Chapter 4: Antidiabetic activities of Phellinus rimosus
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4.A. ANTIDIABETIC ACTIVITY AGAINST ALLOXAN INDUCED HYPERGLYCEMIA IN RATS

4.A.1. INTRODUCTION

Alloxan-induced diabetes is a well-documented model of experimental diabetes. It provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Like and Rossini, 1976). ROS generated from the autoxidation of dialuric acid, produced from the reduction of alloxan, can damage macromolecules such as lipids, proteins, and nucleic acids (Frode and Medeiros, 2008).

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury and primarily caused by ROS. The cytotoxic action of alloxan is associated with the generation of ROS causing oxidative damage (Szkudelski, 2001). These ROS can bind with most normal cellular components; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids (Agarwal and Sohal, 1993; Adachi et al., 1998; Aksenova et al., 1998). It has been suggested that oxidative stress plays an important role in some physiological conditions and in many diseases, including diabetes mellitus (DM), myocardial infarction and carcinogenesis. High reactivity of ROS exerts toxic effects on the pancreatic acinar cells. Sato et al. (1979) reported that plasma TBARS levels increased in diabetic patients due to vascular lesions induced by hyperglycemia. Diabetes manifested by experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative enzymes and thus promote free radical generation (Bayness and Thorpe, 1996). Disturbances of antioxidants defense system in diabetes involves: alteration in antioxidant enzymes (Strain, 1991), impaired glutathione metabolism (McLennan et al., 1991), and decreased ascorbic acid levels (Jennings et al., 1987). Dietary supplements contribute to the prevention of diabetic complications by decreasing LPO and improving antioxidant status. Antioxidants have been reported to reduce the complications in DM by arresting free radical damage (Yokozawa et al., 2002; Fenercioglu et al., 2010).

Mushrooms and entomopathogenic fungi are exemplary sources of natural medicines with antidiabetic activity. A large number of secondary metabolites from
mushroom such as polysaccharides, lectins, polyphenols, alkaloids, terpenes, and antibiotics have an established history of application in medicine (Wasser, 2010). Many investigators have studied the hypoglycemic effect of either the fruiting body or mycelia of various edible/medicinal fungi including *Agrocybe cylindracea*, *Tremella aurantia*, *Cordyceps sinensis*, *Lentinus edodes*, and *Auricularia auricula-judae* (Kiho et al., 1994, 1995, 1996; Yuan et al., 1998; Yang et al., 2002a). Polysaccharides of *Phellinus linteus* and *Phellinus baumi* were reported to possess antidiabetic activity (Hwang et al., 2005; Kim et al., 2010). No study has been carried out on the effect of *P. rimosus* on the antioxidant profile of alloxan induced-diabetic rats. Hence, the present study was aimed to evaluate the effect of *P. rimosus* on lipid peroxidation, blood glucose and nonenzymic and enzymic antioxidants activities in blood, liver, kidney and pancreas of alloxan-induced diabetic rats.

![Alloxan](image)

**Alloxan** (1,3-Diazinane-2,4,5,6-tetrone)

4.A.2. MATERIALS AND METHODS

4.A.2.1. Animals

Male wistar rats weighing (200 ± 25 g) were used in this study.

4.A.2.2. Preparation of the extract

Aqueous ethanol extract of *P. rimosus* was prepared as described previously (2.2.1). The extract was suspended in distilled water and employed for the experiment.

4.A.2.3. Effect of *P. rimosus* against oral glucose tolerance

Effect of *P. rimosus* against the glucose tolerance in rat was evaluated. After an overnight fasting (8-10 hrs) animals were divided into 4 groups with 6 animals each.
Fasting blood glucose level was monitored using ACCU-Chek Integra glucometer (Roche Diagnostics). Group treated with distilled water was taken as control. Other groups were treated with *P. rimosus* (50 mg/kg and 250 mg/kg) and glibenclamide (1 mg/kg) orally. Thirty-min after the drug administration, animals in all the groups were given glucose orally (2 g/kg in solution administered as 2 ml/kg). Blood glucose levels were monitored at 30, 90, 120 min after the glucose administration using glucometer.

**4.A.2.4. Effect of *P. rimosus* against alloxan-induced hyperglycemia**

**4.A.2.4.1. Induction of diabetes**

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of alloxan (140 mg/kg) in saline. Six hours after the alloxan challenge, the animals were administrated orally with a solution of 20% glucose at a dose of 80 mg/rat, followed by glucose solution *ad libitum* for the next 24h to prevent initial alloxan-induced hypoglycemic mortality. Seventy two hours after the alloxan injection, the blood glucose level was measured using the glucometer. Rats with fasting blood glucose levels greater than 250 mg/dL were considered as diabetic (Perfumi and Tacconi, 1996) and selected for further studies.

**4.A.2.4.2. Experimental design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle (distilled water).</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control.</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous-ethanolic extract 50 mg/kg body weight (p.o).</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous-ethanolic extract 250 mg/kg body weight (p.o).</td>
</tr>
<tr>
<td>V</td>
<td>Glibenclamide 1 mg/kg body weight (p.o).</td>
</tr>
</tbody>
</table>

Six healthy rats treated with vehicle (distilled water p.o) was kept as normal, group I. The diabetic rats were randomly divided into four groups consisting of six rats each. Group II was kept as diabetic control. Group III and IV were treated with *P. rimosus* extract 50 and 250 mg/kg body wt. Group V was treated with glibenclamide 1 mg/kg body wt. The extract and glibenclamide were administered by oral gavage once daily for 10 consecutive days. Fasting blood glucose was estimated on 5’th and 10’th days.
after drug treatment. Animals were sacrificed after 10 days’ treatment. Blood was collected directly from the heart of each animal. A part of the blood was stored in heparinized vials and rest is used to separate serum. Pancreas, liver and kidneys were removed and stored at -70˚ until analysis could be completed.

4.A.2.5. Estimation of glucose

Glucose levels were assessed using ACCU-Chek Integra glucometer (Roche Diagnostics).


Non-coagulated (heparin) blood was used for determination of antioxidant status, the SOD (section 2.2.22), GSH (section 2.2.25), GPx (section 2.2.23), CAT (section 2.2.24), were estimated in erythrocyte lysate. The whole blood was used for analyzing Hb using Drabkin’s reagent (section 2.2.21).

4.A.2.7. Estimation of serum lipid profile

The serum samples were subjected to lipid analysis. Triglyceride (TG) (section 2.2.14), total cholesterol (TC) (section 2.2.13), and high-density lipoprotein cholesterol (HDL) (section 2.2.15) were enzymatically determined using commercial kit (Span Diagnostics Pvt., Ltd., India); very low density lipoprotein (VLDL) cholesterol was calculated as TG/5 and low density lipoprotein (LDL) cholesterol was estimated by using (Friedwald et al., 1972) the formula as follows:

\[ \text{LDL-C} = \text{TC} - (\text{HDL} + \text{VLDL}) \]

The arteriosclerosis index (AI) was calculated by the equations as following:

\[ \text{AI} = (\text{total cholesterol} - \text{HDL-C}) / \text{HDL-C} \]

4.A.2.8. Determination of tissue antioxidant status in the liver, kidney and pancreas

10% homogenate of liver, kidney and pancreas were prepared as described in the section (2.2.2) and used for the determination of superoxide dismutase (SOD) (section 2.2.4), catalase (CAT) (section 2.2.6), glutathione peroxidase (GPx) (section 2.2.5), and the levels of glutathione (GSH) (section 2.2.7), lipid peroxidation (section 2.2.8), and total
protein (section 2.2.3). Using a double beam spectrophotometer (SL 164, UV–VIS double beam spectrophotometer, Systronics India Ltd).

4.A.2.9. Determination of liver glycogen content

The glycogen content was determined according to the method of Vander Vries (1954), the details of which are given in section (2.2.9).

4.A.2.10. Histopathology

A portion of the Liver, kidney and pancreatic tissue was fixed in 10% buffered neutral formal saline for histological studies (section 2.2.28). After fixation, tissues were embedded in paraffin, solid sections were cut at 4 - 5 µm thickness and stained with eosin and haemotoxylin. The sections were examined under light microscope and photomicrographs were taken (Magnification X20).

4.A.3. RESULTS

4.A3.1. Hypoglycemic effect against oral glucose tolerance test

Effect of *P. rimosus* against the oral glucose tolerance is presented in Fig. 4.A.1. We found that hypoglycemic effect in the *P. rimosus* treated (250 mg/kg) group was significant (*p*<0.01) and maximum at 90 min after the glucose challenge when compared to that of control group. However, a statistically non-significant difference in the level of blood glucose at 30 min after the glucose challenge was observed between the different treatment groups. Glibenclamide was also improved the glucose tolerance.

4.A.3.2. Hypoglycemic effect against alloxan- induced diabetes

The blood glucose levels of the diabetic control rats remained high throughout the experimental period compared to those of normal group (Table 4.A.1). There was approximately 2.71 fold increase in the glucose in the diabetic control than that of normal group.

Administration of *P. rimosus* (50 and 250 mg/kg) extract for 10 days was effective to reduce the blood glucose level. There was approximately 1.65 and 2.37 fold decrease in the blood glucose in 50 and 250 mg/kg *P. rimosus* treated groups respectively, where as
Fig. 4.A.1: Effect *P. rimosus* and glibenclamide on the oral glucose tolerance test in rats.

Values are mean ± SD, n = 6.
Table 4.A.1: Effect of *P. rimosus* and glibenclamide on alloxan (AL) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>0&lt;sup&gt;th&lt;/sup&gt; day (72 Hr after injection)</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>83.00 ± 4.53</td>
<td>90.00 ± 7.72&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>95.00 ± 6.72*</td>
<td></td>
</tr>
<tr>
<td>AL Diabetic Control</td>
<td>140</td>
<td>330.00 ± 23.51</td>
<td>305.00±16.18&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>295.00 ±18.45*</td>
<td></td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>50</td>
<td>331.00 ± 22.21</td>
<td>240.00 ±12.27***</td>
<td>180.00±13.76***</td>
<td></td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>250</td>
<td>325.00 ± 19.36</td>
<td>190 ± 22.36***</td>
<td>126.00±9.43***</td>
<td></td>
</tr>
<tr>
<td>AL + Glibenclamide</td>
<td>1</td>
<td>336.00 ±17.72</td>
<td>170.00 ±20.19***</td>
<td>131.00±7.45***</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

*** P < 0.001,  * P < 0.05 significantly and  ns P > 0.05 non-significantly different from the blood glucose level before the treatment (0<sup>th</sup> day) and 5 and 10<sup>th</sup> days. (Bonferroni test)
the decrease in glibenclamide (1 mg/kg) treatment was 2.23 fold with respect to diabetic control.

4.A.3.3. Effect of *P. rimosus* on lipid profile of diabetic rats

Serum total cholesterol, triglycerides and LDL-cholesterol levels were significantly elevated (p < 0.001) in diabetic groups in comparison to normal (Table 4.A.2). There were approximately 1.3, 1.29 and 1.71 fold increases in the serum TC, TG and LDL-C respectively in the case of diabetic control group with respect to normal. These significant rises were accompanied by significant declines of plasma HDL by 1.09 fold as compared to the normal. The atherogenic index was significantly increased in diabetic control rats by 2.06 fold as compared with the normal control (Fig. 4.A.2).

The oral administration of *P. rimosus* to diabetic rats at 50 and 250 mg/kg for 10 days, resulted in significant declines in plasma TC, TG and LDL-cholesterol. The fold decrease in the levels of TC, TG and LDL were approximately 0.91, 0.87 and 0.85 in the case of 50 mg/kg 1.07, 1.01 and 1.27 fold in the case of 250 mg/kg treated group respectively compared to the diabetic control. The HDL levels was significantly improved, there was approximately 0.82 and 1.02 fold increase for 50 and 250 mg/kg *P. rimosus* with respect to diabetic control. The AI index was declined after treatment with *P. rimosus* and there was 0.74 and 1.14 fold decreases for 50 and 250 mg/kg *P. rimosus* with respect to diabetic control. Glibenclamide (1 mg/kg) showed 1.02, 0.91 and 1.1 fold decrease for TC, TG and LDL respectively and 0.97 fold increase for HDL with respect to diabetic control group and the AI index was declined to 1.01 fold.

