Vol. 9/2 (2009) 165 - 169

JOURNAL OF NATURAL REMEDIES

Protective effect of *Trianthema portulacastrum* Linn leaves on gentamicin induced nephrotoxicity in rats

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Abstract

Objective: To evaluate the nephro protective effect of ethanolic extract of *Trianthema portulacastrum* leaves in gentamicin induced renal damage in rats. Materials and methods: The nephro protective activity of ethanolic extract of *T.portulacastrum* leaves was evaluated against gentamicin induced renal damage in rats. Various biochemical parameters were assessed and histopathological section of the kidney was taken. The protective effect was further studied from analyzing the potential of the extract to scavenge the free radicals. Results: i.p administration of ethanolic extract of *T.portulacastrum* restored the levels of the biochemical factors determined significantly and exhibited a significant potential to scavenge free radicals with respect to control. Conclusion: The ethanolic extract of *T.portulacastrum* exhibited significant ($P < 0.001$) nephro protective activity.

Key Words: Antioxidant, *Trianthema portulacastrum*, Gentamicin, nephrotoxicity.

1. Introduction

The plant *Trianthema portulacastrum* is a diffuse, prostrate, branched glabrous or papillose, succulent annual herb [1] naturalized in India as a weed in fallow rice fields, river beds, waste lads, railway tracks, common flood and frost throught the year. Abundant during rainy season, often used as a vegetable [2]. Two forms are reported to occur, a red coloured form in which the stem, leaf margin and flowers are red; and a green coloured form, which has a green stem and white flowers [3]. The plant is bitter, hot, analgesic, stomachic, and laxative. Cures bronchitis, heart diseases, diseases of the blood and inflammations [4]. The leaves possess diuretic properties [5, 6]; found useful in edema, dropsy and ascites especially due to early liver, peritoneal and kidney conditions [5].

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2. Materials and Methods

2.1 Preparation of T.portulacastrum leaf extracts (EETP)

Fresh plants were collected from Chennai and authenticated by Botanical Survey of India, Coimbatore, India. Voucher specimen of the plant is deposited for future reference. The leaves were separated and washed with running water to remove any adherent impurities and shade dried. The dried leaves were powdered and extracted with ethanol 90% as the solvent by cold maceration process. The extract was reduced to a molten mass by rotatary vaccum evaporator at 40°C.

2.2 Experimental Animals

Healthy, male albino Wistar rats of either sex each weighing 150 – 200 g was used. The rats were housed in polypropylene cages and maintained under standard conditions. Standard pellet feed and water were provided *ad libitum*. The study was approved by the Institutional Animal Ethical Committee of C.L.Baid Metha college of Pharmacy, Chennai. (IAEC 9- 16/CLBMCP/2006 – 2007 dated 14 - 12 – 2006).

2.3 Nephroprotective Activity

Eighteen animals were used and divided into three groups of six each. They were designated as G1, G2 and G3. G1 served as normal control receiving saline, G2 as negative control receiving Gentamicin 100 mg/kg/day, G3 received concomitant administration of EETP 200 mg/kg/day, and 100 mg/kg /day of gentamicin for 14 days by intraperitonial route [7]. After dosing on 14th day, individual rats were placed in separate metabolic cages for 24 hours for urine collection to determine urine output and urine creatinine content [8]. Blood samples were collected by retro – orbital puncture at the end of these 24 hours. The serum was separated and processed for determination of Blood Urea Nitrogen (BUN) and serum creatinine using commercially available kits of Span Diagnostics ltd. India [9]. Three rats per group were sacrificed and both kidneys were isolated from each rat [10]. The kidneys were processed for histopathological examination [11].

2.4 Histopathological Examination

The kidneys were sectioned longitudinally in two halves and kept in 10 % neutral formalin solution [11]. They were embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and observed under microscope.

2.5 In vitro anti oxidant activity

For all the *in vitro* antioxidant models mentioned below ascorbic acid was used as a reference standard. The concentrations of ascorbic acid were 10, 20, 30, 40, 50 µg/ml and that of the extract were 50, 100, 150, 200, 250 µg/ml.

2.6 DPPH free radical - scavenging activity

The method based on the reduction of a methanolic solution of the coloured free radical 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was used [12]. The methanolic solution of DPPH (0.1mM, 1ml) was incubated with 3 ml of different concentrations of the leaf extract ranging from 50 to 250 µg/ml. Incubation was carried out in room temperature for 30 min. For each concentration, the assay was run in triplicate. At the end of the incubation period, the optical density of each sample was determined at 517 nm [13]. EC_{50} values for both ascorbic acid and the leaf extract were determined.

2.7 Nitric oxide radical scavenging activity

Free radical scavenging activity was evaluated by studying the inhibition of the generation of Nitric oxide from Sodium Nitroprusside using Griess Illosvay reaction [12, 14].

