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Effect of Methanol and Aqueous Leaf Extract of *Mitracarpus Scabrum* in Alloxan Induced Diabetic Rats

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Abstract
Introduction: Diabetes mellitus is serious metabolic disorder affecting large number people worldwide. It is associated with derangement of carbohydrate, protein and lipid metabolism. This study investigate the activity of *Mitracarpus scabrum* leaf extract in alloxan induced diabetic rats.
Material and Methods: Diabetes was induced by a single intraperitoneal injection of alloxan (120 mg/Kg). Rats were divided into four groups. Group I (standard control): treated orally with the reference drug, Glibenclamide. Group II (diabetic Control): diabetic rats untreated. Group III and IV: diabetic rats treated with methanol and aqueous leaf extract of *M. scabrum* (300 mg/kg). After the last treatment, blood samples were collected for estimation of serum glucose, liver enzymes, triacylglycerol, total cholesterol, HDL, LDL and VLDL.
Results: Methanol and aqueous leaves extracts significantly decreased ($P < 0.05$) fasting blood glucose in alloxan-induced diabetic rats from 10.10 ± 4.815 to 5.80±1.608 mmol/l and 18.85±8.33 mmol/l to 12.525±7.463 mmol/l respectively. The extracts also caused a significant ($P < 0.05$) decrease in serum triglyceride, total cholesterol, LDL-cholesterol and increase in HDL-cholesterol.
Conclusion: This results revealed that *M. scabrum* is effective in the management of diabetes mellitus.

Keywords: intraperitoneal injection, Glibenclamide, total cholesterol, serum glucose, triglyceride

Introduction
Diabetes mellitus (DM) is a metabolic disorder that remains a major health problem in the world. It is characterized by relative or absolute deficiency of insulin secretion or insulin resistance that causes chronic hyperglycemia and impaired carbohydrates, lipids, and proteins metabolism [1]. Globally, the number of patients with DM is predicted to have two-fold increase in the next 30 years and consequently it will also increase the number of patients with vascular complication [2]. It is known to affect 3% on average of adult Nigerians [3]. The prevalence in northern Nigerian is 1.6% [4].
Mitracarpus scabrum is a perennial annual herb of about 30cm tall or much smaller and possess rough leaves. The genus *Mitracarpus*, consists of no fewer than 50 species [5]. It is reported that the plant has both antibacterial and antifungal activities [6, 7] Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases including liver diseases [8]. Ischemia, perfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer with relatively little knowledge regarding their modes of action [9]. Just like any other traditional/ herbal medicine *Mitracarpus scabrum* is also used as traditional medicine in Nigeria, the juice from the crushed plant is known to be applied topically for the treatment of skin diseases such as ringworm, lice, itching, craw – craw and other fungi diseases or applied to dressings for fresh cuts, wounds and ulcers [10]. In West Africa the plant is however, used for the treatment of headaches, toothache, amenorrhoea, dyspepsia, hepatic and venereal diseases, leprosy, and skin diseases [11].
Ethnobotanical studies are today recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituent [12]. According to the World Health Organization (WHO), more than 150 plants are known to be used for the treatment of diabetes mellitus and the study of hypoglycemic plants is then encouraged. The ethnobotanical information reports about 800 plants that possess anti-

diabetic potential [13]. But this study was aimed at evaluating the anti-diabetic and hypolipidemic effects of aqueous and methanol leaf extracts of *M. scabrum*.

Materials and Methods

Collection of Plant Material and Extracts Preparation

Fresh leaves of *Mitracarpus scabrum* were collected from Yabo Local Government Area of Sokoto State on 8th February, 2014. The plant sample was authenticated at the Herbarium of Botany unit of Usmanu Danfodiyo University Sokoto were the specimen was deposited. The leaves were allowed to air dry at room temperature and pulverized into fine powder using pestle and mortar. Two hundred and forty (240 g) grams of the sample were extracted with 1600 ml methanol and 140 g was extracted with 1200 ml distilled water for 48hours. The extracts were filtered and dried in a drying cabinet. The percentage yields of both methanol and aqueous extract were 31.0 g and 11.0 g respectively.

Experimental Animals

Healthy Adult albino rats (Wister strains) of both sexes were used for this study. Rats were purchased from the Animal House of Usmanu Danfodiyo University, Sokoto, which were allowed acclimatized to the environment for a period of seven days.

Induction of Diabetes

The rats were injected intraperitoneally with alloxan monohydrate, dissolved in sterile normal saline solution at a dose of 120 mg kg-1 body weight. After a week, rats with moderate hyperglycemia were considered as diabetic and were used for the study.

