ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIOCHEMISTRY

ANTIOXIDANT ACTIVITY AND NEPHROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF *PLEUROTUS EOUS* (BERK.) SACC.: (APK1) PINK EDIBLE OYSTER MUSHROOM

VADIVUKKARASI SASIKUMAR* AND SUDHA. G. MENON

PG and Research Department of Biochemistry, Centre for Biological science, K.S.R. College of Arts and Science, Thokkavadi, Tiruchengode, Tamilnadu, India 637215.
PG and Research Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore 641 029, Tamil Nadu, India.

VADIVUKKARASI SASIKUMAR
PG and Research Department of Biochemistry, Centre for Biological science, K.S.R. College of Arts and Science, Thokkavadi, Tiruchengode, Tamilnadu, India 637215.

ABSTRACT

Pleurotus eous is an edible oyster mushroom currently available in Southern part of India. There is no information about the antioxidant and therapeutic properties of this mushroom. P*leurotus eous* aqueous extract (PEAE) were prepared and analysed for their antioxidant activity by *in vitro* assays such as DPPH, hydroxyl radicals, Ferric Thiocyanate (FTC) and Thiobarbituric acid (TBA) test. The nephroprotective effect was determined by cisplatin administration which induces a marked renal failure in swiss albino mice and on extract treatment at 500 and 1000 mg/kg body weight, the destroyed renal cells were regenerated. The present study reveals the medicinal beneficial effects of this mushroom which have not been explored so far. The active components responsible for these activities have to be determined.

KEYWORDS

Pleurotus, Mushroom, DPPH, Hydroxyl radical, Nephroprotective.

Abbreviation

PEAE- *Pleurotus eous* aqueous extract,h-hours ,min-minutes

1. INTRODUCTION

Cisplatin (Cis diamine dichloroplatinum II) is a highly effective antineoplastic DNA alkylating agent used against a wide variety of cancers (Lynch *et al*., 2005). Higher doses of cisplatin in cancer treatment causes reversible and irreversible side effects including nephrotoxicity, neurotoxicity, bone marrow toxicity, gastrointestinal toxicity and ototoxicity often limit its utility and therapeutic profile (Lynch *et al*., 2005). Cisplatin induces free radical production causing oxidative renal damage, possibly due to depletion of nonenzymic and enzymic antioxidant systems. Some antioxidants have been tested for their ability to protect against cisplatin induced nephrotoxicity in experimental animals (Ajith *et al*., 2007).

Pleurotus eous (Berk.) Sacc is an important genus of edible basidiomycetes which are commonly called as oyster mushroom (Ragunathan *et al*., 1996). These mushrooms have attracted much attention because it's a good source for non-starchy carbohydrates, dietary fiber, moderate quantities of proteins with some essential amino acids, minerals and vitamins (Croan, 2004). Thus, the present manuscript addresses the evaluation of antioxidant properties of three harvesting stages (first day (PEAE1), second day (PEAE2), third day (PEAE3) for studying nephroprotective effects) purchased from Tamilnadu Agricultural University, Coimbatore.

2. MATERIALS AND METHODS

Chemicals

Cisplatin was purchased from Ramakrishna medical hospital, Coimbatore, India. 1, 1-diphenyl-2-picrylhydrazyl (DPPH•), linoleic acid was purchased from Sigma-Aldrich (Bangalore, India). Ferrous sulfate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), hydrogen peroxide, sodium salicylate, ammonium thiocyanate, trichloroacetic acid (TCA), thiobarbituric acid (TBA) and ferrous chloride were obtained from Himedia (Mumbai, India). All other reagents used were of analytical grade. (Sigma- Aldrich, Bangalore, India).

2.1 Extraction of the sample

The PE 1, PE 2 and PE 3 harvested for three consecutive days was dried and 10 g each were extracted by stirring with 100 ml of boiling water at 100°C for 4 hrs, centrifuged at 5000 g for 15 min and filtered through Whatmann No. 1 filter paper. The residue was then extracted twice with 100 ml portions of boiling water, as described above. The combined extracts were freeze dried and labelled as PEAE 1, PEAE 2 and PEAE 3 respectively. The dried extracts were stored at 4°C until analysis.

2.2 Invitro assays

2.2.1 Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

PEAE1-3 (0.5–2.5 mg/ml) was mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank (Chang, 1991). EC_{50} value (mg extract/ml) is the effective concentration at which DPPH radicals

were scavenged by 50% and was obtained by interpolation from linear regression analysis. BHT, BHA, and ascorbic acid were used for comparison.

