Anti-diabetic, Hypolipidemic and Anti-oxidant Activities of Hydroethanolic Root Extract of Rhus Mysurensis Heyne in Streptozotocin Induced Diabetes in Wistar Male Rats

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ABSTRACT

Objectives: The present study was designed to investigate the potential role of hydroethanolic root extracts of Rhus mysurensis (HERM) in the treatment of diabetes along with its antioxidant and hypolipidemic effects were studied in streptozotocin induced diabetes in Wistar rats. Methods: In this study, the anti-diabetic, hypolipidemic and anti-oxidant activities of hydroethanolic root extract of Rhus mysurensis was evaluated by using STZ induced diabetic rats at a dose of 200mg/kg, 400mg/kg and 800mg/kg p.o. daily for 21 days. Blood glucose levels and body weight were monitored at specific time intervals, and different biochemical parameters, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein were also assessed in the experimental animals. Results: Oral administration of hydroethanolic root extracts of Rhus mysurensis (HERM) 400 and 800mg/kg for 21 days significantly decreased the blood glucose level and considerably increased the body weight of diabetic rats. Daily oral treatment with HERM for 3 weeks resulted in reduced serum cholesterol, and triglycerides and improved HDL-cholesterol levels when compared to the diabetic control group. HERM significantly decreased thiobarbituric acid reactive substances (TBARS) and significantly increased superoxide dismutase and catalase in streptozotocin-induced diabetic rats at the end of 21 days of treatment. Conclusions: Hydroethanolic root extract of Rhus mysurensis showed significant anti-diabetic, hypolipidemic and anti-oxidant activities, which provide the scientific proof for its traditional claims. The results also put forward that the HERM is promising for development of standardized phytomedicine for the treatment of diabetes mellitus.

Keywords: Anti-diabetic; anti-oxidant; hypolipidemic; Rhus mysurensis

INTRODUCTION

Diabetes mellitus (DM) is a most common metabolic disorder of endocrine system. It is characterized by hyper-glycaemia resulting from defects in insulin secretion, in insulin action, or both leading to both acute and chronic complications [1,2]. Globally, the incidence of DM is increasing and posing important public health issues.[3] International Diabetes Federation (IDF) estimated the prevalence of DM is approximately 366 million people (8.3%) in 2011 and will rise to 552 million people (9.9%) by 2030. In India, it was around 61.3 million in 2011, rising to 101.2 million by 2030.[4,5] Diabetes mellitus affects 80% of total population of low and middle income countries out of which India and China having larger contribution.[6] It is ninth leading cause of death and encountered around 4.8 million deaths worldwide in 2012. Modern medicines include sulfonylureas, biguanide, thiazolidinedione and α-glycosidase inhibitors and insulin are used for the management of diabetes mellitus.[7] However, these current synthetic drugs are not satisfactory
to cure DM due to less efficacy and several undesirable side effects or contraindications. Therefore, there is a need to develop plant derived anti-diabetic drugs as these are safer, cheaper, and much effective as compared to synthetic drugs. The World Health Organization (WHO) has recommended the screening of medicinal plants for effective treatment of diabetes mellitus and more than 400 plants have been reported till date. Moreover, based on folklore medicine, herbs, spices and several medicinal plants or their extracts have been consumed orally to treat diabetes since ancient times.

**Rhus mysurensis** Heyne [Family: Anacardiaceae], commonly known as Dansara in Rajasthan. It is a dioecious shrub up to 1.5 m tall and found in rocky areas up to 1500 m. It is distributed in north-western and south-western India i.e. S.E. & W. Punjab, Sind and Rajasthan. It is found abundantly in the New Delhi areas of Aravalli mountain range. It is having spinescent branches, trifoliated leaves with 8–18 mm long petioles, obovate leaflets, terminal panicles or from the axils of the upper leaves, much longer than the leaves. Flowers are unisexual, yellowish. Around 1 mm long stamens, included, abortive in female flowers. Glabrous, ovoid shape ovary found in flower. Distinct styles, each with a capitatus stigma. Fruits are yellowish brown, globose, compressed, 4–5 mm broad. Both flowers and leaves are highly scented due to the presence of essential oil. The stem bark of *Rhus mysurensis* is used in tanning industries. Fruits are used for edible purpose in Rajasthan. The root of this plant is used for the treatment of diabetes in Rajasthan based on folklore knowledge. Till date, not even a single study has been performed to explore the pharmacological activity of this plant. The present study was conducted based on the information got from some health practitioners in Sikar district (Rajasthan) of India that decoctions of *R. mysurensis* Heyne roots are effective in the treatment of type 2 Diabetes Mellitus. The present study was undertaken to evaluate the antioxidant, anti-hyperglycaemic and hypolipidemic activity of hydroethanolic root extract of *Rhus mysurensis* Heyne (HERM) in experimental models of streptozotocin (STZ) induced diabetic Wistar rats.

