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COMPARATIVE ANTI-DIABETIC EFFECTS OF ETHANOL EXTRACTS FROM LEAVES, SEEDS and PODS OF *Moringa oleifera* ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The anti-diabetic activity of 30 % ethanol extract of seeds, pods, leaves of *Moringa oleifera* was evaluated on induced diabetic rats for 4 weeks. After the treatment with extracts, the animals were sacrificed by cervical dislocation for the histopathological examination of their pancreas. Saponin and volatile oil were high in pod extract whereas high alkaloids, flavonoids, flavanols and anthraquinones were detected in all extracts. For bioassay, the animals were grouped into A, B, C, D, E, and F corresponding to treatment with 200 mg leave extract per kg body weight, 4 mL seed extract per kg body weight, 300 mg pod extract per kg body weight, 1.0 mL normal saline (diabetic control), 10 mg of metformin per kg body weight, 1.0 mL normal saline (Normal control) respectively. At the end of the treatment, it was found that 300 mg pod extract per kg body weight reduced the blood glucose level from 492.80 mg/dL to 128.30 mg/dL while 200 mg of leave extract per kg body weight reduced blood glucose level from 423.00 to 212.30 mg/dL. It was found that the antihyperglycaemic activity of 300 mg/kg pod extract is comparable to the standard drug. Also the observed activity was more for 300 mg/kg pod extract than 200 mg/kg leave extracts. Moreover at $p \geq 0.005$, there is a statistically significant difference between the blood glucose of the animals before and after they were treated for 28 days with both pod and leave extracts. Both leaves and pod extracts indicated antidiabetic activity, however the seed extract did not exhibit anti-hyperglycaemic activity but animals in treated with seed extract were active throughout the study despite their hyperglycaemic state. It seems the seed extract contains a principle that is capable of ameliorating the other complications of diabetes. This needs to be evaluated in future study.

Keywords: Antidiabetic, Histopathological, Antihyperglycaemic, Bioassay

INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterised by high blood glucose levels due to insufficient or defective insulin, defective beta cells of the islet of Langerhans or lack of sensitivity of tissues to insulin (Mahfuzur, 2012). It is associated with abnormal metabolism of carbohydrates, proteins and lipids leading to unusual lipid profiles and several secondary complications (Uma Devi, *et al* 2006);[i]. It is one of the oldest and earliest diseases described in ancient manuscripts (Ripoll *et al* 2002). It is one of the most common endocrine disorders with a significant morbidity and mortality rate (Patel *et al*, 2012). It is responsible for the causes of 5 % of all deaths globally each year (Vinodmahato *et al*, 2011). In 2019, diabetes was a direct cause of 1.5 million deaths and 48% of all deaths occur before the age of 70. About 460,000 kidney diseases deaths were caused by diabetes, the majority living in -low and-middle –income countries, with projections of the number of people suffering to increase to 300 million by 2025 (WHO, 2023; Jyoti, 2011);). In the United States of America, it is ranked the sixth leading causes of death (Kavishankar *et al*, 2011) and regarded as the primary cause of blindness among people between the ages of 20 and 70 (Manu *et al*2007; Redmond, 2009). There is virtually no report of total recovery from diabetics, rather it has been estimated that 50 % of diabetic patients die of cardiovascular and cerebrovascular diseases (Vinodmahato *et al* 2011). Depending on the pathogenesis, diabetes can be classified into diabetes insipidus, Type I diabetes mellitus (insulin-dependent diabetes mellitus (IDDM)), Type II Diabetes mellitus ("Insulin-Non-Dependent Diabetes Mellitus" (INDDM)), Type 3 Diabetes Mellitus or Gestational Diabetes (Baynest et al, 2015; http://www.diabetes.org/) There are a many redisposing factors for diabetes such as genetic factor; environmental Factors (like exposure to heavy metals or to some poly Aromatic hydrocarbons), viral Infection (Redmond, 2009; Gamble *et al*, 1985) , overweight, obesity and nutrition (Manu et al., 2007), anxiety, aging and stress, pancreatic infection and pancretectomy and auto immunity. Glucose autoxidation in diabetes leads to increased generation of reactive oxidative species and decrease in antioxidant defence mechanisms in the body (Mahfuzur, 2012), which accumulates in the system and cause a lot of complications.

