Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl₄-induced liver injury in rats

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Abstract

This study was undertaken to investigate the putative antioxidant activity of the oyster mushroom *Pleurotus ostreatus* on CCl₄-induced liver damage in male Wistar rats. Intraperitoneal administration of CCl₄ (2 ml/kg) to rats for 4 days resulted in significantly elevated (p < 0.05) serum levels of glutamic oxaloacetic transaminase (SGOT), glutamic pyruvate transaminase (SGPT) and alkaline phosphatase (SALP) compared to controls. In the liver, significantly elevated levels (p < 0.05) of malondialdehyde (MDA) and lowered levels (p < 0.05) of reduced glutathione (GSH) were observed following CCl₄ administration. Quantitative and qualitative analysis of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx) revealed lower activities of these antioxidant enzymes in the liver of CCU-administered rats. An analysis of the isozyme pattern of these enzymes revealed variations in relative concentration presumably due to hepatotoxicity. When rats with CCl₄-induced hepatotoxicity were treated with the extract of *P. ostreatus*, the serum SGOT, SGPT and SALP levels reverted to near normal, while the hepatic concentration of GSH, CAT, SOD and Gpx were significantly increased (p < 0.05) and that of MDA significantly (p < 0.05) lowered, when compared to CCU-exposed untreated rats. Histopathological studies confirmed the hepatoprotective effect conferred by the extract of *P. ostreatus*. These results suggest that an extract of *P. ostreatus* is able to significantly alleviate the hepatotoxicity induced by CCl₄ in the rat.

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Keywords: Antioxidant; Hepatotoxicity; *Pleurotus ostreatus*; Carbon tetrachloride; Free radical; Mushroom

1. Introduction

The genus *Pleurotus* comprises about 40 species (Jose and Janardhanan, 2000) that are commonly referred to as “oyster mushrooms”. They are ubiquitous, being found both in temperate and tropical parts of the world, and are now considered to be the second most important cultivated mushrooms in the world (Chang, 1991). Mushrooms are an important source of nutrients, and of physiologically beneficial, non-toxic medicines (Wasser and Weis, 1999). In fact, mushrooms have been used in folk medicine throughout the world since ancient times. Many medicinal properties have been attributed to mushrooms (Borchers et al., 1999), including inhibition of platelet aggregation (Hokana and Hokana, 1981), reduction of blood cholesterol concentrations (Aletor, 1993), prevention or alleviation of heart disease and reduction of blood glucose levels (Manzi and Pizzoferrato, 2000), and also prevention or alleviation of infections caused by bacterial, viral, fungal and parasitic pathogens (Breene, 1990). Hydnum mushrooms have also been reported to block induced liver lipid peroxidation (Lin et al., 1998). Since research has tended to focus on the dietary value of edible mushrooms, there is relatively little information pertaining to their antioxidant properties and their possible use to treat oxidative stress.

Abbreviations: H: hepatocyte; N: nucleus; SS: sinusoidal space.

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Carbon tetrachloride (CCl₄), a potent hepatotoxic agent is biotransformed to a trichloromethyl radical by the cytochrome P450 system in liver microsomes, and consequently causes lipid peroxidation of membranes that leads to liver injury (Recknagel, 1983; Slater, 1984; McCay et al., 1984). The present study was designed to evaluate the putative antioxidant action of an extract of the oyster mushroom Pleurotus ostreatus in an experimental model of CCl₄-induced hepatotoxicity in Wistar rats

2. Materials and methods

2.1 Preparation of the mushroom extract

The mushroom P. ostreatus was cultivated adopting the "layer spawning" method. Freshly-harvested whole mushrooms were dried in the shade and then finely powdered. Five grams of the powder were extracted with 100 ml of 95% ethanol using Soxhlet apparatus. The residue was filtered and concentrated to a dry mass by vacuum distillation, the filtrate thus obtained was used as mushroom extract.

2.2 Animal experiment

Male albino Wistar rats (150–200 g) procured from the Central Animal House, Raja Muthiah Medical College, Annamalai Nagar, India, were used for the experiments. The animals were acclimatized for 20 days prior to dosing, during which time they had free access to food and water ad libitum. Eighteen such acclimatized rats were randomly divided into three groups of six each. Group I (normals) received only vehicle (olive oil, 1 ml/kg b w) for 4 days, Group II (toxin controls) received vehicle on the first and fourth days, and vehicle and CCl₄ (50% solution of CCl₄ in olive oil, 2 ml/kg b w) on the second and third days. Group III (test rats) received mushroom extract (200 mg/kg b w) on the first and fourth days and mushroom extract and CCl₄ on the second and third days. All administrations were made intraperitoneally. On the fifth day, rats were sacrificed by decapitation, from each rat, blood samples were collected from the inferior vena cava, and the liver was removed. The serum was separated from the blood, and the serum and liver samples were stored at −80 °C until analysis.

2.3 Determination of enzyme levels in serum

Serum glutamyl oxalacetate transaminase (SGOT) and serum glutamyl pyruvate transaminase (SGPT) activities (IU/L) and serum alkaline phosphatase (SALP) activity (IU/L) were measured using standard assay kits (Darmys, Germany) for SGOT and SGPT, Radiant Centrione GmbH (Germany) for SALP.

2.4 Determination of biochemical parameters in liver

2.4.1 Preparation of liver supernates

Prior to biochemical analysis, each liver sample (100 mg/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.0). The homogenate was then centrifuged at 10,000 rpm for 15 min and the supernatant obtained was used for biochemical analysis. All liver parameters were expressed as activity per mg protein. The protein concentration in each fraction was assayed by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

2.5 Determination of lipid peroxidation

The mean malondialdehyde (MDA) content (µmol/mg protein), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TABRS) by the method of Ohkawa et al. (1979)

2.6 Determination of reduced glutathione

The mean reduced glutathione concentration (µmol glutathione/mg protein) in the liver homogenate was determined by the method of Moron et al. (1979)

2.7 Quantitative analysis of enzyme activities

2.7.1 Catalase (CAT)

The mean catalase activity (µmol of H₂O₂ consumed/min/mg protein) was assayed by the method of Sinha (1972).

