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Prevention of cisplatin induced nephrotoxicity by terpenes isolated from *Ganoderma lucidum* occurring in Southern Parts of India

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ABSTRACT

Investigations were carried out to determine the protective effect of terpenes isolated from the fruiting bodies of *Ganoderma lucidum* (Fr) P.Karst against nephrotoxicity caused by the cisplatin, in mice. Intraperitoneal administration of cisplatin (16 mg/kg body wt) resulted in significant nephrotoxicity in mice. Serum urea, creatinine and ALP levels were drastically elevated indicating severe nephrotoxicity . The renal antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and concentration of reduced glutathione (GSH) were depleted by cisplatin injection. The oral administration of terpenes at a dose of 100 mg/kg body weight prevented increase in urea, creatinine levels and ALP activity and also maintained the renal antioxidant defense. The *Ganoderma* terpenes also imparted protection against cisplatin induced renal tissue lipid peroxidation. The results indicated that the total terpenes isolated from *G. lucidum* possessed significant *in vivo* antioxidant activity and rendered protection against cisplatin induced nephrotoxicity. The results suggest the potential therapeutic use of *Ganoderma* terpenes to prevent nephrotoxicity caused during chemotherapy using cisplatin.

1. Introduction

Ganoderma lucidum (Fr) P.Karst, commonly known as Reishi in Japan and Ling Zhi in China is well known for its medicinal properties. *Ganoderma* is a rich source of bitter triterpenes. Currently more than 130 triterpenoids are reported to occur in *G. lucidum* alone (Gao and Zhou, 2003). The vast majority are ganoderic acids and lucidenic acids (Nishitoba et al., 1984). However, it has been reported that some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent (Nishitoba et al., 1986). The biological activities reported for oxygenated triterpenes include bitterness, hepatoprotection and cytotoxicity to hepatoma cells, inhibition of histamine release, angiotensin converting enzyme, cholesterol absorbtion, cholesterol biosynthesis and stimulation as well as inhibition of thrombin induced platlet aggregation (Shiao et al., 1994). Cisplatinum (II) diamine dichloride (Cisplatin) is a potent antineoplastic agent containing the heavy metal platinum, which is extremely effective in the treatment of several types of tumors (Merrin, 1979; Rosenberg et al., 1969; Young et al., 1979; Carter et al., 1987). But one of the less desirable characteristics of cisplatin is its toxicity profile, which includes severe nephrotoxicity and neurotoxicity. Nephrotoxicity has most commonly limited the effective use of this drug.

The clinical consequences of renal damage may include tubular dysfunction, with severe hypocalcaemia, hypokalaemia and hypomagnesaemia, renal salt wasting with severe hyponatraemia and hypovolaemia and rarely the syndrome of inappropriate secretion of antidiuretic hormone (Haskell, 1990). The use of hydration, hypertonic saline, diuretics and other chemotherapeutic agents has rendered protection against cisplatin induced nephrotoxicity (Tognella, 1990). Experimental evidences suggest that reactive oxygen species or free radicals are involved in the nephrotoxicity induced by cisplatin. Free radical mediated oxidative damage may occur in cisplatin induced nephrotoxicity as a consequence of decreased renal levels of antioxidant activity with enhanced lipid peroxidation (Husain et al., 1996, 1998; Somani, 1995). The immunomodulating property of this mushroom provides a promising approach for cancer prevention and its administration is found useful alone or in combination with chemotherapy and radiotherapy (Gao and Zhou, 2003). *G. lucidum* occurring in South India has been reported to possess significant antioxidant activity (Jones, 2000). This indicated its capacity to prevent oxidative stress and the consequent damage caused by free radicals. The major chemical constituent of *Ganoderma* are polysaccharides and terpenoids (Pillai et al., 2006). Methanolic extract of *G. lucidum* has been found to possess significant nephroprotective activity when administered at a high dose

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(Sheena, 2003). We examined the nephroprotective activity of the terpenoids isolated from *G. lucidum* and the findings are reported in this communication.

2. Materials and methods

2.1. Animals

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 (Sai Durga Foods, Bangalore.) and water. Animal experiments were carried out with the approval and guidelines of Institutional Animal Ethical Committee.

