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Original Article

Protective Effect of *Trichosanthes Dioica* Extract Against Gentamicin Induced Nephrotoxicity in Rats

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ABSTRACT

Introduction: Nephropathy is one of the progressive complications prevailed all around the world that is observed which arises due to detrimental effects of metabolites created due to various metabolic and physiological reactions. Gentamicin is one of the drug that may cause nephrotoxicity. **Objectives:** Phytoremedies are being considered as to play deterrent role in the treatment of disease. *T. dioica* is widely distributed in Asian subcontinent and consumed as food. The present work is aimed to study nephroprotective activity of *T. dioica*. **Subjects and Methods:** Dried *T. dioica* fruits were extracted with methanol and the extract was subjected to phytochemical analysis. Acute toxicity was determined as per OECD guidelines. Gentamicin (80 mg/Kg) was used to induce nephrotoxicity. The extract was given orally. Kidney weight was determined and estimation of blood urea nitrogen, serum creatinine, uric acid, catalase, superoxide dismutase, glutathione peroxidase, lipid peroxidation, urinary electrolytes was performed. **Results and Discussion:** Decrement in serum urea and creatinine along with reduction in uric acid was observed. Similarly, restoration of antioxidant enzymes and normalization of urinary electrolytes and kidney weight was observed. Observations from present study divulged that *T. dioica*, which is a good source of antioxidant phytochemicals, is accountable for nephroprotective activity.

Key words: *T. dioica,* Nephroprotective, Histopathology, Uric acid, Creatinine, Urea nitrogen.

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INTRODUCTION

Kidney is regarded as a principal organ which targets to remove toxic effects of drugs, xenobiotics and their metabolites from the body. Oxidative stress raised during this process is responsible for predisposition of glomerular diseases.[1,2] Nephropathy is one of the progressive complications prevailed all around the world that is observed regardless of age, race and location. Such a state arises due to detrimental effects of metabolites created due to various metabolic and physiological reactions. Nephropathy is ranked as one of the ten causes of death worldwide.[2]

Gentamicin, an amino-glycoside antibiotic, is widely recognized for antistaphylococcula activity.[3] Excretion of gentamicin is observed by it accumulation in renal tubules especially in, S1, S2, and S3 segments of the proximal tubules, as well as in the distal tubules and collecting ducts.[4] Such a fact establishes gentamicin to be a nephrotoxic agent. In albino rats, its toxic dose is established as 80 mg/kg[5,6] and thus proves to be fatal as nephrotoxin.

In the current times, there has been considerable interest in discovery of novel drugs from natural origin. Phytoremedies are being considered as to play deterrent role in the treatment of disease. Herbal medicines are rich in polyphenols and flavonoids that are well established as antioxidants.[7]

Trichosanthes dioica Roxb, (Cucurbitaceae), is a perennial herb widely distributed in Asian subcontinent. It is mainly cultivated as a vegetable. *T. dioica* can be considered as a rich source of many medicinally important constituents like alkaloids, glycosides, flavonoids, carbohydrates, fixed oils, steroids, tannins, and phenols. Traditionally leaves juice of *T. dioica* is used as febrifuge, tonic, in alopecia, and in treatment of liver enlargement. According to Ayurveda, it is useful as a diuretic, cardiotonic, antiulcer, laxative etc.[7] Based on the above observations and the presence of diverse phtyoconstituents, the present work is aimed to study nephroprotective activity of *T. dioica*.

MATERIALS AND METHODS

Plant Material

T. dioica (Hindi: Parwal) was purchased from local grocery of Jabalpur and authenticated by Dr. AB Tiwari, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (MP) (Herbarium No: HP/PPHY/301). *T. dioica* was collected in the month of November and dried in shade. It was coarsely powdered and used for preparation of extract.

Extraction

Plant material (100 g) was coarsely ground and subjected to defatting by petroleum ether. The defatted material so obtained was extracted with ethanol (95%). Finally, the extract was dried at 40°C under pressure and stored at 4°C until use. The extract was subjected to phytochemical screening.[8]

Chemicals and Drugs

Gentamicin was purchased from Central Drug House (CDH), India. Silymarin was obtained from Microlabs (Pondicherry, India). All the other chemicals were purchased from CDH, India.