4.A.3.4. Effect of *P. rimosus* on blood antioxidant status

The blood antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (p<0.001) in the diabetic control than that of normal group (Table 4.A.3). There were approximately 1.75, 1.53 and 2.49 fold decreases in the activity of SOD, CAT and GPx in the diabetic control than that of normal group. The *P. rimosus* treatment at 50 and 250 mg/kg for 30 days improved the activities of antioxidant enzymes in the blood. The fold increase in the activity of SOD, CAT and GPx were approximately 1.12, 1.15 and 1.4 fold in the case of 50 mg/kg and 1.33, 1.29 and 2.19 fold in the case of 250 mg/kg.
Table 4.A.2: Effect of *P. rimosus* and glibenclamide on serum lipid levels in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>86.14±12.23</td>
<td>65.30±8.52</td>
<td>34.21±4.24</td>
<td>39.21±7.21</td>
</tr>
<tr>
<td>AL (Diabetic Control)</td>
<td>140</td>
<td>139.12±11.21(^a)</td>
<td>115.43±19.54(^a)</td>
<td>24.23±3.23(^b)</td>
<td>92.53±12.47(^a)</td>
</tr>
<tr>
<td>AL+ <em>P. rimosus</em></td>
<td>50</td>
<td>123.95±14.98(^{ns})</td>
<td>93.41±16.87(^{ns})</td>
<td>27.76±4.86(^{ns})</td>
<td>77.4±15.96(^{ns})</td>
</tr>
<tr>
<td>AL+ <em>P. rimosus</em></td>
<td>250</td>
<td>103.35±15.34(^{**})</td>
<td>85.34±9.85(^{**})</td>
<td>32.14±4.19(^*)</td>
<td>54.53±8.32(^{***})</td>
</tr>
<tr>
<td>AL + glibenclamide</td>
<td>1</td>
<td>109.86±14.23(^*)</td>
<td>87.49±10.32(^*)</td>
<td>30.45±3.93(^{ns})</td>
<td>61.15±11.29(^{**})</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

\(^a\) P < 0.001, \(^b\) P < 0.01 (Bonferroni test) significantly different from normal group.

\(^{**}\) P < 0.001, \(^*\) P < 0.05 significantly and \(^{ns}\) P > 0.05 non significantly different from control group (Bonferroni test)
**Fig. 4.A.2:** Effect of *P. rimosus* on atherogenic index (AI) in alloxan (AL) induced diabetic rats.

Values are mean ± SD, n = 6 animals. \(^a\) P < 0.001, (Bonferroni test) significantly different from normal group. \(***\) P < 0.001, ** P < 0.01 significantly and \(\text{ns}\) P > 0.05 non significantly different from control group (Bonferroni test).

**Fig. 4.A.3:** Effect of *P. rimosus* and glibenclamide treatment on the levels of GSH in the blood of alloxan (AL) induced diabetic rats.

Values are mean ± SD, n = 6 animals. \(^a\) P < 0.001 (Bonferroni test) significantly different from normal group. \(***\) P < 0.001, and ** P < 0.01 (Bonferroni test) significantly different from control group.
Table 4.A.3: Effect of *P. rimosus* on the activities of SOD, CAT and GPx in the blood of alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/g Hb)</th>
<th>CAT (k/g Hb)</th>
<th>GPx (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>755.39±43.37</td>
<td>631.89±58.67</td>
<td>1590±50.00</td>
</tr>
<tr>
<td>AL (Diabetic Control)</td>
<td>140</td>
<td>375.33±29.93(^a)</td>
<td>334.67±39.68(^a)</td>
<td>601.41±16.11(^a)</td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>50</td>
<td>503.21±44.53(^**)</td>
<td>473.25±43.32(^**)</td>
<td>978.97±112.54(^***)</td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>250</td>
<td>603.77±61.23(^***)</td>
<td>522.67±36.93(^***)</td>
<td>1420.18±63.14(^***)</td>
</tr>
<tr>
<td>AL + Glibenclamide</td>
<td>1</td>
<td>660.31±72.99(^***)</td>
<td>548.52±51.77(^***)</td>
<td>1480.73±155.71(^**)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.
\(^a\) P < 0.001 (Bonferroni test) significantly different from normal group.
\(^***\) P < 0.001 and \(^**\) P < 0.01 (Bonferroni test) significantly different from control group.
treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.45, 1.32 and 2.14 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.A.3 shows the levels of reduced glutathione (GSH) in the blood of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.31 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the blood. There was approximately 1.07 and 1.27 fold increase in 50 and 250 mg/kg treated group respectively compared to diabetic control. Glibenclamide (1mg/kg) showed 1.25 fold increase GSH.

**4.A.3.5. Effect of *P. rimosus* on pancreatic antioxidant status**

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered in the pancreas of rat (Table 4.A.4). There was approximately 1.32, 1.21 and 1.37 fold decreases in the activities of SOD, CAT and GPx respectively in the case of diabetic control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 10 days improved the activities of antioxidant enzymes in the pancreas. The fold increase in the activity of SOD, CAT and GPx were approximately 1.11, 0.92 and 1.09 fold in the case of 250 mg/kg and 0.91, 0.82 and 0.92 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.41, 1.01 and 1.16 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.A.4 shows the levels of reduced glutathione (GSH) in the pancreatic tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 2.11 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the pancreatic tissue. There was approximately 1.69 and 1.31 -fold increase in 250 and 50 mg/kg treated group respectively than in the diabetic controls. Glibenclamide (1mg/kg) showed 1.57 fold increase for GSH. The improvement of GSH by *P. rimosus* (250 mg /kg) is slightly higher than that of standard glibenclamide.
Table 4.A.4: Effect of *P. rimosus* on the activities of SOD, CAT and GPx in the pancreas, liver and kidney of Wistar rats.

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Normal</th>
<th>Control</th>
<th><em>P. rimosus</em> 50 mg/Kg</th>
<th><em>P. rimosus</em> 250 mg/kg</th>
<th>Glibenclamide 1 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SOD (U/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>9.42±2.81</td>
<td>3.59±1.43a</td>
<td>6.12±1.57ns</td>
<td>7.59±2.01**</td>
<td>8.09±0.99**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>14.21±1.06</td>
<td>6.33±1.10a</td>
<td>9.10±0.58ns</td>
<td>13.21±3.71**</td>
<td>12.97±3.04**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>11.19±0.61</td>
<td>5.50±0.37a</td>
<td>7.25±1.37ns</td>
<td>9.71±1.13**</td>
<td>9.34±0.83**</td>
</tr>
<tr>
<td><strong>CAT (U/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>100.73±11.59</td>
<td>57.65±15.72a</td>
<td>69.27±8.57ns</td>
<td>79.54±12.36*</td>
<td>83.27±9.72**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>129.60±20.60</td>
<td>61.60±7.80a</td>
<td>84.82±7.50*</td>
<td>112.27±7.14**</td>
<td>115.08±21.18**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>111.39±20.46</td>
<td>63.7±8.56a</td>
<td>72.92±11.07ns</td>
<td>88.22±11.72*</td>
<td>93.89±12.09**</td>
</tr>
<tr>
<td><strong>GPx (U/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>70.36±10.36</td>
<td>37.27±6.16a</td>
<td>47.00±7.12 ns</td>
<td>56.00±8.27**</td>
<td>59.00±8.72**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>99.50±7.77</td>
<td>51.52±12.29a</td>
<td>68.56±8.97ns</td>
<td>81.05±21.65*</td>
<td>84.37±30.07*</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>111.82±10.75</td>
<td>60.43±7.61a</td>
<td>74.27±10.37ns</td>
<td>87.76±21.07*</td>
<td>86.94±13.92*</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

^a^ P < 0.001 (Bonferroni test) significantly different from normal group.

**P < 0.01, * P < 0.05 significantly and ns P > 0.05 non significantly different from control group (Bonferroni test)
Fig. 4.A.4: Effect of *P. rimosus* and glibenclamide treatment on the levels of GSH in the pancreas, liver and kidney of alloxan (AL) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a* P < 0.001 (Bonferroni test) significantly different from normal group. ***P < 0.001, ** P < 0.01 significantly and *ns* P > 0.05 non significantly different from control group (Bonferroni test).
The levels of lipid peroxidation was significantly (p <0.01) higher in control group with respect to normal (Fig. 4.A.5) and it was found to be increased by 1.89 folds. The treatment of *P. rimosus* had significantly (p <0.05) lowered the levels of lipid peroxidation. There was approximately 1.44 and 1.01 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, where as the decrease in glibenclamide (1 mg/kg) treatment was 1.18 fold with respect to diabetic control.

**4.A.3.6. Effect of *P. rimosus* on liver antioxidant status**

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P< 0.001) in the liver tissue of rat (Table 4.A.4). There was approximately 1.77, 1.57 and 1.93 fold decreases in the activities of SOD, CAT and GPx respectively in the case of control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 10 days improved the activities of antioxidant enzymes in the liver. The fold increase in the activity of SOD, CAT and GPx were approximately 1.28, 1.51 and 1.57 fold in the case of 250 mg/kg and 1.15, 1.11 and 1.32 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.34, 1.36 and 1.63 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.A.4 shows the levels of reduced glutathione (GSH) in the liver tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 2.12 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the liver tissue. There was approximately 1.3 and 1.21 -fold increase in 250 and 50 mg/kg treated group respectively compared to diabetic control. Glibenclamide (1mg/kg) showed 1.62 fold increase GSH.

The levels of lipid peroxidation was significantly (p <0.01) higher in control group than the normal (Fig. 4.A.5) and it was found to be increased by 2.01 folds. The treatment of *P. rimosus* had significantly (p <0.01) lowered the levels of lipid peroxidation. There was approximately 1.52 and 1.17 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, where as the fold decrease of glibenclamide (1 mg/kg) was 1.45 fold with respect to diabetic control.
Fig. 4.A.5: Effect of *P. rimosus* and glibenclamide treatment on the levels of lipid peroxidation in the pancreas, liver and kidney of alloxan (AL) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a* P < 0.001 (Bonferroni test) significantly different from normal group. *** P < 0.001, * P < 0.05 (Bonferroni test) significantly different from control group.
4.A.3.7. Effect of *P. rimosus* on kidney antioxidant status

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P < 0.001) in the kidney tissue of rat (Table 4.A.4). There was approximately 1.8, 1.26 and 1.48 fold decreases in the activities of SOD, CAT and GPx respectively in the case of control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 10 days improved the activities of antioxidant enzymes in the kidney. The fold increase in the activity of SOD, CAT and GPx were approximately 1.45, 1.06 and 1.47 fold in the case of 250 mg/kg and 1.01, 0.85 and 1.23 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.47, 1.12 and 1.43 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.A.4 shows the levels of reduced glutathione (GSH) in the kidney tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 2.36 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the kidney tissue. There was approximately 2.01 and 1.34 fold increase in 250 and 50 mg/kg treated group respectively with respect to control. Glibenclamide (1mg/kg) showed 1.95 fold increase GSH.

The levels of lipid peroxidation was significantly (p <0.01) higher in control group than the normal (Fig. 4.A.5) and it was found to be increased by 1.27 folds. The treatment of *P. rimosus* had significantly lowered the levels of lipid peroxidation. There was approximately 1.79 and 0.99 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, whereas the fold decrease of glibenclamide (1 mg/kg) was 1.61 fold with respect to diabetic control.

4.A.3.8. Effect of *P. rimosus* on hepatic glycogen

Hepatic glycogen contents were significantly (p < 0.001) decreased in diabetic control rats with respect to normal and it was found to be decreased by 1.21 folds. However, treatment with *P. rimosus* extract and glibenclamide led to increase in liver glycogen contents over control. There were approximately 0.90, 1.19 and 1.11 fold
Table 4.A.5: Effect of *P. rimosus* and glibenclamide on liver glycogen levels in alloxan (AL) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Liver glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>13.31 ± 2.63</td>
</tr>
<tr>
<td>AL (Diabetic Control)</td>
<td>140</td>
<td>7.63 ± 1.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>50</td>
<td>9.13 ± 1.18&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL + <em>P. rimosus</em></td>
<td>250</td>
<td>11.21 ± 0.81&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>AL + Glibenclamide</td>
<td>1</td>
<td>11.76 ± 1.91&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001 (Bonferroni test) significantly different from normal group.

