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Research Article

Nephroprotective Activity of *Capparis decidua* Aerial Parts Methanolic Extract in Wistar Albino Rats

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Abstract

Background and Objective: Traditionally, medicinal plants play a major role in the treatment of diseases. This study was aimed to investigate the nephroprotective effect of *Capparis decidua* aerial parts methanolic extract against gentamicin induced nephrotoxicity in Wistar albino rats. **Materials and Methods:** Twenty rats were divided into 4 groups of 5 rats each as follows, normal control, gentamicin induced nephrotoxicity injected intraperitoneal (IP) gentamicin 100 mg kg⁻¹, *C. decidua* low dose (CDM200) given 200 mg kg⁻¹ of methanolic extract orally and *C. decidua* high dose (CDM400) given 400 mg kg⁻¹ of methanolic extract orally. Gentamicin was injected concurrently with the plant extract for 7 days. Urea and creatinine were determined to evaluate the nephroprotective action of *C. decidua* aerial parts methanolic extract against gentamicin induced nephrotoxicity. Blood was taken to evaluate complete blood picture. Histopathological examination of kidney sections was also performed. **Results:** The results indicated that the low and high doses of *C. decidua* aerial parts methanolic extract have potential nephroprotective action compared to gentamicin group ($p < 0.05$) as evidenced by the significant decrease in the urea and creatinine levels compared to gentamicin group. Haematological parameters showed no significant differences between the test groups of rats. Rats that received gentamicin exhibited severe renal damage especially in the cortical tubules with presence of eosinophilic tubular cast in the medulla. These changes were less noticed in rats given low and high dosage of *C. decidua* methanolic extract. **Conclusion:** The result indicates the nephroprotective effect of *C. decidua* extract against toxic effects of gentamicin, which supports its folkloric use against renal disorders.

Key words: Nephroprotective, *C. decidua*, methanolic extract, gentamicin, nephrotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nephrotoxicity is one of the most common kidney problems that occur when body is exposed to a drug or toxin. Renal damage leads to the inability of the body to get rid of excess urine and wastes which leads in turn to a rise in blood electrolytes¹. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure such as antibiotics, chemotherapeutic agents and non-steroidal anti-inflammatory drugs (NSAIDs)^{1,2}. Gentamicin is an aminoglycoside antibiotics, used clinically against Gram negative bacteria due to its usefulness and the slow increase in bacterial resistance^{3,4}. However, the main problem associated with gentamicin is its nephrotoxicity therapeutic doses which appear to be a major cause of acute renal failure. The nephrotoxicity of gentamicin limits its long-term clinical use⁵⁻⁷. The medicinal plants play a prominent role against many diseases including renal disorders. A variety of medicinal plants and plants extracts have been reported to be effective as nephroprotective agents². Gentamicin is an aminoglycoside antibiotics, used clinically against Gram negative bacteria due to its usefulness and the slow increase in bacterial resistance. However, the main problem associated with gentamicin is its nephrotoxicity therapeutic doses which appear to be a major cause of acute renal failure. Gentamicin is excreted through kidneys without degradation or metabolic changes. About 5-10% of its dose is concentrated in the proximal tubules^{2,4}. The curative properties of many medicinal plants is due to the presence of various complexes chemical substances called phytochemicals such as alkaloids, glycosides, saponins, resins, flavonoids, tannins, volatile and fixed oils and gums^{8,9}. These phytochemicals have certain biological and physiological effects and act as defense system against various diseases. The beneficial medicinal effects of plant materials result from the combined effects of these phytochemicals¹⁰. Medicinal plants are widely used to control various disorders and characterized by cheapness, safety and non-toxicity compared with synthetic drug^{9,11}. Ancient literature has prescribed various herbs to treat kidney diseases¹². Numerous plants in Sudan are traditionally used to treat a variety of diseases including renal diseases such as *Boscia senegalensis*, gum Arabic, goriander, celery and caraway which used as diuretics⁸. *Capparis decidua* that known in Arabic as Hanbag, Sodab and Tundub belongs to the family Capparaceae. It is a densely branching shrub or small tree with many green vine like apparently leafless branches, hanging in bundles^{13,14}. It is found in the sub-tropical and tropical zones in Africa and Asia. It's distributed in Chad, Sudan, the Arabian Peninsula, Jordan, India, Pakistan, Iran, the Mascarene Island and Natal¹³. Different parts of the

