CHAPTER 7
HEPATO PROTECTIVE EFFECT AND IN VIVO
ANTIOXIDANT ACTIVITY OF THE EXTRACTS OF
PLEUROTUS FLORIDA AND PLEUROTUS SAJOR-CAJU
Drug-induced liver injury is one of the serious complications because liver is central to the metabolic disposition of virtually all drugs and foreign substances (Zimmerman et al., 1993, Kaplowitz, 1991, Farrell, 1994). The liver is involved in a wide variety of metabolic activities such as carbohydrate and lipid metabolism, secretion of bile as well as the synthesis of proteins, although it is often considered as only a detoxification organ. The drugs are usually metabolized without injury to the liver although many fatal and near-fatal drug reactions occur. Some compounds are known to produce metabolites that cause liver injury in a uniform, dose-dependent fashion (Mitchell et al., 1973, Baerg et al., 1970, Klein, 1989). Cirrhosis of the liver is one of the major health problems in developed countries. Cirrhosis of the liver is the fourth leading cause of death in the American adults (Sherlock and Dooley, 1993). The most widely accepted experimental model of cirrhosis is the rat/carbon tetrachloride model, which is remarkably similar to human alcoholic cirrhosis both histologically and systemically (Sundari et al., 1997). The reactive oxygen species and such as superoxide anion, hydrogen peroxide and hydroxyl radical have been implicated in the pathophysiology of various clinical disorders. They play an important role in the inflammation process after intoxication by ethanol/carbon tetrachloride/carrageenan (Yoshikawa et al., 1983, Yuda et al., 1991, Halliwell and Gutteridge, 1984). These radicals and the reactive species derived from them react with cell membrane, induce lipid peroxidation and are responsible for various deleterious effects in cells and tissues where they are
generated (Sies, 1985). The inhibition of free radical generation can serve as a facile model for evaluating the activity of hepatoprotective agents.

Pleurotus mushrooms otherwise known as oyster mushrooms stimulate the activity of antioxidant enzymes and reduces lipid peroxidation when supplemented in rat diet (Bobek et al., 1994). Among the extracts of *P. florida* and *P. sajor-caju* tested for their *in vitro* antioxidant activity, methanolic extract was found to possess significantly higher antioxidant activity. Hence, the methanolic extract was selected for evaluation of the hepatoprotective activity of *P. florida* and *P. sajor-caju*. Since the antioxidant defense play a major a role in hepatoprotection, the studies also envisage to evaluate the *in vivo* antioxidant activity of oyster mushroom extract.

### 7.2 MATERIALS AND METHODS

#### 7.2.1 ANIMALS

Male Wistar rats of 10 weeks old (180 ± 20 g) were used for the study.

#### 7.2.2 Preparation of the methanolic extract

Methanolic extracts of *P. florida* and *P. sajor-caju* were prepared as described in the section 2.2.1

#### 7.2.3 DETERMINATION OF HEPATOPROTECTIVE ACTIVITY OF *P. FLORIDA*

Hepatoprotective activity was determined using CCl₄ induced acute hepatotoxicity in rat models by the method of Nishigaki et al., (1992), with some modifications. Animals were divided into four groups of six animals in each group and treated as follows. Group I (normal) administered with normal saline intraperitoneally (i.p). Group II was given CCl₄ in paraffin oil (0.25ml of
CCl₄ in liquid paraffin (1:1, v/v) per 100g body weight. Group III was given 500 mg/Kg body weight and Group IV 1000 mg/Kg body weight methanolic extract of *P. florida* in normal saline (i.p) 30 min before the injection of CCl₄ (0.25ml of CCl₄ in liquid paraffin per 100g body weight). The administration of the extract was repeated at 24, and 48h after the CCl₄ injection. The group treated with normal saline was kept as normal. The group II treated with CCl₄ alone was kept as control. One hour after the administration of the third dose of extract, animals were sacrificed. Blood was collected from the heart of each animal. Serum was used for the determination of glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase (ALP) and lipid peroxidation (MDA)

Liver was removed from each animal and washed thoroughly in ice-cold saline and homogenate (10 %) was prepared in PBS (50 mM, pH 7) (section 2.2.2). A part of the homogenate was used for the estimation of reduced glutathione (GSH). The remaining homogenate was centrifuged at 10,000 rpm for 10 min in a cooling centrifuge at 4°C, after removal of the cell debris; supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (MDA) (Section 4.2.3.3.1). Protein was determined by the method of Lowry et al., (1951)(Section 4.2.3.3.2).

