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Protective role of *Pleurotus porrigens* (Angel's wings) against gentamicin-induced nephrotoxicty in mice

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Abstract. – *Objectives:* **This study was conducted to quantitatively evaluate the recovery effects of methanolic fraction Angel's wings on gentamicin (GM)-induced nephrotoxicity.**

Material and Methods: **Renal injury was achieved by injecting 100 mg/kg, intraperitoneally of GM in normal saline. Extract were administrated intraperitoneally at doses 200 and 400 mg/kg. Blood samples were examined for serum creatinine, serum urea, and blood urea nitrogen after the 10 consecutive days of treatment.**

Results: **Results show that GM-induced nephrotoxic animal model was successfully prepared. Methanolic fraction of Angel's wings attenuated the gentamicin-induced increase in level of serum creatinine, serum urea, and blood urea nitrogen.**

Conclusions: **The present study shows that the extract at the doses 200 and 400 mg/kg, intraperitoneally offered significant nephroprotective action that is comparable with control group.**

Key Words:

Angel's wings, Creatinine, Gentamicin, Nephroprotective activity, Serum urea.

Introduction

Nephrotoxicity is of critical concern when selecting new drug candidates during the early stage of drug development[1]. Because of its unique metabolism, the kidney is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. In addition, reactive oxygen species (ROS) derived from chemicals or drugs that are exposed to renal cells appear to mediate renal necrosis, although the mechanisms of free radical toxicity are not well understood. Therefore, it is important to understand the role played by antioxidants agents' e.g. phenolic compounds, such as flavonoids, phenolic acids, tannins during drug-mediated toxicity to determine if they can show protective effect against oxidative stress induced by reactive intermediates produced by various chemicals and drugs[2,3]. Lovastatin, a cholesterol-lowering drug derived from *Pleurotus* species, and its analogues are reported to be the best therapeutic agents for correcting hypercholesterolemia[4]. Ethyl acetate and methanol extracts of *Pleurotus florida* have been found to exhibit potent scavenging of hydroxyl radicals and inhibition of lipid peroxidation activities[5]. In our recent article we examined the antioxidant activity of *Pleurotus porrigens*[6]. As far as we know there is no report on nephroprotective effect of *Pleurotus porrigens*.

Materials and Methods

Sample Preparation

Pleurotus porrigens was collected from Sari, Mazandaran, Iran and identified by Dr. B. Eslami (Assistance Professor of plant systematic, Islamic Azad University, Ghaemshahr branch, Iran) where a voucher specimen (No 31) was deposited. The macroscopic descriptions noted included size, shape, color, texture and odor, which were important for the identification mushroom species. Color of the carpophores, shape of cap and stipe, color of flesh and latex, smell and its habitat were also noted.

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Preparation of Extract

The materials were oven dried at 38°C, for 5 days. Dried materials were coarsely ground (2-3 mm) before extraction. Sample was extracted by percolation method using ethyl acetate for 24 hrs at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. This procedure repeated three times. The remaining sample residue consecutive extracted with methanol. Extract was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator until a crude solid extract was obtained.

Animal Model

The study was performed on male NMRI mice of approximately the same age-group and body weight (2-3 weeks; 20-25 g), housed in ventilated animal rooms at a temperature of 24 ± 2°C with a 12 h light/dark cycle and 60 ± 5% humidity. They were fed with standard laboratory animal feed, manufactured by Pasture Institute, Tehran, Iran. Water was provided *ad libitum*. All experiments were performed according to the norms of the Ethical Committee of University of Mazandaran, Babolsar, Iran.

Experimental protocol

Animals were randomly divided into four groups of 10 animals each. Group I was kept as normal control receiving isotonic saline (0.5 ml, i.p.) for 8 consecutive days, and animals of groups II, III and IV were administered gentamicin, manufactured by Daru-pakhsh Co., Iran (100 mg/kg/day, i.p.) for 8 consecutive days, which is well known to produce significant nephrotoxicity in mice[7]. Injections of gentamicin were made daily at 08:00 hours to minimize the circadian variation in nephrotoxicity[8]. Animal of Group II and III received extract (200 and 400 mg/kg/day, i.p.) and group IV received isotonic normal saline (0.5 ml, i.p.) for 10 consecutive days. After the last application, animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood samples were collected via retro-orbital puncture in plain plastic tubes and then centrifuged (900 g for 15 min at 5°C) to separate serum. The serum obtained was stored at –20°C until analysis.

Biochemical Analysis

Blood urea nitrogen (BUN), creatinine (Cr) and serum urea concentration was assessed as markers of nephrotoxicity. BUN, Cr and serum urea were determined using commercially available kits (Sigma, St. Louis, MO, USA).

