Ameliorative potential of aqueous extract of *Pterocarpus marsupium* Roxb bark on diabetes associated metabolic alterations

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Abstract

In this study, have the effect of oral administration of aqueous extract of the bark of *Pterocarpus marsupium* Roxb (PM) for a period of 12 weeks on diabetes associated metabolic alterations in normal and streptozotocin (STZ) induced diabetic rats has been studied. The parameters checked are plasma insulin, glycosylated hemoglobin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), α-glutamyl transferase (α-GT) and creatine kinase (CK). After administration of PM extracts the levels of glycosylated hemoglobin, total cholesterol, triglycerides, LDL –cholesterol were normalized in diabetic rats. It also reversed the elevated levels of AST, ALT, ALP, α- GT and CK to near normal levels. The levels of plasma insulin, glycosylated hemoglobin, and liver glycogen were also restored after PM treatment. The results of this study, indicates that PM possesses desirable effect on diabetes associated metabolic alterations in experimentally induced diabetes mellitus.

Key words

*Pterocarpus marsupium* Roxb, hemoglobin sugar, insulin, lipid profile, serum marker enzymes

Introduction

Diabetes mellitus is found in all parts of the world and rapidly increasing worldwide. This disease is quite alarming in most of the developing countries including India. India has more than 40 million diabetic individuals which represent nearly 20% of total diabetes population worldwide. Many of the currently available antidiabetic agents have number of adverse effects on the body (1) Therefore, managing diabetes without any side effects is still a challenging task for health care providers (2). Hence, the search for more effective and safer hypoglycemic agents with lesser side effects has continued to be an important area of investigation. Numerous diabetes associated metabolic alterations are reported (3-5). Even though antidiabetic activity of crude extracts and purified active constituents of many plants are identified, studies related to the restorative activity of medicinal plants with reference to the diabetes associated altered metabolic functions are very scanty.

Antidiabetic activity of *Pterocarpus marsupium* Roxb (Leguminosae) is already reported (6, 7). The water soluble active constituents isolated from the bark of *P. marsupium* Roxb- (-)-epicatecin, a benzopyran is shown to be very effective in stimulating islets cells of pancreas for insulin secretion (8) and regulation of blood glucose. Number of other phenolic constituents has also been isolated from *P. marsupium* Roxb bark (9). The objective of
the present work was to investigate the restorative activity of PM on STZ-induced metabolic alterations in diabetic rats.

**Materials and Methods**

**Animals:** Male albino rats (Wister strain, weighing 150-200g) were purchased from Tamil Nadu Veterinary Animal Science University, Madhavaram, Chennai and housed under standard husbandry conditions (30°C ± 2°, 60-70% relative humidity and 12h : 12h day night cycle) and allowed standard pelleted rat feed and water *ad libitum*. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC – VIT University).

**Plant material:** The bark of PM was collected from the Morappur forest area, Dharmapuri District, Tamil Nadu during the month of April 2006. A voucher specimen was prepared and submitted to the Forest Department. PM bark was washed with distilled water, shade dried, powdered and stored in an air-tight container until further use.

**Preparation of extract:** The bark PM (100g) was cut in to small slices, powdered and mixed with 500ml of sterile distilled water and a juice was obtained using a Turmix electric extractor. The juice was filtered and the residue was removed. The extract was concentrated under vacuum to get a solid, which was then freeze dried and the yield was calculated.

**Induction of diabetes mellitus in rats:** Diabetes was developed by injecting Streptozotocin (STZ) (Sigma, USA) at a dose of 35mg/kg bodyweight (bw) in 0.1M cold citrate buffer of pH 4.5, interaperitoneally. STZ injected animals exhibited severe glycosuria and hyperglycemia and rats were stabilized over a period of 7 days (10). Diabetes was confirmed in the overnight – fasted rats by measuring blood glucose concentration 96 hr after injection with STZ. The rats with blood glucose above 250 mg/dl were considered to be diabetic and used for the experiment. Control rats were given citrate buffer (pH 4.5).

**Experimental design:** Animals were divided into six groups of six animals each. Group I served as a control; group II had STZ-treated surviving diabetic rats; group III served as a positive control and received tolbutamide (100 mg/kg). Group IV, V and VI comprised the STZ-induced diabetic rats treated with *P. marsupium* Roxb aqueous extract 100, 300 and 500 mg/kg bw/day respectively for 12 weeks, by oral intubation method. Rats were sacrificed at the end of 12 weeks and the blood samples were collected to analyze the effect of PM on biochemical parameters.

**Collection and processing of blood for estimation of glucose and other biochemical parameters:** Plasma insulin concentrations were determined by radioimmunoassay kit (Pharmacia, Uppsala, Sweden) with a betamatic counter (Cronex, Dupont, France). The kit included human insulin as standard and 125I labeled human insulin antibody, which cross-reacts similarly with rat insulin. Total hemoglobin was estimated by the cyanomethaemoglobin method (11) and glycosylated hemoglobin (HbA1C) was estimated by the modified method (12, 13). Serum total cholesterol, triglycerides and serum HDL-cholesterol were determined using commercial kits (Dialab, Austria). Plasma enzymes such as alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1), alkaline phosphatase (ALP; EC 3.1.3.1), α-glutamyl transferase (α-GT; 2.3.2.2) and creatine kinase (CK; 2.7.3.2) activity were measured by using Ecoline kits (E. Merck) in autoanalyser (Microlab- 2000).