**MATERIALS AND METHODS**

**Drugs and chemicals**

The following drugs and chemicals were used in the experiment: glibenclamide and streptozotocin (STZ) were purchased from Sigma-Aldrich, India. Fresh solution of STZ was prepared by dissolving in citrate buffer (0.1 M, pH 4.5). Total cholesterol, high density lipoprotein (HDL) and triglycerides (TC) standard kits were purchased from Merck Specialities Pvt. Ltd, India. All reagents used in this study were of analytical grade.

**Plant material**

*R. mysurensis* roots were collected freshly from the hills of Dantaramgarh, Sikar district (Rajasthan), India in July, 2011. Taxonomic identification was done and a voucher specimen was deposited (voucher specimen number RUBL 20605) at the Department of Botany, University Of Rajasthan, Jaipur, India.

**Experimental animals**

Healthy Male Wistar rats (weighing 200–250 g and age of 3 months) were obtained from the animal house of Gauhati Medical College and Hospital, Guwahati. Animals were housed in polypropylene cages (5 animals per cage), maintained under standard condition (12 h light and 12 h dark cycle; 22–25°C & humidity (60 ± 5%)) and allowed free access to pellet diet and water ad libitum. After randomized grouping and before initiation of the experiment, animals were acclimatized to the laboratory conditions. All procedures complied with the guide for the care and use of laboratory Animals and approved by the institutional animal ethics committee, Gauhati Medical College and Hospital, Guwahati.

**Extraction**

Roots of the plant material was thoroughly washed with distilled water to remove dirt and soil, and dried under shade and optimal ventilation. The plant material was then pulverized and the powdered plant material (700 g) was macerated in water: ethanol (70:30) for 72 h in three successive volumes. The resultant hydro-ethanolic extract was dried under reduced pressure. The extract was evaporated to dryness by warming on a water bath at 60°C and obtained a residue of 64 gm (9.14% yield). The dried extract was kept in a refrigerator until use and used in this study without any further purification.

**Preliminary phytochemical screening**

Standard screening tests of the extract were carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as reducing sugars, alkaloids, steroidal compounds, phenolic compounds, tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones using standard procedures. The results of phytochemical screening of the plant extract are shown in Table 1.
Acute toxicity test

Acute toxicity test was done based on the limit test recommendations of OECD 423 Guideline. On day one, Wistar rat fasted for 3–4h was given 2000 mg/kg of the extract orally. The rat was then kept under strict observation for physical or behavioural changes for 24h, with special attention during the first 4 h. Following the results from the first rat, other four rats were recruited and fasted for 3–4 h and administered a single dose of 2000 mg/kg and was observed in the same manner. These observations continued for further 14 days for any signs of overt toxicity.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test[1,17] was performed in overnight fasted (12h) normal animals. Rats divided into five groups (n = 6) were administered 0.4% CMC solution, hydroethanolic extract (200 mg/kg), hydroethanolic extract (400 mg/kg), hydroethanolic extract (800 mg/kg) and glibenclamide (0.5 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the rat tail vein under light ether anaesthesia (to minimize the distress) at 0, 30, 60 and 120 min of extract administration. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (SD check gold, Standard Diagnostics, Inc., Korea).

