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Evaluation of Anti-diabetic Potential of Aqueous Extract of *Moringa oleifera* Leaf in Alloxan-Induced Diabetic Rats

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ABSTRACT: The anti-diabetic potential of aqueous extract of *Moringa oleifera* leaf was evaluated in alloxan-induced diabetic rats. The rats were grouped into five (5) groups of 10 animals each namely: the Control group, Tween 80-treated, Glibenclamide-treated and *Moringa oleifera*-treated orally at 700 and 900 mg/kg body weight respectively. The results revealed that the extract significantly ($P<0.05$) reduced the fasting blood sugar level as well as the postprandial rise in blood glucose after a heavy glucose meal in normoglycaemic rats. Also administration of the extract reduced fasting blood sugar level. The extract also significantly ($P<0.05$) reduced the serum total cholesterol and triglyceride level but caused an increase in HDL level. It significantly ($P<0.05$) increased the body weight of extract-treated diabetic rats. Sub-chronic study of the effect of the extract showed a significant increase ($P<0.05$) in packed cell volume (PCV), white blood counts in rat induced diabetes which however, attain basal level. The histological studies showed that the diabetic rats with the architecture of the pancreas distorted, was restored to normalcy by the extract. Its LD_{50} was found to be greater than 1000 mg/kg indicating its safety in rats. The result therefore suggests that aqueous extract of *Moringa oleifera* leaf is safe for use via the oral route and also has anti-diabetic potential.

Keywords: Antidiabetic, *Moringa oleifera* leaf, alloxan-induced, glibenclamide-treated

Introduction

The consumption of a variety of local herbs and vegetables by humans is considered to contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of therapeutic agents (Oluwade *et al.,* 2004).

Moringa oleifera is an extremely valued plant, dispersed in many countries of the tropics and subtropics. Most of the parts of the plant have been proven to possess antimicrobial activity (Rockwood *et al.,* 2013). They are known for their pharmacological actions and are also used in the traditional treatment of diabetes mellitus (Babu and Chaudhuri, 2005), hepatotoxicity (Anupama *et al.,* 2013), rheumatism, venomous bites and also for cardiac stimulation (Anupama *et al.,* 2013).

Diabetes mellitus (DM) is defined as a group of metabolic disorders characterized by hyperglycemia resulting from a variable interaction of hereditary and environmental factors due to defects in insulin secretion, insulin action or both (Oyedepo *et al.,* 2013). Hyperglycaemia results either due to defective production or action of insulin which leads to a number of complications; cardiovascular diseases, renal damage, neurological disorder, ocular dysfunction e.t.c. (Oyedepo *et al.,* 2013). The ailment may result in the development of further metabolic and anatomic disturbances among which is lipemia, hypercholesterolaemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes ithe eye, neuropathy, renal disease, and coma (Oyedepo *et al.,* 2013; Andrew *et al.,* 2000).

Dyslipidemia is common in diabetes, as both insulin deficiency and insulin resistance affects enzymes, as well as pathways of lipid metabolism (Lorenzo *et al.,* 2010). Dyslipidemia, as defined by the World Health Organization, is considered in circumstances where the fasting plasma triglyceride is between 150 - 400 mg/dL (1.7 to 4.5 mmol/L), total cholesterol (TC) > 200 mg/dL (>5.2 mmol/L), low-density lipoprotein cholesterol (LDL-C) > 135 mg/dL (>3.5 mmol/L), high-density lipoprotein cholesterol (HDL-C) < 35 mg/dL (<0.9 mmol/L) in men or <40 mg/dL (<1.0 mmol/L) in women, and a ratio of total cholesterol to HDL-cholesterol > 5 (Jisieike-Onuigbo *et al.,* 2011). Characteristic abnormalities of lipids in type 2 diabetes mellitus include elevated triglycerides (TAG) levels, decreased atheroprotective high density lipoprotein cholesterol (HDL-C) levels and increased levels of small dense LDL-C (Ayinla *et al.,* 2011; Beckman *et al.,* 2002).

World Health Organisation (Oyedepo *et al.,* 2013) lay emphasis strongly on the rational use of traditional and natural indigenous medicines, for treating diabetes mellitus. Patients with diabetes mellitus have been treated orally by folklore with a variety of plant extracts (Ayinla *et al.,* 2011). More than 1,200 plants species are used worldwide in diabetes phytotherapy, and experimental studies support the hypoglycaemic activity of a large number of these plants (Ayinla *et al.,* 2011). In addition to correction of blood glucose levels, several plants with hypoglycaemic properties have potential in ameliorating lipid metabolism and abnormalities of diabetes mellitus (Coon and Ernst, 2003).

