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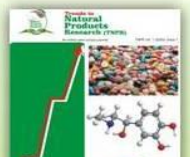

Ameliorative Activity of Ethanol Leaf Extract and Fractions of *Cleome ciliata Schum & Thonn,* in Streptozotocin Induced Diabetic Rats

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Abstract: The present study evaluated the antidiabetic and antilipidemic effects of *Cleome ciliata* leaves in streptozotocin (STZ)-induced diabetic rats. *Cleome ciliata* is a medicinal plant from the Cleomaceae family and is widely used in traditional medicine to treat various ailments. Crude ethanol extraction was done by cold maceration method. The crude ethanol extract was further fractionated using butanol, ethyl acetate and N-hexane. The extract and its fractions were screened for phytochemical constituents. Acute oral toxicity of the extract was done, to ascertain its safety. The crude ethanol leaf extract, n-hexane, ethyl acetate, butanol, and water fractions, were tested for anti-diabetic activity in STZ-induced diabetic rats. Lipid profile was also assessed. Histopathological examination of the pancreas was performed. Phytochemical result indicated the presence of steroids, tannins, saponins, alkaloids, flavonoids, terpenoids, phenols, anthraquinones, cardiac glycosides, reducing sugar, proteins and amino acids. In the oral acute toxicity test no death was recorded up to 5000 mg/kg. *C. ciliata* and fractions evoked a dose related and significant ($p < 0.001$) hypoglycemic activity. The levels of serum total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c) were reduced, and the high-density lipoprotein cholesterol (HDL-c) level was increased. A significant reduction in the serum glycated haemoglobin and a significant increase in serum insulin and liver glycogen were recorded. These results suggest that *C. ciliata* leaves contain hypoglycemic and hypolipidemic principles that could be explored in the treatment of Type 2 diabetes mellitus.

INTRODUCTION

Diabetes is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Hyperglycemia and hyperlipidemia are involved in the development microvascular and macro vascular complications of diabetes, which are the major causes of morbidity and mortality of diabetes (Akah *et al*., 2011). Diabetes has emerged as one of the most serious and common chronic diseases of our times, causing life threatening, disabling and costly complications, and reducing life expectancy (Heald *et al.,* 2019). The global prevalence of diabetes had reached pandemic proportions with the 9th edition of the International Diabetes Federation (IDF) reporting a prevalence of 9% (463 million adults) in 2019 (Heald *et al.,* 2019). The rising prevalence of diabetes has been attributed principally to the ageing of populations (Heald *et al.,* 2019). However, decreasing mortality among those with diabetes due to improving medical care can as well as increase in diabetes incidence in some countries resulting from increasing prevalence of diabetes risk factors, especially obesity, are also important drivers of higher prevalence (Chan *et al.,* 2019; Magliano *et al.,* 2019). Diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglycerides (WHO 2019). These abnormalities occur in many patients despite normal LDL cholesterol levels. These changes are also a feature of the insulin resistance syndrome (also known as the metabolic syndrome), which underlies many cases of type 2 diabetes. There is considerable evidence that hyperglycemia results in the generation of Reactive Oxygen Species (ROS), ultimately leading to increased stress in a variety of tissues (Tiwari et al., 2019).

Cleome ciliata is one of the common annual herbs with compound leaves with leaflets, mostly three, acute at their apices with prickly stems; the fruit is between 2.5 – 6 cm long and the flowers are white, lilac or pink in colour. *Cleome ciliata* is common in southern Nigeria, and grows erect to a height of 30 cm, falls flat on the ground where it continues to grow with flower. It has a slender leaf stalk with trifoliate leaves; the leaflets are net-veined, mostly three, acute at their apices; and elliptical with smooth margin (Asicumpon, 2005).

The plant is juicy, and useful in the management of earache, convulsions and peptic ulcers (Asicumpon, 2005). Leaves are anthelminthic and carminative and their sap is externally applied for chronic otitis media. The leaves of the plant possess antibacterial properties. Preliminary phytochemical investigation suggests the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenols, anthraquinones, glycosides and terpenoids (Okeke and Ezeabara 2018). This study was aimed to evaluate the antidiabetic and antilipidemic activities of ethanol leaf extract and fractions of *Cleome ciliata* in streptozotocin induced diabetic rats

MATERIALS AND METHODS

Animals

Albino rats of either sex were procured from the Department of Veterinary Medicine, University of Nigeria Nsukka. They were kept in the animal house facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu and were given access to water and pelletized vital grower feed *ad libitum*. Animals were handled in conformity with the National Institute of Health Guidelines for the care and use of laboratory animals for research purpose (Pub No. 85-23, revised 1985).

Plant material

Fresh leaves of *C. ciliata* were collected between 6:00 and 7:30 am in the month of February, 2019 in Irri community Isoko South L.G.A, Delta State, Nigeria. Plant sample was validated by expert plant taxonomist from the Department of Botany, Faculty of Life Sciences, Nnamdi Azikiwe University, Awka, Nigeria. A voucher specimen is deposited in the Herbarium of the Department with voucher number "NAU45679" was assigned to it.

