Hypoglycemic, Hematologic and Hypolipidemic Activity of *Mucuna Pruriens* Ethanol Leaf Extract in Alloxan Induced Diabetic Rats

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Authors' contributions

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

Original Research Article

ABSTRACT

Aim: This study investigated the hypoglycemic, hematologic and hypolipidemic potentials of Mucuna pruriens ethanol leaf extract (MELE) in alloxan induced diabetic rats.
Study Design: Experimental Animal Model. The study was conducted in the Physiology Laboratory of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria between November, 2013 and February, 2014.
Methodology: Diabetic rats were divided into 4 groups of 7 animals each (groups 2-5). Group1 comprising of 7 normal rats received 0.2ml normal saline and served as the normal control group. Group 2 received no treatment and served as the diabetic control group. Group 3 was treated with a reference drug, Glibenclamide (5mg/kg) while groups 4 and 5 received 150 and 300mg/kg of MELE respectively. All treatments were done via the oral route and lasted for 21 days.
Results: Alldoses of MELE significantly (P<.05) lowered glucose levels in the diabetic rats with 300mg/kg lowering blood glucose from 311.80 ± 37.10 in diabetic rats to 91.30 ± 2.26 by the end of 21 days. The hypoglycemic effect of MELE compared favorably with that of Glibenclamide. Red blood cells (RBC) counts, packed cell volume (PCV),hemoglobin

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values were all significantly raised (P<.05) in treated rats, while the increased WBC value in diabetic rats was lowered. The levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were significantly (P<.05) decreased in the diabetic treated rats with increase in the levels of high density lipoprotein cholesterol (HDL-C).

Conclusion: These results suggest that MELE contain active principles with hypoglycemic, hematologic and hypolipidemic properties and could be valuable in managing diabetic mellitus and correcting the hematological and lipid profile abnormalities associated with the pathophysiology of the disease.

1. INTRODUCTION

As the days go by, many more wild plants are being exploited for medicinal purposes. To the indigenous users, this may be for reasons of cost, availability, accessibility and effectiveness but to the research scientist it is a sure and easy way to new drugs discovery. Little wonderOjeh et al. [1], reported that the primary aim of sourcing for plants drug through any of the known strategies is mainly to detect the active ingredients in plants that exert definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery. *Mucuna pruriens* is one of such plants that are being exploited. *Mucuna pruriens,*family *Fabaceae*is popularly known as velvet bean or cow-itch in English, Agbala or Ibie by the Igbos of South-eastern Nigeria and Karara by the Hausas of Northern Nigeria is a tropical legume found in Africa, India and the Caribbean. The plant is notorious for the extreme itchiness it produces on contact, particularly with the young foliage and seed pods. It is an annual climbing shrub with long vines that can reach over 15cm in length. The leaves are tripinnate and ovate, with grooves and pointing tips. It is a flowering plant which produces shiny black or brown drift seeds. The plants extracts have been used to treat snake bites, edema, intestinal worm infestations, diabetes, high blood pressure, high cholesterol, muscle pain and rheumatism [2].

Diabetes mellitus (DM) is a common disease associated with increased morbidity and mortality and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia, due to defective insulin secretion, insulin action or both, resulting in impaired carbohydrate, protein and lipid metabolism [3]. Among the pathophysiological anomalies associated with the condition are hyperglycemia and lipid profile abnormalities [3,4] and Anemia [5,6]. Treatment is based on oral hypoglycemic agents and insulin which have so many side effects. This study is therefore designed to evaluate the hypoglycemic, hematologic and hypolipidemic potential of *Mucuna pruriens* ethanol leaf extract (MELE).

2. MATERIALS AND METHODS

2.1 Plant Materials (Collection and Preparation)

Fresh leaves of *Mucuna pruriens* were collected from a farm settlement in Ozuitem, Bende Local Government Area of Abia State, Nigeria and were authenticated by Dr. M.C. Dike of

the Forestry Department, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Sample was deposited in the laboratory with voucher no: MOUAU/CVM/HB006.

The leaves were air dried in the laboratory for 7 days after which they were ground to powder using an electric blender. Thirty five grams (35g) of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent for 48 hours with temperature maintained at 78ºC. At the end of the period, the extract was dried in a laboratory oven at 40ºC to obtain a dried extract.