### 7.2.3.1 DETERMINATION OF SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT) ACTIVITY

SGOT activity was determined according to the method of Reitman and Frankle (1957).
**PRINCIPLE**

Serum containing glutamate oxaloacetate transaminase catalyses the reaction between L-aspartate and \( \alpha \)-ketoglutarate, to form oxaloacetate and glutamate. The unstable oxaloacetate is converted to pyruvate and reacts with 2,4-dinitrophenylhydrazine. The absorbance of the resultant brown colored phenylhydrazone is measured at 505 nm under alkaline conditions.

**PROCEDURE**

0.1 ml of serum was added to 0.5 ml of the buffered substrate (2 mM of \( \alpha \)-ketoglutarate and 100 mM L-aspartate in 100 ml phosphate buffer 0.1M, pH 7.4) at 37°C and incubated for 60 min. After the incubation, 0.5 ml of dinitrophenylhydrazine (19.8 mg/dl \( 1 \) N HCl) was added, mixed well and kept at room temperature for 20 min. 0.4 ml of NaOH was added and read the absorbance after 10 min at 505 nm using the reagent blank. A control tube containing buffered substrate was treated with serum after the incubation at 37°C was also followed in the same manner. The enzyme activity was calculated from the standard (sodium pyruvate, 2 mM) calibration curve. The enzyme activity (U/ml) is converted to IU/l by multiplying with 0.483.

7.2.3.2 **DETERMINATION OF SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT) ACTIVITY**

SGPT activity was determined according to the method of Reitman and Frankle (1957).
PRINCIPLE

Serum containing glutamate pyruvate transaminase catalyses the reaction between L-alanine and α-ketoglutarate, to form pyruvate and glutamate. The pyruvate thus formed was treated with 2,4-dinitrophenylhydrazine. The absorbance of the resultant brown colored phenylhydrazone is measured at 505 nm under alkaline condition.

PROCEDURE

0.1 ml of serum was added to 0.5 ml of the buffered substrate (2 mM of α-ketoglutarate and 100 mM L-alanine in 100 ml phosphate buffer 0.1M, pH 7.4) at 37°C and incubated for 30 min. After the incubation, 0.5 ml of dinitrophenylhydrazine (19.8 mg/dl 1 N HCl) was added, mixed well and kept at room temperature for 20 min. 0.4 ml of NaOH was added and read the absorbance after 10 min at 505 nm using the reagent blank. A control tube containing buffered substrate was treated with serum after the incubation at 37°C was also followed in the same manner. The enzyme activity was calculated from the standard (sodium pyruvate, 2mM) calibration curve. The enzyme activity (U/ml) is converted to IU/l by multiplying with 0.483.

7.2.3.3 DETERMINATION OF SERUM ALKALINE PHOSPHATASE (ALP) ACTIVITY

Serum ALP activity was determined according to the method of Kind and King (1954)
PRINCIPLE

ALP in the serum reacts with disodium phenyl phosphate under alkaline pH 10 release phenol. Phenol reacts with 4-aminoantipyrene in the presence of alkaline oxidizing agent to give a red colored complex, absorbance is measured at 510 nm against reagent blank.

PROCEDURE

0.05 ml of serum was incubated with 0.5 ml of the buffered substrate (1ml of 0.254 g of disodium phenylphosphate dihydrate/dl water mixed with 1ml of the carbonate buffer pH 10) and 1.54 ml of distilled water at 37°C for 15 min. After the incubation, 2 ml chromogen (1ml of 0.6 g 4-aminoantipyrene/dl water and 1ml of potassium ferricyanide 2.4 g/dl water) reagent was added and absorbance measured at 510 nm. Phenol (10 mg %) was used as the standard for the calibration curve. The activity (KA/dl) is converted to IU/l by multiplying with 7.1.

7.2.3.4 DETERMINATION OF SERUM LIPID PEROXIDATION

Serum lipid peroxidation was determined by Ohkawa et al., (1979) after precipitating the protein according to the method of Satoh (1987).

