Hepatoprotective activity of cultured mycelium of Volvariella volvacea

Mathew John “Medicinal properties of cultured mycelium of paddy straw mushroom, volvariella volvacea: Studies on the prevention of oxidative damage and genotoxicity” Thesis. Amala Cancer Research Centre, University of Calicut, 2011
Chapter 7: Hepatoprotective activity of cultured mycelium of Volvariella volvacea
7.1. Introduction

Exposure to drugs and chemicals often induce toxicity to living organisms. Human beings are exposed to these compounds through environmental exposure consumption of contaminated food or during exposure to chemical substances in the occupational environment. In addition, human beings consume a lot of synthetic drugs during diseased condition which are alien to body organs. All this compounds produce a variety of toxic manifestation. Factors determine the toxicity include the pharmacokinetics of the compounds, the metabolic fate of the compound and the target organ ability to respond to the toxic insult. Many organs are capable of metabolizing chemicals to toxic reactive intermediates. The liver is an organ of paramount importance which plays essential role in the metabolism of foreign compounds entering in the body.

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward et al., 1999). Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being. The liver protects the body from potentially injurious substances (endotoxins) absorbed from the intestinal tract as well as bye products of metabolism. The most important in the detoxification process is that of microsomal drug metabolizing system of liver. Unfortunately, the liver is often abused by environmental toxins, poor eating habits and alcohol, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease (Sharma et al., 1991; Subramonium and Pushpangadan, 1999).
Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis (Sherlock and Dooley, 1997). A large number of xenobiotics are reported to be potentially hepatotoxic. Hepatotoxins may react with basic cellular constituents, proteins, lipids, RNA, DNA, and induce various types of pathological symptoms in liver.

Paracetamol or acetaminophen (APAP) is one of the most commonly used analgesics/antipyretics worldwide. The toxic doses of APAP can cause hepatocellular necrosis (Albano et al., 1985). In particular, the mechanism of cell damage is mediated by the metabolic activation of APAP to a highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is able to deplete hepatocellular glutathione (GSH) and to bind covalently with cell macromolecules (Black, 1984). The concentration of intracellular GSH is therefore a vital determinant in the extent of APAP induced hepatic necrosis. The GSH depletion has been suggested to markedly enhance the susceptibility of mitochondrial structural dysfunction from oxidative stress and to induce mitochondrial structural degeneration (Martensson and Meister, 1989). Further, a number of toxic changes have been reported to occur during APAP hepatotoxicity, including calcium dyshomeostasis (Moore et al., 1985), oxidative stress and lipid peroxidation (Nakae et al., 1990) and also protein thiol oxidation (Birge et al., 1988). It may be that one or more of
these toxic mechanisms contribute to the damage to respiratory complexes during APAP hepatotoxicity in addition to direct attack by the toxic metabolite.

Carbon tetrachloride (CCl₄), a selective hepatotoxic chemical agent is one of the most widely used toxins for the experimental induction of liver fibrosis in laboratory animals (Kanter et al., 2005). Through the investigation of acute CCl₄ induced liver damage in animal models, it is now generally accepted that CCl₄ toxicity results from bioactivation of CCl₄ into trichloromethyl free radical by cytochrome P-450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to severe necrosis in the pericentral regions of the liver (Mc Cay et al., 1984; Slater, 1984; Recknagel et al., 1989). The principle causes of CCl₄ induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals leading to liver fibrosis and cirrhosis (Kanter et al., 2005, Lin et al., 1997). CCl₄ also induces hydropic degeneration, centrilobular necrosis, fatty changes, cirrhosis and hepatoma (Smuckler and Barker, 1962). CCl₄-induced damage also produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathological changes (Rajesh and Latha, 2004).

Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety (Batis and Ashwood, 1974). In the last 15 to 20 years, medicinal mushrooms have been subject to various laboratory studies with animals as well as clinical studies with humans. They are thought to be beneficial for a wide variety of hepato disorders, including hepatitis. Hepatoprotective effect of the extracts of mushrooms, namely Dentopolyporous umbellatus,
*Schizophyllum commune* and *Tramella fuciformis* have been reported (Wasser and Weis, 1999; Zhou, 1989). Sugano and co-workers (1982) showed that injection of LEM from *Lentinus edodes* slowed the growth of cancerous liver tumor in rats. A polysasaccharide fraction from the shiitake mushroom demonstrated liver protection action in animals, as well as the ability to improve liver function and enhance the production of antibodies to hepatitis B (Lin and Huang, 1987; Mizoguchi et al., 1987; Amagase, 1987; Mizuno, 1995 a,b). In combination with the polysaccharides from Reishi mushroom (*Ganoderma lucidum*) and Turkey tails (*Trametes versicolor*), lentinan has improved SGPT and completely GPT levels in the livers of mice with toxic hepatitis (Zhang and Luan, 1986; Wasser and Weis, 1997a). Crude extract of Shiitake mushroom cultures have demonstrated liver-protecting actions (Lin, 1987; Hobbs, 1995; Wasser and Weis, 1997b). Hepatoprotective effect was found in the extract of the Maitake mushroom (*Grifola frondosa*) when given to rats (300mg/kg) in a hepatitis model (paracetamol-induced) (Lee, 1992; Ooi, 1993). A polysaccharide derivative from the alcoholic extract of Dendropolyporus umbellatus had demonstrated hepatoprotective effects in mice (Lin and Wu, 1988). Pharmacological activities that may be the result of the protein bound polysaccharide (PSK) from turkey tail mushroom (*Trametes versicolor*) support hepatic function, and indicate on possible prevention of liver cancer (Wang, 1989). In China, *T. versicolor* is considered useful for hepatitis B and chronic active hepatitis (Jianzhe et al., 1987). The chemically modified form of pachyman (kind of polysaccharides), carboxymethylpachyman from *Wolfiporia cocos* was reported to produce an “immediate cure” of chronic viral hepatitis in human clinical studies (Guo et al., 1984; Ding, 1987). So based on these findings the hepatoprotective activity of *V. volvacea* has been studied and the findings are reported in this chapter.
7.2. Materials and methods

7.2.1. Preparation of the extract

Aqueous-ethanol extract of *V. volvacea* was prepared as described in section 3.2.2.

7.2.2. Animals

Male Wistar rats weighing 180 ± 20g were employed for hepatotoxicity studies.

7.2.3. Determination of hepatotoxicity activity

Hepatoprotective activity was determined using carbon tetrachloride (CCl₄) induced chronic and paracetamol induced acute hepatotoxicity.

7.2.3.1. Effect of *V. volvacea* against carbon tetrachloride induced chronic hepatotoxicity.

Hepatoprotective activity against the CCl₄ induced chronic toxicity was determined by the method of Ajith and Janardanan (2002) with some modifications. The animals were divided into 5 groups of six animals each and treated as follows. Silymarin, a clinically used hepatoprotective drug was used as reference. Group I was treated as normal. Group II treated with CCl₄/paraffin oil (1:5, v/v, i.p) three times in a week for 5 weeks (total 15 doses). Group III and IV were treated orally with aqueous ethanolic extract of *V. volvacea* 250 and 500 mg/kg bodyweight, one hour before each CCl₄ administration. Group V was treated with silymarin one hour before each CCl₄ administration. Twenty four hour after the last dose of CCl₄ injection animals were sacrificed. Blood was collected from the heart. Serum was used for the determination of glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP).
7.2.3.2. Effect of *V. volvacea* against APAP induced acute hepatotoxicity
Hepatoprotective activity against the APAP induced-chronic toxicity was determined by the method of Ajith et al. (2007) with some modifications. The animals were divided into five groups of six animals each and treated as follows. Group I - treated with a single dose of acetaminophen (APAP) (3 gm/kg body weight) was kept as control, Group II - treated with distilled water was kept as normal, Group III and IV - treated orally with aqueous-ethanolic extract of *V. volvacea* 250 and 500 mg/kg body weight, Group V - treated with Silymarin 100 mg/kg body weight (p.o). The Silymarin, *V. volvacea* extract and distilled water were administrated orally once daily for 7 days. After 24 hours of last drug administration a single dose of APAP (3gm/kg body weight) was given orally. This was given to all the groups except the group II, which was considered as the normal. Animals were sacrificed exactly after 12 hrs of APAP administration. Blood was collected directly from the heart.