Experimental Design

The experimental animals were divided into four groups of four rats each:

Group I – Standard Control (SC): rats treated orally with the reference drug, Glibenclamide (5 mg/kg body weight/day) which served as the diabetic positive control group.

Group II – Diabetic control (DC), Diabetic rats untreated.

Group III – Diabetic rats treated with methanol extract (DTM), Diabetic rats treated with methanol leaf extract of *Mitracarpus scabrum* (300 mg kg-1 body weight) orally for fourteen (14) days;

Group IV – Diabetic rats treated with aqueous extract (DTA), Diabetic rats treated with aqueous extract of *Mitracarpus scabrum* (300 mg kg-1 body weight) orally for fourteen (14) days.

Administration of Extracts

The treated groups were administered the extracts orally at 300 mg kg-1 body weight per day in the morning hours for 14 days. The animals were maintained on standard laboratory diet and tap water *ad libitum* throughout the period of the study. The animals were weighed before the alloxan injection, at the beginning of the treatment and 24 h after the last treatment. After the last treatment, the rats were fasted overnight and anaesthetized by dropping each in a plastic jar saturated with chloroform vapor. They were then removed from the jar. The animals were sacrifice; blood samples were collected and placed into labeled centrifuge test-tubes to obtain sera.

Biochemical Analysis

Serum glucose level was determined by the glucose oxidase method using Glucometer. 14 Serum total cholesterol level was estimated by the enzymatic method, using cholesterol enzymatic endpoint method assay kit [15] Serum Triacylglycerol (TAG) was done by the method of Trinder [14]. Measurement of Serum High Density Lipoprotein. Serum low density lipoprotein cholesterol (LDL-C) and serum very low density lipoprotein cholesterol (VLDL -C) were calculated according to the Friedewald formula [16]. i. e LDL-C = TC - (HDL-C + TAG/5) and VLDL-C = TAG/5) respectively. Atherogenic index was calculated as the ratio of LDL-C to HDL-C. The serum liver enzymes ALT and AST were assayed using Reitman and Frankel method [17]. While ALP was assayed by Roy method [18].

Data Analysis

The results were expressed as mean ± standard deviation (SD). The data was analyzed using one- way analysis of variance (ANOVA) and differences between samples were determined by Bonferroni compare all pairs of column, using instant statistical software program. *P* values < 0.05 were considered significant.

Results

Table 1: Effect of Methanol and Aqueous Leaf Extracts of Mitracarpus scabrum on the Body Weight (g) of Alloxan Induced Diabetic Rats.

Period	Methanol extract	Aqueous extract
Before alloxan injection	142.75±17.08a	186.0±43.91a
Seven days after alloxan injection	144.0±28.76a	179.0±37.21a
After treatment	167.75±29.74a	180.25±44.38a

Values are mean ± standard deviation (n = 4), means followed by different super script in a column are significantly ($p < 0.05$) different.

Table 2: Effect of Methanol and Aqueous Leaf Extract of Mitracarpus scabrum on Blood Sugar Level in (mmol/l) of Alloxan Induced Diabetic Rats.

Groups	Glucose level before alloxan injection	Glucose level after alloxan injection	Glucose level after treatment
Normal rats (Control)	4.625±0.624a	24.70±11.857b	5.625±2.178a
Diabetic rat (control)	4.825±1.021a	20.8±9.393b	16.05±5.244b
Diabetic treated with methanol extract	5.30±1.203a	10.10±4.815a	5.8±1.608a
Diabetic treated with aqueous extract	4.70±1.013a	18.850±8.33b	12.525±7.463b

Values are mean ± standard deviation (n = 4), means followed by different super script in a column are significantly ($p < 0.05$) different.

Table 3: Effect of Methanol and Aqueous Leaf Extract of *Mitracarpus scabrum* on Lipid Profiles of Alloxan Induced Diabetic Rats.

Parameter (mg/dl)	Diabetic treated With Standard drug (Glibenclamide)	Diabetic control	Diabetic treated with methanol extract	Diabetic treated with aqueous extract
Total cholesterol	76.45±2.43ab	88.58±5.67c	66.99±3.16b	72.58±2.32b
Triglyceride	53.05±15.67c	128.36±13.30a	50.34±3.93c	88.81±7.69b
HDL-C	38.75±6.60c	33.58±6.96a	47.47±2.38c	44.30±6.61ac
LDL-C	27.09±7.20a	29.32±10.58a	9.46±5.33ab	10.52±5.79ab
VLDL-C	10.61±3.13c	25.67±2.66b	10.07±0.79c	17.76±1.54a
Aix	0.732±0.262ab	0.955±0.528a	0.203±0.116b	0.254±0.151b

Values are mean ± standard deviation (n= 4). Mean followed by different superscript in a raw are significantly ($p< 0.05$) different. HDL-C = High Density Lipoprotein Cholesterol: LDL-C= Low density lipoprotein cholesterol: VLDL-C = Very low density lipoprotein cholesterol.

