Comparative Analysis of the Hypoglycaemic and Hypolipidemic Effects of Aqueous Extract of some Ethno medicinal Plants in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Diabetes mellitus is a complex metabolic disorder associated with the development of metabolic complications. This research evaluated the hypoglycemic and hypolipidaemic effects of *Moringa oleifera* (MO), *Treculia africana* (TA) and *Albizzia chevalieri* (AC) plant extracts on diabetes. Albino rats were randomly divided into six (six) main groups; MC, MO, TA, AC, Normal Control (NC) and Diabetic Control (DC) groups. Group MO, TA and AC were further subdivide into three sub groups. Diabetes mellitus was induced by a single dose intraperitoneal injection of alloxan 150 mg/kg body weight. Fasting blood glucose level and lipid profile were assayed using standard methods. Intraperitoneal injection of 150 mg/kg of Alloxan in the albino rats resulted in significant ($p<0.05$) elevation of serum glucose, total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL-C), and low density lipoprotein (LDL-C). Also, there was significant decrease ($p<0.05$) in HDL-C and body weight of the albino rats compared with that of the NC group. Oral administration of MO, TA and AC to diabetic albino rats for 21 days significantly ($p<0.05$) reduced fasting blood glucose level, normalized lipid profile and restore body weight of the albino rats in treated groups

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compared to diabetic control groups. All the plant extracts studied in this research significantly (p<0.05) increase the regeneration of damaged pancreatic β cells. Treatment with MO (800 mg/kg) confirmed highly significant (p<0.05) effect compared to TA and AC.

Keywords: Hypoglycaemia; hypolipidemia; ethnomedicinal plants.

1. INTRODUCTION

Diabetes is a metabolic disease that occurs either when the beta cell of the pancrease does not produce enough insulin (Type I Diabetes) or when the body cannot effectively utilize the insulin it produces (Type II diabetes) (Ali, 1993). Its characterized by persistent elevation of fasting blood glucose level (FBGL) above 200 mg/dl. This is due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Ortiz et al., 2015). In general, the normal range of glucose for most people (fasting adults) is 80 to 110 mg/dl or 4 to 6 mmol/l (where 80 mg/dl is "optimal".) An individual with a consistent range above 126 mg/dl or 7 mmol/l is said to have hyperglycemia, whereas a consistent range below 70 mg/dl or 4 mmol/l is considered hypoglycemic. In fasting adults, blood plasma glucose should not exceed 126 mg/dL. An individual is diagnosed as diabetic when his blood glucose level is chronically ≥126 mg/dL after an overnight fast and ≥200 mg/dL 2 hrs after an oral glucose load of 75 g [1]. In 2014, approximately 8.5% of adult aged 18 years and above are currently suffering from diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths (WHO, 2021). Uncontrolled diabetes leads to a several of complications affecting the vascular system, eyes, nerves and kidneys leading to peripheral vascular disease, nephropathy, neuropathy, retinopathy, morbidity, and/or mortality.

Plants can provide biologically active molecules which leads to the development of structures of modified derivatives with enhanced activity and reduced toxicity. The World Health Organization (WHO) has listed 21,000 medicinal plants used around the world among which, 2,500 species are in India. Plants that contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., are frequently implicated as having antidiabetic effect (Malviya and Malviya 2010). Pharmacological and clinical trials of medicinal plants have shown anti-diabetic effects and repair of *β*-cells of islets of Langerhans (Noor et al., 2008).

Moringa oleifera Lam (Moringaceae) is a highly valued plant distributed in many countries of the tropics and subtropics. *M. oleifera* is a multipurpose tree used as vegetables, spice, cosmetic oil and medicinal plant [2,3]. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, antitumor and antipyretic properties. The aqueous and alcoholic extracts from Moringa flowers have been found to have significant hepatoprotective effect [4] which is attributed to the presence of quercetin [5].

The plant Albizia chevalieri is known in Hausa as Katsari. It is a tree or shrub of 5-12meter tall, often branching low down, trunk up to 30 cm diameter, rounded crown and open. The bark is corky, pale gray, scaly deeply cracked rectangular and thick enough, revealing brown areas when they stand (Alhassane, 2013). The qualitative phytochemical investigation of methanol leaf and bark extracts of Albizia chevalieri revealed the presence of saponins, triterpenes, flavonoids, tannins, and alkaloids (Aliyu et al., 2009).