7.2.3.3. Biochemical analysis
Serum was separated and the activities of SGOT (section 3.2.5.1), SGPT (section 3.2.5.2) and ALP (section 3.2.5.3) were estimated by kinetic method using the kit of Agappae Diagnostic Ltd., India.

7.2.3.4. Determination of antioxidant status in the liver
Livers were excised, washed thoroughly in ice-cold saline to remove the blood. 10% homogenate was prepared in phosphate buffer (0.05 M, pH 7) using a polytron homogeniser. A part of this homogenate was used for the determination of reduced glutathione (GSH) (section 3.2.6). Rest of the homogenate was centrifuged at 10,000 rpm for 20 min for removing the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of superoxide dismutase (SOD).
(section 3.2.5.6), catalase (CAT) (section 3.2.5.7), glutathione peroxidase (GPx) (section 3.2.5.8) and malonaldehyde (MDA) (section 3.2.9). The protein was estimated by the method of Bradford, 1976 (section 3.2.4).

7.2.3.5. Histopathological examination

Pieces of liver from each group were fixed immediately after sacrifice in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol and embedded in paraffin, cut into 4–5 m thick sections and stained with hematoxylin–eosin. The sections were evaluated for the pathological symptoms of hepatotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

7.3. Results

7.3.1. Effect of V. volvacea on the activities of SGPT, SGOT and ALP in APAP induced acute hepatotoxicity

The activities of liver marker enzymes such as SGPT and SGOT, and ALP are given in Table 7.1. Single dose of APAP (3gm/kg) significantly elevated the SGPT, SGOT and ALP activities when compared to the normal animals. There were approximately 1.06, 1.71 and 2.66 fold increases for SGPT, SGOT and ALP respectively for APAP treated group of animals than that of normal group. Treatment of V. volvacea mycelia extract for 7 days prior to APAP administration significantly protected the elevation of transaminases and ALP activities. There was approximately 1.07, 1.29 and 1.60 fold and 1.30, 1.84 and 1.94 fold decrease in the SGPT, SGOT, and ALP activity in the 250 mg/kg and 500 mg/kg body weight extract treated groups respectively than that of APAP control group. The positive standard (Silymarin 100 mg/kg) significantly protected the elevation of transaminases and ALP activities. There were approximately 1.28, 1.03 and 1.21 fold decreases in the case of SGPT and SGOT, and ALP activities respectively in the Silymarin treated group.
Table 7.1. Effect of *V. volvacea* on hepatic transaminases and alkaline phosphatase in rats administered with APAP

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SGPT (IU/l)</th>
<th>SGOT (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (APAP)</td>
<td>3000</td>
<td>91.50 ± 43.59</td>
<td>166.26 ± 12.34</td>
<td>526.40 ± 23.31</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>31.25 ± 14.15**</td>
<td>77.50 ± 12.50**</td>
<td>153.23 ± 35.5**</td>
</tr>
<tr>
<td><em>V. volvacea</em> + APAP</td>
<td>250</td>
<td>39.42 ± 5.17**</td>
<td>88.68 ± 30.21**</td>
<td>269.92 ± 45.43**</td>
</tr>
<tr>
<td><em>V. volvacea</em> + APAP</td>
<td>500</td>
<td>26.15 ± 10.55**</td>
<td>75.20 ± 8.40**</td>
<td>196.14 ± 63.56**</td>
</tr>
<tr>
<td>Silymarin + APAP</td>
<td>100</td>
<td>28.34 ± 8.85**</td>
<td>120.62 ± 28.62**</td>
<td>361.23 ± 56.42**</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6, **p < 0.01 with respect to control.
7.3.2. Effect of *V. volvacea* on the innate antioxidant enzymes during the APAP induced acute hepatotoxicity