2.2. Chemicals

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

2.3. Isolation of total terpenes

The fruiting bodies were collected from the outskirts of Thrissur district, Kerala, South India. The type specimen was deposited in the Herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB. MUBL. 3175).The sporocarps were cut into small pieces, dried at 40–50 °C for 48 h and powdered. Four hundred gram sample of the powdered material was extracted in 4 batches of 100 g each with chloroform using a Soxhlet apparatus for 8–10 h. The extraction was repeated again for the same period of time. The chloroform extracts were combined, dried with anhydrous sodium sulphate, then concentrated at low temperature under vacuum and the solvent was finally evaporated completely. The residue thus obtained was named as total terpene fraction (2 g). The terpene fraction was further purified by column chromatography. The sample was loaded on a silica gel G column and eluted with petroleum ether, then chloroform and finally with chloroform:methanol (90/10). The fractions were analysed by TLC on silica gel using chloroform:methanol (90/10) solvent system. The terpene fractions were detected by anisaldehyde–sulphuric acid and antimony trichloride reagents, and observed under UV. The terpene fractions were combined and again analysed by TLC to determine the purity of the compound. The combined fraction produced only a single spot when analysed by TLC. Pure terpenoid fraction was used for the experiments. The yield was 0.9%.

2.4. Cisplatin induced nephrotoxicity

Animals were divided into four groups of six animals each.

Group I – was given cisplatin (16 mg/kg body wt).
Group II – Normal (normal saline)
Group III – Terpene (50 mg/kg body wt)+Cisplatin (16 mg/kg body wt)
Group IV – Terpene (100 mg/kg body wt)+Cisplatin(16 mg/kg body wt)

Cisplatin was dissolved in normal saline and administered intraperitoneally. Terpene was dissolved in olive oil and was administered orally 1 h before the cisplatin injection. Mice in all groups were sacrificed 72 h after treatment. The blood was collected; serum was separated for creatinine and urea analysis. The kidneys were dissected and stored at −70 °C until analyses were completed. The kidneys were homogenized in 50 mM phosphate buffer (pH 7.0) to give a 10% homogenate. The homogenate was centrifuged at 1000 rpm for 10 min in a cold centrifuge at 0 °C and supernatant was used for enzyme assays and protein determination.

Serum creatinine and urea were estimated by the methods of Brod and Sirota, 1980 and Marshell et al. (1980), method respectively. Tissue homogenate was used for assay of superoxide dismutase (SOD) by the method of Mc Cord and Fridovich (1969), catalase (CAT) by the method of Aebi (1974), glutathione peroxidase (GPx) by the method of Hafemann et al. (1974), reduced glutathione (GSH) by the method of Moron et al. (1979), malondialdehyde (MDA) by the method of Ohkawa (1979) and protein by the method of Bradford (1976) using bovine serum albumin as standard.

2.5. Histopathological observation

A portion of the kidney was fixed in 10% formalin and embedded in paraffin. Six micrometer microtome sections were prepared from each kidney and stained with hematoxylin and eosin. The sections were examined under light microscope.

Table 2
Effect of terpene isolated from *G. lucidum* on cisplatin induced renal catalase activity.

Groups	Treatments (mg/kg body wt)	Catalase U/mg protein
Normal		37.43 ± 7.86
Control (Cisplatin)	16	29.50 ± 9.01
Trepenes+Cisplatin	50	34.49 ± 2.55
Terpenes+Cisplatin	100	41.72 ± 8.47

$P < 0.05$*, 0.01** values are mean ± S.D. of 6 animals in each group. Values compared with control group.

Table 1
Effect of terpenes isolated from *G. lucidum* on cisplatin induced renal urea, creatinine alkaline phosphatase activity in mice.

Groups	Treatment (mg/kg body wt)	Urea (mg/dl)	Creatinine (mg/dl)	Alkaline phosphate (KA/dl)
Normal		48.36 ± 21.92	0.94 ± 1.71	50.91 ± 1.99
Control (Cisplatin)	16	139.5 ± 25.99	3.03 ± 0.90	175.58 ± 30.83
Terpenes+Cisplatin	50	76.01 ± 4.23**	1.96 ± 0.96	97.8 ± 17.30**
Terpenes+Cisplatin	100	50.40 ± 12.45**	0.88 ± 0.2**	57.74 ± 1.81**

$P <$ *0.05, **0·01 values are mean ± S.D. of 6 animals in each group values compared with control group.