



Histopathological Evaluation of Nephroprotective Effect of *Trichosanthes dioica* Roxb. on Gentamicin Induced Nephrotoxicity in Wistar Rats by Colorimetry and Spectrophotometry

Shefali Jayantibhai Chaudhary* and Archana N. Paranjape

Baroda College of Pharmacy, Limda, Vadodara-391760, India.

Date of Receipt- 18/12/2013
Date of Revision- 26/12/2013
Date of Acceptance- 29/12/2013

Address for Correspondence

Baroda College of Pharmacy, Limda, Vadodara-391760, India.

E-mail: shefalic88@yahoo.com

ABSTRACT

Objective: To evaluate the nephroprotective effect of *Trichosanthes dioica* Roxb. on gentamicin induced nephrotoxicity in rats.

Material & method: *Trichosanthes dioica* Roxb. (100 mg/kg, 200 mg/kg, 400 mg/kg body weight) were administered orally to male wistar rats for 10 days simultaneously Gentamicin was administered for five days starting from day six in dose of 80 mg/kg/body weight, i.p. in all groups. Nephrotoxicity was induced by intraperitoneal administration of gentamicin 80 mg/kg/day for 5 days. Each group contains 6 rats. The parameters studied included blood urea nitrogen and serum creatinine. Other parameters are urine output, urinary protein, kidney weight. Histopathological examination was also carried out. **Result:** –It was observed that pretreatment of methanolic extract of *Trichosanthes dioica* fruits significantly protects rat kidney from gentamicin induced nephrotoxicity. Gentamicin-induced glomerular congestion, per tubular and blood vessel congestion, epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the group receiving the fruit extract of *T.dioica* along with gentamicin. This extract also normalized the gentamicin-induced increases in urine and plasma creatinine, blood urea and blood urea nitrogen levels. Additionally, histopathological examination showed that *T.dioica* markedly ameliorated gentamicin induced renal tubular necrosis.

Conclusion: It is concluded that the fruit extract of *Trichosanthes dioica* Roxb. possesses nephroprotective activity against Gentamicin induced nephrotoxicity.

Keywords: *Trichosanthes dioica*, Gentamicin, Serum creatinine,

EXPERIMENTAL DESIGN

MATERIALS AND METHODS

Adult male wistar rats weighing 200–250 g were used. All of them were kept in the same room under a constant temperature ($22\pm 2^\circ\text{C}$) with standard laboratory diet and water ad libitum. The study was conducted after obtaining Institutional animal ethical committee clearance. Procurement of kits: creatinine and BUN kits from span diagnostic, Surat. Procurement of inducer drug (gentamicin) from Torrent Pharmaceuticals, Ahmedabad.

1. Collection and authentication of plant material

The fruits of *Trichosanthes dioica Roxb.* were collected in the month of February 2012 from the local market of Baroda, Gujarat, India. Authentication of plant was done from M N College of Visnagar, Gujarat, herbarium sheet is deposited in Baroda College of Pharmacy, Baroda, Gujarat.

2. Preparation of extract

The fruits of *Trichosanthes dioica Roxb.* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use.

3. Phytochemical Study

The coarse powder was extracted with 1–1.5 liters of methanol by continuous hot Soxhlet apparatus. After completion of extraction, extract was dried under rotary evaporator. The dried extract was stored in a desiccator.

Preliminary phytochemical studies¹⁻⁴

Methanolic extract of the fruits of *Trichosanthes dioica* were subjected to

chemical tests for the identification of their active constituents.

Tests for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

A. Molisch's Test

Filtrate was treated with 2–3 drops of 1% alcoholic α -naphthol solution and 2ml of con. H_2SO_4 was added along the sides of the test tube. Appearance of violet coloured ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolyzed with HCl for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

B. Legal's Test

To the hydrolysate, 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

C. Borntrager's Test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink colour showing the presence of glycosides.

Tests for alkaloids

A small portion of the methanol extract was stirred separately with few drops of dil. HCl and filtered. The filtrate was treated with various reagents as shown for the

presence of alkaloids. Mayer's reagent – Creamy precipitate Dragendroff's reagent– Orange brown precipitate Hager's reagent– Yellow precipitate Wagner's reagent–Reddish brown precipitate

Tests for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Libermann Burchard Test

The residue was dissolved in few drops of acetic acid, 3 drops of acetic anhydride was added followed by few drops of con. H₂SO₄. Appearance of bluish green colour shows the presence of phytosterols.

Tests for fixed oils

Spot test

Small quantity of extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1–2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for gums and mucilages

Small quantity of the extract was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilages.

Tests for saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Tests for proteins and free amino acids

Small quantity of the extract was dissolved in few ml of water and treated with following reagents.

A. Millon's reagent – Appearance of red colour shows the presence of protein and free amino acids.

B. Ninhydrin reagent – Appearance of purple colour shows the presence of proteins and free amino acids.

C. Biuret test – Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

Tests for phenolic compounds and tannins

Small quantity of the extract was taken separately in water and tested for the presence of phenolic compounds and tannins using following reagents.

