

Prevention of nephrotoxicity induced by the anticancer drug cisplatin, using *Ganoderma lucidum*, a medicinal mushroom occurring in South India

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Cisplatin (cisplatinum (II) diamine dichloride) is an anticancer drug extensively used against a variety of cancers. Cisplatin chemotherapy is found to manifest dose-dependent nephrotoxicity. Depletion of renal antioxidant defence system has been suggested to be the main cause of cisplatin-induced nephrotoxicity. Our recent investigations revealed that methanolic extract of *Ganoderma lucidum* possessed significant *in vitro* antioxidant activity. This prompted us to investigate the preventive effect of the methanolic extract of *G. lucidum* to ameliorate cisplatin-induced nephrotoxicity in mice. Nephrotoxicity was assessed by determining the serum creatinine and urea levels and renal antioxidant status in mice after cisplatin administration (16 mg/kg body wt, i.p). Methanolic extract of *G. lucidum* (250 and 500 mg/kg body wt) was administered orally 1 h before cisplatin injection. The extract significantly reduced the elevated serum creatinine and urea levels. Renal antioxidant defence systems, such as superoxide dismutase, catalase, glutathione peroxidase activities and reduced glutathione level, depleted by cisplatin therapy were restored to normal by treatment with the extract. Cisplatin-induced lipid peroxidation was also found markedly reduced by treatment with the extract. The results indicated that methanolic extract of *G. lucidum* rendered significant preventive effect against cisplatin-induced nephrotoxicity. The extract did not interfere with the antitumour activity of cisplatin. The findings suggest the potential therapeutic use of South Indian *G. lucidum* in cancer chemotherapy.

AN understanding of the pathophysiology of cancer and impact of anticancer drugs is of significant importance in the treatment and management of cancer patients. Cisplatin (II) diamine dichloride (cisplatin) is extensively used for the management of oncological disorders, particularly of the ovary, testis, bladder, head and neck¹. Although higher doses of cisplatin are more efficacious for cancer chemotherapy, the high-dose therapy manifests non-haematological toxicities such as nephrotoxicity². A number of chemotherapeutic agents have been reported to render protection against cisplatin-induced nephrotoxicity. However, none of them is known to be clinically effective as a complete protective agent. Several lines of evidence indicate that free radicals are involved in the

nephrotoxicity caused by cisplatin, and the damage is suggested to be the consequence of decreased renal antioxidant enzyme activity with enhanced lipid peroxidation. However, administration of antioxidants has been shown to ameliorate cisplatin-induced nephrotoxicity in animals³.

Mushrooms have a notable place in the folklore throughout the world and in traditions of many cultures⁴. The fruiting body of *Ganoderma lucidum* P. Karst (Figure 1), commonly known as Reishi, has been regarded as a panacea for all types of diseases⁵. A number of species of this medicinal mushroom are found in South India⁶. However, no attempt has been made to investigate the therapeutic potential of *Ganoderma* mushroom occurring in India. Our recent investigations have shown that *G. lucidum* occurring in South India possessed significant antioxidant and antitumour properties⁷. In this communication, we report the preventive effect of the methanolic extract of *G. lucidum* occurring in South India, on nephrotoxicity caused by the administration of a cancer chemopreventive agent, cisplatin.

Male Swiss albino mice 6–8 weeks of age and weighing 25 ± 2 g, were selected from our mouse colony. They were maintained in environmentally controlled conditions with free access to standard food (Lipton, India) and water. Animal experiments were carried out according to the guidelines and with the approval of Animal Ethic Committee.

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

Fruiting bodies of *G. lucidum* were collected from the forest area in the outskirts of Thrissur District, Kerala. The type specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB.MUBL.3175). The fruit-



Figure 1. *Ganoderma lucidum* growing on a tree trunk.

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ing bodies were cut into small pieces, dried at 45–50°C for 48 h, and powdered. Two hundred grams of the powdered material was extracted with petroleum ether using a Soxhlet apparatus. The defatted material was air-dried, then suspended in 70% methanol and boiled for 8 h. The solvent was removed and the extraction repeated. The extracts were combined and filtered through Whatman No. 1 filter paper. The solvent evaporated at low temperature under vacuum and the concentrated extract was finally lyophilized. The methanolic extract thus obtained (8 g) was employed in the experiments. The extract before administration was solubilized in distilled water.