2.2 Animals

Adult albino rats of both sexes (150-180g) obtained from the Animal house of the University of Nigeria, Nsukka were used. They were fed with standard rat feed, with water *ad libitum* but starved for 12 hours prior to the commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory Animals (Pub. No. 85-23, Revised, 1985, as expressed by Akah et al. [3]. The study was conducted at the Physiology Laboratory of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2.1 Acute toxicity study (LD_{50}) determination

Thirty five mice of both sexes weighing 20-25g were divided into 7 groups of 5 mice each and were administered graded I.P doses of *M. pruriens* ethanolic extract in the order 500, 800, 1200 2000, 3000, 4000 and 5000 mg/kg body weight respectively. The mice were kept in aluminum cages and allowed free access to feed and water ad libitum. Observation was made for toxicity signs and number of deaths within 24 hours of administration. LD_{50} was then determined using Karber's method, expressed by Enigide et al. [7] as

$$LD_{50} \quad = \quad LD_{100} - \frac{\sum (Dd \times Md)}{N}$$

Where:

LD_{50} = Dose that killed 50% of animals in a group
LD_{100} = Dose that killed all animals in a group
$\sum(Dd \times Md)$ = Summation of all products of dose difference and mean deaths
N = Number of animals in each group

2.3 Induction of Diabetes

Diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (160mg/kg). Eight days later rats with blood glucose concentration above 190mg/dl were considered diabetic and 35 of such rats were used for the study.

2.4 Blood Glucose Level, Hematological and Lipid Profile Studies

While 7 normal rats were placed in group 1 to serve as the positive control, 28 diabetic oneswere divided randomly into 4 groups (groups 2-5). Group 1 received 0.2ml normal

saline. Group 2 which served as the negative control received no treatment. Group 3 was treated with Glibenclamide (5mg/kg), while groups 4 and 5 received 150 and 300mg/kg of MELE respectively. All treatments were done daily via the oral route and lasted 21 days.

2.5 Acute and Sub-Acute Effect of Meleon Blood Glucose Levels

On the first day of treatment blood was obtained from the tail of each rat in all groups (1-5) by tail snip method prior to and at 2 and 5 hours following treatment and glucose levels were determined for each rat using a glucose meter following standard procedures prescribed by the producer, Roche diagnostic Company, Germany. For the sub-acute studies, the tests were repeated on day 7, 14 and 21.

2.6 Hematological Studies

All rats were sacrificed on the 22nd day and blood was collected by cardiac puncture into EDTA bottles to be used for the determination of hematological parameters including: Red blood cell (RBC) counts, Packed cell volumes (PCV), Hemoglobin (Hb) Concentrations, White blood cell (WBC) counts, White blood cell differential counts, Platelets counts, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC). These parameters were obtained at once for each blood sample using an Automated Hematology Analyzer produced by Mindray Company, China. (MC, 2800)

2.7 Lipid Profile Studies

Four milliliters of each blood sample was centrifuged to obtain a clear plasma which was used to estimate total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) using commercial kits and following standard procedures outlined by the producer, Randox Laboratories, UK.

2.8 Statistical Analysis

Results were expressed as mean $\pm$ SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA), students t-test at 95% level of significance was used to assess significant difference between controls and treated group.

3. RESULTS

3.1 Extract yield and Acute Toxicity

A solid extract weighing 11.6g was obtained after soxhlet extraction which represented a percentage yield of 33.1%, while an Acute toxicity study using Karber's method yielded an LD_{50} value of 1950mg/kg body weight. Acute and sub-acute effects of MELE on Blood glucose levels in diabetic rats. All doses of MELE significantly ($p<0.05$) decreased blood glucose levels in the diabetic treated rats within the 5 hours of the acute study. By the end of the period 150 and 300mg/kg of MELE had reduced glucose levels in diabetic rats by 41.3 and 32.0% respectively. The effect of MELE compared favorably with that of Glibenclamide (5mg/kg) (Table 1). By the end of the 21 days of sub-acute study MELE successfully returned blood glucose levels in diabetic rats to normal values. The result of the sub-acute

effect was also significantly (p<.05) different from that of the negative control group but compared favorably with the effect of Glibenclamide (Table 2).