2.7.2 Superoxide dismutase (SOD)

The mean activity of SOD (units/min/mg protein) was determined by the method of Marklund and Marklund (1974), in which one unit was considered to be the amount of enzyme that inhibited pyrogallol auto-oxidation by 50%.

2.7.3 Glutathione peroxidase (Gpx)

Mean Gpx activity (µmol of glutathione oxidized/min/mg protein) was evaluated by the method of Rotruck, et al. (1973).

2.8 Statistical analysis

The results obtained for each group of rats tested was expressed as the mean ± SD of six values. Statistical analysis of the data was performed by one-way ANOVA.

2.9 Detection of isozymes by electrophoresis

Non-denaturing polyacrylamide gel electrophoresis was performed on liver samples essentially as described by Leemhuis (1970), except that SDS was omitted from all buffers and the samples were not boiled before electrophoresis. The enzymes were run on the basis of equal amounts of protein (70 µg) in a 10% gel for SOD and Gpx and 8% gel for CAT. Electrophoretic separation was performed at 4 °C with a constant power supply of 30 V for stacking gel and 100 V for separating gel. Staining for each enzyme activity was performed thus:

(i) CAT activity was detected by the method of Woodbury et al. (1971). The gel soaked in 5 mM H₂O₂ solution for 10 min was washed with water and stained with a reaction mixture containing 1% potassium ferricyanide (w/v) and 1% ferric chloride. The enzyme appeared as a yellow band superimposed on a dark green background. The reaction was terminated by adding water, and the gel was photographed at once.

(ii) SOD activity was identified by the method of Beauchamp and Fridovich (1971). The gel was soaked in 50 mM Tris–HCl buffer (pH 8) containing 10 mg nitroblue tetrazolium (NBT), 1 mg ethylene diamine tetra acetate acid (EDTA), 2 mg riboflavin (50 ml final volume) and kept in the dark for 30 min. The gel was then placed on an illuminated light box to locate the area of SOD activity, which appeared as a clear zone on a bluish violet background.

(iii) Gpx isozymes were separated by the method of Lim et al. (2002). The gel was soaked in 50 ml of 50 mM Tris–HCl buffer (pH 8) containing 200 µg reduced glutathione, and 8 µl of 30% H₂O₂ for 20 min. The gel was then transferred to 50 ml of 50 mM Tris–HCl buffer (pH 8) containing 25 mg NBT and 25 mg phenazine methosulphate (PMS). The appearance of white bands in the gel was taken to indicate the presence of Gpx isozymes.

Quantification of the isozyme bands for each enzyme studied was performed in a densitometer (G 300 transmittance/reflectance scanning densitometer, Hoefer Scientiftec Instruments, USA)
Table 1
Serum parameters in rats

<table>
<thead>
<tr>
<th>S. no</th>
<th>Groups</th>
<th>SGOT (IU/L)</th>
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<td>3</td>
<td>Mushroom treated (CCL4 + mushroom extract)</td>
<td>265 ± 5.477</td>
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<td>F-value</td>
<td>60.874</td>
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Values are expressed as mean ± SD of six rats in each group. 

P < 0.05 (ANOVA).

SCOT: Serum glutamyl oxalacetate transaminase.

SGPT: Serum glutamyl pyruvate transaminase.

SALP: Serum alkaline phosphatase.

3. Histopathological examinations

Conventional techniques of paraffin-wax sectioning and haematoxylin-eosin staining were used for histological studies (Drury and Wallington, 1980). Slices of fresh liver tissue were cut and fixed in buffered neutral formalin fixative for 24 h. Following fixation, the tissues were washed and processed through an ascending series of alcohol (30%, 50%, 70%, 90% and 100%), cleared in methyl salicylate and infiltrated with wax at 57 °C. The tissues thus cleared were embedded in paraffin. Sections of 6–8 μm thickness were cut, stained by aqueous haematoxylin and alcoholic-eosin and examined by bright-field microscopy at a magnification of 400x.

Nitroblue tetrazolium salt, reduced glutathione, phenazine methosulphate and riboflavin were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents were obtained from Central Drug House Pvt. Ltd., Mumbai, India.

4. Results

4.1. Serum enzyme parameters

A significant increase in the activity of the serum enzymes SGOT, SGPT and SALP was observed in rats receiving CCl4 in vehicle (Group II) when compared to normal (Group I) rats administered vehicle alone (Table 1). However, the activities of these serum enzymes were significantly (P < 0.05) lower in rats treated with the P. ostreatus extract (Group III) than in Group II rats.

4.2. Malondialdehyde concentration in liver

A marked increase in the mean MDA level was found in the liver of Group II (CCl4-exposed) rats relative to normal (Group I) rats (Table 2); this increase was statistically significant (p < 0.05). Treatment with P. ostreatus extract

Table 2
Levels of malondialdehyde and reduced glutathione in liver of rats

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LPO - μmol of MDA produced/mg protein.

GSH - μg of reduced glutathione/mg protein.

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LPO: Lipid peroxidation.

GSH: Reduced glutathione.

MDA: Malondialdehyde.

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CAT: Catalase
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Gpx: Glutathione peroxidase
in Group III rats was found to result in a significant \((p < 0.05)\) lowering of the mean MDA concentration, presumably by limiting lipid peroxidation in the hepatic tissue.

4.3. Reduced glutathione concentration in liver

\(\text{CCI}_4\) administration in Group II rats resulted in a marked decrease (relative to normal) in the level of reduced glutathione in liver (Table 2); this decrease was statistically significant \((p < 0.05)\). Treatment with \(P. \text{ostreatus}\) extract in Group III rats resulted in a significantly higher concentration of GSH \((p < 0.05)\) than that in Group II.

4.4. Activities of CAT, SOD and Gpx in liver

A significant decrease in CAT and SOD activity was observed in the liver of \(\text{CCI}_4\)-administered (Group II) rats when compared to normal (Group I) rats that had received vehicle alone (Table 3). Treatment with the extract of \(P. \text{ostreatus}\) appeared to exert a beneficial effect since the activities of CAT and SOD were significantly \((p < 0.05)\) higher in liver of Group III than in Group II rats. No such significant difference between mean Gpx activity in the different groups of rats was observed.