Preparation of plant extract

Fresh leaves of *C. ciliata* were washed with tap water and air dried at room temperature for two weeks. Dried leaves were pulverized by using mechanical grinder and a total of 7.2 kg was extracted by cold-macerated by using 80 % ethanol for a period of 48 hours, with occasional agitation. Filtrate was recovered with the aid of a muslin cloth. The filtrate recovered was concentrated with an evaporator. Fractionation of the crude extract was done using ethyl acetate, n-hexane and butanol in their order of polarity.

Phytochemical screening

Phytochemical evaluation for presence of phytoconstituents was done by following the method described by Harborne (1973).

Acute toxicity test

This test was performed in two phases (Lorke 1983). Three doses of the extract (10, 100 and 1000 mg/kg) were administered to three groups of mice (n/gp=3) in the first phase. The animals were observed for 24 hours. In the second stage, four doses 2000, 3000, 4000 and 5000 mg/kg were administered to four groups of mice (n/gp=1). The animals were observed for 24 hours for mortality and adverse effects.

Induction of diabetes

This study was done by following the method described by Mourad *et a*l. (2017). Overnight fasted Wistar albino rats (150-155g) were induced diabetes by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 50 mg/kg. After the induction, they had free access to food and water. The animals were allowed to drink 5 % glucose solution overnight to overcome the hypoglycaemic shock for 18 h. The development of diabetes was confirmed after 72 h of the streptozotocin injection. The animals having fasting blood glucose levels more than 200 mg/dl were considered diabetic and used for the experiment.

Experimental design

Three days after induction of diabetes, animals were divided into 13 groups of 10 rats each. Non diabetic animals served as normal control. The animals were treated orally for 28 days as follows: Group 1 (Normal control) received 5 % Tween 80 (5 ml/kg), Group 2 (Diabetic control) received 5 % Tween 80 (5 ml/kg), Group 3 received metformin (100 mg/kg), Groups 4 and 5 received 250 and 500 mg/kg of ethanol crude extract, respectively, Groups 6 and 7 received 250 and 500 mg/kg of ethyl acetate fraction, respectively, Groups 8 and 9 received 250 and 500 mg/kg of n-hexane fraction, respectively, Groups 10 and 11 received butanol fraction 250 and 500 mg/kg respectively while Groups 12 and 13 received aqueous fraction 250 and 500 mg/kg respectively. Fasting blood glucose level was monitored once every week for the 28 days of treatment. On the 28th day, the animals were fasted for 12 h, anesthetized using diethyl ether and sacrificed.

Blood glucose determination

Fasting blood glucose (FBG) was determined before the start of the experiment. The initial and final body weights were measured. Blood glucose level was determined from the tail vein on days 0, 7, 14, 21 and 28th day using accu-check glucometer machine. On the day 28, blood was collected via retro orbital plex under mild ether anaesthesia from overnight fasted rats into a dry test tube and allowed to coagulate at ambient temperature for 30 m. Serum was centrifuged at 2000 rpm for 10 m; the clear supernatant was used for the analysis of various biochemical parameters. Liver tissues were excised, blotted, weighed and stored in formalin for histopathology study.

Determination of serum total cholesterol

Serum total cholesterol (TC) was evaluated using Randox commercial assay kits following the methods described by Ezeigbo (2010). One millilitre (1 ml) of the working cholesterol reagent was added into tubes labeled blank, standard and test groups. Ten microlitres of standard cholesterol reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula below

$$\text{Total cholesterol in sample } \left({}^{\text{mg}}/_{\text{dl}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Determination of serum triglyceride

Serum triglyceride was evaluated by using Randox commercial assay kits following the methods described by Tietz (2014). One millilitre (1 ml) of the working triglyceride reagent was added into tubes labeled blank, standard and test groups. Ten microlitres of standard triglyceride reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula below;

$$\text{Triglyceride } \left({}^{\text{mg}}/_{\text{dl}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Determination of serum high density Lipoprotein cholesterol (HDL-cholesterol)

Serum HDL-cholesterol was evaluated by using Randox commercial assay kits by following the methods developed by National Institute of Health Consensus Development Conference Statement

(1992). One hundred microlitres (100 ul) of samples and standard cholesterol reagent were dispensed into test tubes containing 250 ul of HDL cholesterol precipitate (R1). The mixture was centrifuged at 4000 rpm for 10 minutes. Thereafter, 100 ul of samples and standard supernatants were added to another set of test tubes labelled samples and standard containing cholesterol reagent. The mixture was incubated for 10 m at room temperature and absorbance of standard and samples were measured against reagent blank at 500 nm within 60 m using Spectrophotometer. HDL-cholesterol level in sample was calculated using the formula below;

$$\text{HDL} - \text{Cholesterol}\left(^{\text{mg}}/_{\text{dl}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x } \text{Concentration of Standard}$$

Determination of serum low density Lipoprotein cholesterol (LDL-cholesterol)

Low density lipoproteins (LDL) cholesterol in serum was calculated by using the equation described by Friedewald *et al*. (2017). The Friedewald's equation estimates the value of HDLC using the values of total cholesterol, triglyceride and HDL cholesterol.

