Invited Special Paper

ANNALS OF MEDICINE & SURGERY

OPEN

Alteration in oxidative/nitrosative disparity and nephroprotective effect of hydroethanolic extract of *Ocimum tenuiflorum* L. in gentamicin-induced acute kidney injury

Neeraj Thakur, PhD[a,b,*], S.K. Shukla, PhD[a], Mahesh Kumar, PhD[a], Chethan G.E., PhD[b,*], Alok Singh, PhD[a,c], Radhika, PhD[a], Anand Kumar Singh, PhD[a,d], Kruti Debnath Mandal, PhD[e], M. Saminathan, PhD[f], Priyanka Choudhary, PhD[g], U.K. De, PhD[h], Kalyan Sarma, PhD[b]

Background: Antibacterial, antioxidant, and antilipidemic properties of *Ocimum tenuiflorum* are well known from previous studies. This study was designed to study the phytochemical constituents, antioxidant efficacy, *in vivo* nephroprotective activity, and immunomodulatory potential of *Ocimum tenuiflorum* hydroethanolic extract in gentamicin-induced acute kidney injury (AKI) rat model.

Methods: *Ocimum tenuiflorum* extract was given for 8 days to gentamicin-induced toxicity (100 mg/kg) in rats. Nephroprotective and immunomodulatory efficacy of *O. tenuiflorum* extract was evaluated based on urine and serum biochemistry, blood and tissue oxidative stress indices, cytokine levels, kidney injury biomarkers, and histopathology.

Results: Gentamicin toxicity resulted in a reduction in catalase, glutathione reductase, superoxide dismutase, and interleukin-10 levels in blood and tissue homogenates, while an increase in serum creatinine, blood urea nitrogen, lipid peroxide, tumor necrosis factor-alpha, cystatin C, kidney injury molecule-1, and gamma-glutamyl transpeptidase levels. Treatment with *O. tenuiflorum* ameliorated oxidative stress, cytokine imbalance, and kidney injury; however, the results were almost similar to standard drug. Furthermore, histopathological analysis of kidney, liver, and heart tissues confirmed the organoprotective efficacy of *O. tenuiflorum* extract.

Conclusion: The present findings demonstrate the curative efficacy of *O. tenuiflorum* in gentamicin-induced AKI, probably mediated through phenolic and flavonoid phytoconstituents, antioxidant properties, and down-regulation of inflammatory cytokines. Therefore, future studies may be established to evaluate its efficacy and safety for clinical trials.

Keywords: acute kidney injury, cystatin C, gamma-glutamyl transpeptidase, kidney injury molecule-1, *Ocimum tenuiflorum* L., oxidative stress

[a]*Department of Veterinary Medicine, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar, Uttarakhand, India,* [b]*Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India,* [c]*Department of Teaching Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, ANDUA&T, Kumarganj, Ayodhya, Uttar Pradesh, India,* [d]*Livestock Farm Complex (Veterinary Medicine), College of Veterinary and Animal Sciences, Nauner, Datia, Madhya Pradesh, India,* [e]*Veterinary Clinical Complex, Faculty of Veterinary and Animal Sciences, RGSC-Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh, India,* [f]*Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India,* [g]*Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Rampura Phul, Bathinda, Punjab, India and* [h]*Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India*

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

**Corresponding author. Address: Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram 796015, India. Tel.: +91 9882262629. E-mail: niju.thakur92@gmail.com (N. Thakur); Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram 796015, India. Tel.: +91 6395004921. E-mail: chethanvetmed@gmail.com (Chethan G. E.).*

Annals of Medicine & Surgery (2025) 87:2674–2688

Introduction

Exposure to toxins, drugs, and other compounds is the main reason behind *in vivo* acute kidney injury (AKI)[1]. AKI, a reversible condition where there is a quick decline in renal function manifested by elevated serum creatinine (SCr) and blood urea nitrogen (BUN) concentrations, has replaced the word acute renal failure[2]. Even with the use of numerous pharmacologic agents, the mortality rate of patients with AKI has remained at 25–70%. AKI can be of pre-renal, renal, or post-renal origin, and among these pre-renal causes account for 40–70% of all the cases[3]. AKI is estimated to be the cause of over 2 million deaths worldwide each year. In order to fully comprehend the pathophysiological significance and mechanisms of AKI, comprehensive models must be used, as they present significant challenges in both human and veterinary medicine. Human medicine is expanding its understanding of AKI due to novel findings in epidemiology, pathophysiology, and biomarkers[4]. Animal models, including laboratory animals and bigger mammals, offer cutting-edge tools for experimental research that complement the investigation of AKI mechanisms,

Received 11 February 2025; Accepted 20 March 2025

Published online 2 April 2025

http://dx.doi.org/10.1097/MS9.0000000000003230

HIGHLIGHTS

- *Ocimum tenuiflorum* L. extract showed anti-inflammatory effects in gentamicin-induced acute kidney injury by decreasing lipid peroxidation and increasing antioxidant enzymes (glutathione and catalase) levels in urine, serum, and tissue homogenates.
- Immunomodulatory effects of *O. tenuiflorum* L. were evident from a decrease in tumor necrosis factor-alpha and an increase in interleukin-10 levels in urine, serum, and tissue homogenate.
- Kidney injury biomarker (kidney injury molecule-1, cystatin C, and gamma-glutamyl transpeptidase) levels were reduced in experimental animals after treatment with *O. tenuiuflorum* L. extract suggesting nephroprotective effects.

toxin-induced damage, and genetic predispositions[5]. Researchers endeavor to elucidate the complex nature of AKI and convert their findings into clinical breakthroughs to alleviate its influence on health outcomes in various species by utilizing human and animal models. This holistic approach enables the development of more potent methods of diagnosis and therapeutic interventions to enhance outcomes for patients with AKI. AKI rarely has a single, identifiable pathogenesis, and mostly heterogeneous etiologies are involved. Endoplasmic reticulum stress in beta cells of the pancreas leads to reduced insulin levels, and as a result, it predisposes to AKI development[6]. Soluble mediators like cytokines are released from injured kidney cells and attract leukocytes to the site of injury in AKI caused by ischemia/reperfusion injury[7]. Tumor necrosis factor-alpha (TNF-α), an inflammatory cytokine, can stimulate inflammatory cells and aggravate inflammatory response in AKI, and interleukin-10 (IL-10), an anti-inflammatory cytokine, can help in reducing the inflammatory response[8]. AKI has been diagnosed with a number of biomarkers that are in various stages of development and validation. Kidney injury molecule-1 (KIM-1) is a type 1 membrane protein that can cleave and rapidly enter tubule lumen after kidney injury and can be detected in urine[9]. Cystatin C (CysC) is an endogenous cysteine proteinase inhibitor that is secreted into urine when there is acute tubular epithelial damage. Gamma-glutamyl transpeptidase (GGT) is a brush border enzyme localized in proximal renal tubule and released into urine as a result of tubular cell damage[10].