<sup>**</sup> P < 0.01, <sup>*</sup> P < 0.05 significantly and <sup>ns</sup> P > 0.05 non significantly different from control group (Bonferroni test)
Fig. 4.A.6: Histopathology of kidney in alloxan induced diabetic rats

Normal

Diabetic control

Glibenclamide (1 mg/kg)

P. rimosus (250 mg/kg)
Fig. 4.A.7: Histopathology of liver in alloxan induced diabetic rats

Normal

Diabetic control

Glibenclamide (1 mg/kg)  P. rimosus (250 mg/kg)
Fig. 4.A.8: Histopathology of pancreas in alloxan induced diabetic rats

Normal  
Diabetic control

Glibenclamide (1 mg/kg)  
P. rimosus (250 mg/kg)
increases for *P. rimosus* 50, 250 and glibenclamide 1mg/kg treated group respectively than that of diabetic control (Table 4.A.5).

4.A.3.9. Histopathological observation

Histopathological evaluation reveals that kidney of normal group rats showed normal architecture of glomerulus and tubules. Kidney of alloxan induced rats showed vacuolar degenerative changes in the epithelial cells, cellular proliferation with fibrosis and thickening of capillary walls. Animals treated with *P. rimosus* and glibenclamide apparently showed normal architecture of glomeruli and tubules similar to the normal group (Fig. 4.A.6).

The normal group revealed regular morphology of liver parenchyma cells with intact hepatocytes. In case of alloxan treated group distortion in the arrangement of cells around the central vein, mononuclear inflammatory infiltrations, vacuolation, and development of fibrosis in the degenerate cells were noted. Diabetic rats treated with *P. rimosus* and glibenclamide showed reduced fibrosis (Fig. 4.A.7).

Alloxan was known to destroy the β cells of the pancreas. Histopathological evaluation reveals that alloxan caused extensive damage in pancreatic β-cells, such as a decrease of islet cells’ number, cell necrosis. Administration of *P. rimosus* extract and glibenclamide to alloxan-induced diabetic rats restored partially the pancreatic tissue damages (Fig. 4.A.8).

4.A.4. Discussion

According to World Health Organization, around 171 million people worldwide were suffering from diabetes in 2000. This figure is predicted to double by 2030 (Wild et al., 2004). Diabetic retinopathy, nephropathy and cardiovascular disease are among the most common complications of diabetes. Around 85% of all diabetics eventually develop diabetic retinopathy, which is the commonest cause of blindness in the fourth and seventh decades of life (Tewari and Venkatesh, 2004). The present study was undertaken to evaluate the antidiabetic, hypolipidemic and antioxidant status of *P. rimosus* in alloxan induced diabetes. To the best of our knowledge this is the first report of antidiabetic activity of the mushroom, *P. rimosus*. 
In the present investigation, diabetes control rats exhibited significantly elevated fasting blood glucose level as compared to normal control rats. Maintenance of blood glucose level in diabetic rats with extract treatment indicated the effectiveness of the extract. Mechanism of action of the standard drug glibenclamide is well established. Binding of glibenclamide with its receptor leads to the closure of the potassium channels which opens calcium channels for influx of Ca\(^{2+}\) ions into the cytoplasm and release of insulin from the pancreatic islets. These K+ channels are responsive to ATP/ADP ratio and close when the ratio increases because of an increase in glucose metabolism (Panten et al., 1996; Luzi et al., 1997). With chronic glibenclamide treatment, insulin production is not increased and may return to pretreatment values, but insulin efficacy continues and is thought to involve extra pancreatic mechanisms to increase insulin sensitivity in target tissues. This also leads to decrease hepatic glycogenolysis, gluconeogenesis and blood-glucose concentrations.

It has been demonstrated that ROS are involved in alloxan-induced diabetes. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β cells (Szudelski, 2001). The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals. This loss is likely to stem from kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered (Lenzen et al., 1996). Alloxan rapidly affects the functions of pancreatic β-cells; this can be explained by the low levels of antioxidants in the pancreas to scavenge the generated ROS. It has been reported that alloxan activates nuclear factor-kappa B (NF-κB) 30 min after the injection of alloxan (Ho et al., 2000). The activated NF-κB up-regulates the expression of several genes, including that of inducible nitric oxide synthase and tumor necrosis factor-α (Ho et al., 2000). These proteins can amplify further generation of ROS and could destroy the pancreatic β-cells. Insulin resistance also results from the increased serine phosphorylation of IRS, which may reduce its ability to undergo phosphorylation of its tyrosine residues and ultimately accelerate the degradation of IRS-1 (Evans et al., 2005). The oxidative stress-mediated activation of the JNK pathway can lead to nucleocytoplasmic translocation...
of an important transcription factor for the insulin gene, that is, pancreatic and duodenal homeobox factor-1 (also known as IDX-1 / STF-1 / IPF1), and thus is likely to be involved in the progression of β-cell dysfunction in diabetes (Kaneto et al., 2005).

Diabetes mellitus is usually associated with an increase in plasma lipids levels, the risk factor for coronary heart diseases (Davidson, 1981; Al-Shamaony et al., 1994). A decrease of serum lipid concentration through drug therapy or dietary measures seems to decrease the risk of vascular diseases (Rhoads et al., 1976). It has been demonstrated that insulin deficiency in diabetes mellitus led to a variety of derangements in metabolic and regulatory processes, which in turn provoked a lipid accumulation of total cholesterol and triglycerides in diabetic patients (Goldberg, 1981). The abnormally high concentration of serum lipids observed in alloxan induced diabetic rats in the present study was mainly due to the increase in the mobilization of free fatty acids from the peripheral fat depots (Bopanna et al., 1997).

In the present study, alloxan treatment significantly increases lipid peroxides and decreases nonenzymetic antioxidant levels and antioxidant enzymes activities in the pancreatic, liver and kidney of rats. These results confirm the previous reports that alloxan-induced diabetes is accompanied by an increased generation of free radicals (Sefi et al., 2010). One reason for the elevated lipid peroxidation in alloxan induced diabetes is the reduction in the levels of glutathione, a potent endogenous antioxidant. This study reveals that the increased blood glucose level was closely associated with the elevated lipid peroxidation. The ROS scavenging capacity by antioxidants is decreased in diabetes such that constant oxidative stress develops and oxidation of lipids, proteins and other macromolecules such as DNA is increased. Augmentation of tissue antioxidative capacity would also attenuate lipid peroxidation through this mechanism (Ohkawa et al., 1979).

The diabetogenic action of alloxan can be prevented by the SOD, CAT and other hydroxyl radical scavengers, such as ethanol and dimethyl urea; hence there is evidence to suggest that the incidence of diabetes involves superoxide anion and hydroxyl radicals (Ames et al., 1993). The deleterious effects of superoxide anion and hydroxyl radicals can be counteracted by antioxidant enzymes, such as SOD, CAT and GPx. We have observed
the decrease in SOD, CAT, GPx activities and GSH levels in blood, liver, kidney and pancreas of diabetic rats.

GSH is a major endogenous antioxidant which counteracts free radical mediated damage. It is known to participate in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates (Nicotera and Orrenius, 1986). A marked depletion in the GSH content of the blood, liver, kidney and pancreas was observed in alloxan induced diabetic rats. Thus, the decrease in GSH levels in the diabetic rats might reflect a direct reaction between GSH and the free radicals generated by hyperglycemia in diabetes mellitus. Treatment with \textit{P. rimosus} extract shows a significant restoration in GSH content of blood (Fig. 4.A.3) and pancreatic, hepatic and renal tissues of diabetic rats (Fig. 4.A.4). These results indicate that the \textit{P. rimosus} extract could either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or both effects.

The decrease in SOD activity in diabetic control rats could result from inactivation by hydrogen peroxide or by glycosylation of the enzyme which have been reported to occur in diabetes (Ravi et al., 2004). CAT involves in the reduction of hydrogen peroxide. Decrease in CAT activity could result from inactivation by superoxide radical and glycation of the enzyme. Moreover, CAT is known to be involved in detoxification of high hydrogen peroxide concentrations. Following the treatment of \textit{P. rimosus} extract for 10 days, the activities of both SOD and CAT was augmented in diabetic rats (Table 4.A.3 and Table 4.A.4) which could be attributed to the strong antioxidant property of the \textit{P. rimosus} extract. GPx catalyses the reduction of hydrogen peroxide to non-toxic compounds (Carlberg and Mannervik, 1975). Administration of \textit{P. rimosus} also increased the activities of GPx in diabetic conditions.

Increased TBARS in pancreatic, hepatic and renal tissues is an index of enhanced lipid peroxidation in type 2 diabetes, which might be due to enhanced production or decrease destruction of ROS (Anuradha and Selvam, 1993; Shirwaikar et al., 2004). In the present study, the MDA levels, a lipid peroxidation product and a marker of oxidative stress were elevated significantly in diabetic animals. Treatment with \textit{P. rimosus} significantly decreased the MDA levels (Fig. 4.A.5).
Glucose synthesis in the rat liver was impaired during diabetes. The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and the availability of insulin. The reduced glycogen store in the diabetic rats has been attributed to the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. This is probably due to lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthase systems. In the present study, there was a significant decrease in the liver glycogen in diabetic rats. Treatment with *P. rimosus* significantly increased the glycogen levels of the diabetic animals (Table 4.A.5). This prevention of depletion of glycogen in the liver is possibly due to stimulation of insulin release from β cells.

Our study reveals that one mechanism of antihyperglycemia action of *P. rimosus* may be through its scavenging ability to protect the liver, kidney and pancreatic islets from the free radicals—generated in hyperglycemic status. Alloxan was known to destroy the β cells of the pancreas. Histopathological evaluation reveals that alloxan caused extensive damage in pancreatic β-cells, such as a decrease of islet cells’ number, cell necrosis. In fact, the cytotoxic action of this diabetogenic agent is mediated by the formation of ROS (Heikkila et al., 1976) with a simultaneous massive in cytosolic calcium concentration (Kim et al., 1994; Park et al., 1995). Administration of *P. rimosus* extract to alloxan-induced diabetic rats restored partially the normal pancreatic islets (Fig. 4.A.8).

Phytochemical analysis using standard techniques indicates the presence of polysaccharides, quinones, phenolics, terpenenoids and saponins in the aqueous-ethanol extract of *P. rimosus*. HPTLC analysis revealed the presence of several minor and major constituents. A number of polysaccharides isolated from various sources have been used in traditional Chinese medicine for anti-diabetes, most of which performed a good effect. Examples are panaxan, laminaran, coixan, pachymaran, anemarn, moran, lithosperman, trichosan, saciharan, ephedran, abelmosan, atractan. Kim et al. (2010) demonstrated the anti-diabetic activity of polysaccharides isolated from *Phellinus linteus* in non-obese diabetic mouse. Hypoglycemic effect of crude exopolysaccharides produced by a medicinal mushroom *Phellinus baumii* has also been demonstrated (Hwang et al., 2005). Some of the phenolic compounds have been isolated from herbs used in traditional Chinese medicines for anti-diabetes. Most of them showed mechanism to improve the function of β
cells of pancreatic islet. *P. rimosus* is a rich source of polysaccharides and phenolic compounds. The results of the study thus indicate the possible use of *P. rimosus* for effective management of diabetes.
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4.B.4. DISCUSSION
4.B. ANTIDIABETIC ACTIVITY AGAINST STREPTOZOTOCIN INDUCED HYPERGLYCEMIA IN RATS.

4.B.1. INTRODUCTION

Diabetes mellitus is characterized by absolute or relative deficiencies of insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism (Dukworth, 2001). STZ-induced hyperglycemia is a widely applied experimental diabetic model because of the ability of STZ to selectively target and destroy insulin-producing pancreatic islet \( \beta \)-cells. The intracellular action of STZ induces DNA strand breaks in pancreatic islet \( \beta \)-cells and results in islet cell death (Morgan et al., 1994), thus reducing insulin secretion. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (Huang et al., 2005). Disturbances of antioxidants defense system in diabetes involves: enhancement of lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism (Bagri et al., 2009). Earlier studies have shown that treatment with antioxidant reduces diabetic complications (Wohaieb and Godin, 1987). Efforts to discover antioxidants as useful drug candidates to combat diabetic complications are going on relentlessly.