4.5. Isozyme patterns in liver

An analysis of the electrophoretic pattern of catalase isozymes in the liver tissue (Figs. 1a and 1b) revealed one band with slight variations in the staining intensity of the band among the three groups of rats; the liver of normal (Group I) rats exhibited a single band of high intensity (band area 230.99), while that of Group III rats (treated with \(P. \text{ostreatus}\) extract) exhibited a single band of similar intensity (band area 224.61); however, liver homogenates of Group II rats (administered \(\text{CCI}_4\) in vehicle) revealed a single band of notably decreased intensity (216.27).

Two isoforms of SOD were detected in the liver tissue of all three groups of rats. Isoform SOD1 appeared as a band of essentially similar intensity in the liver of all three groups (band area 140, 138.8 and 139.2 in Groups I, II and III, respectively). Group I and III rats exhibited SOD2 bands of equivalent staining intensity (band area 123.6 and 122.25, respectively) while Group II rats exhibited an SOD2 band of decreased intensity (band area 118.75) (Figs. 2a and 2b).

Four isoforms of Gpx enzyme were noted in the liver tissue of all three groups of rats. The staining intensity of the Gpx1 and Gpx4 isoforms was essentially similar in all three
groups of rats (Figs. 3a and 3b). However, the staining intensity of the Gpx2 isoform was less in Group II rats (band area 134.75) compared to that in Group I (normal) rats (band area 142.6) and Group III (P. ostreatus-treated) rats (band area 140.72). The Gpx3 isoform exhibited a similar phenomenon (reduced staining intensity [band area 102] in Group II rat livers compared to Group I [band area 110] and Group III [band area 109] rats).

Fig 2b. Densitometric pattern of superoxide dismutase isoenzyme in liver tissue. L1 - normal; L2 - CCl4; L3 - CCl4 + mushroom extract; S1 - SOD1; S2 - SOD2.

Fig 3a. Electrophoretic pattern of Gpx isoenzyme in liver tissue. L1 - normal; L2 - CCl4; L3 - CCl4 + mushroom extract; G1 - Gpx1; G2 - Gpx2; G3 - Gpx3; G4 - Gpx4.

Fig 3b. Densitometric pattern of Gpx isoenzyme in liver tissue. L1 - normal; L2 - CCl4; L3 - CCl4 + mushroom extract; G1 - Gpx1; G2 - Gpx2; G3 - Gpx3; G4 - Gpx4.
4.6 Histopathological examinations

When compared to the histoarchitecture of the liver of Group I (normal) animals (Fig 4), liver cells of Group II rats (exposed to CCl4) revealed extensive damage, characterized by the disruption of the lattice nature of the hepatocyte, damaged cell membranes, degenerated nuclei, disintegrated central vein and damaged hepatic sinusoids (Fig 5). In Group III rats (exposed to CCl4 and mushroom extract), only minimal disruption of the hepatic cellular structure was observed (Fig 6).

5. Discussion

The Pleurotus species of oyster mushroom are cultivated on a commercial scale worldwide, including in India, since these have been proven to be a good source of almost all essential amino acids. Levostatin, a cholesterol-lowering drug derived from Pleurotus species, and its analogues are reported to be the best therapeutic agents for correcting hypercholesterolemia (Endo, 1988). Jose and Janardhanan (2000) have reported that the ethyl acetate and methanol extracts of Pleurotus florus exhibited potent hydroxyl radical scavenging and lipid peroxidation inhibition activities. The concentrations of cysteine, methionine and aspartic acid are reported to be higher in Pleurotus than those in other edible mushrooms, such as Agaricus bispora (brown), A. bispora (white), and Lentinula edodes (Mattila et al., 2002). In addition, Pleurotus ostreatus has also been reported to possess excellent reducing power of ferric ions (Lin, 1999). Hence the present investigation was carried out to test the antioxidant potential of Pleurotus ostreatus in an animal model of experimentally induced oxidative stress.

Carbon tetrachloride (CCl4) is a well-known hepatotoxic agent. The basis of its hepatotoxicity lies in its biotransformation by the cytochrome P450 system to two free radicals. The first metabolite, a trichloromethyl free radical, forms covalent adducts with lipids and proteins, can interact with O2 to form a second metabolite, a trichloromethylperoxy free radical, or can remove hydrogen atoms to form chloroform. This sequence of events leads to lipid peroxidation of membranes and consequent liver injury. In response to this hepatocellular injury, "activated" hepatic Kupfer cells release increased quantities of active oxygen species and other bioactive agents (ElSissi et al., 1993).

Since free radicals play such an important role in CCl4-induced hepatotoxicity, it seems logical that compounds that neutralize such radicals may have an hepatoprotective effect. Indeed, various natural products have been reported to protect against CCl4-induced hepatotoxicity (Hsiao et al., 2003). The medicinal herb Artemisia campestris has been found to scavenge free radicals and thence to exert a hepatoprotective effect against CCl4-induced liver injury (Aniya et al., 2000). Similarly, ginsenosides are reported to contribute to protection against CCl4-induced hepatotoxicity in rats, ostensibly by their antioxidant properties (Jeong et al., 1996).

In the present study, an experimental model of acute hepatotoxicity in Wistar rats was induced by intraperitoneal administration of CCl4. Direct evidence of this hepatotoxicity was noted in the occurrence of alterations in various hepatic parameters. Increased MDA concentration, decreased GSH level, decreased activities of antioxidant enzymes (CAT, SOD, Gpx) in Group II rats (administered CCl4 alone) in comparison to Group I (normal rats). The increase in levels of serum enzymes (SGOT, SGPT and SALP) in Group II rats also suggested hepatotoxicity in these rats. The mushroom extract was administered in a concentration of 200 mg/kg body weight. This concentration of the extract was chosen since the results obtained by other workers in earlier related studies suggested that this dose would be effective. Jose and Janardhanan (2000) observed that when an extract of the mushroom Pleurotus florus was administered in an intraperitoneal dose of 250, 500 and 1000 mg/kg body weight, it inhibited the growth of solid tumour in a dose-dependent manner. In another study, Yadav and Dixit (2003) reported that leaves of Kalanchoe pinnata Pers., when administered intraperitoneally in a dose of 200 mg/kg body weight, exhibited hepatoprotective activity in albino rats with CCl4-induced hepatotoxicity. For these reasons, we administered the extract of Pleurotus ostreatus in a dose of 200 mg/kg body weight.