plant have been widely used in traditional system of medicine to treat a variety of disorders, such as coughs, asthma, inflammation, fever, boils, cardiac troubles, diabetes, fertility problems, muscular injuries, parasitic infection and constipation¹⁵. In Sudan *C. decidua* is used in swelling, jaundice and infection of joint¹⁴. Scientific reports demonstrated that *C. decidua* have insecticidal effect¹⁶. Hepatoprotective activities, hypercholesterolemia, anti-depressant, anthelmintic, purgative, antimicrobial and anti-inflammatory activities^{13,15,17-21}. The objective of this study was to investigate the nephroprotective activity of *Capparis decidua* aerial parts methanolic extract against gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

Study area: The study was carried out at Veterinary Medicine and Surgery Department, College of Veterinary Medicine, Sudan University of Science and Technology, Sudan. This research project was conducted from 2014-2015.

Plant material: *C. decidua* aerial parts were collected from Arkaweit area in Khartoum State and identified by a taxonomist in Medicinal and Aromatic Plants, Traditional Medicine and Research Institute (MAPTMRI), National Center of Research (NCR), Khartoum, Sudan. The plant material was dried at room temperature and ground into powder.

Extraction: The powdered aerial parts of *C. decidua* were extracted by soxhlet apparatus using methanol 98%. The solvent was then collected and evaporated under reduced pressure using rotary evaporator apparatus²².

Experimental animals: Twenty Wistar albino rats of both sexes 97-189 g in weight were purchased from Medicinal and Aromatic Plants, Traditional Medicine and Research Institute (MAPTMRI), National Center of Research (NCR), Khartoum, Sudan. The rats were kept in the Laboratory Animal House in Sudan University of Science and Technology, College of Veterinary Medicine. The animals were maintained under standard environmental condition (i.e., relative humidity: 40-60%, temperature: 24±2°C and 12 h light-dark cycle) and fed with mash feed consisting of flour, meat, edible oil, sodium chloride, vitamins, minerals with free access to water.

This study was approved by the Scientific Research Committee of the College of Veterinary Medicine, Sudan University of Science and Technology (SUST) in accordance with good clinical practice and international guidelines for animal use in experimentations.

Nephroprotective activity

Grouping and dosing: Rats were divided into 4 groups of 5 rats each, Group 1: Served as control (CON), Group 2: Received nephrotoxic drug gentamicin (GEN) (Interchemie, Holland) at a dose of 100 mg/kg/day intraperitoneal (IP) for 7 days. Group 3: Rats were injected with gentamicin at a dose of 100 mg kg⁻¹/day IP concurrently with 200 mg kg⁻¹ orally of *C. decidua* aerial parts methanolic extract (CDM200) for 7 days. Group 4: Animals were injected with gentamicin IP at a daily dose of 100 mg kg⁻¹ simultaneously with 400 mg kg⁻¹ of *C. decidua* methanolic extract (CDM400) for 7 days.

Sample collection: Blood was obtained from the retro-orbital plexus using capillary tubes. Samples for haematological analysis was collected in tubes containing EDTA as anticoagulant and analyzed immediately. Those for biochemical analysis were collected in plain glass tubes and left at room temperature for 30 min to coagulation and then centrifuged at 3000 rpm for 15 min. The serum was stored at -20°C until used.

Haematological parameters: White blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and platelet (PLT) were measured using automatic analyzer (Sysmex-kx-2/N, Spain).

Biochemical analysis: The concentration of serum urea and creatinine were measured by Mindray autoanalyzer using commercial kit.

Body weight of rats: Body weights of animals were recorded at day 0 and 7.

Postmortem: At the end of the experiment, rats were euthanized and examined for presence of pathological lesions the kidneys were carefully inspected and removed. The relative weight of each kidney was calculated with the formula²³:

$$\text{Relative weight} = \frac{\text{Kidney weight}}{\text{Body weight}} \times 100$$

Histopathology: Small pieces of kidneys were taken at postmortem and preserved in 10% buffered formalin for histopathological examination. Fixed kidney tissue was processed and sectioned (4-5 µm) following the conventional histopathological methods and stained with Hematoxylin and Eosin Stain²⁴.