A. Dil. FeCl₃ solution (5%) – violet colour

B. 1% solution of gelatin containing 10% NaCl – white precipitate

C. 10% lead acetate solution – white precipitate.

Tests for flavonoids

A.

With aqueous Sodium hydroxide solution: Blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (flavonones)

B. With Con. H₂SO₄

Yellow orange colour (anthocyanins), yellow to orange colour (flavones), orange to crimson (flavonones)

C. Shinoda's test

Small quantity of the extract was dissolved in alcohol and to that a piece of magnesium followed by Con. HCl drop wise was added and heated. Appearance of magenta colour shows the presence of flavonoids.

4. Acute toxicity study

To study the effect of *Trichosanthes dioica* Roxb on gentamicin induced nephrotoxicity in rats (Protocol No.: 984/11/12). Approved by CPCSEA department on 07/01/2012.

Plant authentication certificate has been approved on 22/01/2012.

Acute oral toxicity study was done according to OECD-423 guidelines. Albino wistar rats (100–200g) were used. Dose given orally was=2000mg/kg. 3 rats were used for each group. Mortality and general behavior was observed continuously for: initial 4 hrs, intermittently for next 6 hrs, then again at 24 hrs and 48 hrs, following the drug administration.

5. Experimental design⁵

Animals were divided in to seven groups. Each group was contained six animals.

Model used was Gentamicin induced nephrotoxicity.

Group 1: Control with normal saline (5 ml/kg)

Group 2: Gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days.

Group 3: Methanolic extract of *T.dioica* was administered in dose of 100mg/kg (p.o) for ten days and simultaneously administered

gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Group 4: Methanolic extract of *T.dioica* was administered in dose of 200mg/kg (p.o) for ten days and simultaneously administered gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Group 5: Methanolic extract of *T.dioica* was administered in dose of 400mg/kg by (p.o) for ten days and simultaneously administered gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Group 6: Standard group: methylprednisolone administered in dose 100mg/kg, s.c. for ten days and gentamicin simultaneously administered for five days starting from day six.

Group 7: Methanolic extract of *T.dioica* (400mg/kg/body weight, p.o), daily for ten days.

At the end of experimental period, all the animals were sacrificed under diethyl ether anaesthesia. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 4000 rpm for 15 min and analyzed for various biochemical parameters.

6. Assessment of kidney function

Biochemical parameters i.e., Estimation of urinary output, urinary protein, Blood urea and Creatinine were analyzed according to the reported methods. The kidney was removed, weighed and morphological changes were observed. A portion of kidney was fixed in 20% formalin for histopathological studies.

7. Statistical analysis

The values were expressed as Mean±SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Turkey multiple

comparison tests. P values < 0.05 were considered as significant.

8. Biochemical parameters

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Semi Autoanalyser.

1) Urine output

Urine output was measured 24 hr before and after the completion of treatment.

2) Serum creatinine

Principle: Creatinines in a protein free solution react with alkaline picrate and produces a red colored complex, which is measured colorimetrically.

Reagents (supplied in the kit):

Reagent 1: Picric acid

Reagent 2: Sodium hydroxide, 0.75N

Reagent 3: Stock Creatinine Standard, 150 mg %

Procedure

Preparation of working solution– Working standard– 0.1 ml of reagent 3 (stock creatinine standard) was diluted to 10 ml with purified water and mixed well.

For spectrophotometer:

A control is the absorbance of control;
A test is the absorbance of sample

Step A. Deproteinization of test sample

See Table No. 1

Mixed well, kept in a boiling water bath exactly for one min. Cool immediately under running tap water and filtered.

Step B. Color development

See Table No. 2

Mixed well and allowed to stand at R.T. exactly for 20 min and measure immediately the optical density of blank (B), standard (S) and test (T) against purified

water on a colorimeter with a green filter. Measure the O.D. at 520 nm.

Calculation

Serum creatinine in mg/100ml = $(\text{O.D. test} - \text{O.D. blank}) \div (\text{O.D. std} - \text{O.D. blank}) \times 3.0$

3) BUN

Principle: urea reacts with hot acidic diacetyl monoxime in presence of thiosemicarbazide and produces a rose–purple colored complex, which is measured colorimetrically.

Reagents (supplied in the kit):

Reagent 1: Urea reagent

Reagent 2: Diacetyl monoxime (DAM)

Reagent 3: Working Urea Standard, 30 mg%

Preparation of working solution

Solution 1: 1 ml of reagent was diluted to 1 to 5 ml with purified water.

Procedure

For spectrophotometer

See Table No. 3

Mixed well and kept the tubes in the boiling water exactly for 10 min. Cool immediately under running water for 5 min, mixed by inversion and measured the color intensity within 10 min using a green filter against blank. Measure O.D. at 525 nm.