Mitochondria are the principal source of ROS in cells and impairment of mitochondrial function is intrinsically related to diabetes (Brownlee, 2001). Renal mitochondria were tightly coupled during diabetes. Recent studies have shown that mitochondrial-derived oxidants are mediators of molecular signalling and are implicated in mitochondrial-dependent apoptosis (Gogvadze and Zhivotovsky, 2007). Involvement of mitochondria in hyperglycemia-induced apoptotic pathways has been observed in diabetic neuropathy (Schmeichel et al., 2003), impaired kidney function (Verzola et al., 2002), and myocardial abnormalities (Cai et al., 2002). Nevertheless, the effect of hyperglycemia on renal mitochondrial respiratory complex function has not been thoroughly investigated. Rosca et al (2005) demonstrated that renal Complex-III was a target for glycation and
inhibition during chronic diabetes. A study by Katyare and Satav (2005) showed that respiration rates and ATPase activity were elevated in diabetic renal mitochondria.

In addition to hyperglycaemia, several other factors such as dyslipidemia or hyperlipidemia are involved in the development of cardiovascular complications related to diabetes which are the major causes of morbidity and mortality (Markku Laakso, 1995; Nabel, 2003). Previous studies showed that diabetes is associated with profound alterations in the serum lipid and lipoprotein profiles and with an increased risk for coronary heart disease (Ansar et al., 2011). Abnormalities in fatty acid metabolism may cause inappropriate accumulation of lipids and inflammatory mediators in muscle and liver as well as impairing β cell function (so-called "lipotoxicity"). This will cause inhibition of insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 and development of insulin resistance (Yu et al., 2002). Lowering serum lipid levels through dietary changes or drug therapy is associated with a decrease in the risk of vascular disease and related complications. Although, oral hypoglycemic agents and insulin are the mainstays in the treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and significantly fail to alter the course of diabetic complications (Rang and Dale, 1991).

*Phellinus* is a large and widely distributed genus of the family Hymenochetaceae (Donk). Polysaccharides of *Phellinus linteus* and *Phellinus baumi* were reported to possess antidiabetic activity (Hwang et al., 2005; Kim et al., 2010). Investigations on the pharmacological activities of *P. rimosus* are fragmentary. No study has been carried out on the antidiabetic effect of *P. rimosus* in the streptozotocin induced-diabetic rats. Hence, the present study was aimed to evaluate the effect of *P. rimosus* on lipid peroxidation, blood glucose, serum insulin, and nonenzymic and enzymic antioxidants activities in blood, liver, kidney and pancreas and the activities of mitochondrial dehydrogenases as well as on respiratory chain complexes in the renal mitochondria of streptozotocin induced diabetic rats.
4.B.2. MATERIALS AND METHODS

4.B.2.1. Animals
Male wistar rats weighing (250 ± 20 g) were used in this study.

4.B.2.2. Preparation of the extract

Aqueous ethanol extract of *P. rimosus* was prepared as described previously (2.2.1). The extract was suspended in distilled water and employed for the experiment.

4.B.2.3. Effect of *P. rimosus* against streptozotocin-induced hyperglycemia

4.B.2.3.1. Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of streptozotocin (STZ) (45 mg/kg) in citrate buffer (pH 4.5). Six hours after the STZ challenge, the animals were administrated orally with a solution of 20% glucose at a dose of 80 mg/rat, followed by glucose solution *ad libitum* for the next 24 h to prevent initial STZ-induced hypoglycemic mortality. Seventy two hours after the STZ injection, the blood glucose level was measured using ACCU-Chek Integra glucometer (Roche Diagnostics). Rats with fasting blood glucose levels greater than 300 mg/dL were considered as diabetic (Ramesh et al., 2010) and selected for further studies.

Streptozotocin (2-deoxy-2-([methyl(nitroso)amino]carbonyl)amino)-β-D-glucopyranose)
### 4.B.2.3.2. Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle (distilled water).</td>
</tr>
<tr>
<td>II</td>
<td>Single dose of STZ (45 mg/kg body weight, i.p.) as control.</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous-ethanolic extract 50 mg/kg body weight (p.o).</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous-ethanolic extract 250 mg/kg body weight (p.o).</td>
</tr>
<tr>
<td>V</td>
<td>Glibenclamide 1 mg/kg body weight (p.o).</td>
</tr>
</tbody>
</table>

Rats were treated with vehicle (distilled water p.o) was kept as normal, group I. The diabetic rats were randomly divided into four groups consisting of six rats each. Group II was kept as diabetic control. Group III and IV were treated with *P. rimosus* extract 50 and 250 mg/kg body wt. Group V was treated with glibenclamide 1 mg/kg body wt. The extract and glibenclamide were administered by oral gavage once daily for 30 consecutive days. Fasting blood glucose was estimated on 10’th and 20’th and 30 th days after drug treatment. Animals were sacrificed after 30 days treatment. Blood was collected directly from the heart of each animal. A part of the blood was stored in heparinized vials and rest is used to separate serum. Pancreas, liver and kidneys were removed and stored at -70° until analysis could be completed.

#### 4.B.2.4. Estimation of glucose

Glucose levels were assessed using ACCU-Chek Integra glucometer (Roche Diagnostics).

#### 4.B.2.5. Estimation of Insulin

The serum insulin levels were estimated using the Access ultrasensitive insulin assay kit (Beckman Coulter, USA)

**Principle**

The Access Insulin assay is a simultaneous one-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with mouse monoclonal anti-insulin alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-insulin antibody (Allauzen et al., 1994, 1995). The serum or plasma insulin binds to the antibody on the solid phase, while the conjugate reacts with a different
antigenic site on the insulin molecule. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of insulin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

4.B.2.6. Evaluation of antioxidant status in blood

Non-coagulated (heparin) blood was used for determination of antioxidant status. SOD (section 2.2.22), CAT (section 2.2.24), GSH (section 2.2.25), GPx (section 2.2.23), were estimated in erythrocyte lysate. The whole blood was used for analyzing Hb using Drabkin’s reagent (section 2.2.21).

4.B.2.7. Determination of tissue antioxidant status in the liver, kidney and pancreas

10% homogenate of liver, kidney and pancreas were prepared as described in the section (2.2.2) and used for the determination of superoxide dismutase (SOD) (section 2.2.4), catalase (CAT) (section 2.2.6), glutathione peroxidase (GPx) (section 2.2.5), and the levels of glutathione (GSH) (section 2.2.7), lipid peroxidation (section 2.2.8), and total protein (section 2.2.3), using a double beam spectrophotometer (SL 164, UV–VIS double beam spectrophotometer, Systronics India Ltd).

4.B.2.8. Estimation of serum lipid profile

The serum samples were subjected to lipid analysis. Triglyceride (TG) (section 2.2.14), total cholesterol (TC) (section 2.2.13), and high-density lipoprotein cholesterol (HDL) (section 2.2.15) were enzymatically determined using commercial kit (Span Diagnostics Pvt., Ltd., India); very low density lipoprotein (VLDL) cholesterol was calculated as TG/5 and low density lipoprotein (LDL) cholesterol was estimated by using (Friedwald et al., 1972) the formula as follows:

\[
\text{LDL-C} = \text{TC} - (\text{HDL} + \text{VLDL})
\]

The arteriosclerosis index (AI) was calculated by the equations as following:

\[
\text{AI} = (\text{total cholesterol} - \text{HDL-C}) / \text{HDL-C}
\]
4.B.2.9. Determination of the effect of *P. rimosus* on the liver marker enzymes

The serum samples were subjected to assay for hepatic marker enzymes such as serum glutamate pyruvate transaminase (SGPT) (section 2.2.17), serum glutamate oxaloacetate transaminase (SGOT) (section 2.2.16) and alkaline phosphatase (ALP) by kinetic method using the kit of Agappae Diagnostic Ltd., India using a double beam spectrophotometer (Systronics India Ltd, Hyderabad, India). The transaminases activities were determined as change in absorbance/min at 340 nm. Serum ALP activity was determined from the rate of release of paranitrophenol at 405 nm by the methods described in section (2.2.18).

4.B.2.10. Determination of liver glycogen content

The glycogen content was determined according to the method of Vander Vries (1954), the details of which are given in section (2.2.9).

4.B.2.11. Determination of the effect of *P. rimosus* on the renal function test

The serum samples were subjected to assay for renal markers such as creatinine and urea. There activities were measured by kinetic method using the kit of Agappe Diagnostic Ltd, India as described in the section 2.2.20 and 2.2.19.

4.B.2.12. Isolation of mitochondria from the kidney tissues

Kidney tissues were washed thoroughly in ice cold saline to remove the blood. They were gently blotted between the folds of a filter paper and weighed in an analytical balance. About 10% of the homogenates of each rat kidney sample was prepared in 50 mmol/l phosphate buffer (pH 7.0) containing 0.25 mol/l (w/v) sucrose. Mitochondria were isolated from the kidney homogenate by differential centrifugation according to the method described in section 2.2.10. Mitochondrial fraction was frozen and thawed 3 to 5 times and the supernatant after centrifugation at 3000g was used for the enzyme analysis.

The supernatant (approximately 3 mg/ml protein) was used for the determination of activities of manganese-superoxide dismutase (Mn SOD) (section: 2.2.4) and glutathione peroxidase (GPx) (section: 2.2.5) using a double beam spectrophotometer (2202-Systronics India Ltd, Hyderabad, India).


The level of reduced glutathione in the mitochondrial fraction was determined by the method described in the section 2.2.7.

4.B.2.15. Determination of lipid peroxidation in the renal mitochondria

The level of lipid peroxidation measured as malondialdehyde equivalents formed in the mitochondrial fraction was determined by the method described in the section 2.2.8.

4.B.2.16. Determination of activities of the Krebs cycle dehydrogenases in the renal mitochondria

4.B.2.16.1. Determination of activity of the isocitrate dehydrogenase (ICDH) activity

ICDH activity was estimated according to the method of Fatania et al. (1993) as described in the section 2.2.11.1. The activity was expressed as micromoles of NAD reduced/min/mg protein using extinction coefficient 6.3 mM$^{-1}$cm$^{-1}$.

4.B.2.16.2. Determination of α-ketoglutarate dehydrogenase (α-KGDH) activity

α-KGDH activity was estimated by the method of Reed and Mukherjee (1969) as described in the section 2.2.11.2. The activity was expressed as μmoles of NAD (reduced/min/mg protein) using extinction coefficient 6.3 mM$^{-1}$cm$^{-1}$.

4.B.2.16.3. Determination of succinate dehydrogenase (SDH) activity

SDH activity was estimated by the method of Nulton-Persson and Szweda (2001) as described in the section 2.2.11.3. The activity was calculated using the extinction coefficient of DCPIP (19.1 mM$^{-1}$ cm$^{-1}$) and expressed as μmoles of DCPIP reduced/min/mg protein.
4.B.2.16.4. Determination of malate dehydrogenase (MDH) activity

MDH activity was estimated by the method of Mehler et al. (1948) as described in the section 2.2.11.4. The activity was expressed as μmoles of NADH oxidized/min/mg protein using the extinction coefficient of NADH 6.3 mM⁻¹ cm⁻¹.

4.B.2.17. Determination of activities of the respiratory chain complexes in the cardiac mitochondria

4.B.2.17.1. Determination of complex I activity

Estimated by the method of Janssen et al. (2007) described in the section 2.2.12.1. The activity was expressed as μmoles of DCPIP reduced/ min/mg protein (extinction coefficient of DCPIP is 19.1mM⁻¹ cm⁻¹)

4.B.2.17.2. Determination of complex III activity

Complex III activity was estimated by the method of Krahenbuhl et al. (1991) by preparing Decyl ubiquinol as described in the section 2.2.12.2. Activity of complex III was expressed as micromoles of ferricytochrome-C reduced/min/mg protein.

4.B.2.17.3. Determination of complex IV activity

Complex IV activity was determined by the method of Capaldi et al. (1995) by preparing ferrocytochrome C as described in the section 2.2.12.3.

4.B.2.17.4. Determination of ATP level

ATP level in the mitochondrial fraction was measured using the colorimetric/fluorimetric kit purchased from Biovision, USA.

Principle

ATP is the primary energy currency of living systems. Virtually all energy requiring processes utilize the chemical energy stored in the phosphate bond of ATP. ATP is formed exclusively in the mitochondria and a variety of genetic diseases can affect ATP formation in the mitochondria. The assay utilizes the phosphorylation of glycerol generates a product that is easily quantified by colorimetric (λmax = 570 nm) method.

