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Aqueous-ethanolic extract of morel mushroom mycelium *Morchella esculenta*, protects cisplatin and gentamicin induced nephrotoxicity in mice

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ABSTRACT

Morchella esculenta (L) Pers. is an excellently edible and delicious morel mushroom found growing in the temperate forests. The mycelium of this mushroom is widely used as a flavouring agent. The current investigation was undertaken to explore the protective effect of the aqueous-ethanol extract of cultured mycelium of *M. esculenta* against cisplatin and gentamicin induced acute renal toxicity in *Swiss albino* mice. Cisplatin and gentamicin when administered induced a marked renal failure, characterized by a significant increase in serum urea and creatinine concentrations. Treatment with the extract at 250 and 500 mg/kg body weight decreased the cisplatin and gentamicin induced increase in serum creatinine and urea levels. Treatment with the extract also restored the depleted antioxidant defense system. The decreased activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) in the kidneys consequent to cisplatin and gentamicin administration was significantly elevated. The enhanced renal antioxidant defense system also prevented the tissue lipid peroxidation. The experimental results suggest that aqueous-ethanol extract of morel mushroom, *M. esculenta* mycelium protected cisplatin and gentamicin induced nephrotoxicity possibly by enhancing renal antioxidant system. The findings thus suggest the potential therapeutic use of morel mushroom mycelium as a novel nephroprotective agent.

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). Although higher doses of cisplatin are more efficacious for the treatment of cancer (Cozzaglio et al., 1990; Di et al., 1990; Gandara et al., 1991) many 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). Primary targets of cisplatin in kidney are proximal straight and distal convoluted tubules where it accumulates and promotes cellular damage, by multiple mechanisms including oxidative stress, DNA damage and apoptosis (Safirstein et al., 1987; Schaaf et al., 2002; Cummings and Schnellmann, 2002; Xiao et al., 2003). Several lines of evidence suggest the role of reactive oxygen species (ROS) in the pathogenesis of nephrotoxicity (Baliga et al., 1998; KrishnaMohan et al., 2006). Cisplatin induces free radical production causing oxidative renal damage, possibly due to depletion of non-enzymatic and enzymatic antioxidant systems.

Gentamicin, a typical aminoglycoside antibiotic is widely used in clinical practices for the treatment of life threatening gram-negative infections. This antibiotic generally causes drug-induced dose-dependent nephrotoxicity in 10–20% of therapeutic courses (Karahan et al., 2005). Gentamicin induced nephrotoxicity is characterized by direct tubular necrosis, without morphological changes in glomerular structures (Cuzzocrea et al., 2002; Eisenberg et al., 1987). Gentamicin generates hydrogen peroxide in rat renal cortex mitochondria and can also enhance the generation of reactive oxygen species (ROS) (Yanagida et al., 2004; Karahan et al., 2005). Abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Baliga et al., 1998; Kehrer, 1993; Parlakpinar et al., 2005). The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of gentamicin mediated nephrotoxicity (Karahan et al., 2005). Gentamicin also acts as an iron chelator and the iron–gentamicin complex is a potent catalyst of radical generation (Yanagida et al., 2004).

Mushrooms are nutritional food as well as source of physiologically beneficial and non-toxic medicines. Since ancient times they have been used in folk medicine throughout the world. Mushrooms contain a large number of biologically active components that offer health benefits and protection against many degenerative diseases. A number of medicinal mushrooms have recently been reported to possess significant antioxidant activity

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(Jose et al., 2002; Jones and Janardhanan, 2000; Ajith et al., 2002; Lakshmi et al., 2004; Ekanem and Ubengama, 2002). Some of the most recently isolated and identified substances from mushrooms have been reported to possess significant cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective and antidiabetic activities (Oii, 2000).

Members of the genus *Morchella*, commonly known as morels are one of the most highly priced edible mushrooms in the world (Negi, 2006; Duncan et al., 2001). *M.orchella esculenta* is an excellently edible mushroom growing in temperate regions. In India, species of *Morchella* locally known as 'Guchhi' are found growing in the forests of Jammu and Kashmir and Himachal pradesh. The preparations from these mushrooms are reported to be used in health care and medicinal purposes among traditional hill societies (Prasad et al., 2002). Since commercial cultivation of morel mushrooms for the fruiting body production has not been largely successful till now, the cultured mycelium is extensively used as a flavouring agent. In this communication, we report the nephroprotective activity of the aqueous-ethanol extract of cultured mycelium of *M. esculenta* (L) Pers.

2. Materials and methods

2.1. Chemicals

Glutathione (GSH), 5,5′ dithio-dinitro bisbenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), nitroblue tetrazolium (NBT) and thiobarbituric acid (TBA) riboflavin and sodium azide were purchased from SRL, Mumbai, hydrogen peroxide (H_2O_2), EDTA, *n*-butanol, ascorbic acid, pyridine from Merck India Ltd., Mumbai, India and 2,7-dichlorofluorescein diacetate from Sigma–Aldrich. Cisplatin (Samarath Pharma, Pvt. Ltd., Mumbai, India), Gentamicin (Biochem Pharmaceutical Industries, Mumbai, India) and vitamin E (Evion 400 of Merck India Ltd., Mumbai, India) were purchased from the Amala Hospital Pharmacy. All other chemicals and reagents used were of analytical grade.