Calculation

Serum/plasma: urea in mg/100ml, (A) = $(\text{O.D. of test}) \div (\text{O.D. of std.}) \times 30$

Blood urea nitrogen in mg/100ml = $[(A) \times 30 \times 20] \div 100$

4) Protein in urine

Measure amount of total protein in urine by grading as: +1, +2, +3, +4.

+1 – Minimum amount of protein

+2 – Greater than +1 amount

+3 – Greater than +2 amount

+4 – Maximum amount of protein

5) Weight of kidney

Weight the kidney in all groups of animals 24 hrs before and after completion of 10 days of treatment.

9. Determination of renal tissue injury

The kidneys were fixed in phosphate-buffered 10% formalin and then embedded in paraffin wax, sectioned (4–5µm) and stained with Hematoxylin and Eosin (H&E).

The severity of the histological lesion was graded from 0 to 4, by a blindfold fashion (all slices were evaluated by two blind-folded pathologists and the results were collectively introduced in analysis and explanation), as the following:

0; no sign of necrosis (no damage), 1+; necrosis of individual cells, 2+; necrosis of all cells in adjacent proximal convoluted tubules, with survival of surrounding tubules, 3+; necrosis confined to the distal third of the proximal convoluted tubule with a band of necrosis extending across the inner cortex. 4+; necrosis affecting all the three segments of the proximal convoluted tubule⁵.

RESULTS

1. The results of preliminary phytochemical studies of the two different plant extracts are presented in Table 4

Table 4: Data showing preliminary phytochemical screening of the methanolic extract of *Trichosanthes dioica* Roxb.

+ Present – Absent

2. Acute toxicity study

No toxic symptoms and mortality were found in both the doses during this study.

3. Biochemical parameters:

Serum Creatinine

In Gentamicin treated group of animals the concentration of serum creatinine was considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating with methanol extract of *T.dioica* (group 3, 4 & 5) showed significant decrease ($p<0.001$) in concentration of serum creatinine compared to Gentamicin treated group (group 2). Standard group and group 7 showed significant decreased ($p<0.001$) in concentration of serum creatinine compared to disease control group. (Table 5)

Blood urea nitrogen

In Gentamicin treated group of animals the concentration of blood urea nitrogen was considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating (group 3, 4 & 5) with methanol extract of *T.dioica* showed significant decrease ($p<0.001$) in concentration of BUN as compared to gentamicin treated group. Standard group and group 7 showed significant decreased ($p<0.001$) in concentration of blood urea nitrogen compared to disease control group.(Table 6)

Protein in urine

In disease control group the amount of protein in urine was found more than the normal control. In test group (group 3, 4, 5) protein amount was found to decrease than the disease control. In Test group 5 (400 mg/kg) more decrease in the amount of protein was found as compared to test group 3 (100 mg/kg) and 4 (200 mg/kg). (Table 7)

Urine output

Urine output was decrease in disease control than normal control. In test groups the

urine output was increase than disease control group.(Table 8)

4. Kidney weight

In gentamicin treated group of animals weight of kidneys were considerably increased compared to normal animals (group1) and treating (group 3, 4 & 5) with methanol extract of *T.dioica* showed significant decrease ($p<0.001$, $p<0.05$) in kidney weight. Standard group and group 7 showed significant decreased ($p<0.001$) in concentration of kidney weight compared to disease control group.(Table 9)

5 Histopathological studies

Group 1: Control with normal saline (5 ml/kg) (p.o)

Grade: **0** – no sign of necrosis (no damage)

Group 2: Gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days

Grade: **4+**; necrosis affecting all the three segments of the proximal convoluted tubule.

Group 3: Methanolic extract of *T.dioica* was administered in dose of 100mg/kg (p.o) for ten days and simultaneously administered gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Grade: **2+**; necrosis of all cells in adjacent proximal convoluted tubules, with survival of surrounding tubules.

Group 4: Methanolic extract of *T.dioica* was administered in dose of 200mg/kg (p.o) for ten days and simultaneously administered gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Grade: **2+**; necrosis of all cells in adjacent proximal convoluted tubules, with survival of surrounding tubules.

Group 5: Methanolic extract of *T.dioica* was administered in dose of 400mg/kg (p.o) for

ten days and simultaneously administered gentamicin for five days starting from day six in dose of 80 mg/kg/body weight, i.p.

Grade: **1+**; necrosis of individual cells.

Group 6: Standard group:

Methylprednisolone administered in dose 100mg/kg, (s.c.) for ten days and gentamicin simultaneously administered for five days starting from day six.

Grade: **2+**; necrosis of all cells in adjacent proximal convoluted tubules, with survival of surrounding tubules.

Group 7: Methanolic extract of *T.dioica* (400 mg/kg/body weight, p.o), daily for ten days.

Grade: no sign of necrosis (no damage).