Glibenclamide is an oral anti-diabetic often used by patient with type 2 diabetes. It belongs to the class of sulfonylureas among other classes of oral anti-diabetics. Glibenclamide acts on β-cells stimulating insulin and thus reducing plasma glucose. Action is increased in the elderly and in patients with renal disease and contraindicated in pregnancy. Adverse effect is severe hypoglycaemia which can be prolonged and severe (Rang *et al.,* 2005).

The study therefore was carried out to investigate the anti-diabetic potential of *Moringa oleifera* leaf extract in alloxan-induced diabetic rats.

Materials and Methods

Experimental Animals:

One hundred (100) male albino Wistar rats with body weight within the range 80-120 g were selected for the experiments. Animals were obtained from the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. The animals were housed in polypropylene cage at normal temperature and were fed on pellet diet and water *ad libitum*.

Plant Material and Authentication

Moringa oleifera leaf was procured from Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State. The plant was authenticated by a taxonomist in the Department of Botany at the University of Ilorin and a voucher specimen (UIH 001/1008) was prepared and deposited at the University Herbarium.

Sample Preparation:

Five hundred (500 g) of *Moringa oleifera* leaf was macerated with two litres of distilled water at room temperature for 24 hrs with occasional shaking. The resulting extract was filtered using muslin cloth and the filtrate was concentrated using rotary evaporator under reduced pressure to give a semi-solid residue which is then dried in the oven at 60^0C and a greenish powder weighing 200 g was obtained.

Acute Toxicity Test:

The acute toxicity and lethality test of the aqueous extract of *Moringa oleifera* leaf in rats was estimated using the method of Lorke as described by (Okoli *et al.,* 2010), and the animals were observed continuously after every 2 hr under the following profiles:

- Behavioral Profile: Alertness, Restlessness
- Neurological Profile: Pain Response, Touch Response, Gait
- Autonomic Profile: Defecation and urination

After a period of 24 hr, the animals were observed for any lethality or death.

Induction of Diabetes:

Diabetes was induced according to the principle of Aruna *et al*., as described by (Okoli *et al.,* 2010) with slight modification. A single intraperitoneal injection of alloxan monohydrate (150 mg/kg b.w.) was given and after day 5, blood was drawn from the rat by tail snipping to determine blood glucose level. Animals with blood glucose level ≥ 225 mg/dl were considered diabetic and used for the study.

Initial screening of the aqueous extract of *Moringa oleifera* leaf for evaluating its glyceamic potential was done with a range of doses (700 and 900 mg/kg body weight) given orally to a group of normoglyceamic rats by conducting fasting blood sugar and oral glucose tolerance test. The hypoglyceamic, anti-diabetic and anti-dyslipidemic effects of the extract were also assessed.

Assessment of Normoglyceamic Activity:

Animals fasted overnight were randomly divided into five groups (n=5) and received oral administration of distilled water (2 ml/kg), 20% v/v tween 80 (2 ml/kg), glibenclamide (0.5 mg/kg) and *Moringa* leaf extract (700 and 900 mg/kg) respectively. The blood glucose levels of the animals were measured prior to treatment (pre-treatment) and at 0.5, 1, 2, and 4 hr after extract administration.

Oral Glucose Tolerance Test:

Animals fasted for 16 hr but with free access to water were randomly divided into five groups (n=5) and received oral administration of distilled water (2 ml/kg), 20% v/v tween 80 (2 ml/kg), glibenclamide (0.5 mg/kg) and *Moringa* leaf extract (700 and 900 mg/kg) respectively. Ninety minutes later, the rats were fed with glucose (4 g/kg). The blood glucose level of each animal was measured before (0) and at 30, 60, 90, 120, 150 and 180 min after glucose load.

Assessment of Hypoglycaemic Activity:

Animals were fasted for 8 hr but with free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) levels of the rats were determined using Accu chek glucometer. Subsequently, diabetes was induced and the animals were randomly divided into five groups (n=10) and received oral administration of distilled water (2 ml/kg), 20% v/v tween 80 (2 ml/kg), glibenclamide (0.5 mg/kg) and *Moringa* leaf extract (700 and 900 mg/kg) respectively. Blood glucose was measured before (0) and 0.5, 1, 2 and 4 hr after treatment.

