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Hypoglycemic, hematologic and hypolipidemic activity of *Jatropha tanjorensis* ethanol leaf extract in alloxan induced diabetic rats

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ABSTRACT

This study investigated the hypoglycemic, hematologic and hypolipidemic potentials of Jatropha tanjorensis ethanol leaf extract (JELE) in alloxan induced diabetic rats using experimental animal model. Group1 comprising of 7 normal rats received 0.2ml normal saline and severed as the normal control group. Alloxan induced diabetic rats were divided into 4 groups of 7 animals each (groups 2-5). Group 2 received no treatment and served as the diabetic control. Group 3 was treated with a reference drug, Glibenclamide (5mg/kg) while groups 4 and 5 received 150 and 300mg/kg of JELE respectively. All treatments were done via the oral route and lasted for 21 days. Results obtained indicate that all doses of JELE significantly (P<0.05) lowered glucose levels in the diabetic rats with 300mg/kg lowering blood glucose from 311.80 ± 37.10 in diabetic rats to 95.95 ± 2.90 by the end of 21 days. The hypoglycemic effect of JELE compared favorably with that of Glibenclamide. Red blood cells (RBC) counts, packed cell volume (PCV), hemoglobin 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<0.05) decreased in the diabetic treated rats with increase in the levels of high density lipoprotein cholesterol (HDL-C). These results suggest that JELE contains active principles with hypoglycemic, hematologic and hypolipidemic properties and could be of value in the management of diabetic mellitus and associated anemia and lipid abnormalities.

Key words: Glucose, Hypoglycemic, Hypolipidemic, *Jatropha tanjorensis*, Rats.

INTRODUCTION

In many parts of the world, the exploitation of wild plants for medicinal purposes is been encouraged. This may be for reasons of cost, availability, accessibility and effectiveness. Beyond these reasons however, ethno pharmacology seems to be a major link between research scientists and new drug discoveries, since in most cases it narrows down the researcher's job, making it easy for him to pursue a clear cut objective. No wonder [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. *Jatropha tanjorensis* is one of such plants that are being investigated for their physiological and pharmacological dynamics. The plant belonging to family *Euphorbiaceae* is a common weed of field crops in the higher rainfall forest zones of West

Africa. In Nigeria, it is commonly called hospital too far or catholic vegetable. The leaf is a commonly consumed vegetable in many parts of southern Nigeria [2], where it is considered a natural remedy against diabetes mellitus. Extracts from the leaves of the plant have also been used to treat malaria infection and hypertension in some parts of Nigeria [2, 3].

The plant leaves were initially and popularly consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume and hence employed in anemia treatment. However the plants popularity has been doused by unproven claims that the whitish latex emanating from the leaf, stem and stalk may be toxic to man [4].

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 [5]. Among the pathophysiological anomalies associated with the condition are hyperglycemia and lipid profile abnormalities [5, 6] and Anemia [7, 8]. Treatment is based on oral hypoglycemic agents and insulin which in most cases have so many side effects. This study is therefore designed to evaluate the hypoglycemic, hematologic and hypolipidemic potentials of *Jatropha tanjorensis* ethanol leaves extract (JELE).

MATERIALS AND METHODS

2.1 Plant materials (Collection and Preparation)

Fresh leaves of *Jatropha tanjorensis* were collected from a settlement in Owerri-aba, Ugwunagbo Local Government Area of Abia State, Nigeria. The leaves were dried under shade in the laboratory for 7 days after which they were ground to powder using an electric blender. Thirty five (35) grams 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 70^0C. At the end of the period, the extract was dried in a laboratory oven at 40^0C to obtain a dried extract weighing 9.6g and represented a yield of 27.40%.

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 [5]. 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.