Low density cholesterol (mg/dl) =
(total cholesterol (TC) minus high-density lipoprotein (HDL)-cholesterol minus triglycerides (TGs)/5 in mg/dl)

Very low-density lipoprotein cholesterol (VLDL-c) estimation was determined from:

VLDL = TG/5

Serum insulin, glycogen and glycated haemoglobin assay

Serum insulin level, liver glycogen and Glycated haemoglobin level were estimated were by the microplate method using Randox commercial kit as described by Carroll *et a*l. (1956).

Statistical analyses

Results obtained from this study were presented as mean ± Standard error of mean (SEM) of sample replicates (n=10). Raw data were analysed using one-way analyses of variance (ANOVA), followed by Dunnett's multiple comparisons test using Statistical Package for Social Science (SPSS, version 25). $p < 0.05$, $p < 0.01$, $p < 0.001$ was established to be statistically significant.

RESULTS

Phytochemical analysis

The results of the phytoconstituent of the extract and fractions revealed the presence of: steroids, tannins, saponins, alkaloids, flavonoids, terpenoids, phenols, anthraquinones, cardiac glycosides, reducing sugar, proteins and amino acids (Table 1).

Acute Toxicity

There were no deaths after 24 hours of administration of varying doses of the extract up to 5000 mg/kg. The LD_{50} was therefore above 5000 mg/kg.

Effect of ethanol extract and fractions of blood glucose level in diabetic rats

High blood glucose (>200 mg/dl) recorded 72-hour post administration of STZ was an indication of diabetic state. The STZ treated group, had significant ($p < 0.05$) increase in blood glucose at the 10^{th} hour. The extract treatment (250 and 500 mg/kg) as well as metformin (100 mg/kg) at the 10^{th} hour significantly ($p < 0.001$) reduced blood glucose compared with diabetic group. The highest reduction in blood glucose level occurred with ethyl acetate fraction (500 mg/kg) this was followed by ethanol crude extract (500 mg/kg). Water fraction (250 mg/kg) had the least reduction in blood glucose level (Table 2).

Effect of ethanol extract and fractions of on blood glucose level in diabetic rats after 28 days' treatment

Oral administration of ethanol crude extract and fractions at the dose (250 and 500 mg/kg) significantly ($p < 0.001$) reduced blood glucose level from day 14^{th} till day 28th. Ethyl acetate fraction (500 mg/kg) had the highest ($p < 0.001$) reduction in blood glucose level on the 28^{th} day. Ethanol crude extract at the same dose significantly ($p < 0.001$) reduced blood glucose level. The least reduction in blood glucose occurred with water fraction 250 mg/kg (Table 3).

Effect of ethanol crude extract and fractions on serum lipid profile

Administration of the extract and fractions caused significant ($p < 0.001$) reduction in serum cholesterol, TG, LDL, VLDL and significantly ($p < 0.001$) increased HDL with ethyl acetate fraction having the best activity. N-hexane fraction had the least activity (Table 4).

Effect of ethanol extracts and fractions *on* serum insulin level, liver glycogen and glycated hemoglobin

The STZ diabetic induced group had significant ($p < 0.001$) decrease in the serum insulin, liver glycogen and increase in glycated hemoglobin. Administration of the extract and fractions for 28 days significantly ($p < 0.001$) increased the serum insulin, liver glycogen level and significantly reduced glycated hemoglobin when compared to control group. Ethyl acetate fraction had the best activity followed by ethanol crude extract while water fraction gave the least activity (Table 5).

Histological studies

The results showed a rejuvenation in histoarchitectural structure of the pancreas of extract and fractions treated groups. Normal control pancreas reveals acinar pattern structure with pyknotic nuclei of some acinar cells (Figure 1). The acinar cells which stained strongly are arranged in lobules with prominent nuclei.

The islet cells are seen embedded within the acinar cells and surrounded by a fine capsule (Plate A). The pancreas of the untreated diabetic rats (Plate B) showed some acinar cells with islet-cells with congested pyknotic nuclei with visible lymphocytic infiltrates. Histoarchitecture was deeply affected. Metformin treatment, reavealed secreterory acini with bulky pancreatic islet. The nucleus appears pyknotic (Plate C). Treatment with the extract and fractions (Plates D-M**)** restored the histoarchitecture of the pancreas considerably.