Assessment of Anti-Diabetic Activity:

Animals were fasted for 8 hr but allowed free access to water. The basal fasting blood glucose (FBG) of rats was determined and diabetes was induced. The animals were randomly divided into five groups (n=10) and received oral administration of distilled water (2 ml/kg), 20% v/v tween 80 (2 ml/kg), glibenclamide (0.5 mg/kg) and *Moringa* leaf extract (700 and 900 mg/kg) respectively. The treatments were administered orally to the animals once daily for 28 days. Blood glucose was measured before (pre-treatment) and on days 7, 14 and 28 after commencement of treatment. The body weight of each animal was also measured on these days. Animals were sacrificed on day 28 by light anesthesia and blood samples were collected through jugular puncture. The blood was then centrifuged at 300 *rpm* for 5 min to collect plasma.

Determination of Plasma Lipid Profiles:

Plasma triglycerides, and total cholesterol levels were measured at the end of the treatment using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories, UK, in which the CHOD-PAP method of Trinder (Okoli *et al.,* 2010) was employed. Absorbance was measured at 500 nm. The phosphotungstate precipitation method of Richmond (Okoli *et al.,* 2010) as applied in Randox kit was used for the determination of HDL-cholesterol.

Haematological Parameters:

Packed cell volume (PCV), white blood cell count and white blood differentials were determined using standard operating procedure as described by Adeneye and Benebo (2007). Blood samples were collected through tail snipping and measurements were taken before (basal) and after the induction of diabetes (pre-treatment) as well as on days 14 and 28 after commencement of treatment.

Histological Studies on Pancreas of Aqueous *Moringa* Leaf Extract (AMLE)-Treated Diabetic Rats:

The effect of AMLE on tissue architecture of the pancreas of treated diabetic rats were evaluated by histological studies.

Statistical Analysis:

Data were analyzed using one way Anova and the results were expressed as mean ± SEM. The results were further subjected to Duncan post hoc test for multiple comparisons and differences between means were significantly different at $P<0.05$.

Results

Acute Toxicity Test:

The behavior of the treated rats appeared normal with no toxic effect at dosage of 1000 mg/kg body weight as well as no lethality.

Normoglycaemic Effect of AMLE:

The extract showed a significant reduction ($P<0.05$) in fasting blood glucose levels in normoglycaemic rats when compared with the control and pre-treatment values. Maximum reduction occurred at 2 hr post-treatment (Table 1).

Table 1: Effect of AMLE on blood glucose of normoglycaemic rats

Treatment	Fasting Blood Glucose Level (mg/dl)				
	Pre-Treatment	0.5h	1h	2h	4h
Control	67.00 ± 2.89	73.33 ± 2.19	71.67 ± 1.45	70.67 ± 1.20	68.00 ± 3.05
Tween 80 (2ml/kg)	75.67 ± 6.01	94.00 ± 16.52	102.33 ± 26.83	67.67 ± 4.37*	72.33 ± 4.26
Glibenclamide (0.5 mg/kg)	89.33 ± 7.13	78.67 ± 1.20	69.00 ± 5.03	61.00 ± 6.51*	64.64 ± 1.67*
AMLE (700 mg/kg)	79.00 ± 7.00	87.67 ± 5.24	67.67 ± 2.33*	62.00 ± 2.00*	67.00 ± 9.54*
AMLE (900 mg/kg)	77.00 ± 3.21	67.67 ± 6.33*	69.00 ± 5.51	65.00 ± 4.04*	55.00 ± 8.89*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent reduction in fasting blood glucose levels in normoglyceamic rats relative to the control and pre-treatment values.

Effect of AMLE on Oral Glucose Tolerance:

Following oral administration of glucose load, the postprandial blood glucose level rose at 60 min. Post-treatment with the extract showed a significant reduction ($P<0.05$) in blood glucose levels up to 180 min. At 180 min, the blood glucose level of tween and extract-treated rats were below basal levels when compared with the control and glibenclamide-treated rats (Table 2).

Table 2: Effect of AMLE on oral glucose tolerance of normoglycaemic rats

Treatment	Blood Glucose Level (mg/dl)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	83.00 ± 3.21	78.67 ± 2.80	78.33 ± 2.40	71.67 ± 4.10	71.67 ± 4.41	71.33 ± 6.36	63.67 ± 2.33
Tween 80 (2ml/kg)	99.67 ± 7.06	484.00 ± 33.2	100.33 ± 12.1	73.00 ± 9.07*	70.67 ± 4.10*	75.00 ± 6.03*	72.67 ± 9.28*
Glibenclamide (0.5 mg/kg)	93.67 ± 2.8	330.67 ± 93.6	136.67 ± 60.1	190.67 ± 41.0	142.67 ± 63.6	57.67 ± 70.2*	138.00 ± 26.6
AMLE (700 mg/kg)	97.00 ± 2.31	112.67 ± 23.3	82.00 ± 11.02*	73.67 ± 7.31*	110.00 ± 30.0	83.67 ± 4.81*	70.00 ±4.16*
AMLE (900 mg/kg)	86.00 ± 1.15	176.33 ± 59.3	79.67 ± 2.40*	72.67 ± 1.33*	86.33 ± 14.15	67.67 ± 7.54*	66.33 ± 5.46*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represents reduction in blood glucose levels relative to control and 0 min.