The body has enzymatic and non-enzymatic systems that defend against oxidative damage, but antioxidant enzyme levels get depleted in AKI[11]. The primary elements of the antioxidant system are superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) reductase, glutathione peroxidase (GPx), and antioxidant vitamins A, E, and C. The sequential removal of peroxides and free radicals and the mutual shielding of enzymes are the foundations for the synergistic and cooperative interactions of antioxidants. The manifestation of oxidative stress results from the inadequate availability of antioxidant enzymes or excessive formation of reactive oxygen species/reactive nitrogen species (ROS/RNS), which leads to the oxidation of the polyunsaturated fatty acids in the red blood cell (RBC) membrane and lipid peroxidation[12]. ROS/RNS generation has been linked to pathophysiology of renal damage[13]. Any substance with antioxidant or anti-inflammatory activity will have a preventive effect against kidney damage, as oxidative stress and inflammatory damage have been primarily linked to the development of AKI[2]. Management of AKI is mostly supportive with renal replacement therapy, including life support measures like hemodialysis and peritoneal dialysis. Since there are not many specific nephroprotective therapies available, preventing kidney damage in intensive care remains a significant concern and seeking alternative therapeutic measures becomes a necessity[14]. Medicinal plants have always been an integral part of traditional medicinal systems. Various medicinal plants like *Astragalus membranaceus, Orthosiphon stamineus*, and *Azima tetracantha* are reported to have nephroprotective activity[2]. *Ocimum tenuiflorum* L. (Krishna Tulsi), also known as *Ocimum sanctum* L., is one of the oldest herbs within the *Ocimum* genus in the Lamiacae family. It is a purple-green-leaved monotype of common basil, a well-known medicinal plant that grows in the wild as well as in Indian houses. It is grown as a perennial herb in tropical and subtropical regions of Asia, Africa, South, and Central America. *Ocimum tenuiflorum* L. has been traditionally used by people of several states of India, including Uttarakhand and Himachal Pradesh, for the management of affections related to the urinary system, treatment of cold and cough, kidney stones, and hyperglycemia[15]. The whole plant is crushed and juice is consumed for remedy from the affection. Pharmacological activities of *O. tenuiflorum* L. include antibacterial, antilipidemic, antioxidant, and protective action against diabetic nephropathy and renal damage[16]. Antioxidant and renoprotective effects of some constituents of herbal remedies have protective effects against renal injuries[17]. The present study was undertaken to evaluate the organoprotective efficacy of *O. tenuiflorum* L. hydroethanolic extract in gentamicin-induced AKI Wistar albino rat model. Amelioration of oxidative stress, cytokine imbalance, and kidney injury were considered as primary outcome measures. We hypothesized that supplementation of *O. tenuiflorum* L. will alter the ROS/RNS levels, pro-inflammatory/anti-inflammatory cytokine levels, urine and serum biochemical profiles, and urine and serum concentrations of kidney injury biomarkers like KIM-1, CysC, and GGT.

Methods

Plant material and extraction

Ocimum tenuiflorum L. plant collection was done from the vicinity of Pantnagar, Uttarakhand, India. Identification of the plant was done by the Botanical Survey of India, NRC, Dehradun, India (No. BSI/NRC/Tech./Herb.(Ident.)pkd/2021-22/250). The plant name has been checked with http://www.worldfloraonline.org on 1 August 2024. The whole plant was dried and then cut into small pieces, washed in distilled water to eliminate dirt, air dried, and then pulverized uniformly with an electric grinder. The powdered plant material was overnight steeped in hydroethanolic (1:1) solvent and then extracted using a magnetic stirrer for 6 h at room temperature. Whatman filter paper (No. 40) was used to filter, and solvent was extracted by using a rotary evaporator. The extract was vacuum sealed and refrigerated until used. The yield of *O. tenuiflorum* L. from extract was 24.8%, with dark green, thick, and sticky residue.

Quantitative analysis of extract for phytochemicals

Total phenol content

The concentration of phenolics in plant extract was assessed using the Folin–Ciocalteu reagent with slight modifications from the procedure described by Biglari *et al*[18].

Total flavonoid content

The total flavonoid content was estimated using a modified aluminum chloride colorimetric assay[19].

Total flavanol content

The total flavanol content in plant extract was estimated according to the method given by Kumaran and Karunakaran[20].

Study of in vitro *antioxidant activity*

DPPH scavenging assay and total antioxidant capacity

The antioxidant activity of *O. tenuiflorum* L. plant extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and total antioxidant capacity (TAC) was estimated according to the phosphomolybdenum method described by Asnaashari *et al*[21] and Prieto *et al*[22], respectively.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) analysis of *O. tenuiflorum* L. hydroethanolic extract and powder was carried out by outsourcing at the Council of Scientific and Industrial Research–Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India.

In vivo *evaluation of nephroprotective activity*

Study animals

Wistar albino male rats (IVRI2CQ), 12–13 weeks old, weighing around 150–200 g, were acquired from Laboratory Animal Section, Indian Council for Agricultural Research–Indian Veterinary Research Institute, Izatnagar, and utilized for the experiment after approval from Institute Animal Ethics Committee (IAEC) wide letter number IAEC/CVSc/VMD/341 following international, national, or institutional guidelines for humane treatment of animals. An acclimatization period of 2 weeks was provided to animals in the laboratory animal house of the institute. Throughout the study period, the rats were housed in clean polypropylene cages at room temperature of 25 ± 2 °C with a 12 h light/12 h dark cycle at the laboratory animal house of the institute. The animals were fed a standard diet and had access to *ad libitum* water.

Experimental design

The nephroprotective and antioxidant activity of the plant material was determined in the gentamicin-induced AKI rat model described earlier[23]. Twenty-four male rats were randomly divided into four groups each containing six animals. Group I (Gp. I) served as a healthy control and was not given any treatment. Group II (Gp. II) rats served as disease control and received gentamicin at 100 mg/kg body weight (BW) intra-peritoneally (IP) for 8 days. Group III (Gp. III) rats received gentamicin along with standard herbal drug Cystone® (Himalaya Pharmaceutical Company, India) at 100 mg/kg BW orally by gastric gavage for 8 days. Group IV (Gp. IV) received gentamicin along with hydroethanolic (1:1) extract of *O. tenuiflorum* L. [Effective Concentration$_{50}$ (EC_{50})] at 400 mg/kg BW orally through gastric gavage for 8 days (The EC_{50} was calculated from the earlier study: Neeraj Thakur)[24].

Sampling

A 24-h urine sample was collected from individual animals by housing them in metabolic cages on days 0, 4, and 8 of the study. Part of each urine sample was acidified using 5 M hydrochloric acid to pH 2. Urine samples were then centrifuged at 1500 g for 10 minutes to remove debris, and supernatants were stored at –20 °C till analysis. Blood samples were collected on days 0, 4, and 8 of the study by venipuncture of the tail vein of rats into vials containing heparin and clot activator. Blood samples in clot-activator containing vials were allowed to clot and centrifuged at a speed of 3000 revolutions per minute (rpm) for 20 minutes at 4 °C to separate serum and stored at –20 °C until analysis. Animals were sacrificed at the end of study (day 8), and kidney, heart, and liver tissue samples were collected for histopathological analysis and the evaluation of oxidative stress indices. One half of the kidney was preserved in 2.5% glutaraldehyde for electron microscopy.

Urine and serum biochemistry

Urine urea nitrogen (UUN), creatinine, total protein, albumin, and sodium and potassium levels in urine and BUN, creatinine, total protein, albumin, and sodium and potassium concentrations in serum were estimated by using commercially available kits as per the manufacturer's instructions (Tulip Diagnostics Private Limited, Goa, India) spectrophotometrically using Thermo Scientific Spectro 20D+ spectrophotometer.

Estimation of oxidative/nitrosative stress indices

Oxidative stress indices in blood

Preparation of RBC suspension and RBC hemolysate. Approximately 3 mL of blood samples were collected from rats in vacutainers containing heparin (10 IU/mL) as an anticoagulant. Plasma and buffy coats were removed after centrifugation at 3000 rpm for 10 minutes. RBCs were washed thrice in an ice-cold isotonic normal saline solution (NSS). RBC pellet was diluted in a 1:10 ratio with ice-cold distilled water for preparing 10% hemolysate, and leftover RBC pellet was diluted with ice-cold NSS in a 1:1 ratio to get RBC suspension.