Serum transaminases and alkaline phosphatase have long been considered as sensitive indicators of hepatic injury (Molander et al., 1955). Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells (Zimmerman and Seef, 1970). Leakage causes a decrease in levels of GOT, GPT and ALP in hepatic cells but increase in levels of serum GOT, GPT and ALP (Yadav and Dixit, 2003). This may explain the increase in levels of SGOT, SGPT and SALP observed in Group II rats in the present study. Treatment with the extract of Pleurotus ostreatus, as occurred in Group III rats, appears to have protected against hepatic injury due to CCl4, as suggested by the normal levels of SGOT, SGPT and SALP in this group.

Glutathione is a major, non-protein thiol in living organisms which performs a key role in co-ordinating innate antioxidant defence mechanisms. It is involved in the maintenance of normal structure and function of cells, probably by its redox and detoxification reactions (Gueen, 1995). Reduced glutathione (GSH) plays a key role in the detoxification of the reactive toxic metabolites of CCl4, liver necrosis is initiated when reserves of GSH are markedly depleted (Recknagel et al., 1991). Williams and Burk, 1990). Thus, the reduced levels of GSH (relative to normals) observed in the present investigation in Group II rats (administered CCl4) are consistent with the results of other workers. Anand et al. (1996) have suggested that lowered GSH levels may occur due to increased utilization of GSH by antioxidant enzymes such as Gpx which scavenge H2O2. Interestingly, in the present study, Group III rats...
(administered the mushroom extract) had a mean GSH level that was significantly higher than that in Group II rats and close to the value obtained in Group I (normal rats). The P. ostreatus extract appears to have conferred some hepatoprotection to Group III rats, maintaining GSH levels at near normal values.

Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA) (Vaca et al., 1988). MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage involving a series of chain reactions (Ohkawa et al., 1979). It reacts with thiobarbituric acid, producing red-coloured products. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. It has been hypothesized that one of the principal causes of CCL4-induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCL4 (Recknagel et al., 1989, 1991). The observation of elevated levels of hepatic MDA in Group II rats (administered CCL4 alone) in the present study is consistent with this hypothesis. Thus, the maintenance of near normal levels of hepatic MDA in Group III rats (administered CCL4 and mushroom extract) is of great interest since it provides additional evidence to suggest a hepatoprotective role for P. ostreatus extract.

Living tissues are endowed with innate antioxidant defence mechanisms, such as the presence of the enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx). A reduction in the activities of these enzymes is associated with the accumulation of highly-reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Reedy and Lokesh, 1992; Krishnakantha and Lokesh, 1993; Sheela and Angusti, 1995). Administration of CCL4 leads to generation of peroxyl radical, O₂⁻, which is associated with inactivation of CAT and SOD enzymes. This probably explains the significantly reduced activities of CAT SOD and Gpx observed by us in rats challenged with CCL4 (Group II). In rats receiving CCL4 and P. ostreatus extract (Group III), the activities of CAT, SOD and Gpx were significantly higher than in Group II rats, and very similar to the values noted in normal (Group I) rats. This suggests a hepatoprotective effect by the P. ostreatus extract, which is a very encouraging finding. The extract possibly confers this protective effect by dampening the generation of free radicals that is induced by CCL4.

The utilization of multiple isoforms of enzymes is believed to be one of the primary control mechanisms that regulates cellular metabolism (Sang et al., 2005). However, there is a paucity of data on the regulation of expression of antioxidant enzyme isoforms by oxidative stress, an aspect that deserves study. This was done in the present investigation. Our studies on the electrophoretic pattern of hepatic catalase isozymes revealed one band of high intensity in normal (Group I) rats and a single band of almost similar intensity in rats administered CCL4 and P. ostreatus extract (Group III), however, in Group II rats (administered CCL4 alone) the single band observed was of decreased staining intensity. In the case of hepatic SOD, where two isoforms (SOD1 and SOD2) were noted, a difference in the pattern of expression of the isoforms was noted: the SOD1 isoform appeared essentially the same in all three groups of rats whereas SOD2 expression was essentially the same in Group I and III rats but decreased in Group II rats (challenged in CCL4 alone). Similarly, out of the four isoforms of Gpx expressed in the liver, Gpx1 and Gpx4 isozymes were essentially similar in all three groups of rats whereas in the case of isoforms Gpx2 and Gpx3, a decreased expression was observed in Group II rats (administered CCL4 alone) compared to that seen in Group I (normal) and Group III (administered CCL4 and P. ostreatus extract) rats. Such a pattern of differential expression of enzyme isoforms has been noted in barley shoot and root exposed to saline stress (Sang et al., 2005).

Histopathological studies were performed to provide direct evidence of the hepatotoxicity of CCL4, and of the hepatoprotective effect of the extract of P. ostreatus. Marked disruption of the structure of hepatocytes was noted in liver tissue of Group II rats (exposed to CCL4 alone). Only minimal disruption of the structure of hepatocytes was noted in liver tissue of Group III rats (exposed to CCL4 and mushroom extract); this minimal disruption of the hepatocyte structure complemented the results of the liver enzyme studies (SGOT, SGPT and SALP activities and MDA levels approximated to the levels in normal rats).

The results of the present study suggest that an extract of the oyster mushroom P. ostreatus is able to confer protection against acute hepatotoxicity induced by administration of CCL4 in Wistar rats. In rats receiving the mushroom extract and CCL4 near normal levels of hepatic constituents (MDA, GSH, CAT, SOD, and Gpx) and serum enzymes (SGOT, SGPT, and ALP) were maintained, in contrast to alterations in all these parameters in rats receiving CCL4 alone. Whether a similar protective effect is obtained in a chronic model of hepatotoxicity requires evaluation. The dose range of the mushroom extract that would exert beneficial effects in liver injury should be determined. Also, the possibility of developing a dietary oral supplement containing this extract or its active principle is being investigated since this would be of considerable practical relevance. In spite of these limitations, the present investigation is important in presenting data suggesting considerable promise for the mushroom P. ostreatus as a hepatoprotective agent in CCL4-induced oxidative stress in the rat.