People value plants due to the ancient conviction that plants were / are created to supply man with food, medical treatment, and other effects, as long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (Khan et al, 2023). Plants have been found to have pancreatic beta cell regenerating capacity, some stimulate insulin release, and some others are found to have active principles with insulinomimetic properties or capable of stimulating tissue's sensitivity to insulin (Kavishankar, 2011). Among other plants that have been evaluated for anti-diabetic properties are A*loe vera, A Loebarbadensis, Allium sativa, Ocimum sanctum,* and *Moringa spp (*Prakash, 2009, Jyoti *et al*, 2011) and several thousands of plants. *Moringa oleifera*, has long been used as folklore to treat various diseases and as a nutritional supplement. There are about 13 species in the *Moringa* family but the most widely known and widely cultivated species of all the 13 species is *Moringa oleifera (*Khawaja ,2010) . In different parts of English-speaking countries, it is called by

different names such as Drumstick tree, Horseradish tree, West Indies Bean (Manzoor *et al* 2007; Manzoor *et al,* 2007)); In Nigeria it is known as *Ewe igbale* (in yoruba), *Zogale* (in Hausa), in igbo it is called *okwe oyibo* while Fulani calls it *gawara*. In addition, people gave it some special names such as Tree of life, miracle tree and Mother best-friend because of its nutritional, socio-economic and therapeutic significance (Khan, *et al* 2023).

Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, anti-aging, and anti-inflammatory properties used for nutrition and healing (Khawaja, 2010)[ii]. It is one of the richest plant sources of Vitamins A, B, C, D, E and K, vital minerals (Ca, Cu, Fe, K, Magnesium, Mn, and Zn) with over 40 natural anti-oxidants and all essential amino acids (Jed, 2005; Khawaja, 2010). *Moringa* could be a good source of nutritional supplements for these malnourished children (Babu, 2006; Duke, 1987). *Moringa* has been used in the traditional medicine passed down for centuries in many cultures around the world for skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhoea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation, diabetes, and pregnancy. The healing properties of *Moringa* oil have been documented by ancient cultures. Moringa oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. *Moringa* oil has been used in skin preparations and ointments since Egyptian times [iii] . According to a scientific report, when the leaves of *Moringa* were added to cattle feed, their daily weight gain increased considerably. The milk production increased by 43% when the feed of cows was supplemented with 15 to 17 kilograms of fresh *Moringa* leaves daily. It increased by 58 % when the feed was supplemented with 2 kg dry matter. When supplemented with 3 kg dry matter per day, milk production increased by 65 %. Imagine what would be possible if milk production in developing countries could be increased in this way. It could prevent the untold suffering of people with protein deficiency (Francis *et al,* 1991; Foidl, *et al* 2001).

An extensive phytochemical screening done by Josephine showed that the aqueous extract of the leaves of *Moringa oleifera* contain a wide range of phytochemicals such as alkaloids, catechol tannin, garlic tannin, steroids, triterpenoids flavonoids, saponin, anthraquinone, and reducing sugar. Other works indicate that it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates reported to have hypotensive, anticancer, and antibacterial activity. Fahey Jed and co-workers at John Hopkin University found that the antimicrobial compounds isolated from *Moringa oleifera are* effective against *Helicobacter pylori E.coli*, *Pseudomonas aeroginosa*, *staphylococcus aureus* and *salmonella typhii*. (Jed, W, 2005, Galan *et al,* 2004; Vinoth, *et al* 2012). Much work has been done on the various parts of the plant, but none of them compared the abilities of the various parts, the fulcrum of this article.

MATERIALS AND METHODS

Chemicals and instruments

The chemicals were of analytical reagent grade, products of Sigma Chemical Company (St. Louis, MO) and tablet of metformin® were locally purchased. The absorbance was recorded by UV-Visible spectrophotometer (Agilent 8453 t technologies, Hewlett-Packard, Germany) with Agilent Chem Station software (Agilent, Palo Alto, USA) running on a Compaq compatible personal computer (Hewlett-Packard, Obregon, Mexico). The Chem Station also consists of an interface bus for data acquisition and a pin writer HP DeskJet 5652 printer. Water bath (RE300B, Baloworld Scientific Ltd, UK), Rotary evaporator (Telsar, Model Cryodos-8, Spain), Oven (Search Tech Instrument, Model 9101 ISA, China), Freeze dryer (Yorog Model RE-52, Japan), Randox Glu-PAP kits (Bayer Diagnostic, India), Weighing balance (Metler Toledo, AL 2004, China), Oxalate fluoride bottle, Spectrophotometer

Animals

One hundred and twenty (120) Wister rats of both sexes (130 - 150 g) were used for the study. The animals were cared for following Institute of Laboratory Animal Research (ILAR) guidelines. The animals were housed in a properly ventilated animal centre of the College of Medicine of the University of Lagos, Nigeria. They were allowed to acclimatize for 4 weeks before to experimentation under standard laboratory conditions 12:12 h dark/light cycle) with access to a commercial pellet diet and water ad libitum.

Sample Preparation

Moringa oleifera leaves, pods and seeds, harvested from Ilorin (Kwara State), identified and authenticated at the University Herbarium in the Department of Botany, University of Lagos by Mr. O. O. Oyebanji then vouchered by Miss Sulihat. The Herbarium number assigned is LUH 5905. They were washed air-dried at room temperature on the laboratory benches in the Department of Pharmaceutical Chemistry, University of Lagos. The dried samples were pulverized separately with an electric blender and stored in a container protected from light.