Induction of diabetes

Diabetes mellitus was induced in 12h overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ at the dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5).[19] After that animals were left aside for 4h and then 5% glucose solution was placed in the cages for 24 hours. Diabetes was confirmed after 72h of STZ administration by checking the blood glucose levels. The mortality within 7 days after STZ injection was found to be 18% and 39 animals out of remaining 50 animals were found diabetic. The diabetic animals were monitored for stabilization of blood glucose level for seven days and study was started on the next day (day 0). Only those animals having blood glucose levels > 200 mg/dl were selected and used for the current study.[19] The body weight and plasma blood glucose levels were measured before and towards end of the experiment.

Experimental design and drug treatment

In the experiment a total of 36 rats (6 normal; 30 STZ diabetic surviving rats) were used. The rats were divided into six groups of six rats each.

Group I: Normal control rats, received a single injection of citrate buffer (vehicle); Group II: Diabetic control rats, received oral gavage of 0.4% CMC once daily for three weeks; Group III: Diabetic rats treated with RM hydroethanolic extract at a dose of 200 mg/kg bw; Group IV: Diabetic rats treated with RM hydroethanolic extract at a dose of 400 mg/kg bw; Group V: Diabetic rats treated with Glibenclamide at a dose of 0.5 mg/kg bw dissolved in 0.4% CMC once daily for three weeks. The extract was dissolved in 0.4% CMC and administered orally in Group III, Group IV, and Group V once daily for three weeks (Figure 1).

At the end of the study, the animals were euthanized between 0900–1100 h to minimize diurnal variation. The changes in body weight and blood glucose levels of all the groups were measured at weekly intervals i.e. 0, 7, 14 and 21 day during the study. Fasting blood glucose level was

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<p>| Table 1. Phytochemical screening of hydro-ethanolic root extract of Rhus myrsineus. Where, − = negative; + = weakly positive; ++ = moderately positive; +++ = strongly positive |
|-------------------------------|-------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Chemical Tests/reagents</th>
<th>Findings/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorf's reagent/Meyer’s reagent</td>
<td>–</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>Biuret test</td>
<td>+</td>
</tr>
<tr>
<td>Triterpene steroids</td>
<td>Sulphuric acid reagent</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride reagent</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>Fehling’s reagent</td>
<td>–</td>
</tr>
<tr>
<td>Non-reducing sugar</td>
<td>Iodine solution</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Acid-alcohol/solid magnesium/amyl-alcohol</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager’s test, BPC</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Lieberman’s test/Keller-Killiani test</td>
<td>+</td>
</tr>
</tbody>
</table>
estimated by glucose oxidase – peroxidase method (SD check gold, Standard Diagnostics, Inc., Korea). Finally on day 21, the blood samples were collected through cardiac puncture under mild diethyl ether anesthesia from rats. Then the blood samples were centrifuged at 3000 rpm for 10 min in cold centrifuge at 20°C to obtain serum. Lipid profile [total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride] levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India) with the help of UV-Visible Spectrophotometer (Thermo scientific).

Low density lipoprotein (LDL) concentration was calculated from the formula of Friedwald et al.

Estimation of liver biochemical parameters

Measurement of lipid peroxidation

Liver was homogenized in 2.5% 50 mM PBS buffer pH 7.0 using polytron homogenizer after incubation in triton × 100 for 20 min. Homogenate was used for the measurement of thiobarbituric acid reactive substance at an absorbance of 535 nm according to the method of Zhang, 2004.

Measurement of SOD and catalase activity

Liver homogenate was centrifuged at 4°C, 17,500 × g for 10 min, resulted supernatant was used for the measurement of SOD activity by haematoxylin auto oxidation method and catalase activity by hydrogen peroxide degradation method.

Statistical analysis

All data were expressed as Mean ± SEM. Between and within group analysis was carried out using one way ANOVA followed by Tukey’s post hoc test and level of significance was set at p < 0.05. For data processing, Graph Pad Prism data analysis software was used.

Figure 1. Pictorial representation of the experimental design of the HERM study.
RESULTS

Extraction

The percentage yield of ethanolic extract of the dried roots of *Rhus mysurensis* was found to be 9.14% (w/w). The extract was dark-brown semisolid at room temperature and solidified when stored in a refrigerator. Extract returned to semisolid state on re-exposure to room temperature.