Reduced GSH. The estimation of GSH in blood was done by the 5-5′dithiobis(2-nitrobenzoic acid) (DTNB) method in RBC suspension as described by Prins and Loos[25].

Lipid peroxide. The lipid peroxide (LPO) level in RBC hemolysate was determined by the method given by Placer *et al*[26].

Catalase. CAT activity in RBC hemolysate was estimated using the method of Bergmayer[27], with hydrogen peroxide (H_2O_2) as a substrate.

Superoxide dismutase. The SOD level in blood was estimated using the method of Madesh and Balasubramanian[28].

Oxidative/nitrosative indices in tissue homogenate

Preparation of tissue homogenates. Tissue section (200 mg) of kidney, heart, and liver was collected in ice-cold phosphate buffer saline (PBS) (pH 7.4) and under cold conditions; tissue homogenates were prepared using a tissue homogenizer. Tissue homogenates were centrifuged at 3000 rpm for 10 minutes, the supernatant obtained was employed for the estimation of LPO/malondialdehyde (MDA) and nitric oxide (NO). For GSH estimation, organ samples were collected in separate solution containing 0.02 M EDTA.

Reduced glutathione. The GSH level was determined by estimating free –SH groups, using the DTNB method suggested by Sedlak and Lindsay[29].

Malondialdehyde. Extent of lipid peroxidation was estimated in terms of MDA concentration and was determined by the thiobarbituric acid method given by Rehman[30].

Nitric oxide. NO content in tissue homogenate was estimated by using Griess reagent as per method given by Green *et al*[31].

Immunomodulatory potential of *O. tenuiflorum* extract. TNF-α (catalog no. KB3145) levels in urine and serum and IL-10 (catalog no. k11-0109) levels in serum and tissue homogenate were measured by using commercially available enzyme linked immunosorbant assay kits according to the manufacturer's instructions (Krishgen BioSystems, Mumbai, India).

Kidney injury biomarkers. CysC (catalog no. k11-0146), KIM-1 (catalog no. k11-0550) (Krishgen BioSystems, Mumbai, India), and GGT (Aspen Laboratories, Pvt. Ltd., Delhi) were estimated by commercially available kits following the manufacturer's guidelines.

Histopathology. Kidney, liver, and heart tissue samples (5 mm) were collected and fixed in 10% formalin. Tissue samples were processed for histopathological examination as per the standard protocol.

Electron microscopy. Fixation of collected tissue samples (1–1.5 mm) was done in 2.5% glutaraldehyde for 12 h at 4 °C for ultrastructural studies by electron microscope. Fixed tissues were then washed at 4 °C in 0.1 M PBS (pH 7.2) three times for 15-minutes. Washed tissues were then dehydrated in ascending grades of alcohol for 20 minutes each at 4 °C, followed by washing in absolute alcohol. In a liquid carbon dioxide chamber, dehydrated tissues were dried at 31.5 °C at 1100 psi. After that, dried tissues were mounted on aluminum stubs with adhesive tape and were gold coated by JFC-1600 auto-fine sputter gold coater. Coated tissues were viewed at 80 kV under a scanning electron microscope JSM-6610 LV (JEOL Ltd., Japan).

Statistical analysis

The data were analyzed using one-way analysis of variance using Statistical Package for the Social Sciences (SPSS) version 27. Data (n = 6 in each group) were analyzed to calculate the mean, standard error of mean (SEM), and significance of difference ($P < 0.05$), if any, between mean values of different groups and days. Graphs were drawn in GraphPad Prism version 9.

Results

Quantitative analysis of O. tenuiflorum *hydroethanolic extract for phytochemical screening*

Total phenol, flavonoids, and flavanol contents

Total phenols were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract, while flavanol and total flavonoid contents were estimated in terms of quercetin equivalents (QE) (Table 1).

Quantification of bioactive compound by HPLC

HPLC analysis of hydroethanolic extract showed the presence of rosmarinic acid (Fig. 1). Compound was identified by comparing its retention time with standard reference compound (Table 2).

In vitro *evaluation of antioxidant activity*

DPPH assay

DPPH radical scavenging activity of hydroethanolic extract of *O. tenuiflorum* L. was compared with standard antioxidant (ascorbic acid), and results have been presented in (Table 3).

Total antioxidant capacity

TAC was determined by the phophomolybdenum method, and results are presented in (Table 4).

In vivo *estimation of oxidative/nitrosative stress indices in gentamicin-induced AKI*

Ocimum tenuiflorum L. extract increases antioxidant enzymes and reduces lipid peroxidation in blood

In disease control (Gp. II), GSH, SOD, and CAT levels were significantly ($P < 0.05$) reduced, while LPO levels were elevated in blood when compared to healthy control (Gp. I) on day 4 and 8. Treatment groups with standard treatment (Gp. III) and *O. tenuiflorum* L. (Gp. IV) showed a significant ($P < 0.05$) increase in GSH, SOD, and CAT levels, and a significant ($P <$

Table 1

Total phenolic content, total flavonoid, and total flavanol content in prepared extracts

Plant extract	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total flavanol content (mg QE/g)
Ocimum tenuiflorum L.	215.74 ± 1.38	64.26 ± 2.17	116.32 ± 1.52

GAE, QE.

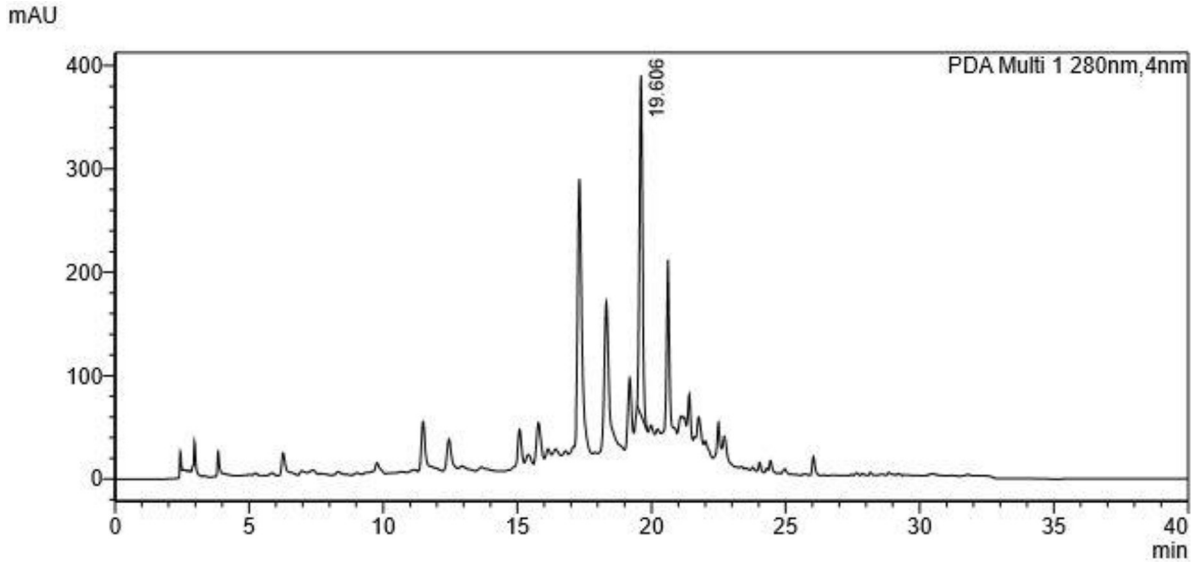

Figure 1. High-performance liquid chromatography chromatogram of *O. Tenuiflorum* hydro-ethanolic extract. Compound identification was done by comparing retention time with standard compounds.