Extraction of Sample

Each sample was separately extracted by warm maceration method with 2 Litre of 30 % aqueous ethanol solution for 48 hours. The resultant extract was filtered with cheesecloth and concentrated in a rotary evaporator at 60 ^{0} C under reduced pressure at 3000 rpm. The reduced volumes were freeze-dried to obtain a solid mass which were stored in amber bottles at 4 ^{0}C until used. The leaves (657.50 g) yielded 85.70 g (13.03 %) of solid mass; the pod (310.50 g) yielded 36.50 g (11.76 %) of solid mass and the seed (410.00g) yielded 69.20 ml, 94.8 g (23.12 %) of yellowish oil.

Qualitative Phytochemical Screening

Standard analytical procedures as described by; (Sofowora, 1993; AOAC. 2015; Mbang *et al,* 2020) were adopted for the identification of phytochemical constituents.

Determination of Blood Glucose

Trinder's method was adopted (Trinder, 1969; AOAC. (2015).) as cited by Halima et al (Halima *et al,* 2011,David *et al,* 4th Edition) which is based on the Glucose-Oxidase-Peroxidase Principle ( ) Blood samples was collected from the eyes of diabetic and normoglycaemic animals separately into a fluoride oxalate bottle using a sterilized capillary tube. The blood samples were centrifuged at 2500 rpm for 15 minutes and the plasma was into separate a plain bottle with the aid of a Pasteur pipette (Mbang *et al*, 2020). The plasma was kept in the fridge at 4 ^{0}C until assay. Subsequently, 20 µL of each plasma was pipetted using a cuvette with the aid of a micropipette followed by 200 µL of freshly prepared Randox reagent. The mixture was mixed thoroughly, incubated at room temperature for 10 minutes and the absorbance was

taken against the reagent blank at a wavelength of 546 nm. The procedure was repeated for 20 µL of standard glucose.

Oral Glucose Tolerant Test (OGTT) for Normoglyceamic Rats

An effective dose of 200 mg/kg, established for *Moringa* leaves extract was adopted (Dolly, 2009, Manohar, *et al* 2012). However, there was none for the seed and pod. Therefore a pre-treatment experiment was conducted to establish the effective dose with seed and pod extracts by OGTT method. The animals were divided into 8 groups (A-H) of 15 rats each fasted for 10 hours overnight after which the Fasting Blood Glucose level was taken. Group A was administered with 1 mL normal saline as control group, B (standard group) was administered with 10 mg of metformin per kg body weight, group C was administered with 200 mg of *Moringa pod* extract per kg body weight, group D was administered with 300 mg of pod extract per kg body weight, group E administered with 400 mg of pod extract per kg body weight, group F was administered with 2 mL of seed extract per kg body weight, Group G was administered with 3 mL of seed extract per kg body weight while Group H was administered with 4 mL of seed extract per kg body weight. Basal blood glucose level (BGL) was taken 90 minutes after dosing with the prepared variable extracts and recorded as '0-hour' value. The rats were then loaded with 2000 mg glucose per kg body weight by oral administration using oral cannula and their glucose tolerance was studied for 4 hours at regular intervals f 1 hour each. The percentage reduction in blood glucose at different intervals after glucose load was estimated (Oshinubi *et al*, 2006) using the relationship;

$$\frac{P_0 - P_I}{P_0} \times 100\%$$

Where P_0= initial plasma glucose or post glucose loading, P_i = interval plasma glucose. The percentage change in the plasma glucose of each group was compared with each other.

Induction of Experimental Diabetes

Another set of albino Wistar rats were grouped into six (groups A- F) containing 15 animals per group. Groups A- E were induced by a single intraperitoneal injection of freshly prepared solution of alloxan-monohydrate by administering a volume that corresponds with a dose of 100 mg per kg body weight calculated for each animal from;

Volume of alloxan solution = $\frac{W \times D}{C}$

Where W= weight of animals in kg, D = dose of alloxan to be administered, C = concentration of alloxan monohydrate solution prepared (Okeke, 2006). The animals were observed within 72 hours for diabetic symptoms such as frequent urination, thirst, blood glucose above 200 mg/dL and loss of weight. Basal blood glucose of the animals was taken and their treatment with the appropriate extract began as soon as they were noticed to be diabetic. The diabetic study was carried out for 28 days with oral administration of extracts once per day with the aid of an oral cannula (Dolly, 2009; Okeke, *et al*, 2006). Each group was treated as designed; A was treated with 200 mg of *Moringa* leaves extract per kg body weight, B was treated with 4 mL of seed oil per kg body weight, C was treated with 300 mg of pod extract per kg body weight, D (diabetic control) was treated with 1 mL normal saline, E (Standard) was administered with 10 mg of metformin per kg body weight while F (Normal control) was administered with 1 ml normal saline. At every interval of 7 days, FBG was obtained by collecting blood samples from the eyes and analysed.