Table 1: Phytochemical constituents of ethanol extract and fractions of *C. ciliata* leaf

Phytochemicals	Ethanol Crude extract	N-hexane fraction	Ethyl Acetate fraction	Butanol fraction	Water fraction
Flavonoids Shinoda test Alkaline reagent test	+++	++	+++	+	+
Alkaloids Wagner's test	++	+	++	++	+
Steroids Liebermann-Burchard test	+++	+++	++	++	-
Phenols Ferric chloride test Lead acetate test	+++	+++	+++	++	+++
Terpenoids Salkowski test	++	+++	++	+	++
Anthroquinone Borntrager s test	+	+	++	++	+
Saponin Frothing test	+++	+++	+++	+++	+++
Tannins Gelatin test	+++	+++	+++	++	+
Carbohydrates Iodine test	+	+	++	+	+
Proteins & Amino acids Ninhydrin test Millon's test	+	++	++	+	+
Reducing sugar	++	++	++	+	++
Resins	++	+	+	+	+
Cardiac Glycosides Keller- Killani test Liebermann's test	+++	+++	+++	++	+++

(-) => Not Present, (+) => Faintly Present, (++) => moderately present, (+++) => Highly present

Table 2: Effect of ethanol extract and fractions of blood glucose level in diabetic rats

Group	Treatment	Blood glucose level (mg/dL)					
		0 hr.	**2 hrs.**	**4 hrs.**	**6hrs.**	**8hrs.**	**10hrs.**
1	Normal Control	84.21±0.62	83.22±0.19 (1.18%)	80.46±1.03 (4.45%)	82.45±0.54 (2.09%)	85.55±0.39 (0.78%)	81.37±0.15 (3.37)
2	DC (STZ 50mg/kg)	266.51±0.59#	269.63±1.36# (-1.17%)	293.55±2.12# (-10.15%)	299.64±0.89# (-12.43%)	303.89±0.87# (-14.03%)	312.18±0.87# (-17.14)
3	Met. 100mg/kg	278.98±0.54	245.37±0.21 (12.03%)	187.54±0.32 (21.70%)	109.43±0.42*** (60.74%)	92.64±0.66** (66.80%)	86.24±0.38** (69.09%)
4	ECE 250mg/kg	278.32±0.76	267.34±0.78 (3.95%)	245.44±0.21 (11.81%)	135.58±0.36*** (51.19%)	120.34±0.54** (56.76%)	109.54±0.56** (60.64%)
5	ECE 500mg/kg	289.20±0.17	271.27±1.29 (6.20%)	167.56±0.81* (42.06%)	121.14±1.34** (58.11%)	114.19±0.63** (60.51%)	98.33±0.58** (65.99%)
6	NHF 250mg/kg	276.55±0.42	255.31±0.18 (7.68%)	235.57±0.72 (14.81%)	146.36±0.63* (47.08)	123.15±0.31** (55.47%)	119.48±1.11** (56.79%)
7	NHF 500mg/kg	286.43±1.44	265.32±0.23 (7.37%)	224.27±0.40 (21.70%)	137.56±0.84** (51.97%)	119.67±0.61** (58.22%)	111.54±0.32** (61.06)
8	EAF 250mg/kg	280.81±0.54	256.78±0.36 (8.56%)	199.53±0.48 (28.94%)	123.54±0.40** (56.01%)	111.77±0.34** (60.19%)	91.16±0.60** (67.54)
9	EAF 500*mg/kg	289.97±0.19	241.42±0.34 (16.74%)	159.89±1.38* (44.86%)	113.65±0.89** (60.81%)	90.12±1.69** (68.92%)	83.68±0.46*** (71.14%)
10	BTF 250mg/kg	274.89±1.67	268.56±0.16 (2.34%)	243.76±0.44 (11.32%)	198.78±0.24 (27.69%)	135.35±0.12* (50.76%)	125.54±0.64** (54.33%)
11	BTF 500mg/kg	273.99±0.75	260.32±0.33 (4.98%)	240.86±0.17 (12.09%)	163.23±0.56* (40.42%)	126.54±0.59* (53.82%)	117.25±0.11** (57.23%)
12	WF 250mg/kg	269.89±0.64	258.88±0.38 (4.08%)	245.44±0.60 (9.06%)	198.49±0.45 (26.46%)	155.12±0.77* (42.52%)	130.64±0.87** (51.59%)
13	WF 500mg/kg	278.34±0.31	254.17±0.21 (8.68%)	235.33±0.11 (15.45%)	157.44±1.05* (43.43%)	136.66±0.47* (50.90%)	120.68±0.25** (56.64%)

#p < 0.001 with diabetic control from normal control. *p < 0.05; **p < 0.01; ***p < 0.001. compared with respective diabetic control. n=10 DC =Diabetic control, Met= Metformin, ECE= Ethanol crude extract, NHF= N-hexane fraction, EAF= Ethyl acetate fraction, BTF= butanol fraction and WF= water fraction.

Table 3: Effect of ethanol extracts and fractions on blood glucose level in STZ induced diabetic rats after 28 days treatment.