0.05) decrease in LPO levels in comparison to Gp. II on days 4 and 8 (Fig. 2).

Ocimum tenuiflorum L. extract enhances antioxidant enzymes and reduces oxidative/nitrosative stress in tissue homogenates

Kidney, liver, and heart tissue homogenates in Gp. II had significantly ($P < 0.05$) reduced levels of GSH as compared to Gp. I and elevated levels of MDA and NO as compared to Gp. I. Hydroethanolic extract of *O. tenuiflorum* L. (Gp. IV) significantly ($P < 0.05$) enhanced GSH and reduced MDA and NO levels as compared to Gp. II (Fig. 3).

In vivo *evaluation of nephroprotective activity of hydroethanolic extract of* O. tenuiflorum *L.*

Ocimum tenuiflorum L. extract increases urine creatinine, UUN, serum total protein, albumin, and sodium levels and reduces SCr, BUN, urine total protein, urine albumin, and urine sodium levels in gentamicin-induced AKI

Urine total protein, albumin, sodium, SCr, and BUN levels were significantly ($P < 0.05$) elevated, and urine creatinine, UUN, serum total protein, albumin, and sodium levels were significantly ($P < 0.05$) reduced in Gp. II on days 4 and 8 as compared to Gp. I (Tables 5 and 6). Treatment with *O. tenuiflorum* L. (Gp. IV) significantly ($P < 0.05$) decreased urine total protein, albumin, sodium, SCr, and BUN levels, and significantly ($P < 0.05$) increased urine creatinine, UUN, serum total protein, albumin, and sodium levels similar to standard treatment (Gp. III) on days 4 and 8.

Ocimum tenuiflorum L. extract downregulates TNF-α and upregulates IL-10 expression in gentamicin-induced AKI

TNF-α levels were significantly ($P < 0.05$) elevated in urine as well as serum of disease control (Gp. II) when compared to healthy control (Gp. I) on days 4 and 8 (Fig. 4). TNF-α levels in Gp. III and IV were significantly ($P < 0.05$) reduced after treatment in comparison to Gp. II on days 4 and 8. Serum IL-10 levels were significantly ($P < 0.05$) decreased in Gp. II as compared to Gp. I on days 4 and 8. Treatment with *O. tenuiflorum* L. (Gp. IV) improved serum IL-10 levels, and results were comparable to standard treatment (Gp. III) (Fig. 4c). IL-10 levels in tissue homogenates of kidney, liver, and heart were significantly ($P < 0.05$) decreased in Gp. II when compared to Gp. I. In treatment groups (Gps. III and IV), these values were statistically similar and significantly ($P < 0.05$) higher compared to Gp. II (Fig. 5).

Ocimum tenuiflorum L. extract attenuates kidney injury biomarkers in gentamicin-induced AKI

CysC, KIM-1, and GGT levels in serum and urine were significantly ($P < 0.05$) higher in Gp. II as compared to Gp. I on days 4 and 8. *Ocimum tenuiflorum* L. treatment (Gp. IV) ameliorated kidney injury and attenuated CysC and KIM-1 levels in serum and urine, and GGT levels in urine. There was no significant difference in serum GGT levels among Gps. II, III, and IV (Tables 7 and 8).

Ocimum tenuiflorum L. extract reduces inflammation and damage to renal tissue, hepatocytes, and cardiomyocytes

In healthy control (Gp. I), normal glomerulus and proximal convoluted tubule (PCT) were seen lined by cuboidal cells in cortex, and normal collecting ducts in medulla [hematoxylin and eosin (H&E) ×100] (Fig. 6a and b). In disease control (Gp. II),

Table 2

HPLC analysis of hydroethanolic extract of *Ocimum tenuiflorum* L.

Compound	Retention time (min)	Area	% Content
Rosmarinic acid	19.606	2 599 104	0.13

HPLC, high-performance liquid chromatography.

Table 3

DPPH radical scavenging activity

Ascorbic acid			*Ocimum tenuiflorum* L.		
Concentration (µg/mL)	% Inhibition	IC_{50}	Concentration (µg/mL)	% Inhibition	IC_{50}
10	50.24 ± 1.98	7.155	10	47.21 ± 2.11	12.608
20	61.08 ± 2.11		20	55.13 ± 1.87	
30	75.34 ± 0.82		30	64.08 ± 1.77	
40	83.81 ± 0.71		40	73.31 ± 1.35	
50	90.25 ± 0.53		50	78.55 ± 0.41	
60	97.22 ± 0.32		60	85.26 ± 0.49	

DDPH, 2,2-diphenyl-1-picrylhydrazyl.

PCT had severe tubular degeneration, epithelial vacuolation, tubular dilatation, eosinophilic proteinaceous material, and basophilic cast in tubular lumen (Fig. 6c). Distal convoluted tubule (DCT) and collecting ducts of medulla showed tubular degeneration and tubular dilatation with eosinophilic cast in lumen (Fig. 6d). In standard treatment (Gp. III), PCT revealed mild tubular degeneration, tubular epithelial vacuolation and dilatation, and absence of eosinophilic proteinaceous material (H&E ×100) (Fig. 6e). DCT and collecting ducts showed mild degeneration, tubular vacuolation, and dilatation (H&E ×100) (Fig. 6f). In the *O. tenuiflorum* L. treatment group (Gp. IV), PCT showed mild tubular degeneration and dilatation, and no proteinaceous material and cast in tubular lumen (Fig. 6g). DCT showed mild tubular degeneration, dilatation, and cellular debris in lumen (Fig. 6h). Hepatocytes were arranged in cords with eosinophilic cytoplasm, and sinusoids were lined by endothelial cells and Kupffer cells in healthy control (Gp. I) (H&E ×200) (Fig. 7a). In disease control (Gp. II), liver showed marked dilatation of sinusoidal spaces, hepatocyte atrophy, and Kupffer cell hypertrophy (H&E ×200) (Fig. 7b). In standard treatment (Gp. III), mild dilatation of sinusoidal spaces was observed (H&E ×100) (Fig. 7c). In the *O. tenuiflorum* L.-treated group (Gp. IV), liver showed mild vacuolar degeneration and necrosis of hepatocytes (H&E ×200) (Fig. 7d). The longitudinal section of myocardium consisted of branching fibers of cardiac myocytes and intercalated discs in Gp. I (H&E ×200) (Fig. 8a). In Gp. II, transverse sections of myocardium revealed degeneration of cardiac myocytes (H&E ×200) (Fig. 8b). In Gp. III, the longitudinal section of myocardium showed mild degeneration of cardiac myocytes (Fig. 8c). In the group treated with *O. tenuiflorum* L. (Gp. IV), the longitudinal section of myocardium consisted of branching fibers of cardiac myocytes and intercalated discs (H&E ×200) (Fig. 8d).

Scanning electron microscopy

Kidney tissue of healthy control (Gp. I) appeared normal and pathological changes were absent. A tuft of microvilli from squamous cells lining the tubule was seen (Fig. 9a). In disease control (Gp. II), basal lamina of PCT filled with debris and erythrocytes were evident (Fig. 9b). Tuft of microvilli from squamous cells and few erythrocytes were noticed in the *O. tenuiflorum* L. treatment group (Fig. 9c).