Histopathological Examination

After 28 days, the rats were sacrificed by cervical dislocation and the pancreases were extracted from the liver and fixed in 10 % formalin solution for sectioning (5 µm), staining with haematoxylin and eosin to enable histopathological examination. The photo-micrograph of each pancreas was taken observed and interpreted.

Data Analysis;

Graph Pad Prism software was used for statistical analyses of data. Data obtained for OGTT and anti-diabetic studies were tabulated as the mean ±SEM and subjected to statistical analyses using t-test and ANOVA as applicable. At the end of the experiment, the results obtained for each group were compared based on Dun's Multiple Comparison Method as well as a Turkey multiple Comparison Method using Student's t-Test. Differences were considered significant at $P < 0.001$ or $P<0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

The extracts of leaves, seeds, and pods were screened for some selected phytochemical constituents using standard methods. The results are shown in Tables 1-5.

Table 1: Phytochemical Screening

Constituents	Leaves extract	Seeds extract	Pods
Alkaloids	+++	+++	+++
Flavonoids	+++	++	++
Flavanol	++	+	++
Steroidal	++	++	-
Triterpenoidal	++	++	-
Saponin	-	-	++
Tannin	++	-	-
Cardiac glycosides	+	-	++
Free anthraquinone	++	+	+
Anthraquinone-o-glycoside	+++	+	++
Anthraquinone–c-glycoside dianthrones	++	++	++
Free Reducing sugar	+	+++	+++
Glycosides	++	++	++
Volatile oil	-	+++	+

Key; below detection limit = -, low detection = +, moderate detection = ++, abundant detection = +++

Assessement of Effective Dose by OGTT

Table 2: Oral Glucose Tolerance Test (OGTT)

Groups	FBG (mg/dl)	0hour (mg/dl)	1hour (mg/dl)	2hour (mg/dl)	3hour (mg/dl)	4hour (mg/dl)
A (1ml saline)	79.63±1.26	78.73±1.04	130.6±0.74	118.10±1.74	112.90±2.23	96.76±1.16
B (standard)	91.75±6.12	87.99±6.07	112.1±1.13	98.43±2.43	75.42±2.58	63.88±1.18
C(200g/kg Pod)	82.70±3.32	71.82±0.87	135.4±7.76	111.40±1.51	91.07±5.46	73.77±1.48
D(300mg/kg Pod)	77.05±1.78	69.60±0.80	134.5±3.28	109.20±2.99	84.48±3.43	70.58±0.52
E (400mg/kg pod)	79.49±2.98	72.60±1.51	125.4±3.97	113.40±1.51	83.63±2.17	71.12±2.13
F(2ml/kg seed oil)	75.78±1.38	76.16±1.21	162.0±4.48	164.90±3.80	162.20±5.36	150.30±0.79
G (3ml/kg seed oil)	83.97±2.47	82.33±1.82	165.5±3.38	162.70±4.45	154.10±4.28	152.20±7.31
H (4ml/kg seed oil)	83.20±4.08	80.52±3.98	142.5±3.50	136.50±3.11	132.30±3.13	131.30±2.98

Table 3: Percentage Change in Blood Glucose at intervals

Group	Treatment	2hours	3 hours	4hours
A	1ml normal saline	9.59%	13.60%	25.94%
B	10 mg/kg metformin	12.17%	32.71%	43.00%
C	200 mg/kg pod extract	17.69%	32.72%	45.49%
D	300 mg/kg pod extract	18.84%	37.72%	47.54%
E	400 mg/kg pod extract	9.55%	33.31%	43.29%
F	2 ml/kg seed extract	-1.84%	-0.14%	7.19%
G	3 ml/kg seed extract	1.69%	6.91%	8.07%
H	4 ml/kg seed extract	4.22%	7.20%	7.86 %

Table 4: Anti-diabetic

Week Group	WK 0 (mg/dl)	WK 0 PPBG(mg/dl)	WK 1 FBG(mg/dl)	WK 2 FBG(mg/dl)	WK 3 FBG(mg/dl)	WK 4 FBG(mg/dl)
A leaves	*423.0+43.9	482.4+ 35.2	339.3+23.2	298.0+8.5	259.7+ 21.2	*212.3+ 3.2
B	501.0+ 0.7	574.2 +31.7	455+ 51.28	498.0+ 67.4	557.3+ 32.1	416.5+ 15.5
C	**492.8+ 5.2	547.2+ 20.8	263.0+ 4.3	165.7+ 3.5	139.0+ 2.19	**128.3+ 2.2
D	449.4+ 4.3	455.8 + 26.7	478.3+ 28.2	470.7 +19.4	533.3+ 15.4	569.0 + 0
E	**489.4+ 1.9	537.8+ 55.5	141.0+ 4.1	129.7+ 3.4	127.0 + 1.5	**121.0+1.2
F	101.3+ 3.5	107.0 + 2.5	103.8+ 11.1	89.5 + 3.0	88.5+ 2.2	116.8+ 16.3