2.2.3 Scavenging ability on hydroxyl radicals

Hydroxyl scavenger ability was measured according to the method of Croan, 2004. OH radicals were generated from $FeSO_4$ and hydrogen peroxide. The radical scavenging activity of PEAE1-3 was determined by their ability to hydroxylate salicylate. The reaction mixture (3 ml) contained 1 ml $FeSO_4$ (1.5mM), 0.7 ml hydrogen peroxide (6 mM), 0.3 ml sodium salicylate (20 mM) and varying concentrations of PEAE1-PEAE3. After incubation for 1 h at 37˚C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as scavenging activity = $[1-(A_1-A_2)/A_0]$ X 100%, Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without sodium salicylate.

2.2.4 Total antioxidant activity

2.2.4.1 Ferric Thiocyanate (FTC) and Thiobarbituric acid (TBA) test

FTC test was conducted according to the method described by Shimad *et al*., 1992. In this study, 4 mg of PEAE1-3 were individually dissolved in 4 ml of ethanol. Then, the extract solutions were mixed with 2.52% linoleic acid (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml) and distilled water (3.9 ml). These mixtures were then kept in screwcap containers at 40˚C in the dark. In order to determine the FTC values, 0.1 ml of these mixtures was respectively added into 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of ferrous chloride solution (in 3.5% HCl) to the reaction mixture, the absorbance of the samples was read at 500 nm by using a spectrophotometer. This procedure was repeated every 12 h until the control sample reached its maximum absorbance value.

TBA test (Mackeen *et al*., 2000) was also conducted after the control sample reached its maximum absorbance value in FTC. 1.0 ml of 20% TCA and 2.0 ml of 0.67% TBA were added to 2 ml of sample acquired from FTC. The sample was then placed in boiling water for 10 min. After cooling under the running tap water, the sample was centrifuged at 3000g for 30 min. Finally, the absorbance of supernatants at 532 nm was measured by using a spectrophotometer.

2.3 Nephroprotective effects

2.3.1 Test animals

Female Swiss albino mice of six weeks were purchased from Small Animal Breeding Centre, College of Veterinary and Animal Science, Mannuthy, Kerala, India. The animals were kept for a week under environmentally controlled conditions with free access to standard food (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. Mice weighing 25 ± 2 g were used for the experiments. All animal experiments were carried out according to the guidelines and approval of institutional animal ethic committee (IAEC), Government of India.

2.3.2 Experimental design

2.3.2.1 Cisplatin induced nephrotoxicity

Six groups of six animals in each group were selected for the study. Group I treated with (vehicle) distilled water (Normal control). Group II injected with a single dose of cisplatin (12 mg/kg body weight, i.p.) (Toxic control). Group III and IV were treated with PEAE 3 (500 and 1000 mg/kg body weight) and group V received vitamin E 250 mg/kg body weight. The PEAE3 and vitamin E were administrated orally 1 h before and 24 and 48 h after cisplatin injection. Seventy two hours after cisplatin injection, animals were anaesthetized with chloroform and sacrificed (Ajith *et al*., 2002).

2.3.2.2 Biochemical analysis

Kidneys were excised after sacrificing the animals and washed with ice-cold saline (0.89%) and 10% homogenate was prepared in phosphate buffer (0.05 M, pH 7) using a homogenizer at 4°C. Urea, γ-GT by kit method, Creatinine (Owen *et al*., 1954), uric acid (Caraway, 1963), TBARS (Niehius and Samuelsson, 1968) and hydroperoxides were assayed by the method of Jiang *et al*., 1992. The values are expressed as nmoles of TBARS formed/min/mg protein and mmoles/g tissue for hydroperoxides.

2.3.2.3 Determination of antioxidant status

Glutathione- S-transferase and reduced glutathione (GSH) in the tissue was determined by the method of Moron *et al*., 1979, Vitamin C (Omaye *et al*., 1979), vitamin E (Varley, 1976) Glutathione peroxidase (GPx) activity was determined by the method of Ellman *et al*., 1959. Superoxide dismutase (SOD) activity was assayed according to the method of Das *et al*., 2000 and catalase (CAT) by the method of (Sinha, 1972). The protein was estimated by the method of Lowry *et al*., 1957.

2.3.2.4 Histopathological examination

Small piece of kidney from each group were preserved in 10% formalin and stained with Ehrlich's hematoxylin. The sections were evaluated for the pathological symptoms of nephrotoxicity.

3. Statistical analysis

The data were expressed as mean ± S.D from triplicate determination. Statistical differences at $p<0.05$ between the stages were analyzed by ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS 10.0 software.