Discussion

Gentamicin is a broad-spectrum aminoglycoside antibiotic that has been linked to nephrotoxicity and ototoxicity[32]. Gentamicin has been employed in various models for nephrotoxicity studies. Various antioxidants have been studied to protect renal physiological character and function, but to date, no antioxidant has been proven to be clinically efficient. With this concern, focus has been drawn toward the utilization of natural antioxidant sources, which can ameliorate renal injury and serve as alternate medicine to synthetic antioxidants[33]. Numerous natural products with therapeutic importance have been approved and made available as medications or are presently undergoing testing as potential new medications. The majority of immunocompromising medications, including mycophenolic acid, cyclosporine A, rapamycin, tacrolimus (FK506), and fingolimod (FTY720), are derived from natural compounds[34]. India has been acknowledged as a rich source of medicinal plants since ancient times. *Ocimum tenuiflorum* L. is commonly grown in the Indian sub-continent for its religious sanity and diverse medicinal properties[35]. Total phenolics and total flavonoid in hydroethanolic (50% ethanol) extract of the plant were calculated as GAE, which was found to be higher than previously reported levels in the butanol fraction and ethanol extract. It proves that hydroethanol is a better solvent for fractionation of polyphenolics and flavonoids. In phytochemical analysis of the hydroethanolic extract utilizing HPLC, rosmarinic acid has been isolated and quantified as a biologically active compound. In the present study, the percent content of rosmarinic acid present in raw material of *O. tenuiflorum* L. was 0.13%, which is consistent with the findings of other researchers, who reported 0.116% rosmarinic acid[36]. Phytochemical studies have depicted that oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and β-caryophyllene are some of the main chemical constituents of *O. tenuiflorum*. Various flavanoid compounds like esculin, caffeic acid, quercetin, and luteolin have been identified in extracts of *O. tenuiflorum* L[37]. Phenolic compounds can neutralize free radicals by easily donating electrons or hydrogen atoms. The number and position of hydroxyl groups alter the antioxidant capacity of phenolics, and rosmarinic acid has four phenolic hydroxyl groups attributing to *in vitro*

Table 4

Total antioxidant capacity of hydroethanolic extract of *Ocimum tenuiflorum* L.

Extract	Total antioxidant capacity in ascorbic acid equivalent (mg AAE/g extract)
O. tenuiflorum	63.17 ± 2.47

Table 5

Alterations in urine biochemistry on different days in disease control (Group II), standard treatment (Group III), and EC_{50} of *O. tenuiflorum* L. (Group IV) in comparison to healthy control (Group I)

Day	Healthy control ($n = 6$)	Disease control ($n = 6$)	Standard treatment ($n = 6$)	*O. tenuiflorum L.* EC_{50} ($n = 6$)
Urine creatinine (mg/dL)				
Day 0	33.513 ± 0.440^{Ax}	32.508 ± 0.929^{Ax}	32.435 ± 1.047^{Ax}	34.522 ± 0.432^{Ax}
Day 4	33.275 ± 0.398^{Ax}	24.531 ± 1.182^{By}	28.915 ± 0.690^{Cy}	29.240 ± 0.613^{Cy}
Day 8	32.968 ± 0.756^{Ax}	16.769 ± 0.638^{Bz}	25.224 ± 0.343^{Cz}	26.501 ± 0.408^{Cz}
Urine urea nitrogen (UUN) (mg/dL)				
Day 0	708.365 ± 6.628^{Ax}	689.807 ± 14.117^{Ax}	713.590 ± 8.317^{Ax}	699.135 ± 9.278^{Ax}
Day 4	714.051 ± 5.296^{Ax}	602.555 ± 3.910^{By}	686.627 ± 8.604^{Cx}	680.639 ± 5.156^{Cx}
Day 8	711.846 ± 3.978^{Ax}	388.416 ± 8.539^{Bz}	570.937 ± 6.011^{Cy}	571.680 ± 4.590^{Cy}
Urinary total protein (g/dL)				
Day 0	0.164 ± 0.002^{Ax}	0.160 ± 0.003^{Ax}	0.164 ± 0.001^{Ax}	0.159 ± 0.002^{Ax}
Day 4	0.162 ± 0.002^{Ax}	0.212 ± 0.004^{By}	0.181 ± 0.003^{Cy}	0.186 ± 0.002^{Cy}
Day 8	0.165 ± 0.002^{Ax}	0.317 ± 0.005^{Bz}	0.221 ± 0.004^{Cz}	0.213 ± 0.006^{Cz}
Urinary albumin (g/dL)				
Day 0	0.085 ± 0.002^{Ax}	0.084 ± 0.002^{Ax}	0.084 ± 0.001^{Ax}	0.085 ± 0.002^{Ax}
Day 4	0.081 ± 0.002^{Ax}	0.123 ± 0.005^{By}	0.100 ± 0.004^{Cy}	0.103 ± 0.005^{Cy}
Day 8	0.081 ± 0.002^{Ax}	0.170 ± 0.003^{Bz}	0.116 ± 0.003^{Cz}	0.118 ± 0.002^{Cy}
Urinary sodium (µmol/mL)				
Day 0	1.35 ± 0.02^{Ax}	1.33 ± 0.02^{Ax}	1.33 ± 0.02^{Ax}	1.37 ± 0.01^{Ax}
Day 4	1.34 ± 0.03^{Ax}	1.60 ± 0.02^{By}	1.41 ± 0.02^{ACx}	1.40 ± 0.02^{ACx}
Day 8	1.34 ± 0.03^{ACx}	2.15 ± 0.05^{Bz}	1.47 ± 0.04^{Cx}	1.31 ± 0.03^{Ax}
Urinary potassium (µmol/mL)				
Day 0	0.036 ± 0.001^{Ax}	0.036 ± 0.001^{Ax}	0.037 ± 0.002^{Ax}	0.037 ± 0.001^{Ax}
Day 4	0.038 ± 0.001^{Ax}	0.028 ± 0.001^{By}	0.032 ± 0.001^{Cxy}	0.032 ± 0.001^{Cx}
Day 8	0.040 ± 0.001^{Ax}	0.017 ± 0.001^{Bz}	0.026 ± 0.001^{Cy}	0.028 ± 0.001^{Cxy}

The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$).

lipid peroxidation inhibition and radical scavenging activity. Radical scavenging activity depicted that *O. tenuiflorum* L. extract contains active constituents that can impart hydrogen to a free radical and nullify the potentially damaging entity. TAC of hydroethanolic extract of *O. tenuiflorum* L. indicated the presence of powerful reducing agents in extract. It has been

Table 6

Alterations in serum biochemistry on different days in disease control (Group II), standard treatment (Group III), and EC_{50} of *O. tenuiflorum* L. (Group IV) in comparison to healthy control (Group I)