Statistical Analysis

The values are presented as means ± SD. Differences between group means were estimated using a one-way ANOVA followed by Duncan's multiple range test. Results were considered statistically significant when $p<0.05$.

Results

Results are shown in Table I. Gentamicin (100 mg/kg) when injected for 8 consecutive days caused significant ($P<0.001$) increase in serum creatinine (94.31%) and serum urea (100.3%). The *Pleurotus porrigens* extract-treated mice (400 mg/kg) differed from normal control mice by an elevated concentration of serum creatinine (24.22%, $P<0.01$), serum urea (32.1%, $P>0.05$)

Table I. Effect of methanolic fraction of Pleurotus porrigens on serum creatinine, serum urea, and BUN levels in gentamicin-induced nephrotoxic mice.

Groups	Serum creatinine mg/dl	Serum urea mg/dl	Blood urea nitrogen mg/dl
Gentamicin control (100 mg/kg, i.p.)	42.75 ± 0.96***	119.56 ± 5.22***	52.50 ± 5.50**
Normal	22.00 ± 2.94	59.68 ± 6.31	38.50 ± 3.50
Extract-treated (200 mg/kg, i.p.)	35.33 ± 1.25 **	90.14 ± 1.09***	41.00 ± 1.00*
Extract-treated (400 mg/kg, i.p.)	27.33 ± 0.94 *	78.87 ± 3.45**	36.33 ± 2.52 ns

Values are Means ± SD (n = 5). Data for normal animals are considered as base-line data. ns = not significant, *$P<0.05$, **$P<0.01$ and ***$P<0.001$ versus control.

and blood urea nitrogen ($P>0.05$). The extract at the concentration of 400 mg/kg, decreased blood urea nitrogen level more than normal group (5.6%, $P>0.05$). In 200 mg/kg treated mice, biochemical parameters were different from normal control mice by an elevated concentration of serum creatinine (60.59%, $P<0.001$), serum urea (51.03%, $P<0.05$) and blood urea nitrogen ($P>0.05$).

Discussion

Gentamicin, aminoglycoside antibiotic was isolated from *Micromonospora purpurea* in 1963 and, being active against *Pseudomonas aeruginosa* and *Serratia marcescens*, is widely used in the treatment of life-threatening infections[9]. Nephrotoxicity is a major complication of the gentamicin administration. Thus amelioration of nephrotoxicity would enhance its clinical use[9]. Some antioxidant agents that have been used to ameliorate gentamicin induced nephrotoxicity in rats include deferoxamine, methimazole, vitamin E, vitamin C diethyl dithiocarbamate, L-histidinol, thymoquinone[10]. But none of these compounds have proved to be clinically efficient to provide complete protection in patients. Recently, interest has considerably increased in finding naturally occurring antioxidant that are able to ameliorate cisplatin and gentamicin induced nephrotoxicities, to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis[11]. In this study, we have explored the possible protective role of *Pleurotus porrigens* extract on gentamicin-induced renal injury. The results of the present study indicate that gentamicin 100 mg/kg/day gentamicin for 8 days administration brought about a significant increase in BUN, serum creatinine and urea. Extract at concentration of 400 mg/kg showed therapeutic effects. The induced effects of gentamicin were significantly reversed by the extract. It seems that this plant has the potential to be used to ameliorate gentamicin nephrotoxicity. Reactive oxygen species (ROS) including hydroxyl radicals have been implicated in the etiology of gentamicin-induced nephrotoxicity[10,12]. Walker and Shah[13] have showed that gentamicin *in vitro* enhances the generation of hydrogen peroxide by renal cortical mitochondria and that iron chelators and hydroxyl-radical scavengers protect against gentamicin-mediated renal damage[14]. Recently, we have reported good antioxidant and chelating activity of *Pleurotus porrigens* extract[6]. So, the possible mechanism of nephroprotection of *Pleurotus porrigens* may be attributed to its antioxidant and free radical-scavenging properties that may be result of presenting phytochemical compounds such as phenols and flavonoids. Other mechanism by which *Pleurotus porrigens* extract ameliorates gentamicin-induced nephrotoxicity remains to be elucidated.

Conclusion

The present study showed that methanolic fraction of *Pleurotus porrigens* has nephroprotective action of gentamicin in male albino mice and hence can be deemed to be a good bioagent for the treatment of acute renal injury induced by nephrotoxins. Further investigations of individual compounds and characterization of bioactive compounds of *Pleurotus porrigens* responsible for the observed significant nephroprotective efficacy is needed.

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