Key: mean values ± SEM, n = 5, *p ≥ 0.05, **p ≥ 0.001

Group A was treated with 200 mg of *Moringa* leave extract per kg body weight, group B was treated with 4 ml of *Moringa* seed extract per kg body weight, C was treated with 300 mg of *Moringa* pod extract per kg body weight, D (diabetic control) was treated with 1 ml normal saline, E (standard) was administered with 10 mg of metformin per kg body weight while F (Normal control) was administered with 1 ml normal saline.

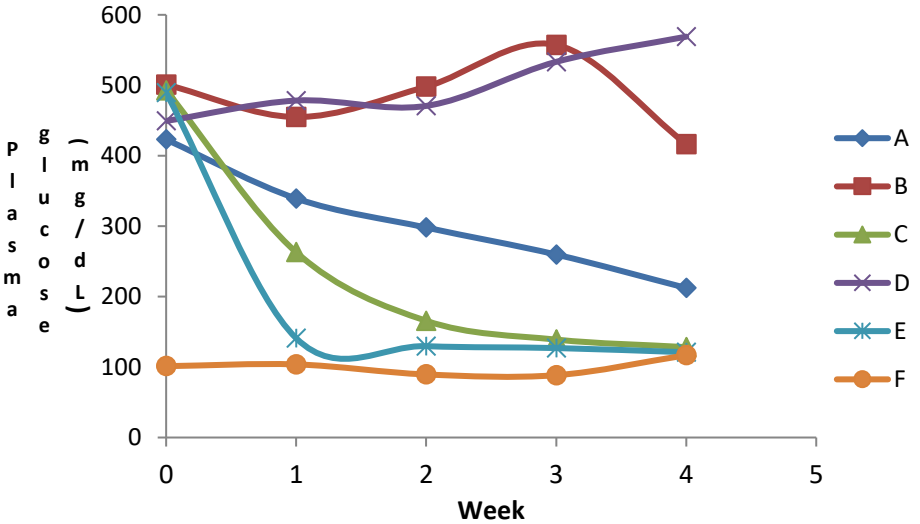
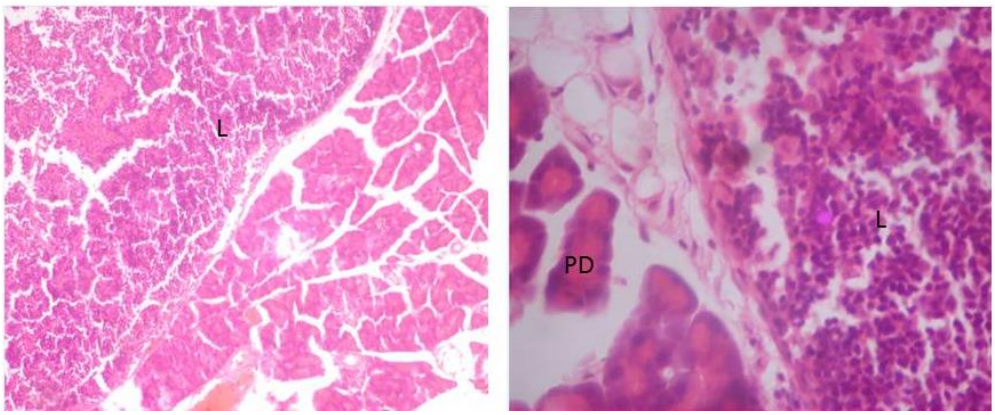


Figure 1: Graphical illustration of the Anti-hyperglycaemia activity

Key; Group A = 200 mg/Kg leave extract, Group B = 4 mL/ kg seed extract, Group C = 300 mg/Kg pod extract, Group D = negative control, Group E = standard Control (10 mg/kg metformin) and Group F= Norman control.