Day	Healthy control ($n = 6$)	Disease control ($n = 6$)	Standard treatment ($n = 6$)	*O. tenuiflorum L.* EC_{50} ($n = 6$)
Creatinine (mg/dL)				
Day 0	0.805 ± 0.008^{Ax}	0.874 ± 0.033^{Ax}	0.867 ± 0.040^{Ax}	0.799 ± 0.036^{Ax}
Day 4	0.822 ± 0.005^{Ax}	1.312 ± 0.003^{By}	1.169 ± 0.031^{Cy}	1.138 ± 0.018^{Cy}
Day 8	0.830 ± 0.019^{Ax}	2.055 ± 0.052^{Bz}	1.289 ± 0.007^{Cz}	1.298 ± 0.005^{Cz}
BUN (mg/dL)				
Day 0	18.629 ± 0.256^{Ax}	17.345 ± 0.470^{Ax}	19.236 ± 0.576^{Ax}	19.128 ± 0.565^{Ax}
Day 4	18.899 ± 0.147^{Ax}	31.906 ± 0.394^{By}	23.263 ± 0.243^{Cy}	22.747 ± 0.309^{Cy}
Day 8	18.784 ± 0.210^{Ax}	69.341 ± 0.507^{Bz}	36.915 ± 0.338^{Cz}	36.698 ± 0.307^{Cz}
Total protein (g/dL)				
Day 0	6.855 ± 0.099^{Ax}	6.891 ± 0.140^{Ax}	6.916 ± 0.064^{Ax}	6.898 ± 0.069^{Ax}
Day 4	6.812 ± 0.158^{Ax}	5.128 ± 0.105^{By}	6.326 ± 0.035^{Cy}	6.392 ± 0.078^{Cy}
Day 8	7.071 ± 0.061^{Ax}	4.165 ± 0.057^{Bz}	5.893 ± 0.059^{Cz}	5.797 ± 0.114^{Cz}
Albumin (g/dL)				
Day 0	4.166 ± 0.040^{Ax}	4.215 ± 0.054^{Ax}	4.210 ± 0.041^{Ax}	4.255 ± 0.056^{Ax}
Day 4	4.224 ± 0.051^{Ax}	3.335 ± 0.070^{By}	4.012 ± 0.210^{Acy}	3.995 ± 0.093^{Acy}
Day 8	4.099 ± 0.067^{Ax}	2.378 ± 0.092^{Bz}	3.624 ± 0.032^{Cy}	3.619 ± 0.101^{Cy}
Sodium (mmol/L)				
Day 0	139.89 ± 1.09^{Ax}	137.26 ± 1.38^{Ax}	138.31 ± 0.82^{Ax}	136.22 ± 1.51^{Ax}
Day 4	139.12 ± 1.53^{Ax}	120.02 ± 1.88^{By}	129.38 ± 0.86^{Cy}	128.84 ± 0.70^{Cy}
Day 8	138.18 ± 1.34^{Ax}	88.38 ± 1.51^{Bz}	116.60 ± 1.85^{Cz}	112.07 ± 2.63^{Cz}
Potassium (mmol/L)				
Day 0	3.780 ± 0.040^{Ax}	3.901 ± 0.039^{Ax}	3.821 ± 0.026^{Ax}	3.750 ± 0.036^{Ax}
Day 4	3.921 ± 0.022^{Ax}	4.363 ± 0.065^{By}	3.927 ± 0.052^{Ax}	3.980 ± 0.042^{Axy}
Day 8	3.974 ± 0.045^{Ax}	5.751 ± 0.057^{Bz}	4.249 ± 0.051^{Cxy}	4.240 ± 0.021^{Cz}

The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$).

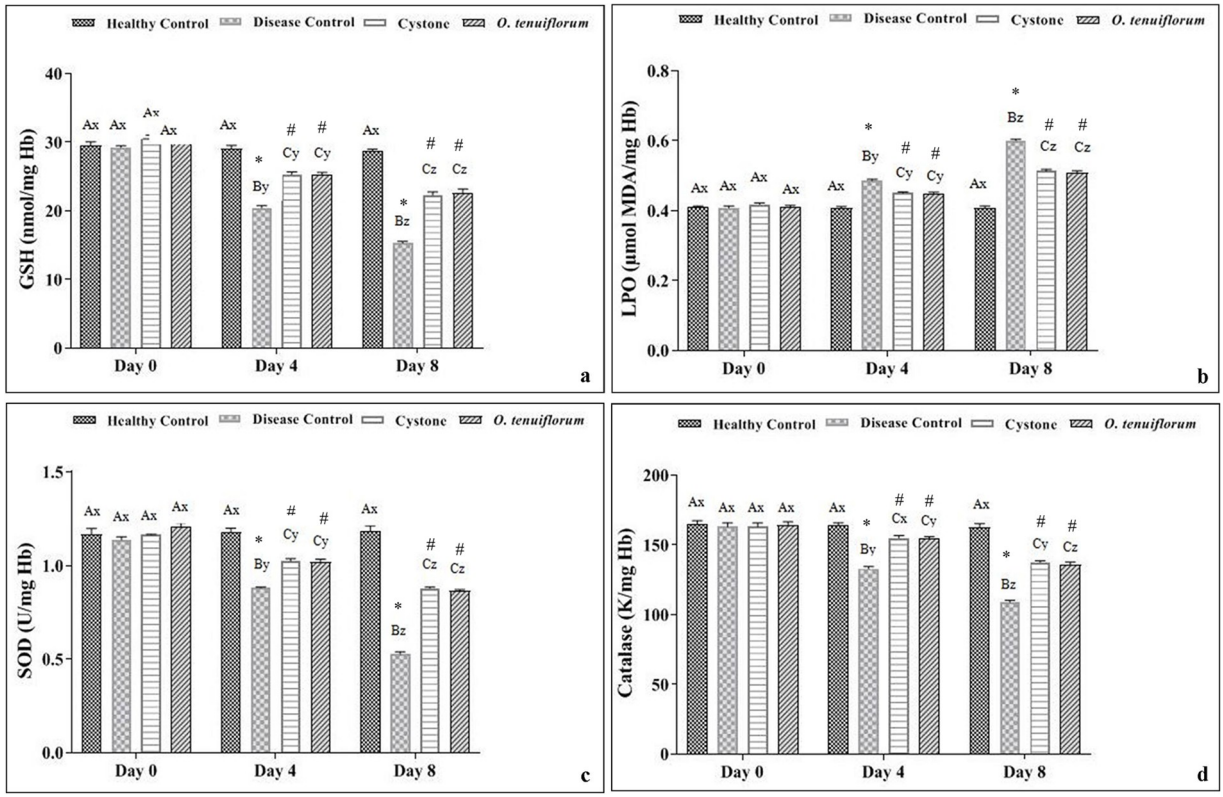

Figure 2. *Ocimum tenuiflorum* hydro-ethanolic extract improves antioxidant indices in blood in gentamicin-induced acute kidney injury: (a) blood GSH levels; (b) blood LPO levels; (c) blood SOD levels, and (d) blood catalase levels. The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$), $^{*}P < 0.05$ versus healthy control, $^{\#}P < 0.05$ versus disease control. GSH, glutathione; LPO, lipid peroxide; SEM, standard error of mean; SOD, superoxide dismutase.

shown that the phosphomolybdate-reducing ability of the extracts of numerous medicinal plants is positively linked with their polyphenol and flavonoid content[38].

