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

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**Abstract**

**Introduction:** Diabetes mellitus is a serious metabolic disorder affecting large numbers of people worldwide. It is associated with derangement of carbohydrate, protein and lipid metabolism. This study investigated 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
diabetic potential \[^{[13]}\]. But this study was aimed at evaluating the anti-diabetic and hypolipidemic effects of aqueous and methanol leaf extracts of *Mitracarpus 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 48 hours. 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 Glucoometer. 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.

<table>
<thead>
<tr>
<th>Period</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before alloxan injection</td>
<td>142.75±17.08a</td>
<td>186.0±43.91a</td>
</tr>
<tr>
<td>Seven days after alloxan</td>
<td>144.0±28.76a</td>
<td>179.0±37.21a</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td>167.75±29.74a</td>
<td>180.25±44.38a</td>
</tr>
</tbody>
</table>

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 (mmol/l) of Alloxan Induced Diabetic Rats.

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

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

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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 after injection, as well as decreased body weight (Table 1). 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 that of treated groups. The rats orally administered with aqueous and methanol extract of Mitracarpus scabrum showed decrease in serum blood sugar level when compared to 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].

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].

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

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Diabetic treated with Standard drug (Glibenclamide)</th>
<th>Diabetic control</th>
<th>Diabetic treated with methanol extract</th>
<th>Diabetic treated with aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>76.45±2.43ab</td>
<td>88.58±5.67c</td>
<td>66.99±3.16b</td>
<td>72.58±2.32b</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>53.05±15.67c</td>
<td>128.36±13.30a</td>
<td>50.34±3.93c</td>
<td>88.81±7.69b</td>
</tr>
<tr>
<td>HDL-C</td>
<td>38.75±6.60c</td>
<td>33.58±6.96a</td>
<td>47.47±2.38c</td>
<td>44.30±6.61ac</td>
</tr>
<tr>
<td>LDL-C</td>
<td>27.09±7.20a</td>
<td>29.32±10.58a</td>
<td>9.46±3.53ab</td>
<td>10.52±5.79ab</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>10.61±3.13c</td>
<td>25.67±2.66b</td>
<td>10.07±0.79c</td>
<td>17.76±1.54a</td>
</tr>
<tr>
<td>Aix</td>
<td>0.732±0.262ab</td>
<td>0.955±0.528a</td>
<td>0.203±0.116b</td>
<td>0.254±0.151b</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n= 4). Mean followed by different superscript in a raw are significantly (p< 0.05) different.

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

<table>
<thead>
<tr>
<th>Parameter (U/L)</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Diabetic treated with methanol extract</th>
<th>Diabetic treated with aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>17.79±9.01a</td>
<td>43.74±7.50b</td>
<td>25.85±5.12a</td>
<td>10.63±4.41a</td>
</tr>
<tr>
<td>AST</td>
<td>32.34±11.27a</td>
<td>49.54±41.82a</td>
<td>64.49±33.47a</td>
<td>32.52±12.13a</td>
</tr>
<tr>
<td>ALP</td>
<td>14.38±7.14a</td>
<td>26.33±5.25ab</td>
<td>30.32±5.57ab</td>
<td>38.96±15.32b</td>
</tr>
</tbody>
</table>

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

### 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
References