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Oyster mushroom extract protects antioxidant defence system in cisplatin induced nephrotoxicity in mice

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SUMMARY

Cisplatin is a highly effective and extensively used anticancer drug. Higher doses of cisplatin manifest acute nephrotoxicity and this is one of the limiting factors of this drug in cancer chemotherapy. The effect of the oyster mushroom extract to ameliorate cisplatin (*cis* platinum (II) diammine dichloride) induced nephrotoxicity and restoration of antioxidant defence system in mice was investigated. The investigations showed that prior administration of methanolic extract of *Pleurotus florida* at a dose of 500 and 1000mg/Kg body weight significantly reduced elevated serum creatinine and urea levels and increased superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in the kidney, consequent to cisplatin treatment, in a dose dependent manner. The extract restored the decreased reduced glutathione (GSH) activity and increased malondialdehyde (MDA) level due to cisplatin administration. The results thus indicated that oyster mushroom extract rendered significant protection against cisplatin induced nephrotoxicity and depletion of antioxidant defence system in a dose dependent manner. Since oyster mushrooms are excellently edible and non-toxic, the finding reported here is of significant use in cancer chemotherapy.

Key words: Medicinal mushroom; Oyster mushroom; *Pleurotus florida*; Antioxidant defence; Nephroprotection

INTRODUCTION

Cisplatin (*Cis* platinum (II) diammine dichloride) is a highly effective and extensively used anticancer drug against a variety of cancers. Higher doses of cisplatin are more efficacious for the treatment. However high dose chemotherapy of this drug manifests acute nephrotoxicity, ototoxicity, and other toxicities (Bodenner *et al*.,1986; Hamers, 1993). This deleterious side effect is a limiting factor for the use of this anticancer drug. A number of chemotherapeutic agents have been reported to render protection against cisplatin induced nephrotoxicity (Tognella, 1990). However, none of them is known to be clinically effective as a complete protective agent. Several lines of evidence suggest that free radicals are involved in the nephrotoxicity of cisplatin and the damage is the consequence of decreased renal antioxidant enzyme activity with the enhanced lipid peroxidation (Ajith *et al*., 2002). Administration of antioxidants has been shown to ameliorate cisplatin induced nephrotoxicity in animals (Babu *et al*., 1995).

Medicines of fungal origin have a notable place among the therapeutic agents. Mushrooms are macrofungi. The medicinal properties of mushrooms have long been recognized by oriental cultures and folklore. Oyster mushrooms (*Pleurotus* species) are excellently edible and commercially cultivated mushrooms in many parts of the world. *Pleurotus* species have been reported to possess hypoglycemic, antithrombotic, hypotensive, hypolipidemic, antitumor and immunomodulatory activities (Chang, 1996, Jose *et al*., 2002).

We have earlier reported that oyster mushroom, *Pleurotus florida* Eger. possessed potent *in vitro* free

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radical scavenging activity (Jose and Janardhanan, 2000). Since cisplatin chemotherapy resulted in significant inhibition of renal antioxidant activity, we considered it desirable to evaluate the effect of this culinary medicinal mushroom extract to ameliorate cisplatin induced nephrotoxicity.

MATERIALS AND METHODS

Animals

Male Swiss albino mice of age 6-7 weeks (25 ± 2 g) were used for the studies. The animals were maintained under environmentally controlled conditions with free access to standard food and water according to the guidelines recommended by Animal Ethical Committee.

Preparation of the extract

Fruit bodies of *Pleurotus florida* were obtained from the mushroom cultivation and demonstration unit of Integrated Rural Technology Centre (IRTC), Palakkad, Kerala, India.The mushrooms were dried at 40-50^{o}C and powdered. The powdered material (200 g) was defatted with petroleum ether and then extracted with methanol in a Soxhlet apparatus for 8-10 h. The methanol extract was evaporated to dryness under vacuum. The residue thus obtained (2.15 g) was dissolved in saline and used for the studies.

Chemicals

Reduced glutathione (GSH), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), Diacetyl monoxime (DAM) were purchased from Sisco Research Lab,Pvt. Ltd Mumbai. Cisplatin was purchased from Dabur India Ltd, New Delhi. All other chemicals and reagents used in the studies were of analytical grade.

Cell line

Daltons lymphoma ascites (DLA) cell line was obtained from Cancer Institute, Adayar, Chennai and maintained in mice in our laboratory.

