Chapter 5: Hypolipidemic effects of *Phellinus rimosus*
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5.4. DISCUSSION


5.1. INTRODUCTION

Hypercholesterolemia and hypertriglyceridemia are major risk factors either, alone or together for the development of coronary artery disease and the progression of atherosclerosis (Lusis, 2000). High levels of low-density lipoprotein (LDL) accumulate in the extracellular sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis, hypertension, obesity, diabetes, functional depression in some organs, etc (Bierman et al., 1966; Catapano et al., 2000; Lusis, 2000). Furthermore, free-radical-mediated peroxidative modification of polyunsaturated fatty acids of LDL and very-low-density lipoprotein (VLDL) is thought to be contributed in the progression of atherosclerotic lesions. 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).

Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidaemia in animal models in order to screen natural or chemical drugs (Schurr et al., 1972) and to study cholesterol and triacylglycerol metabolism (Zeniya and Reuben, 1988). The accumulation of plasma lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity (Hayashi et al., 1981).

In hyperlipidemic conditions, enzymatic as well as non-enzymatic antioxidative defence systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbic acid, and reduced glutathione (GSH) are altered leading to ROS mediated damage (Araujo et al., 1995). Oxidative stress is an early event in the evolution of hyperlipidemia, and it has been suggested that appropriate support for enhancing antioxidant supply in subjects with abnormally elevated lipid levels can attenuate the course of the disease (Yang et al., 2008).

The cause of hyperlipidemia has been thought to be related to increased lipid synthesis, decreased lipid clearance from the blood or a combination of these two processes. Consequently, one method to lower blood lipid levels would be to inhibit the synthesis of cholesterol or triglyceride. Such agents have been developed and currently
serve as therapeutics for hyperlipidemia. Inhibitors of the rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, include pravastatin-Na, simvastatin and atorvastatin: These drugs effectively lower the serum cholesterol levels and are widely used to treat patients with hypercholesterolemia (Moghadasuan et al., 2000). These lipid lowering drugs that are used to treat hyperlipidemia are known to possess side effects (Chattopadhyaya et al., 1996). For example, some studies reported some patients were unable to tolerate statin treatments, due to musculoskeletal symptoms and other side effects (Rader, 2001). Therefore, there is an urgent need to have drugs with lipid lowering and antioxidant activities with no side effects. Natural products are the best claimed option (Jain et al., 2010).

Traditionally, edible mushrooms have been prescribed in Oriental medicine due to their hypocholesterolemic effects (Sun et al., 2007). In general, the intake of edible mushroom reduces the cardiovascular risk (Kaneda and Tokuda, 1966; Bobek and Galbavy, 1999; Yamada et al., 2002; Mori et al., 2008). The hypocholesterolemic effect of edible mushrooms has been reported, in an early work by Kaneda and Tokuda (1966), who studied cholesterol lowering properties of *Lentinus edodes*, *Auricularia polytricha*, *Flammulina velutipes* and *Agaricus bisporus*. Additionally, investigations from Bobek and coworkers (Bobek et al., 1991, 1993, 1998) were focused on the hypocholesterolemic effects of *Pleurotus ostreatus*, which revealed lipid peroxidation inhibition and suppression of the activity of HMG-CoA reductase in both normocholeolemic and hypercholesterolemic animal models. Indeed, some competitive inhibitors of HMG-CoA reductase have been isolated from different mushrooms such as eritadenine from *L. edodes* (Chibata et al., 1969) and meviol from *Pleurotus* sp. (Gundecimerman, 1993).

Investigations on the pharmacological activities of *P. rimosus* are fragmentary. Presence of antioxidant and anti-inflammatory activities of *P. rimosus* might be clinically relevant in the management of heart and circulation health complications. No studies evaluating the hypolipidemic effects *P. rimosus* have been made so far. Therefore, this study was aimed to determine the hypolipidemic effects of *P. rimosus* in triton WR-1339 and high cholesterol diet induced hyperlipidemic rats.
5.2. Materials and methods

5.2.1. Animals

Male wistar rats weighing (190 ± 25 g) were used in this study.

5.2.2. 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.

5.2.3. Determination of hypolipidemic activity

Hypolipidemic activity of *P. rimosus* extract was determined by triton WR-1339 induced and high cholesterol diet induced hyperlipidemia models in rats.

5.2.3.1. Hypolipidemic effect against Triton WR-1339 induced hyperlipidemia

Animals were divided into 5 groups of 6 animals each and treated as follows:

- Group-I: Vehicle (distilled water).
- Group-II: Hyperlipidemic control rats.
- Group III: Aqueous-ethanol extract of *P. rimosus* 50 mg/kg body weight (p.o).
- Group IV: Aqueous-ethanol extract of *P. rimosus* 250 mg/kg body weight (p.o).
- Group V: Atorvastatin 2.5 mg/kg body weight (p.o).