**Conflict of interest statement**

There are no conflicts of interest.

**Acknowledgements**

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References


Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats

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**Abstract**

This study was undertaken to investigate the putative antioxidant activity of the oyster mushroom *Pleurotus ostreatus*, on lipid peroxidation and antioxidant status of major organs of aged (24 month old) rats when compared to young (4 month old) rats. Elevated levels of malondialdehyde (MDA) and significantly lowered levels of reduced glutathione (GSH) and of vitamins C and E were observed in the liver, kidneys, heart, and brain of aged rats when compared to values in young rats. Quantitative analysis of the activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx) revealed significantly lower values in the liver, kidneys, heart, and brain of aged rats. An analysis of isozyme pattern of these enzymes in aged rats also revealed variations in relative concentration, presumably due to oxidative stress. Administration of the extract of *P. ostreatus* to aged rats resulted in elevated levels of reduced glutathione and vitamins C and E and in increased activities of CAT, SOD and Gpx so that the values in most of these parameters did not differ significantly from those in young rats. In addition, the level of MDA was lowered on administration of mushroom extract to aged rats. These results suggest that treatment with an extract of *P. ostreatus* can improve the antioxidant status during ageing therein minimizing the occurrence of age-associated disorders associated with involvement of free radicals.

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**Keywords** Antioxidants Mushroom *Pleurotus ostreatus* Ageing Free radicals

1. Introduction

Ageing is characterized by a progressive decline in biochemical and physiological functions of various tissues and organs in an individual. The causes of this decline are still unclear but it has been proposed that increased oxidative stress, disturbances in energy metabolisms, and a primary dysregulation of the immune system might play an important role (Prolla and Mattson, 2001). According to the free-radical theory of ageing (Harman, 1956) and its modern version, the oxidative stress theory of ageing, the disruption of the delicate balance between generation of reactive oxygen species (ROS) and antioxidant scavenging systems with increasing age could lead to a shift to an oxidative cellular milieu. Hence, ageing is now considered to be associated with an increase in oxidative damage to biomolecules (Ames et al., 1993; Goto et al., 2001) and also enhanced levels of inflammation (Miller, 1996; Johnson et al., 1999; Cheng et al., 2002). Reactive oxygen species are believed to be usually generated in aerobic cells and aerobic organisms are provided with antioxidant defense systems that could avert damage due to oxidative stress (Fridovich, 1978; Sies, 1985). The major antioxidant defense systems are composed of antioxidant enzymes and biological antioxidants, the former include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx), and the latter include reduced glutathione (GSH), vitamin C and vitamin E (Klivenyi et al., 2000). The increased oxidative damage observed during ageing might be due to the insufficiency of antioxidants (Reiter, 1995).
Supplementation of antioxidants could conceivably protect the human body from free radicals and ROS effects and retard the progress of many chronic diseases as well as lipid peroxidation (Lai et al., 2001; Gulcin et al., 2003). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tert-butyldihydroquinone. However, BHA and BHT are suspected to be responsible for liver damage and carcinogenesis (Wich, 1988; Sheiwin et al., 1990). Thus, compounds (particularly from natural sources) that have potential antioxidant properties are being sought.

Mushrooms have long constituted an integral part of the normal human diet. These are reported to contain relatively large amounts of vitamins A, C and β-carotene, all of which have protective effects because of their antioxidant properties (Mucia et al., 2002). Mushrooms also contain many phenols, which are very efficient scavengers of peroxo radicals (Mucia et al., 2002). The extract of the mushroom, 

\textit{Pleurotus ostreatus}, has been reported to remove the hyper-oxide radical, the main factor in the human aging process (Liu et al., 1997). P. ostreatus is reported to contain higher concentrations of cystine, methionine and aspartic acid than other edible mushrooms, such as \textit{Agaricus bisporus} (brown), \textit{A. bispora} (white) and \textit{Lentinus edodes} (Matilla et al., 2002). Levostatin, a cholesterol-lowering drug derived from \textit{Pleurotus} species, and its analogues are reported to be the best therapeutic agents for correcting hypercholesterolemia (Endo, 1988). Ethyl acetate and methanol extracts of \textit{P. flouida} have been found to exhibit potent scavenging of hydroxyl radicals and inhibition of lipid peroxidation activities (Jose and Janardhanan, 2000). In addition, \textit{Pleurotus ostreatus} has also been reported to possess excellent reducing power on ferrocenium ions (Lin, 1999). Hitherto, research has tended to focus on the dietary value of edible mushrooms, however, there is relatively little information pertaining to their antioxidant activity and their possible use to inhibit oxidative stress during aging. The present study was designed to have a two-pronged approach (a) to confirm the occurrence of oxidative stress in aged rats, and (b) to determine whether an extract of the mushroom \textit{P. ostreatus} enhances the antioxidant status in aged rats.

2. Materials and methods

2.1 Preparation of the mushroom extract

The mushroom \textit{P. ostreatus} was cultivated adopting the "layer spawning" method. Freshly harvested whole mushrooms were shade-dried and then finely powdered. Five grams of the powder was extracted with 100 ml of 95% ethanol using a Soxhlet apparatus (an apparatus which is used to remove or concentrate substances that may otherwise be very difficult to remove or concentrate if a manual process is used). The material thus obtained was filtered, and the resulting filtrate was concentrated to a dry mass by vacuum distillation, this was used as mushroom extract.

2.2 Animal experiment

Male albino \textit{Wistar} rats weighing approximately 80-110 g (4 month old) and 300-375 g (24 month old), procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai Nagar, India, were used for the experiments. The animals were acclimatized for 20 days prior to dosing, during which time they had free access to food and water ad libitum. Eighteen such acclimated rats were divided into three groups of six each: Group I, normal young (4 month old) rats; Group II, normal aged (24 month old) untreated rats; Group III, normal aged rats treated with mushroom extract (200 mg/kg bw administered intraperitoneally for 21 days). On the 22nd day, rats were sacrificed by decapitation, the liver, kidneys, heart, and brain were removed from each rat and stored at -80 °C until further analysis.