Procedure

The reagents used were from the Biovision, USA. The ATP reaction mixture for one reaction contains ATP buffer (44μl), ATP probe (2μl), ATP converter (2μl) and
developer mix (2µl) to a final volume of 50 µl reaction mixture. To this reaction mixture add mitochondrial tissue protein (approximately 20µg), mix well. Incubate at room temperature for 30 minutes, after incubation make up the volume to 1 ml with distilled water. Measure the O.D at 570 nm. A standard graph was also prepared using the different concentrations of the ATP standard.

ATP conc (pmol/mg protein) = $\frac{TS}{SV}$

Where, $TS =$ ATP amount in the reaction from standard curve.

$SV =$ Sample volume added in to sample wells.

4.B.2.18. Histopathology

A portion of the Liver, kidney and pancreatic tissue was fixed in 10% buffered neutral formal saline for histological studies (section 2.2.28)

4.B.3. RESULTS

4.B.3.1. Hypoglycemic effect against STZ-induced diabetes

The blood glucose of the diabetic rats after STZ treatment was significantly higher than that in normal rats. The blood glucose levels of the diabetic control rats remained high throughout the experimental period compared to those of normal group. Thirty days after STZ injection blood glucose in the diabetic control rats was 3.81 fold higher than that of normal rats. Administration of $P. rimosus$ (250 and 50 mg/kg) extract for 30 days effectively reduce the blood glucose level. There was approximately 2.13 and 1.57 fold decrease in the blood glucose in 250 and 50 mg/kg $P. rimosus$ treated groups respectively. The decrease in glibenclamide (1 mg/kg) treatment was 2.4 fold with respect to diabetic control (Table 4.B.1).

4.B.3.2. Effect of $P. rimosus$ and glibenclamide on insulin level in diabetic rats

Serum insulin levels were significantly decreased in diabetic control rats with respect to normal ($p < 0.001$). There was approximately 2.1 fold decrease in the insulin level. However, treatment with $P. rimosus$ extract or glibenclamide increased insulin level over control. There were approximately 0.83, 1.23 and 1.6 fold increases for $P. rimosus$ 50, 250 and glibenclamide 1mg/kg treated group respectively than that of diabetic control (Fig. 4.B.1).
Table 4.B.1: Effect of *P. rimosus* and glibenclamide on streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Fasting blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0&lt;sup&gt;th&lt;/sup&gt; day (72 Hr after injection)</td>
</tr>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>87.00 ± 4.25</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>378.00 ± 24.63</td>
</tr>
<tr>
<td>STZ+<em>P. rimosus</em></td>
<td>50</td>
<td>405.00 ± 43.27</td>
</tr>
<tr>
<td>STZ + <em>P. rimosus</em></td>
<td>250</td>
<td>393.00 ± 28.65</td>
</tr>
<tr>
<td>STZ+Glibenclamide</td>
<td>1</td>
<td>414.00 ±27.79</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

*** P < 0.001, **P<0.01, * P < 0.05 significantly and ns P > 0.05 non-significantly different from the blood glucose level before the treatment (0<sup>th</sup> day) and 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> days. (Bonferroni test)
Fig. 4.B.1: Effect of *P. rimosus* and glibenclamide on insulin level in streptozotocin (STZ) induced diabetic rats.

Values are mean $\pm$ SD, $n = 6$. $^a P < 0.001$ (Bonferroni test) significantly different from normal group. *** $P < 0.001$ significantly and $^\text{ns} P > 0.05$ non significantly different from control group (Bonferroni test).

Fig. 4.B.2: Effect of *P. rimosus* and glibenclamide treatment on the levels of GSH in the blood of streptozotocin (STZ) induced diabetic rats.

Values are mean $\pm$ SD, $n = 6$ animals. $^a P < 0.001$ (Bonferroni test) significantly different from normal group. ** $P < 0.01$, * $P < 0.05$ significantly and $^\text{ns} P > 0.05$ non significantly different from control group (Bonferroni test).
4.B.3.3. Effect of *P. rimosus* on body weight

Body weight of streptozotocin-induced diabetic rats was found to be significantly \( p < 0.001 \) less compared to normal rats (Table 4.B.2). After 30 days treatment with *P. rimosus* or glibenclamide, the body weight had significantly \( p < 0.01 \) increased compared to diabetic control.

4.B.3.4. Effect of *P. rimosus* on blood antioxidant status

The blood antioxidant enzymes such as SOD, CAT and GPx were lowered significantly \( p<0.001 \) in the diabetic control than that of normal group (Table 4.B.3). There were approximately 2.05, 1.29 and 1.39 fold decreases in the activity of SOD, CAT and GPx in the diabetic control than that of normal group. The *P. rimosus* treatment at 50 and 250 mg/kg for 30 days improved the activities of antioxidant enzymes in the blood. The fold increase in the activity of SOD, CAT and GPx were approximately 1.24, 0.94 and 0.99 fold in the case of 50 mg/kg and 1.63, 1.12 and 1.25 fold in the case of 250 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.65, 1.11 and 1.31 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.B.2 shows the levels of reduced glutathione (GSH) in the blood of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.23 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the blood. There was approximately 1.04 and 1.16 fold increase in 50 and 250 mg/kg treated group respectively compared to diabetic control. Glibenclamide (1 mg/kg) showed 1.13 fold increase GSH.

4.B.3.5. Effect of *P. rimosus* on pancreatic antioxidant status

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered in the pancreas of rat (Table 4.B.4). There was approximately 1.59, 1.48 and 2.09 fold decreases in the activities of SOD, CAT and GPx respectively in the case of diabetic control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 30 days improved the activities of antioxidant enzymes in the pancreas. The fold increase in
Table 4.B.2: Effect of *P. rimosus* on body weight in streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Body weight (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Normal (STZ)</td>
<td>Vehicle</td>
<td>263.63±15.31</td>
<td>281.63±12.23</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>45</td>
<td>261.75±21.03</td>
<td>171.00±7.10a</td>
<td></td>
</tr>
<tr>
<td>STZ + <em>P. rimosus</em></td>
<td>50</td>
<td>269.00±21.69</td>
<td>220.00±25.32*</td>
<td></td>
</tr>
<tr>
<td>STZ + <em>P. rimosus</em></td>
<td>250</td>
<td>258.60±31.39</td>
<td>239.50±35.75***</td>
<td></td>
</tr>
<tr>
<td>STZ + Glibenclamide</td>
<td>1</td>
<td>264.33±8.08</td>
<td>233.00±27.17**</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.
a P < 0.001 (Bonferroni test) significantly different from normal group.
*** P < 0.001, ** P < 0.01, * P < 0.05 significantly different from control group (Bonferroni test)
Table 4.B.3: Effect of *P. rimosus* on the activities of SOD, CAT and GPx in the blood of streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/g Hb)</th>
<th>CAT (k/g Hb)</th>
<th>GPx (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>986.27±59.99</td>
<td>436.37±42.34</td>
<td>584.28±64.85</td>
</tr>
<tr>
<td>STZ (Diabetic Control)</td>
<td>45</td>
<td>475.27±25.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264.17±39.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>334.74±36.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+<em>P. rimosus</em></td>
<td>50</td>
<td>601.37±43.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323.42±34.53&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>405.11±35.21&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>250</td>
<td>789.39±55.29&lt;sup&gt;***&lt;/sup&gt;</td>
<td>384.52±47.63&lt;sup&gt;***&lt;/sup&gt;</td>
<td>518.41±48.92&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+ Glibenclamide</td>
<td>1</td>
<td>823.74±89.93&lt;sup&gt;***&lt;/sup&gt;</td>
<td>359.75±21.77&lt;sup&gt;**&lt;/sup&gt;</td>
<td>548.25±57.27&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001 (Bonferroni test) significantly different from normal group.

<sup>***</sup> P < 0.001, <sup>**</sup> P < 0.01, <sup>*</sup> P < 0.05 significantly and <sup>ns</sup> P>0.05 non significantly different from control group (Bonferroni test)
Table 4.B.4: Effect of *P. rimosus* on the activities of SOD, CAT and GPx in the pancreas, liver and kidney of diabetic rats.

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Normal</th>
<th>(STZ) Diabetic Control</th>
<th>STZ + <em>P. rimosus</em> 50 mg/Kg</th>
<th>STZ + <em>P. rimosus</em> 250mg/kg</th>
<th>STZ + Glibenclamide 1mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>Pancreas</td>
<td>6.40±1.50</td>
<td>3.01±0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.95±1.27&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.18±0.91&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.90±0.47&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>8.32±1.77</td>
<td>4.09±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.10±1.36&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.71±0.53&lt;sup&gt;**&lt;/sup&gt;</td>
<td>5.70±1.19&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>8.94±1.31</td>
<td>4.92±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.23±0.07&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>7.75±0.80&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.21±1.76&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>Pancreas</td>
<td>56.36±6.29</td>
<td>29.16±4.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.38±6.30&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>45.03±6.24&lt;sup&gt;**&lt;/sup&gt;</td>
<td>49.26±9.26&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>116.99±25.28</td>
<td>51.34±15.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.33±17.09&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>95.33±21.05&lt;sup&gt;**&lt;/sup&gt;</td>
<td>87.89±20.41&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>93.45±5.72</td>
<td>49.34±3.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.96±12.55&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>76.3±12.55&lt;sup&gt;**&lt;/sup&gt;</td>
<td>70.62±13.64&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>Pancreas</td>
<td>47.21±8.43</td>
<td>16.89±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.29±5.79&lt;sup&gt;*&lt;/sup&gt;</td>
<td>37.35±5.04&lt;sup&gt;***&lt;/sup&gt;</td>
<td>31.62±1.67&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>55.87±18.26</td>
<td>21.5±6.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.22±16.35&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>48.65±17.02&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.63±11.89&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>60.27±7.45</td>
<td>25.62±4.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.69±7.60&lt;sup&gt;*&lt;/sup&gt;</td>
<td>51.33±10.43&lt;sup&gt;***&lt;/sup&gt;</td>
<td>47.72±7.32&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001, <sup>b</sup> P < 0.01 (Bonferroni test) significantly different from normal group.

<sup>***</sup> P < 0.001, <sup>**</sup> P < 0.01, <sup>*</sup> P < 0.05 significantly and <sup>ns</sup> P > 0.05 non significantly different from control group (Bonferroni test)
the activity of SOD, CAT and GPx were approximately 1.37, 1.13 and 1.73 fold in the case of 250 mg/kg and 0.87, 0.89 and 1.1 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 1.45, 1.18 and 1.59 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.B.3 shows the levels of reduced glutathione (GSH) in the pancreatic tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.48 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the pancreatic tissue. There was approximately 1.23 and 0.89 fold increase in 250 and 50 mg/kg treated group respectively than in the diabetic controls. Glibenclamide (1mg/kg) showed 1.14 fold increase for GSH. The improvement of GSH by *P. rimosus* (250 mg /kg) is slightly higher than that of standard glibenclamide.

The levels of lipid peroxidation was significantly (p <0.001) higher in control group with respect to normal (Fig. 4.B.4) and it was found to be increased by 1.66 folds. The treatment of *P. rimosus* lowered the levels of lipid peroxidation. There was approximately 1.34 and 0.88 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, where as the decrease in glibenclamide (1 mg/kg) treatment was 1.18 fold with respect to diabetic control.

**4.B.3.6. Effect of *P. rimosus* on liver antioxidant status**

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P< 0.001) in the liver tissue of rat (Table 4.B.4). There was approximately 1.37, 1.38 and 1.33 fold decreases in the activities of SOD, CAT and GPx respectively in the case of control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 30 days improved the activities of antioxidant enzymes in the liver. The fold increase in the activity of SOD, CAT and GPx were approximately 1.29, 1.11 and 1.13 fold in the case of 250 mg/kg and 0.78, 0.79 and 0.63 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 0.94, 1.01 and1.03 fold increase for SOD, CAT and GPx respectively with respect to control group.
Fig. 4.B.3: Effect of *P. rimosus* and glibenclamide treatment on the GSH levels in the pancreas, liver and kidney of streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals.  

\[ a \] P < 0.001 (Bonferroni’s t-test) significantly different from normal group.  

\[ *** \] P < 0.001,  

\[ ** \] P < 0.01,  

\[ * \] P < 0.05 significantly and  

\[ \text{ns} \] P > 0.05 non significantly different from control group (Bonferroni’s t-test).
Fig. 4.B.4: Effect of *P. rimosus* and glibenclamide treatment on the levels of lipid peroxidation in the pancreas, liver and kidney of streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a P < 0.001* (Bonferroni test) significantly different from normal group. ***P < 0.001*, *P < 0.05* significantly and *ns P>0.05* non significantly different from control group (Bonferroni test).
Fig. 4.B.3 shows the levels of reduced glutathione (GSH) in the liver tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.45 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the liver tissue. There was approximately 1.13 and 0.99 -fold increase in 250 and 50 mg/kg treated group respectively compared to diabetic control. Glibenclamide (1mg/kg) showed 1.15 fold increase GSH.