Hypoglycaemic Effect of AMLE:

The extract showed a significant reduction ($P<0.05$) in blood glucose levels in diabetic rats when compared with the pre-treatment values. Maximum reduction occurred at 2 hr post-treatment (Table 3).

Table 3: Hypoglycaemic effect of AMLE on blood glucose level of diabetic rats

Treatment	Blood Glucose Level (mg/dl)				
	Pre-Treatment	0.5h	1h	2h	4h
Control	67.00 ± 2.89	73.33 ± 2.19	71.67 ± 1.45	70.67 ± 1.20	68.00 ± 3.05
Tween 80 (2ml/kg)	415.60 ± 62.69	424.80 ± 55.37	406.80 ± 44.17	304.60 ± 20.36*	390.20 ± 74.65*
Glibenclamide (0.5 mg/kg)	475.60 ± 46.23	337.00 ± 691.30*	350.00 ± 69.30*	321.40 ± 59.51*	276.60 ± 74.14*
AMLE (700 mg/kg)	594.60 ± 4.70	387.80 ± 36.78*	484.20 ± 59.18*	347.00 ± 80.09*	411.80 ± 47.78*
AMLE (900 mg/kg)	405.60 ± 73.63	286.00 ± 66.89*	327.80 ± 70.93*	305.60 ± 96.52*	308.20 ± 83.48*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent reduction in blood glucose levels in rats relative to the pre-treatment values.

Anti-Diabetic Effect of AMLE:

The extract showed a significant reduction ($P<0.05$) in blood glucose levels following sub-chronic administration when compared with the diabetic pre-treatment values. At day 28, the extract reduced the blood glucose level better than the glibenclamide (Table 4).

Table 4: Effect of AMLE on blood glucose level of diabetic rats

Treatment	Blood Glucose Level (mg/dl)				
	Pre-Treatment	0.5h	Diabetic Post-treatment		
	Pre-Treatment (Basal)	Diabetic (Pre-treatment)	Day 7	Day 14	Day 28
Control	87.80 ± 6.65	87.60 ± 6.72	86.00 ± 7.23	88.00 ± 7.45	88.40 ± 7.68
Tween 80 (2ml/kg)	90.40 ± 2.73	325.80 ± 26.67	122.00 ± 12.76*	194.20 ± 19.31*	121.60 ± 7.49*
Glibenclamide (0.5 mg/kg)	74.60 ± 3.60	341.80 ± 41.99	206.20 ± 9.89*	127.40 ± 19.95*	106.20 ± 20.20*
AMLE (700 mg/kg)	85.60 ± 5.10	381.60 ± 53.53	201.20 ± 8.27*	111.00 ± 17.67*	103.30 ± 19.27*
AMLE (900 mg/kg)	405.60 ± 73.63	286.00 ± 66.89*	327.80 ± 70.93*	305.60 ± 96.52*	308.20 ± 83.48*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent reduction in blood glucose levels in rats relative to diabetic pre-treatment values.

Effect of AMLE on Lipid Profile of Diabetic Rats:

The extract showed a significant reduction ($P<0.05$) in TAG and cholesterol levels when compared with diabetic pre-treatment values and a significant increase in HDL levels when compared with the diabetic pre-treatment values. The extract evoked a better effect at 900 mg/kg than glibenclamide (Table 5).

