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RESEARCH ARTICLE

Nephroprotective activity of *Macrothelypteris oligophlebia* rhizomes ethanol extract

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Abstract

Context: *Macrothelypteris oligophlebia* (Bak.) Ching (Thelypteridaceae) is a Chinese herbal medicine used traditionally for the treatment of diseases such as edema, boils, burns, and roundworms. However, research about the nephroprotective potential of this plant is not available.

Objective: Present study was designed to evaluate the protective effect of ethanol extract of *M. oligophlebia* rhizomes (EMO) on gentamicin (GM)-induced nephrotoxicity.

Materials and methods: Rats were intraperitoneal (i.p.) injected with GM (100 mg/kg) to induce nephrotoxicity and simultaneously EMO (250 and 500 mg/kg) was orally given to GM-treated rats for 8 days. Blood urea nitrogen (BUN), serum creatinine (Cr), malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were evaluated in renal tissues. Histopathological analysis was used for evaluation of the renal damage.

Results: Administration with GM-induced renal dysfunction in rats. Pre-treatment with EMO (500 mg/kg) significantly decreased the levels of BUN, Cr, MDA and NO (decreased BUN from 12.71 ± 1.28 to 7.19 ± 0.23 mmol/l, Cr from 39.77 ± 5.34 to 19.17 ± 0.90 μmol/l, MDA from 5.60 ± 0.37 to 2.63 ± 0.24 nmol/ml, and NO from 868.17 ± 22.67 to 589.51 ± 8.83 μmol/ml), and also restored the activities of renal antioxidant enzymes (SOD, CAT, and GSH-Px) (restored SOD from 1.59 ± 0.17 to 2.94 ± 0.13 U/mg protein, CAT from 3.22 ± 0.34 to 10.57 ± 0.27 U/mg protein, and GSH-Px from 9.11 ± 1.29 to 20.72 ± 1.83 U/mg protein).

Discussion and conclusion: Our results suggest that the rhizomes of *M. oligophlebia* potentially have a protective role in renal tissue against oxidative stress in acute renal failure.

Keywords: Gentamicin, renal injury, nephrotoxicity, renal oxidative stress

Introduction

Gentamicin (GM), an aminoglycoside class of bactericidal antibiotic, is effective against Gram-negative bacterial infections (Al-Qarawi et al., 2008). However, the clinical use of GM is limited by its major drawback, acute renal failure (ARF), accounting for 10–20% of all cases of nephrotoxicity (Erdem et al., 2000). GM-induced nephrotoxicity is characterized by increased levels of serum creatinine (Cr) and blood urea nitrogen (BUN), decreased glomerular filtration rate (GFR) and morphological alterations (Soliman et al., 2007; Balakumar et al., 2010).

A growing body of experimental evidence both *in vitro* and *in vivo* demonstrates that GM-induced renal injury is believed to involve the generation and release of reactive oxygen species (ROS) in the renal cortex (Yanagida et al., 2004; Polat et al., 2006; Yamam & Balikci, 2010). This is considered as one of the important mechanisms for GM-induced nephrotoxicity and other deleterious effects (Khan et al., 2009). ROS, like super oxide anion, hydroxyl radical and hydrogen peroxide, exhibit beneficial effects on cellular responses and immune function at moderate concentration, but at high levels, ROS can produce

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cell injury including peroxidation of membrane lipids, proteins denaturation and DNA damage (Pham-Huy et al., 2008; Sandeep & Nair, 2010). ROS have been shown to affect several biological processes which are potentially important in glomerular disease and also in the pathogenesis of ischemic renal injury (Shah, 1984; Shah et al., 1987; Parlakpinar et al., 2006). To the best of our knowledge, GM also acts as an iron chelator which is capable of catalyzing free radical formation (Yanagida et al., 2004). Hence, drugs that serve as free radical scavengers or iron chelators may effectively prevent nephrotoxicity induced by GM.

Herbal medicine has a long history in the treatment of renal injury. A number of crude herbal extract, such as *Phyllanthus amarus* Schum. and Thonn. (Euphorbiaceae), *Sida rhomboidea*. Roxb (Malvaceae), and *Nigella sativa* Linn. (Ranunculaceae) have been reported to prevent GM-induced nephrotoxicity (Begum et al., 2006; Adeneye & Benebo, 2008; Thounaojam et al., 2010).