Treculia Africana (African bread fruit) belongs to the mono specific genus Treculia decen [6]. Analysis of the hexane extract of Treculia Africa seeds indicate that it contains a stearine solid fat fraction, resembling that of palm-kernel oil (Akubor and Badifu, 2004).

2. METHODOLOGY

2.1 Collection and Identification of Plant Materials

The fresh *M. Oleifera* (horseradish tree), *Albizzia chevalieri* leaves and *Trecullia africana* seeds were obtained from their natural habitat in Mal. Sani Nasiru Farm, Yankaba village, Kaura Namoda local government, Silami of Sokoto State and Umuidi community of Anambara State, Nigeria respectively. The samples were identified and authenticated by the Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda, Zamfara State Nigeria using a

standard procedure and the voucher number were deposited.

2.2 Preparation of Samples

The samples were thoroughly washed to remove dust and the drained parts were air dried. The samples were pounded using pestle and wooden mortar until powder was obtained. 500 g of each powdered sample was soaked in 2.5 L of distilled water and agitated intermittently for 24 hours using platform shaker. The solution was then filtered with filter paper (Number 1) to obtain the aqueous extract. It was then allowed to dry in an oven at 100 °C to obtain the crude extract [7]. The extracts were stored in an air tight container for further work. The required doses of 200 mg, 400 mg and 800 mg/kg body weights were obtained by reconstituting the stored extract using distilled water [8].

2.3 Experimental Animals

Thirty-six (36) male albino rats weighing between 100 – 200 g were used for this study. The rats were kept at animals' house under normal environmental conditions and maintained with free access to pelletized growers feed, and access to water *ad libitum.* The albino rats were allowed to acclimatize for 14 days. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal Welfare Act, 1999).

2.4 Experimental Design

By the end of the 14 days' acclimatization period, the animals were randomly assigned into six main groups of three rats each. They were labelled as Normal Control (NC), Diabetic Control (DC) Metformin Treated (MC), *M. Oleifera* (MO), *Trecullia Africana* (TA) and *Albizzia chevalieri* (AC). The NC group received water and feed only and serve as Normal control (NC). Diabetes was induced in all the other groups. The DC group was not treated and it serve as Diabetic control. The MO, TA and AC group were further subdivide into three subgroups with three albino rats in each group. They were designated as MO_1, MO_2, MO_3, TA_1, TA_2, TA_3, and AC_1, AC_2, AC_3 and 200, 400 and 800 mg/kg doses of MO, TA and AC were administered respectively. Group MO received *M. Oleifera* extract, TA group received *T. Africana* extract while AC group were treated with *A. chevalieri* extract. The extracts

were orally administered to induced diabetic albino rats once daily for a period of 21 days.

2.5 Induction of Diabetes

All rats, except the Normal Control Group were intraperitoneally injected with 150 mg/kg body weight of the prepared alloxan. After seventy-two hours of alloxan administration, the albino rats were fasted overnight and diabetes was confirmed from the rats by measuring their fasting blood glucose level with the aid of a single touch glucometer. Rats that have fasting blood glucose level >7.0 mmol/l (126 mg/dl) were considered diabetic and included in the study [9].

2.6 Collection of Blood Sample

After 3 weeks of treatment with the different extracts, the albino rats were fasted overnight. The rats where anesthetized by placing them in a seal cotton wool soaked in diethyl ether inhalation jar. The albino rats were sacrificed by decapitation (at the end of 3 weeks of treatment) and blood samples were obtained and centrifuged at 4000 ×g for 10 min at 4 °C. The supernatant was kept at 37 ^{0}C for further biochemical measurements. Fasting blood sugar and Lipid profile were then estimated.

2.7 Determination of Biochemical Parameters

2.7.1 Estimation of serum glucose level

Serum glucose was estimated by glucose oxidase/ peroxidase method using the Randox kit [10].

2.7.2 Procedure

Test tubes were set up in triplicates and labelled as blank, test and standard. 10µl of serum standard (5. 5 mmol/L) and10µl of distilled water were respectively pipetted into the test tubes. Each test tube was then followed by 1000 µl of glucose reagent. The tubes were mixed properly, incubated at 37 ^{o}C for 10 minutes and the absorbance of standard and tests read against the blank at 500nm using spectrophotometer.

Calculation: The glucose concentration was calculated using the relation:

$$Serum\ glucose\ (mmol/L) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times conc\ of\ standard$$

2.7.3 Estimation of serum total cholesterol

Serum total cholesterol (TC) was estimated by enzymatic method using Randox kit [11].