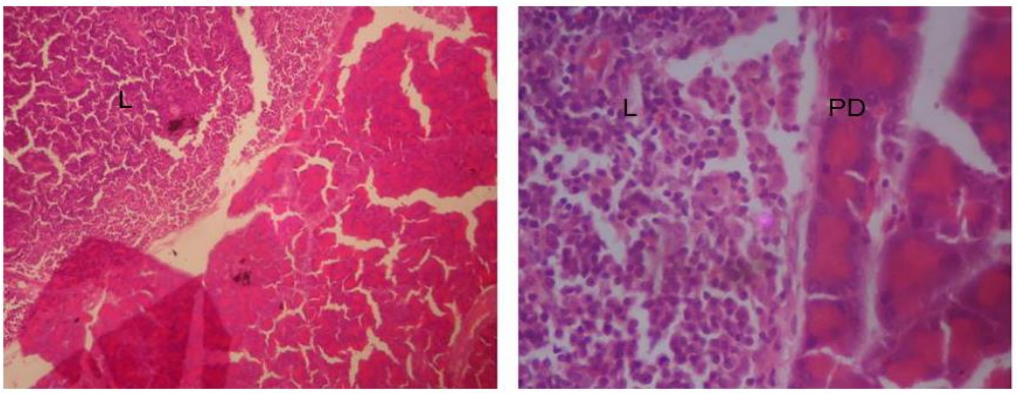
Histopathological Photo-Micrograph

The following information was derived after a careful examination of the photo-microgram of the pancreas of Wister rats used in the various groups. The magnification used is x 10 and x 40 in each case.