Group	Treatment	Blood glucose level (mg/dL)				
		0 day	**7th day**	**14th day**	**21st day**	**28th day**
1	Normal Control	86.28±0.72	82.65±0.87 (5.32%)	83.83±0.78 (2.84%)	81.93±0.76 (5.04%)	84.51±1.17 (2.05%)
2	DC (STZ 50mg/kg)	354.92±0.41#	391.22±0.15# (-10.23%)	429.82±0.34# (-21.10%)	487.15±0.89# (-21.10%)	512.66±0.87# (-44.44)
3	Met. 100mg/kg	327.35±0.39	313.16±0.49* (26.72%)	211.21±0.67** (50.58%)	115.19±0.18*** (73.05%)	90.31±0.47*** (78.87%)
4	ECE 250mg/kg	449.85±0.66	316.14±0.89* (29.72%)	210.16±0.56** (53.28%)	141.11±0.51*** (68.63%)	102.46±0.37*** (77.22%)
5	ECE 500mg/kg	456.17±0.49	309.33±0.47* (32.19%)	187.49±0.81** (58.99%)	115.32±0.41*** (74.72%)	94.53±1.39*** (79.28%)
6	NHF 250mg/kg	458.37±0.79	321.31±1.44* (29.90%)	222.62±0.17** (51.43%)	153.54±0.74*** (66.50%)	107.12±0.83***(7
7	NHF 500mg/kg	456.99±0.50	312.47±1.42* (31.62%)	190.51±0.77** (58.31%)	119.65±0.10*** (73.82%)	96.65±1.24*** (78.85%)
8	EAF 250mg/kg	487.43±0.47	299.58±0.62* (38.54%)	136.77±0.68** (71.94%)	120.74±0.89*** (75.23%)	92.16±0.87*** (81.09%)
9	EAF 500mg/kg	562.89±0.47	367.49±0.62* (34.71%)	208.21±0.38** (63.01%)	109.11±0.22*** (80.62%)	80.10±0.87*** (85.77%)
10	BTF 250mg/kg	398.89±0.20	267.45±1.22* (32.95%)	235.65±0.89* (40.92%)	143.13±0.53*** (64.12%)	110.37±0.27*** (72.33%)
11	BTF 500mg/kg	399.65±0.88	271.24±0.77* (32.13%)	231.47±0.75* (42.08%)	121.33±1.42*** (69.64%)	102.43±0.28*** (74.37)
12	WF 250mg/kg	394.97±1.42	245.56±0.89* (37.83%)	200.31±1.47** (49.28%)	126.82±0.78*** (49.28%)	115.32±0.43*** (70.80%)
13	WF 500mg/kg	397.90±0.70	269.90±0.63* (32.17%)	205.54±0.53** (48.34%)	131.32±0.69*** (66.99%)	108.15±0.75*** (66.99%)

#p < 0.001 with diabetic control from normal control. *p < 0.05; **p < 0.01; ***p < 0.001. compared with respective diabetic control. n=10

DC =Diabetic control, Metf= Metformin, ECE= Ethanol crude extract, NHF= N-hexane fraction, EAF= Ethyl acetate fraction, BTF= butanol fraction and WF= water fraction.

Table 4: Effect of ethanol extracts and fractions on serum lipid profile in STZ induced diabetic rats**.**

Group	Treatment	Changes in mg/dL level				
		TC	**TG**	**HDL**	**LDL**	**VLDL**
1	Normal Control	75.52±1.96	83.00±0.47	95.83±0.78	24.63±0.87	17.47±0.23
2	DC (STZ 50mg/kg)	183.33±0.41#	199.44±0.15#	39.22±0.34#	96.32±1.08#	62.65±0.31#
3	Met.100mg/kg	76.48±0.47	85.12±0.49*	92.67±0.88**	24.31±0.71***	18.43±0.13***
4	ECE 250mg/kg	90.53±0.39	96.59±0.16*	80.67±1.87**	31.78±0.84***	24.75±0.45***
5	ECE 500mg/kg	86.67±1.52	92.90±0.89*	87.88±0.47**	26.70±0.86***	20.77±0.67***
6	NHF 250mg/kg	99.32±0.11	112.65±0.39*	65.56±0.33**	35.76±1.06***	36.99±0.83***
7	NHF 500mg/kg	88.99±0.20	99.87±0.65*	69.32±0.12**	39.16±0.87***	29.39±0.82***
8	EAF 250mg/kg	80.64±0.41	87.56±0.49*	86.90±1.37**	29.45±0.64***	18.92±0.39***
9	EAF 500mg/kg	76.44±0.47	80.77±0.41*	94.59±0.31**	24.54±0.78***	18.10±0.68***
10	BTF 250mg/kg	91.54±0.78	98.79±0.76*	78.65±0.76*	33.87±1.59***	28.69±0.59***
11	BTF 500mg/kg	88.65±0.31	93.11±0.87*	83.87±0.75*	29.45±0.80***	24.58±0.58***
12	WF 250mg/kg	93.76±0.65	98.99±0.23*	72.55±0.55**	33.98±0.78***	30.19±1.08***
13	WF 500mg/kg	88.79±1.44	103.23±0.69*	83.89±0.53**	31.44±0.54***	27.87±0.99***

#p < 0.001: with diabetic control from normal control. *p < 0.05; **p < 0.01; ***p < 0.001. compared with respective diabetic control. n=10
DC =Diabetic control, Met= Metformin, ECE= Ethanol crude extract, NHF= N-hexane fraction, EAF= Ethyl acetate fraction, BTF= butanol fraction and WF= water fraction.