Cisplatin induced nephrotoxicity

Animals were divided into 4 groups of six animals each. Group I (normal) administered with normal saline intraperitoneally (i.p). Group II was given 16 mg/Kg cisplatin i.p (16 ml/Kg). Group III and IV were given 500 mg/Kg and 1000 mg/Kg body weight methanolic extract of *P. florida* in normal saline (i.p) 30 min before the injection of cisplatin (16 mg/kg body weight, i.p). Mice of all groups were sacrificed 3 days after the treatments under diethyl ether anesthesia (Somani *et al*., 2000). The blood from each of them was collected and serum was separated for creatinine (Brod and Sirota, 1980) and urea (Marshell *et al*., 1980) analyses. The kidneys of the animal were dissected and immediately stored at -70^{o}C until analysis were completed. The kidneys were homogenized in 50 mM Phosphate buffer (pH -7) to give a 10% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0^{o}C and the supernatant was used for superoxide dismutase (SOD) (Mc Cord and Fridovich, 1969), catalase (CAT) (Aebi, 1974), glutathione peroxidase (GPx) (Hafemann *et al*., 1974) reduced glutathione (GSH) (Moron and De Pierre, 1979), malondialdehyde (MDA) (Ohkawa *et al*., 1979) and protein (Lowry *et al*., 1951) determinations.

Effect of pretreatment of *P. florida* extract on antitumor activity of cisplatin

Efficacy of cisplatin on antitumor activity in concomitant administration of methanolic extract of *P.florida* was determined. Animals were injected with 1×10^{6} viable cells of Daltons Lymphoma Ascites (DLA) in phosphate buffered saline in the right groin and divided into 3 groups of 6 animals each. After 24 hr of tumor implantation, animals were treated as follows; Group 1 treated with saline intraperitonealy was kept as control, group 2 treated with *P.florida* (1000 mg/Kg, i.p) 1h before cisplatin injection (3 mg/Kg body weight i.p) and group 3 was given cisplatin injection (3 mg/Kg body weight i.p). The treatment continued for 10 consecutive days. At the end of 5th week, the animals were sacrificed and tumors extirpated and weighed. The percent inhibition was calculated using the formula $(1\text{-}B/A)\times 100$ where, A is average tumor weight of the control group and B is that of treated.

Phytochemical analysis

Preliminary chemical examination of the extract was carried out to ascertain the major chemical

constituents. The extract was subjected to anthrone test(Yemm and Wills, 1954) and also for phenol-sulphuric acid reaction (Dubois *et al.*, 1956) to determine polysaccharide component of the extract and the protein content was detected by Lowry-Folin test (Lowry *et al.*, 1951). A portion of the extract was dissolved in a small quantity of methanol and subjected to TLC on silica gel G using chloroform: methanol (90:10) as solvent system. The spots were identified by examining under U.V and spraying with specific reagents (Harbone, 1973).

Statistical analysis

Experimental data were expressed as mean ± SD. Student's *t* test was applied for expressing the significance and *P* value less than 0.05 was considered as significant.

RESULTS

Serum creatinine levels registered a seven fold increase in cisplatin treated mice compared to normal group. Administration of methanolic extract of *P.florida* (500 and 1000 mg/Kg body weight) significantly reduced the increase in serum creatinine levels to almost normal (Table 1). Serum urea level also increased over five fold in cisplatin treated group than the normal. The methanolic extract of *P.florida* (500 and 1000 mg/Kg body weight) significantly reduced the increase in serum urea level in a dose dependent manner (Table 1).

Superoxide dismutase, catalase and glutathione peroxidase activities in the kidney significantly decreased (45, 72 and 79%) in cisplatin treated mice compared to the normal. Administration of methanolic extract of *P.florida* (500 and 1000 mg/Kg body weight) prior to cisplatin treatment significantly enhanced these enzyme activities compared to cisplatin alone (Fig. 1, 2 and 3).

The concentration of renal reduced GSH was significantly decreased (47%) in cisplatin treated group compared to normal. Administration of methanolic extract of *P.florida* (500 and 1000 mg/Kg body weight) prior to cisplatin treatment could increase the concentration of GSH significantly (Table 2).

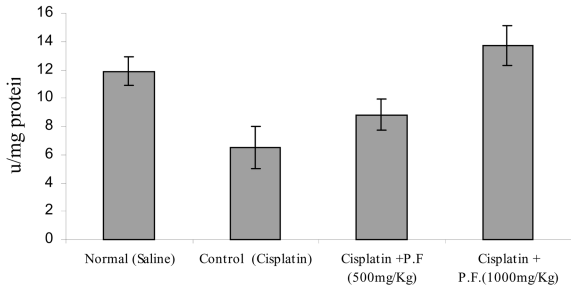

Fig. 1. Super oxide dismutase in normal, cisplatin treated, cisplatin plus *Pleurotus florida* (P.F) (500 and 1000 mg/kg) treated mice in the kidney 3 days after cisplatin administration. Values are expressed as mean ± SD (n=6, 4, 6, 6 respectively). $^{*}P<0.001$ with respect to normal, $^{**}P<0.05$ and $^{***}P<0.001$ with respect to control.

