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Effect of methanolic extract of aerial parts of *Merremia tridentata* on gentamicin-induced nephrotoxicity in rats

Sreedevi Adikay*, R.Radha, Bharathi Koganti

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila, Viswavidyalayam, Tirupati-517502, (INDIA)

E-mail: srideviturupati@rediff.com.

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ABSTRACT

Methanolic extract of aerial parts of *Merremia tridentata* (300 & 600 mg/kg, p.o.) was screened for its nephroprotector activity against gentamicin-induced (40mg/kg) renal damage in curative and prophylactic models. Nephrotoxicity was assessed by measuring serum markers level (blood urea nitrogen, serum creatinine) and urinary protein excretion. Gentamicin-induced renal toxicity characterized by significant elevation of blood urea nitrogen, serum creatinine and high urinary protein excretion. Among the two regimens, more pronounced activity was observed in curative model. In curative model, methanolic extract showed dose dependent activity. Animals which received prophylactic treatment exhibited moderate protection against gentamicin-induced kidney damage. Present results suggested methanolic extract showed nephroprotector activity against gentamicin-induced nephrotoxicity. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Merremia tridentata;
Nephrotoxicity;
Gentamicin;
Serum markers;
Urinary protein.

INTRODUCTION

Gentamicin is an aminoglycoside antibiotic commonly used in treating of life threatening gram-negative bacterial infections. However 30% of the patients treated with gentamicin for more than seven days show some signs of nephrotoxicity^[1] and serious complications resulting from gentamicin-induced nephrotoxicity are limiting factor for its clinical usage. Although the mechanism of gentamicin-induced nephrotoxicity is not clear, several *in vivo* and *in vitro* studies suggested that the oxygen free radicals play an important role^[2]. Previous reports evidenced that substances like arabicgum^[3] lycopen^[4], Caffeic acid phenyl ester^[5], curcumin^[6] have been effectively reduced the gentamicin-induced renal damage. Further Plants which contain antioxidant principles such as *Pongamia pinnata*^[7] partially reduced the nephrotoxicity induced by gentamicin. *Merremia tridentata* (F;Convolvulaceae) is one such plant which is a small glabrous plant found in south India. The plant

reported to be used in traditional medicine to treat several ailments like rheumatism, haemorrhoids, inflammation and urinary disorders by tribes of Chittoor District (Andhra Pradesh, India)^[8]. Detailed literature survey showed the absence of any experimental data to justify the nephroprotective role of this plant. Hence the present study was focused on the pharmacological validation and justification of its use in the traditional system of medicine.

MATERIALS AND METHODS

Plant material

Aerial parts of the *Merremia tridentata* (*M.tridentata*) were collected from Chandragiri, Chittoor District, A.P., India in the month of August, 2005 and authenticated by botanist Dr. Madhavachetti, Herbarium keeper, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. And a herbarium specimen has been deposited in the Depart-

ment of pharmacognosy, Institute of pharmaceutical Technology, Sri Padmavathi University, Tirupati, Andhra Pradesh, India. The plant material was shade dried and powdered.

Drugs and chemicals

Gentamicin was purchased from Sigma (M.O., U.S.A.), urea, creatinine, protein estimation kits were procured from Dr. Reddy's laboratories, Hyderabad, A.P., India.

Preparation of methanolic Extract: The shade dried coarsely powdered aerial parts were (1kg) exhaustively extracted with methanol in Soxhlet's apparatus. The solvent was removed under reduced pressure (yield 35%) and the extract was used for pharmacological studies.

Phytochemical studies

Preliminary phytochemical screening revealed the presence of steroids, phenolic compounds and triterpenoids^[9].

Pharmacological studies

Animals

The study was performed on Wistar strain albino rats of either sex (120 days) weighing 150-200g. They were maintained on standard diet (Gold Mohar pellets, Bangalore) and water was given *ad libitum*. They were housed in polypropylene cages and were acclimatized to laboratory environment for about a week. The study was conducted after obtaining Institutional ethical committee clearance.

Acute toxicity studies

Animals were divided into groups and each group containing six animals first group received vehicle (control) and remaining groups received increasing doses of (30, 100, 300, 600, 1000 and 3000mg/kg) methanolic extract of aerial parts of *M.tridentata* suspended in 1% tween 80. The animals were observed continuously for 2 hrs for the gross behavioral changes and then intermittently once every 2 hrs. and finally at the end of 24 and 72 hrs to note any other toxic signs including death.

Experimental protocol

Animals were divided into 7 groups of 6 animals each and put on treatment schedule described in TABLE 1. To induce nephrotoxicity in rats, gentamicin was administered at 40 mg/kg, subcutaneously, for 13 days.

Methanolic extract was prepared in 1% tween 80 (40mg/ml) and extract was administered orally by gastric intubation.