The activities of hepatic innate antioxidant activities such as SOD, CAT and GPx were lowered significantly in the APAP control group of animals than that of normal group (Table 7.2). There was approximately 1.04, 0.97 and 1.2 fold decrease for SOD, CAT, and GPx respectively in the APAP control than that of normal group. The treatment with the mycelia extract significantly protected the hepatic antioxidant status. There were approximately 0.88, 0.76 and 1.09 fold increase of SOD, CAT and GPx activities respectively for 250 mg/kg extract treated group and 1.29, 1.0 and 1.5 fold increase respectively for 500 mg/kg extract treated group than that of control group. Similarly, Silymarin administration significantly protected the hepatic antioxidant status against the APAP induced hepatic damage. There was approximately 1.13, 0.84 and 1.02 fold increase for SOD, CAT, GPx respectively in the Silymarin treated group than that of control group.

7.3.3. Effect of *V. volvacea* on level of GSH during the APAP induced acute hepatotoxicity

APAP significantly decreased the level of GSH (Fig.7.1). There was 1.35 fold decreases in the level of GSH for APAP than that of control groups. But the treatment with the extract significantly enhanced the level of GSH. There was approximately 1.08 and 1.48 fold increase in the level of GSH for 250 and 500 mg/kg extract treated group than that of APAP control. Similarly, Silymarin (100 mg/kg) significantly enhanced the level of GSH than that of control. There was approximately 0.85 increase in the level of GSH in the Silymarin (100 mg/kg) treated group than that of control group.
Table 7.2. Effect of *V. volvacea* on the activities of hepatic antioxidant enzymes in rats administered with APAP

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (APAP)</td>
<td>3000</td>
<td>8.20 ± 1.28</td>
<td>36.32 ± 19.14</td>
<td>11.70 ± 1.30</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>13.63 ± 3.73**</td>
<td>75.49 ± 21.17**</td>
<td>22.70 ± 6.60**</td>
</tr>
<tr>
<td><em>V. volvacea</em> + APAP</td>
<td>250</td>
<td>10.64 ± 2.31 ns</td>
<td>63.02 ± 20.62 ns</td>
<td>20.10 ± 5.90*</td>
</tr>
<tr>
<td><em>V. volvacea</em> + APAP</td>
<td>500</td>
<td>15.54 ± 3.28**</td>
<td>70.94 ± 13.45**</td>
<td>26.70 ± 7.20**</td>
</tr>
<tr>
<td>Silymarin + APAP</td>
<td>100</td>
<td>11.25 ± 0.50 ns</td>
<td>59.21 ± 12.73 ns</td>
<td>16.20 ± 2.90 ns</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6., **P<0.01, *P<0.05, nsP>0.05 with respect to control.
Figure 7.1. Effect of *V. volvacea* on the levels of GSH and lipid peroxidation in rats administrated with APAP

Values are the mean ± SD; n = 6, **P<0.01, nsP>0.05 with respect to control.
Figure 7.2

Histopathology of liver in APAP induced acute hepatotoxicity

NORMAL

APAP CONTROL

VV 250 mg/kg + APAP

VV 500 mg/kg + APAP

SILYMARIN 100 mg/kg + APAP
7.3.4. Effect of *V. volvacea* on lipid peroxidation during the APAP induced acute hepatotoxicity

The APAP treatment significantly elevated the hepatic lipid peroxidation levels than that of normal group (Fig. 7.1). There was approximately 1.29 fold increase in the level of lipid peroxidation in the APAP control than that of normal group. The administration of *V. volvacea* extract significantly decreased the lipid peroxidation level. There was approximately 0.56 and 1.41 fold decrease in the case of 250 and 500 mg/kg extract treated group than that of APAP control. Similarly, the Silymarin (100 mg/kg) significantly decreased the lipid peroxidation than that of APAP control. There was approximately 1.29 fold decrease in the lipid peroxidation level in the Silymarin treated group than that of APAP control.