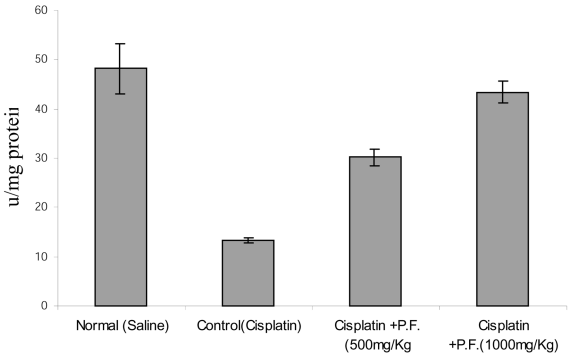

Fig. 2. Catalase activity in normal, cisplatin treated, cisplatin plus *Pleurotus florida* (P.F) (500 and 1000 mg/kg) treated mice in the kidney 3 days after cisplatin administartion. Values are expressed as mea n ±SD (n =6, 4, 6 ,6 respectively). $^{*}P<0.001$ with respect to normal and $^{**}P<0.001$ with respect to control.

Table 1. Levels of urea and creatinine in the serum of mice

Groups	Serum urea (mg/dl)	Serum Creatinine (mg/dl)
Normal(Saline)	61.8 ± 4.6	0.41 ± 0.11
Control(Cisplatin)	370.4 ± 29.2[a]	3.48 ± 1.40[a]
Cisplatin +*P.florida* (500 mg/Kg)	99.0 ±9.4[b]	0.77 ± 0.07[b]
Cisplatin +*P.florida* (1000 mg/Kg)	70.5 ± 14.2[b]	0.50 ± 0.10[b]

Values are mean ± SD (n = 4 for control (see text) and n = 6 for other groups),
[a]$P<$ 0.001 vs normal [b]$P<$0.001vs Control

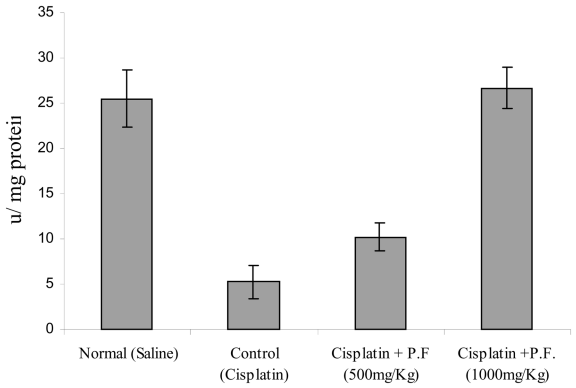

Fig. 3. Glutathione peroxidase in normal, cisplatin treated, cisplatin plus *Pleurotus florida* (P.F) (500 and 1000 mg/kg) treated mice in the kidney 3 days after cisplatin administartion. Values are expressed as mean ± SD (n=6, 4, 6, 6 respectively). $^{*}P<0.001$ with respect to normal, $^{**}P<0.005$ and $^{***}P<0.001$ with respect to control.

The concentration of renal malondialdehyde significantly increased (48%) in cisplatin treated group compared to control. The cisplatin treatment after the administration of methanolic extract of *P.florida* (500 and 1000 mg/Kg body weight) registered a significant decrease in malondialdehyde levels compared to the cisplatin alone (Table 2).

Administration of methanol extract of *P. florida* prior (30 min) to the injection of cisplatin did not interfere with the tumor reducing activity of cisplatin. Cisplatin plus methanol extract and cisplatin alone reduced the tumor development by 98% and 95% respectively compared to the control group. The tumor weight of the animals in the control group was 11.25 ± 1.00 g whereas the same for the animals treated with cisplatin plus extract and cisplatin alone was 0.170 ± 0.09 g and 0.51 ± 0.21 g, respectively (Table 3).

The phytochemical analysis of the extract showed that it reacted with anthrone reagent and also with phenol-sulphuric acid reagent and produced typical colour reactions indicating the presence of polysaccharide. The extract also responded to Lowry-Folin test indicating the presence of protein. The results thus indicated that a protein-bound polysaccharide is the major component of the extract. TLC analysis indicated the presence of traces of flavonoids and terpenes in the extract.