Preliminary phytochemical screening

Phytochemical screening of the crude extract of *R. mysurensis* revealed the presence of various secondary metabolites (Table 1). Cardiac glycosides, reducing sugars, steroidal compounds and phenolic compounds, tannins, saponins and flavonoids were detected in the crude extract.

Acute toxicity study

Acute toxicity study of the hydroethanolic extract of *R. mysurensis* did not reveal any behavioral, neurological, autonomic or physical changes such as alertness, motor activity, restlessness, convulsions, coma, diarrhea and lacrimation. Besides, the extract did not cause mortality in the animals at a dose of 2000 mg/kg during the observation time. Thus, the median lethal dose (LD50) of the plant extract is said to be greater than 2000 mg/kg, indicating a good safety margin.

Oral glucose tolerance test in normal rats (OGTT)

Oral glucose tolerance test in normal rats showed that all three doses of HERM i.e. 200, 400 and 800 mg/kg prevented significantly (*P* < 0.01) the increase in blood glucose levels after 30 min of 2 g/kg glucose administration as compared to control. No significant effect was shown by HERM extract at 60 and 120 min. Glibenclamide significantly blocked (*P* < 0.01) the increase in blood glucose levels after glucose administration at 30 min and 60 min (Table 2).

Effect on fasting blood glucose level

After STZ injection, diabetic animals showed significant rise in fasting blood glucose (FBG) level as compared to normal controls. Daily treatment of the extract for prolonged duration (21 days) produced decrease in blood glucose levels in diabetic rats in a dose dependent manner. These fall in FBG were significant (*P*<0.01) when compared to diabetic control. Blood glucose level at 21st days was (213.67 ± 6.51), (197.67 ± 14.68) and (136.17 ± 8.40) mg/dL at the doses of 200, 400 and 800 mg/kg of HERM respectively. At the end of study (on 21st day) HERM extract at 800 mg/kg BW treated group decreased the FBG level significantly (53.33%) as compared to diabetic control. The effects of HERM extract on the FBG level of normal and diabetic animals is also shown in Table 3.

Effect on body weight

The body weight change of experimental animals during study is shown in Table 4. There was significant reduction in body weight of diabetic control rats as compared to normal control rats. At the termination of study (on 21st day), there was significant (*P*<0.01) increase in body weight in HERM treated group in dose dependent fashion and in glibenclamide treated group when compared with diabetic control rats.

Effect on lipid profile

Oral administration of HERM showed dose dependent hypolipidemic activity. It reduced plasma cholesterol, triglyceride, LDL in STZ treated rats. In addition, to hypolipidemic activity, HERM also produced a noteworthy dose dependent increase in level of high density lipoproteins (HDL). High density lipoprotein (HDL) is commonly referred to as good cholesterol possessing the ability to reverse cholesterol transport and also protect LDL from oxidation, thereby minimizing the deleterious consequences of LDL oxidation. HERM 800 mg/kg showed highest decrease in the level of plasma cholesterol, triglyceride, LDL whereas increase in HDL level in STZ treated rats as compared to the left over groups of HERM (Table 5).