2.3 Biochemical analyses

Samples of liver, kidneys, heart, and brain (100 mg/ml buffer) were homogenized in 50 mM phosphate buffer (pH 7.0), and then centrifuged at 10,000 rpm for 15 min, the supernatant thus obtained was used for biochemical analysis. All parameters were expressed as activity per mg protein (a) The protein concentration in each fraction was determined by the method of Lowry et al. (1951), using crystalline bovine serum albumin as standard. (b) The malondialdehyde (MDA) content (nmoles/mg protein), a measure of lipid peroxidation, was assayed in liver, kidneys, heart, and brain as thiobarbituric acid-reacting substance (TBARS) following the method of Ohkawa et al. (1979). (c) Reduced glutathione (GSH) was estimated by the method of Morten et al. (1979), virtually the entire non-protein sulphydryl content of cells is in the form of reduced glutathione 5,5'-Dithio(2-nitrobenzoic acid) (DTNB) is a disulfide compound that is readily reduced by sulphydryl compounds forming a highly colored yellow anion, the optical density of this yellow substance is measured at 412 nm. (d) Ascorbic acid was measured by the method of Omave et al. (1979). Ascorbic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenyl hydrazine to form bis-2,4-dinitrophenyl hydrazine, this undergoes further rearrangement to form a product with an absorption maximum at 520 nm. Thiourea provides a reducing medium which helps to prevent interference from non-ascorbic acid chromogens. (e) Vitamin E was estimated by the method of Desai (1984). In this method, ferrous ions are reduced to ferrous ions in the presence of tocopherol, resulting in the formation of a pink-colored complex with bathophenanthroline. Orthophosphoric acid is added as a chelating agent to reduce carotene interference by preventing its oxidation and stabilization of color, by binding excess ferric ions, thus preventing their photochemical reduction. (f) The activity of catalase (CAT) was measured by the method of Sinha (1972). The principle of this method is that dichromate in acetic acid
is reduced to chromic acid when heated in the presence of H$_2$O$_2$, with the formation of pericholic acid as an unstable intermediate, the chromic acetate thus produced is measured at 570 nm. The activity of superoxide dismutase (SOD) was measured as the degree of inhibition of autooxidation of pyrogallol at an alkaline pH (Marklund and Marklund, 1974). Glutathione peroxidase (Gpx) was assayed by measuring the amount of reduced glutathione (GSH) consumed in the reaction mixture according to the method of Rotrucc et al. (1973).

2.4 Detection of isozymes by electrophoresis

Non-denaturing polyacrylamide gel electrophoresis (native-PAGE) was performed on samples of liver, kidney, heart and brain essentially as described by Laemmli (1970), except that SDS was omitted from all buffers and the samples were not boiled before electrophoresis. The enzymes were run on the basis of equal amounts of protein (70 μg) in a 10% gel for SOD and Gpx and 8% gel for CAT. Electrophoretic separation was performed at 4°C with a constant power supply of 50 V for stacking gel and 100 V for separating gel. Staining for the activity of each enzyme was performed separately as follows:

(i) CAT activity was detected by the method of Woodbury et al. (1971). Here, the gel was soaked in 5 mM H$_2$O$_2$ solution for 10 min and then washed with water and stained with a reaction mixture containing 1% potassium ferricyanide (w/v) and 1% ferric chloride. The enzyme appeared as a yellow band superimposed on a dark green background. The reaction was terminated by adding water and the gel was photographed at once.

(ii) SOD activity was identified by the method of Beaucamp and Fridovich (1971). The gel was soaked in 50 mM Tris HCl buffer (pH 8) containing 10 mg nitroblue tetrazolium (NBT), 1 mg ethylene diamine tetraacetic acid (EDTA) and 2 mg riboflavin (50 ml final volume), and kept in the dark for 30 min. The gel was then placed on an illuminated light box to locate the area of SOD activity, which appeared as a clear zone on a bluish-violet background.

(iii) Gpx isozymes were separated by the method of Lin et al. (2002). The gel was soaked in 50 ml of 50 mM Tris–HCl buffer (pH 8) containing 200 mg reduced glutathione, and 8 μl of 30% H$_2$O$_2$ for 20 min. The gel was then transferred to 50 ml of 50 mM Tris HCl buffer (pH 8) containing 25 mg NBT and 25 mg phenazine methosulphate (PMS). The appearance of white bands in the gel was taken to indicate the presence of Gpx isozymes.

2.5 Materials

Nitroblue tetrazolium salt, reduced glutathione, phenazine methosulphate and riboflavin were purchased from Himedia Laboratories Pvt Ltd, Mumbai, India. All other chemicals and solvents were obtained from Central Drug House Pvt Ltd, Mumbai, India.

2.6 Statistical analysis

The results obtained for each group of rats tested were expressed as the means ± SD of six values. Statistical analysis of the data was performed by Student’s t test.

3. Results

An increase in the mean MDA level, a measure of lipid peroxidation, was found in the liver, kidneys, heart and brain of aged untreated (Group II) rats relative to young (Group I) rats (Tables 1a and 1b). Treatment of aged (Group III) rats with extract of P. ostreatus was found to lower the mean MDA concentration so that it approached that of normal young rats, presumably by limiting lipid peroxidation in the tissues of the major organs.

In aged untreated rats (Group II) a significant decrease (relative to young rats) in the level of GSH was observed in the liver, kidneys, heart and brain tissues (Tables 1a and 1b). Administration of the extract of P. ostreatus to aged rats in Group III elevated GSH to levels that approximated the levels in young rats.

A significantly lower levels of vitamins E was observed in the liver, kidneys, heart and brain of aged untreated (Group II) rats, when compared to levels in normal young (Group I) rats (Tables 1a and 1b). A similar significantly lower concentration of vitamin C was noted in liver and heart (and a decrease in kidney and brain) of Group II rats, compared to levels in Group I rats. However, the levels of vitamins C and E in liver, kidneys, heart and brain of aged rats administered P. ostreatus extract (Group III) were found to be at near normal levels that is the levels found in young (Group I) rats (Tables 1a and 1b).