The levels of lipid peroxidation was significantly (p <0.001) higher in control group than the normal (Fig. 4.B.4) and it was found to be increased by 1.49 folds. The treatment of *P. rimosus* had lowered the levels of lipid peroxidation. There was approximately 1.05 and 0.77 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, where as the fold decrease of glibenclamide (1 mg/kg) was 1.01 fold with respect to diabetic control.

4.B.3.7. Effect of *P. rimosus* on kidney antioxidant status

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P< 0.001) in the kidney tissue of rat (Table 4.B.4). There was approximately 1.24, 1.66 and 1.75 fold decreases in the activities of SOD, CAT and GPx respectively in the case of control group with respect to normal. The *P. rimosus* treatment at 250 and 50 mg/kg for 30 days improved the activities of antioxidant enzymes in the kidney. The fold increase in the activity of SOD, CAT and GPx were approximately 1.14, 1.21 and 1.32 fold in the case of 250 mg/kg and 1.01, 1.04 and 1.12 fold in the case of 50 mg/kg treated group respectively compared to the diabetic controls. The standard reference drug glibenclamide (1 mg/kg) showed 0.89, 1.09 and 1.33 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 4.B.3 shows the levels of reduced glutathione (GSH) in the kidney tissue of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.46 fold in the case of control group with respect to normal. Treatment with *P. rimosus* extract improved the levels of GSH in the kidney tissue. There was approximately 0.89 and 0.72 fold increase in 250 and 50 mg/kg treated group respectively with respect to control. Glibenclamide (1mg/kg) showed 0.8 fold increase GSH.
The levels of lipid peroxidation was significantly (p <0.001) higher in control group than the normal (Fig. 4.B.4) and it was found to be increased by 1.87 folds. The treatment of *P. rimosus* had significantly lowered the levels of lipid peroxidation. There was approximately 1.45 and 1.09 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, whereas the fold decrease of glibenclamide (1 mg/kg) was 1.35 fold with respect to diabetic control.

4.B.3.8. Effect of *P. rimosus* on the antioxidant enzymes in the renal mitochondria

In the study, the activities of renal mitochondrial innate antioxidant activities such as MnSOD and GPx were lowered significantly (p < 0.01) in the diabetic control than that of normal group (Table 4.B.5). There was approximately 1.92 and 1.8 fold decreases in the activities of MnSOD and GPx in the diabetic control than that of normal group. But the treatment of *P. rimosus* at 250 mg/kg significantly (p < 0.001) protected the renal mitochondrial antioxidant status. There was approximately 1.77 and 1.49 fold increases respectively in the activities of MnSOD and GPx for *P. rimosus* 250 mg/kg treated group than that of diabetic control. The treatment of *P. rimosus* 50 mg/kg had only non significant changes in the activities of MnSOD and GPx, but the mean values were higher than that of diabetic control. Similarly, glibenclamide enhanced the renal mitochondrial antioxidant system by enhancing the activities of MnSOD and GPx. There was approximately 1.32 and 1.15 fold increase for MnSOD and GPx activities in the glibenclamide treated group than that diabetic control.

4.B.3.9. Effect of *P. rimosus* on level of renal mitochondrial GSH

Fig. 4.B.5 shows the levels of reduced glutathione (GSH) in the renal mitochondria of normal and experimental animals. The level of reduced glutathione was found to be decreased 1.31 fold in the case of diabetic control group with respect to normal group. Treatment with *P. rimosus* extract improved the levels of GSH in the kidney tissue. There was approximately 1.13 and 0.99 -fold increase in 250 and 50 mg/kg treated group respectively compared to diabetic control. Similarly glibenclamide (1mg/kg) showed 1.27 fold increase in the mitochondrial GSH than that of diabetic control.
Table 4.B.5: Effect of *P. rimosus* on the renal mitochondrial antioxidant status (MnSOD and GPx) in streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>10.17±1.73</td>
<td>43.25±5.45</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>3.81±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.29±3.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>50</td>
<td>6.60±1.31&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>26.39±5.72&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>250</td>
<td>9.19±1.41&lt;sup&gt;***&lt;/sup&gt;</td>
<td>37.63±6.45&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>STZ+Glibenclamide</td>
<td>1</td>
<td>8.50±2.77&lt;sup&gt;***&lt;/sup&gt;</td>
<td>34.18±9.79&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001 (Bonferroni test) significantly different from normal group.

<sup>***</sup> P < 0.001, <sup>**</sup> P < 0.01 significantly and <sup>ns</sup>P > 0.05 non significantly different from control group (Bonferroni test)
Fig. 4.B.5: Effect of *P. rimosus* and glibenclamide treatment on the levels of renal mitochondrial GSH in the streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a* $P < 0.001$ (Bonferroni test) significantly different from normal group. ***$P < 0.001$ significantly and ns $P > 0.05$ non significantly different from control group (Bonferroni test).

Fig. 4.B.6: Effect of *P. rimosus* and glibenclamide treatment on the levels lipid peroxidation in the renal mitochondria of streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6. *a* $P < 0.001$ (Bonferroni test) significantly different from normal group. **$P < 0.01$, *$P < 0.05$ significantly and ns $P > 0.05$ non significantly different from control group (Bonferroni test).
4.B.3.10. Effect of *P. rimosus* on renal mitochondrial lipid peroxidation

The levels of mitochondrial lipid peroxidation was significantly (p < 0.001) higher in diabetic control group than that of normal group (Fig. 4.B.6) and it was found to be increased by 1.31 folds. The treatment of *P. rimosus* had lowered the levels of lipid peroxidation. There was approximately 1.3 and 1.14 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, where as the fold decrease of glibenclamide (1 mg/kg) was 1.29 fold with respect to diabetic control.

4.B.3.11. Effect of *P. rimosus* on lipid profile of diabetic rats

Serum total cholesterol, triglycerides and LDL-cholesterol levels were significantly elevated (p < 0.001) in diabetic groups in comparison to normal (Table 4.B.6). There were approximately 1.35, 1.72 and 1.2 fold increases in the serum TC, TG and LDL-C respectively in the case of diabetic control group with respect to normal. These significant rises were accompanied by significant declines of plasma HDL by 1.12 fold as compared to the normal. The atherogenic index were significantly increased in diabetic control rats by 4.32 fold as compared with the normal control (Fig. 4.B.7).

The oral administration of *P. rimosus* to diabetic rats at 50 and 250 mg/kg for 30 days, resulted in significant declines in plasma total cholesterol, TG and LDL-cholesterol. The fold decrease in the levels of total cholesterol, triglycerides, and LDL were approximately 1.02, 1.01 and 0.99 in the case of 50 mg/kg 1.12, 1.37 and 1.31 fold in the case of 250 mg/kg treated group respectively compared to the diabetic control. The HDL levels was significantly improved, there was approximately 0.79 and 1.02 fold increase for 50 and 250 mg/kg *P. rimosus* with respect to diabetic control. The AI index was declined after treatment with *P. rimosus* and there was 1.54 and 2.59 fold decreases for 50 and 250 mg/kg *P. rimosus* with respect to diabetic control.

The standard reference drug glibenclamide (1 mg/kg) showed 1.13, 1.29 and 1.11 fold decrease for total cholesterol, triglycerides and LDL respectively and 0.85 fold increase for HDL with respect to HFD control group and the AI index was declined to 2.01 fold.
Table 4.B.6: Effect of *P. rimosus* and glibenclamide on serum lipid levels in STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>83.79±14.76</td>
<td>57.80±9.63</td>
<td>39.82±7.53</td>
<td>31.41±8.92</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>175.81±20.08(^a)</td>
<td>148±20.09(^a)</td>
<td>26.31±5.73(^a)</td>
<td>119.89±23.28(^a)</td>
</tr>
<tr>
<td>STZ + <em>P. rimosus</em></td>
<td>50</td>
<td>140.52±13.59(^*)</td>
<td>111.52±15.83(***)</td>
<td>29.92±4.72(^\text{ns})</td>
<td>87.1±14.43(^*)</td>
</tr>
<tr>
<td>STZ + <em>P. rimosus</em></td>
<td>250</td>
<td>114.41±18.62(***)</td>
<td>82.27±8.98(***)</td>
<td>37.32±4.79(^*)</td>
<td>60.21±9.62(***)</td>
</tr>
<tr>
<td>STZ + glibenclamide</td>
<td>1</td>
<td>124.36±12.82(***)</td>
<td>90.23±8.83(***)</td>
<td>33.25±5.93(^\text{ns})</td>
<td>72.86±12.72(***)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

\(^a\) P < 0.001 (Bonferroni test) significantly different from normal group.

\(***\) P < 0.001, \(**\) P < 0.01, \(*\) P < 0.05 significantly and \(\text{ns}\) P > 0.05 non significantly different from control group (Bonferroni test).
Fig. 4.B.7: Effect of *P. rimosus* on atherogenic index (AI) in streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. a P < 0.001 (Bonferroni test) significantly different from normal group. *** P < 0.001, ** P < 0.01 (Bonferroni test) significantly different from control group.

Fig. 4.B.8: Effect of *P. rimosus* on LDL/HDL ratio in streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. a P < 0.001 (Bonferroni test) significantly different from normal group. *** P < 0.001 and * P < 0.05 (Bonferroni test) significantly different from control group.
4.B.3.12. Effect of *P. rimosus* on the activities of SGPT, SGOT and ALP

Serum activities of transaminases, SGPT and SGOT, and ALP are presented in Table 4.B.7. Single dose of streptozotocin (45 mg/kg) significantly (*p* < 0.001) elevated the SGPT, SGOT and ALP activities when compared to the normal control group of animals. There were approximately 1.67, 1.24 and 1.61 fold increases for SGPT, SGOT and ALP respectively in the diabetic control than that of normal control group. Treatment of both the doses of *P. rimosus* 50 and 250 mg/kg for 30 days significantly (*p* < 0.01) protected the elevation of transaminases and ALP activities. There were approximately 1.01, 0.97 and 0.99 fold decreases for *P. rimosus* 50 mg/kg treated group and approximately 1.22, 1.2 and 1.35 fold decreases respectively for *P. rimosus* 250 mg/kg in the activities of SGPT, SGOT, and ALP than that of diabetic control. Similarly, there was approximately 1.29, 1.21 and 1.27 fold decreases respectively in the activities of SGPT, SGOT, and ALP for glibenclamide treated group than that of diabetic control.

4.B.3.13. Effect of *P. rimosus* on the activities of urea and creatinine

Single dose of streptozotocin (45 mg/kg) significantly (*p* < 0.001) elevated the urea and creatinine levels when compared to the normal group of animals. There were approximately 1.75 and 1.46 fold increases for urea and creatinine levels respectively in the diabetic control than that of normal group. Treatment of both the doses of *P. rimosus* 50 and 250 mg/kg for 30 days significantly (*p* < 0.001) protected the elevation of urea and creatinine. There were approximately 1.11 and 1.09 fold decreases for *P. rimosus* 50 mg/kg treated group and approximately 1.39 and 1.3 fold decreases respectively for *P. rimosus* 250 mg/kg in the levels of urea and creatinine than that of diabetic control (Table 4.B.8). Similarly, there was approximately 1.45 and 1.27 fold decreases respectively in the urea and creatinine levels for glibenclamide treated group than that of diabetic control.