Three test tubes were set up and labelled as blank, test and standard. Into the labelled test tubes, 10 µl of serum standard (200 mg/dl) and 10µl of distilled water were respectively pipetted into the test tubes. 1000 µl of the total cholesterol reagent was added to each of the test tube. The content of the tubes was agitated to ensure proper mixing and incubated at 37°C for 5 minutes. The absorbance of the standard and test were read against the blank at 500 nm.

Calculation: Cholesterol concentration was obtained using the relation:

Serum total cholesterol (mg/dl) =
$$\frac{Absorbance\,of\,Test}{Absorbance\,of\;Standard} \times \text{Conc of}$$
Standard

2.7.4 Estimation of serum HDL - C

This was done by enzymatic method [12] using Randox Kit. Into centrifuge tubes, 200 µl of serum and 500 µl of precipitant (0.55 mmol/L phosphotungastic acid and 25 mmol/l Magnesium Chloride) were added, mixed and allowed to stand for 10 minutes at room temperature. The tubes were centrifuged for 10 minutes at 4000 rpm. The supernatant was collected and used for the analysis. Three test tubes were then set up and labelled blank, standard and test. The tubes were agitated and incubated for 5 minutes at 37^{0}C. The absorbance of the samples and standard were measured against the reagent blank at 500 nm.

Calculation: The HDL-C concentration was obtained from the relation:

Serum HDL-C (mg/dl)   =
$$\frac{Absorbance\,of\,Test}{Absorbance\,of\;Standard} \times \text{Conc. of}$$
Standard

2.7.5 Estimation of serum triglyceride

This was assayed using enzymatic method as described by Tietz [13] using Randox Kit. The tubes were agitated and incubated at 37°C

for 5 minutes and the absorbance of the standard and tests were read at 500 nm against the blank.

Calculation: The TG levels were calculated using the relation:

Serum TG (mg/dl) =
$$\frac{Absorbance\,of\,Test}{Absorbance\,of\;Standard} \times \text{Conc. of}$$
Standard

2.7.6 Estimation of serum LDL – C

This was estimated using Friedewald formula [14].

LDL - C (mg/dl) =   TC – (HDL - C) + (
$$\frac{TG}{5})$$

2.7.7 Estimation of serum VLDL – C

This was estimated using Friedewald formula [14].

LDL - C (mg/dl) = $\dfrac{TG}{5}$

3. RESULTS AND DISCUSSION

3.1 Effect of Administration of Different Concentration of the Medicinal Plants on Body Weight Changes

The result indicated that intraperitoneal injection of alloxan into albino rats resulted in significant (p<0.05) decrease in body weight of the albino rats (95.00±9.78) compared with that of the normal control (173.25±12.57). The results of the repeated treatment with different ethno medicinal plants (MO, TA and AC) and Metformin for three weeks resulted in significant (p<0.05) increase in body weight of all the albino rats in the treated groups compared to DC group. MO$_3$ in a dose dependent manner demonstrated the most significant effect of restoring body weight (144.50±9.45) of alloxan induced diabetic rats followed by TA (134.75±12.24), while AC (120.25±19.40) has the lowest significant (p<0.05) effect compared to MO and TA respectively.

Table 1. Effect of administration of different concentration of the medicinal plants on body weight changes in rats

GRP	Body Weight Before Alloxan Induction (g)	Body Weight After Alloxan Induction (g)			
		Initial day	7th Day	14th Day	21st Day
NC	146.25±14.03	149.50±13.58	163.00±12.81	170.00±13.45	173.25±12.57d
DC	120.00±9.59	119.00±10.68	107.00±11.15	105.75±10.81	95.00±9.78c
MC	150.25±15.01	135.00±14.67	137.50±15.13	141.00±15.07	143.25±14.13a
MO1	140.75±15.56	123.75±15.04	127.50±15.76	130.25±15.51	135.25±15.81e
MO2	141.25±11.43	125.75±12.15	130.00±11.48	131.25±10.31	135.75±11.71f
MO3	142.25±9.72	128.00±11.26	136.25±9.97	142.75±9.62	144.50±9.45g
TA1	136.75±15.47	125.75±12.65	128.75±13.11	131.50±12.97	135.50±13.61h
TA2	140.00±18.05	130.50±17.86	133.00±18.08	135.75±17.65	137.50±16.94i
TA3	143.25±12.25	125.50±12.14	130.50±11.12	132.75±11.39	134.75±12.24j
AC1	120.50±17.99	110.25±17.71	112.00±16.84	113.75±17.32	114.25±17.08k
AC2	118.75±14.84	110.25±15.62	112.50±15.50	114.75±15.57	116.75±14.72l
AC3	125.25±21.03	117.50±20.29	115.00±20.17	118.25±19.83	120.25±19.40m