To investigate cisplatin-induced nephrotoxicity, animals were divided into four groups of six animals each. Group 1 was kept as normal. Group 2 was given cisplatin (16 mg/kg body weight, i.p.)⁸. Groups 3 and 4 were given methanolic extract of *G. lucidum* (250 and 500 mg/kg body wt; p.o), 1 h before the cisplatin injection (16 mg/kg body wt, i.p). Mice in all groups were sacrificed 72 h after treatment. Blood was collected directly from the heart; serum was separated for creatinine and urea analyses. The kidneys were dissected and stored at –70°C until the analyses could be 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 in a cold centrifuge at 0°C and the supernatant was used for enzyme assay and protein determination. Serum creatinine and urea were estimated by the method of Brod and Sirota⁹ and Marshall *et al.*¹⁰, respectively. The activity of superoxide dismutase (SOD) was assayed by the method of McCord and Fridovich¹¹, catalase (CAT) by the method of Beers and Sizer¹², glutathione peroxidase (GPX) by the method of Hafemann *et al.*¹³, levels of reduced glutathione (GSH) by the method of Moron *et al.*¹⁴ and malondialdehyde (MDA) by the method of Ohkawa *et al.*¹⁵ using 1,1,3,3-tetramethoxypropane as standard. The protein content was estimated by the method of Lowry *et al.*¹⁶ using bovine serum albumin as standard.

To determine the efficacy of antitumour activity of cisplatin with pretreatment of the extract, the animals were divided into three groups of six animals each. Animals in all groups were injected with 1×10^6 viable cells of Dalton's lymphoma ascites (DLA) in phosphate-buffered saline. DLA cell line was originally obtained from Cancer Institute, Adyar, Chennai and maintained in our laboratory. Twenty-four hours after tumour implantation, animals were treated as follows. Group 1 administered with vehicle (distilled water, p.o) was kept as control. Group 2 was administered with methanolic extract of *G. lucidum* (500 mg/kg body wt) orally and then with cisplatin (3 mg/kg body wt, i.p) after, one hour, once daily. Group 3 was given cisplatin (3 mg/kg body wt, i.p) alone, once daily. The treatments in all groups were continued for 10 consecutive days. At the end of the 5th week, animals were sacrificed, the tumour was extirpated and weighed.

The per cent inhibition was calculated using the formula $(1-B/A) \times 100$, where A is the average tumour weight of the control group and B that of the treated group¹⁷.

Experimental data were expressed as mean \pm SD. Student's 't' test was applied for expressing the significance, and P values less than 0.05 were considered as significant.

Serum creatinine and urea levels were significantly elevated ($P < 0.001$) in the cisplatin-treated animals compared to the normal group. The increase of serum creatinine and urea levels was 7 and 5.7-fold, respectively. Treatment of animals with methanolic extract of *G. lucidum* significantly reduced the elevated levels of serum creatinine and urea. The extract treatment was able to lower the serum creatinine and urea to almost normal level (Table 1).

Renal SOD activity decreased significantly ($P < 0.001$) in cisplatin-treated animals compared to the normal

Table 1. Serum creatinine and urea level in normal, cisplatin (16 mg/kg, i.p) and cisplatin + methanolic extract of *Ganoderma lucidum* (GLME; 250 mg/kg and 500 mg/kg body wt, p.o) treated mice

Group	Treatment (mg/kg)	Urea (mg/dl)	Creatinine (mg/dl)
Normal	–	52.51 \pm 3.8	0.408 \pm 0.039
Cisplatin	16	300.49 \pm 28.51 ^a	2.98 \pm 0.290 ^a
Cisplatin + GLME	250	148.6 \pm 5.68 ^b	1.08 \pm 0.080 ^b
Cisplatin + GLME	500	99.15 \pm 11.14 ^b	0.442 \pm 0.069 ^b

Values are mean \pm SD; $n = 6$.

All values are significant.

^a $P < 0.001$ with respect to normal; ^b $P < 0.001$ with respect to cisplatin group.