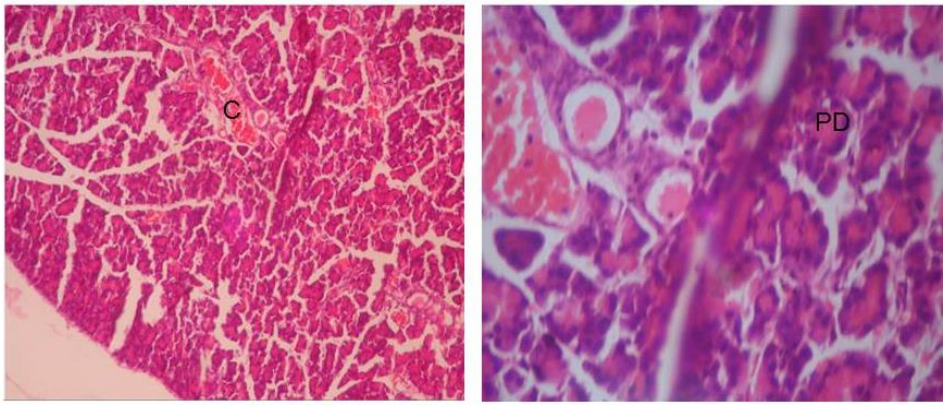
(a): x 10 (b): x 40

Figure 2: Photomicrograph of Group A



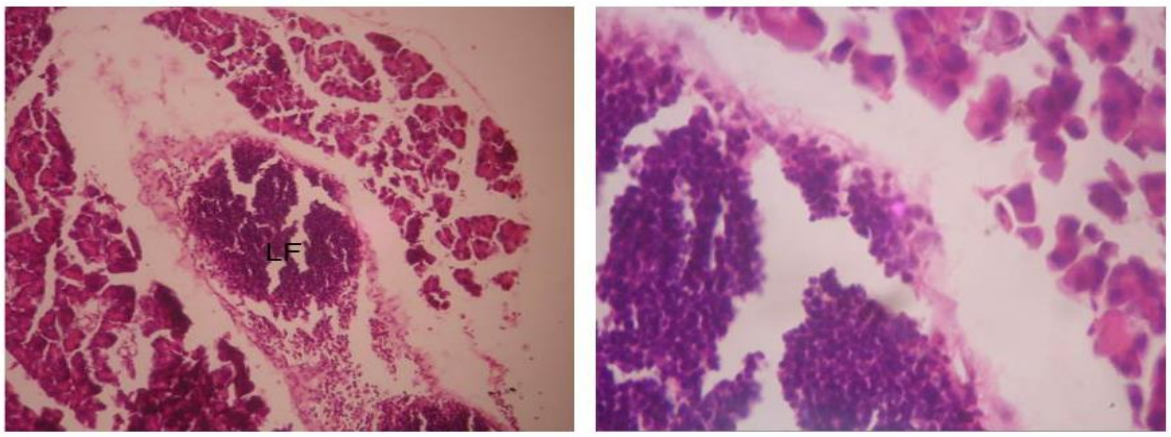
(a) x 10 (b) x 40

Figure 3: Photo-micrograph of the pancreas of Group B



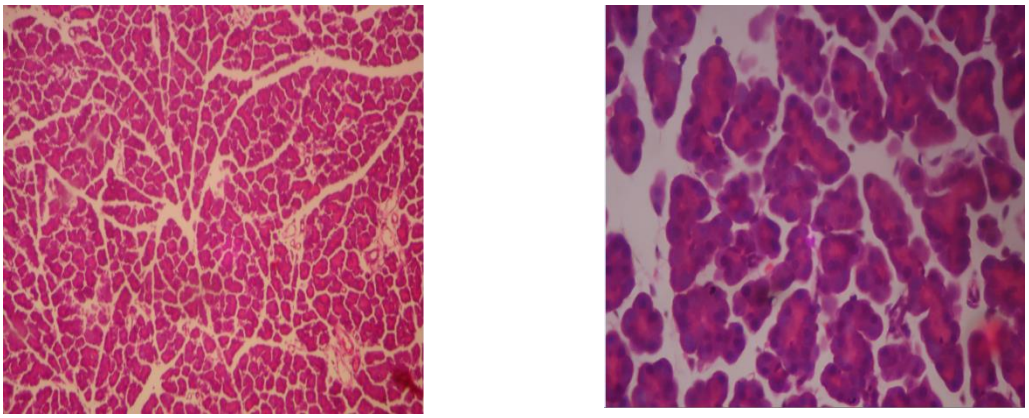
(a) x 10 (b) x 40

Figure 4: Photo-micrograph of Group C



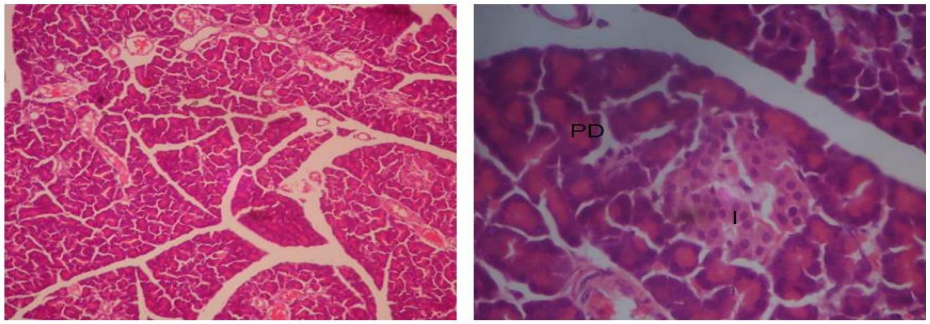
(a) x 10 (b) x 40

Figure 5: Photo-micrograph for the pancreas of group D



(a) x 10 (b) x 40

Figure 6: Photo-micrographs for the pancreas of group E



(a) x 10 (b) x 40

Figure 7: Photo-micrograph of the pancreas of group F

Discussion

The plant has been linked with medicinal significance due to its phytochemical contents (Josephine *et al* 2010; Jed, 2005). Table 1 above shows that alkaloids, flavonoids, flavanols and anthraquinones are high in the three samples. Cardiac glycosides are detected in leave and pod extract only. However, volatile oil content is high in seed extract, low in pod extract and below the detection limit in leave extract. Only pod extract has high saponin content.

We can deduce from Table 2 (OGTT) that 300 mg of pod extract per kg body produced better results.Viewing Table 3, the percentage of blood glucose inhibition of 300mg/kg body weight was 18.84 %, 37.72 % and 47.54 % at 2 hours, 3 hours and 4 hours respectively. For seed extract, 4 mL of seed extract per kg body weight which corresponds to the highest percentage change of 4.22 % , 7.20 % and 7.86 at 2 hours 3hr and 4 hours respectively after glucose load was found to be most suitable for the experiment. This was adopted in the antidiabetic study.

Table 3 shows the percentage reduction in blood glucose. **A**s shown, the standard drug (10 mg/kg body weight) reduced the blood glucose from 489.40 mg/dL to 121.00 mg/dL after 28 days of treatment. On the other hand 300 mg pod extract per kg body weight reduced the blood glucose level from 492.80 mg/dl to 128.30 mg/dL after 28 days of treatment whereas 200 mg of leave extract per kg body weight was able to reduce the blood glucose level from 423.00 to 212.30 mg/dL. However, the onset of action for metformin (10 mg/kg) is observed in the second week of the treatment this is earlier than that of the

pod. The result obtained for seed is erratic as seen on the graph (Figure 6)

Statistical analysis of the data showed that at **P < 0.05**, there was a statistically significant decrease in the blood glucose of the standard group, group A and group C between the first week and the 4th week of treatment. However, at $p \geq 0.001$ there is no statistical significant decrease in the blood glucose of group A but there is a statistically significant decrease in the blood glucose of the standard group and group C. Also by using Dun's comparison method ($P < 0.05$) there is no statistically significant difference between the anti-diabetic effects of the standard drug (metformin) and 300 mg of pod extract per kg body weight.

Fig 1 is a curve showing the result of the antidiabetic study. From the curve, seed extract (B) has no antiglyceamic activity as obvious from the rising pattern of curve B. Curves A, C and E drop until their values become almost stable. This clearly showed that both leaves and pods extracts have good antidiabetic activity comparable to metformin. However drastic falling nature of curve E indicated that the onset action of metformin is higher. The curve also clearly confirms that *Moringa oleifera* pod extract has more antidiabetic potency than the leave extract.

Figure 2 is the photomicrograph of a diabetic rat treated with 200 mg/kg aqueous ethanol extract of the leaves. The pancreatic ducts (PD) are degenerated and florid chronic inflammatory cells (predominantly lymphocytes, L) are seen. Although, there is rejuvenation of the organ since the pancreatic architecture here appears to be better than that of untreated diabetic rats Fig. 6).