Animals

Healthy adult male wistar albino rats (5-6 month; 150-200 g) were used. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 30°C; and 35-60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai India) and water *ad libitum*. All the animal experimental protocols were approved by Institutional Animal Ethics Committee (Approval No: SRIT-P/IEAC/PPHY/301).

Acute Toxicity Study

Acute toxicity studies were performed according to Organization for Economic Cooperation and Development (OECD) guidelines.[9] Rats

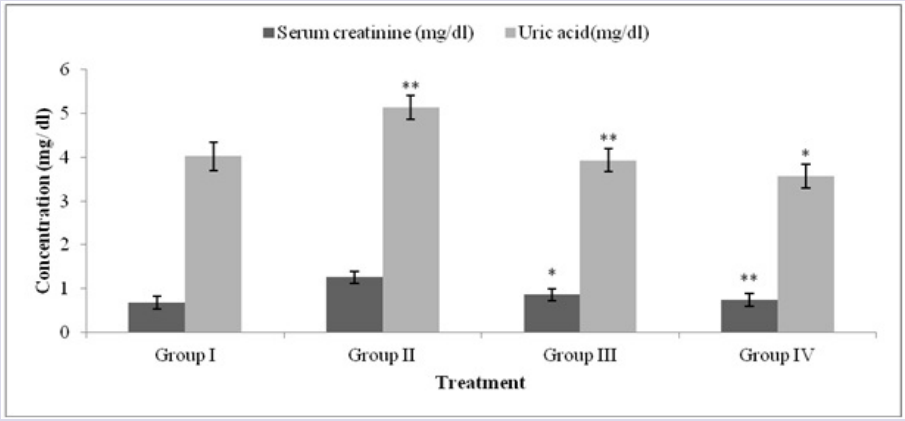

Figure 1: Effect of ethanol extract of *T. dioica* on serum creatinine and uric acid in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of five animals in each group. Extract-treated groups compared with the control group. Significance considered as *P<0.01 and **P<0.001.

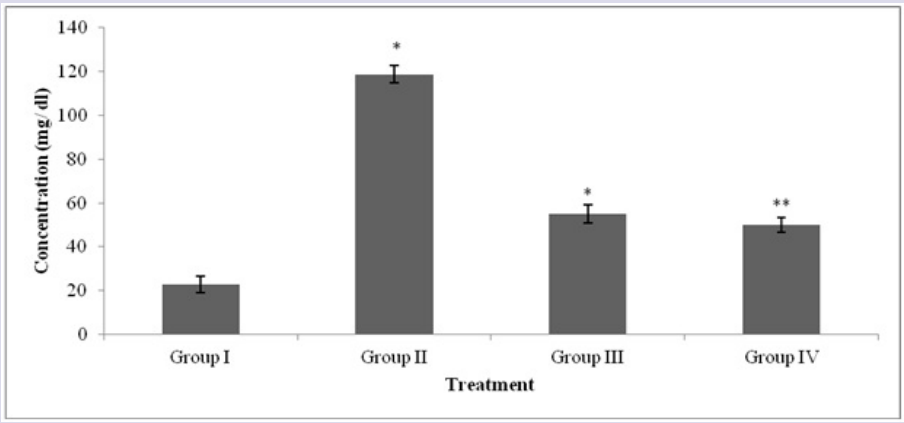

Figure 2: Effect of ethanol extract of *T. dioica* on blood urea in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of five animals in each group. Extract-treated groups compared with the control group. Significance considered as *P<0.01 and **P<0.001.

weighing between 150 and 200 g were used. The animals were fasted for 4 h with free access to water only. *T. dioica* extract was administered orally in doses of 2,000 mg/kg rats and observed over 14 days for mortality and physical/behavioral changes. Thus a dose of 250 and 500 mg/kg of extract was used in this study. The extract was suspended in 1% carboxymethyl cellulose and administered to animals via oral route.

Experimental Protocol

Group I served as control and received distilled water p.o. for 8 days. Group II served as gentamicin group. The gentamicin treated group received 100 mg/kg/day gentamicin by the intraperitoneal (i.p.) route. Group III and IV received 200 and 400 mg/kg body weight (of extract of *T. dioica*) along with gentamicin. After dosing on the day 8, individual rats were placed in separate metabolic cages for 24 h for urine collection to determine urine creatinine content.