Statistical analysis: The data was analyzed by one way analysis of variance (ANOVA) (SPSS version 15) and the results were expressed as mean ± SEM. A value of p < 0.05 was considered as statistically significant²⁵.

RESULTS

Haematological parameters: There were no significant differences in all haematological parameters except that MCV were significantly decreased in gentamicin group (Table 1).

Biochemical parameters

Urea levels: Gentamicin (GEN) group exhibited a significant (p > 0.05) increase in urea levels compared to control animal. Rats treated with low and high doses of *C. decidua* aerial parts methanolic extract (CDM200 and 400) had reduced the elevation of urea levels compared to GEN group. By contrast no significant difference was noted in serum urea levels among plant extract treated groups (Table 2). Simultaneous administration of *C. decidua* aerial parts methanolic extract and gentamicin was unable to reduce urea levels to that of control and the difference of level of urea still significantly higher (p < 0.01) than the control animal.

Creatinine levels: Rats that received gentamicin showed significant elevation (p < 0.05) in the values of creatinine compared with control and treated rats. The values of creatinine in treated groups (200 and 400 mg kg⁻¹) was found to be reduce towards control level (Table 2).

Table 1: Haematological effects of methanolic extract of *C. decidua* aerial parts on gentamicin induce nephrotoxicity in rats

Treatments	WBC (× 10 ³ µL)	RBC (× 10 ⁶ µL)	PCV (%)	Hb (g dL ⁻¹)	MCV (fL)	MCH (Pg)	MCHC (g dL ⁻¹)	PLT
CON	6.46 ± 1.14 ^a	6.33 ± 0.43 ^a	37.70 ± 2.35 ^a	11.78 ± 0.54 ^a	59.84 ± 0.67 ^a	18.68 ± 0.60 ^a	31.24 ± 0.79 ^a	1063.60 ± 183.03 ^a
GEN	8.70 ± 1.32 ^a	6.61 ± 0.61 ^a	36.30 ± 2.81 ^a	11.75 ± 0.67 ^a	55.15 ± 0.96 ^b	18.00 ± 0.75 ^a	32.55 ± 0.83 ^a	1062.00 ± 86.79 ^a
CDM200	8.34 ± 0.46 ^a	6.60 ± 0.39 ^a	40.10 ± 2.16 ^a	12.76 ± 0.56 ^a	60.66 ± 0.51 ^a	19.36 ± 0.41 ^a	31.90 ± 0.48 ^a	822.00 ± 86.76 ^a
CDM400	7.90 ± 0.81 ^a	6.46 ± 0.17 ^a	38.16 ± 1.14 ^a	12.18 ± 0.25 ^a	59.36 ± 1.92 ^a	18.58 ± 0.22 ^a	31.98 ± 0.36 ^a	953.40 ± 68.31 ^a

WBC: White blood cells, RBC: red blood cells, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration, MCH: Mean corpuscular haemoglobin, PLT: Platelet, CON: Control, GEN: Gentamicin, CDM200: *Capparis decidua* aerial parts methanolic extract low dose, CDM400: *Capparis decidua* aerial parts methanolic extract high dose, values are expressed as Mean ± SD, means within the same column with different superscripts are significantly different at p < 0.05 (n = 5)