Fig. 3 is the photo-micrograph showing the structure of the pancreas of a diabetic rat administered with an extract from *Moringa seed*. There are aggregates of florid lymphocytic infiltrates as a result of the inflammation of the organ. The pancreatic ducts PD and the islet cells were damaged and there is no sign of healing and regeneration.

Figure 4 shows the photo-micrograph of a diabetic rat treated with 300 mg/kg aqueous ethanol extract of *moringa oleifera* pod. It shows pancreatic duct PD lined by normal islet cells and very few lymphocytic infiltrate is seen. This appearance shows that there is a marked rejuvenation of the organ following the administration of the extract. Figure 5 below is the photo-micrograph for the pancreas of group D (an untreated diabetic rat). A florid aggregate of lymphocytes is seen forming lymphoid follicles LF. The pancreatic ducts PD are scattered and the islet cells are relatively damaged. Figure 6 is the photo-micrographs for the pancreas of group E (standard group), diabetic rats treated with 10 mg/kg metformin. There is rejuvenation of the pancreas and the islet cell. The pancreatic duct PD and the islet cells are seen. No lymphocytic infiltrate and no inflammation. Figure 7 is the photo-micrograph showing the architecture of the pancreas of a healthy rat from the normoglyceamic group F. Normal appearing pancreatic duct is seen with well-defined islet cells, no inflammation, and hence no lymphocytic infiltrate;

CONCLUSION

The study was carried out by inducing laboratory rats with diabetes. The condition was evident by increased blood glucose, increased volume of water taken, decreased food intake, loss of weight, persistent urination and weakness. OGTT assessment backed up with statistical evaluation and histopathological examination of the pancreas as shown in the photomicrographs. Figures 2-7) show that group A and C experienced pancreatic rejuvenation. That is, ethanol extract of Pod and leave exhibit antidiabetic activity. However, the effect of 300 mg/kg pod extract on the pancreas of group C appears to be more than that of 200 mg/kg leave extract on group B. The presence of active principle in the extracts may potentiate glucose-induced insulin secretion from existing β-cells, rejuvenation of β-cells or cause a reduction in the glucose generation from glycogen, thus decreasing serum glucose levels in treated diabetic rats. Phytochemical screening indicates the presence of flavonoids. Some flavonoids especially flavonols (e.g quercitin and kaefemprol) are reported to act as insulin secretagogues or insulinomimetic agents probably by influencing the pleiotropic mechanisms to attenuate diabetic complications (Rajnish *et al* 2012) Finally, it was found during the study that blood glucose level of rats (whether diabetic or normal) is erratic. This is indicated by high standard error in all the results obtained for the diabetic study and by fluctuating FBG obtained in some of the groups examined. On the otherhand the seed appears not to exhibit anti-hyperglycaemic activity but it contains an active principle that is capable of ameliorating the other complications of diabetes mellitus. This is obvious from the way the animals treated with seed extract remain active despite their hyperglycaemia condition throughout the experiment period. Since the plant is edible with a very low toxicity level (LD_{50} = 1300 mg/kg body weight for pod and LD_{50} = 1500 mg/kg body weight for leaves), it is ideal for patients with impaired blood glucose.

LIST OF ABBREVIATIONS

FBG; fasting Blood glucose, BBG; Basal Blood Glucose, OGTT- Oral Glucose Tolerance Test

RECOMMENDATION

During the pre-treatment stage (by OGTT), some of the rats died as a result of the volume of blood collected from the fasted animals as required by the method adopted. This method may not be the best for a study involving blood glucose determination at intervals within a short time. This is because such determination would require serial blood collections within a short time leading to draining and dehydration of the starved animals.

Secondly, further comparative anti-diabetic study should be carried out on the other parts of the plant; this should include its root and bark. The study should involve bioactivity – guided isolation, characterisation and structural elucidation of the various active principles. Probably this might lead to a novel oral anti-diabetic agent. Also, the subsequent study should include biochemical and haematological parameters since research findings show that diabetes has effect on these parameters. Therefore it is expected that a potent anti-diabetic preparation should have a positive effect on these parameters. Although the seed does not reduce the blood glucose level, animals in that group remain more active and healthier in spite despite their high blood glucose. The effect of *Moringa* seed on the other complications of diabetes should be investigated. Finally, *Moringa oleifera* should be incorporated into the meals of diabetic individuals and there should be general public sensitization about the importance of *Moringa oleifera*

ETHICAL APPROVAL

The animals were cared for in accordance with Institute of Laboratory Animal Research (ILAR) guidelines. *Moringa oleifera* leaves, pods and seeds, harvested from Ilorin (Kwara State, Nigeria), identified and authenticated at the University Herbarium in the Departmentof Botany, University of Lagos by Mr. O. O. Oyebanji then vouchered by Miss Sulihat. The Herbarium number assigned is LUH 5905.

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