Group I treated with vehicle (distilled water) served as normal, in all other groups hyperlipidemia was induced by a single intraperitoneal (ip) injection of triton WR 1339 (300 mg/kg b.w.) dissolved in normal saline (pH 7.4) (Okazaki et al., 1990). Group II was kept as control, without any treatment. Group III and IV were treated with *P. rimosus* extract 50 and 250 mg/kg body wt. Group V was treated with atorvastatin 2.5 mg/kg body wt. The extract and atorvastatin were administered by oral gavage one hour before the triton administration and 24 hour after treatment animals were sacrificed. Blood was collected directly from the heart of each animal and the serum was separated and used for the estimation of serum lipid profile. Liver samples were removed and stored at -70° until analysis could be completed.
5.2.3.2. Hypolipidemic effect against high cholesterol diet induced hyperlipidemia

Animals were divided into 5 groups of 6 animals each and treated as follows

- **Group-I**: Vehicle (distilled water).
- **Group-II**: Hyperlipidemic control rats.
- **Group III**: Aqueous-ethanol extract of *P. rimosus* 50 mg/kg body weight (p.o).
- **Group IV**: Aqueous-ethanol extract of *P. rimosus* 250 mg/kg body weight (p.o).
- **Group V**: Atorvastatin 2.5 mg/kg body weight (p.o).

Group I animals received a basal unsupplemented chow diet. All other groups of animals were fed with a diet containing 1% cholesterol and 0.5% cholic acid, i.e. high cholesterol diet (HCD) for 30 days. The composition of diets was detailed in Table 5.1. Cholic acid was added to improve the cholesterol absorption from the intestine. Rats treated with vehicle (distilled water p.o) were kept as normal, group I. Group II was kept as HCD control. Group III and IV were treated with *P. rimosus* extract 50 and 250 mg/kg body wt. Group V was treated with atorvastatin 2.5 mg/kg body wt. The extract and atorvastatin were administered by oral gavage once daily for 30 consecutive days. Twenty-four hours after the completion of drug administration, the animals were sacrificed by cervical decapitation. Heparin and plain blood samples were collected directly from the heart and serum was separated. The heart and liver were excised immediately and kept at -70°C for the determination of enzymatic and non-enzymatic antioxidant status.

5.2.4. 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:

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

The arteriosclerosis index (AI) was calculated by the equations as following:
### Table 5.1: Composition of high cholesterol and normal diet (g/kg diet)

<table>
<thead>
<tr>
<th></th>
<th>High cholesterol diet (HCD)</th>
<th>Normal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>250</td>
<td>300</td>
</tr>
<tr>
<td>Gingly oil cake</td>
<td>235</td>
<td>250</td>
</tr>
<tr>
<td>Black gram husk</td>
<td>240</td>
<td>290</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
<td>-----</td>
</tr>
<tr>
<td>Bile salt</td>
<td>5</td>
<td>-----</td>
</tr>
<tr>
<td>Hydrogeneated ground nut oil</td>
<td>100</td>
<td>-----</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
AI = (total cholesterol - HDL-C) / HDL-C

5.2.5. Hepatic and cardiac lipid peroxidation and antioxidant status

10% homogenate of liver and heart were prepared as described in the section (2.2.2) and used for the determination of 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) photocolorimetrically using a double beam spectrophotometer (SL 164, UV–VIS double beam spectrophotometer, Systronics India Ltd).

5.2.6. Hepatic HMG-CoA reductase activity

Hepatic HMG-CoA reductase activity was measured from the HMG –CoA / mevalonate ratio. HMG-CoA was determined by its reaction with hydroxylamine hydrochloride at alkaline pH and subsequent colorimetric measurement of the resulting hydroxamic acid by formation of complexes with ferric salts at 540 nm. Mevalonate was estimated by reaction with the same reagent but at pH 2.1. At this pH, the lacton form of mevalonate readily reacts with hydroxylamine hydrochloride to form the hydroxamate. The ratio between HMG-CoA and mevalonate is inversely proportional to HMG-CoA reductase activity (i.e. an increase in ratio indicates decreased activity) (Rao and Ramakrishnan, 1975).

Freshly excised liver tissue was immediately washed with saline, blotted on filter paper, weighed and then cut into small pieces and homogenized in saline/arsenate solution to give a 10% (w/v) liver homogenate. Mix equal volumes of the fresh 10% tissue homogenate and diluted perchloric acid. Allow to stand for 5 min and centrifuge (2000 rpm, 10 min). Treat 1.0 ml of filtrate with 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA), mix, and after 5 min add 1.5 ml of ferric chloride reagent to the same tube and shake well. Take readings after 10 min at 540 nm vs. a similarly treated saline/arsenate blank.
5.2.7. Histopathology

A portion of the liver tissue was fixed in 10% buffered neutral formalin saline for histological studies (section 2.2.28).

5.3. RESULTS

5.3.1. Effect of *P. rimosus* on serum lipid profile of Triton WR-1339 induced hyperlipidemic rats

Serum total cholesterol, triglycerides and LDL-cholesterol levels were significantly elevated (p < 0.001) in triton treated groups in comparison to normal (Table. 5.2). There were approximately 5.57, 9.9 and 4.91 fold increases in the total cholesterol, triglycerides, and LDL-cholesterol respectively in the case of control group with respect to normal. These significant rises were accompanied by significant declines of plasma HDL by 1.52 fold. The atherogenic index was significantly increased in hypercholesterolemic control rats by 6.51 fold as compared with the normal (Fig. 5.1).