7.3.5. Histopathological observations

Histopathological examination of livers challenged with APAP showed centrilobular necrosis, ballooning degeneration, inflammatory infiltration of lymphocytes and fatty changes. The effects of APAP toxicity in the liver sections of rats treated with the extract were reduced moderate to normal (Fig. 7.2).

7.3.6. Effect of *V. volvacea* on the activities of SGPT, SGOT and ALP in CCl₄ induced chronic hepatotoxicity

The activities of liver marker enzymes such as SGPT and SGOT, and ALP are given in Table 7.3. The chronic hepatotoxicity by CCl₄ three times in a week for 5 weeks (total 15 doses) significantly elevated the SGPT, SGOT and ALP activities when compared to the normal animals. There were approximately 4.91, 1.71 and 1.53 fold increases for SGPT, SGOT and ALP respectively for CCl₄ treated group of animals than that of normal group. Treatment of *V. volvacea* mycelia extract along with CCl₄ administration significantly protected
Table 7.3. Effect of *V. volvacea* on hepatic transaminases and ALP in rats after chronic CCl₄ administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SGPT (IU/l)</th>
<th>SGOT (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>38.8 ±11.79**</td>
<td>99.67 ± 8.23**</td>
<td>92.21 ± 21.49**</td>
</tr>
<tr>
<td>Control (CCl₄)</td>
<td>-</td>
<td>280.5 ± 32.22</td>
<td>196 ± 11.73</td>
<td>195.7 ± 212.82</td>
</tr>
<tr>
<td><em>V. volvacea</em>+</td>
<td>250</td>
<td>109.5 ± 16.40**</td>
<td>156 ± 6.2”</td>
<td>135.86 ± 19.74**</td>
</tr>
<tr>
<td>CCl₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. volvacea</em>+</td>
<td>500</td>
<td>67 ± 14.80**</td>
<td>140 ± 6.2”</td>
<td>109.47 ± 46.2”</td>
</tr>
<tr>
<td>CCl₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silymarin+</td>
<td>100</td>
<td>46 ± 18.5”</td>
<td>110.67 ± 6.02”</td>
<td>92.86 ± 1.64”</td>
</tr>
<tr>
<td>CCl₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6, **P<0.01 with respect to control.
the elevation of transaminases and ALP activities. There was approximately 1.97, 1.13 and 1.12 fold and 3.04, 1.26 and 1.11 fold decrease in the SGPT, SGOT, and ALP activities in the 250 mg/kg and 500 mg/kg body weight extract treated groups respectively than that of CCl₄ control group. The positive standard (Silymarin 100 mg/kg) significantly protected the elevation of transaminases and ALP activities. There were approximately 3.85, 1.58 and 1.84 fold decreases in the case of SGPT and SGOT, and ALP activity respectively in the Silymarin treated group than that of CCl₄ control.

7.3.7. Effect of *V. volvacea* on the innate antioxidant enzymes during the CCl₄ induced chronic hepatotoxicity

The activities of hepatic innate antioxidant activities such as SOD, CAT and GPx were lowered significantly in the CCl₄ control group of animals than that of normal group (Table 7.4). There was approximately 1.62, 1.26 and 1.71 fold decrease for SOD, CAT, and GPx respectively in the CCl₄ control than that of normal group. The treatment with the mycelia extract significantly protected the hepatic antioxidant status. There were approximately 1.23, 1.13 and 1.24 fold increase of SOD, CAT and GPx activities respectively for 250 mg/kg extract treated group and 1.46, 1.13 and 1.42 increases respectively for 500 mg/kg extract treated group than that of control group. Similarly, Silymarin administration significantly protected the hepatic antioxidant status against the CCl₄ induced hepatic damage. There was approximately 1.28, 1.07 and 1.32 fold increase for SOD, CAT, GPx respectively in the Silymarin treated group than that of CCl₄ control group.