2.2. Test animals

Female Swiss albino mice, six weeks old purchased from Small Animal Breeding Centre, College of Veterinary and Animal Science, Mannuthy, Kerala, India 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).

2.3. Production of mushroom mycelium

Culture of *M. esculenta* obtained from Microbial Type Culture Collection, Institute of Microbiology, Chandigarh, India, (MTCC 1795) was used for the studies. The fungus was grown in submerged culture on potato-dextrose broth (PDB) for the production of mycelial biomass. After ten days of growth in submerged culture the fungal biomass was harvested, washed thoroughly and dried at 40–50 °C (Janardhanan et al., 1970).

2.4. Preparation of extracts

The dried mushroom mycelia were powdered and extracted with hot aqueous-ethanol (ethanol/water 50/50 v/v) for 8–10 h. The extract was concentrated and solvent completely evaporated under vacuum. The residue (6%) thus obtained was employed for the experiments.

2.5. Experimental design

2.5.1. Cisplatin induced nephrotoxicity

Animals were divided into six groups of six animals in each group. Group I treated with (vehicle) distilled water was kept as normal. Group II injected with a single dose of cisplatin (12 mg/kg body weight, i.p.) was kept as control. Group III and IV were treated with aqueous-ethanolic extract of *M. esculenta* mycelium (250 and 500 mg/kg body weight), and group V received vitamin E 250 mg/kg body weight. The extract 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 ether and sacrificed. The blood was collected directly from the heart of each animal (Ajith et al., 2002).

2.5.2. Gentamicin induced nephrotoxicity

Animals were divided into six groups of six animals in each group. Group I treated with (vehicle) distilled water was kept as normal. Group II injected with gentamicin (50 mg/kg body weight, i.p.) for six days was kept as control (Karahan et al., 2005). Group III and IV were treated with aqueous-ethanolic extract of *M. esculenta* mycelium (250 and 500 mg/kg body weight), and group V received vitamin E 250 mg/kg body weight. The extract and vitamin E were administrated orally 1 h before every gentamicin injection and continued for six days. Twenty-four hours after the last doze of gentamycin, animals were anaesthetized with ether and sacrificed. The blood was collected directly from the heart of each animal.

2.6. Biochemical analysis

Creatinine was estimated using diagnostic kit (Span Diagnostics Ltd., India) and urea by diacetylmonoxime (DAM) reagent diagnostic kit (Span Diagnostics Ltd., India). Tissue lipid peroxidation (MDA) was assayed by the method of Ohkawa et al. (1979).

2.6.1. Determination of antioxidant status in the liver

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 polytron homogenizer at 4 °C. Reduced glutathione (GSH) in the tissue was determined by the method of Moron et al. (1979), Glutathione peroxidase (GPx) activity was determined by the method of Hafemann et al. (1974) and Glutathione-S-transferase (GST) activity by the method of Habig et al., 1974. Tissue superoxide dismutase (SOD) activity was assayed according to the method of McCord and Fridovich, 1969 and tissue catalase (CAT) activity by the method of (Beer and Sizer, 1952). The protein was estimated by the method of (Bradford, 1970).

2.6.2. Isolation of kidney glomeruli

Kidney glomeruli were isolated by the method of Takemoto with slight modifications (Takemoto et al., 2002).The kidneys were removed, minced into 1-mm 3 pieces, and digested in collagenase (1 mg/ml collagenase A, 100 U/ml deoxyribonuclease I in HBSS) at 37 °C for 30 min with gentle agitation. The collagenase-digested tissue was gently pressed through a 100 μm cell strainer using a flattened pestle and the cell strainer was then washed with 5 ml of HBSS. The filtered cells were passed through a new cell strainer without pressing and the cell strainer washed with 5 ml of HBSS. The cell suspension was then centrifuged at 200*g* for 5 min. The supernatant was discarded and the cell pellet was resuspended in 2 ml of HBSS.

2.6.3. Measurement of ROS in kidney glomeruli

Endogenous amounts of ROS were measured by a fluorometric assay with 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (Bondy and Guo, 1994). DCFH-DA is a stable, nonfluorescent molecule that is de esterified within cells to the ionized free acid, DCFH. This is trapped within cells and thus accumulated (Bass et al., 1983). DCFH is then oxidized in the presence of ROS (superoxide anion, hydrogen peroxide, and hydroxyl radical) to the highly fluorescent 2′,7′-dichlorofluorescein (DCF) (Scott et al., 1988). Cells were incubated at 37 °C for 45 min in PO_4 buffer (50 mM, pH 7) containing 10 μM DCFH-DA (prepared in DMSO) in a final volume of 1 ml and fluorescence was quantified using Fluorescence spectrometer (Nanodrop fluorospectrometer, Wilmington, USA) at excitation 488 nm and emission 525 nm.

2.7. Histopathological examination

Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol and embedded in paraffin, cut into 4–5 m thick sections and stained with hematoxylin–eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

2.8. Statistical analysis

All experimental data were expressed as mean ± S.D. The statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnett's *t*-test using InStat3 software. $p < 0.01$ was considered to be significant.

3. Results

3.1. Effect of the mushroom extract on serum urea and creatinine concentrations

Serum urea and creatinine concentrations were significantly increased in the cisplatin and gentamicin alone treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity (Figs. 1 and 2). Treatment with the extract