DISCUSSION

The use of gentamicin, an aminoglycoside antibiotic with a wide spectrum of activities against Gram-positive and Gram-negative bacterial infections but with high preference for latter is equally associated with nephrotoxicity as its side effect.⁶⁻⁸ Thus gentamicin induced nephrotoxicity is well established experimental model of drug induced renal injury.^{9,10} Many animal experiments have demonstrated overwhelmingly, the positive correlation between oxidative stress and nephrotoxicity.¹¹ Gentamicin induces nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomyelinase and phospholipases in addition to causing oxidative stress.^{10,12}

Drug induced nephrotoxicity are often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis.¹³ So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animals and humans.¹⁴ In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance.¹⁵ Creatinine

derives from endogenous sources by tissue creatinine breakdown.¹⁶ Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. In the present study drug induced nephrotoxicity were established by single daily intraperitoneal injection of the gentamicin, for 10 days. This toxicity characterized by marked elevation in the circulating levels of blood urea, serum creatinine and histological features of tubulonephritis in the disease control rats when compared to untreated rats. However these changes were inhibited by pretreatment with single daily graded doses of *T.dioica* extract for 10 days. Oral administration of fruit extract significantly decreased the urea and creatinine levels in all treatment group compare to toxic group.

It was established that gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR.¹⁷ Urine output of disease control group was decreased compared to normal control. Urine output of test groups was increased compared to disease control.

Gentamicin is known to decrease the activities of catalase, glutathione peroxidase and the level of reduced glutathione.¹⁸ Thus it can be assumed that the nephroprotection showed by *T.dioica* extract in gentamicin induced nephrotoxicity may be mediated through its potent antioxidant effect.

From the histopathological results of present study it is evident that the degree of necrosis of proximal tubules is decreased by treatment with *T.dioica* which was compared with standard drugs.

The amount of protein in urine sample was found more in disease control group than normal control. Amount of protein in urine of test groups was decreased than disease control group. The decrease was more predominant in

higher dose group of *T.dioica* indicating positive nephroprotective effect of *T.dioica*.

Kidney weight of disease control group was increased than normal control. Weight of kidney of test group was decreased than disease control group. The decrease was more predominant in higher dose group of *T.dioica*.

The findings suggest the potential use of *T.dioica* as a therapeutically useful nephroprotective agent. Further studies are required to determine its mechanisms of action of nephroprotection in order to use it for treatment of drug induced nephrotoxicity.

CONCLUSION

It is concluded that the fruit extract of *Trichosanthes dioica* possesses nephroprotective activity against gentamicin induced nephrotoxicity.

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Table 1. Deproteinization of test sample

Serum/plasma/dilute urine	0.5 ml
Purified water	0.5 ml
Reagent 1: Picric Acid	3.0 ml

Table 2. Color development

Items	Blank (B)	Standard (S)	Test (T)
Filtare/Supernatant (From Step A.)	–	–	2.0 ml
Working standard	–	0.5 ml	–
Purified water	0.5 ml	–	–
Reagent 1: picric acid	1.5 ml	1.5 ml	–
Reagent 2: sodium hydroxide, 0.75 N	0.5 ml	0.5 ml	0.5 ml

Table 3. For spectrophotometer

Items	Blank (B)	Test (T)	Standard (S)
Solution 1	2.5 ml	2.5 ml	2.5 ml
Sample	–	0.01 ml	–
Reagent 3: working urea standard, 30 mg%	–	–	0.01 ml
Reagent 2: Diacetylmonoxime (DAM)	0.25 ml	0.25 ml	0.25 ml

Table 4. Phytoconstituents

Phytoconstituents	Methanolic extract
Carbohydrates	++
Glycosides	++
Fixed oils and fats	+
Gums and mucilage	–
Proteins and amino acids	–
Saponins	–
Tannins	+
Steroids	+
Flavonoids	++
Alkaloids	++

Table 5. Effect of 80 mg/kg/day intraperitoneal Gentamicin and graded oral *T.dioica* on serum creatinine in treated rats for 10 days

Group	Treatment	Dose	Creatinine level (mg/dl) (Mean±SEM)
1	Normal control	5 ml/kg normal saline	0.5767 ± 0.02155
2	Nephrotoxicity control	80 mg/kg gentamicin (i.p.)	7.472 ± 0.3283 ⁺⁺⁺
3	Test group 1	100 mg/kg fruit extract (p.o)	1.002 ± 0.04729 ^{***}
4	Test group 2	200 mg/kg fruit extract (p.o)	1.028 ± 0.04199 ^{***}
5	Test group 3	400 mg/kg fruit extract (p.o)	0.7767 ± 0.07732 ^{***}
6	Standard group	100 mg/kg (s.c.)	0.8983 ± 0.1328 ^{***}
7	Administered only <i>T.dioica</i> fruit extract	400 mg/kg (p.o)	0.6017 ± 0.01493 ^{***}

All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I) : A

Table 6. Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *T.dioica* on BUN in treated rats for 10 days