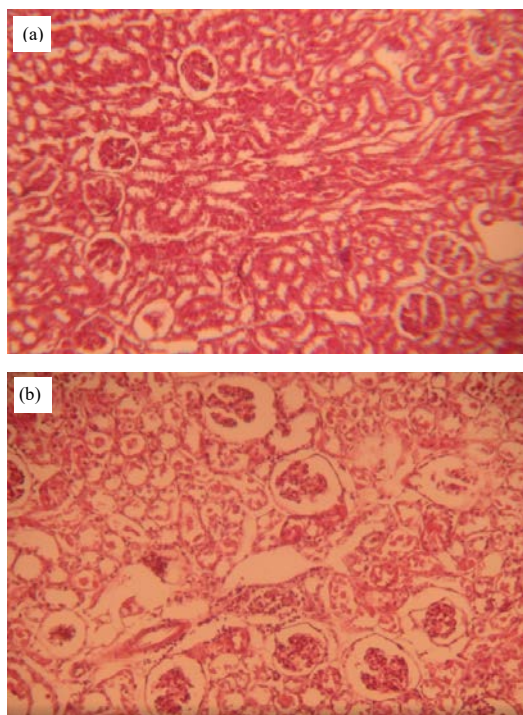


Fig. 1(a-b): (a)Control, showing normal histological appearance and (b) Section of kidney of rat treated with gentamicin showing destroyed tubules in renal cortex, dilation in bowman spaces some of them appeared shrinkaged and enucleated tuft
10X, H and E stain

Table 2: Change of urea and creatinine levels after administration of *C. decidua* aerial parts methanolic extract and gentamicin in rats

Treatments	Urea (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)
CON	34.00±2.02 ^c	0.54±0.07 ^b
GEN	71.40±3.17 ^a	3.10±0.68 ^a
CDM200	56.50±7.19 ^b	1.20±0.22 ^b
CDM400	53.80±3.60 ^b	0.94±0.07 ^b

CON: Control, GEN: Gentamicin, CDM200: *Capparis decidua* aerial parts methanolic extract low dose, CDM400: *Capparis decidua* aerial parts methanolic extract high dose, values are expressed as Mean±SD, means within the same column with different superscripts are significantly different at p<05 (n = 5)

Table 3: Change in body weights of rats after administration of *C. decidua* aerial parts methanolic extract and gentamicin in rats

Treatments	Day 0 (g)	Day 7 (g)
CON	146.0±12.94 ^a	150.0±12.31 ^a
GEN	149.3±28.39 ^a	143.7±23.45 ^a
CDM200	144.6±7.23 ^a	146.0±7.60 ^a
CDM400	142.6±7.81 ^a	148.2±7.73 ^a

CON: Control, GEN: Gentamicin, CDM200: *Capparis decidua* aerial parts methanolic extract low dose, CDM400: *Capparis decidua* aerial parts methanolic extract high dose, values are expressed as Mean±SD, means within the same column with different superscripts are significantly different at p<05 (n = 5)

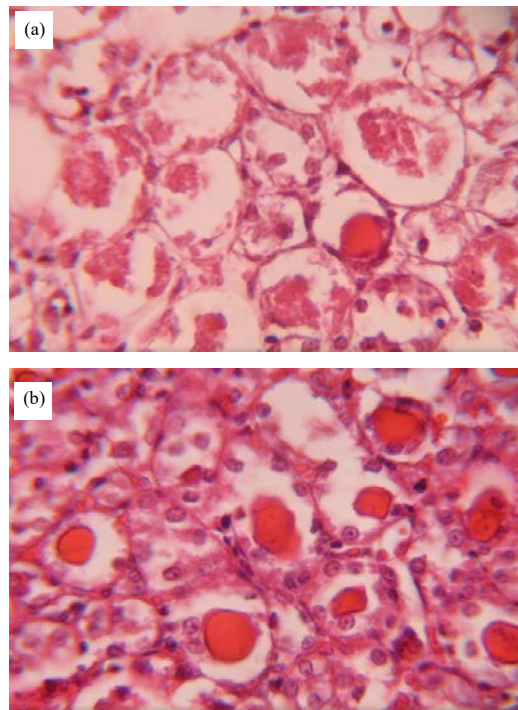


Fig. 2(a-b): Sections of kidney of rat treated with gentamicin, (a) Showing desquamation of cellular tubules with presence of eosinophilic casts and (b) Eosinophilic casts
25X, H and E stain

Table 4: Relative kidney weights after administration of *C. decidua* aerial parts methanolic extract and gentamicin in rats

Treatments	Right (g)	Left (g)
CON	0.34±0.02 ^b	0.32±0.01 ^b
GEN	0.46±0.05 ^a	0.46±0.06 ^a
CDM200	0.46±0.01 ^a	0.45±0.02 ^a
CDM400	0.50±0.03 ^a	0.49±0.02 ^a

CON: Control, GEN: Gentamicin, CDM200: *Capparis decidua* aerial parts methanolic extract low dose, CDM400: *Capparis decidua* aerial parts methanolic extract high dose, values are expressed as Mean±SD, means within the same column with different superscripts are significantly different at p<05 (n = 5)

Body weight of rats: There was no significant differences (p>0.05) in body weight between all experimental groups (Table 3).