The oral administration of *P. rimosus* to rats at 50 and 250 mg/kg 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 1.57, 1.58 and 1.8 in the case of 50 mg/kg and 1.95, 1.83 and 2.73 fold in the case of 250 mg/kg treated group respectively compared to the hyperlipidemic control. The HDL levels was significantly improved, there was approximately 1.19 and 1.31 fold increase for 50 and 250 mg/kg *P. rimosus* with respect to control. The AI index was declined after treatment with *P. rimosus* and there was 1.32 and 1.69 fold decreases for 50 and 250 mg/kg *P. rimosus* with respect to hyperlipidemic control.

The standard reference drug atorvastatin (2.5 mg/kg) showed 2.46, 1.98 and 4.29 fold decreases for total cholesterol, triglycerides and LDL respectively and 1.37 fold increases for HDL with respect to hyperlipidemic control group. The AI index was declined to 2.45 fold with atorvastatin. The antihyperlipidemic effect exhibited by the *P. rimosus* extract at the oral dose of 250 mg/kg was more comparable to the standard drug atorvastatin (2.5 mg/kg).
Table 5.2: Effect of *P. rimosus* on serum lipid levels in Triton WR-1339 induced hyperlipidemic 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>86.75 ±10.07</td>
<td>73.27±8.33</td>
<td>36.73±4.77</td>
<td>35.81±5.98</td>
</tr>
<tr>
<td>Triton Control</td>
<td>300</td>
<td>394.67 ± 33.17</td>
<td>747.52±57.54</td>
<td>24.10±5.74</td>
<td>221.27±23.63</td>
</tr>
<tr>
<td>Triton + <em>P. rimosus</em></td>
<td>50</td>
<td>235.74±35.43</td>
<td>455.59±52.29</td>
<td>28.70±5.54ns</td>
<td>115.86±20.19ns</td>
</tr>
<tr>
<td>Triton + <em>P. rimosus</em></td>
<td>250</td>
<td>189.98±28.48</td>
<td>406.72±29.57</td>
<td>31.90±5.81ns</td>
<td>76.45±13.21***</td>
</tr>
<tr>
<td>Triton + atorvastatin</td>
<td>2.5</td>
<td>151.41±22.29</td>
<td>365.19±38.41</td>
<td>33.20±6.12ns</td>
<td>47.39±9.64***</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6 animals.

* P < 0.001 and ** P < 0.01 (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).
Fig. 5.1: Effect of *P. rimosus* on atherogenic index (AI) in Triton WR-1339 (TRI) induced hyperlipidemic rats.

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

Fig. 5.2: Effect of *P. rimosus* on LDL/HDL ratio in Triton WR-1339 (TRI) induced hyperlipidemic rats.

Values are mean ± SD, n = 6 animals. $^a$ P < 0.001 (Bonferroni test) significantly different from normal group. $^{***}$ P < 0.001 (Bonferroni test) significantly different from control group.
5.3.2. Effect of *P. rimosus* on lipid profile of high cholesterol diet induced hyperlipidemic rats

The high-fat diet resulted in significant increase of plasma lipids including the total cholesterol, triglycerides and LDL-cholesterol. There were approximately 2.21, 2.14 and 2.86 fold increases in the total cholesterol, triglycerides, and LDL-cholesterol respectively in the HCD control group with respect to normal. These significant rises were accompanied by significant declines of plasma HDL by 1.33 fold. The atherogenic index was significantly increased in HCD treated rats by 5.3 fold as compared with the normal (Table 5.3).

The oral administration of *P. rimosus* to HCD rats at 50 and 250 mg/kg for 30 days, resulted in significant decline in plasma total cholesterol, TG and LDL-cholesterol. The fold decrease in the levels of total cholesterol, triglycerides, and LDL were approximately 1.34, 1.15 and 1.52 in the case of 50 mg/kg and 1.68, 1.42 and 2.27 fold in the case of 250 mg/kg treated group respectively when compared to the HCD control. The HDL levels was significantly improved, there was approximately 1.26 and 1.46 fold increase for 50 and 250 mg/kg *P. rimosus* with respect to HCD control. The AI index was declined after treatment with *P. rimosus* and there was 2.24 and 3.8 fold decreases respectively for 50 and 250 mg/kg *P. rimosus* treated groups with respect to HCD control (Fig. 5.3).

The standard reference drug atorvastatin (5 mg/kg) showed 1.83, 1.55 and 2.71 fold decrease for total cholesterol, triglycerides and LDL respectively and 1.53 fold increase for HDL with respect to HCD control group. The AI index was declined to 5 fold with atorvastatin. The antihyperlipidemic effect exhibited by the *P. rimosus* extract at the oral dose of 250 mg/kg was more pronounced.