Blood collection

A period of 24 hours after the last treatment, the animals were anesthetized with ether and by bilateral prilumbal vertical incisions; blood was collected by cardiac puncture. Blood so collected was kept at room temperature for about half an hour to clot. Serum from coagulated blood was separated and stored before biological analysis.

Physiological, Biochemical and electrochemical analysis

Kidney weight

The kidney was removed, weighed and morphological changes were observed.

Kidney function test

Blood urea nitrogen[11] and serum creatinine[12] were estimated by methods reported elsewhere.

Uric acid estimation

Uric acid was estimated by a method reported by Trinder.[13]

Kidney antioxidant assessment status

Catalase,[14] superoxide dismutase,[15] glutathione peroxidase[16] and lipid peroxidation[17] were estimated by reported methods.

Urinary electrolytes

Estimation of urinary electrolytes viz. Na^+ and K^+ was performed by flame photometry.[18]

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was carried out by using one-way ANOVA followed by Dunnet'st test and p<0.001 was considered significant.

RESULTS

Acute toxicity studies

T. dioica extract did not show any toxicity at a dose of 2000 mg/kg as evidenced by observations. No signs of abnormal behavior or mortality were observed during the study period. Thus, dose of 250 mg/ kg and 500 mg/ kg of extract were selected for further studies.

Biochemical studies

In the gentamicin treated group of rats, concentration of serum urea and creatinine were significantly increased as compared to the normal animals (Group I) which indicate severe nephrotoxicity. Treatment (Group III & IV) with T. dioica extract showed significant decrease ($p<0.001$) in concentration of creatinine and serum urea as compared to gentamicin treated (Group II) (Figure 1 and 2). On the other hand, the concentration of uric acid was increased considerably in the gentamicin treated groups (group II) as comparable with control group (Group I). Treatment with *T. dioica* extract significantly ($p<0.05$) decreaseed the uric acid levels in experimental rats (Group 3 & 4) ($p<0.01$) compared to Gentamicin treated group (Group II) (Figure 1).

Urinary sodium and potassium and kidney weight

In gentamicin treated group of rats, the concentration of urinary sodium and potassium considerably decreased ($p<0.001$) as compared to normal animals. Along with this, treatment (Group III & IV) with the *T. dioica* extract significantly ($p<0.01$) increased the concentration of urinary sodium and potassium compared to gentamicin treated group (Group II) (Figure 3).

Kidney weight

In the gentamicin treated group of animals,the weight of the kidneys were considerably increased compared to normal animals (Group I). The *T. dioica* extract treatment groups (Group III&IV) showed significant decreases ($p<0.001$) in kidney weight (Figure 3).

Kidney antioxidant status

There was a significant diminution of activity of catalase, superoxide dismutase and glutathione peroxidase in gentamicin treated animals (Group II) when compared to normal animals (Group I) (Figure 4). Treatment with *T. dioica* extract significantly restored the level of catalase, superoxide dismutase and glutathione peroxidase compared to genta-

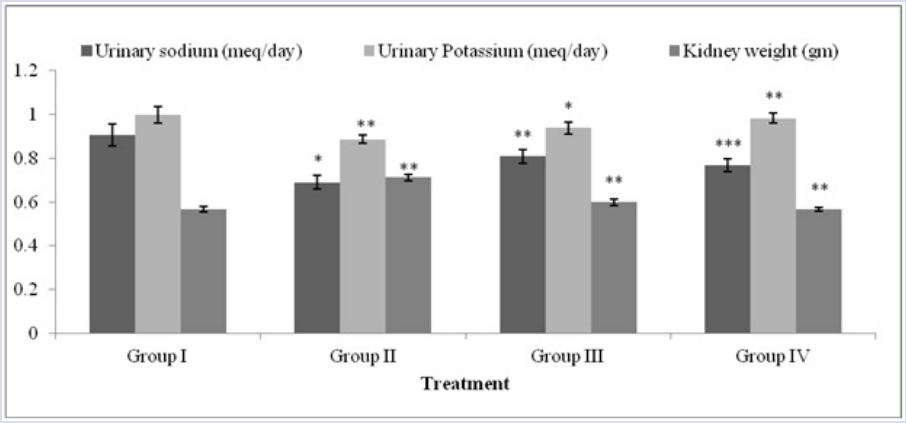

Figure 3: Effect of extract of *T. dioica* on urinary sodium, urinary potassium and kidney weight in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of five animals in each group. Extract-treated groups compared with the control group. Significance considered as *P<0.01 and **P<0.001.