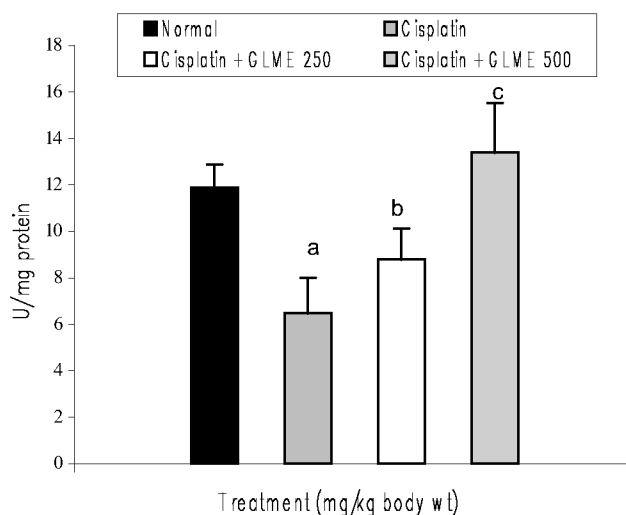


Figure 2. Renal super oxide dismutase activity in normal, cisplatin (16 mg/kg), cisplatin plus *G. lucidum* (GLME; 250 and 500 mg/kg) treated mice in the kidney, three days after cisplatin administration. Values are expressed as mean \pm SD. ^a $P < 0.001$ with respect to normal, ^b $P < 0.05$, and ^c $P < 0.001$ with respect to control.

group (Figure 2). Administration of methanolic extract of *G. lucidum* prior to cisplatin treatment restored the level of SOD in a dose-dependent manner in animals of the cisplatin-treated group.

The renal GSH concentration decreased by over 40% ($P < 0.001$) in cisplatin-treated animals compared to the normal group. However, the administration of methanolic extract of *G. lucidum* at a concentration of 500 mg/kg body wt prior to cisplatin treatment increased the renal GSH concentration to the normal level (Table 2).

The concentration of MDA increased by over 50% ($P < 0.001$) in cisplatin-treated animals compared to the normal group, indicating the enhanced activity of lipid peroxidation. Administration of methanolic extract of *G. lucidum* at a concentration of 500 mg/kg body wt decreased MDA concentration to the normal level (Table 2).

Renal CAT and GPX activities were also decreased significantly ($P < 0.001$) after administration of cisplatin (Figures 3 and 4). Administration of methanolic extract

of *G. lucidum* prior to cisplatin treatment restored the activities of CAT and GPX to normal levels.

Treatment with the methanolic extract of *G. lucidum* prior to cisplatin injection did not interfere with the anti-tumour efficacy of cisplatin (Table 3). The average weight of tumour in the cisplatin and the extract (500 mg/kg body wt) plus cisplatin-treated animals was 0.48 ± 0.06 and 0.24 ± 0.02 g, respectively. The tumour weight in the control animals was 10.2 ± 0.18 g. The per cent inhibition of tumour growth by cisplatin and cisplatin plus *G. lucidum* extract (500 mg/kg body wt) was 95 and 97%, respectively. This indicated that the extract did not interfere with the antitumour activity of cisplatin.

Earlier experimental studies have shown that a minimum dose of cisplatin (5 mg/kg body wt, i.p) was sufficient to induce nephrotoxicity in rats³. The present study was carried out using higher doses of cisplatin (16 mg/kg body wt, i.p). This corresponds to the dose of cisplatin normally used in clinical practice⁸. This dose is also found to produce nephrotoxicity and ototoxicity. Several protective agents have been evaluated against cisplatin-induced nephrotoxicity in experimental and clinical stud-

Table 2. Level of GSH and lipid peroxidation (MDA) in the kidney of mice treated with normal, cisplatin (16 mg/kg body wt., i.p), cisplatin + methanolic extract of *G. lucidum* (GLME; 250 mg/kg and 500 mg/kg body wt, p.o) treated mice

Group	Treatment (mg/kg)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Normal	—	12.4 ± 0.88	0.37 ± 0.11
Cisplatin	16	7.3 ± 1.32^a	0.83 ± 0.08^a
Cisplatin + GLME	250	10.6 ± 0.28^b	0.54 ± 0.05^b
Cisplatin + GLME	500	12.3 ± 2.25^b	0.39 ± 0.02^b

Values are mean \pm SD; $n = 6$.

All values are significant.

^a $P < 0.001$ with respect to normal group; ^b $P < 0.001$ with respect to cisplatin group.