Values are expressed as mean ± S.E.M; Mean values having different superscript letter in the same column are significantly different at (p<0.05)

Very significant (p<0.05) decrease in body weight of the Alloxan- induced diabetic albino rats observed before the commencement of oral administration of the ethno medicinal treatment (Table 1) could be due to the destruction of ß-cells of the islets of Langerhans of the pancreatic cells by Alloxan. This leads to insulin deficiency thereby decreasing cellular glucose metabolism and increasing degradation of structural proteins thus affecting the body weight of the albino rats. This agrees with findings by other researchers [15]. Gradual significant body weight gain (p<0.05) observed in an alloxan induced diabetic albino rats treated with different ethno medicinal plants in a dose dependent manner for the period of three weeks (Table 1) could be due to the ability of the plants to neutralize or inhibit the toxic effect of alloxan on ß-cells of the islets of Langerhans of the pancreatic cells thereby restoring its normal function.

3.2 Effect of Administration of Medicinal Plants on Serum Glucose Level

As shown in Table 2, intraperitoneal injection of allozan (150 mg/bw) in albino rats significantly (p<0.05) raised the serum glucose level to 270.84±2.3 mg/dl compared to NC, 63.13±1.18. Treatment of alloxan induced diabetic albino rats with different ethno medicinal plants (MO, TA and AC) in a dose dependent manner for three (3) weeks led to significant decrease (*p*<0.05) in serum glucose level when compared with the diabetic control group (DC).

It is observed that the significant difference (p<0.05) in serum glucose level exist between

MO, TA and AC treated groups. MO has the highest (p<0.05) hypoglycaemic effect (108.8±1.40) when compared with hypoglycaemic potential of TA (149.4±1.50) and AC (121.6±1.55). Metformin demonstrated the most significant hypoglycaemic effect when compared to MO, TA and AC. Also, there is no significant (*p*>0.05) difference observed in serum glucose level between normal control group (63.13±1.18) and that of Metformin treated group (62.76±2.35).

Table 2. Effect of administration of different concentration of the medicinal plants on serum glucose level

GROUP	Glucose level (mg/dl)
[NC]	63.13±1.18[a]
[DC]	270.84 [b]
[MC]	62.76±2.35[a]
[MO1]	150.6±0.58 [c]
[MO2]	130.56±0.61[d]
[MO3]	108.8±1.40[e]
[TA1]	168.04±2.23[f]
[TA2]	155.2±0.78[g]
[TA3]	149.4±1.50[h]
[AC1]	162.02±2.17[i]
[AC2]	145.6±3.60[j]
[AC3]	121.6±1.55[k]

Values are expressed as mean ± S.E.M. Mean values having different superscript letter in the same column are significantly different at (p<0.05)

The result revealed that ethno medicinal plants at higher dose demonstrated the most significant

hypoglycaemic effect than the lower doses use in this study. It is also observed that oral administration of MO extract in a dose dependent manner revealed higher significant hypoglycaemic effect (P<0.05) when compared to TA and AC extracts. The potential hypoglycaemic activity of MO is almost similar to that of Metformin effect. The hypoglycaemic effect of the medicinal plants might be attributed to the role of phytochemical constituents present in plants as observed by some researchers. Quercetin-3-0 (-6''- malonyl glucoside), Quercetin-3-O- glucoside and kaempferol-3-O (-6''-malonyl glucoside) were reported as hypoglycaemic agent due to their inhibitory activity on α- glucosidase enzyme [16], this might delay the formation of glucose from carbohydrate, thus reduced postprandial hyperglycaemia. Kankara et al., (2016) reported the inhibitory effect of the leave and seed chloroform extracts of *Moringa* against α-glucosidase activity.