Table 5: Effect of AMLE on lipid profile of diabetic rats

Lipid Profile	Treatment	Pre-Diabetic (Basal)	Diabetic (Pre-Treatment)	Diabetic (Post-Treatment) 14 Days	Diabetic (Post-Treatment) 28 Days
Triglyceride Concentration	Control	142.01 ± 5.97	146.24 ± 6.82	156.26 ± 7.24	159.63 ± 0.75
	Tween Control	162.43 ± 7.01	191.78 ± 2.31	147.01 ± 1.78*	189.16 ± 2.80
	Drug Contro	172.76 ± 7.48	187.10 ± 2.31	177.76 ± 7.48*	1169.56 ± 7.05*
	AMLE (700 mg/kg)	165.23 ± 6.57	187.29 ± 3.24	182.34 ± 6.96	74.39 ± 0.93*
	AMLE (900 mg/kg)	168.01 ± 6.45	178.13 ± 6.50	170.05 ± 6.50	161.57 ± 15.10*
Cholesterol Concentration	Control	112.01 ± 3.20	122.24 ± 4.82	126.26 ± 5.24	129.63 ± 0.75
	Tween Control	102.43 ± 2.01	114.60 ± 1.23	108.80 ± 0.40*	118.16 ± 2.80
	Drug Control	122.76 ± 5.48	193.60 ± 0.47	156.80 ± 2.62*	149.40 ± 2.24*
	AMLE (700 mg/kg)	125.23 ± 5.57	180.20 ± 5.47	164.90 ± 2.56*	151.10 ± 2.99*
	AMLE (900 mg/kg)	128.01 ± 6.45	158.53 ± 3.38	156.99 ± 2.66	95.11 ± 1.39*
HDL Concentration	Control	100.94 ± 6.90	88.96 ± 2.29	92.05 ± 1.93	100.30 ± 1.93
	Tween Control	73.10 ± 2.77	74.77 ± 0.65	79.31 ± 1.48	93.10 ± 4.77*
	Drug Control	112.76 ± 7.48	97.92 ± 1.33	104.69 ± 3.39*	104.15 ± 0.51*
	AMLE (700 mg/kg)	115.23 ± 6.57	13.80 ± 0.33	86.34 ± 2.47*	109.88 ± 0.25*
	AMLE (900 mg/kg)	108.01 ± 6.45	11.95 ± 0.19	67.21 ± 1.72*	94.32 ± 2.24*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent reduction in blood lipid levels relative to diabetic pre-treatment values.

Haematological Parameters

The extract showed a significant reduction ($P<0.05$) in blood haematological parameters when compared with the diabetic pre-treatment values (Table 6).

Table 6: Effect of AMLE on the haematological parameters of diabetic rats.

Groups	Days	PCV (%)	WBC (x109/L)	NEU (%)	EOSIN (%)	LYMPH (%)
Normal Control	Basal	35 ± 0.58	3.0 ± 0.03	60 ± 0.00*	1.67 ± 0.33*	40 ± 0.00*
	Pre-Treatment	27 ± 1.56*	5.1 ± 0.33*	57 ± 0.67*	1.0 ± 0.00*	32 ± 0.00*
	14 Days	31 ± 0.67*	7.9 ± 0.10*	68 ± 0.00*	0.0 ± 0.00*	38 ± 0.00*
	28 Days	34 ± 0.58*	6.2 ± 0.58*	60 ± 0.00*	0.0 ± 0.00*	40 ± 0.00*
Tween Control	Basal	46 ± 0.88*	5.2 ± 0.11*	62 ± 1.45*	1.0 ± 0.00*	40 ± 1.45*
	Pre-Treatment	30 ± 0.76	3.2 ± 0.15	57 ± 1.76	1.0 ± 0.00*	37 ± 1.45*
	14 Days	34 ± 1.16*	4.2 ± 0.15*	60 ± 1.20*	1.0 ± 0.00*	40 ± 1.45*
	28 Days	40 ± 0.67*	5.3 ± 0.15*	60 ± 0.00*	1.0 ± 0.00*	41 ± 1.33*
Drug Control	Basal	43 ± 0.88*	4.3 ± 0.57*	62 ± 0.88*	1 ± 0.33*	37 ± 1.45*
	Pre-Treatment	29 ± 1.21	3.3 ± 0.57	54 ± 3.22	0 ± 0.00*	30 ± 0.45
	14 Days	35 ± 1.20*	7.1 ± 0.73*	63 ± 2.40*	0 ± 0.00*	37 ± 1.45*
	28 Days	32 ± 4.89*	4.3 ± 0.64*	60 ± 0.88*	1 ± 1.0*	37 ± 0.67*
700 mg	Basal	40 ± 2.89*	5.8 ± 0.69*	60 ± 0.81*	0 ± 0.00*	40 ± 0.33*
Moringa	Pre-Treatment	30 ± 0.97	4.8 ± 0.20	54 ± 3.30	0 ± 0.00*	30 ± 0.45
oleifera	14 Days	30 ± 1.16*	6.6 ± 0.33*	64 ± 2.30*	0 ± 0.00*	40 ± 0.00*
	28 Days	35 ± 5.18*	5.8 ± 0.20*	60 ± 1.16*	1 ± 0.05*	40 ± 1.33*
900 mg	Basal	52 ± 2.52*	5.0 ± 0.72	60 ± 0.88*	1 ± 0.33*	39 ± 0.33*
Moringa	Pre-Treatment	32 ± 34	4.8 ± 0.72*	42 ± 0.73	1 ± 0.33*	30 ± 0.30
oleifera	14 Days	34 ± 2.08*	6.4 ± 1.73*	62 ± 1.73*	1 ± 0.3*	37 ± 1.45*
	28 Days	33 ± 0.33*	5.0 ± 0.29*	60 ± 1.68*	1 ± 0.58*	40 ± 0.00*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent reduction in haematological parameters relative to diabetic pre-treatment values.