PRINCIPLE

Lipids were isolated by precipitating them with serum protein using 0.02 % trichloroacetic acid. The level of lipid peroxidation was measured as malondialdehyde by reacting with TBA in acetic acid solution. The reaction product was assayed by measuring absorption at 532 nm.
PROCEDURE

To 0.5 ml serum, 2.5 ml of 0.02 % TCA was added and the tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rev./min for 10 min, the precipitate was washed once with 0.05 M H₂SO₄. The precipitate was suspended in distilled water and estimated the TBARS by procedure given under tissue lipid peroxidation determination (section 3.2.2.2). The result was expressed as nmol/ml of serum.

7.2.3.5 DETERMINATION OF TISSUE REDUCED GLUTATHIONE (GSH)

Reduced glutathione in the tissue was determined according to the method of Moron et al., (1979).

PRINCIPLE

The acid soluble sulfhydryl groups (non-protein thiols of which more than 93% is reduced glutathione) forms a yellow colored complex with dithionitrobenzene (DTNB). The absorbance of the colored complex was measured at 412 nm.

PROCEDURE

0.5 ml of the tissue homogenate was mixed with 0.1 ml of 25 % TCA and kept on ice for few minutes. These were then subjected to centrifugation at 3000 g for few minutes to settle the precipitate. 0.3 ml of the supernatant was mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8) and 2 ml of 0.6 mM DTNB (prepared in 0.2 M buffer, pH 8). The absorbance of yellow color obtained was measured after 10 min at 412 nm against a blank which contained 0.1 ml of 5% TCA in place of the supernatant. A standard graph was prepared using different concentrations of GSH (10-50 nmoles) in 0.3 ml of 5% TCA.
The GSH content was calculated with the help of this standard graph and expressed as n mole/mg protein.

7.2.3.6 DETERMINATION OF TISSUE SUPEROXIDE DISMUTASE ACTIVITY

Superoxide dismutase activity was determined according to the method of McCord and Fridovich (1969).

**PRINCIPLE**

Illumination of riboflavin solution in the presence of EDTA causes a reduction of the flavin. It then re-oxidizes and simultaneously reduces oxygen to $\text{O}_2^-$, which is allowed to react with a detector molecule NBT, reduced the NBT to a formazan blue. The SOD in the sample will inhibit the formazan production.

**PROCEDURE**

0.01 ml of the homogenate was mixed with 0.2 ml of 0.1 M EDTA (containing 0.0015% NaCN), 0.1 ml of 1.5 mM NBT and phosphate buffer (67 mM, pH 7.8) in a total volume of 2.6 ml. After adding 0.05 ml of riboflavin, the absorbance of the solution was measured against distilled water at 560 nm. Illuminated all the tubes uniformly for 15 min and absorbance of the blue color formed were measured again. Percent of inhibition was calculated after comparing absorbance of sample with the absorbance of control (the tube containing no enzyme activity). The volume of the sample required to scavenge 50% of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/mg protein.
7.2.3.7 DETERMINATION OF TISSUE CATALASE (CAT) ACTIVITY

Tissue Catalase activity was determined according to the method of Beer and Siezer (1952).

**PRINCIPLE**

Catalase catalyses the decomposition of H₂O₂. In the ultraviolet range H₂O₂ shows a continual increase in absorption with decreasing wavelength. The decomposition of H₂O₂ can be followed directly by the decrease in extinction at 240 nm.

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

**PROCEDURE**

0.1 ml of the tissue homogenate (approximately 0.1 mg protein) was mixed with 1.9 ml of the phosphate buffer (0.5 M, pH 7). The decrease in extinction was measured at 240 nm, 1 min interval for 3 min immediately after adding 0.1 ml of 10 mM H₂O₂ solution in buffer. A sample control was placed in the reference cuvette containing 0.1 ml of tissue homogenate and 2.9 ml of the buffer. Activity of catalase was calculated using the \( \mu \)molar extinction coefficient of 43.6 cm⁻¹.

\[
\text{nmoles of H}_2\text{O}_2 \text{ decomposed/min/mg protein} \quad \text{or} \quad \frac{\text{nmoles}}{\text{U/mg protein}} = \frac{\Delta A/\text{min} \times 1000 \times 3}{40 \times \text{mg protein in sample}}
\]

7.2.3.8 DETERMINATION OF TISSUE GLUTATHIONE PEROXIDASE (GPx) ACTIVITY

Glutathione peroxidase activity was determined according to the method of Hafemann et al., (1974).
PRINCIPLE

The activity of GPx was determined by measuring the decrease in GSH content after incubating the sample in the presence of \( \text{H}_2\text{O}_2 \) and \( \text{NaN}_3 \).