Macrothelypteris oligophlebia (Bak.) Ching (Thelypteridaceae) is widely distributed in southwestern China. Its rhizomes have been used as a folk medicine mainly for the treatment of diseases such as edema, boils, burns, and roundworms (Editorial Board of China Herba, 1998). Study on the chemical constituents of this species has been conducted previously and several flavonoids were isolated, including kaempferol, rutin and kaempferol-3-*O*-β-rutinoside (Hori et al., 1987). However, reports on the pharmacological effects of *M. oligophlebia* have not been documented. The present study was designed to investigate the nephroprotective activity of ethanol extract of *M. oligophlebia* rhizomes against GM-induced ARF *in vivo*.

Materials and methods

Chemicals

Commercial kits for measure BUN and Cr were obtained from Jiancheng Biotechnology Institute (Nanjing, China). Bovine serum albumin and gentamicin (GM) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade and purchased from Sinopharm chemical Reagent Co. Ltd. (Shanghai, China).

Plant material

The air-dried rhizomes of *M. oligophlebia* were collected during June 2009 in the Jiangxi province, China and identified by Prof. Ceming Tan, Forest Plants Specimen Mansion. A voucher specimen (MTF0812) was deposited in College of Pharmacy, Huazhong University of Science and Technology.

Preparation of extract

The air-dried rhizomes of *M. oligophlebia* (1.5 kg) were ground and extracted with 75% ethanol at 95°C for 2 h. The resulting solvent was concentrated in an evaporator to obtain a crude extract and the yield was 10.4 g/100 g of the raw material.

For animals test, an appropriate weight of ethanol extract of *M. oligophlebia* rhizomes (EMO) was dissolved with distilled water to get two dosages (250 and 500 mg/kg body weight).

Phytochemical studies

The ethanol extract of *M. oligophlebia* rhizomes were subjected to column chromatography over silica gel and Sephadex LH-20 repeatedly. The isolated compounds were identified according to the spectral techniques and compared with the previous data reported in the literature.

Test animals

Male Wister rats, weighing 200 ± 20 g each, were purchased from the Research Center of Laboratory Animals, Tongji Medical Center, Huazhong University of Science and Technology, Wuhan, China. The animals were placed under a 12 h light-dark cycle at controlled temperature (23 ± 2°C) and humidity (60 ± 5%). They were fed with standard mice chow and tap water ad libitum and acclimated 7 days before experiment. All animals were fasted 12 h prior to experiment but free access to water. All experiments were conducted in accordance with the Chinese Community guidelines for the use of experimental animals and approved by the Huazhong University of Science and Technology Committee on Animal Care and Use.

Gentamicin-induced acute renal toxicity in rats

Animals were divided into four groups, each group comprises of eight rats. Group I (control group) animals were injected intraperitoneal (i.p.) with saline for 8 days. Group II (GM-treated group) animals were injected i.p. with GM (100 mg/kg body weight) for 8 days (Nitha & Janardhanan, 2008; Thounaojam et al., 2010). Group III and IV animals were administered orally with EMO (250 and 500 mg/kg body weight) 2 h prior to injection i.p. with GM for the same period.

After the last once of the treatment, all animals were sacrificed. Blood samples were collected to determine the serum levels of BUN and Cr. Kidney of each rat was quickly removed, harvested two pieces through a midline incision; one piece was fixed in formalin for pathological examination, the other piece was frozen at –80°C for further enzymatic analysis.

Assessment of renal function

BUN and Cr levels in serum samples were assayed by using standard diagnostic kits.

Measurement of renal antioxidants

Kidneys were rinsed immediately with 0.9% cold physiological saline and then homogenized with ice cold phosphate buffer (1:10, w/v) using a homogenizer. The obtained homogenate was centrifuged at 800 rpm for 10 min at 4°C, the resulting supernatant was further centrifuged at 10,500 rpm for 20 min at 4°C to yield the post

mitochondrial supernatant, which was used to determine the superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), nitric oxide (NO) and glutathione peroxidase (GSH-Px) (Paglia & Valentine, 1967; Marklund & Marklund, 1974; Zhong et al., 2009; Mohan et al, 2010; Koppula & Choi., 2011). Protein concentrations in tissue homogenates were determined by the method described previously using bovine serum albumin as the standard (Bradford, 1976).

Histopathological evaluation

The tissue samples were fixed in 10% neutral buffered formalin for 1 day, dehydrated in ascending graded series of alcohol and embedded in paraffin, 6 μm of the tissue sections were stained with hematoxylin and eosin. The kidney tissues were examined under light microscope to evaluate their pathological symptoms such as lymphocyte infiltration, necrosis and cytoplasmic vacuolization.

Statistical analysis

Data were represented as mean ± standard deviation (S.D.). Results were analyzed by one-way ANOVA followed by the Dunnett *post hoc test*. Differences were considered significant at $p < 0.05$.