In the present study, there was a significant reduction in the activity of SOD, CAT, and GSH and a significant increase in LPO levels in erythrocytes of disease control as compared to healthy control on days 4 and 8. According to various studies, oxygen-free radicals are to be blamed for gentamicin-induced AKI[39]. Under physiological circumstances, the body produces GSH more frequently than oxidized form glutathione disulfide (GSSG); when the ratio between the two forms is upset, oxidative stress results. GSSG is converted to GSH by the primary antioxidant enzyme GPx, which is mostly produced by the kidney. Depression of antioxidative factors in AKI can be due to the generation of ROS in circulation. Lipid peroxidation occurs as a result of free-radical-induced tissue injury resulting in alteration of activities of various membrane-bound enzymes[12,13,40]. Treatment with *O. tenuiflorum* L. showed a significant ($P < 0.05$) increase in GSH, SOD, and CAT levels and a significant ($P < 0.05$) decrease in LPO levels in comparison to disease control. SOD, GSH, and micronutrients like selenium, copper, and zinc play an important role in preventing free-radical formation and as a result maintain cellular integrity[41]. When exposed to high levels of oxidative stress, polyphenols effectively prevent biological systems from deteriorating by absorbing, quenching, and neutralizing free radicals as well as acting as reducing agents

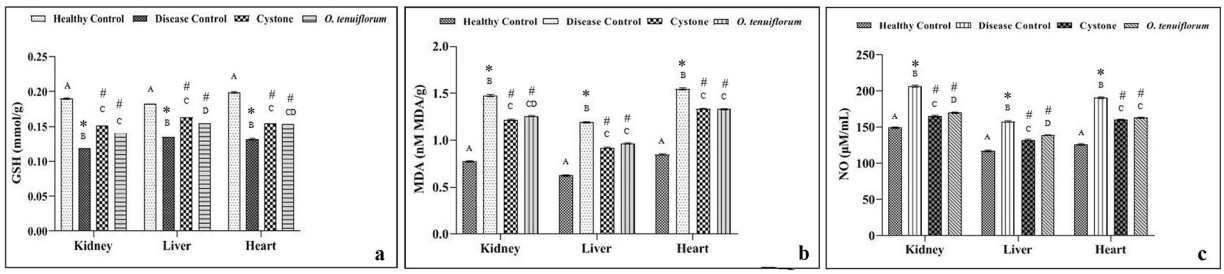

Figure 3. *Ocimum tenuiflorum* hydro-ethanolic extract improves antioxidant/nitrosative indices in tissue homogenate in gentamicin-induced acute kidney injury: (a) tissue GSH levels in kidney, liver, and heart; (b) tissue MDA levels in kidney, liver, and heart; and (c) tissue NO levels. The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day differ significantly ($P < 0.05$), $^{*}P < 0.05$ versus healthy control, $^{\#}P < 0.05$ versus disease control. GSH, glutathione; MDA, malondialdehyde; NO, nitric oxide; SEM, standard error of mean.

Table 7

Alterations in serum levels of kidney injury biomarkers on different days in disease control (Group II), standard treatment (Group III), and EC_{50} of *O. tenuiflorum* L. (Group IV) in comparison to healthy control (Group I)

Day	Healthy control ($n = 6$)	Disease control ($n = 6$)	Standard treatment ($n = 6$)	*O. tenuiflorum* L. EC_{50} ($n = 6$)
Cystatin C (pg/mL)				
Day 0	0.805 ± 0.010^{Ax}	0.812 ± 0.008^{Ax}	0.808 ± 0.010^{Ax}	0.811 ± 0.009^{Ax}
Day 4	0.819 ± 0.010^{Ax}	0.869 ± 0.016^{By}	0.835 ± 0.006^{Cy}	0.842 ± 0.007^{ACy}
Day 8	0.827 ± 0.015^{Ax}	1.133 ± 0.015^{Bz}	0.888 ± 0.008^{Cy}	0.877 ± 0.007^{ACz}
KIM-1 (ng/mL)				
Day 0	192.23 ± 4.14^{Ax}	192.75 ± 4.34^{Ax}	194.46 ± 4.04^{Ax}	194.12 ± 5.82^{Ax}
Day 4	202.61 ± 6.34^{Ax}	472.59 ± 21.04^{By}	312.82 ± 10.30^{Cy}	290.66 ± 5.58^{Cy}
Day 8	211.07 ± 5.55^{Ax}	1339.13 ± 34.41^{Bz}	496.73 ± 8.29^{Cz}	488.72 ± 6.14^{Cz}
GGT (IU/L)				
Day 0	17.87 ± 0.21^{Ax}	17.07 ± 0.08^{Ax}	17.15 ± 0.14^{Ax}	17.33 ± 0.13^{Ax}
Day 4	17.10 ± 0.18^{Ax}	19.53 ± 0.22^{By}	18.55 ± 0.08^{Cy}	18.19 ± 0.21^{Cx}
Day 8	17.22 ± 0.19^{Ax}	20.12 ± 0.33^{By}	19.04 ± 0.07^{By}	19.02 ± 0.09^{Bxy}

GGT, gamma-glutamyl transpeptidase; KIM-1, kidney injury molecule-1.

The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$).

and metal chelators. Plant polyphenols and flavonoids may be significant in influencing the activities of metalloenzymes and regulating the redox status of the cell due to their metal ion-reducing capacity[42]. The flavonoids from *O. tenuiflorum* L. were discovered to have exceptional anti-oxidant action *in vivo*, which reduced lipid peroxidation and protected lymphocytes from oxidative stress brought on by radiation[43].

Gentamicin administration resulted in AKI indicated by an increase in urine total protein, urine albumin, urine sodium, SCr, BUN, and TNF-α levels and a decrease in urine creatinine, UUN, serum total protein, serum albumin, serum sodium, and IL-10 levels in disease control in comparison to healthy control due to impairment of renal function. A decrease in urine creatinine and UUN levels suggests decreased tubular excretion and improper elimination of urea[44]. Increased total protein and albumin excretion in urine has been linked to damage to the principal site of drug accumulation, the proximal tubular cells. Increased accumulation of nitrogenous waste products in the body due to deranged renal filtration functions in kidney damage can lead to an increase in SCr and BUN. Pro-inflammatory cytokine-like TNF-α is up-regulated in ischemic renal injuries, leading to neutrophil influx. IL-10, an anti-inflammatory cytokine, can suppress the leukocyte activation and decrease pro-inflammatory cytokine and chemokine production. TNF superfamily of cytokines members controls the proliferation, differentiation, and death of cells, along with other cellular responses. Both TNF-α and Fas ligand play a role in renal damage in several studies of renal injury. IL-10 participates in compensatory anti-inflammatory response syndrome and is considered as the most important anti-inflammatory cytokine. The relationship between TNF-α and oxidative damage is complex; TNF-α has been known to increase ROS and RNS formation in kidney diseases leading to increased urinary albumin excretion, which can be correlated with the findings of the present study[45]. The *O. tenuiflorum* L.-treated group resulted in the reduction of urine total protein, urine albumin, urine sodium, SCr, BUN, and TNF-α levels and the elevation of urine creatinine, UUN, serum total protein, serum albumin, serum sodium, and IL-10 levels in comparison to disease control. Renoprotective activity of *O. tenuiflorum* L. can be attributed to the presence of

Table 8

Alterations in urine levels of kidney injury biomarkers on different days in disease control (Group II), standard treatment (Group III), and EC_{50} of *O. tenuiflorum* L. (Group IV) in comparison to healthy control (Group I)