4.B.3.14. Effect of *P. rimosus* on hepatic glycogen

Hepatic glycogen contents were significantly decreased in diabetic control rats with respect to normal (*p* < 0.001). However, treatment with *P. rimosus* extract or glibenclamide led to increase in liver glycogen contents over diabetic control. There were
Table 4.B.7: Effect of *P. rimosus* on the activity of liver function enzymes in streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>ALP (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>95.32 ± 16.80</td>
<td>45.67 ± 4.37</td>
<td>105.13 ± 18.81</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>191.67 ± 11.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.36 ± 17.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>182.99 ± 19.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + STZ</td>
<td>50</td>
<td>154.47 ± 26.29**</td>
<td>74.25 ± 7.5**</td>
<td>146.58 ± 21.04*</td>
</tr>
<tr>
<td><em>P. rimosus</em> + STZ</td>
<td>250</td>
<td>123.92 ± 9.01***</td>
<td>58.93 ± 8.9***</td>
<td>120.99 ± 15.26***</td>
</tr>
<tr>
<td>Glibenclamide + STZ</td>
<td>1</td>
<td>130.73 ± 10.31***</td>
<td>56.57 ± 7.99***</td>
<td>124.47 ± 10.79***</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001, (Bonferroni test) significantly different from normal group.

<sup>***</sup> P < 0.001, <sup>**</sup> P < 0.01, and <sup>*</sup> P < 0.05 (Bonferroni test) significantly different from control group.
Table 4.B.8: Effect of *P. rimosus* on the serum urea and creatinine concentration in streptozotocin (STZ) induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>48.85±3.28</td>
<td>0.60±0.14</td>
</tr>
<tr>
<td>Diabetic Control (STZ)</td>
<td>45</td>
<td>99.91±9.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + STZ</td>
<td>50</td>
<td>73.7±6.48&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.86±0.14&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + STZ</td>
<td>250</td>
<td>58.13±6.70&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.73±0.11&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide+STZ</td>
<td>1</td>
<td>60.75±1.28&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.75±0.11&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

<sup>a</sup> P < 0.001, (Bonferroni test) significantly different from normal group.

<sup>***</sup> P < 0.001 (Bonferroni test) significantly different from control group.
approximately 0.99, 1.34 and 1.15 increases for *P. rimosus* 50, 250 and glibenclamide 1mg/kg treated group respectively than that of diabetic control (Fig 4.B.9).

### 4.B.3.15. Effect of *P. rimosus* on the activities of mitochondrial dehydrogenases

The activities of mitochondrial dehydrogenases such as ICDH, α-KGDH, SDH and MDH were significantly declined in the diabetic control than that of normal group (Table 4.B.9). There was approximately 1.73, 1.17, 1.36 and 1.14 fold decreases in the activities of ICDH, α-KGDH, MDH and SDH respectively than that of normal group. The treatment with *P. rimosus* significantly (*p* < 0.01) enhanced the activities of mitochondrial enzymes than that of diabetic control group. There were approximately 0.90, 0.87, 0.89 and 0.86 fold increases in the activities of ICDH, α-KGDH, SDH and MDH respectively for *p. rimosus* 50 mg/kg treated group and approximately 1.39,0.99, 1.23 and 1.06 fold increases in the activities of ICDH, α-KGDH, SDH and MDH respectively for *P. rimosus* 250 mg/kg treated group than that of diabetic control group. Whereas, the fold increases for glibenclamide were respectively 1.33, 1.06, 1.12 and 1.11 for ICDH, α-KGDH, SDH and MDH than that of diabetic control group.

### 4.B.3.16. Effect of *P. rimosus* on the activities of respiratory chain complexes

The activities of respiratory chain complexes such as complex I, III and IV were also significantly (*p* < 0.001) declined in the diabetic control than that of normal group (Table 4.B.10). There were approximately 1.83, 1.77 and 2.17 fold decreases in the activities of complex I, III and IV respectively in the diabetic control group than that of normal group. But, the administration of *P. rimosus* (250 mg/kg) significantly enhanced the activities of complex I, III and IV than that of diabetic control. There were approximately 1.57, 1.35 and 1.62 fold increases in the activities of complex I, III and IV respectively in the *P. rimosus* 250 mg/kg treated group and approximately 1.12, 1.57 and 1.31 fold increases respectively in the *P. rimosus* 50 mg/kg treated group than that of diabetic control. Similarly, the treatment with glibenclamide significantly enhanced the activities of complex I, III, and IV than that of diabetic control. The increase in activities of complex I, III and IV were approximately 1.31, 1.33 and 1.87 fold than that of diabetic control.
Fig. 4.B.9: Effect of *P. rimosus* on liver glycogen levels in streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a* P < 0.001 (Bonferroni test) significantly different from normal group. ***P < 0.001, *P < 0.05 significantly and ns P > 0.05 non significantly different from control group (Bonferroni test).

Fig. 4.B.10: Effect of *P. rimosus* treatment on renal mitochondrial ATP level in streptozotocin (STZ) induced diabetic rats.

Values are mean ± SD, n = 6 animals. *a* P < 0.001 (Bonferroni test) significantly different from normal group. ***P < 0.001, **P < 0.01 significantly and ns P > 0.05 non significantly different from control group (Bonferroni test).
Table 4.B.9: Effect of *P. rimosus* and glibenclamide on the krebs cycle dehydrogenases in the kidney of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>ICDH (μmoles of NAD⁺ reduced/min/mg protein)</th>
<th>α-KGDH (μmoles of NAD⁺ reduced/min/mg protein)</th>
<th>SDH (μmoles of DCIP reduced/min/mg protein)</th>
<th>MDH (μmoles of NADH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>259.37±16.65</td>
<td>57.86±9.83</td>
<td>79.65±15.04</td>
<td>6381.15±912.20</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>113.49±25.79^a</td>
<td>31.96±8.70^a</td>
<td>41.15±6.25^a</td>
<td>4286.23±524.93^a</td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>50</td>
<td>177.31±51.34*</td>
<td>40.07±4.73^ns</td>
<td>49.3±7.20^ns</td>
<td>4900.99±706.27^ns</td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>250</td>
<td>208.7±14.17***</td>
<td>47.87±7.65*</td>
<td>67.89±9.50***</td>
<td>5772.5±689.43*</td>
</tr>
<tr>
<td>STZ+glibenclamide</td>
<td>1</td>
<td>234.41±49.65***</td>
<td>50.95±7.99**</td>
<td>57.99±5.27*</td>
<td>5874.35±487.19**</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6. ^a^ P < 0.001 (Bonferroni test) significantly different from normal group. ^***^ P < 0.001, ^**^ P < 0.01, ^*^ P < 0.05 significantly and ^ns^ P > 0.05 non significantly different from control group (Bonferroni test).

Units: Isocitrate dehydrogenase (ICDH) — μmoles of NAD⁺ reduced/min/mg protein; α – Ketoglutarate dehydrogenase (α - KGDH) — μmoles of NAD⁺ reduced/min/mg protein; Succinate dehydrogenase (SDH) — μmoles of DCIP reduced / min / mg protein; Malate dehydrogenase (MDH) — μmoles of NADH oxidized/min/mg protein.
Table 4.B.10: Effect of *P. rimosus* and glibenclamide treatment on the activity of respiratory chain complexes I, III and IV in the kidney of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Complex I</th>
<th>Complex III</th>
<th>Complex IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>53.19±10.61</td>
<td>25.21±6.12</td>
<td>21.09±4.90</td>
</tr>
<tr>
<td>(STZ) Diabetic Control</td>
<td>45</td>
<td>15.74±7.34</td>
<td>9.25±1.49</td>
<td>6.29±1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.05</em></td>
<td><em>P &lt; 0.05</em></td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>250</td>
<td>45.22±9.03</td>
<td>19.75±5.12</td>
<td>15.65±3.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ns</em></td>
<td><em>ns</em></td>
<td><em>ns</em></td>
</tr>
<tr>
<td>STZ+ <em>P. rimosus</em></td>
<td>50</td>
<td>29.8±3.96</td>
<td>14.31±3.21</td>
<td>12.17±2.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>P &lt; 0.01</strong></td>
<td><strong>P &lt; 0.05</strong></td>
<td><strong>P &lt; 0.05</strong></td>
</tr>
<tr>
<td>STZ + glibenclamide</td>
<td>1</td>
<td>36.94±6.62</td>
<td>18.69±4.59</td>
<td>17.33±3.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P &lt; 0.05</em></td>
<td><em>P &lt; 0.05</em></td>
<td><em>P &lt; 0.05</em></td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6.

*P < 0.001 (Bonferroni test) significantly different from normal group.

**P < 0.001, *P < 0.01, *P < 0.05 significantly and ns P > 0.05 non significantly different from control group (Bonferroni test).

Units: Complex I — μ moles of DCIP reduced/min/mg protein; Complex III — μ moles of ferricytochrome C reduced/min/mg protein; Complex IV — μ moles of ferrocytochrome C oxidized/min/mg protein.
Fig. 4.B.11: Histopathology of kidney in streptozotocin induced diabetic rats

Normal
Diabetic control
Glibenclamide (1 mg/kg)  P. rimosus (50 mg/kg)

P. rimosus (250 mg/kg)
Fig. 4.B.12: Histopathology of liver in streptozotocin induced diabetic rats

Normal  Diabetic control

Glibenclamide (1 mg/kg)  P. rimosus (50 mg/kg)

P. rimosus (250 mg/kg)
Fig. 4.B.13: Histopathology of pancreas in streptozotocin induced diabetic rats

Normal
Diabetic control
Glibenclamide (1 mg/kg)

P. rimosus (50 mg/kg)

P. rimosus (250 mg/kg)
4.B.3.17. Effect of *P. rimosus* on mitochondrial ATP level

The mean value of ATP level in the renal mitochondria of rat declined in diabetic control group. The ATP level elevated in the *P. rimosus* and glibenclamide treated groups (Fig. 4.B.10).

4.B.3.18. Histopathological observation

Histopathological evaluation reveals that kidney of normal group rats showed normal architecture of glomerulus and tubules. Kidney of STZ induced diabetic control rats showed vacuolar degenerative changes in the epithelial cells, cellular proliferation with fibrosis, thickening of capillary walls and hypertrophy of glomerular tuft. Animals treated with *P. rimosus* and glibenclamide apparently showed normal architecture of glomeruli and tubules similar to the normal group (Fig. 4.B.11).

The normal group revealed regular morphology of liver parenchyma cells with intact hepatocytes. In case of STZ treated group distortion in the arrangement of cells around the central vein, mononuclear inflammatory infiltrations, vacuolation, and development of fibrosis in the degenerate cells were noted. Diabetic rats treated with *P. rimosus* and glibenclamide showed reduced fibrosis (Fig. 4.B.12).

STZ was known to destroy the β cells of the pancreas. Histopathological evaluation reveals that alloxan caused extensive damage in pancreatic β-cells, such as a decrease of islet cells’ number, cell necrosis. Administration of *P. rimosus* extract and glibenclamide to streptozotocin-induced diabetic rats restored partially the pancreatic tissue damages (Fig. 4.B.13).

4.B.4. DISCUSSION

Streptozotocin (STZ) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β-cell necrosis. The action of STZ on mitochondria generates $O_2^-$ anions, which leads to diabetic complications (Anderson et al., 1974; Szkudelski, 2001). The present study was undertaken to evaluate the hypoglycemic, hypolipidemic and antioxidant properties of *P. rimosus* in STZ induced diabetes.
In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. STZ causes a massive reduction in insulin release inducing hyperglycemia. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus (Latner, 1958). Administration of *P. rimosus* significantly lowered the elevated serum glucose level as compared to diabetic control (Table 4.B.1).

Treatment with *P. rimosus* significantly elevated the serum insulin levels in diabetic rats (Fig. 4.B.1). The possible mechanism by which the *P. rimosus* exhibiting the antihyperglycemic action in diabetic rats may be by increasing the pancreatic secretion of insulin from the existing β-cells as it is evidenced by the significant increase in the level of insulin in diabetic rats after the treatment of *P. rimosus*. Similarly glibenclamide also increased the insulin levels.

Diabetic rats showed reduction in their body weights (30 days after the induction of diabetes) when compared to normal rats, which could be due to poor glycemic control. The excessive catabolism of protein to provide aminoacids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in diabetic untreated rats. In the present study, diabetic rats treated with *P. rimosus* showed an increase in body weight as compared to the diabetic control (Table 4.B.2). Rise in insulin levels upon treatment with *P. rimosus* in diabetic rats resulted in improved glycemic control, which prevented the loss of body weight.

Diabetes is associated with hyperlipidemia (Chase and Glasgow, 1976) which is one of the major cardiovascular risk factors. Clinical trials have demonstrated that the increase in plasma low density lipoprotein cholesterol (LDL-C) levels is implicated in the early development and progression of atherosclerosis. However, high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction (Martin et al., 1986). Triglycerides (TGs) may also be a risk factor, especially in individuals with diabetes (West et al., 1983). It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids such as total cholesterol (TC) and triglyceride.
(TG) in diabetic patients (Goldberg, 1981). Diabetic patients have problems in packaging cholesterol and tend to have higher serum TG levels. Our data were in line with this notion as the diabetic rats exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TC, TG, low density lipoprotein cholesterol (LDL-C), and reduction of high-density lipoprotein cholesterol (HDL-C) levels.