7.3.8 Effect of *V. volvacea* on level of GSH during the CCl₄ induced chronic hepatotoxicity

CCl₄ significantly decreased the level of GSH (Fig.7.3). There was 1.10 fold decreases in the level of GSH for CCl₄ than that of control groups. But the
Table 7.4. Effect of *V. volvacea* on the activities of hepatic antioxidant enzymes in rats after chronic CCl₄ administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>9.41 ± 1.23**</td>
<td>68.84 ± 13.00**</td>
<td>19.79 ± 3.50**</td>
</tr>
<tr>
<td>Control (CCl₄)</td>
<td>-</td>
<td>4.33 ± 0.77</td>
<td>39.38 ± 5.07</td>
<td>8.91 ± 0.61</td>
</tr>
<tr>
<td><em>V. volvacea</em>+ CCl₄</td>
<td>250</td>
<td>7.79 ± 1.59**</td>
<td>51.49 ± 1.39ns</td>
<td>14.39 ± 2.55*</td>
</tr>
<tr>
<td><em>V. volvacea</em>+ CCl₄</td>
<td>500</td>
<td>8.26 ± 0.88**</td>
<td>57.75 ± 7.70**</td>
<td>18.24 ± 4.70**</td>
</tr>
<tr>
<td>Silymarin+ CCl₄</td>
<td>100</td>
<td>7.39 ± 0.81**</td>
<td>56.32 ± 8.55**</td>
<td>15.73 ± 3.13**</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6., **P<0.01, *P<0.05, nsP>0.05 with respect to control.
treatment with the extract significantly enhanced the level of GSH. There was approximately 1.06 and 1.02 fold increase in the level of GSH for 250 and 500 mg/kg extract treated group than that of APAP control. Similarly, Silymarin (100 mg/kg) significantly enhanced the level of GSH than that of control. There was approximately 1.14 increases in the level of GSH in the Silymarin treated group than that of CCl₄ control group.

7.3.9 Effect of *V. volvacea* on lipid peroxidation during the CCl₄ induced chronic hepatotoxicity

The CCl₄ treatment significantly elevated the hepatic lipid peroxidation levels than that of normal group (Fig.7.3). There was approximately 1.23 fold increase in the level of lipid peroxidation in the CCl₄ control than that of normal group. The administration of *V. volvacea* extract significantly decreased the lipid peroxidation level. There was approximately 0.96 and 1.13 fold decrease in the case of 250 and 500 mg/kg extract treated group than that of CCl₄ control. Similarly, the Silymarin significantly decreased the lipid peroxidation than that of CCl₄ control. There was approximately 1.16 fold decrease in the lipid peroxidation level in the Silymarin treated group than that of CCl₄ control.

7.3.10 Histopathological Observations

Histopathological examination of livers challenged with CCl₄ showed severe necrosis, fatty infiltration and fibrosis in the hepatocytes. The liver sections of rats treated with the extract showed well preserved architecture (Fig.7.4).