Table 1. Acute Effect of MELE on blood glucose level in diabetic rats

Group	Treatment	0HR	2HRS	5HRS
		Glucose level (mg/dL)	**Glucose level mg/dL**	**Glucose level mg/dL**
1	Normal control	110.40 $\pm$7.10	107.80 $\pm$4.52	101.40 $\pm$3.90
2	Diabetic control	311.80 $\pm$37.10	307.80 $\pm$34.30	311.60 $\pm$51.40
3	Glibenclamide 5mg/kg	288.20 $\pm$37.50*	206.00 $\pm$37.00*	150.60 $\pm$10.50*
4	MELE, 150mg/kg	230.00 $\pm$10.24*	238.60 $\pm$4.67*	135.50 $\pm$9.15*
5	MELE, 300mg/kg	224.20 $\pm$19.10*	220.40 $\pm$17.40*	152.40 $\pm$ 8.82*

*P<.05 versus diabetic control

Table 2. Sub-acute effect of MELE on blood glucose level in diabetic rats

Group	Treatment	Day 7	Day 14	Day 21
		Glucose level in mg/dL	**Glucose level inmg/dL**	**Glucose level in mg/dL**
1	Normal control	98.40 $\pm$4.69	102.00 $\pm$2.29	103.40 $\pm$2.29
2	Diabetic control	377.40 $\pm$56.30	323.20 $\pm$27.87	318.00 $\pm$25.30
3	Glibenclamide 5mg/kg	104.40 $\pm$6.34*	80.80 $\pm$5.21*	82.13$\pm$4.80*
4	MELE, 150mg/kg	110.80 $\pm$7.77	94.40 $\pm$5.45*	97.11$\pm$3.84*
5	MELE, 300mg/kg	108.60 $\pm$3.18*	90.60 $\pm$4.27*	91.30$\pm$2.26*

*P<.05 versus diabetic control

3.2 Effects of MELE on Hematological Parameters

All doses of MELE significantly (P<0.05) raised RBC, PCV, and HB values (Table 3) and lowered WBC counts in the diabetic treated rats. MCV, MCH, MCHC, Platelets, Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils values were not significantly affected (Tables 3 and 4).

3.3 Effect of MELE on Lipid Profile in Diabetic Rats

The elevated total cholesterol, triglycerides, LDL-C and VLDL-C in diabetic rats were significantly (P< .05) lowered by all doses of MELE while the lowered HDL-C was raised (Table 5).

Table 3. Effect of MELE on RBC, PVC, HB, MCV, MCH and MCHC in diabetic rats

Group	Treatment	RBC x 10^{12} per liter	PCV(%)	HB (g/dL)	MCV (fL)	MCH (pg)	MCHC(g/dL)
1	Normal control	6.80±0.26	40.97±2.43	12.4±0.53	60.23±3.67	18.23±0.29	30.50±1.71
2	Diabetic control	4.77±0.17	30.10 ±1.81	10.03±0.31	63.40 ±3.96	21.03±1.14	33.50±2.20
3	Glibenclamide,5mg/kg	5.10±0.25	30.97±1.61	10.30±0.26	60.73±4.32	20.20±0.18	33.26±1.05
4	MELE, 150mg/kg	6.79±0.70*	39.87±0.60*	12.70 ±0.30*	58.73±1.32*	18.70±0.57*	31.83±0.26
5	MELE, 300mg/kg	7.25±0.74*	41.90±1.37*	13.07±033*	57.83±1.28*	18.07±0.13*	31.23±0.91

P<.05 versus diabetic control

Table 4. Effects of MELE on platelet counts, WBC and differential WBC counts in diabetic rats

Group	Treatment	Platelets x 10^9/L	WBC x 10^9/L	Lymphocytes %	Neutrophils %	Midcells (Eosinophils, Monocytes & Basophils) %
1	Normal control	909 ± 227	9.76±2.97	46.57±5.93	33.87±7.98	19.70±1.63
2	Diabetic control	611 ± 102	29.63±2.29	51.31±0.57	27.43±2.06	21.57±2.03
3	Glibenclamide, 5mg/kg	571 ± 103	17.7±0.44*	46.97±1.88*	29.77±0.24*	23.20±1.68
4	MELE, 150mg/kg	610 ± 48	9.73±0.24*	53.40±5.10	29.07±1.44	17.53±5/86
5	MELE, 300mg/kg	737± 16	7.30±1.34*	35.60±2.17*	41.17±4.14*	23.23±2.115