Pathological findings: No salient pathological changes were seen in experimental rats except in the kidney of the treated groups showed enlarged pales kidneys.

The relative weight of kidneys was significantly increased in gentamicin group (GEN) and in rats receiving the plant extract (CDM200 and CDM400) compared to control as shown in Table 4.

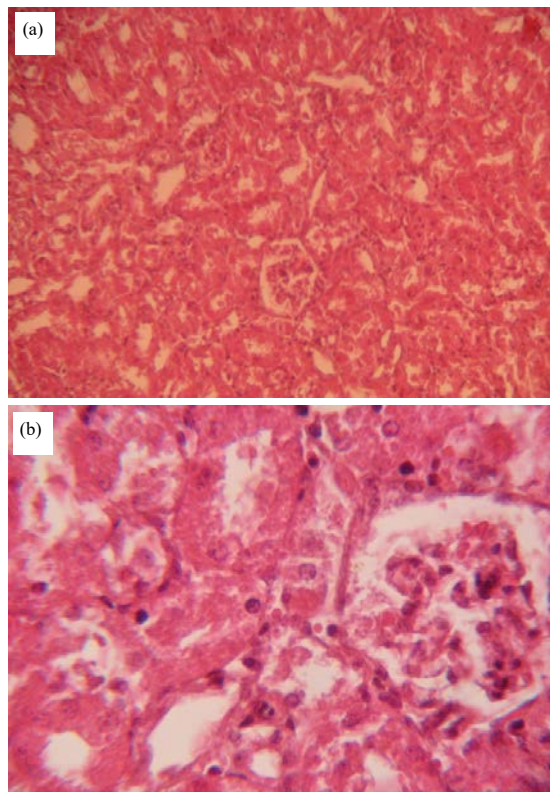


Fig. 3(a-b): Sections of kidney of rat treated with 200 mg kg⁻¹ (a) Showing normal glomeruli with dilated bowman spaces and (b) cytoplasmic swelling in cortical tubular cell epithelium
10X, 25X, H and E stain

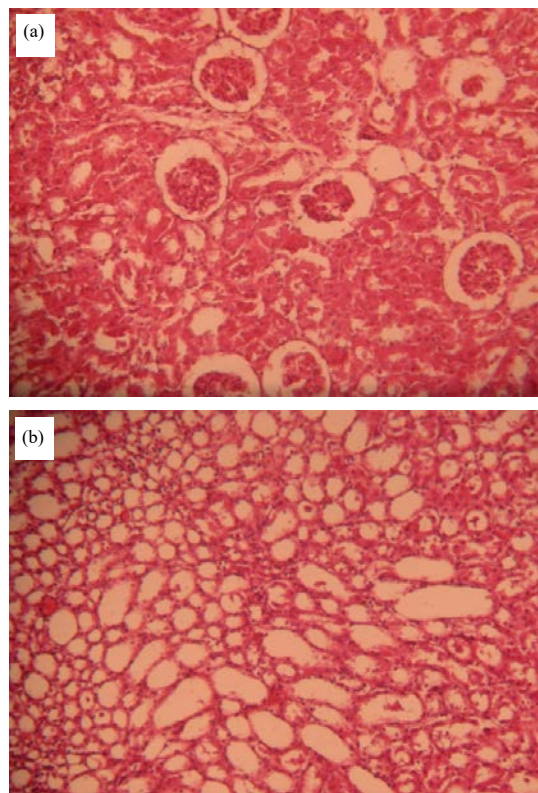


Fig. 4(a-b): Section of kidney of rat treated with 400 mg kg⁻¹ group, dilated (a) Glomeruli with dilated bowman spaces, swelling tubular epithelium and (b) medullary tubules with small eosinophilic cast
10X, 4X, H and E stain