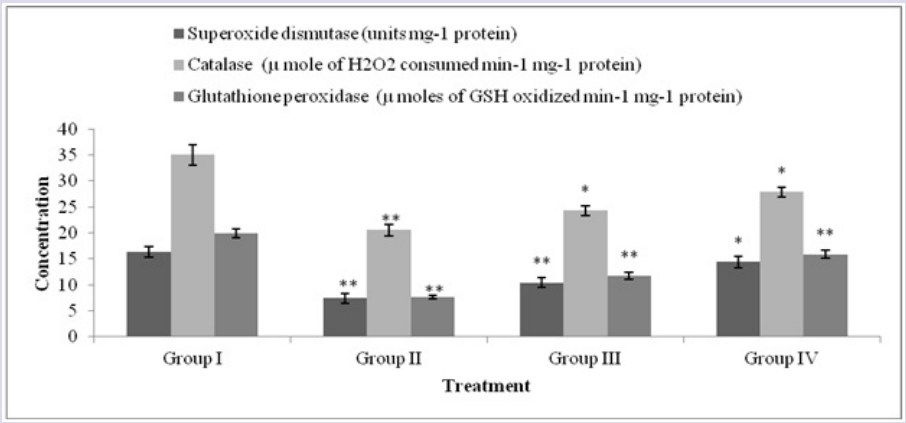

Figure 4: Effect of ethanol extract of *T. dioica* on superoxide dismutase, catalase and glutathione peroxidase in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of five animals in each group. Extract-treated groups compared with the control group. Significance considered as *P<0.01 and **P<0.001.

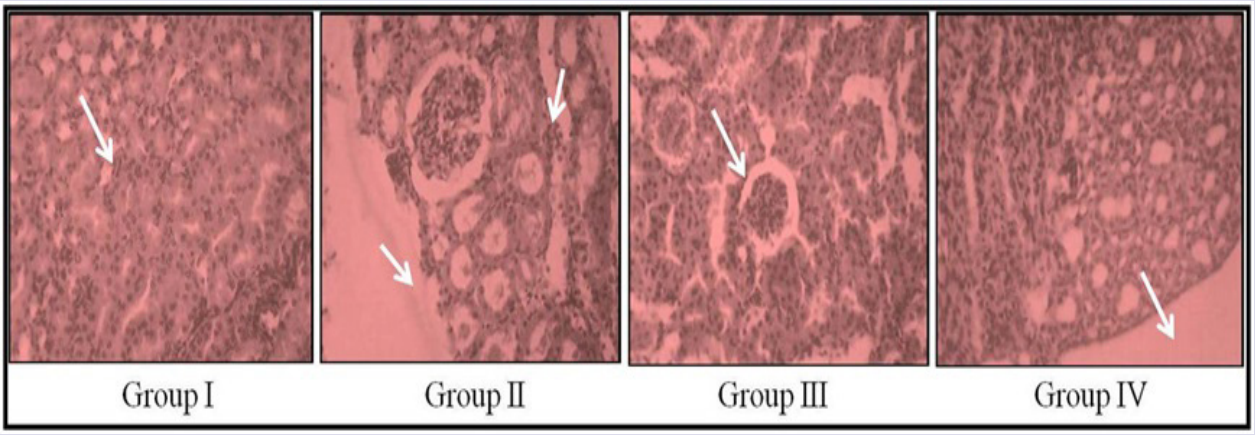

Figure 5: Evidence for the protective effect of *T. dioica* in rats treated with gentamicin; (a) control, (b) toxicant, (c) extract treated 250 mg/kg p.o., and (d) extract treated 500 mg/kg p.o.

micin treated rats (Group II). Nevertheless, considerable augmentation in the malondialdehyde was seen in case of Gentamicin treated animals (Group I). Treatment with *T. dioica* extract significantly reduced the level of malondialdehyde (Figure 4).