4. RESULTS

4.1 Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Table 1 depicts the radical scavenging activity of PEAE extracts. PEAE3 showed higher radical scavenging activity followed by PEAE1 and PEAE2. EC_{50} values were 1.4, 1.24, 1.35 mg/ml (50% inhibition) respectively. In comparison, at 0.2 mg/ml, scavenging abilities of BHA, BHT and ascorbic acid were 86.88 %, 98.08 % and 98.09 % respectively (Data not shown). These results clearly indicated that PEAE3 had effective and powerful antioxidant activity.

Table 1
Scavenging activity (%) of DPPH radical of aqueous extract of Pleurotus eous

Extracts	Sample concentration (mg/ml)				
	0.5	1.0	1.5	2.0	2.5
PEAE1	22.93 ± 0.55^{ax}	43.74 ± 1.43^{bx}	64.33 ± 1.59^{cx}	78.24 ± 3.52^{dx}	83.76 ± 2.08^{ex}
PEAE2	24.09 ± 1.23^{ay}	35.77 ± 1.18^{by}	54.88 ± 1.35^{cy}	77.07 ± 3.86^{dx}	80.68 ± 1.52^{dy}
PEAE3	22.33 ± 1.10^{ax}	35.87 ± 0.88^{by}	55.03 ± 1.10^{cy}	77.25 ± 2.32^{dx}	84.76 ± 1.68^{ex}

4.2 Hydroxyl radical scavenging activity

The ability of PEAE1-3 to scavenge hydroxyl radicals was shown in Table 2. The EC_{50} values were found to be 2.8, 2.7, 2.2 mg/ml (50% inhibition) respectively. The scavenging power of PEAE1-3 steadily increased with increasing sample concentration. In comparison, PEAE 3 had a greater tendency to scavenge hydroxyl radical.

Table 2
Hydroxyl radical scavenging activity (%) of aqueous extract of Pleurotus eous

Extracts	Sample concentration (mg/ml)				
	2	4	6	8	10
PEAE1	16.22 ± 0.39^{ax}	34.12 ± 1.66^{bx}	53.38 ± 2.15^{cx}	70.27 ± 1.79^{dx}	78.04 ± 2.93^{ex}
PEAE2	30.74 ± 1.35^{ay}	41.55 ± 1.05^{by}	57.43 ± 1.76^{cy}	67.91 ± 3.40^{dy}	82.09 ± 2.09^{ey}
PEAE3	37.64 ± 0.03^{az}	49.45 ± 1.40^{bz}	54.61 ± 1.32^{cx}	68.63 ± 1.80^{dy}	83.39 ± 2.10^{ey}

Values are expressed as mean ± SD (n =3). Means within the same row with different letters (a-e) and means within thesame column with different letters (x-z) not sharing common superscript letters differ significantly at 5% level by DMRT.

4.3 Total antioxidant activity (FTC and TBA tests)

Figure 1 exemplifies the effects of different concentrations (0.5–2.0g/mL) of PEAE1-3 on lipid peroxidation of linoleic acid emulsion. These results clearly indicated PEAE 3 had effective and powerful antioxidant activity.

Figure 2 summarize the effects of different concentrations (0.5–2.0g/mL) of PEAE1-3 on reducing ability using the potassium ferricyanide reduction method. PEAE 2 had effective reducing power when compared to others.

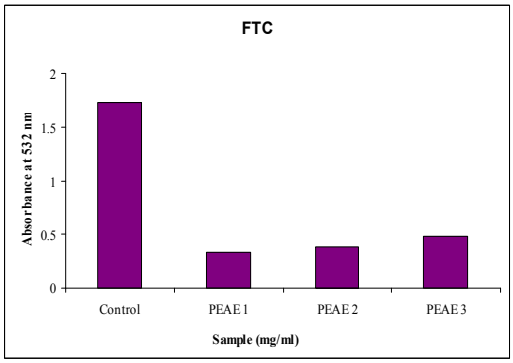


Fig. 1
FTC assay

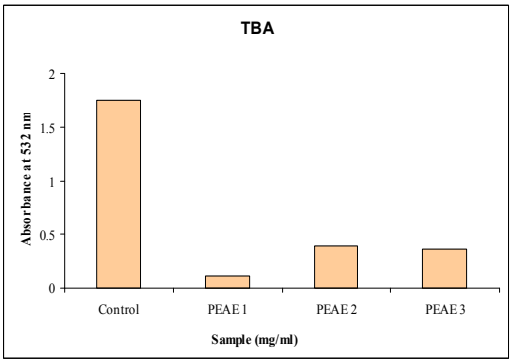


Fig. 2
TBA inhibitory activity

4.4 Nephroprotective effects

4.4.1. Biochemical analysis

The activity of PEAE3 on the levels of serum biochemical constituents in cisplatin treated mice was represented in figure 3 and 4. Cisplatin induced animals showed an elevated levels of urea, creatinine, uric acid coupled with decreased levels of protein. Treatment with two different doses (500 and 1000 mg/kg b.wt.) of PEAE3 and vitamin E altered all the biochemical constituents to near normal levels indicating its significant ($p<0.05$) nephroprotective effects. The experimental results revealed that the nephroprotective

activity of the PEAE (1000 mg/kg b.wt.) is comparable to that of vitamin E (250 mg/kg b.wt.).