Results

Phytochemical studies

The isolated compounds were identified as matteucinol, naringenin, naringenin-4′-*O*-glucoside, protoapigenone, 5,7-dihydroxy-6,8-dimethyl flavanone and protoapigenin-4′-*O*-β-D-glucoside. All these components belong to the flavonoid group.

Effect of EMO treatment on renal function

As shown in Table 1, serum levels of BUN and Cr were marked higher in GM-treated group as compared to the control group, indicating the formation of severe nephrotoxicity. Pre-treatment with EMO significantly attenuated the increased levels of BUN and Cr induced by GM.

Effect of EMO treatment on kidney tissue enzymes

The activities of SOD, NO, CAT, MDA, and GSH-Px in each group were shown in Table 1. In comparison with the control group, rats that only treated with GM significantly elevated the concentrations of MDA and NO, and decreased the activities of SOD, CAT and GSH-Px. Administration of EMO to rats resulted in a marked reduction in MDA and NO levels, and an increase in SOD, CAT and GSH-Px activities.

Histopathological observations

Histology of kidney sections from rats treated with GM alone showed obvious features of ARF (Figure 1B), as compared to the control group (Figure 1A). From the picture, marked tubular necrosis in renal cortex was observed. Pre-treatment with 500 mg/kg dose of EMO provided the best histological protection against GM-induced renal tubular damage.

Discussion

Preliminary phytochemical studies on EMO revealed that flavonoids were its characteristic constituents. Flavonoids are polyphenolics that commonly occur in herbal medicine. They have been proposed to have the ability to scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative stress that give rise to deteriorative disease (Jadeja et al., 2009; Mishra et al., 2010). These six known flavonoids obtained from EMO were isolated for the first time from this plant. Naringenin and protoapigenone have been shown to possess antioxidant, nephroprotective, neuroprotective and antitumor activities (Badary et al., 2005; Youdim & Bakhle, 2006; Huang et al., 2010). In this study, the nephroprotective activity of ethanol extract of *M. oligophlebia* rhizomes was evaluated for the first time.

The nephroprotective activity of EMO was assessed on renal injury induced by GM in rats. GM is widely used to investigate nephroprotective activity in animal models. One mechanism of GM-induced nephrotoxicity is believed to involve in renal oxidative stress (Khan et al., 2009; Balakumar et al., 2010; Yaman & Balikci, 2010). Moreover, this underlying mechanism has been supported by the fact that administration of several components with antioxidant properties, free radical scavengers and antioxidant enzyme are capable of preventing GM-induced nephrotoxicity (Balakumar et al., 2010). This, for instance, including vitamin E (Varzi et al., 2007),

Table 1. Effect of EMO on BUN, Cr, SOD, MDA, CAT, NO and GSH-Px activities in rats treated with GM.

Treatment	BUN (mmol/l)	Cr (μmol/l)	SOD (U/mg protein)	MDA (nmol/ml)	CAT (U/mg protein)	NO (μmol/ml)	GSH-Px (U/mg protein)
Control (normal saline)	5.72 ± 0.43	17.56 ± 3.32	2.94 ± 0.21	2.99 ± 0.24	10.63 ± 0.52	593.50 ± 12.19	23.33 ± 0.85
GM (100 mg/kg)	12.71 ± 1.28**	39.77 ± 5.34*	1.59 ± 0.17*	5.60 ± 0.37*	3.22 ± 0.34*	868.17 ± 22.67*	9.11 ± 1.29*
GM + EMO (250 mg/kg)	8.59 ± 0.46**	24.69 ± 2.10*	2.56 ± 0.15*	3.14 ± 0.22*	9.97 ± 0.38*	555.37 ± 17.32*	15.05 ± 3.02*
GM + EMO (500 mg/kg)	7.19 ± 0.23**	19.17 ± 0.90	2.94 ± 0.13	2.63 ± 0.24	10.57 ± 0.27	589.51 ± 8.83	20.72 ± 1.83

Values are mean ± S.D.

BUN, blood urea nitrogen; CAT, catalase; Cr, serum creatinine; EMO, ethanol extract of *Macrothelypteris oligophlebia* rhizomes; GM, gentamicin; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase.

*$p < 0.05$ when compared with the control group.