Histopathological studies

Histopathological studies also reveal the protective effect of *T. dioica*extract on the kidney of gentamicin treated rats (Figure 5). In the gentamicin treated group, glomerular and tubular damage, along with destruction of the tubular lumen, was observed (Group II). The *T. dioica* treated group revealed amelioration of renal lesions. Such an effect was more insightful in rats treated with 500 mg/kg of methanol extract of *T. dioica* extract (Figure 5).

DISCUSSION

Kidneys are extremely susceptible to deterioration, due to reactive oxygen species which are produced as a result of oxidative stress produced by various toxicants.[19] Thus, a series of reactions are initiated that predispose to various detrimental changes in kidney architecture leading to nephropathy.[20] Nephrotoxicity due to drugs is usually observed due to their accretion in renal cortex which is based on their 'affinity' towards kidney along with 'drug trapping' by them. Drugs like gentamicin, paracetamol and industrial and environmental toxicants can produce injury to kidney due to the generation of free radicals.[21] Due to the progression of oxidative stress, innate defense mechanism may be impaired and less effective. In such case, if such oxidative affront continues, activity of innate system is hampered[22] leading to progression of renal damage.

In the case of gentamicin, nephrotoxicity is produced in a two step process. In the early step, the drug is transported and gets accumulated in proximal tubular cells of nephrons. In the later stage, adverse interaction between these polycationic drugs along with oxidative stress predisposes to cellular damage of nephrons.[23-4] Antioxidant supplementation during such condition may prove to be useful.[25] Phytomedicines could be regarded as novel source of naturally occurring antioxidants.[26-30] Rutin, quercetin, and apigenin, are some of the common plant derived antioxidants.[31]

The present work focused on the assessment of the protective effect of *T. dioica* on gentamicin-induced nephrotoxicity in wistar rats. In this study, treatment with gentamicin caused nephrotoxicity which is obvious as observed by biochemical and histological studies. Treatment with extract caused impediment in augmentation of creatinine, blood urea nitrogen, and urea in serum. Histopathological observations on kidney of normal control rat confirmed healthy anatomical kidney architecture. Treatment with gentamicin in rats caused presence of inflammatory collections and cell necrosis. The *T. dioica* extract treated group revealed the presence of no necrosis along with minimal inflammatory surroundings. Hence, a gross histopathological analysis revealed the protective effect of *T. dioica* extract on kidney.

CONCLUSION

In conclusion, *T. dioica* is good source of numerous phytoconstituents. Phytoconstituents like phenolics, terpenoids and flavonoids present in plant are responsible for such protective effect. Previously, *T. dioica* triterpenoids demonstrated antiproliferative activity due to involvement of possible antioxidant role.[32] Due to such effect, *T. dioica* demonstrated nephroprotective activity. Still, more systematic studies are required to confirm such effect.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

CDH: Central Drug House; **OECD:** Organization for Economic Cooperation and Development; **Na:** Sodium; **K:** Potassium.

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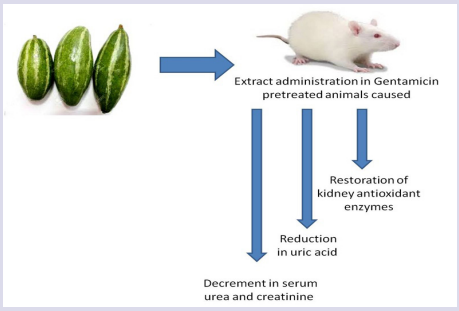
PICTORIAL ABSTRACT



SUMMARY

- Ayurvedic literature quotes use of Trichosanthes dioica as diuretic, cardiotonic, antiulcer, laxative.
- *Trichosanthes dioica* is also a good antioxidant.
- Trichosanthes extract administration in experimental animals caused decrement in serum urea and creatinine along with reduction in uric acid was observed.
- Restoration of antioxidant enzymes and normalization of urinary electrolytes and kidney weight was observed.
- Mechanism of protective effect is due to antioxidant effect, prevention of inflammation and necrosis.

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