Assessment of renal function

Blood urea nitrogen {(BUN) diacetyl monoxine method}, Serum creatinine {(SC) alkaline picrate method} were estimated by the methods as reported by Godkar^[10]. Urine was collected on days 14 and 24 for 6hrs by use of metabolic cages and analyzed for urinary protein (sulphosalicylic acid method)^[11].

Statistical analysis

Values are expressed as means \pm s.e.m. Results were compared by ANOVA followed by student's Newman-Keuls test. Statistical significance was set at $P < 0.001$.

RESULTS AND DISCUSSION

Acute toxicity studies

Administration of the methanolic extract of *M.tridentata* produced no toxic effects up to 3 g/kg in rats even after 72 hrs.

Effect of methanolic extract on gentamicin-induced nephrotoxicity

Treatment of methanolic extract of aerial parts of *M.tridentata* for 10 days (Group 7) did not cause any significant changes in serum markers level and excretion of protein in urine when compared with control animals. Hence, extract of *M.tridentata* did not show any deteriorative effects on kidney.

TABLE 1: Treatment schedule-nephroprotector activity

Group	Treatment	No. of days	Day of withdrawal of blood/urine	Purpose
1	Tween 80	1- 23	24	Control
2	Tween 80 + Gentamicin	1- 13	14	Prophylactic control
3	Methanolic extract (600mg/kg)+ Gentamicin	1-13 1 - 13	14	Prophylactic activity
4	Gentamicin + Tween 80	1-13 14 -23	24	Curative control
5	Gentamicin + Methanolic extract(300 mg/kg)	1-13 14 -23	24	Curative activity
6	Gentamicin +Methanolic extract(600 mg/kg)	1 - 13 14-23	24	Curative activity
7	Methanolic extract(600 mg/kg)	1 - 10	11	Effect of extract on kidney

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TABLE 2: Effect of methanolic extract on gentamicin-induced nephrotoxicity

Group	BUN(mg/dl)	SC(mg/dl)	U _p (mg/24hr)
1	30.8±0.4	0.6±0.03	7.0±0.04
2	41.0±0.4 ^a	1.3±0.03 ^a	14.4±0.04 ^a
3	34.2±0.3 ^b	0.7±0.02 ^b	8.0±0.04 ^b
4	44.8±0.5 ^a	1.7±0.09 ^a	16.5±0.07 ^b
5	33.8±0.3 ^c	0.9±0.02 ^c	8.7±0.08 ^a
6	28.0±0.3 ^c	0.6±0.02 ^c	7.2±0.02 ^c
On day 13	93.8±6.4 ^a	2.1±0.08 ^a	18.2±0.3 ^c

Values were expressed mean ± SEM each group containing 6 animals (one-way ANOVA followed by Student's Newman-Keuls post hoc test); ^aP<0.001 compared to normal control; ^bP<0.001 compared to prophylactic control; ^cP<0.001 compared to curative control

Effect of methanolic extract of aerial parts of *M.tridentata* on gentamicin-induced renal toxicity was listed in TABLE 2.

Gentamicin is an aminoglycoside antibiotic which has been reported to cause nephrotoxicity in man and in experimental animals. Morphological studies suggested that gentamicin nephrotoxicity is cited with necrosis of proximal tubular cells^[12,13]. Acute tubular necrosis is a prominent feature of gentamicin nephrotoxicity. It is clinically assessed by elevation of Blood Urea Nitrogen (BUN), Serum Creatinine (SC), Proteinuria^[14]. Other clinical indices of renal function impairment including Oliguria, azotemia and uremia.

Daily subcutaneous administration of gentamicin (40 mg/kg) alone for days 13 in Group-2 animals caused renal impairment characterized by significant elevation of serum markers level and increased the excretion of protein when compared to normal control animals. In curative regimen, animals which received both gentamicin and extract reversed the effects that are caused by gentamicin i.e., reduced the level of serum markers and decreased the urinary protein excretion when compared to Group-2 animals and the protection was dose dependent.

Animals which received prophylactic treatment of methanolic extract of aerial parts of *M.tridentata* partially but significantly protected the gentamicin-induced effects.

The present study revealed that subcutaneous administration of gentamicin (40mg/kg) caused significant nephrotoxicity as characterized by increase in BUN, SC levels and urinary protein excretion was increased. Animals which received curative treatment at 600mg/kg dose exhibited higher nephroprotector activity.

Earlier reports on phytochemical studies on aerial parts of *M.tridentata* reported to contain flavonoids such as Diosmetin, Luteolin and their glycosides^[15]. Fla-

vonoids exhibits several biological effects such as anti-inflammatory, antihepatotoxic and antiulcer actions^[16,17]. They are potent antioxidants and have free radical scavenging abilities^[18]. Hence the possible mechanism by which the aerial parts of the extract exerts nephroprotection could be attributed to its free-radical scavenging property. Further investigation is needed to determine the exact phytoconstituents that are responsible for its nephroprotector activity.

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