Body Weight:

The extract showed a significant increase ($P<0.05$) in body weight when compared with pre-diabetic values (Table 7).

Table 7: Effect of sub-chronic administration of AMLE on body weight of diabetic rats.

Treatment	Body weight (g)			
	Pre-diabetic (Basal)	Diabetic Post-treatment		
		Day 7	Day 14	Day 28
Control	81.53 ± 8.74	103.40 ± 8.39*	122.60 ± 7.36*	156.60 ± 6.10*
Tween 80 (2ml/kg)	82.06 ± 9.19	106.40 ± 7.88*	115.60 ± 8.27*	132.80 ± 7.17*
Glibenclamide (0.5mg/kg)	84.58 ± 5.45	126.80 ± 22.92*	107.20 ± 7.07*	131.40 ± 7.22*
AMLE (700 mg/kg)	74.73 ± 5.26	82.40 ± 6.43*	99.40 ± 12.99*	107.80 ± 12.81*
AMLE (900 mg/kg)	77.45 ± 8.43	83.80 ± 11.32*	89.80 ± 13.37*	104.60 ± 12.62*

Values are expressed as mean ± SEM (n = 5). Values with superscripts * represent increase in body weight relative to pre-diabetic (Basal).

Histological Studies:

The effect of the extract on pancreatic tissues of diabetic-treated and glibenclamide-treated were shown in plates 1-5.

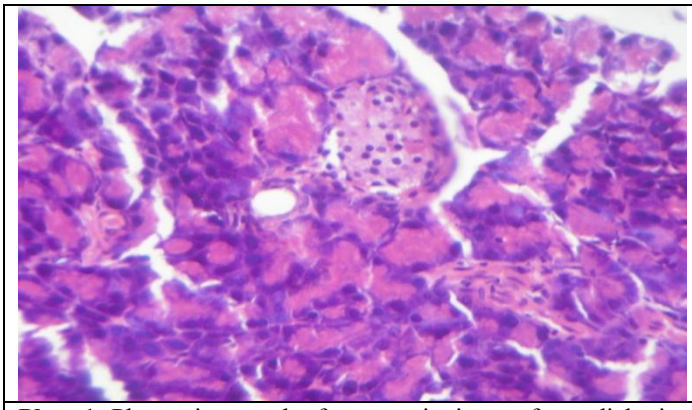

Plate 1: Photomicrograph of pancreatic tissue of non-diabetic control (H and E x400).

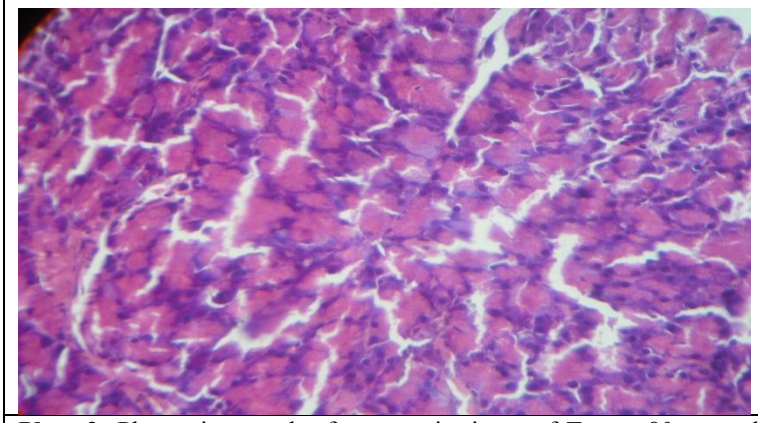

Plate 2: Photomicrograph of pancreatic tissue of Tween-80 control group (H and E x400). There was no visible change in the architecture of the pancreas of animals administered tween-80 when compared with the non diabetic group.