Significantly lower activities of CAT, SOD and Gpx enzymes were noted in the liver, kidneys, heart and brain of aged untreated (Group II) rats when compared to the values in young (Group I) rats (Tables 2a and 2b). In aged rats that had been administrated extract of P. ostreatus (Group III), the activities of these enzymes were maintained at near-lower levels, that is, there were no significant differences compared with the values noted in Group I rats (except in the activity of Gpx in the liver and kidney tissues).

3.1 Isozyme pattern of enzymes in liver, kidney, heart and brain

An analysis of the electrophoretic pattern of catalase isozymes in liver, kidney, heart and brain tissues...
Table 1a

Effect of extract of mushroom (*P* ostreatus) on malondialdehyde (MDA) reduced glutathione (GSH) and vitamins C and E in liver and kidney of young and aged rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tissues</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Young rats</td>
<td>Aged rats</td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>0.91 ± 0.02</td>
<td>1.32 ± 0.09 NS</td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td>10.31 ± 0.35</td>
<td>7.2 ± 0.48*</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>2.8 ± 0.15</td>
<td>1.89 ± 0.08*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>1.65 ± 0.15</td>
<td>1.22 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of six rats in each group

MDA: nmoles of MDA produced/mg protein; GSH: μg of reduced glutathione/mg protein; vitamins C and E: μg/mg protein

NS not significant

*p < 0.05

Table 1b

Effect of extract of mushroom (*P* ostreatus) on malondialdehyde (MDA) reduced glutathione (GSH) and vitamins C and E in heart and brain of young and aged rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tissues</th>
<th>Heart</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Young rats</td>
<td>Aged rats</td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>1.29 ± 0.35</td>
<td>1.48 ± 0.33 NS</td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td>12.10 ± 1.19</td>
<td>8.40 ± 1.45*</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>1.51 ± 0.40</td>
<td>1.01 ± 0.53*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>1.18 ± 0.02</td>
<td>0.986 ± 0.07</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of six rats in each group

MDA: nmoles of MDA produced/mg protein; GSH: μg of reduced glutathione/mg protein; vitamins C and E: μg/mg protein

NS not significant

*p < 0.05

Table 2a

Effect of extract of mushroom (*P* ostreatus) on catalase (CAT) superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver and kidney of young and aged rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tissues</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Young rats</td>
<td>Aged rats</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td>73.37 ± 4.44</td>
<td>39.66 ± 6.33*</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td>13.87 ± 2.27</td>
<td>9.8 ± 0.108*</td>
</tr>
<tr>
<td>GPx</td>
<td></td>
<td>62.36 ± 2.88</td>
<td>42.63 ± 0.90</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of six rats in each group

CAT: μmoles of H2O2 utilized/min/mg protein; SOD: U/mg protein; GPx: μmoles of GSH oxidized/mn/mg protein

NS not significant

*p < 0.05

(Fig 1a–c) revealed a single band with variations in the staining intensity of the band among the three groups of rats, the samples of liver, kidney, heart and brain of young (Group I) rats exhibited a single band of high intensity (band area 220, 223, 161 87 and 112 76, respectively), while those of Group III rats (aged rats treated with *P* ostreatus extract) exhibited a single band of almost equal intensity (band area 212 37, 221 87 159 91 and 109 87, respectively), however, homogenates of liver, kidneys, heart and brain of group II (aged untreated) rats revealed a single band of lower intensity (band area 189 12, 180 24, 135 09 and 98 54, respectively) (Fig 1a–c).

Two isoforms of SOD were detected in the liver, kidneys, heart and brain of all three groups of rats. Isoform
Table 2b
Effect of extract mushroom (P. ostreatus) on catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in heart and brain of young and aged rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tissues</th>
<th>Heart</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Young rats</td>
<td>Aged rats</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td>47.8 ± 4.09</td>
<td>39.98 ± 2.21</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td>11.55 ± 1.62</td>
<td>7.96 ± 1.79</td>
</tr>
<tr>
<td>GPx</td>
<td></td>
<td>51.97 ± 3.78</td>
<td>38.39 ± 2.63</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of six rats in each group.

CAT, μmoles of H₂O₂ utilized/min/mg protein. SOD, U/mg protein.
GPx, μmoles of GSH oxidized/min/mg protein.

NS, not significant.

* p < 0.05.

Five isoforms of the Gpx enzyme were noted in the liver of all three groups of rats. In rats of Group S I, II and III, the staining intensity of Gpx 1 was essentially similar (band area 175. 39, 171.74 and 173.17, respectively); similar phenomenon was noted for Gpx isoform 3, (band area 168.55, 164. 57 and 166.98, respectively) and Gpx isoform 45 (band area 156.71, 151.65 and 153.45, respectively) (Fig. 3a); however, the staining intensity of the Gpx 2 isoform was less in Group II (band area 110.09 and 96.13, respectively) rats than that in Group I (band area 124.88 and 117.60) and in Group III (band area 118.24 and 101.18) rats (Fig. 3a). Similarly, four isoforms of the Gpx enzyme were noted in kidney tissues of all three groups of rats.
Lipid peroxidation, a process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, only low levels of lipid peroxides occur in body tissues. The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation (Rikans and Hornbrook, 1997). MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage (Ohkawa et al., 1979). Ageing has been reported to be associated with increased disruption of membrane lipids leading to subsequent formation of peroxide radicals (Niki et al., 1993). In the present investigation, such a disruption of membrane lipids possibly accounted for the observed increase in MDA levels in the organs of aged (Group II) rats when compared to young (Group I) rats. In addition, insufficient levels of antioxidants to scavenge peroxo radicals during ageing (Sohal et al., 1990; Wei, 1998) could also have contributed to the elevated level of MDA in the aged rats. These data from the present investigation corroborate findings of earlier investigations (Arivazhagan and Panneerselvam, 2000; Balu et al., 2005; Augustyniak et al., 2005). Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species on lipids, DNA and proteins (Halliwell, 1996). The observed reduction in the MDA level in aged rats following administration of the mushroom extract is one indicator of the antioxidant activity of *P. ostreatus*.