\[
\text{H}_2\text{O}_2 + 2 \text{GSH} \rightarrow 2\text{H}_2\text{O} + 2 \text{GSSG}
\]

PROCEDURE

Tissue homogenate (approximately 0.5 mg protein) was incubated with 0.1 ml of 5 mM GSH, 0.1 ml of 1.25 mM \( \text{H}_2\text{O}_2 \), 0.1 ml of 25 mM \( \text{NaN}_3 \) and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37°C for 10 min. The reaction was stopped by adding 2 ml of 1.65 % \( \text{HPO}_4^{2-} \) and the reaction mixture was centrifuged at 1500 rpm for 10 min. 2 ml of the supernatant was mixed with 2 ml 0.4 M \( \text{Na}_2\text{HPO}_4 \) and 1 ml of 1 mM DTNB. The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 min at 37°C. A sample without the tissue homogenate processed in the same way was kept as the blank.

One unit of enzyme activity was defined as decrease in log GSH by 0.001/min after subtraction of the decrease in log GSH per minute for the non-enzymatic reaction and is expressed as units/mg protein.

7.2.3.9 HISTOPATHOLOGICAL EXAMINATION

Portions of the liver from the experimental animals were fixed in 10 % formalin and then embedded in paraffin. Microtome sections 6 \( \mu \text{m} \) thickness were prepared from each portion of liver and stained with hematoxylin-eosin. The sections were examined for the pathological symptoms of hepatotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration etc.
7.2.4 DETERMINATION OF HEPATOPROTECTIVE ACTIVITY USING METHANOLIC EXTRACT OF *P. SAJOR-CAJU*

To determine hepatoprotective effect of *P. sajor-caju*, all the experiments described above were repeated using methanolic extract of this mushroom. Activities of serum transaminases (GOT, GPT), Alkaline phosphates (ALP), lipid peroxidation (MDA) hepatic antioxidants such as SOD, GPx, CAT, lipid peroxidation (MDA) and GSH were determined. Histopathological examination of the liver was also carried out.

7.3 RESULTS

7.3.1 HEPATOPROTECTIVE ACTIVITY OF THE EXTRACT AGAINST ACUTE CCl₄ TOXICITY

The activities of SGPT, SGOT and ALP of animals 48 hours after intoxication with CCl₄ are presented in Table 7.1. The results indicated that SGOT, SGPT and ALP activities were significantly enhanced by CCl₄ injection. The treatment of methanolic extract of *P. florida* at 500 and 1000mg/kg body weight significantly decreased SGOT and SGPT activities. The effect was significantly high at 1000 mg/Kg treatment. The activity of serum ALP in the extract treated groups of animals was also significantly reduced compared to the CCl₄ treated control group (Table 7.1).

The increased SGPT, SGOT and ALP levels after intoxication with CCl₄ was also significantly reduced when treated with methanolic extract of *P. sajor-caju*. The effect was significantly high at 1000mg/kg treatment (Table 7.2).
7.3.2 EFFECT ON ANTIOXIDANT STATUS IN LIVER

The activities of hepatic SOD, CAT and GPx, were decreased significantly in the group of animals treated with CCl₄ compared to the normal animals (Table 7.3). The activity of SOD in the CCl₄ injected animals was 16.5 ± 2.1 U/mg protein. Treatment of *P. florida* extract (1000 mg/kg body wt.) prior to the CCl₄ challenge enhanced the activity to 20.2 ± 2.9 U/mg protein. The activities of CAT and GPx in the CCl₄ alone treated animals were 43.25 ± 3.6 and 15.80 ± 0.80 U/mg protein respectively. The activity was restored to normal when CCl₄ challenged group of animals were treated with *P. florida* extract.

Methanolic extract of *P. sajor-caju* also significantly elevated the reduced SOD, CAT and GPx activities of animals challenged with CCl₄ in a dose dependent manner (Table 7.4). Methanolic extract of *P. sajor-caju* at a concentration of 1000mg/kg body weight significantly enhanced these enzyme activities.

The level of hepatic GSH was also elevated significantly when animals were treated with *P. florida* and *P. sajor-caju* extracts prior to the CCl₄ challenge compared to the normal group of animal (Fig 7.1 and 7.2).