Histopathological examination: Examination of the kidney sections of control rats revealed normal histological appearance (Fig. 1 and 2). The administration of gentamicin caused significant renal damage especially in the cortex tubules. Bowman spaces were significantly dilated and some of the glomerular tufts appeared shrunken or completely missing. Some medullary tubules showed desquamation and eosinophilic casts. Focal interstitial mononuclear cell infiltration was also seen in the medulla (Fig. 3). Kidney sections of rats that received low dose (200 mg kg⁻¹) of *C. decidua* methanolic extract displayed normal glomeruli, some of them showing dilated bowman spaces, cell swelling of cortical tubular epithelium were seen (Fig. 4). Kidney of rats given high dose (400 mg kg⁻¹) of *C. decidua* methanolic extract showed separated disrupted dilated tubules in cortex and medulla. Dilatations of bowman spaces with shrinkage of glomerular tuft were seen. Casts were seen in medullary tubules (Fig. 4).

DISCUSSION

This study was conducted to investigate the nephroprotective activity of *Capparis decidua* aerial parts methanolic extract against gentamicin induced nephrotoxicity in rats. Rats treated with gentamicin exhibited a marked elevation in serum urea and creatinine, which reflect a significant functional impairment of kidney in gentamicin group, this is in agreement with previous reports^{4,25}. Gentamicin toxicity has been induced as a result of the release of the oxidants in kidney which destructing the nephrons. Gentamicin leads to increase in the production of hydrogen peroxide by renal cortical mitochondria²⁶.

In renal disorders, the serum urea rises because the rate of urea production exceeds the rate of clearance. Creatinine is regularly derived from endogenous sources by tissue creatinine breakdown²⁷. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine²⁸.

Administration of *C. decidua* methanolic extract produced nephroprotective effect as evidenced by its ability to decrease serum urea and creatinine levels significantly compared to gentamicin group. However, haematological parameters were not affected in all treated group, except rats' injected gentamicin showed decreased MCV levels. The relative kidney weights of treated groups were significantly increased compared to normal control.

In histopathology, injection of gentamicin produced obvious renal damage especially to the renal cortex tubules. Glomerular congestion, especially in glomerular tufts, desquamation of tubules epithelium, inflammatory cells infiltrations were clearly seen. This is in the agreement with various previous studies^{6,25,29}. The rats receiving the *C. decidua* methanolic extract along with gentamicin displayed less change in renal tubules.

These damages could be due to increase the production of free radicals which causes oxidative stress to renal tubules. *C. decidua* aerial parts methanolic extract exhibited nephroprotective effect which was clearly indicated the less pathological changes and reduced urea and creatinine levels. The protection effect seems to be better in rats receiving the low dose (200 mg kg⁻¹) compared to the high dose (400 mg kg⁻¹).

Recent studies have shown that natural antioxidants obtained from different alternative systems of medicine display a wide range of biological activities⁴.

Some nephroprotective plants have been stated of inhibiting harmful effects of nephrotoxic agents in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects²⁸. Phytochemicals such as alkaloids and flavonoids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity^{2,28,30}.

The protection offered by the extract could have been due to the antioxidant effect of the plant or the presence of flavonoids and alkaloids. The inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical has been investigated³¹.

The plant sources are rich of antioxidants, phytoconstituents are capable to terminate free radical reactions and prevent the body from oxidative damage³².

CONCLUSION

In conclusion, this study demonstrated that *C. decidua* aerial parts may have a potential nephroprotective action against gentamicin induced nephrotoxicity in rats. The nephroprotective effect appears to be better with low dose

(200 mg kg⁻¹) than high dose (400 mg kg⁻¹). Further studies should be done to explore the exact compound(s) responsible for this action.

SIGNIFICANCE STATEMENT

This study discovers that *C. decidua* multipurpose tree, can be beneficial for treatment of renal disorders. The study will help the researcher in discovering a new source of nephroprotective agent. This is due to the promising activity of aerial parts of this plant in protecting the damage induced by gentamicin in rats. The study also confirmed its vital role in future for therapeutic fields.

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