5.3.3. Effect of *P. rimosus* on hepatic HMG-CoA reductase activity

The hepatic HMG-CoA reductase activity was significantly lowered in the *P. rimosus* treated group than in the hyperlipidemic control groups both in triton (Table 5.4) and high cholesterol diet models (Table 5.5).
Table 5.3: Effect of *P. rimosus* and atorvastatin on serum lipid levels of high cholesterol diet (HCD) induced hyperlipidemic 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>93.00 ± 8.05</td>
<td>83.37 ± 11.51</td>
<td>36.47 ± 7.21</td>
<td>39.45 ± 13.98</td>
</tr>
<tr>
<td>HCD Control</td>
<td>------</td>
<td>245.11 ± 21.11(^a)</td>
<td>219.29 ± 15.14(^a)</td>
<td>27.29 ± 6.61(^{ns})</td>
<td>175.2 ± 21.71(^a)</td>
</tr>
<tr>
<td>HCD + <em>P. rimosus</em></td>
<td>50</td>
<td>157.24 ± 9.49(^{***})</td>
<td>164.59 ± 12.01(^{***})</td>
<td>34.59 ± 6.71(^{ns})</td>
<td>89.85 ± 11.13(^{***})</td>
</tr>
<tr>
<td>HCD + <em>P. rimosus</em></td>
<td>250</td>
<td>124.41 ± 8.49(^{***})</td>
<td>132.91 ± 9.57(^{***})</td>
<td>40.17 ± 5.81(^*)</td>
<td>57.19 ± 10.13(^{***})</td>
</tr>
<tr>
<td>HCD + atorvastatin</td>
<td>2.5</td>
<td>109.74 ± 12.19(^{***})</td>
<td>122.65 ± 8.41(^{***})</td>
<td>42.33 ± 8.27(^*)</td>
<td>42.66 ± 10.29(^{***})</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6 animals.

\(^a\) P < 0.001 significantly and \(^{ns}\) P > 0.05 non significantly different from normal group (Bonferroni test).

\(^{***}\) P < 0.001, \(^*\) P < 0.05 significantly and \(^{ns}\) P > 0.05 non significantly different from control group (Bonferroni test).
Fig. 5.3: Effect of *P. rimosus* on atherogenic index (AI) in high cholesterol diet (HCD) induced hyperlipidemic rats.

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

Fig. 5.4: Effect of *P. rimosus* on LDL/HDL ratio in high cholesterol diet (HCD) induced hyperlipidemic rats.

Values are mean ± SD, n = 6 animals. $^a$ P < 0.001 (Bonferroni test) significantly different from normal group. $^{***}$ P < 0.001 (Bonferroni test) significantly different from control group.
Table 5.4: Effect of *P. rimosus* on HMG Co A/Mevalonate ratio in the liver of Triton WR-1339 induced hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>HMGCoA/Mevalonate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>2.6±0.21</td>
</tr>
<tr>
<td>Triton Control</td>
<td>300</td>
<td>1.05±0.17(^a)</td>
</tr>
<tr>
<td>Triton+ <em>P. rimosus</em></td>
<td>50</td>
<td>1.13±0.25(^\text{ns})</td>
</tr>
<tr>
<td>Triton+ <em>P. rimosus</em></td>
<td>250</td>
<td>1.76±0.27(^***)</td>
</tr>
<tr>
<td>Triton+ atorvastatin</td>
<td>2.5</td>
<td>1.97±0.33(^***)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6 animals.

\(^a\) 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).
Table 5.5: Effect of *P. rimosus* on HMG Co A/Mevalonate ratio in the liver of high cholesterol diet (HCD) induced hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>HMGCoA/Mevalonate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>1.18±0.19</td>
</tr>
<tr>
<td>HCD Control</td>
<td>---------</td>
<td>2.5±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + HCD</td>
<td>50</td>
<td>2.9±0.21&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + HCD</td>
<td>250</td>
<td>3.2±0.45&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atorvastatin + HCD</td>
<td>2.5</td>
<td>3.54±0.39&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6 animals.
<sup>a</sup> P < 0.001 (Bonferroni test) significantly different from normal group.
<sup>***</sup> P < 0.001, <sup>*</sup> P < 0.05 significantly and <sup>ns</sup>P > 0.05 non significantly different from control group (Bonferroni test).
5.3.4. Effect of *P. rimosus* on hepatic antioxidant status

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P< 0.001) in the liver of rat treated with HCD (Table 5.6). There was approximately 1.16, 1.27 and 1.35 fold decreased 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 0.94, 1.03 and 1.27 fold in the case of 250 mg/kg and 0.89, 0.92 and 0.95 fold in the case of 50 mg/kg treated group, respectively compared to the HCD controls. The standard reference drug atorvastatin (2.5 mg/kg) showed 0.81, 1.06 and 1.23 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 5.5 shows the levels of reduced glutathione (GSH) in the liver tissue of normal and HCD treated rats. The level of GSH was found to be decreased 1.11 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 1.07 and 0.83 -fold increase in 250 and 50 mg/kg treated group, respectively compared to HCD control. Atorvastatin (2.5 mg/kg) showed 1.12 fold increases in GSH.