2.3 Acute toxicity study (LD_{50})

Thirty five mice of both sexes weighing 20-25g were divided into 7 groups of 5 mice and were assigned graded oral doses of *Jatropha tanjorensis* in the order 500 1000, 2000, 3000, 4000, 5000 and 6000 mg/kg body weight. The mice were kept in aluminum cages and allowed free access to feed and water. Observation was made for toxicity signs and number of deaths at the end of 24 hours. LD_{50} was determined according to Karber's method, as expressed by Enigide *et al.*, (2013) [9].

2.4 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.

2.5 Blood glucose level, hematological and lipid profile studies

While 7 normal rats were placed in group 1 to serve as the normal control, 28 diabetic ones were divided randomly into 4 groups (2-5) of 7 rats each. Group 2 which served as the diabetic control received no treatment. Group 3 was treated with Glibenclamide (5mg/kg), while groups 4 and 5 received 150 and 300mg/kg of JELE respectively. All treatments were done daily via the oral route and lasted 21 days.

2.6 Acute and sub-acute effect of JELE on 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.

2.7 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 –MC-2800 (Mindray Company, China).

2.8 Lipid Profile Studies

A portion 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.

2.9 Statistical Analysis

Results were expressed as mean ± 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.

RESULTS

3.1 Acute toxicity

Seven groups of 5 rats each were administered varying doses of *JELE*. No death was recorded at the end of the 24 hours of study, even at the highest dose of 6000 mg/kg body weight. The mice instead had normal disposition both physically and mechanically with no observed signs of toxicity.

3.2 Acute and sub-acute effects of JELE on Blood glucose levels in diabetic rats

All doses of JELE significantly (P<0.05) lowered 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 JELE had reduced glucose levels in diabetic treated rats by 42.75 and 41.58% respectively. The effect of JELE compared favorably with that of Glibenclamide (Table 1). By the end of the 21 days of sub-acute study the glucose levels of all diabetic rats treated with JELE returned to about normal values and was also significantly (P<0.05) different from that of the diabetic control group but compared favorably with that of Glibenclamide (Table 2).

Table 1: Acute Effect of JELE on Blood Glucose Level in Diabetic Rats

		0HR	2HRS	5HRS
Group	**Treatment**	**Glucose level (mg/dL)**	**Glucose level mg/dL**	**Glucose level mg/dL**
1	Normal control	110.40 ±7.10	107.80 ±4.52	101.40 ±3.90
2	Diabetic control	311.80 ±37.10	307.80 ±34.30	311.60 ±51.40
3	Glibenclamide 5mg/kg	288.20 ±37.50*	206.00 ±37.00*	150.60 ±10.50*
4	JELE, 150mg/kg	251.20 ±6.56*	213.40 ±5.49*	143.80 ±24.70*
5	JELE, 300mg/kg	222.00 ±13.34*	207.80 ±11.40*	129.70 ± 15.50*

**P< .05 versus diabetic control*

Table 2: Sub-acute effect of JELE on blood glucose level in diabetic Rats

		Day 7	Day 14	Day 21
Group	**Treatment**	**Glucose level in mg/Dl**	**Glucose level in mg/dL**	**Glucose level in mg/dL**
1	Normal control	98.40 ±4.69	102.00 ±2.29	103.40 ±2.29
2	Diabetic control	377.40 ±56.30	323.20 ±27.87	318.00 ±25.30
3	Glibenclamide 5mg/kg	104.40 ±6.34*	80.80 ±5.21*	82.13 ±4.80*
4	JELE, 150mg/kg	117.00 ±3.66	103.00 ±2.84*	101.30±2.90*
5	JELE, 300mg/kg	116.80 ±3.51*	93.80 ±4.25*	95.95 ±3.81*

**P< .05 versus diabetic control*

3.2 Effects of JELE on Hematological Parameters

All doses of JELE significantly (P<0.05) increased RBC, PCV, and HB values (Table 3) and lowered WBC counts in the diabetic treated rats. MCV, MCH, MCHC, Platelets, Lymphocytes, Neutrophils and Midcells (monocytes, eosinophils and basophils) were all significantly (P< 0.05) affected and tilted towards normal values (Tables 3 and 4).