7.4. Discussion

In living system, liver is considered to be highly sensitive to toxic agents. Hepatic dysfunction due to investigation or inhalation to hepato toxins such as acetaminophen, cadmium chloride, ethanol, CCl₄, allyl alcohol are
Figure 7.3. Effect of *V. volvacea* on the levels of GSH and lipid peroxidation in rats administrated with CCl₄

Values are mean ± S.D, n=6, **P<0.01 with respect to control.
Figure 7.4

Histopathology of liver in $\text{CCL}_4$ induced chronic hepatotoxicity

NORMAL

$\text{CCL}_4$ CONTROL

VV 250 mg/kg + $\text{CCL}_4$

VV 500 mg/kg + $\text{CCL}_4$

SILYMARIN 100 mg/kg + $\text{CCL}_4$
increasing worldwide. The liver protects the body from potentially injurious substances absorbed in the intestinal tract and also the toxic byproducts of metabolism. One of the leading causes of death in developed countries is disease due to liver toxicity. Liver injuries induced by APAP and CCl₄ are two commonly used models for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the level of increased cytoplasmic enzymes (SGPT and SGOT) in circulation (Sallie et al., 1991). APAP and CCl₄ are reported to increase the SGPT, SGOT and ALP activities, and exacerbated oxidative injury (Ajith and Janardhanan, 2002; Ajith et al., 2007; Fakurazi et al., 2008; Padhy et al., 2007; Sabir and Rocha, 2008). The rise in activities of SGPT, SGOT and ALP has been attributed to damage structural integrity of the liver (Chenoweth and Hake, 1962), because these enzymes are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991).

The results of this study reveal that the *V. volvacea* mycelia extract (250 and 500 mg/kg) have preventive activity on APAP and CCl₄ induced acute and chronic hepatotoxicity in a dose dependent manner. The amelioration of liver toxicity by the extract is evident from its significant effect on the SGOT, SGPT and ALP activities. These findings are also confirmed by histopathological observations. The positive standard used in both the studies, Silymarin, is a well-established hepatoprotective drug reported to revert abnormal alterations of these enzymes in various drug induced hepatotoxicity (Pradhan and Girish, 2006; Upadhyay et al., 2007). The ability of silymarin in preventing drug induced hepatotoxicity is associated with its ability to act as a radical scavenger, thereby protecting membrane permeability (Song et al., 2006).

To prevent the oxidative damage, tissues have constructed an antioxidant defense system that includes non-enzymatic antioxidants (e.g., GSH, uric acid,
bilirubin, and vitamin- C) and enzymatic antioxidants such as SOD, CAT, GPx and Glutathione reductase (GR) (Halliwell and Gutteridge, 1990). SOD is the first enzyme involved in the antioxidant defense by lowering the steady state of O$_2^\cdot$. CAT is a hemeprotein, localized in the peroxisomes and catalyses the decomposition of H$_2$O$_2$ to water and oxygen. GPx, a selenoenzyme, present predominantly in liver and catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Venukumar and Latha, 2002). Therefore, the enzymatic antioxidants activities and/or the inhibition of free radicals generation are important in terms of protecting the liver from APAP and CCl$_4$ induced damage (Arulkumaran et al., 2009). These antioxidant enzymes are effortlessly inactivated by lipid peroxides or free radical, which results in decreased activities of these enzymes in APAP and CCl$_4$ induced hepatic toxicity (Ajith and Janardhanan, 2002; Ajith et al., 2002; Ajith et al., 2007; Padhy et al., 2007; Sabir and Rocha, 2008).

The results of the present study indicate that SOD, CAT and GPx activities are significantly decreased in the liver in response to APAP and CCl$_4$ induced treatment alone compared with normal group of rats, implying increased oxidative damage to the liver. On the contrary, SOD, CAT and GPx activities are significantly elevated by administration of V. volvacea extract to both APAP and CCl$_4$ intoxicated rats, suggesting that it has the ability to restore/maintain the activity of hepatic enzymes in APAP and CCl$_4$ damaged liver.