The levels of lipid peroxidation was significantly (p <0.001) higher in HCD group than the normal (Fig. 5.6) and it was found to be increased by 1.27 folds. The treatment of *P. rimosus* had lowered the levels of lipid peroxidation. There was approximately 1.14 and 0.82 fold decrease in the lipid peroxidation in 250 and 50 mg/kg *P. rimosus* treated groups respectively, whereas, the fold decrease of atorvastatin (5 mg/kg) was 1.05 fold with respect to HCD control.

5.3.5. Effect of *P. rimosus* on cardiac antioxidant status

The activities of the antioxidant enzymes such as SOD, CAT and GPx were lowered significantly (P< 0.001) in the heart tissue of rat (Table 5.6). There was approximately 1.37, 1.24 and 1.62 fold decreased in the activities of SOD, CAT and GPx respectively in the case of control group with respect to normal. The *P. rimosus* treatment at 50 and 250 mg/kg for 30 days improved the activities of antioxidant enzymes in the
Table 5.6: Effect of *P. rimosus* on the activities of SOD, CAT and GPx in the liver and heart of high cholesterol diet (HCD) induced hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Normal</th>
<th>HCD Control</th>
<th>HCD + <em>P. rimosus</em> 50 mg/Kg</th>
<th>HCD + <em>P. rimosus</em> 250mg/kg</th>
<th>HCD + Atorvastatin 2.5mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>Liver</td>
<td>15.32±1.17</td>
<td>9.79±2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.9±1.05&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>14.03±2.54&lt;sup&gt;*&lt;/sup&gt;</td>
<td>13.3±3.39&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>9.27±0.87</td>
<td>4.97±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.63±2.41&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>8.17±1.67&lt;sup&gt;*&lt;/sup&gt;</td>
<td>8.00±2.11&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>Liver</td>
<td>156.57±16.23</td>
<td>90.63±19.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114.5±12.56&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>134.29±31.21&lt;sup&gt;*&lt;/sup&gt;</td>
<td>142.23±25.41&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>121.94±12.61</td>
<td>75.73±12.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.39±9.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>109.63±20.45&lt;sup&gt;*&lt;/sup&gt;</td>
<td>105.47±23.30&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>Liver</td>
<td>89.96±6.99</td>
<td>53.75±7.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.45±6.32&lt;sup&gt;*&lt;/sup&gt;</td>
<td>81.63±3.97&lt;sup&gt;***&lt;/sup&gt;</td>
<td>78.75±2.60&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>71.80±2.20</td>
<td>40.54±2.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.93±4.62&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>63.09±11.27&lt;sup&gt;***&lt;/sup&gt;</td>
<td>58.49±4.52&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).
Fig. 5.5: Effect of *P. rimosus* and atorvastatin treatment on the levels of GSH in the liver and heart of high cholesterol diet induced hyperlipidemic rats. Values are mean ± 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).

Fig. 5.6: Effect of *P. rimosus* and atorvastatin treatment on lipid peroxidation levels in the liver and heart of high cholesterol diet (HCD) induced hyperlipidemic 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 \(\text{ns} P > 0.05\) non significantly different from control group (Bonferroni test).
heart. The fold increase in the activity of SOD, CAT and GPx were approximately 0.69, 0.91 and 1.08 in the case of 50 mg/kg treated group and 1.07, 1.01 and 1.21 in the case of 250 mg/kg respectively when compared to the HCD controls. The standard reference drug atorvastatin (5 mg/kg) showed 0.93, 0.94 and 1.26 fold increase for SOD, CAT and GPx respectively with respect to control group.

Fig. 5.5 shows the levels of reduced glutathione (GSH) in the heart tissue of normal and HCD treated rats. The level of GSH was found to be decreased 1.19 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 1.05 and 0.86 -fold increase in 250 and 50 mg/kg treated group respectively compared to HCD control. Atorvastatin (5 mg/kg) showed 1.04 fold increase in GSH.

The levels of lipid peroxidation was significantly (p <0.001) higher in control group than the normal (Fig. 5.6) and it was found to be increased by 1.5 folds. The treatment of P. rimosus lowered the levels of lipid peroxidation. There was approximately 1.21 and 0.94 fold decrease in the lipid peroxidation in 250 and 50 mg/kg P. rimosus treated groups respectively, where as the fold decrease of atorvastatin (5 mg/kg) was 1.1 fold with respect to HCD control.