Effect on Oxidative parameters

The levels of TBARS were significantly (*P*<0.001) increased in STZ control animals as compared to normal control group. Treatment with HERM 400 mg/kg (*P*<0.001) and 800 mg/kg (*P*<0.001) significantly reduced the TBARS levels when compared with STZ control animals in dose related manner (Figure 2). The level of SOD was significantly (*P*<0.001) depleted in STZ control group as compared with normal control group. Reduced SOD level was found to be dose dependently elevated towards normal level upon administration of HERM as compared with STZ control group. Moreover, HERM 400 and 800 mg/kg showed statistically significant elevated levels of SOD when compared with STZ control group (Figure 3). There was significant (*P*<0.001) reduction in
Table 2. Effect of hydroethanolic root extract of \textit{R. mysurensis} on oral glucose tolerance test. All the values were expressed as mean ± SEM (n = 6); *** \( P < 0.001 \). a vs control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oral Glucose Tolerance Test</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>75.1 ± 3.17</td>
<td>108.3 ± 2.15</td>
<td>101.3 ± 1.83</td>
<td>82.0 ± 2.65</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td></td>
<td>71.2 ± 3.21</td>
<td>84.4 ± 2.11(^{**})a</td>
<td>96.6 ± 2.71</td>
<td>80.3 ± 2.46</td>
</tr>
<tr>
<td>HERM (200mg/kg)</td>
<td></td>
<td>73.5 ± 3.14</td>
<td>89.5 ± 2.21(^{***})a</td>
<td>97.5 ± 2.87</td>
<td>79.6 ± 2.27</td>
</tr>
<tr>
<td>HERM (400mg/kg)</td>
<td></td>
<td>78.3 ± 4.10</td>
<td>93.3 ± 1.99(^{**})a</td>
<td>95.0 ± 2.58</td>
<td>82.3 ± 2.27</td>
</tr>
<tr>
<td>HERM (800mg/kg)</td>
<td></td>
<td>73.1 ± 3.30</td>
<td>82.1 ± 2.81(^{**})a</td>
<td>78.6 ± 2.21(^{***})a</td>
<td>74.6 ± 2.33</td>
</tr>
</tbody>
</table>

Table 3. Effect of hydroethanolic root extract of \textit{R. mysurensis} on fasting blood glucose level in STZ induced diabetic rats. All the values were expressed as mean ± SEM (n = 6); \( *** P< 0.001, \ * P<0.05 \). a vs control & b vs Diabetic control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting Blood Glucose Level (g/dl)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>76.33 ± 4.60</td>
<td>77.0 ± 4.37</td>
<td>75.3 ± 3.83</td>
<td>77.5 ± 4.35</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td></td>
<td>295.6 ± 15.0</td>
<td>4(^{***})a</td>
<td>308.8 ± 14.0(^{**})a</td>
<td>313.3 ± 14.2(^{2***})a</td>
</tr>
<tr>
<td>HERM (200mg/kg)</td>
<td></td>
<td>287.8 ± 11.56</td>
<td>266.8 ± 11.54</td>
<td>241.1 ± 9.8(^{**})b</td>
<td>213.6 ± 6.5(^{**})b</td>
</tr>
<tr>
<td>HERM (400mg/kg)</td>
<td></td>
<td>311.5 ± 13.07</td>
<td>256.8 ± 13.7(^{6*})b</td>
<td>220.1 ± 13.6(^{1***})b</td>
<td>197.6 ± 14.6(^{2***})b</td>
</tr>
<tr>
<td>HERM (800mg/kg)</td>
<td></td>
<td>291.8 ± 13.06</td>
<td>225.3 ± 11.7(^{3***})b</td>
<td>174.6 ± 10.7(^{3***})b</td>
<td>136.1 ± 8.4(^{3***})b</td>
</tr>
<tr>
<td>Glibenclamide (0.5mg/kg)</td>
<td></td>
<td>291.8 ± 13.06</td>
<td>203.0 ± 12.10</td>
<td>160.3 ± 9.64</td>
<td>120.6 ± 7.37</td>
</tr>
</tbody>
</table>

Table 4. Effect of hydroethanolic root extract of \textit{R. mysurensis} on body weights in STZ induced diabetic rats. All the values were expressed as mean ± SEM (n = 6); \( *** P< 0.001, \ ** P<0.001, \ * P<0.05 \). a vs control & b vs Diabetic control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight (g)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>230.6 ± 3.94</td>
<td>234.6 ± 4.63</td>
<td>239.3 ± 4.55</td>
<td>243.2 ± 3.56</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td></td>
<td>230.3 ± 5.01</td>
<td>214.1 ± 4.7(^{a})</td>
<td>203.5 ± 4.1(^{3***})a</td>
<td>197.1 ± 4.0(^{2***})a</td>
</tr>
<tr>
<td>HERM (200mg/kg)</td>
<td></td>
<td>224.0 ± 4.18</td>
<td>223.0 ± 4.04</td>
<td>227.1 ± 4.4(^{**})b</td>
<td>230.8 ± 4.0(^{**})b</td>
</tr>
<tr>
<td>HERM (400mg/kg)</td>
<td></td>
<td>225.1 ± 4.87</td>
<td>221.0 ± 4.97</td>
<td>228.1 ± 4.7(^{3***})b</td>
<td>234.3 ± 5.4(^{2***})b</td>
</tr>
<tr>
<td>HERM (800mg/kg)</td>
<td></td>
<td>221.3 ± 4.34</td>
<td>220.1 ± 4.20</td>
<td>229.6 ± 5.4(^{**})b</td>
<td>236.8 ± 5.6(^{2***})b</td>
</tr>
<tr>
<td>Glibenclamide (0.5mg/kg)</td>
<td></td>
<td>227.6 ± 3.90</td>
<td>226.0 ± 3.96</td>
<td>234.6 ± 4.2(^{**})b</td>
<td>240.5 ± 4.6(^{2***})b</td>
</tr>
</tbody>
</table>