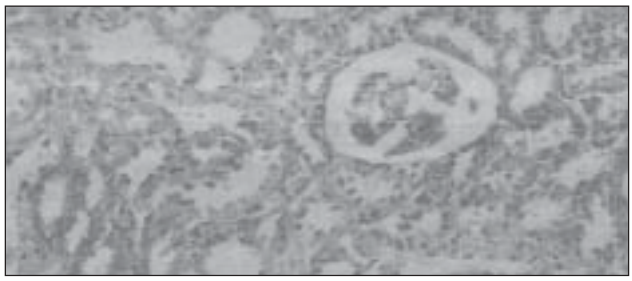

Fig. 1. Group I Positive Control receiving saline showing normal architecture

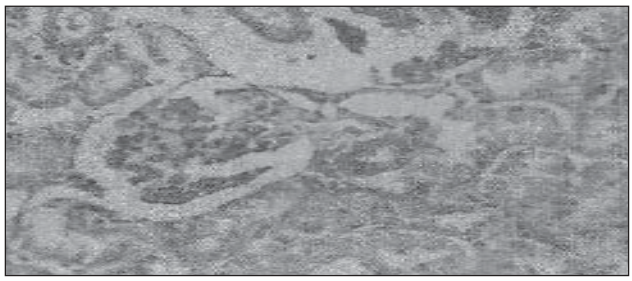

Fig. 2. Group II Gentamicin treated showing glomerular and peritubular congestion

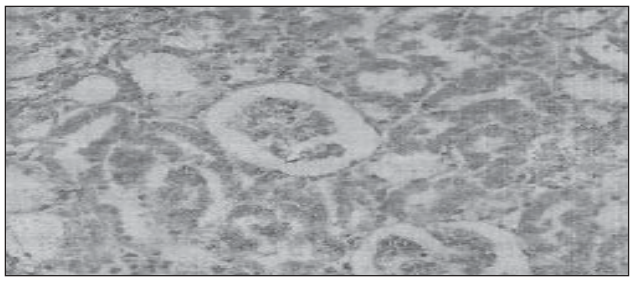

Fig. 3. Group III Gentamicin + Ethanolic extract of *T.portulacastrum* treated showing restored cell damage to near normalcy

Table 1: Parameters studied for the nephroprotective activity of the ethanol extract of leaves of *Trianthema portulacastrum.*

Parameter	Control (Mean±SEM)	Gentamicin (Mean±SEM)	Gentamicin and *T.portulacastrum* (Mean±SEM)
Body weight (g)	173 ± 1.6	152 ± 1.7^{*a}	168 ± 1.8
Weight of Kidney (g)	0.54 ± 0.03	0.69 ± 0.04	0.51 ± 0.021
Urine volume (ml)	2.8 ± 0.17	2.1 ± 0.12	5.6 ± 0.17^{*b}
Urine creatinine (mg/dl)	94 ± 1.9	243 ± 1.5^{*a}	101 ± 1.3
Serum creatinine (mg/dl)	0.68 ± 0.012	1.03 ± 0.021^{*a}	0.63 ± 0.017
Blood urea (mg/dl)	40 ± 0.56	109 ± 0.51^{*a}	47 ± 0.62
Blood urea nitrogen	18 ± 0.32	51 ± 0.28^{*a}	22 ± 0.33

n = 6 in each group, ^{*a} P<0.05 when compared to control (group I), ^{*b} P<0.05 when compared to Group II. Data analyzed by Dunnet's *'t'* test.

Table 2: EC_{50} concentrations of the ethanol extract of *Trianthema portulacastrum* and ascorbic acid in antioxidant models.

Antioxidant activity	EC_{50} of standard ascorbic acid (µg/ml)	EC_{50} of Ethanol extract of *T.portulacastrum* (µg/ml)
DPPH free radical scavenging activity	14.5	110.25
Nitric oxide scavenging activity	19.75	96

In this assay 1.0 ml of Sodium Nitroprusside (5 mM) in phosphate buffered saline (PBS) was mixed with 3.0 ml of different concentrations ranging from 50 to 250 µg/ml of the leaf extract dissolved in distilled water. This assay mixture was then incubated at room temperature for 150 minutes. These solutions were treated with Griess' reagent and the optical density of the resultant chromophore was determined spectrophotometrically at 546 nm and compared with the absorbance of the standard solutions of ascorbic acid. The experiment was run in triplicate. As a blank, assay mixture without extract and ascorbic acid were determined.

2.8 Statistical analysis

The data obtained was analyzed using one-way ANOVA followed by Dunnet's t test. $p < 0.05$ was considered significant.

3. Results and Discussion

Urine creatinine, serum creatinine, blood urea, blood urea nitrogen and the weights of the kidneys were found to be significantly increased in rats treated with only gentamicin; the treatment with ethanol extract of the *T.portulacastrum* was found to protect the tested animals from such effects of gentamicin (Table 1). Urine volume was significantly increased in the group treated with the leaf extract. The body weight of the negative control groups was found to be reduced significantly in comparison with positive saline control and *T.portulacastrum* treated group. The Histopathological sections of the negative control showed peritubular congestion and blood vessel congestion resulting in inflammations in the renal cells. Concurrent administration with the extract reduced such changes induced by gentamicin (Fig. 1, 2 & 3). The *in vitro* antioxidant activities studied revealed that the extract possesses significant potential to scavenge free radicals (Table 2).

Various studies have shown that gentamicin induces renal damage by free radical generation [15]. Gentamicin in addition to oxidative stress also alters the lysosomal membranes and activates phospholipases [16]. Hence, synthetic and natural antioxidants and free radical scavengers are claimed to provide nephroprotection due to gentamicin injury. Hence, the probable mechanism of nephroprotection by *Trianthema portulacastrum* may be attributed to its antioxidant and free radical scavenging property.

To conclude, the study has shown that the leaves of *Trianthema portulacastrum* possess marked nephroprotective activity with minimal toxicity and thus has a promising role in the treatment of renal injury induced by nephrotoxins, especially gentamicin. Further isolation of active components and its nephroprotective activity in chronic renal failure model have to be evaluated.

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