Glutathione is a major, non-protein thiol in living organisms which performs a key role in co-ordinating the innate antioxidant defense mechanisms. It is involved in the maintenance of the normal structure and function of cells, probably by its redox and detoxification reactions (Gueeri, 1995). In the present study, level of GSH was found to be lower in aged (Group II) rats than in young (Group I) rats. Similar decreases in the levels of reduced glutathione in liver (Liu and Choi, 2000) and brain (Arockia Rani and Panneerselvam, 2001) of aged rats have also been reported. Anand et al. (1996) have suggested that lowered GSH levels may result from increased utilization of GSH by antioxidant enzymes such as Gpx, which scavenge H$_2$O$_2$. Interestingly, in the present study, when mushroom extract was administered to aged rats (Group III) the GSH level was found to be very similar to that in young (Group I) rats. This again strongly suggests the antioxidant potential of the extract of this mushroom. An increase in the levels of GSH in aged rats treated with grape seed extract as a source of antioxidant has also been reported (Balu et al., 2005).

Circulating antioxidants such as vitamins C and E are non-enzymatic scavengers of free radicals. Vitamin E reacts with lipid peroxo radicals, acting as a chain terminator of lipid peroxidation, and protects the cellular structures from attack by free radicals (Arivazhagan et al., 2000), while vitamin C facilitates the maintenance of vitamin E levels at optimum concentrations. A decrease in ascorbic acid level in leucocytes and in blood of rats with an increase in age has been reported by Jayachandran and Panneerselvam (1995). Decrease in vitamin E level of aged human platelets has also been reported (Verical et al., 1992). In the present study, significantly lower (relative to young rats) levels of vitamins C and E were recorded in aged rats; however, in aged rats that were treated with mushroom extract of *P. ostreatus*.
The levels of these vitamins were essentially similar to the levels in young rats. A similar increase in vitamins C and E levels in liver of aged rats treated with a potent antioxidant, green tea extract, has also been reported (Augustyniak et al. 2005).

Lung tissues are endowed with innate antioxidant defense mechanisms, including the enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx). CAT has been shown to be responsible for the detoxification of significant amounts of H₂O₂ (Cheng et al., 1981). SOD catalyzes the removal of superoxide radical O₂⁻, which would otherwise damage the membrane and biological structures. Gpx catalyzes the reduction of H₂O₂ to H₂O and O₂. A reduction in the activity of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Reedy and Lokes, 1992, Krishnananthi and Lokes, 1993, Sheela and Angusti, 1995). A reduction in the activity of these enzymes in aging has been well-documented (Barja de Quirgga et al., 1990, Matsuo et al., 1992, Kalasvelu and Panneerselvam, 1998). Anvazhagan et al. (2000), Balu et al. (2005), Valls et al. (2005). So also, in the present study, significantly lower activities of these enzymes were recorded in aged rats when compared to young rats. Administration of mushroom extract markedly elevated the levels of these antioxidant enzymes to that of young rats, indicating the antioxidant potential of the mushroom P. ostreatus.

Supplementation of the diet with fruits and vegetables is reported to be beneficial in reversing the deleterious effects of aging on neuronal communication and behavior (Joseph et al., 2005). Augustyniak et al. (2005) reported that the administration of green tea extract increased the activities of CAT, SOD, and Gpx in the liver of aged rats. Balu et al. (2005) reported that supplementation of the diet with grape seed extract could improve the antioxidant status in the central nervous system of aged rats. So also in the present investigation, administration of P. ostreatus extract appears to have brought about a remarkable improvement in the activity of antioxidant enzymes in aged rats.

The utilization of multiple isoforms of enzymes is believed to be one of the primary control mechanisms that regulate cellular metabolism (Sang et al., 2005). However, there is a paucity of data on the regulation of expression of the isoforms of antioxidant enzymes during oxidative stress, an aspect that deserves study. An attempt was made to evaluate the pattern of isoforms during aging and following supplementation with mushroom extract. Our studies on the electrophoretic patterns of antioxidant enzymes in liver, kidney, heart and brain revealed characteristic isoform patterns. In general, the number of bands and the staining intensity of isoforms was less in aged untreated (Group II) rats than in the two other groups, tissue-specific variations were also noted. Such a pattern of differential expression of enzyme isoforms has been noted in barley shoot and root exposed to saline stress (Sang et al., 2005). The modification in the pattern of enzyme isoforms during stress has been attributed to some shift in gene expression (El-baky et al., 2003). Interestingly in the present study, the decreased intensity of CAT and SOD2 isoform and complete absence of Gpx 2 and 3 isoforms in brain of Group II rats suggests that the brain tissue may be particularly vulnerable to oxidative stress. A possible reason for this could be that since the brain is rich in non-heme iron, it could be catalytically involved in the production of oxygen free radicals (Subbarao and Richardson, 1990). Another possible explanation is that the presence of unsaturated fatty acids in high concentrations makes the brain tissue a good substrate for the occurrence of peroxidation reactions (Ogawa, 1994).

The variation in the antioxidant enzyme isoform profile observed in the present investigation during aging may possibly be due to an alteration in gene expression. Since direct genomic action of melatonin has been well-documented (Becker-Andre et al., 1994, Wiesenberg et al., 1995, Steinhiller et al., 1995), supplementation with melatonin during aging has been proposed as a means of increasing the activity of antioxidant defense system (ADS) gene with a view to promoting the synthesis of antioxidant enzymes (Srinivasan, 1999). In the present study, a similar phenomenon possibly occurred, resulting in the increased intensity of staining of antioxidant enzyme isoforms following supplementation with mushroom extract.

In conclusion, administration of an extract of the oyster mushroom P. ostreatus appeared to protect the liver, kidneys, heart and brain of aged Wistar rats against oxidative stress by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymatic and non-enzymatic antioxidants. It remains to be seen whether a similar protective effect can be demonstrated in humans during the process of aging. An extract of this mushroom can be conveniently incorporated in the diet as a nutritional supplement, thereby augmenting the body’s defenses against oxidative stress.

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