Table 5: Effect of ethanol extracts and fractions on serum insulin level, liver glycogen and glycated haemoglobin levels in STZ induced diabetic rats

Group	**Treatment**	**Serum insulin(µU/ml)**	**Glycated haemoglobin (%)**	**Liver glycogen(mg/gm)**
1	Normal Control	129.89±0.86	2.65±0.76	14.69±0.80
2	DC (STZ 50mg/kg)	57.54±0.78#	7.82±0.79#	5.78±0.47#
3	Met. 100mg/kg	126.76±0.54***	2.87±0.39***	13.89±0.45***
4	ECE 250mg/kg	120.56±1.08***	4.30±0.65***	12.66±0.69**
5	ECE 500mg/kg	125.60±1.54***	2.88±0.55***	14.13±0.55***
6	NHF 250mg/kg	120 .11±0.41***	4.46±0.87**	11.88±0.69**
7	NHF 500mg/kg	124.49±0.35***	3.44±0.17**	13.69±0.38***
8	EAF 250mg/kg	123.55±0.87***	3.04±0.90***	12.96±0.88**
9	EAF 500mg/kg	129.74.00±1.38***	2.59±0.62***	14.70±0.26***
10	BTF 250mg/kg	99 .89±0.54**	4.79±0.47***	10.35±0.76*
11	BTF 500mg/kg	118.61±0.46**	3.51±1.44**	13.55±0.55***
12	WF 250mg/kg	89.89±0.42**	4.86±0.76**	8.84±0.59
13	WF 500mg/kg	105.58±1.78**	3.97±0.67**	10.12±0.90

#$p < 0.001$ diabetic control from normal control. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. compared with respective diabetic control. n=10 DC =Diabetic control, Metf= Metformin, ECE= Ethanol crude extract, NHF= N-hexane fraction, EAF= Ethyl acetate fraction, BTF= butanol fraction and WF= water fraction.

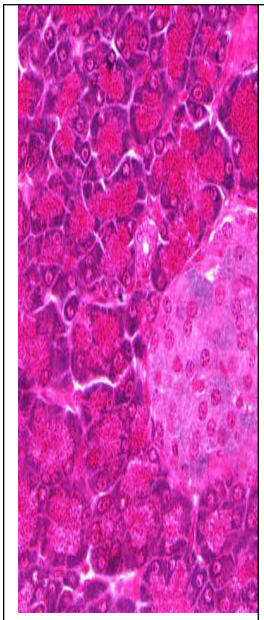	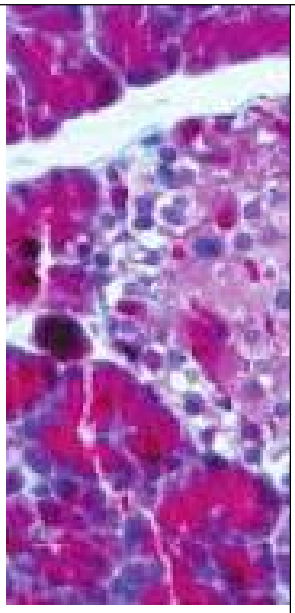	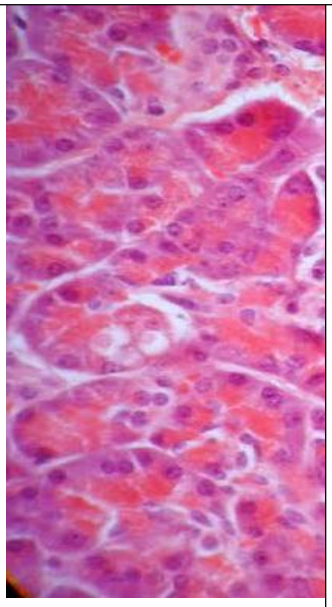	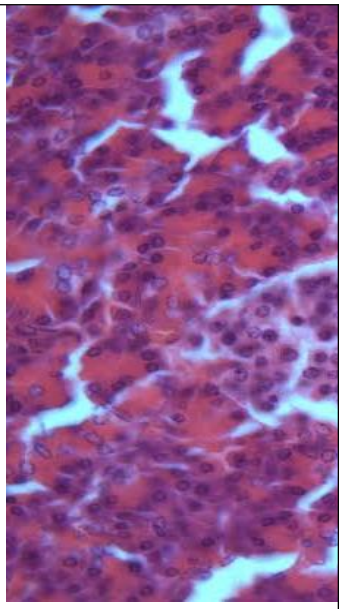
Plate A: Normal control pancreas	Plate B: Diabetic rat not treated	Plate C: 100mg/kg Metformin	Plate D: Ethanol extract 250 mg/kg
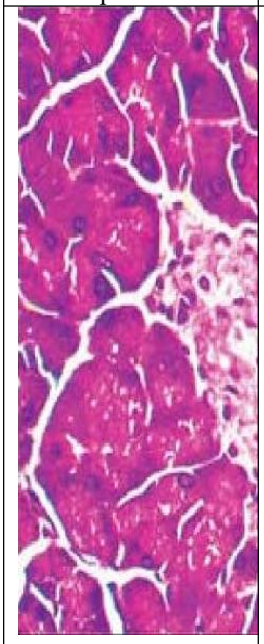	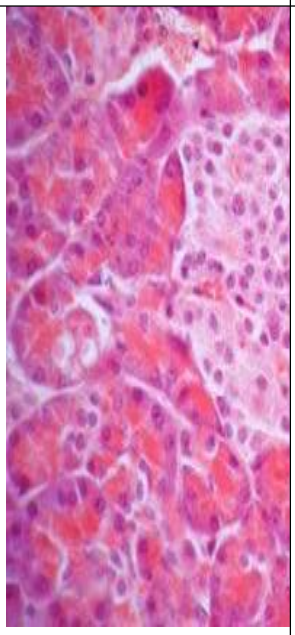	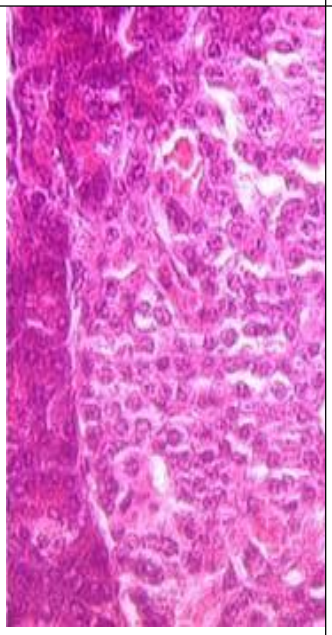	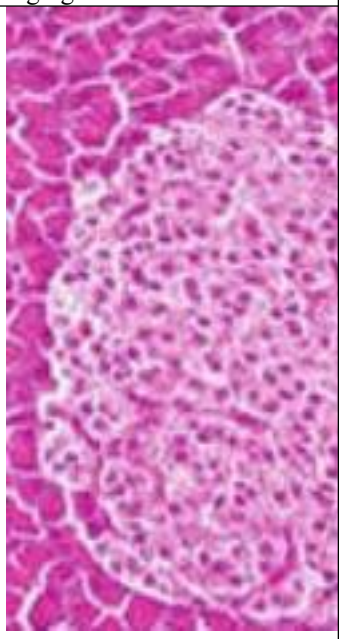
Plate E: Ethanol extract 500 mg/kg	Plate F: N-hexane fraction 250 mg/kg	Plate G: N-hexane fraction 500 mg/kg	Plate H: Ethylacetate 250 mg/kg