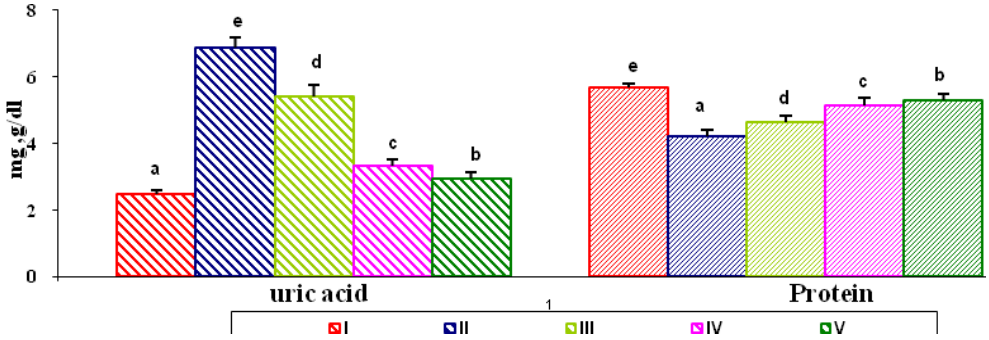


Fig.3

Effect of PEAE3 on the levels of uric acid and protein in serum on cisplatin induced nephrotoxicity.

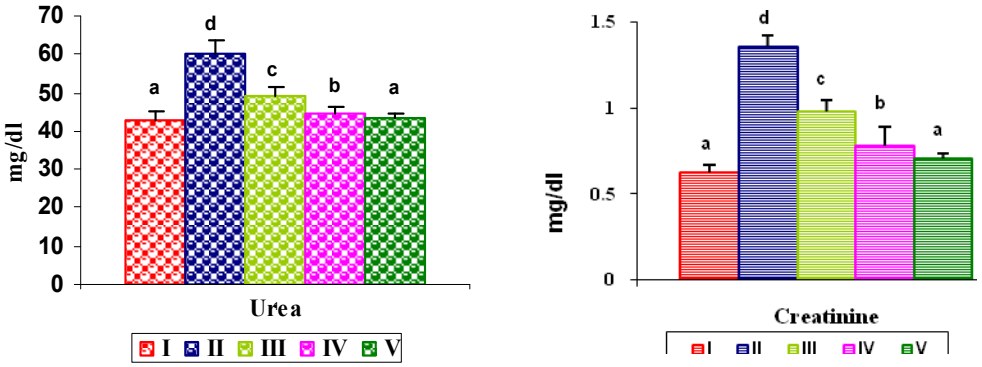


Fig.4

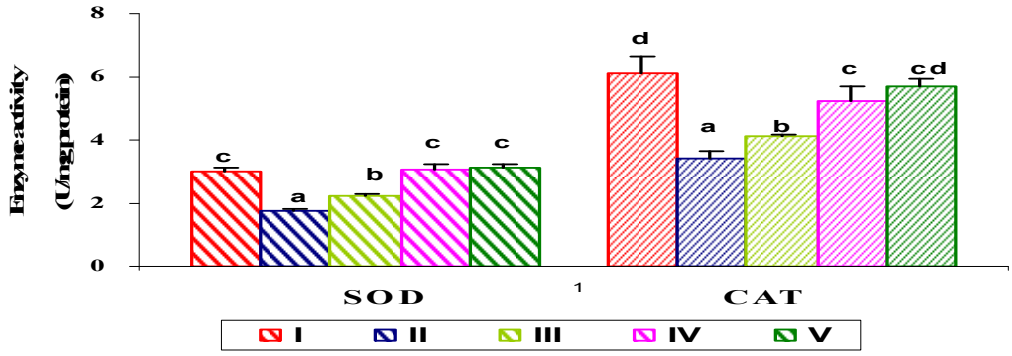
Effect of PEAE3 on the levels of urea and creatinine in serum on cisplatin induced nephrotoxicity.