Group	Treatment	Dose	Blood urea nitrogen (BUN) level (mg/dl) (Mean±SEM)
1	Normal control	5 ml/kg normal saline	12.587±0.2086
2	Nephrotoxicity control	80 mg/kg gentamicin (i.p.)	26.455±0.8684 ⁺⁺⁺
3	Test group 1	100 mg/kg fruit extract (p.o)	19.617±0.6105 ^{***}
4	Test group 2	200 mg/kg fruit extract (p.o)	17.785±0.3372 ^{***}
5	Test group 3	400 mg/kg fruit extract (p.o)	14.517±0.3740 ^{***}
6	Standard group	100 mg/kg (s.c.)	14.045±0.6034 ^{***}
7	Administered only <i>T.dioica</i> fruit extract	400 mg/kg (p.o)	013.555±0.3306 ^{***}

All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I) : ANOVA followed by Tukey–Kramer’s test.

Table 7. Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *T.dioica* on urinary protein in treated rats for 10 days

Group	Treatment	Dose	Protein in urine (grade) (Mean±SEM)
1	Normal control	5 ml/kg normal saline	0.333±0.2108
2	Nephrotoxicity control	80 mg/kg gentamicin (i.p.)	3.333±0.333 ⁺⁺⁺
3	Test group 1	100 mg/kg fruit extract (p.o)	1.667±0.2108 ^{***}
4	Test group 2	200 mg/kg fruit extract (p.o)	1.667±0.2108 ^{***}
5	Test group 3	400 mg/kg fruit extract (p.o)	1.333±0.2108 ^{***}
6	Standard group	100 mg/kg (s.c.)	1.333±0.2108 ^{***}
7	Administered only <i>T.dioica</i> fruit extract	400 mg/kg (p.o)	1.500±0.2236 ^{***}

All the values are expressed as mean±SEM (n=6) in each group.

Where, ^{***}p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I) : ANOVA followed by Tukey–Kramer’s test.

Table 8. Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *T.dioica* on urine output in treated rats for 10 days

Group	Treatment	Dose	Urine output level (ml/day) (Mean±SEM)
1	Normal control	5 ml/kg normal saline	8.190±0.2987
2	Nephrotoxicity control	80 mg/kg gentamicin (i.p.)	3.215±0.3080 ⁺⁺⁺
3	Test group 1	100 mg/kg fruit extract (p.o)	6.617±0.2475 ^{***}
4	Test group 2	200 mg/kg fruit extract (p.o)	6.955±0.3257 ^{***}
5	Test group 3	400 mg/kg fruit extract (p.o)	6.055±0.2402 ^{***}
6	Standard group	100 mg/kg (s.c.)	7.943±0.3507 ^{***}
7	Administered only <i>T.dioica</i> fruit extract	400 mg/kg (p.o)	6.850±0.3137 ^{***}

All the values are expressed as mean±SEM (n=6) in each group.

Where, ^{***}p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I) : ANOVA followed by Tukey–Kramer’s test.

Table 9. Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *T.dioica* on kidney weight in treated rats for 10 days

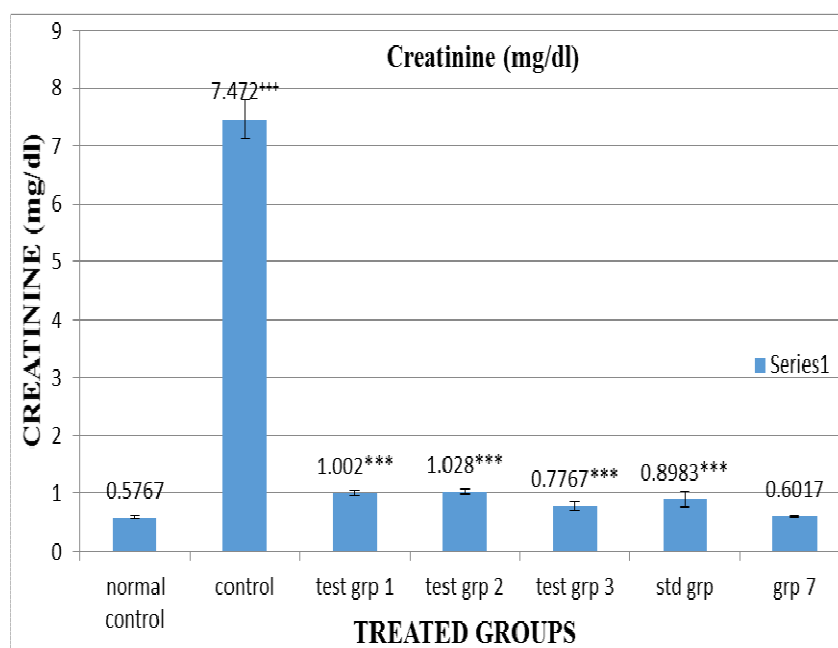
Group	Treatment	Dose	Kidney weight (gm) (Mean±SEM)
1	Normal control	5 ml/kg normal saline	0.5307±0.02755
2	Nephrotoxicity control	80 mg/kg gentamicin (i.p.)	0.7543±0.02798 ⁺⁺⁺
3	Test group 1	100 mg/kg fruit extract (p.o)	0.6267±0.01787 [*]
4	Test group 2	200 mg/kg fruit extract (p.o)	0.6318±0.03222 [*]
5	Test group 3	400 mg/kg fruit extract (p.o)	0.5353±0.02948 ^{***}
6	Standard group	100 mg/kg (s.c.)	0.5375 ±0.03600 ^{***}
7	Administered only <i>T.dioica</i> fruit extract	400 mg/dl (p.o)	0.5247±0.01475 ^{***}

All the values are expressed as mean±SEM (n=6) in each group.