3.3 Effect of Administration of Different Medicinal plants on Serum Lipid Profile

The intraperitoneal injection of alloxan in albino rats resulted in significant increase (*p*<0.05) of the serum total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) level. A significant decrease (*p*<0.05) in serum HDL-C level in the alloxan induced diabetic groups was observed when compared with that of the NC group (Table 3). The was attributed to the increase breakdown of triglycerides and mobilization of free fatty acids (FFA) from the adipose tissue. This agrees with the findings of other researchers' [16]. They observed an increase in serum triglycerides and total cholesterol levels in alloxan diabetic rats. Severe diabetes mellitus associated with insulin deficiency might activate hormone-sensitive lipases; glucagon and catecholamines thereby stimulating lipolysis which is accompanied with a reduced LDL-receptor resulting in high concentration of serum LDL cholesterol in diabetic subjects. Treatment of alloxan induced diabetic albino rats with MO, TA and AC in a dose dependent manner for three weeks demonstrated significant (*p*<0.05) decrease in serum TC, TG, VLDL-C and LDL-C level while serum HDL-C level significantly increased (*p*<0.05) when compared with the diabetic control group (DC). The MO_3 treated group

demonstrated the highest significant (p<0.05) hypolipidemic effect followed by TA and AC. Also, the result revealed non-significant differences (p>0.05) of serum TC, TG, VLDL-C and LDL-C levels in MO_3 treated group as compared with the normal control (NC) and Metformin treated groups (MC). This hypolipidemic effect observed might be due to the ability of the phytochemical constituent of the medicinal plant to restore the function of the pancreas. The result also showed higher level of serum HDL-C in the MO groups followed by TA treated group while AC treated had the lowest serum HDL-C level, but still significant (p<0.05) when compared with the diabetic control group.

The result in Table 3 shows that oral administration of aqueous extract of MO, TA and AC in dose dependent manner for 21 days to diabetic rats resulted in significant (p<0.05) reduction in TC, TG, LDL-C and VLDL-C. A significant (p<0.05) increase of serum HDL-C level was observed when compared with the diabetic control (DC) rats. This finding prove the hypolidaemic activity of the MO, TA and AC tested in this study. Oral administration of MO, AF and AC extracts to the alloxan induced diabetic albino rats ameliorated the diabetic complications by significant reduction of the serum levels of lipid profile and significant elevation of HDL-C. The hypolipodemic activity observed might be due to the restoration of the pancreatic β-cells activity to secrete insulin.

3.4 Effect of oral Administration of the Medicinal Plants on the Pancreas of Alloxan Induced Diabetic Albino Rats

The histological results of the effect of treatment with MO, TA and AC on histological changes in the pancreases of diabetic albino rats are shown in Table 4 and Plates 1 to 6. Treatment of alloxan induced diabetic albino rats with MO, TA and AC revealed the regeneration of the number of islet in the stained pancreases. The number of islet per square centimetre seen in MO_3 treated group ranges from one to three or more islet as compared to DC and MC groups. The number of islet in TA and AC the treated groups ranged from zero to two islets (- to ++). However, treatment of alloxan induced diabetic albino rats with TA and AC at low doses for three weeks demonstrated mild improvement in the number of islet when compared to DC group.

Table 3. Effect of administration of different concentrations of the medicinal plants on serum lipid profile

	Lipid Profile (mg/dl)				
GRP	TC	TG	HDL	VLDL	LDL
NC	72.35±1.37[a]	77.25±1.67[a]	48.05±1.31[c]	15.44±0.33[a]	8.85±1.30[a]
DC	104.59±2.88[e]	94.10±2.30 [c]	22.67±1.24[a]	18.82±0.46[c]	63.09±3.25[e]
MC	69.58±1.81[a]	78.51±1.36[a]	43.29±3.52[c]	15.70±0.27[a]	10.59±3.97[a]
MO$_1$	85.91±4.48[b]	86.89±3.37[b]	38.84±1.91[b]	17.37±0.67[b]	29.67±2.95[c]
MO$_2$	79.09±1.69[ab]	80.19±1.65[a]	42.58±1.52[c]	16.03±0.32[a]	20.47±1.15[b]
MO$_3$	76.26±3.33[a]	76.42±1.15[a]	47.85±1.28[c]	15.28±0.23[a]	13.12±2.27[a]
TA$_1$	88.04±0.84[c]	90.69±4.44[b]	37.54±1.09[b]	18.13±0.88[b]	32.36±0.97[c]
TA$_2$	93.45±2.79[d]	90.39±2.21[b]	39.24±0.878[b]	18.07±0.44[b]	36.13±3.49[d]
TA$_3$	84.51±2.53[b]	82.42±5.20[a]	42.68±1.75[c]	16.48±1.04[a]	25.34±3.81[b]
AC$_1$	90.27±2.44[dc]	106.83±3.83[d]	38.88±0.68[b]	21.36±0.76[c]	30.03[c] ±1.62[c]
AC$_2$	86.57±3.73[b]	95.09±5.22[c]	37.24±1.49[b]	18.89±1.01[c]	30.43±5.56[c]
AC$_3$	89.81±2.48[c]	90.82±1.44[b]	39.32±.815[b]	18.16±0.28[b]	32.32±1.63[c]