Table 5. Effect of hydroethanolic root extract of \textit{R. mysurensis} on serum lipid profile in STZ induced diabetic rats. All the values were expressed as mean ± SEM (n = 6); \( *** P< 0.001, \ ** P<0.001, \ * P<0.05 \). a vs control & b vs Diabetic control. CH=Cholesterol, TGs=Triglycerides, HDL=High density lipoproteins, LDL=Low density lipoproteins

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total-CH (mg/dl)</th>
<th>TGs (mg/dl)</th>
<th>HDL-CH (mg/dl)</th>
<th>LDL-CH (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.72 ± 3.06</td>
<td>81.02 ± 3.80</td>
<td>34.05 ± 1.70</td>
<td>29.48 ± 3.78</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>168.69 ± 6.0(^{**})a</td>
<td>140.95 ± 5.8(^{**})a</td>
<td>19.53 ± 2.2(^{**})a</td>
<td>120.97 ± 5.9(^{**})a</td>
</tr>
<tr>
<td>HERM (200mg/kg)</td>
<td>132.88 ± 3.8(^{**})b</td>
<td>120.39 ± 5.2(^{2***})b</td>
<td>23.13 ± 1.46</td>
<td>85.68 ± 4.8(^{**})b</td>
</tr>
<tr>
<td>HERM (400mg/kg)</td>
<td>116.21 ± 2.9(^{**})b</td>
<td>113.47 ± 4.2(^{3***})b</td>
<td>25.85 ± 1.51</td>
<td>67.67 ± 3.6(^{**})b</td>
</tr>
<tr>
<td>HERM (800mg/kg)</td>
<td>91.82 ± 3.0(^{**})b</td>
<td>99.22 ± 3.4(^{3***})b</td>
<td>29.61 ± 2.2(^{3***})b</td>
<td>42.24 ± 3.8(^{3***})b</td>
</tr>
<tr>
<td>Glibenclamide (0.5mg/kg)</td>
<td>87.46 ± 4.68</td>
<td>101.77 ± 3.55</td>
<td>29.32 ± 2.46</td>
<td>37.81 ± 6.81</td>
</tr>
</tbody>
</table>
catalase activity in STZ control group compared with normal group. The administration of 400 and 800mg/kg doses of HERM recovered CAT activity significantly (\(P<0.001\)) towards normal when compared with STZ control animals (Figure 4).

DISCUSSION

The present study for the first time reports the antioxidant, antihyperglycemic and hypolipidemic activities of HERM in STZ-induced diabetic Wistar rats. The results of this
study revealed that HERM at doses of 400 and 800mg/kg significantly normalized elevated blood glucose level and restored serum and liver biochemical parameters towards normal values. Streptozotocin (STZ) is a nitrosourea compound produced by Streptomyces achromogenes, which specifically induces DNA strand breakage in β-cells causing diabetes mellitus. This leads to insulin deficiency which in turn increases the blood sugar level. The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. Persistent hyperglycemia, the common characteristic of diabetes can cause most diabetic complications. In all patients, treatment should aim to lower blood glucose to near-normal levels.