The atherosclerotic index (AI), defined as the ratio of TC to HDL-C, is a diagnostic indicator of the risk of atherosclerosis development. Administration of *P. rimosus* provides a beneficial action on rat lipid metabolism in regard to the reduction of AI. In fact, the AI was deceased after *P. rimosus* treatment (Fig. 4.B.7). It is also desirable to have higher plasma HDL and lower LDL-cholesterol to prevent atherogenesis, since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. Again, the administration *P. rimosus* extract significantly suppressed the higher values of LDL-C/HDL-C ratio (Fig. 4.B.8).

Treatment with *P. rimosus* for 30 days was effective to produce a significant reduction in the TC, TG, LDL-C and increase in HDL-C levels in diabetic rats (Table 4.B.6). These results indicate that *P. rimosus* has a lipid-lowering effect on the diabetic rats. Maintenance of serum lipid profiles recommended the effectiveness of the extract against experimental type 2 diabetic rats.

STZ-induced diabetes is a well-documented model of experimental diabetes. STZ-diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Like and Rossini, 1976). The roles of oxidative stress and antioxidants in organs and tissues damage have been studied extensively in experimental diabetes and diabetic patients (West, 2000). Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins and the subsequent oxidative degradation (Mahboob et al., 2005). Increased oxidative stress is involved in diabetes. There is evidence that glycation itself induces the generation of oxygen-derived free radicals in diabetic condition (Senthilkumar et al., 2006). The ROS scavenging capacity by antioxidants is decreased in diabetes such that constant oxidative stress develops and oxidation of lipids, proteins and other macromolecules such as DNA is
increased. Augmentation of plasma antioxidative capacity would also attenuate lipid peroxidation through this mechanism (Ohkawa et al., 1979).

Significant biochemical and molecular changes occur in mitochondria of the liver, kidneys, and brain with experimentally-induced diabetes. Hyperglycemia-induced generation of superoxide anion by mitochondria is considered as the initial trigger for a vicious cycle of oxidative stress in diabetes (Brownlee, 2001). Accumulation of peroxidation products in mitochondria is associated with a decrease in electron transport chain activity that impairs cellular energy metabolism and viability (Orrenius, 2007). Specific examples of mitochondrial alterations associated with oxidative stress include mitochondrial swelling, decreased transmembrane potential, decreased respiratory control ratio, uncoupling of oxidative phosphorylation, and reduced cytochrome c oxidase activity (Venditti et al., 2007). Mitochondria are one of the primary targets of ROS generated during the oxidative stress induced in diabetes, (Brignone et al., 1991) which in turn damages mitochondrial DNA. The depletion of the intracellular concentration of nicotinamide adenine dinucleotide-oxidized (NAD+), electron transport, and ATP formation can be ascribed to the activation of the nuclear enzyme poly (ADP-ribose) polymerase, which is stimulated by the ROS-induced DNA damage (Shen et al., 2004; Ceriello et al., 2006) Hence, in pancreatic beta cells, defects in mitochondrial ATP production can impair the glucose metabolism and thus affect the secretion of insulin, which can result in glucose tolerance (Maechler and Wollheim, 2001). A redox imbalance in pancreatic β-cells can also cause apoptotic cell death.

There is clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals (Grankvis et al., 1981). Antioxidative enzymes form the first line of defense against ROS in the organism. The diabetogenic action of STZ can be prevented by by antioxidant enzymes, such as SOD, CAT and GPx.

Earlier workers had reported a decrease in the activities of these antioxidant enzymes (SOD, CAT, GPx and GST) in the plasma and liver of diabetic rats (Anuradha and Selvam, 1993). We have also observed the decrease in GSH levels (Fig. 4.B.2 and Fig. 4.B.3) and SOD, CAT and GPx, activities in liver, kidney, pancreas and blood in diabetic
rats (Table 4.B.3 and Table 4.B.4). Similarly mitochondrial SOD, GPx (Table 4.B.5) and GSH levels in kidney was also found to be decreased (fig. 4.B.5). This could be attributed to higher levels of superoxide radicals and hydrogen peroxide as indicated by increased ROS levels in these rats, which reduced the antioxidant enzymes activities.

Administration of *P. rimosus* has been reported to prevent or attenuate the decrease of tissue antioxidant enzymes, provide cellular protection against ROS (Ajith and Janardhanan, 2002). Antioxidant treatment was reported to alleviate the oxidative injury by enhancing SOD and GPx activities and increasing the mRNA expression of the two antioxidant enzymes (Pang et al., 1999). Following the treatment with *P. rimosus* extract the activities of SOD, CAT, GPx and GSH level was augmented in blood, pancreas, liver and kidneys of diabetic rats. Similarly, MnSOD, GPx and GSH level in kidney mitochondria was also improved. This could be attributed to the strong antioxidant property of the *P. rimosus* extract. It is possible that the effect of *P. rimosus* on SOD and GPx was associated with its induction on the expression of genes of the antioxidant enzymes.

Level of lipid peroxidation in the liver, pancreas and kidney of STZ induced diabetic rats were found to be increased. A possible explanation for the enhancement of lipid peroxidation products in STZ treated rats may be due to decreased levels of antioxidant system. As a result of these metabolic events peroxidation reactions are accelerated leading to cellular injury (Gokkusu and Mostafazadeh, 2003). Rats treated with *P. rimosus* showed decreased activities of the MDA levels suggesting that *P. rimosus* is capable of protecting the tissues from lipid peroxidation (Fig 4.B.4). Similarly, lipid peroxidation in kidney mitochondria also found to be decreased (Fig 4.B.6)

Mitochondrial electron transport chain (ETC) is considered as a major intracellular source of ROS (Boveris and Chance, 1973). The high percentage of polyunsaturated fatty acids in the mitochondria membrane makes it particularly susceptible to damage induced by ROS. In the present study, high oxidative stress in mitochondria of kidney in the STZ induced diabetes is indicated by the increased level of LPO. Mitochondrial membrane lipid peroxidation results in irreversible loss of mitochondrial functions such as mitochondrial respiration, oxidative phosphorylation and ion transport (Bacon and Britton, 1990). The
mitochondrial enzymes (ICDH, SDH, MDH and α-KGDH) catalyse the oxidation of several substrates through the tricarboxylic acid (TCA) cycle, yielding reducing equivalents which are channeled through the respiratory chain for the synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation. Inhibition of these enzymes by ROS may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy (Capetenaki, 2002).

ICDH is NADP dependent and controls the mitochondrial redox balance and the subsequent oxidative damage. SDH is a component of electron chain and is bound to the inner mitochondrial membrane. Le-Quoc et al. (1981) have reported that SDH loses its activity, when vicinal thiol groups are oxidized. The activities of these dehydrogenases were reduced in the kidney mitochondria of STZ induced diabetic rats. The decreased activities of these enzymes results in decreased NADH/NAD ratio. The dehydrogenases of TCA cycle enzymes could have been affected by the free radicals in diabetic condition. *P. rimosus* treatment could partially restore the activities of these enzymes to normal in the kidney mitochondria (Table 4.B.9). Increase in the activities of these TCA cycle enzymes in *P. rimosus* pretreated rats shows better utilization of energy yielding intermediates by TCA cycle.

Earlier studies in diabetic rats Hu et al. (2009) have observed decreased expression and activity of complexes II and IV in cardiomyocytes exposed to high glucose. Sukhdev and Rajat, (2011) also reported reduced activities of mitochondrial complexes in diabetic brain while N-acetylcysteine supplementation was observed to reverse the loss in the activity of mitochondrial electron transport chain enzymes. Renal Complex-III appears to be an early and specific mitochondrial target during experimental type-1 diabetes (Shankar et al., 2009). Complex-III is centrally located in the electron transfer process and has been implicated as one of the major sites for superoxide generation in the mitochondria during diabetes. Partial inhibition of Complex-III during conditions of increased respiration would decrease the transfer of electrons from ubiquinol to Complex-III, and increase the half-life of ubisemiquinone, which leads to generation of superoxide. The impairment of complex I and III activities may increase the electron leakage from the ETC, generating more
superoxide radical and perpetuating a cycle of oxygen radical-induced damage to mitochondrial membrane constituents (Paradies et al., 2002).

In the current study, the activities of complexes I, III and IV were declined significantly in the STZ treated diabetic rats than that of normal rats. This is assumed to be due to the excess generation of ROS by STZ and the treatment of *P. rimosus* significantly enhanced the activity complex I, III and IV which is ascribed to the free radical scavenging activity of *P. rimosus* (Table 10). Thus *P. rimosus* elevated the renal mitochondrial ATP level in the rat by enhancing the Krebs cycle enzyme activity as well as the activity of complexes I and IV.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites (Rej, 1978). SGOT, SGPT and ALP are reliable markers of liver function. Ohaeri (2001) found that liver was necrotized in STZ-induced diabetic rats. Therefore, an increase in the activities of SGOT, SGPT and ALP in plasma might be mainly due to the leakage of these enzymes from the liver into the blood stream (Navarro et al., 1993) which gives an indication of the hepatotoxic effect of STZ. Treatment of the diabetic rats with *P. rimosus* caused reduction in the activity of these enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes (Table 7). These results are in agreement with those obtained by Eliza et al. (2009) in rats.

Diabetic rats showed significantly increased levels of urea and creatinine in the serum, which are considered as significant markers of renal dysfunction. In the present study, the significant reduction in the levels of serum urea and creatinine in the diabetic treated rats indicated that the *P. rimosus* prevents the progression of renal damage in diabetic rats (Table 4.B.8).

The histopathological analysis of kidney, liver and pancreas of diabetic control rats revealed tissue damages. It may be noted that several authors reported such histopathological changes in kidney, liver and pancreas tissues of mice exposed to streptozotocin (Atsuyo et al., 2008; Palsamy et al., 2010). Administration of *P. rimosus* extract to STZ induced diabetic rats restored partially the normal architecture (Fig. 4.B.11, Fig. 4.B.12 and Fig. 4.B.13).
Glycogen is the primary intracellular storable form of glucose. The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and the availability of insulin. The regulation of glycogen metabolism in vivo occurs by the enzymes glycogen synthase and glycogen phosphorylase. The reduced glycogen store in the diabetic rats has been attributed to the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. This is probably due to lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthetase systems (Shirwaikar et al., 2004). In the present study, there was a significant decrease in the liver glycogen in diabetic rats. Treatment with *P. rimosus* significantly increased the glycogen levels of the diabetic animals and this may be because of the reactivation of glycogen synthase system by the insulin (Fig. 4.B.9).

The previous studies from our lab have demonstrated the profound in vitro superoxide and hydroxyl radical scavenging, as well as lipid peroxidation inhibiting activities of extract of *P. rimosus* (Ajith and Janardhanan, 2002). Phytochemical screening of the *P. rimosus* extract showed the presence of polysaccharides and polyphenols. The presence of these constituents might also be responsible for exhibited activity. The findings of the present study shows a number of positive effects of *P. rimosus* on rats with STZ induced disturbances in lipoprotein profile, antioxidant status, and blood glucose level. Furthermore, *P. rimosus* also has the hepatoprotective and renal protective properties. Thus, *P. rimosus* is beneficial in the control of diabetes, abnormalities in lipid profiles and oxidative stress.

We have also demonstrated that early stages of diabetes induced alterations in respiratory chain complexes, ATP synthesis, and renal dysfunction. These diabetes-induced alterations in activities of mitochondrial complexes and energy status could contribute to the underlying role of oxidative stress in the pathogenesis of diabetic nephropathy. The administration of *P. rimosus* had significantly protected the renal mitochondria by enhancing the renal antioxidant status and also showed considerable increase of TCA enzymes such as ICDH, α-KGDH, MDH and SDH activities and respiratory chain complexes I, III and IV thereby restoring the mitochondrial functional status in diabetic rats. Finally, strategies to limit the extent of renal mitochondrial damage during hyperglycemia (by therapeutic agents that will specifically modulate mitochondrial function) might prevent or inhibit the development of nephropathy in the diabetic population.