Figure 1a: Photomicrographs of histological Pancreas of STZ induced diabetic and non-diabetic rats after 28 days treatment. Staining H.E. 400X

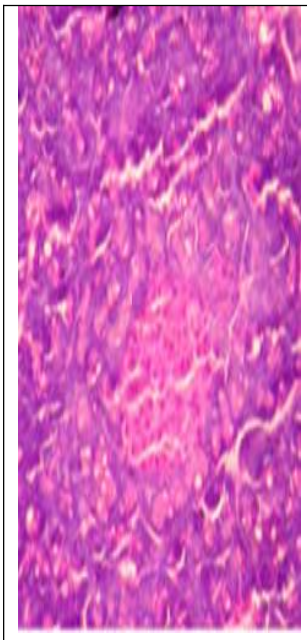	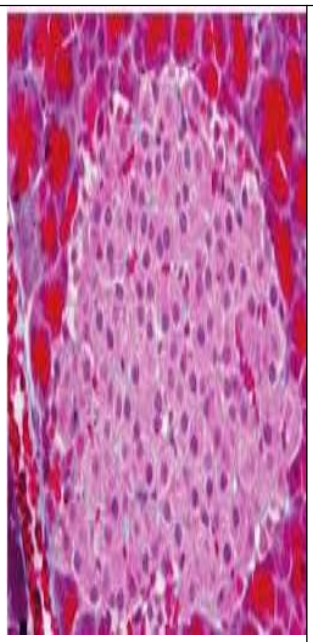	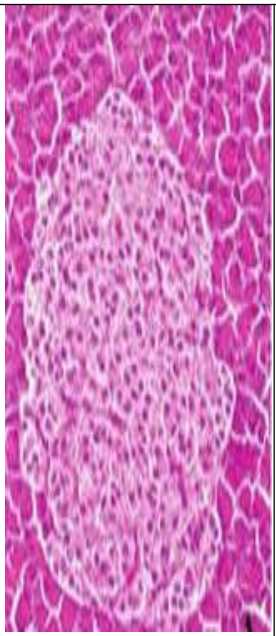	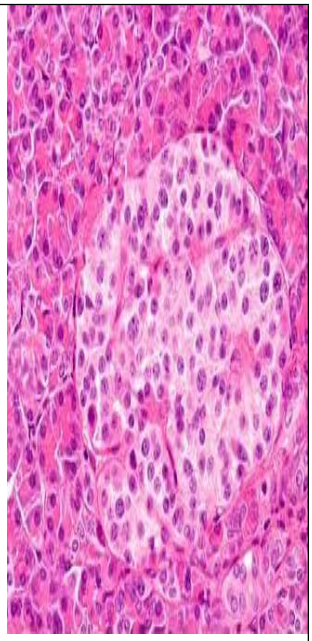
Plate I: ethyl acetate fraction 500mg/kg	Plate J: Butanol fraction 250 mg/kg	Plate K: Butanol fraction 500 mg/kg	Plate L: water fraction 250 mg/kg
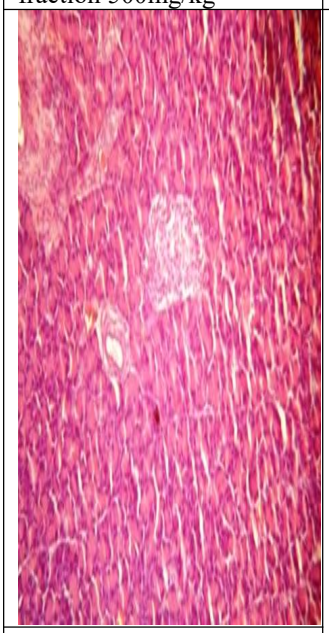			
Plate M: Water fraction 500 mg/kg			