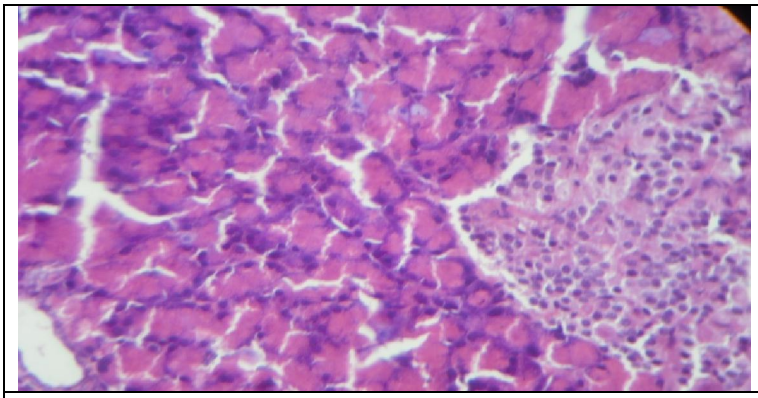

Plate 3: Photomicrograph of pancreatic tissue of drug control group (H and E x400). There was no visible change in the architecture of the pancreas of animals administered glibenclamide when compared with the non diabetic group.

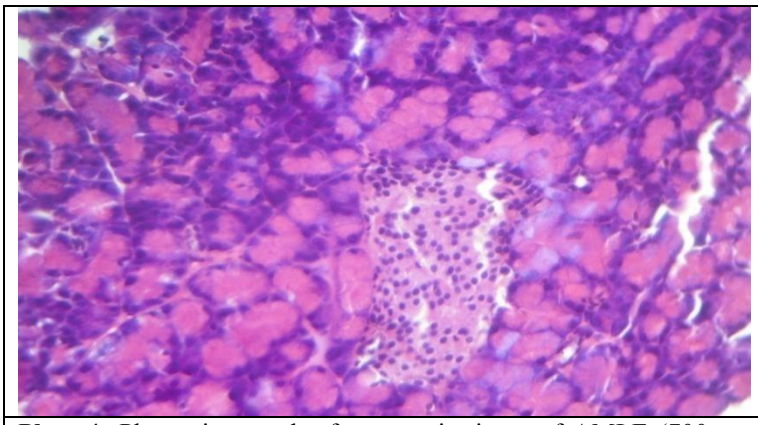

Plate 4: Photomicrograph of pancreatic tissue of AMLE (700 mg/kg) group (H and E x400). There was no visible change in the architecture of the pancreas of animals administered *Moringa* extract (700 mg/kg) when compared with the non diabetic group.

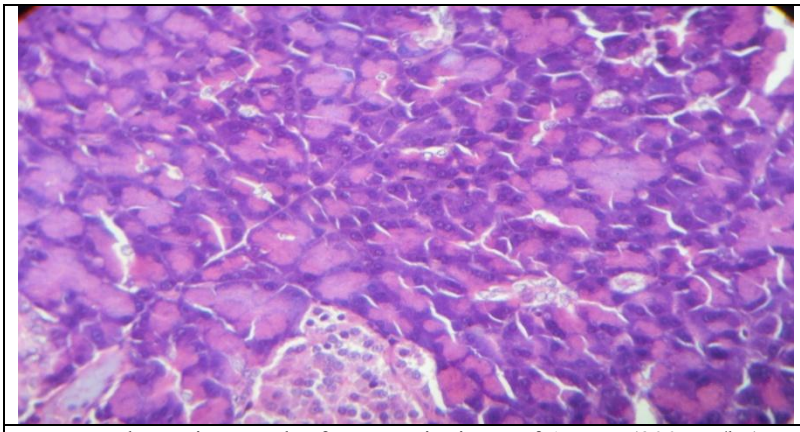

Plate 5: Photomicrograph of pancreatic tissue of AMLE (900 mg/kg) group (H and E x400). There was no visible change in the architecture of the pancreas of animals administered *Moringa* extract (900 mg/kg) when compared with the non diabetic group.

Discussion

Diabetes Mellitus (DM) poses a significant threat to the health and quality of life of an individual. Due to increase complications and mortality in DM, adequate glyceamic controls might be useful in its management. In this study, anti-diabetic potential of aqueous extract of *Moringa oleifera* leaf in alloxan-induced diabetic rats showed that a single oral administration of the extract reduced the fasting blood glucose levels as well as suppressed the postprandial rise in blood glucose of normal rats after a heavy glucose meal.

A number of investigators have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids posses' hypoglycemic effects in various experimental animals model (Goji *et al.,* 2009). However, in line with the hypothesis of Marles and Farnsworth (1995) which stipulates that plant which contains terpenoid posseses hypoglycemic activities in diabetic and normal mammal, then it could be thus inferred that aqueous *Moringa oleifera* leaf extract possess hypoglycemic activity.