4.4.2. TBARS and hydroperoxides

The changes in the levels of TBARS and hydroperoxides in control and experimental animals are depicted in figure 5. Cisplatin administration produced a two fold increase in the levels of TBARS and hydroperoxides, as compared to control rats. The levels of the TBARS and hydroperoxides were significantly ($p<0.05$) reversed on treatment with the PEAE3 in a dose dependent manner. The inhibition of lipid peroxidation at the dose of 1000 mg/kg was comparable to that of reference drug vitamin E.

Fig.5

Effect of PEAE3 on the levels of TBARS and hydroperoxides in kidney on cisplatin induced nephrotoxicity.



4.4.3. Determination of antioxidant status

The activities of renal CAT, SOD (figure 6) and GPx, GST (table 3) in the cisplatin and PEAE3 treated group of animals. Renal enzymic antioxidants was decreased significantly ($p<0.05$) in the cisplatin treated group of animals compared to normal group. Treatment with the PEAE3 (500 and 1000 mg/kg b.wt) prevented the drug induced decline of all the enzyme activities. The PEAE (1000 mg/kg b.wt) and vitamin E showed restoration of all the enzyme activities to near normal.

Figure 7 represents the levels of GSH and vitamin C in the kidney of control and experimental groups of mice. A significant ($p<0.05$) decrease in nonenzymic antioxidant levels was observed in cisplatin treated group. The non-enzymic antioxidant levels in the mushroom treated group at a dose of 500 and 1000 mg/kg b.wt were significantly higher than the cisplatin treated group. Administration of the PEAE3 (1000 mg/kg b.wt) showed a better nephroprotective activity than the 500 mg/kg b.wt dose. The effect of the reference drug vitamin E on nonenzymic antioxidant levels was also reversed to near normal.

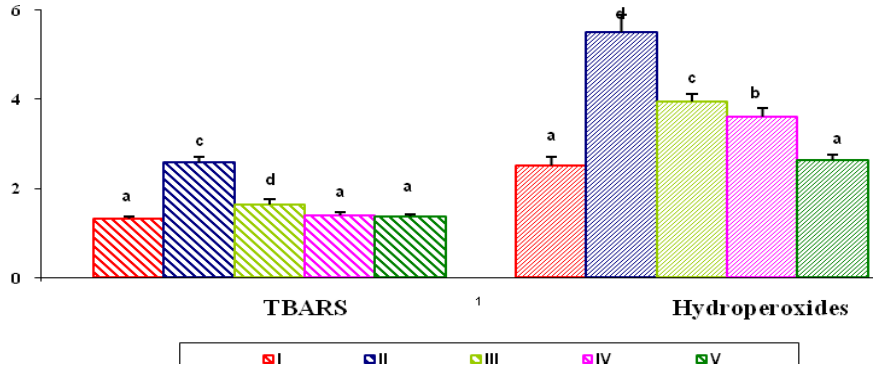


Fig.6

Effect of PEAE3 on the activities of SOD and CAT in kidney on cisplatin induced nephrotoxicity.

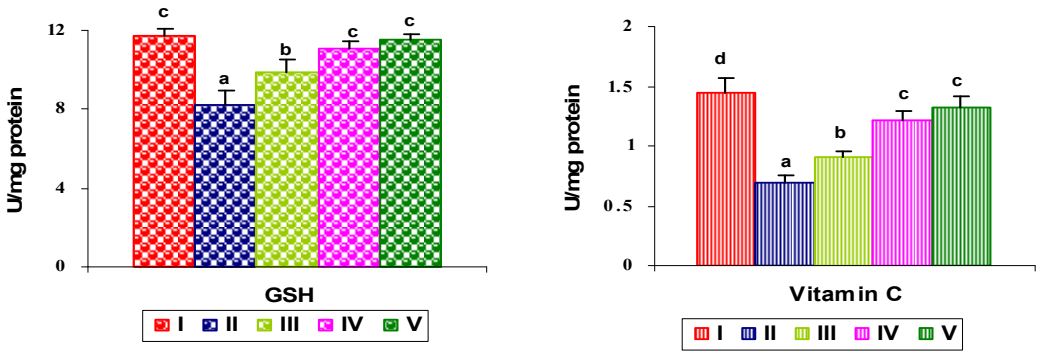


Fig.7
Effect of PEAE3 on the GSH and vitamin C in kidney on cisplatin induced nephrotoxicity.