Table 3. Antitumour activity of cisplatin (3 mg/kg body wt, i.p) and cisplatin + methanol extract of *G. lucidum* (GLME; 500 mg/kg body wt, p.o) against DLA-induced solid tumour model in mice

Group	Treatment (mg/kg)	Tumour wt during 5th week (g)	Percentage inhibition
Control	Vehicle	10.2 ± 0.18	—
Cisplatin	3	0.48 ± 0.06^a	95.3
Cisplatin + GLME	500	0.24 ± 0.02^a	97.6

Values are mean \pm SD; $n = 6$.

All values are significantly different from the control group. ^a $P < 0.001$ with respect to control.

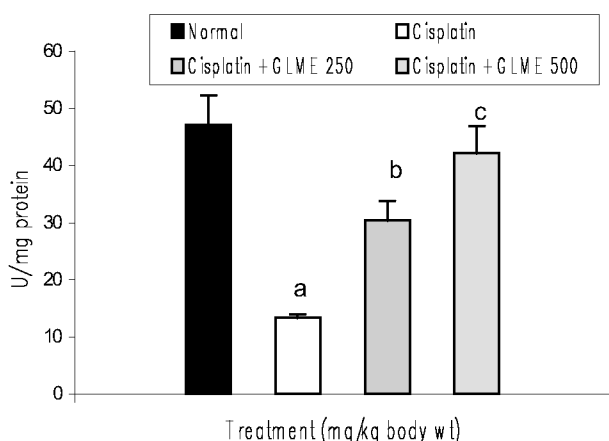


Figure 3. Renal catalase activity in normal, cisplatin (16 mg/kg), cisplatin plus *G. lucidum* (GLME; 250 and 500 mg/kg) treated mice, three days after cisplatin administration. Values are expressed as mean \pm SD. ^a $P < 0.001$ with respect to normal, ^b $P < 0.05$, and ^c $P < 0.001$ with respect to control.

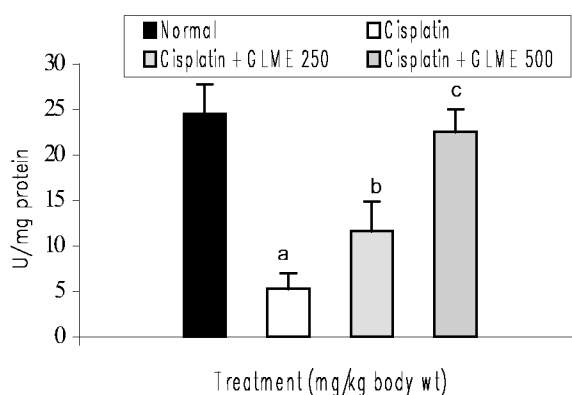


Figure 4. Renal glutathione peroxidase activity in normal, cisplatin treated, cisplatin plus *G. lucidum* (GLME; 250 and 500 mg/kg) treated mice, three days after cisplatin administration. Values are expressed as mean \pm SD. ^a $P < 0.001$ with respect to normal, ^b $P < 0.05$, and ^c $P < 0.001$ with respect to control.

ies. They include diethyldithiocarbamates¹⁸, glutathione³, glycine¹⁹, methionine²⁰, procaine and procainamide²¹. However, none of these compounds has proved to be clinically efficacious as complete protection in patients. Cystone, a polyherbal ayurvedic preparation has been reported to render protection against cisplatin-induced nephrotoxicity without interfering with its antitumour activity²². Graded doses of lipoic acid were found to effectively prevent cisplatin-induced nephrotoxicity by preventing the decrease in renal antioxidant defence system and the increase in lipid peroxidation⁸. Naturally-occurring antioxidants such as sodium malate²³ and silibinin²⁴ have also been reported to reduce cisplatin-induced nephrotoxicity. The results of the present study show that renal SOD, CAT, GPX activities and reduced GSH level significantly decreased in the cisplatin-treated animals compared to the normal group. These observations support the hypothesis that the mechanism of nephrotoxicity in cisplatin-treated animals is related to depletion of antioxidant defence system. Treatment with *G. lucidum* extract (250 and 500 mg/kg body wt, p.o) prior to cisplatin administration prevents the depletion of renal antioxidants.