In the anti-diabetic activity studies, daily oral administration of the extract for 28 days revealed a gradual but sustained reduction in blood glucose levels in diabetic rats. According to Oyedepo *et al.* 2013, aqueous extract of *Moringa oleifera* leaf has been shown to normalize the high blood glucose level in diabetic rats by the 28th day of the experiment. Treatment with the extract also reduced mortality of diabetic rats from hyperglycaemia and prolonged their survival. In this study, the diabetic non-treated control animals all died on day 14 post-induction of diabetes (data not shown) whereas the extract-treated group survived beyond the period of the experiment. Since a better activity has been achieved in severely diabetic rats with damaged islet, it is possible that aqueous extract of *Moringa* leaf has some direct effect. The increase in the total cholesterol and TAG and the decrease in HDL levels of diabetic rats observed in this study are in accordance with earlier report in diabetic subjects (Ayinla *et al.*, 2011). Diabetes-induced hyperlipidemia has been attributed to excess mobilization of fat from the adipose due to under-utilization of glucose (Nimenibo-Uadia, 2003). Chronic oral administration of the extract also revealed a significant reduction in TAG, and cholesterol levels as well as the increase in HDL levels of the diabetic-treated and normoglycaemic rats when compared with the control rats supports the findings of Coon and Ernst (Coon and Ernst, 2003) who stated that most hypoglycemic plants have potentials of ameliorating diabetic lipid metabolism anomalies.

This hypolipidemic effect of *Moringa oleifera* might be due to its chemical composition, which reveals the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals (Ayinla *et al.*, 2011; Ezekwe and Obidoa, 2001). Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its fecal excretion (Rotimi *et al.,* 2001). The increased bile acid excretion might have been offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol. Although the precise mechanism through which the leaf extract exerted it hypolipidemic effect is not clearly known nor studied, it is suggested from the findings that the control of glycaemia might have been contributory since control of glycaemia is a major determinant of total cholesterol and triglyceride (Ayinla *et al.,* 2011).

Diabetic dyslipidaemia is marked by increased triglycerides, cholesterol and low density lipoprotein (LDL) particles of altered composition and decreased high density lipoprotein (HDL), which constitutes an important cardiovascular risk factor in diabetics (Agrawal *et al*., 2006). It has been found that reduction in total cholesterol and triglycerides through dietary or drug therapy can help in preventing diabetic complications as well as improving lipid metabolism in diabetic patients (Okoli *et al.,* 2009).

Also significant improvement in the haematological parameters on a long term treatment with extract for 28days suggests the favorable effects of the aqueous extract of *Moringa oleifera* leaf. Sub-chronic administration of the extract also showed weight gain in diabetic-treated compared to control rats.

Histopathological analysis showed that after the long administration of the extract both at 700 and 900 mg/kg body weight, the extract was able to restore the architecture of the pancreas damaged by alloxan. (Plates 1-5). Alloxan owes its diabetogenic potential to destruction of β -cells of the islet (Szudelski, 2001; Fröde and Medeiros, 2008) which consequently impairs insulin secretion and leads to hyperglycaemia. Extract treatment may have restored the integrity and possibly, functions of the damaged pancreatic tissues. Glibenclamide used as a reference hypoglycaemic drug did not cause any such effect. The precise mechanism of this tissue repair is yet to be known.

However, due to the tremendous implication of oxidative stress (Hayden *et al*., 2005; Leung and Leung, 2008) which leads to the damage to the pancreas, it seems reasonable to suggest that the antioxidant (Tasaduq *et al.,* 2003) and radical scavenging effects (Jagetia and Baliga, 2004) of *Moringa oleifera* leaf may play a significant role in protecting pancreatic tissues from oxidants including those generated by alloxan. Alloxan destroys insulin-producing pancreatic β-cells through the production of reactive oxygen species which then cause tissue damage (Lee *et al.,* 2008). The hypoglycaemic effect of the extract may be responsible for protection confer on the pancreas from the deleterious effect of chronic hyperglycaemia. Rather than reflecting a direct tissue repair effect, it is likely that the extract, through antioxidative and hypoglycaemic effects, protected the already compromised pancreas from further assault or pancreatic damage which may allow the natural repair processes to proceed and restore the tissues. However, it is unknown if the repaired tissues also had their functions fully or partially restored since the blood glucose level of the animals did not return to normalcy or pre-treatment levels as at the end of the experiment. A return to basal or pre-treatment levels might indicate a full restoration of insulin secretion by the repaired pancreatic tissues (Okoli *et al.*, 2009).

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