Where, *p<0.05, ***p<0.001 when compared to gentamicin treated group (i.e. group II):

ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I): ANOVA followed by Tukey–Kramer’s test.

**Figure 1.** Effect of *T.dioica* on serum creatinine

All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p<0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, ⁺⁺⁺p<0.001 when compared to control group (i.e. group I) : ANOVA followed by Tukey–Kramer’s test.

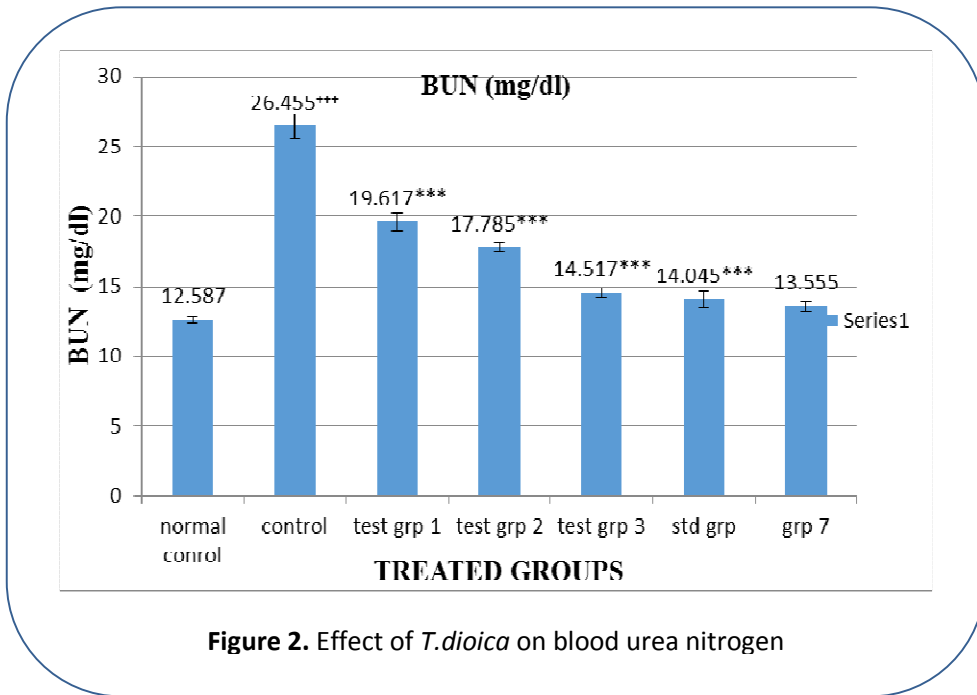


Figure 2. Effect of *T.dioica* on blood urea nitrogen

All the values are expressed as mean±SEM (n=6) in each group. Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test. While, +++p<0.001 when compared to control group (i.e. group I) : ANOVA followed by Tukey–Kramer’s test.