* P< .05 versus diabetic control*

Table 5. Effect of MELE on lipid profile in diabetic rats

Group	Treatment	Total Cholesterol mg/dL	Triglyceridesmg/dL	HDL-C mg/dL	LDL-C mg/dL	VLDL-C mg/dL
1	Normal control	81.29±0.32	37.97±0.64	40.26±0.73	33.44±0.84	7.59±0.13
2	Diabetic Control	158.10±3.27	70.83±0.82	17.05±0.53	126.90 ±3.43	14.17±0.18
3	Glibenclamide 5mg/kg	52.10±1.69*	41.68±0.42*	35.32±0.51*	8.44±1.97*	8.34±0.08*
4	MELE, 150mg/kg	68.23±0.26*	39.46±0.23*	31.39±0.60*	28.95±0.73*	7.89±0.05*
5	MELE, 300mg/kg	52.25±0.70*	29.76±0.47*	22.18±0.33*	24.12±0.65*	5.95±0.10*

* P<.05 versus diabetic control*

4. DISCUSSION

A percentage yield of 33.1% attests to the high amount of phytochemicals present in *Mucuna pruriens* leaves, while an LD_{50} value of 1950mg/kg body weight indicate that MELE has mild toxicity and could well be tolerated at low doses. This informed the dose design for this work and ensured that minimal doses which could produce significant effects were used. The administration of Alloxan monohydrate successfully induced hyperglycemia in the rats used. Hyperglycemia is usually the first sign in the development of diabetes mellitus. Alloxan monohydrate achieved this effect by selectively destroying the pancreaticbeta cells of the islets of langerhans in the rats. This marked degeneration of the islets lowered insulin secretion with reduction in the rate of conversion of glucose to glycogen, the result of which is the marked increase of sugar levels in the diabetic rats. These results obtained agree with already existing literature by Eddouks et al. [8], who reported that Alloxan induces diabetes by destroying the beta cells of the pancreas which are involved in the synthesis, storage and release of insulin, the peptide hormone regulating carbohydrate and lipid metabolism leading to high blood sugar levels. These high sugar levels confirm the development of diabetes mellitus [5].

All doses of MELE significantly (P<.05) lowered blood glucose levels within the 5 hours of acute study and returned the blood glucose levels in diabetic rats to normal values by the end of 21 days of treatment. The effect of MELE compared favorably with that of Glibenclamide, a reference hypoglycemic drug used. The results therefore suggest that MELE contain active principles with hypoglycemic properties. The extract may have achieved this hypoglycemic effect by increasing insulin selection and peripheral utilization of glucose in diabetic rats, inhibition of endogenous glucose production, inhibition of intestinal glucose absorption and/or regenerating existing beta cells. These mechanisms have all been reported to be responsible for lowering blood sugar levels [8,9,10].

Results of the hematological studies indicate that anemia is a pathophysiology associated with diabetes mellitus as the diabetic rats had significantly (P< .05) lowered RBC, PCV, HB, MCH, MCV and MCHC values when compared to the normal control rats. The results agree with [5,6] and [10], who all reported that in diabetes mellitus, there is the development of anemia, particularly, the hypochromic type, due to fall in the iron content of the body resulting from oxidation stress associated with the condition. All doses of MELE restored the values of these parameters to normal in the diabetic treated rats by the end of the 21 days of treatment, suggesting that MELE has anti-anemic activity. This effects of MELE could be due to its high iron content [6] and/or the ability to improve bone marrow functions [11].

In the lipid profile studies, the elevated total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) with decreased high density lipoprotein cholesterol (HDL-C) observed in the untreated diabetic group agreed with existing literature report that the development of diabetes mellitus is usually accompanied by marked increase in blood cholesterol, triglycerides, LDL-C, VLDL-C and a reduction in HDL-C. [3].The lowering of cholesterol, triglycerides, LDL-C and VLDL-C and increase in HDL-C observed in the groups treated with 150 and 300mg/kg of MELE is indicative of the presence of principles with hypolipidemic properties in the extract. [12] had reported the presence of saponin in the leaf extract of *Mucuna pruriens* and saponin has been reported to possess the ability to reduce blood cholesterol [13].

5. CONCLUSION

The results obtained from this study has shown that the ethanol leaf extract of *Mucuna pruriens* (MELE) may contain principles with hypoglycemic properties and may also be a safe and potent agent capable of restoring to normal, the hematological and lipid profile anomalies associated with diabetes mellitus and so could be used for such purposes.

Competing Interest: Authors hereby declare that no competing interest exist as far as this work is concerned.

Ethical Approval: Authors declare that this work is not against public interest and that all animal experiments were conducted in line with the NIH guidelines for care and use of laboratory animals, Pub. No. 85-23, Revised, 1985 and approved by the University's Animal Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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