Vernacular names (In Nigeria): tsarkiyar kuusuu (Hausa), Agogo igun (Yoruba), Aran umon (Ibibio) (Burkill, 1985).

Table 5: Percentage change in hematological parameters

Treatment group	Dose (mg/kg)	PCV	T.WBC	Neutrophils	Lymphocytes
Extract	100	18.54	45.15	7.14	9.22
	200	28.93	51.26	10.36	20
Glibenclamide	5	9.30	0.0	7.01	10.43
Distilled water (5ml/kg)		9.30	11.41	0.71	2.78

PCV- packed cell volume, T.WBC- total white blood cell, n=5

Table 7: Percentage change in serum biochemical parameters

Treatment group	Dose (mg/kg)	Cholesterol (%)	Triglycerides (%)	HDL (%)	LDL (%)	Urea (%)	Creatinine (%)
Extract	100	33.0	7.87	27.48	48.72	29.0	7.55
	200	23.40	0.85	30.15	32.09	21.58	10.64
Glibenclamide	5	18.10	2.14	2.29	0.55	8.26	5.43
Distilled water (5mg/kg)	-	1.51	0.932	0.92	3.13	1.43	4.67

HDL- high density lipoprotein, LDL-low density lipoprotein

CONCLUSION

From the results obtained, *C. micranthum* no doubt has a potent non dose dependent antidiabetic effect, in which the 100 mg/kg was found to be more potent than the 200 mg/kg. In addition to the antidiabetic effect, it was also established that the plant also has other effects such as hemanitic, immune boasting, lipid lowering effect and has no nephrotoxic effect. Work is ongoing on activity guided fractionation and isolation of the antidiabetic constituent(s).

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CONFLICT OF INTEREST

None

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