**Toxicity studies:** The aqueous extract was administered orally to different groups of rats (n=6) in doses ranging from 100mg- 1g /kg of BW/day to 2-5g/kg of bw/day. The rats were observed for any lethal effects.
Statistical analysis: Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT). All the results were expressed as mean ±SD for six rats in each group and \( P<0.05 \) was considered as significant.

Results

The yield of aqueous extract of PM bark was found to be 3.9 % (w/v). The PM extract treated rats appeared normal. No toxic effect was reported up to 20 to 50 times of the effective dose of the aqueous extract and there were no deaths in any of these groups. The effect of aqueous extract of PM bark on changes in plasma glucose, insulin, total hemoglobin, glycosylated hemoglobin and liver glycogen is given in Table 1. In diabetic rats significantly \( (F>0.05; P<0.001) \) decreased levels of liver glycogen, plasma insulin and total hemoglobin and increased levels of glucose and glycosylated hemoglobin was observed when compared to untreated normal rats. Oral administration of aqueous extract of PM bark significantly \( (F>0.05; P<0.001) \) increased the levels of liver glycogen, plasma insulin and total hemoglobin and restored glycosylated hemoglobin to near normal level when compared to untreated normal control rats. Table 2 presents the levels of serum lipids in normal and in diabetic rats. Total cholesterol, triglycerides and LDL cholesterol levels were significantly \( (F>0.05; P<0.001) \) increased in diabetic rats with significant decrease in HDL cholesterol level when compared to untreated control rats. Oral administration of aqueous extract of PM had significant \( (F>0.05; P<0.001) \) effect in restoring the levels of serum lipids to near normal level with mild increase in HDL cholesterol level. The effect of aqueous extract of PM on the activities of serum enzymes is given in Table 3. The elevated levels of AST, ALT, ALP, GT and CK in diabetic rats were restored to near normal levels on treatment with PM extract.

Table 1. Effect of aqueous extract of *P. marsupium* Roxb bark on plasma glucose, insulin, haemoglobin, glycosylated haemoglobin and hepatic glycogen in normal and streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg bw/day)</th>
<th>Plasma glucose (g/dL)</th>
<th>Plasma insulin (μU/ml)</th>
<th>Total haemoglobin (g/dL)</th>
<th>Glycosylated hemoglobin (%)</th>
<th>Liver glycogen (mg/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Citrate buffer</td>
<td>69±3.19</td>
<td>15.62±1.3</td>
<td>15.50±0.5</td>
<td>5.5±0.4</td>
<td>10.75±0.74</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>STZ</td>
<td>288±3.28*</td>
<td>6.89±1.0*</td>
<td>12.95±1.0*</td>
<td>7.2±0.5*</td>
<td>5.69±0.69*</td>
</tr>
<tr>
<td>Diabetic + Tolbutamide</td>
<td>100</td>
<td>197±2.98</td>
<td>13.90±1.4</td>
<td>14.10±1.1</td>
<td>4.5±0.3</td>
<td>8.23±0.12</td>
</tr>
<tr>
<td>Diabetic + <em>P. marsupium</em></td>
<td>100</td>
<td>130±12.3</td>
<td>12.14±2.6</td>
<td>14.30±0.5</td>
<td>4.2±0.7</td>
<td>9.74±0.74</td>
</tr>
<tr>
<td>Normal</td>
<td>Citrate buffer</td>
<td>120±11.2</td>
<td>13.58±2.5</td>
<td>15.95±0.9</td>
<td>4.9±0.1</td>
<td>10.45±0.18</td>
</tr>
</tbody>
</table>
Each value is mean ± SD for six rats in each group.

STZ- Streptozotocin (35mg/Kg bw in 0.1M cold citrate buffer, pH 4.5)

*Values of diabetic control differ significantly when compared to positive control and drug treated groups and normal control at $F > 0.05$ (ANOVA) and $P< 0.05$ (DMRT).

### Table 2. Effect of aqueous extract of *P. marsupium* Roxb bark on serum total cholesterol, triglycerides, HDL and LDL levels in normal and streptozotocin- induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Citrate buffer</td>
<td>80±2.13</td>
<td>84±1.0</td>
<td>57.7±1.1</td>
<td>32.2±1.5</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>STZ</td>
<td>126±2.9*</td>
<td>125±2.5*</td>
<td>26.0±1.5*</td>
<td>46.1±2.1*</td>
</tr>
<tr>
<td>Diabetic + Tolbutamide</td>
<td>100</td>
<td>80±1.5</td>
<td>87±1.9</td>
<td>29.5±1.8</td>
<td>33.2±2.5</td>
</tr>
<tr>
<td>Diabetic + <em>P. marsupium</em></td>
<td>100</td>
<td>80±2.8</td>
<td>84±1.3</td>
<td>27.9±1.9</td>
<td>34.1±2.4</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>79±2.4</td>
<td>82±1.5</td>
<td>29.1±1.6</td>
<td>33.9±2.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>77±2.3</td>
<td>82±1.0</td>
<td>30.8±1.5</td>
<td>32.5±2.7</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for six rats in each group.