Table 3: Effect of JELE 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	JELE, 150mg/kg	7.18 ±0.15*	40.37±0.50*	13.23 ±0.35*	56.20 ±0.61*	18.47±0.57*	31.63±0.29
5	JELE, 300mg/kg	6.85±0.57*	37.63±2.31*	11.90±0.78*	55.23±2.53*	17.50±0.47*	31.63±0.57

**P<.05 versus diabetic control*

TABLE 4:Effects of JELE 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	JELE, 150mg/kg	850 ± 95.30	10.50±1.77*	44.53±4.33	36.80±3.27	18.67±6.90
5	JELE, 300mg/kg	744 ± 82.90	8.60±0.24*	52.40±5.61*	26.50±6.05*	21.10±0.70

** P< .05 versus diabetic control*

3.3 Effect of JELE 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 5:Effect of JELE on lipid profile in diabetic rats

Group	Treatment	Total Cholesterol mg/Dl	Triglycerides mg/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	JELE, 150mg/kg	57.62 ±0.28*	33.95 ±0.63*	20.85 ±0.76*	29.98 ±0.52*	6.79 ±0.13*
5	JELE, 300mg/kg	57.27 ±0.20*	37.90 ±0.38*	23.22 ±0.46*	26.47 ±0.42*	7.58 ±0.08*

** P< .05 versus diabetic control*

DISCUSSION

The Acute toxicity (LD_{50}) results indicates that JELE is a plant extract of low toxicity and could be well tolerated even at high doses, as all animals administered the extract during the acute toxicity studies looked physically strong and emotionally relax with no observed signs of toxicity even at a dose of 6000mg/kg body weight. at low to moderate doses and accounts for their use in most parts of the world for the management of diseases. The result agrees with [3], who reported that animals administered *Jatropha tanjorensis* showed no overt signs of distress or toxicity even at a dose of 8000mg/kg body weight. This accounts for plant's use over the years in traditional medicine to treat diseases without any reported case of toxicity or death [10].

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 pancreatic beta 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. [11], had 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. This high sugar levels confirms the development of diabetes mellitus [7].

All doses of JELE significantly (P< .05) lowered blood glucose levels within the 5 hours of acute study and returned the blood glucose levels in diabetic rats to about normal values by the end of 21 days of treatment. Result therefore suggests that JELE contain active principles with hypoglycemic properties. This hypoglycemic effect may have been achieved 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 [11, 12, 13].

Anemia was found to indeed be associated with diabetes mellitus as the diabetic control rats all had significantly (p< .05) lowered RBC, PCV, HB, MCH, MCV and MCHC values when compared to the normal control rats. The results agree with [7, 8, and 14], who 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 JELE restored the values of these parameters to normal in the diabetic treated rats after days of treatment, suggesting that the extract has anti-anemic activity, attributable to its high iron content [8] 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 diabetic control group also suggest that the development of diabetes mellitus is usually accompanied by anomalies in body lipid composition. This finding agrees with existing literature report that the development of diabetes mellitus is usually followed by marked increase in blood cholesterol, triglycerides, LDL-C, VLDL-C and a reduction in HDL-C [5]. The lowering of cholesterol, triglycerides, LDL-C and VLDL-C and increase in HDL-C observed in the rats treated with 150 and 300mg/kg of JELE indicates presence of principles with hypolipidemic properties in the extract. [15] had reported the presence of saponin and flavonoids in the leaf extract of *Jatropha tanjorensis* and both substances have been implicated in blood cholesterol lowering [15, 16].

CONCLUSION

The results obtained from this study indicate that ethanol leaf extract of *Jatropha tanjorensis* (JELE) contain principles with hypoglycemic properties and could be a very safe and potent agent to be employed in the fight against diabetes mellitus and its associate hematological and lipid profile anomalies.

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