Table 4: Effect of Methanol and Aqueous Leaf Extract of *Mitracarpus scabrum* on Serum Liver Enzymes of Alloxan Induced Diabetic Rats.

Parameter (U/L)	Normal control	Diabetic control	Diabetic treated with methanol extract	Diabetic treated with aqueous extract
ALT	17.79±9.01a	43.74±7.50b	25.85±5.12a	10.63±4.41a
AST	32.34±11.27a	49.54±41.82a	64.49±33.47a	32.52±12.13a
ALP	14.388±7.14a	26.338±5.25ab	30.325±4.57ab	38.965±15.32b

Values are mean ± standard deviation (n= 4). Mean followed by different superscript in a raw are significantly ($p< 0.05$) different. ALT= Alanine amino transferse, AST= Aspartate amino transferase, ALP= Alkaline Phosphatase

Discussion

The present study was focused in observing the anti-diabetic effect of aqueous and methanol leaf extract of *Mitracarpus scabrum*. The overall comparison of the antidiabetic effect of *Mitracarpus scabrum* aqueous leaf extract with that of methanol extract were carried out.

After the administration of alloxan, there was increase in the blood glucose levels, volume of water taken by the albino rats, and reduction in the content of food taken. The results showed that the intraperitoneal administration of alloxan to rats significantly increased blood glucose levels seven days (7) after injection, as well as decreased body weight (Table 1 and 2). Weight loss is a main sign of diabetes but its mechanism is not clear. It could be due to many factors that include loss of appetite, increased muscle waste and loss of tissue proteins [19, 20]. The alloxan administration caused diabetic condition, by destroying the beta-cells of the islets of langerherns, which produce insulin [21]. The situation was characterized by the increase in the blood glucose concentration.

Groups which were administered orally aqueous and methanol leaf extract showed decrease in serum blood sugar level when compared to the diabetic group (Table 2). Repeated oral administration of methanol and aqueous extract of *Mitracarpus scabrum* leaf into diabetic rats for 14 days caused significant ($p < 0.05$) reduction in serum blood glucose levels. The level of glucose in diabetic control group remains all time high when compared to the treated groups. Plant's extract may act on blood glucose through different mechanisms, these effects might have been due to the increased release of insulin from remnant β-cells and/or regenerated β-cells. [22, 23] Restored insulin sensitivity [24] Interference on absorption of dietary carbohydrates as well as disaccharides in small intestine [25] or facilitate utilization of glucose by peripheral tissues mediated by GLUT-4, an insulin dependent glucose transporter [26].

The serum level of cholesterol in diabetic control group was increased when compared to the treated groups (Table 3). The treated groups administered with the aqueous and methanol leaf extract of *Mitracarpus scabrum* showed a significant ($p < 0.05$) decreased level of serum cholesterol when compared with the diabetic group. However, there was no significant difference ($p > 0.05$) between the group treated with methanol extract and group treated with standard diabetic drug (Glibenclamide).

The serum level of triglycerides in diabetic control group was also increased when compared to that of treated groups. The rats orally administered with aqueous and methanol extract of *Mitracarpus scabrum* showed significant ($p < 0.05$) decreased in the serum level of triglycerides when compared to the diabetic group. The results of this study corroborated with an earlier study [27]. when studying the hypoglycemic effect of *Leptadenia hastata.* The non-significant difference ($p > 0.05$) between the methanol treated group and standard diabetic drug (Glibenclamide) was observed.

There was also a remarkable increase in the serum HDL-Cholesterol levels of both the methanol and aqueous extract treated rats as against the standard drug and untreated rats. The increase in serum lipid profiles may be a consequence of increased lipids breakdown/ Peroxidation and mobilization of free fatty acids were reported to arise from peripheral deposits [28].

The serum levels of biomarkers enzymes (Table 4) of the diabetic group treated with methanol extract increases (AST) and aqueous treated group (ALP) as against the standard drug and untreated diabetic group respectively. The elevation of serum biomarker enzymes such as ALT, AST and ALP has been observed in diabetic rats indicating impaired liver function that may be due to hepatic damage induced by hyperglycemia [29].

Conclusion

The study has revealed the ability of *Mitracarpus scabrum* leaf extracts in reducing blood glucose level in diabetic rats. Thus: *Mitracarpus scabrum* may be used as antidiabetic agent. Although methanol extract seems to be more potent than the aqueous extract. Further study to isolate, characterize the active ingredients and to evaluate the toxicological effect of these extracts are recommended.

Conflict Of Interest

Authors have no conflict of interest

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