**$P < 0.01$ when compared with the control group.

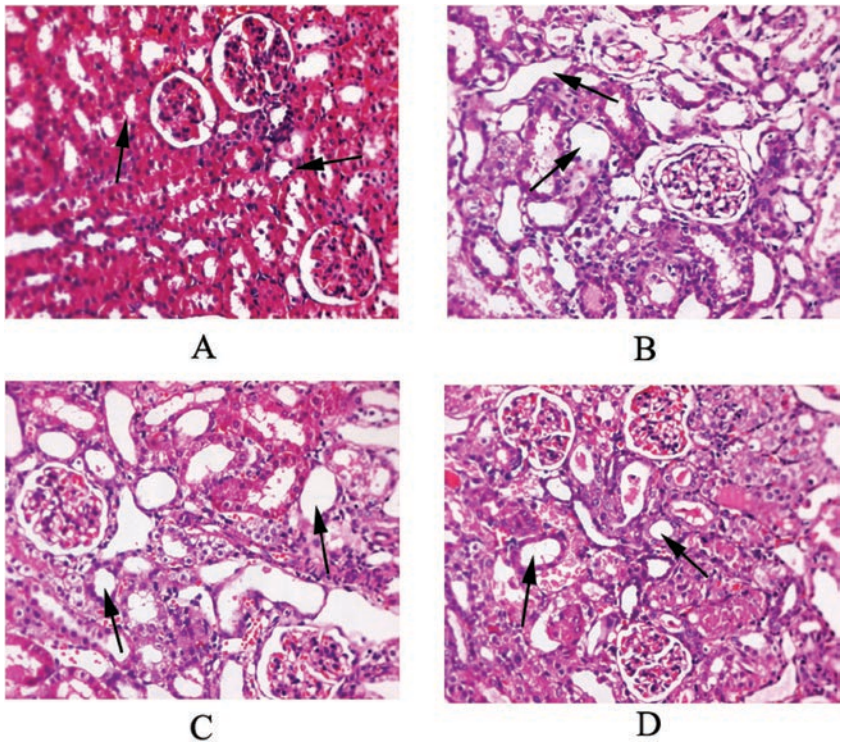

Figure 1. Representative imagines of kidney from rats. (A) Control group: section shows normal tubules; (B) Gentamicin (100 mg/kg) treated group: tubule shows extensive and marked necrosis; (C) Gentamicin + EMO (250 mg/kg) treated group: tubules show limited damage; (D) Gentamicin + EMO (500 mg/kg) treated group: tubule reveals slight degenerative change. EMO, ethanol extract of *Macrothelypteris oligophlebia* rhizomes.

N-acetylcysteine (Mazzon et al., 2001), SOD mimetic (Cuzzocrea et al., 2002), and curcumin (Farombi & Ekor, 2006). Furthermore, our results confirm the hypothesis that ARF is related to renal oxidative injury.

In the present study, we have shown that administration of GM at a dose of 100 mg/kg eventuated in renal dysfunction indicated by the elevated levels of Cr and BUN as well as histopathological evaluation which revealed marked and extensive tubular necrosis throughout the cortex. Oral administration of EMO was found to have a protective potential against GM-induced nephrotoxicity. Increased levels of Cr and BUN, accompanied by the tubular necrosis in renal tissue were significantly prevented by EMO, suggesting an almost normalization of kidney function.

The activities of the renal antioxidant enzymes, such as SOD, CAT and GSH-Px, obviously depleted in the GM-treated alone group compared to the control group. This deficiency in renal antioxidant defense enzymes could aggravate the renal oxidative damage. SOD is an enzyme associated with copper, zinc, and manganese which are essential for enzyme activity (Sharma, 1985). It is said that SOD has the distinct ability to neutralize superoxide, one of the most damaging free radical substances in nature. The decreased SOD activity is not enough to scavenge the superoxide anion produced during the normal metabolic process and cause the initiation and propagation of lipid peroxidation in GM-treated group (Nitha & Janardhanan, 2008). The activities of CAT and GSH-Px also decreased in the GM-treated group, which resulted in the decreased ability of renal to scavenge toxic hydrogen peroxide and lipid peroxidation (Badary et al., 2005; Nitha & Janardhanan, 2008). Therefore, the significant increase in the level of MDA, as marker of lipid peroxidation, was observed in the GM-treated group. Pre-treatment with EMO to rats reduced the depletion of SOD, CAT and GSH-Px activities, resulting in reduction of the renal MDA. In addition, our experimental data indicated that the increasing generation of NO was also reduced by EMO, and renal NO played a vital role in the regulation of GFR (Safa et al., 2010). The results obtained in this study suggested that EMO was capable of ameliorating GM-induced renal damage.

Conclusion

In summary, the results of the present study demonstrated that ethanol extract of *M. oligophlebia* rhizomes showed significant nephroprotective activity against GM-induced ARF in rats. Additionally, further studies are essential to find out the active compounds from EMO that are responsible for its nephroprotective role.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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