STZ- Streptozotocin (35mg/Kg bw in 0.1M cold citrate buffer, pH 4.5)

*Values of diabetic control differ significantly when compared to positive control and drug treated groups and normal control at $F > 0.05$ (ANOVA) and $P< 0.05$ (DMRT).
The present investigation was to evaluate the efficacy of the aqueous extract of PM on STZ-induced metabolic changes in diabetic rats. Decreased Hb content observed in diabetic rats might be due to increased formation of glycosalated Hb. Generally total haemoglobin level is much below the normal level in diabetic subjects (14) and HbA1c level has been reported to be increased in patients with diabetes mellitus (15). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (16). The level of HbA1c is always monitored as a reliable index of glycemic control in diabetes (17). Elevated levels of HbA1c and reduced levels of Hb observed in our study reveal that diabetes animals had prior high blood glucose level. Administration of aqueous extract of PM bark (500mg/kg bw/day) had brought back the elevated HbA1c levels to near normal level. It has already been reported that decreased liver glycogen content was due to insulin deficiency and associated glycogenolysis process (18). The possibility of restoration of glycogen content in STZ-induced diabetic rats by the administration of PM extract may be due to increased insulin secretion and reactivation of glycogen synthase enzyme system. Hypercholestralemia and hypertriglyceridemia in STZ-induced diabetic rats are well documented. Insulin deficiency leads to increased serum lipids because of increased lipolysis (19). The elevated levels of serum total cholesterol, triglycerides and LDL cholesterol were significantly decreased after treatment with PM. Similar findings were also reported with the methanolic extract of the bark of PM (6). In STZ-induced animals a change in the serum enzymes is directly related to changes in the

Table 3. Effect of aqueous extract of *P. marsupium* Roxb bark on serum marker enzymes AST, ALT, ALP, γGT and CK in normal and streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg bw/day)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>γ GT (U/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Citrate buffer</td>
<td>210±8.0</td>
<td>181±1.5</td>
<td>233±1.7</td>
<td>12.54±1.0</td>
<td>352±1.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>STZ</td>
<td>230±8.0*</td>
<td>185±1.5*</td>
<td>240±1.2*</td>
<td>13.95±1.2*</td>
<td>630±1.3*</td>
</tr>
<tr>
<td>Diabetic + Tolbutamide</td>
<td>100</td>
<td>222±7.9</td>
<td>182±1.2</td>
<td>231±1.2</td>
<td>12.65±1.6</td>
<td>352±1.2</td>
</tr>
<tr>
<td>Diabetic + <em>P. marsupium</em> Roxb</td>
<td>100</td>
<td>210±7.2</td>
<td>182±0.7</td>
<td>233±2.4</td>
<td>12.21±1.8</td>
<td>352±1.5</td>
</tr>
<tr>
<td>Roxb</td>
<td>300</td>
<td>216±7.0</td>
<td>181±0.8</td>
<td>234±1.6</td>
<td>12.32±1.5</td>
<td>353±1.4</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>224±8.0</td>
<td>182±1.1</td>
<td>235±1.5</td>
<td>12.54±1.4</td>
<td>354±1.5</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for six rats in each group.

STZ- Streptozotocin (35mg/Kg bw in 0.1M cold citrate buffer, pH 4.5)

Values of diabetic control differ significantly when compared to positive control and drug treated groups and normal control at F > 0.05 (ANOVA) and P< 0.05 (DMRT).
metabolic functions of AST, ALT, and ALP, γ-GT and CK. It has been reported that the increased levels of transaminases under insulin deficiency (20) were responsible for the increased gluconeogenesis and ketogenesis during diabetes. The increased levels of serum AST, ALT ALP have already been reported to be associated to liver dysfunction and leakage of these enzymes to the liver cytosol into the blood stream in diabetes (21). Reduction in the activity of AST, ALT, and ALP, γ- GT and CK in PM extract treated diabetic rats indicates the alleviating role of the aqueous extract against STZ–induced haptocellular necrotic changes.

Our observations are in well agreement with the reports of several workers that STZ-induced diabetes mellitus and insulin deficiency leads to increased levels of cholesterol and triglycerides (22), and increased levels of alkaline phosphatase and transaminases (23). Further, the antidiabetic active principle of PM, (-)-Epicatechin has been reported to be safe even at higher doses (24). From this study, it can be concluded that administration of aqueous extract of PM bark is beneficial in normalizing the altered carbohydrate metabolism in diabetes, and protects liver by restoring the enzyme levels and also improves the lipid metabolism as well.

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References