Table 3. Effect of cisplatin, cisplatin + methanol extract of *P.florida* (1000 mg/Kg body wt) on solid tumour

Groups	Weight of tumor (gms)	%inhibition
Control	11.25 ± 1.00	----
Cisplatin +Extract	0.170 ± 0.09[a]	98%
Cisplatin	0.510 ± 0.21[a]	95%

Values are mean ± SD (n = 6), $^{a}P<0.001$ vs normal

DISCUSSION

Results of the investigation show that methanolic extract of *P.florida* rendered significant protection against cisplatin induced nephrotoxicity in mice. Several lines of evidence indicate that free radicals and reactive oxygen species are involved in cisplatin induced oxidative stress because of depletion of reduced GSH concentration and antioxidant enzyme activity in the kidneys (Hannemann and Baumann, 1988). The decrease in superoxide dismutase activity after cisplatin administration might be due to the loss of copper and zinc, which are essential for enzyme activity (Sinet and Carber, 1981). Cisplatin has been demonstrated to induce the loss of copper and zinc in the kidneys. The decreased superoxide dismutase activity is insufficient to scavenge the superoxide anion produced during

Table 2. Concentrations of MDA and GSH activity in the kidney

Groups	MDA(n mole/mg protein)	GSH(n mole/mg protein)
Normal(Saline)	0.41 ± 0.07	12.8 ± 0.5
Control(Cisplatin)	0.79 ± 0.04[a]	6.7 ± 1.3[a]
Cisplatin+*P.florida*(500 mg/Kg)	0.50 ± 0.04[b]	10.8 ± 0.9[b]
Cisplatin + *P.florida* (1000 mg/Kg)	0.40 ± 0.04[b]	12.5 ± 2.1[c]

Values are mean ± SD (n = 4 for control (see text) and n = 6 for other groups),
$^{a}P< 0.001$ vs normal, $^{b}P<0.001$ and $^{c}P < 0.005$ vs Control.

the metabolic process. The superoxide anion can thus cause initiation and progression of lipid peroxidation.

The activity of catalase and glutathione peroxidase also decreased after cisplatin administration. This resulted in the decreased ability of the kidney to scavenge toxic H_2O_2 and lipid peroxides. The restoration of renal superoxide dismutase, catalase and glutathione peroxidase activities by pretreatment of *P. florida* extract suggests that the oyster mushroom extract is capable to protect these enzymes even three days after cispaltin administration.

GSH depletion can markedly increase the toxicity of cisplatin. Therefore lipid peroxidation due to cisplatin administration is a consequence of GSH depletion and impaired antioxidant enzyme activities. The increased GSH levels render protection, which is evident from the treatment of the extract prior to cisplatin administration.

The mortality rate was reduced in the extract pretreated group (n=6) compared to the cisplatin treated group (n=4). These observations support the conclusion that part of the mechanism of nephrotoxicity in cisplatin treated animals is due to depletion of antioxidant system. The findings thus supports the use of phytotherapeutants that possess antioxidant activity to ameliorate cisplatin-induced nephrotoxicity during the course of chemotherapy.

Chemical analysis of the methanolic extract of *P. florida* showed a protein bound polysaccharide as the major component. TLC analysis also indicated the presence of the traces of terpenes and flavonoids in the extract. Nevertheless, the nephroprotective activity of the extract can be attributed to the protein bound polysaccharide as both methanol and aqueous extracts possessed significant antioxidant activity (Jose and Janardhanan, 2000).

Earlier experimental studies have shown that a maximum dose of 5 mg/Kg body weight (i.p) of cisplatin induced severe nephrotoxicity in rats (Boogaard *et al*., 1991). In our experimental studies relatively higher dose of cisplatin (16 mg/Kg body weight, i.p), which corresponds to higher therapeutic doses of cisplatin currently being used in clinical practice was used.

Nephroprotection by various chemotherapeutic agents is being evaluated against higher doses of cisplatin in clinical practice (Cozzagilo *et al*., 1990). The present study reveals the profound nephroprotective effects of oyster mushroom, *Pleurotus florida* against the toxic renal effects that occur with higher doses of cisplatin treatment in mice.

In summary, a single dose of cisplatin leads to significant inhibition of renal antioxidant enzyme activity, increased serum urea, creatinine and enhanced tissue lipid peroxidation activities three days after treatment. However, the graded doses of methanolic extract of *P. florida* restored the antioxidant enzyme activity in a dose dependent manner. The treatment also decreased serum urea, creatinine levels and tissue lipid peroxidation. Oyster mushrooms are excellently edible and non-toxic and the results thus suggest the potential use of their extracts in cancer chemotherapy.

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