The decrease in SOD activity after cisplatin administration might be due to the loss of copper and zinc, which are essential for enzyme activity²⁵. Cisplatin has been demonstrated to induce the loss of copper and zinc in the kidneys. The decreased SOD activity is insufficient to scavenge the superoxide anion produced during the normal metabolic process. The superoxide anion can cause initiation and progression of lipid peroxidation.

The activity of CAT and GPX is also found to decrease after cisplatin administration. This resulted in the decreased ability of the kidney to scavenge toxic H₂O₂ and lipid peroxides. Restoration of renal SOD, CAT and GPX activities by pretreatment with *G. lucidum* extract suggests that the extract is capable of protecting the enzymes even three days after cisplatin administration. GSH depletion can markedly increase the toxicity of cisplatin. The increased GSH levels render protection, which is evident from the extract plus cisplatin-treated group of animals.

The concentration of MDA is observed to be significantly increased in the cisplatin-treated animals compared to the normal group. Administration of *G. lucidum* extract along with cisplatin decreased MDA to the normal level compared to cisplatin-treated animals. Depletion of renal GSH can markedly increase the toxicity of cisplatin, probably due to the augmentation of lipid peroxidation. The present investigation also indicates that pretreatment with *G. lucidum* extract does not interfere with the antitumour efficacy of cisplatin. The tested doses of methanolic extract of *G. lucidum* show no signs of toxicity in mice. Survival rate of animals treated with cisplatin and *G. lucidum* extract supports the nephroprotective effect of this mushroom.

Free radicals are known to play an important role in cisplatin-induced nephrotoxicity. The free radicals and reactive oxygen species induce oxidative stress in kidneys^{26,27}. Due to cisplatin administration, platinum-sulphydryl group complexes formed are taken up by renal cells and stabilized by intracellular GSH for several hours. In case of intracellular GSH depletion, the complexes undergo rapid transformation to reactive metabolites²⁸. Thus GSH depletion results in increased toxicity of cisplatin. GSH depletion also results in lipid peroxidation and this seems to be the prime factor that permits lipid peroxidation and impaired antioxidant enzyme activities. These observations support the conclusion that the mechanism of nephrotoxicity in cisplatin-treated rats is related to depletion of antioxidant systems. Thus, nephroprotection by the mushroom extract might be directly related to its antioxidant activity.

Preliminary phytochemical analysis indicates that the major constituent of the extract is a polysaccharide. This conclusion is based on the positive reaction of the extract to anthrone test²⁹ and also to phenol-sulphuric acid reagent³⁰. A number of species of *Ganoderma* are used for medicinal purposes, the most important among them being *G. lucidum* and *G. applanatum*. Over 250 species have been described worldwide; most of them based on variable and overlapping characters³¹. Their morphological characters are insufficient to distinguish interspecific and intraspecific variants. The two recognizable groups are *Ganoderma lucidum* (Curt.: Fr.) P. Karst and *G. applanatum* (Pers.) Pat. complex. Fruiting bodies of *G. lucidum* were used in the present study. This strain was found growing on the trunks of living *Caesalpinia coriaria* Wild. trees in the Amala Hospital Campus, Thrissur. The fruiting bodies were lignicolous, annual and mostly sessile. The pileus surface was shining, laccate and purplish-brown in colour. The specimen was identified with the help of the available literature and the identification was confirmed by K. M. Leelavathy, Department of Botany, University of Calicut. The voucher specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras as mentioned earlier.

The experimental results reveal that the methanolic extract of *G. lucidum* could help prevent nephrotoxicity manifested consequent to cisplatin chemotherapy. The effect is mainly due to the capacity of the extract to restore renal antioxidant defence system. Our earlier investigations have shown that methanolic extract of *G. lucidum* occurring in South India possessed significant antioxidant and antitumour activities⁷. Treatment of mice at a dose of 500 mg/kg body wt for a period of ten days after the implantation of the tumour was found to reduce 97.7% of tumour load. This indicates that the antioxidant activity of the mushroom extract does not interfere with its antitumour property. Hence, a combined therapy with *G. lucidum* extract and cisplatin would be more benefi-

cial than cisplatin alone. The experimental findings thus suggest the potential therapeutic use of South Indian *G. lucidum* mushroom in cancer chemotherapy.

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