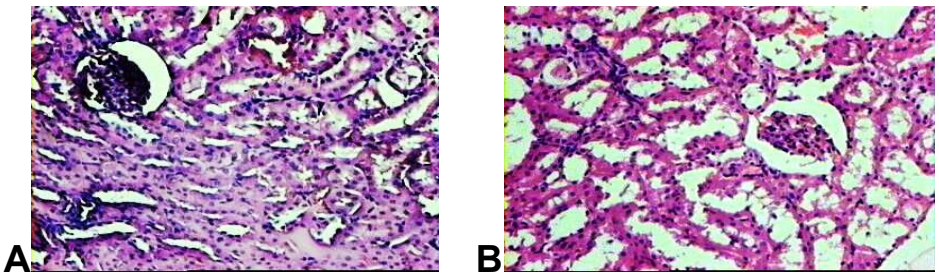
Table 3
Effect of PEAE3 on the activities of kidney enzymic antioxidants in cisplatin treated mice

Parameters	Normal control	CP (12)	PEAE (500)+ CP	PEAE (1000) + CP	Vit E (250) + CP
GST	83.94 ± 0.87^{c}	70.23 ± 0.35^{a}	78.74 ± 3.06^{b}	82.69 ± 4.46^{c}	83.51 ± 2.42^{c}
GPx	2.01 ± 0.04^{d}	1.28 ± 0.08^{a}	1.69 ± 0.07^{b}	1.85 ± 0.04^{c}	1.94 ± 0.07^{d}

Values are expressed as mean ± SD, n=6. Values within the same row not sharing common superscript letters (a-e) differ significantly at p<0.05 by DMRT. Units: GPx-µmol of glutathione oxidized/min/mg protein; GR - µmole of glutathione utilized/min/ mg protein; GST - µmole of CDNB-GSH conjugate formed/min/mg protein.

4.4.5. Histopathological studies

The photomicrograph showing the protective effect of PEAE3 against histopathological alterations induced by cisplatin in the kidney tissue of different experimental groups (Figure 8). **5.**



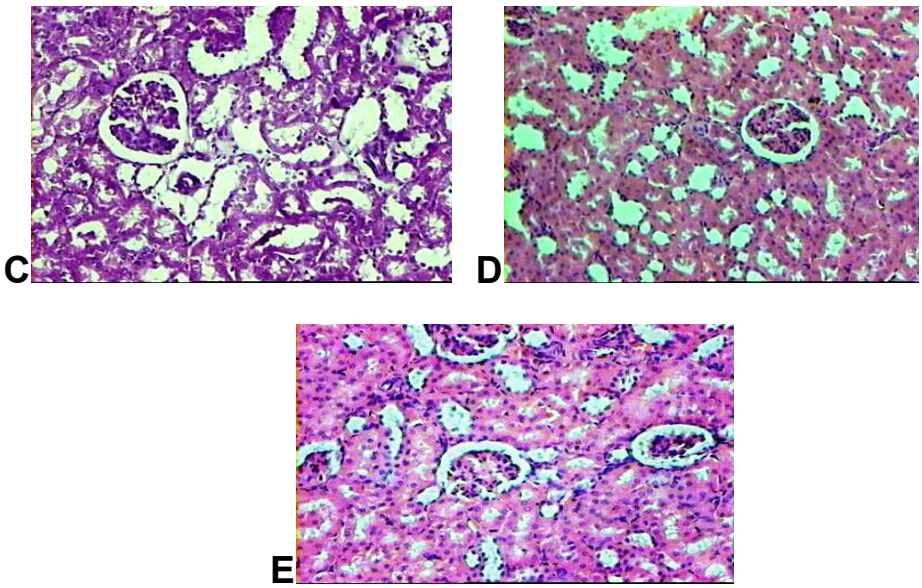


Fig.8. (A) Histological structure of normal control showed normal glomeruli, proximal and distal convoluted tubules, interstitium and blood vessels. (B) CP control showed normal glomeruli, distal convulated tubules and blood vessels. The proximal convoluted tubules showed protein casts and severe sloughing of tubular epithelial cells into the lumen. The interstitium showed mild edema. (C) In PEAE3 (500 mg/kg b.wt) treated, the proximal convoluted tubules showed protein casts and minimal sloughing of epithelial cells into the lumen. The distal convoluted tubules and blood vessels appear normal, the interstitium showed mild edema. This indicates mild improvement in histopathological changes. (D) PEAE3 1000 mg/kg b.wt showed normal glomeruli and protein casts at proximal convoluted tubules but there is no evidence of sloughing of epithelial cells into the lumen. The distal convoluted tubules and blood vessels appear normal. The interstitium showed mild edema (E) Vitamin E treated mice showed normal glomeruli. The proximal convoluted tubules do not show protein casts or sloughing of epithelial cells into the lumen. The distal convoluted tubules, interstitium and blood vessels appear normal.