GSH acts as a non-enzymatic antioxidant in detoxification pathway that reduces the reactive toxic metabolites of APAP and CCl$_4$. Normally GSH contributes significantly to the intracellular antioxidant defensive system as it is a powerful consumer of superoxide, singlet oxygen, and hydroxyl radicals (Miesel and Zuber, 1993). The breakdown of the GSH-dependent antioxidant
defensive system increases the intracellular flux of oxygen free radicals (Miesel and Zuber, 1993) creating an oxidative stress and initiating apoptosis. APAP is a powerful inducer of cytochrome P-450 and produces a highly reactive quinone-imine, which combines with sulphahydroxy groups of proteins and cause rapid depletion to intracellular GSH (Jollow et al., 1974; Rathbun et al., 1996) and this depletion of GSH is a critical determinant for cell survival and death in oxidative stress conditions (Comporti, 1989; Hidaka et al., 2007). Therefore, it appears that GSH conjugation is critically essential to decrease the toxic effect of APAP. In the present study, the hepatic content of GSH was significantly decreased in APAP intoxicated rats compared with normal group of rats. However, treatment with V. volvacea extract significantly elevated the GSH level to APAP -intoxicated rats, suggesting that V. volvacea could protect the APAP -induced depletion of hepatic GSH.

The initiation of oxidative stress-related various tissue injuries, cell death, and the progression of many acute and chronic diseases are generally believed to be an important underlying cause of increased lipid peroxidation (Halliwell, 1997). There are numerous reports showing that lipid peroxidation by free radical derivatives of APAP is one of the principal mechanisms of APAP -induced liver damage leading to disturbance of cell membrane integrity (Olaleye and Rocha, 2008; Omololu et al., 2009). MDA is a major reactive aldehyde that formed during the peroxidation of biological membrane polyunsaturated fatty acid (Vaca et al., 1988). The elevation of MDA levels in the liver imply enhanced peroxidation leading to tissue damage and breakdown of the antioxidant defense mechanisms to prevent the formation of superabundant free radicals (Naik et al., 2003). In the present study, APAP and CCl₄ induced toxicity causes an increase in MDA levels of liver tissue. By contrast, treatment with V. volvacea extract significantly reverses these changes. The extract administration caused a significant decrease in MDA
levels as compared to the APAP and CCl₄-induced toxicity group, suggesting that *V. volvacea* mycelia extract could protect the APAP and CCl₄-induced lipid peroxidation in rats.

It is generally thought that CCl₄ toxicity is due to reactive free radical (CCl₃·), which is generated by its reductive metabolism by hepatic cytochrome P450. The reactive intermediate is believed to cause lipid peroxidation and breakdown of cellular membranes (De Groot and Sies, 1989). Recent experimental studies have investigated the role of antioxidative vitamins, minerals, drugs and plant-derived compounds in the prevention and therapy of liver fibrosis (Parola et al., 1992).

The antioxidant systems such as antioxidant vitamins (A, C and E), SOD, CAT, GSH, ceruloplasmin and GPx protect the cells against lipid peroxidation, which is the base of many pathologic processes (Williams, 1984; Bray and Bettger, 1990). The GSH antioxidant system consists of an array of non-enzymic and enzymic reaction pathways involving the neutralization of free radical species. Preturbation of the GSH status of a biological system has been reported to lead to serious consequence (Sreepriya et al., 2001). GPx utilizes it for the decomposition of lipid hydroperoxides and other reactive oxygen species (ROS) and glutathione-S-transferase (GST) maximizes the conjugation of free radicals and various lipid hydroperoxides to GSH to form water-soluble products that can be easily excreted out (Ahmed et al., 2000). Ohta et al. (1995) have reported the decreased activities of SOD and CAT after the administration of single dose of CCl₄. In our study, decline in the activities of SOD, CAT, GPx and GSH levels in CCl₄ administered rats and recovery to near normalcy in extract treated groups revealed that oxidative stress elicited by CCl₄ intoxication has been nullified due to the antioxidant effect of the aqueous ethanolic extract of *V. volvacea*.
In conclusion, the ethanolic extract of *V. volvacea* mycelia possessed significant hepatoprotective effect against APAP and CCl₄ induced acute hepatotoxicity in rats. The effect might be mediated to its significant antioxidant activity. The findings suggest the therapeutic potential of the cultured mycelia of *V. volvacea* for liver protection.