The lipid peroxidation level was enhanced significantly in the control group of animals, which were challenged with CCl₄ compared to the normal group of animals (Fig 7.3 and 7.4). Treatment of animals with the extracts of *P. florida* and *P. sajor-caju* prior to CCl₄ challenge reduced the MDA level significantly.
7.3.3 HISTOPATHOLOGICAL EXAMINATIONS

Histopathological examination of the liver of the animals treated with CCl₄ showed centrilobular necrosis, inflammatory infiltration of lymphocytes and fatty changes. Treatment of the rats with the extract of *P. florida* and *P. salor-caju* (500 and 1000 mg/kg body wt) prior to CCl₄ challenge prevented hepatic damage to a great extent (Fig 7.5 & 7.6).
Table 7.1: Effect of methanolic extract of *P.jloricin* on serum GOT, GPT and ALP activities consequent to CCl₄ challenge

<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>125.67 ± 3.99&lt;sup&gt;d&lt;/sup&gt;</td>
<td>68.48 ± 2.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>124.50 ± 4.14&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (CCl₄/paraffin)</td>
<td>0.25ml / 100g body weight</td>
<td>471.17 ± 8.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>253.83 ± 3.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>332.83 ± 4.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol Extract + CCl₄</td>
<td>500</td>
<td>204.67 ± 5.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>215.33 ± 6.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>219.50 ± 5.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>41.50 ± 3.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>107.67 ± 11.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.67 ± 4.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd=9.554 (SGOT), lsd= 5.031 (SGPT), lsd= 5.312 (ALP)

Table 7.2. Effect of methanolic extract *P.sajor-caju* on serum GOT, GPT and ALP activities consequent to CCl₄ challenge

<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>126.00 ± 3.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>69.08 ± 2.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>126.00 ± 6.10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (CCl₄/paraffin)</td>
<td>0.25ml / 100g body weight</td>
<td>473.50 ± 5.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>256.00 ± 14.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>320.87 ± 4.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol Extract + CCl₄</td>
<td>500</td>
<td>215.33 ± 11.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>256.17 ± 8.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>219.50 ± 5.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>143.50 ± 6.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>107.67 ± 11.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.50 ± 3.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 14.24 (SGOT), lsd= 10.08 (SGPT), lsd= 6.580 (ALP)
Table 7.3. Effect of methanolic extract *P. florida* on liver SOD, GPx and CAT activities consequent to CCl₄ challenge

<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>22.2 ± 3.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>64.5 ± 4.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.48 ± 1.81&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (CCl₄/paraffin (1:1))</td>
<td>0.25ml / 100g body weight</td>
<td>16.5 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.25 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.80 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol Extract</td>
<td>500</td>
<td>18.5 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.89 ± 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.80 ± 1.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>21.2 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.5 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.50 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals
Any two values having a common letter are not significantly different at 5% level, lsd=1.985(SOD), lsd= 9.876 (CAT), lsd= 1.451 (GPX).

Table 7.4. Effect of methanolic extract *P. sajor-caju* on liver SOD, GPx and CAT activities consequent to CCl₄ challenge

<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>24.20 ± 3.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>74.50 ± 4.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.42 ± 1.41&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (CCl₄)</td>
<td>0.25ml / 100g body weight</td>
<td>17.32 ± 2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.86 ± 5.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.50 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol Extract</td>
<td>500</td>
<td>19.20 ± 1.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.5 ± 4.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.50 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>23.12 ± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.54 ± 3.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.18 ± 1.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals
Any two values having a common letter are not significantly different at 5% level, lsd=1.865(SOD), lsd= 10.06 (CAT), lsd= 1.821 (GPX).
Fig 7.1: Effect of methanolic extract of *P. flordia* on hepatic GSH level consequent to CCl₄ challenge

A: Control B: Normal, C: Methanolic extract of *P. flordia* (500 mg/Kg body weight) D: Methanolic extract of *P. flordia* (1000 mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.747
Fig 7.2: Effect of methanolic extract of *P. sajor-caju* on hepatic GSH level consequent to CC14 challenge

A: Control, B: Normal C: Methanolic extract of *P. sajor-caju* (500 mg/Kg body weight) D: Methanolic extract of *P. Sajor-caju* (1000 mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.339
Fig 7.3: Effect of methanolic extract of *P. florida* on serum and tissue MDA level of liver consequent to CCl₄ challenge

A: Normal, B: Control, C: Methanolic extract of *P. florida* (500 mg/Kg body weight) D: Methanolic extract of *P. florida* (1000 mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.269 (serum MDA), lsd= 0.294 (tissue MDA)
Fig 7.4: Effect of methanolic extract of *P.sajor-caju* on serum and tissue MDA level of liver consequent to CCl₄ challenge