5.3.6. Effect of P. rimosus on the activities of SGPT, SGOT and ALP

Serum activities of transaminases, SGPT and SGOT are presented in Table 5.7. Feeding high cholesterol diet significantly elevated the SGPT and SGOT activities when compared to the normal control group of animals. There was approximately 1.19 and 1.09 fold increased activity for SGPT and SGOT respectively in the HCD control than that of normal group. Treatment of both the doses of P. rimosus 50 and 250 mg/kg for 30 days protected the elevation of transaminases activities. There were approximately 0.89 and 1.02 fold decreased activity for P. rimosus 50 mg/kg treated group and approximately 0.87 and 0.95 fold decreased respectively for P. rimosus 250 mg/kg in the activities of SGPT and SGOT than that of HCD control. Similarly, there were approximately 0.98 and 1.01 fold decreased in the activities of SGPT and SGOT respectively for glibenclamide treated group than that of HCD control.
Table 5.7: Effect of *P. rimosus* on the activity of liver function enzymes in high cholesterol diet (HCD) induced hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>50.12±10.43</td>
<td>71.65±9.66</td>
</tr>
<tr>
<td>HCD Control</td>
<td>---------</td>
<td>80.05 ± 7.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.94±10.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + HCD</td>
<td>50</td>
<td>73.72±7.39&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>91.89±8.37&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. rimosus</em> + HCD</td>
<td>250</td>
<td>61.35±9.25&lt;sup&gt;*&lt;/sup&gt;</td>
<td>82.04±11.16&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>atorvastatin+HCD</td>
<td>2.5</td>
<td>65.85±7.49&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>78.74±9.54&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6 animals.
<sup>a</sup> P < 0.001 and <sup>b</sup> P < 0.01 (Bonferroni test) significantly different from normal group.
<sup>*</sup> P < 0.05 significantly and <sup>ns</sup> P > 0.05 non significantly different from control group (Bonferroni test).
Table 5.8: Effect of *P. rimosus* on body weight (g) in high cholesterol diet (HCD) induced hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>180.50±15.67</td>
</tr>
</tbody>
</table>
| HCD Control        | ------           | 180.00±19.72 | 270.00±21.25  
| HCD + *P. rimosus* | 50               | 187.00±16.17 | 255.00±21.13  
| HCD+ *P. rimosus*  | 250              | 178.00±24.39 | 235.00±18.99  
| HCD + Atorvasttin  | 2.5              | 191.32±17.25 | 241.33±19.25  

Values are mean ± SD, n = 6.

\( ^a \) 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. 5.7: Histopathology of liver in high cholesterol diet induced hyperlipidemic rats

Normal
HCD control

Atrorvastatin (2.5 mg/kg)  P. rimosus (50 mg/kg)

P. rimosus (250 mg/kg)
5.3.7. Effect of *P. rimosus* on body weight

Initial body weights of the five groups were not significantly different (180±25 g); however, after 4 weeks, the body weight gains were significantly lower in the *P. rimosus* treated group than that of the HCD control group (Table 5.8). The rats fed with high-fat diet showed marked increases in weight from 180 ± 19.72 to 270 ± 21.25.

5.3.8. Histopathological observations

Histopathological evaluation reveals that a significant accumulation of fat droplets was observed in the liver of HCD control group rats. A comparable decrease in the lipid droplets was observed in *P. rimosus* and atorvastatin treated groups, the architecture of hepatocytes was found to be very similar to that of normal group (Fig. 5.7).

5.4. Discussion

Cardiovascular disease is a leading cause of global mortality, accounting for almost 17 million deaths annually (Smith et al., 2004); atherosclerosis, in particular, is the main contributor for the pathogenesis of myocardial and cerebral infarction. It is well established that elevated blood lipid levels constitute the major risk factor for atherosclerosis (Castelli et al., 1986). An increased cholesterol level in the plasma may cause coronary atherosclerosis to develop (Chien et al., 2007). In addition, serum TG is also considered an important risk factor for atherosclerosis (Jones et al., 2000; Hopkins et al., 2005). Elevated levels of plasma TG and LDL-cholesterol, accompanied by reduced HDL-cholesterol levels, are often associated with an increased risk of coronary heart disease, when there is excess LDL in the blood, it is deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. Moreover, in animal experiments, the LDL of hypercholesterolemic rabbits was more susceptible to oxidative modification than that of normo lipidemic rabbits (Nenseter et al., 1994). This oxidative modification of LDL is causally involved in the initiation and promotion of atherosclerosis (Steinberg, 1993). Thus, an increased cholesterol level can be a significant predictor of the development of coronary artery disease, and serum LDL-cholesterol levels should be used as the basis for initiating and monitoring treatment of patients with elevated blood cholesterol (Schaefer et
al., 1995). According to these studies, lowering of serum TC and LDL-cholesterol levels is important for reducing the risk of atherosclerosis.

The non-ionic detergent, Triton WR-1339, has been widely used to block the uptake of triacyl glycerol-rich lipoproteins from plasma by peripheral tissues in order to produce acute hyperlipidemia in animal models. This model is widely used for a number of different aims (Fiser et al., 1974; Kalopssis et al., 1980) and, in particular, in rats it has been used for screening natural or chemical hypolipidaemic drugs (Schurr et al., 1972) because it is convenient in terms of length of treatment period and handling. With this aim, many medicinal plants have been assessed for their hypolipidemic activity against triton WR-1339-induced hyperlipidemic model (Khanna et al., 2002). Schurr et al. (1972) demonstrated that a parenteral administration of a dose of triton WR-1339 to adult rats induced hyperlipidaemia. The maximum plasma triglycerides and total cholesterol were reached at 20 h, followed by a decline to normal values.