DISCUSSION

There is continuing interest on the screening of medicinal plants with a view to determine new sources of natural antioxidants (Kahkonen *et al*., 1999). Many types of antioxidants with different functions play their role in the defense network. It is also evident that the antioxidant supplementation helps in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (Rose *et al*., 1982). The better scavenging ability of PEAE might be due to hydrogen donating components extracted from PEAE. The above results revealed PEAE were effective free radical scavengers, acting possibly as primary antioxidants.

Natural antioxidant can be used to replace the synthetic antioxidant in the food industry such as BHT, BHA and TBHQ, which may possess mutagenic activity (Skrinjar *et al*., 2007). With the radical scavenging activity, consumption of mushrooms might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses.

Hydroxy radicals are known to be capable of abstracting hydrogen atoms from membrane and bring about peroxide reactions of lipids (Lo, 2005). Overall the scavenging might be due to the active hydrogen donating ability of hydroxyl substitutions.

The trend of thiobarbituric acid reactive substances inhibitory activity of PEAE1, PEAE2 and PEAE3 is rather similar to the trend of FTC test in this study. This suggests that reduction of thiobarbituric acid reactive substances in the PEAE might due to the lower hydroperoxides accumulation in the respective samples, previously. Besides, secondary antioxidant compounds that might be present in these PEAE may also contribute to the

inhibition of hydroperoxides decomposition in these samples. PEAE exhibited *in vitro* antioxidant activity by inhibiting the oxidation of linoleic acid in both FTC and TBA methods.

Kidney represents the major control system maintaining body homeostatsis. Serum creatinine and urea are useful biomarkers in evaluating the extent of kidney damage (Levey *et al*., 1999). Total protein concentration is likely to be decreased if there is inhibition of protein synthesis or if degradation of protein is promoted (Heidenreich *et al*., 1999). Serum biochemical parameters such as urea and creatinine were found to be elevated significantly after cisplatin administration, clearly indicating renal impairment. (Ravi *et al*., 1995)

GST and GPx are GSH dependent antioxidant enzymes. The reduction observed in the activities of GPx and GST accompanied with cisplatin induced injury might be due to decreased availability of its substrate, reduced glutathione (Karthikeyan *et al*., 2007). GST catalyses the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids, as well as the metabolism of xenobiotics (Valavanidisa *et al*., 2006). The decreased activity of CAT and GPx resulted in decreased ability of the kidney to scavenge toxic H_2O_2 and lipid peroxides. Restoration of renal SOD, CAT and GPx activities by treatment with mushroom extract suggests that the extract is capable of protecting the enzymes after cisplatin administration. GSH acts as a non-enzymic antioxidant that reduces H_2O_2, hydroperoxides (ROOH) and xenobiotic toxicity (Kadiska *et al*., 2000). Cisplatin can cause the generation of oxygen free radicals, such as hydrogen peroxide, superoxide anions and hydroxyl radicals. The hydroxyl radical is capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. These radicals can evoke extensive tissue damage, reacting with macromolecules, such as membrane lipids, proteins and nucleic acids (Conklin and Nicolson, 2008).

In conclusion, the results of the present study indicate that the PEAE (1000 mg/kg b.wt) possesses profound nephroprotective activity. The experimental results also reveal that the nephroprotective activity of the extract is comparable to that of vitamin E. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the aqueous extract of mushroom, a novel therapeutically useful nephroprotective agent.

It was shown that β-glucan from maitake (*Grifola frondosa*) also possesses the nephroprotective effects against cisplatin induced nephrotoxicity, which supports our study (Masuda *et al*., 2009).

6. CONCLUSION

Our results concluded that P*leurotus eous* possesses antioxidant and nephroprotective effects. P*leurotus eous* can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals.

7. ACKNOWLEDGEMENTS

The author thanks The management of kongunadu arts and Science College, KSR college of Arts and Science, Tiruchengode, India for their encouragement. The author also thanks Dr.P.Lakshman, Professor, Department of Plant Pathology, Tamilnadu Agricultural University, Coimbatore for his support during the period of work.