Figure 1b: Photomicrographs of histological Pancreas of STZ induced diabetic and non-diabetic rats after 28 days treatment. Staining H.E. 400X

DISCUSSION

Diabetes is characterized by high concentrations of blood sugar, which can cause serious complications, such as organ failures and/or complications in the kidneys, eyes, and cardiovascular system (Akah *et al.,* 2011). Therefore, the treatment methods mainly focus on reducing fluctuations in blood sugar levels and their related complications (Ellappan *et al.,* 2013).

The results of this study revealed that the crude ethanol extract and fractions of *C. ciliata* reduced elevated blood sugar level in rats. The extract and fractions contain biologically active metabolites that were reported to have antihyperglycemic effect (Fawzy *et al.,* 2018, Akah *et al.,* 2011). Several studies have reported that flavonoids, alkaloids and phenolic compounds possess bioactive antidiabetic moieties (Li *et al.,* 2014, Fawzy *et al.,* 2018, Oliver-Bever, 2018). In fact, studies have also showed that alkaloids could reduce plasma glucose concentration through the stimulation of pancreatic β- cells of Langerhans to produce insulin, preventing the absorption of glucose from the intestine or enhancing the glucose uptake in peripheral tissues (Babu *et al.,* 2007, Gayathri *et al.,* 2009).

Hyperlipidemia is one of the common complications of diabetes which is characterized by increase in serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low-density lipoprotein (VLDL) and decrease in high density lipoproteins (HDL) (Zhang *et al.,* 2017). The most characteristic lipid abnormality in diabetics is hypertriglyceridaemia, with or without associated increase in plasma cholesterol. Another serious abnormality is azotemia, a condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy (Zhang *et al.,* 2017).

Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to the underutilization of glucose (Krishnakumar *et al*., 2018). The regression of the diabetic state by the extract and fractions may have increased the utilization of glucose, thereby depressing the mobilization of fat. TG, LDL, and HDL are associated with lipolysis, transport of plasma cholesterol, and atherosclerotic tendency, respectively (Mironova *et al.,* 2019). The increase in serum levels of TC, TG, HDL, LDL and the decrease in the serum HDL level of the e STZ-diabetic rats suggest that the animals may be predisposed to dyslipidaemia and other traits of metabolic syndrome. (Ezeja *et al.,* 2014). Thus, the reversal of the hyperlipidaemic traits by the plant extract is an indication of the ameliorative effects of the diabetic complication. This result is in agreement with that of Daisy et al. (2019) who reported that increasing insulin secretion after administration of *G. sylvestre* extract led to a decrease in cholesterogenesis and

fatty acid synthesis. Similar results have been reported by other workers (Shigematsu *et al.*, 2017, Mall *et al.*, 2019, Wang *et al.,* 2019). Furthermore, Luo *et al*. (2017) stated that the ability of *G. sylvestre* water extract to prevent obesity is by improving the cholesterol metabolism and inhibiting polyphagia.

Normalization of serum insulin, liver glycogen and glycated haemoglobin level is a good determinant of diabetic control. HbA1c is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus (Ezeja*et al.,* 2014). Proglycation is a non-enzymatic reaction between excess glucose present in the blood and free ammonia groups on the globin component of haemoglobin (Ezeja *et al.,* 2014). Measurement of HbA1c, Serum insulin, and liver glycogen level provides information of long-term glycaemic status and correlates with various complications related to diabetes mellitus (Wang *et al.,* 2019).

CONCLUSIONS

This study has provided evidence that *Cleome ciliata* leaves contain bioactive compounds with anti-hyperglycemic and antilipidemic activities, and could be explored for the treatment of type 2 diabetes.

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