In the present study reduction of plasma total cholesterol in HCD treated rats after treatment with *P. rimosus* (Table 5.3) was associated with a decrease of its LDL fraction which is a major, potentially modifiable risk factor of cardiovascular diseases and the target of many hypocholesterolemic therapies. The finding suggests that the cholesterol-lowering activity of *P. rimosus* appears to be due to the enhancement of LDL-C catabolism through hepatic receptors, as demonstrated by Khanna et al. (2002). Similarly in triton WR 1339 treated rats also the treatment with *P. rimosus* significantly decreased the TC and LDL levels (Table 5.2).

It is also recently reported that TG play a key role in the regulation of lipoprotein interactions to maintain normal lipid metabolism. Moreover, these higher serum TG levels have been attributed mainly to an increased population of small, dense LDL deposits which are very atherogenic (Austin et al., 1994) and enhanced cholesteryl ester mass transfer from apolipoprotein B-containing lipoproteins (VLDL and LDL) (Guerin et al., 2001). TGs have also been proposed to be a major determinant of cholesterol esterification, its transfer and HDL remodelling in human plasma (Murakami et al., 1995).

*P. rimosus* significantly suppressed the elevated blood concentrations of TGs (Table 5.3) in HCD rats. The result suggests that the extracts are able to restore, at least
partially, the catabolism of triglycerides. The underlying mechanism of this activity is not elucidated by the present study. However, as hypothesised by many works (Sudheesh et al., 1997; Perez et al., 1999; Xie et al., 2007), the restoration of catabolic metabolism of TG could be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL). Similarly in triton WR 1339 treated rats also the treatment with *P. rimosus* significantly decreased the TG levels (Table 5.2).

HDL carries cholesterol and cholesterol esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing cholesterol levels in the blood and peripheral tissues, and in inhibiting atherosclerotic plaque formation in the aorta. In the present study, HDL-C levels were significantly increased in animals treated with *P. rimosus* compared to the control group (Table 5.3). The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of hepatic triglyceride lipase (HTL) activity on HDL which may lead to a rapid catabolism of blood lipids through extrahepatic tissues (Sudheesh et al., 1997; Anila and Vijayalakshmi, 2002). Similarly in triton WR 1339 treated rats also the treatment with *P. rimosus* significantly increased the HDL levels (Table 5.2).

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 in HCD (Fig 5.3) and triton treated rats (Fig 5.1). Similar results were reported by others when studying the hypolipidemic effect of natural products (Cherng and Shih, 2005). This ameliorative action was due to the plasma lipid-lowering activity of the extract.

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 in HCD (Fig 5.4) and triton WR- 1339 treated rats (Fig 5.2) showing the beneficial effect of this mushroom in preventing atherosclerosis incidence.
Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis, nephritis, Alzheimer’s disease and cancer (Baker et al., 2004; Mullan et al., 2004). Oxidative stress is a causative factor that links hyperlipidaemia with the pathogenesis of atherosclerosis (Young and Mceneny, 2001). Oxidative stress occurs when the production of free radicals exceeds the capacity of the natural antioxidant system. High-fat diets have been shown to increase free radical production in vivo (Slim et al., 1996; Dobrian et al., 2000), followed by oxidative stress and hypercholesterolemia (Ohkawa et al., 1979). During the metabolism of dietary cholesterol, it is delivered to hepatocytes, where a substantial amount of ROS are generated (Erdincler et al., 1997). Therefore, feeding a high-fat diet leads to an increase in free radical production, which elevates lipid peroxides (Harrison et al., 2003).

A hypercholesterolemic diet changes the in vivo antioxidant status by increasing the generation of oxygen free radicals, which exert their cytotoxic effect by causing lipid peroxidation (Prasad and Kalra, 1993), furthermore, an increase of TBARS levels in animals fed with a high cholesterol diet has been previously reported (Prasad and Kalra, 1993; Dhuley, 1999; Shunkla et al., 2004). Accordingly, we have also observed increased TBARS levels in hypercholesterolemic rats. Treatment with *P. rimosus* extract prevented the lipid peroxidation (Fig 5.6).

Atherosclerosis is a progressive disease involving both the large and medium-sized arteries. It is a common factor in many cardiac complaints, but it can affect different organs. Also, the occurrence of antioxidant effects is not organ specific, so that we evaluated these effects on liver and heart which is the most common experimental model for this kind of study (Frei and Higdon, 2003; Hatipoglu et al., 2004; Sun et al., 2004; Auger et al., 2005). It was reported that during mild oxidative stress, tissues respond by activating antioxidant mechanisms (Halliwell, 1994) and that high oxidative stress levels, most likely depress the antioxidant defenses. In agreement with previous reports (Daniel et al., 1998; Mary et al., 2002), we observed a decrease in the activities of the antioxidant enzymes SOD, CAT and GPx in animals maintained on high cholesterol diet, compared to those maintained on a normal diet. Such decreases may be associated to the production of α-, β-unsaturated aldehydes during lipid peroxidation. These compounds have the ability to increase oxidative stress by promoting the cellular consumption of GSH and by
inactivating selenium-dependent GPx (Kinter and Roberts, 1996). Moreover, the reaction of these products with amino acid residues of proteins may cause oxidative modification of antioxidant enzymes (Bosch-Morell et al., 1999) and other products resulting from the polyunsaturated fatty acid damage that may cause protein breakdown (Esterbauer et al., 1991).