REFERENCES

1. Ajith, T.A., Jose, N. and Janardhanan, K.K. 2002. Amelioration of cisplatin induced nephrotoxicity in mice by ethyl acetate extract of a polypore fungus, *Phellinus rimosus*. J. Exp. Clin. Cancer. Res. 21, 213-217.
2. Ajith, T.A., Usha, S. and Nivitha, V. 2007. Ascorbic acid and α-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice: a comparative study. Clin. Chim. Acta. 375, 82-86.
3. Caraway, W.T. 1963. Uric acid. In: Seligson, D. (Ed.), Standard methods of Clinical Chemistry. Academic Press, New York. 4, 239-247.
4. Chang, S.T. (1991). Cultivated mushrooms. Handbook of Applied Mycology. Marcel Dekker. New York, 3: 221-240.
5. Conklin, K.A. and Nicolson, G.L. 2008. Molecular replacement in cancer therapy: reversing cancer metabolic and mitochondrial dysfunction, fatigue and the adverse effects of cancer therapy. Curr. Cancer Ther. Rev. 4, 66-76.
6. Croan, S.C. (2004). Conversion of conifer wastes into edible and medicinal mushrooms. Forest Products Journal, 54: 68-76.
7. Das, S., Vasight, S., Snehlata, R., Das, N. and Srivastava, L.M. 2000. Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. Curr. Sci. 78, 486-487.
8. Ellman, G.C. 1959. Tissue sulfhydryl groups. Archi. Biochem. Biophys. 82, 70-77.
9. Heidenreich, O., Neininger, A., Schratt, G., Zinck, R., Cahill, M.A. and Engel, K. 1999. MAPKAP kinase 2 phosphorylates serum response factor *in vitro* and *in vivo*. J. Biol. Chem. 274, 14434 -14443.
10. Jiang, Z. Y., Hunt, J.V. and Wolft, S. D. 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem. 202, 384-389.
11. Kadiska, M.B., Gladen, B.C. and Baird, D.D. 2000. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl_4 poisoning? Free Radic. Biol. Med. 28, 838-845.
12. Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S and *et al*. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47:3954–62.
13. Karthikeyan, K., Sarala Bai, B.R. and Niranjali Devaraj, S. 2007. Cardioprotective effect of grape seed proanthocyanidins on isoproterenol induced myocardial injury in rats. Int. J. Cardiol. 115, 326-333.
14. Lo, S.H. (2005). Quality evaluation of *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* and their antioxidant properties during post harvest storage. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.
15. Lowry, O.H., Roseobrough, N.J., Farr, A.L. and Randall, R.J. 1957. Protein measurement with the Folin's phenol reagent. J. Biol. Chem. 193, 265-275.
16. Lynch, E.D., Gu, R., Pierce, C. and Kil, J. 2005. Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. Hear. Res. 201, 81-89.
17. Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z. and Amran, M. (2000). Antimicrobial, antioxidant, antitumor promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff ex. T. Anders. J. Ethnopharmacol. 72: 395-402.
18. Masuda, Y., Inoue, M., Miyata, A., Mizuno, S. and Nanba, H. 2009. Maitake β-glucan enhances therapeutic effect and reduces myelosupression and nephrotoxicity of

cisplatin in mice. Int. Immunopharmacol. 9, 620-626.
19. Moron, M.S., Defierre, J.W. and Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem. Biophys. Acta. 582, 67-68.
20. Niehius, W.G. and Samuelsson, D. 1968. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem. 6, 126-130.
21. Omaye, S.T., Turnball, T.D. and Sauberlich, H.E. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods. Enzymol. 62, 1-11.
22. Owen, J.A., Iggo, J.B., Scandrett, F.J. and Stemart, J.P. 1954. Determination of creatinine in plasma or serum, a critical examination. Biochem. J. 58, 426-431.
23. Ragunathan, R., Gurusamy, R., Palaniswamy, M. and Swaminathan, K. (1996). Cultivation of *Pleurotus* spp. on various agro residues. Food Chem. 55: 139-144.
24. Ravi, R.S., Somini, S.M. and Rybak, L.P. 1995. Mechanism of cisplatin ototoxicity. Antioxidant system. Pharmacol. Toxicol. 76, 386-394.
25. Rose, W.M., Creighton, M.O., Stewart, D.H.P.J., Sanwal, M., Trevithick, G.R., 1982. In vivo effects of vitamin E on cataractogenesis in diabetic rats. Can. J. Ophtalmol.17, 61.
26. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
27. Sinha, A. K. 1972. Colorimetric assay of catalase. Anal. Biochem. 47, 389-394.
28. Skrinjar, M., Kolar, M.H., Jelśek, N., Hras, A.R., Bezjak, M. and Knez, Z. (2007). Application of HPLC with electrochemical detection for the determination of low levels of antioxidants. J. Food Comp. Anal. 20: 539-545.
29. Valavanidisa, A., Vlahogiannia, T., Dassenakisb, M. and Scoullosb, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178 - 189.
30. Varley, H. 1976. Practical Clinical Biochemistry, Arnold-Heinemann Publishers Pvt. Ltd., 4, 52.