Hyperglycemia was observed after 48 hours of STZ induction. Treatment with HERM in STZ-induced diabetic rats started reducing fasting blood glucose levels in a dose dependent manner after 7 days and made them normoglycemic after 21 days. The antihyperglycemic effect of HERM at a dose of 800mg/kg was found to be comparable to the effect exerted by the reference drug glibenclamide at a dose of 0.5mg/kg. Normal healthy animals were found to be stable in their body weight whereas diabetic animals showed reduction in body weight. The losses in weights of diabetic animals were due to the increased muscle wasting and loss of tissue proteins. In this study, the reduction of body weight was diminished by extracts after 21 days of treatment in a dose dependent manner.

Since lipid abnormalities accompanying with premature atherosclerosis is the major cause of cardiovascular diseases in diabetic patients, therefore ideal treatment for diabetes, in addition to glycemic control, should have a favourable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes. From this point of view, it is encouraging that a regular administration of HERM extract for 21 days nearly normalized lipid profile in diabetic animals. The dose of 800mg/kg not only lowered TC, TG and LDL but also enhanced the cardio-protective lipid HDL. The LD50 of the extract is high (no death even with 15 times of effective dose) indicating high margin of safety. The fall of 50 and 75% in plasma sugar of severely diabetic group after 7 and 14 days of treatment of most effective dose further confirms our findings.

Oxidative stress in diabetes mellitus has been shown to coexist with impairment in the endogenous antioxidant status. Our study results showed that HERM strongly restored liver antioxidant parameters and decreased lipid peroxidation in diabetic animals. The reduction in liver antioxidant status during diabetes may be the result of counteraction against increased formation of lipid peroxides. A conspicuous increase in the concentration of TBARS in STZ-induced diabetic rats indicated greater lipid peroxidation leading to tissue injury and failure of the endogenous antioxidant defence mechanisms to prevent overproduction of free radicals. Lipid peroxidation is generally measured in terms of TBARS as a biomarker of oxidative stress. Treatment with HERM for 21 days inhibited hepatic lipid peroxidation in diabetic rats as shown by the reduction of TBARS levels towards normal levels, suggesting that HERM could improve the pathologic condition of diabetes by inhibiting lipid peroxidation in STZ treated rats.

Enzymatic antioxidant mechanisms play an important role in the elimination of free radicals (ROS). A reduction in the activities of these enzymes results in the accumulation of superoxide anion and hydrogen peroxides which would have otherwise been effectively scavenged by these enzymes. HERM treatment for 21 days significantly recovered the hepatic SOD and CAT activities towards normal in a dose dependent manner, indicating a protective role of the extract. This may be attributed to the presence of phytochemicals such as phenol and flavonoids. This is further supported by evidence indicating the use of natural extracts from plant source in reducing the risk of oxidative stress, due to their rich source of phytochemicals.

Phytochemical investigation of HERM reveals the presence of sterols, saponins, coumarins, quinones, tannins, flavonoids. These principles are documented to be bioactive for the management of diabetes. It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas beta cell regeneration ability. Sterols have also shown to decrease blood sugar in experimental animal models. Thus, the significant antidiabetic HERM may be due to the presence of more than one antihyperglycemic principle and their synergistic properties.

In the present study, the administration of HERM to STZ-induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of biochemical profile including lipid contents, as compared to diabetic control rats. Moreover, HERM treatment resulted in significant modulation of lipid peroxidation, endogenous enzymatic antioxidant and detoxification status. Hence, it can be concluded that the hydroethanolic extract of R. myurensis root is remarkably effective against streptozotocin-induced diabetes in Wistar rats.
possibly by virtue of its augmenting the endogenous antioxidant mechanisms. Further pharmacological and biochemical investigations should be done to elucidate the mechanism of the anti-diabetic and hypolipidemic effect of *R. myrsinensis*.

**CONCLUSION**

In conclusion, the present study demonstrates that HERM (400 and 800 mg/kg, p.o.) at the dose levels tested reveals potent anti-diabetic, hypolipidemic and antioxidant activities in STZ-induced diabetes in male Wistar rats and also shows the improvement in oral glucose tolerance in glucose-loaded normal rats without inducing hypoglycemic state. The agent with these multiple beneficial effects viz., anti-diabetic, hypolipidemic, and antioxidant properties without causing hypoglycemia would be of greater therapeutic benefit in the management of diabetes associated with abnormalities in lipid profiles and merits further detailed investigation to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

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