Epidemiological evidence from many studies overwhelmingly supports the fact that supplement of antioxidants is significantly associated with a reduction in the level of oxidized lipoprotein (Ohkawa et al., 1979). Our results showed that administration of \textit{P. rimosus} could significantly counteract the decline in the activity of antioxidant enzymes (SOD, CAT and GSH-Px) (Table 5.6) and the levels of non-enzymic antioxidant (GSH) in rats fed HCD diet (Fig 5.5). Considering the endogenous stress-related markers (SOD, GPx and CAT), our results suggest that the \textit{P. rimosus} extract could improve efficiency of superoxide radical conversion to hydrogen peroxide by increase the SOD activity following deactivation of hydrogen peroxide by GPx. Increased CAT activity is considered to indicate the excessive hydrogen peroxide levels when the detoxification capacity of glutathione system becomes insufficient.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA) is a key rate limiting enzyme involved in the cholesterol biosynthetic pathway (Goldstein and Brown, 1990). Therefore, inhibition of HMG-CoA reductase decreases intracellular cholesterol biosynthesis (Steinberg et al., 1989). HMG CoA reductase inhibitors are the most commonly prescribed class of lipid lowering drug. HMG CoA reductase inhibitors have a favorable profile in the reduction of lipoprotein concentrations. The ratio (absorbance of HMG-CoA/absorbance of mevalonate) is taken as an index of the activity of HMG-CoA reductase required to convert HMGCo-A to mevalonate. If cholesterol biosynthesis is decreased in a clinical condition, this ratio will increase, and vice versa. In the present study, hepatic HMG-CoA reductase activity was significantly decreased (HMG CoA / Mevalonate ratio increased) after 30 days in animals maintained on a high-fat diet compared to normal. The decrease has been ascribed to inhibition of HMG-CoA reductase activity by exogenous cholesterol, resulting in up regulation in order to supply other essential products of synthetic pathways downstream of the enzyme. Our results revealed
that the HMG-CoA reductase activity in hyperlipidemic rats receiving *P. rimosus* were markedly reduced compared to that of the HCD control (Table 5.5).

In triton control group cholesterol synthesis was increased and the HMG-CoA/mevalonate ratio was decreased compared to the normal group. Treatment with *P. rimosus* increased the HMG-CoA/mevalonate ratio compared to the triton control group (Table 5.4). From the results, it is evident that cholesterol-lowering effect of *P. rimosus* and atorvastatin are due to the decreased cholesterol biosynthesis by inhibiting the activity of HMG-CoA reductase.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites (Rej, 1978). In order to rule out a possible damage to the liver by the hypercholesterolemic diet, we evaluated the serum transaminases activity. AST and ALT levels remain the most useful tests for the detection hepatic cell damage, because both are present in high concentrations in hepatocytes. These enzymes leak into the circulation when hepatocytes or their cell membranes are damaged. It has been reported that treatment with a high concentration of cholesterol can cause liver damage (Bolkent et al., 2004). Rats fed with high cholesterol diet lead to increased activities of AST and ALT in serum, while treatment with *P. rimosus* leads to decreased activity of these enzymes. This observation indicates that fatty infiltration and degeneration of liver cells caused by fat-cholesterol feeding were significantly reduced by *P. rimosus* (Table 5.7).

Liver histological examination of the normal group showed normal cell architecture (Fig. 5.7), while significant morphological changes were observed in the HCD control group. A comparable decrease in the hepatic lipid droplets was observed in *P. rimosus* (250 mg/kg) and atorvastatin treated groups, the architecture of hepatocytes was found to be very similar to that of normal group. It demonstrated the protective effect of *P. rimosus*.

Flavonoids and tannins, a heterogeneous group of ubiquitous plant polyphenols, exhibit different pharmacological activities, including hypolipidemic and anti-atherogenic effects (Del Bas et al., 2005). Preliminary phytochemical screening revealed the presence of saponins and polyphenol in the *P. rimosus* extract. Several studies show that saponins possess hypolipidemic activity and this has been reported to increase the lipoprotein lipase
activity, which helps to remove free fatty acids from circulation, causing decrease in cholesterol level (Sidhu and Oakenful, 1990).

Flavonoids and other polyphenols may also contribute to the hypolipidemic activity by increasing the cholesterol metabolism and by modulating the enzymes involved in cholesterol metabolism, such as HMG CoA reductase, lecithin cholesterolacyl transferase, cholesterol 7a-hydroxylase (CYP7A1) and acyl-CoA:cholesterol acyltransferase (Chen et al., 2003). It may be suggested that the hypolipidemic and antioxidant activity of *P. rimosus* might be correlated to these compounds. This result is considered important for the treatment of hyperlipidemia-induced atherosclerosis and apparently validates the medicinal use of *P. rimosus*. 