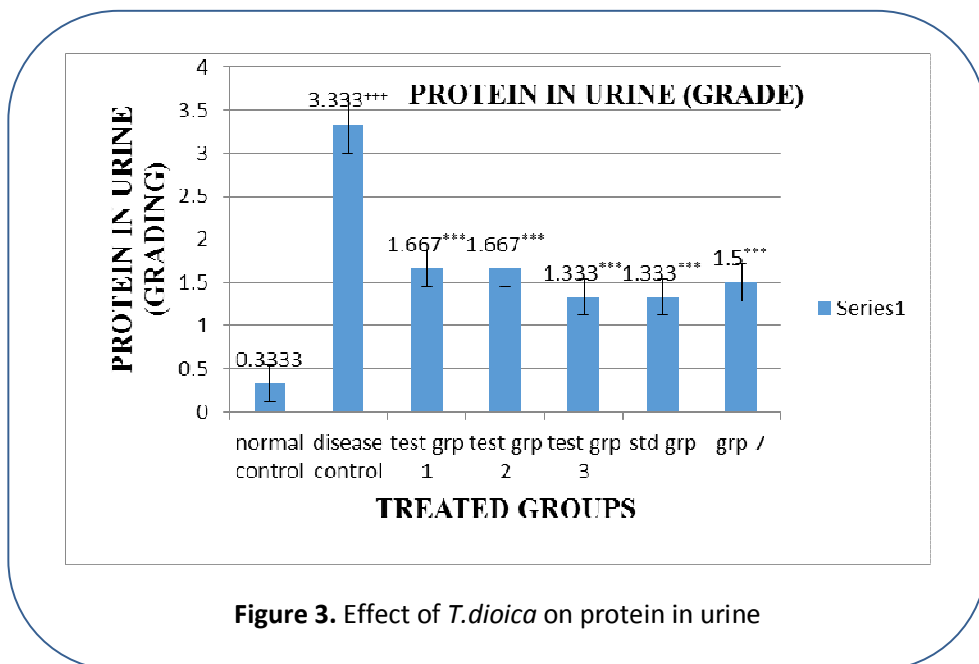


Figure 3. Effect of *T.dioica* on protein in urine

All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, +++p<0.001 when compared to control group (i.e. group I): ANOVA followed by Tukey–Kramer’s test.

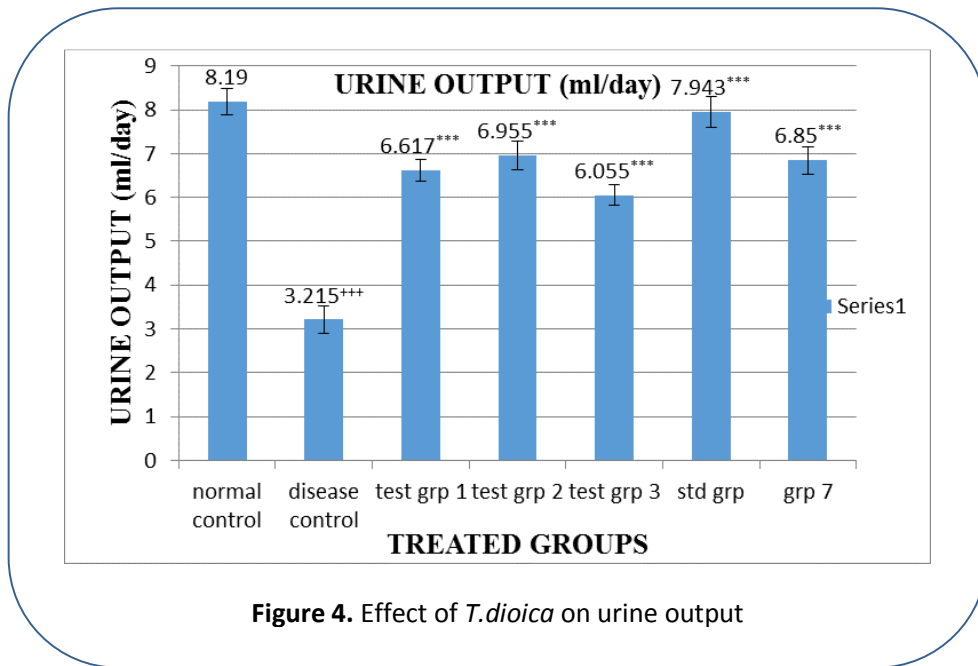
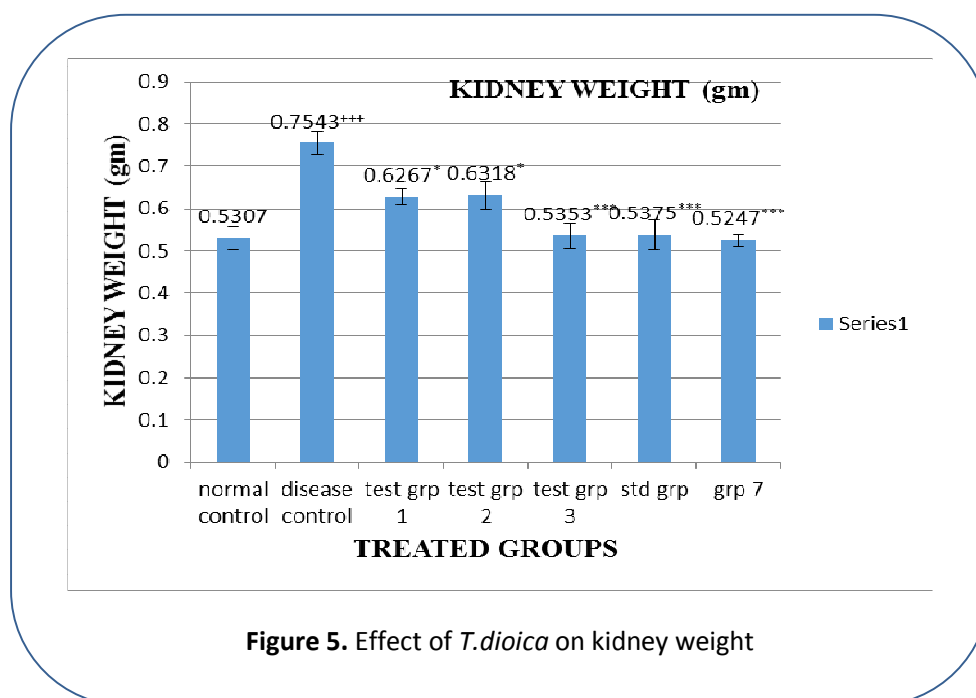


Figure 4. Effect of *T.dioica* on urine output

All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, +++p<0.001 when compared to control group (i.e. group I): ANOVA followed by Tukey–Kramer’s test.