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly different at (p<0.05)
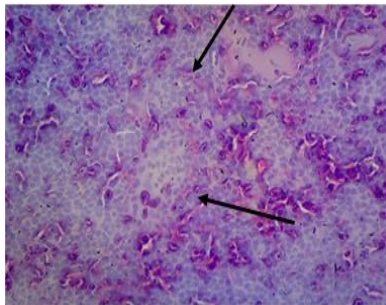

Plate 1: Light Photomicrograph of Normal Rat Pancreas. Showing Pancreas with [+++] Islets per low power field (x200).
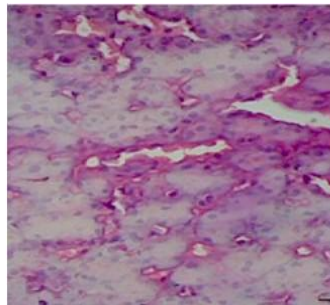

Plate 2: Light Photomicrograph of Diabetic Rat Pancreas. Showing Pancreas with [-] Islets per low power field (x200).
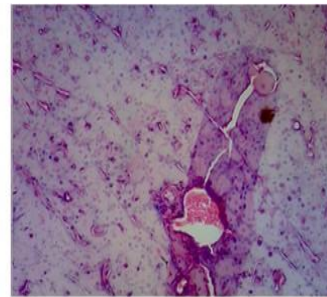

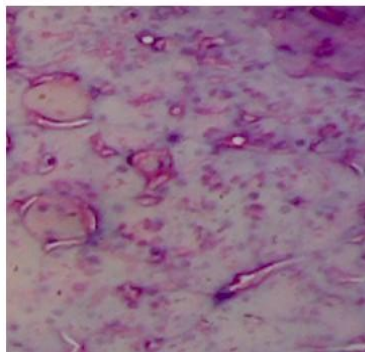**Plate 3:** Light Photomicrograph of MC group Pancreas. Showing Pancreas with [+] Islets per low power field (x200).

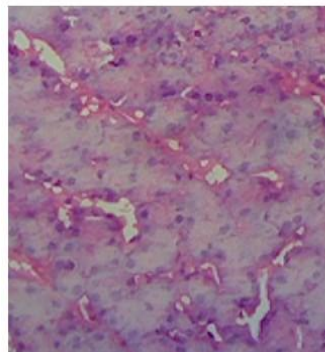**Plate 4:** Light Photomicrograph of MO group Pancreas. Showing Pancreas with [+++] Islets per low power field (x200).

Plate 5: Light Photomicrograph of TA group Pancreas. Showing Pancreas with [-] Islets per low power field (x200).
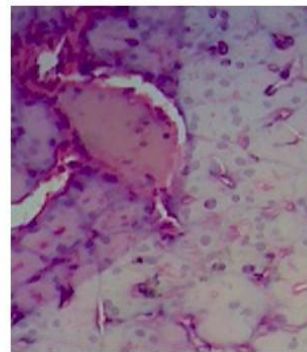

Plate 6: Light Photomicrograph of AC group Pancreas. Showing Pancreas with [+] Islets per low power field (x200).

Table 4. Effect of administration of different concentration of the medicinal plants on number of islets per square centimetre on the stained pancreatic tissues

Group	Number of Islets		
NC	+	++	+++
DC	-	-	-
MC	+	-	++
MO$_2$	+	++	-
MO$_3$	-	+	+++
TA$_2$	-	+	-
TA$_3$	+	++	-
AC$_2$	+	-	-
AC$_3$	+	++	-

Key: (-); No islet seen, (+); one islet, (++); two islets, (+++), three or more islets seen per low power field (x200)

4. CONCLUSION

The results obtained showed that the medicinal plants studied in this research possess antidiabetic activity and could be considered as potential sources for antidiabetic drugs.

ETHICAL APPROVAL

Experiments were carried out with prior permission from the Institutional Animal Ethical committee, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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