A: Normal, B: Control, C: Methanolic extract of *P.Sajor-caju* (500 mg/Kg body weight) D: Methanolic extract of *P.Sajor-caju* (1000 mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.410 (serum MDA), lsd= 0.235 (tissue MDA)
Fig 7.5: Hepatoprotective activity of methanolic extract of *P. florida* against CCl₄ induced acute hepatotoxicity in rats. Skin sections stained with H&E. a) Normal b) CCl₄/Paraffin oil c) Methanol extract (500mg/kg Body weight) + CCl₄ d) Methanol extract (1000mg/kg Body weight) + CCl₄
Fig 7.6: Hepatoprotective activity of methanolic extract of *P.sajor-caju* against CCl\textsubscript{4} induced acute hepatotoxicity in rats. Skin sections stained with H&E. a) Normal b) CCl\textsubscript{4}/Paraffin oil c) Methanolic extract (500mg/kg Body weight) + CCl\textsubscript{4} d) Methanolic extract (1000mg/kg Body weight) + CCl\textsubscript{4}
7.4. DISCUSSION

In living system, liver is considered to be highly sensitive to toxic agents. Hepatic dysfunction due to ingestion or inhalation of hepatotoxins such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride, allyl alcohol are 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 diseases due to liver toxicity.

The experimental results show that CCl₄ administration induces liver damage. This is evident from significant elevation of hepatic marker enzymes GOT, GPT and ALP. Elevation of these enzymes in serum is due membrane damage. The results of the present study show that the administration of methanolic extract of *P. florida* and *P. sajor-caju* significantly reduced the increase in serum GOT, GPT and ALP. The elevation of SGPT in the serum indicates the hepatocyte necrosis. The first step in the biotransformation of carbon tetrachloride is catalysed by cytochrome P-450 enzymes (mainly CYP2E1), leading to the formation of the reactive trichloromethyl radical. The radical, is oxidised further forming the even more reactive trichloromethylperoxyl radical, which can react further to form phosgene. Phosgene may be detoxified by reaction with water to produce carbon dioxide or with glutathione or cysteine. Formation of chloroform and dichlorocarbene occurs under anaerobic conditions (McGregor and Lang, 1996). Covalent binding to macromolecules and lipid peroxidation occur via reactive metabolic
intermediates of carbon tetrachloride in particular the trichloromethylperoxyl radical. In experiments models using mice and rats, carbon tetrachloride is found to be capable of inducing hepatomas and hepatocellular carcinomas. The doses capable of inducing hepatic tumours are higher than those inducing cell toxicity. It is likely that the carcinogenicity of carbon tetrachloride is secondary to its hepatotoxic effects (Anonymous, 1999).

Hepatotoxicity may be due to the enhanced lipidperoxidation, depletion of superoxide dismutase and catalase activities and altered immunological changes induced by various chemical agents or direct damage to the cell (Delev 1995, Farrel 1998, Kaplowitz et al., 1989). Irrespective of the mechanisms of injury it is clear that ultimate hepatic necrosis is brought about by increased lipid peroxidation.

The antioxidant status of the liver is also altered in CCl₄ treated animals. The decline of antioxidant status is responsible for the increased lipid peroxidation, which leads to loss of membrane fluidity, integrity and finally cell functions of liver (Halliwell and Gutteridge, 1989, Smith et al., 1987). The treatment of the animals with extracts prior to the CCl₄ injection increased the hepatocyte SOD, CAT and GPx activity and effectively prevented the radical mediated loss of membrane integrity. Hence the extract treated animals show reduced transaminase activity and MDA level in serum.

The results of the present investigations indicate that methanolic extracts of P.florida and P.sajor-caju possess significant hepatoprotecting properties. Hepatoprotective effects of extracts of a number of mushrooms
Grifola frondosa, Schizophyllum commune, Ganoderma lucidum and Tremella fuciformis have been reported (Wasser and Weis, 1999). However, the present studies reveal that the significant free radical scavenging activity of the methanolic extract of P. florida and P. sajor-caju appears to be responsible for rendering hepatoprotection against CCl₄ challenge. Thus the major contributing factor of hepatoprotection against the toxic chemicals by the oyster mushroom extracts might be due to their profound free radical scavenging or antioxidant activity.