All the values are expressed as mean±SEM (n=6) in each group.

Where, ***p< 0.001 when compared to gentamicin treated group (i.e. group II): ANOVA followed by Tukey–Kramer’s test.

While, +++p<0.001 when compared to control group (i.e. group I): ANOVA followed by Tukey–Kramer’s test.

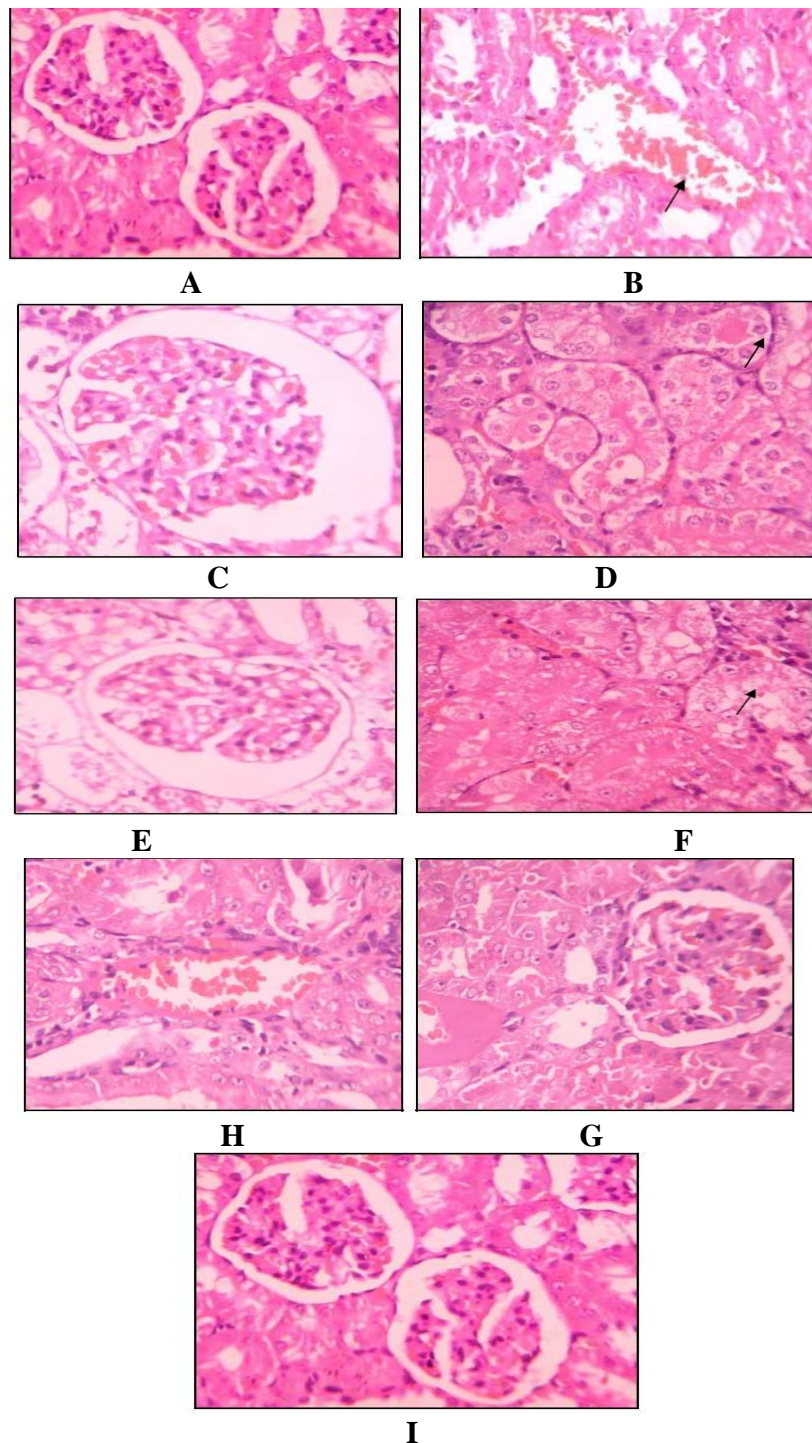


Figure 6. Histopathological study of kidney A–normal group, B–normal saline, C & D–80 mg/kg gentamicin induced toxicity (disease control), E–test group at a dose 100 mg/kg (group 3), F– test group at dose 200 mg/kg (group 4) , H–test group at dose 400 mg/kg (group 5), G–standard group (Vit C–dose 200 mg/kg), I–group 7 (only extract)