Day	Healthy control ($n = 6$)	Disease control ($n = 6$)	Standard treatment ($n = 6$)	*O. tenuiflorum* L. EC_{50} ($n = 6$)
Cystatin C (pg/mL)				
Day 0	4.085 ± 0.041^{Ax}	4.141 ± 0.023^{Ax}	4.104 ± 0.039^{Ax}	4.076 ± 0.035^{Ax}
Day 4	3.975 ± 0.124^{Ax}	4.779 ± 0.071^{By}	4.325 ± 0.042^{Cy}	4.179 ± 0.059^{ACx}
Day 8	4.052 ± 0.051^{Ax}	9.119 ± 0.118^{Bz}	4.979 ± 0.021^{Cz}	4.589 ± 0.018^{ACy}
KIM-1 (ng/mL)				
Day 0	1719.73 ± 21.52^{Ax}	1738.32 ± 17.33^{Ax}	1730.89 ± 15.29^{Ax}	1728.45 ± 15.21^{Ax}
Day 4	1724.42 ± 19.77^{Ax}	2102.66 ± 25.27^{By}	1863.24 ± 17.27^{Cy}	1819.88 ± 22.91^{ACy}
Day 8	1718.31 ± 21.09^{Ax}	3192.21 ± 30.02^{Bz}	2153.27 ± 36.63^{Cz}	2088.31 ± 33.12^{Cz}
GGT (IU/L)				
Day 0	5.12 ± 0.12^{Ax}	5.40 ± 0.09^{Ax}	5.18 ± 0.04^{Ax}	5.21 ± 0.09^{Ax}
Day 4	5.36 ± 0.10^{Ax}	8.82 ± 0.13^{By}	6.03 ± 0.05^{Cy}	6.10 ± 0.04^{Cy}
Day 8	5.39 ± 0.12^{Ax}	15.21 ± 0.22^{Bz}	9.28 ± 0.23^{Cz}	9.09 ± 0.19^{Cz}

KIM-1, kidney injury molecule-1; GGT, gamma-glutamyl transpeptidase.

The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$).

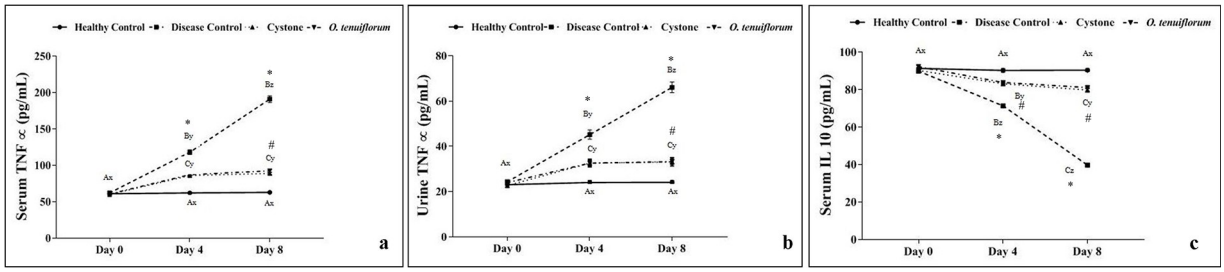

Figure 4. *Ocimum tenuiflorum* hydro-ethanolic extract reduces serum and urine TNF-α and improves serum IL-10 levels in gentamicin-induced acute kidney injury: (a) serum TNF-α levels; (b) urine TNF-α levels, and (c) serum IL-10 levels. The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day and superscripts x, y, and z between the days within a group differ significantly ($P < 0.05$), $^{*}P < 0.05$ versus healthy control, $^{\#}P < 0.05$ versus disease control. IL-10, interleukin-10; TNF-α, tumor necrosis factor-alpha; SEM, standard error of mean.

rosmarinic acid, carvacrol, and eugenol. Rosmarinic acid present in *O. tenuiflorum* L. has anti-inflammatory and anti-oxidative activity[46]. Anti-inflammatory property of rosmarinic acid is attributed to its ability to inhibit lipoxygenases and cyclooxygenases, and its interference in the complement cascade. Nuclear factor-kappa-B (NF-κB) plays an important role in augmenting the expression of pro-inflammatory cytokines. Rosmarinic acid reduces NF-κB p65 nuclear translocation in proximal tubular cells and TNF-α expression[47,48]. Carvacrol has mucomembrane protective activity and can protect renal parenchyma against damage, and eugenol shows anti-inflammatory effects by inhibiting TNF-α and resulting in reduced albuminuria[49,50].

Kidney, liver, and cardiac tissue homogenates showed a reduction in GSH and IL-10, while MDA and NO levels were elevated after gentamicin administration, which indicates induction of oxidative stress and inflammation. Gentamicin can induce increased production of H_2O_2 in kidney, liver, and cardiac mitochondria leading to peroxidative damage[51]. Similar nephrotoxic effects due to oxidative stress have also been reported by Barhoma[50]. Inflammatory cytokines, activated leukocytes, urine toxins, and oxidative stress brought on by AKI may all contribute to liver diseases. Animals that received the *O. tenuiflorum* L. treatment (Gp. IV) had higher GSH values and decreased MDA and NO levels than disease control (Gp. II), and these values were similar to the cystone treatment group (Gp. III). Rosmarinic acid is known to increase the activity of glutathione transferase, SOD, GPx, and CAT in kidneys[52]. Rhamnetin is a flavonoid found in seeds, flowers, and leaves of many plants and is reported to have antioxidant and renoprotective effects[53].

The microscopic examination of renal histology showed severe tubular degeneration, epithelial vacuolation, tubular dilatation, and eosinophilic proteinaceous material in tubular lumen of animals treated with gentamicin, and these were consistent with findings of other researchers. Dilated hepatic sinusoidal spaces with hepatocyte atrophy, fragmentation and degeneration of cardiac myocytes, and enlarged interfibrillar gaps were also observed in disease control. Earlier reports have depicted gentamicin-induced hepatotoxicity. Gentamicin leads to myocardial fiber hypertrophy and widening of intracellular space between myocardial fibers, which can occur due to myocardial contractile fibers being replaced by fibrous tissue[54]. Cardiac muscle fiber fragmentation with loss of muscle striations is noticed in gentamicin toxicity[51]. ROS/RNS generation can be an additional contributing factor to the severity of

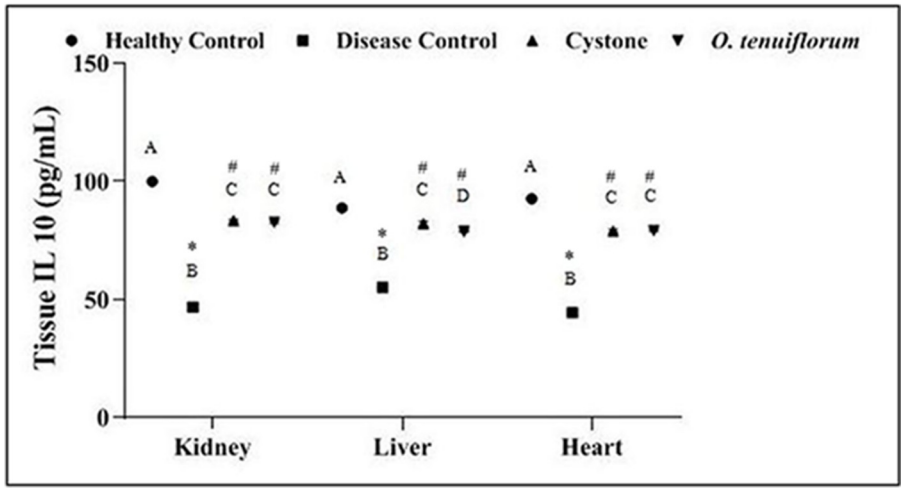

Figure 5. *Ocimum tenuiflorum* hydro-ethanolic extract increases tissue homogenate IL-10 levels in kidney, liver, and heart in gentamicin-induced acute kidney injury. The values have been expressed as mean ± SEM. Superscripts A, B, and C between the groups within a day differ significantly ($P < 0.05$), $^{*}P < 0.05$ versus healthy control, $^{\#}P < 0.05$ versus disease control. IL-10, interleukin-10; SEM, standard error of mean.

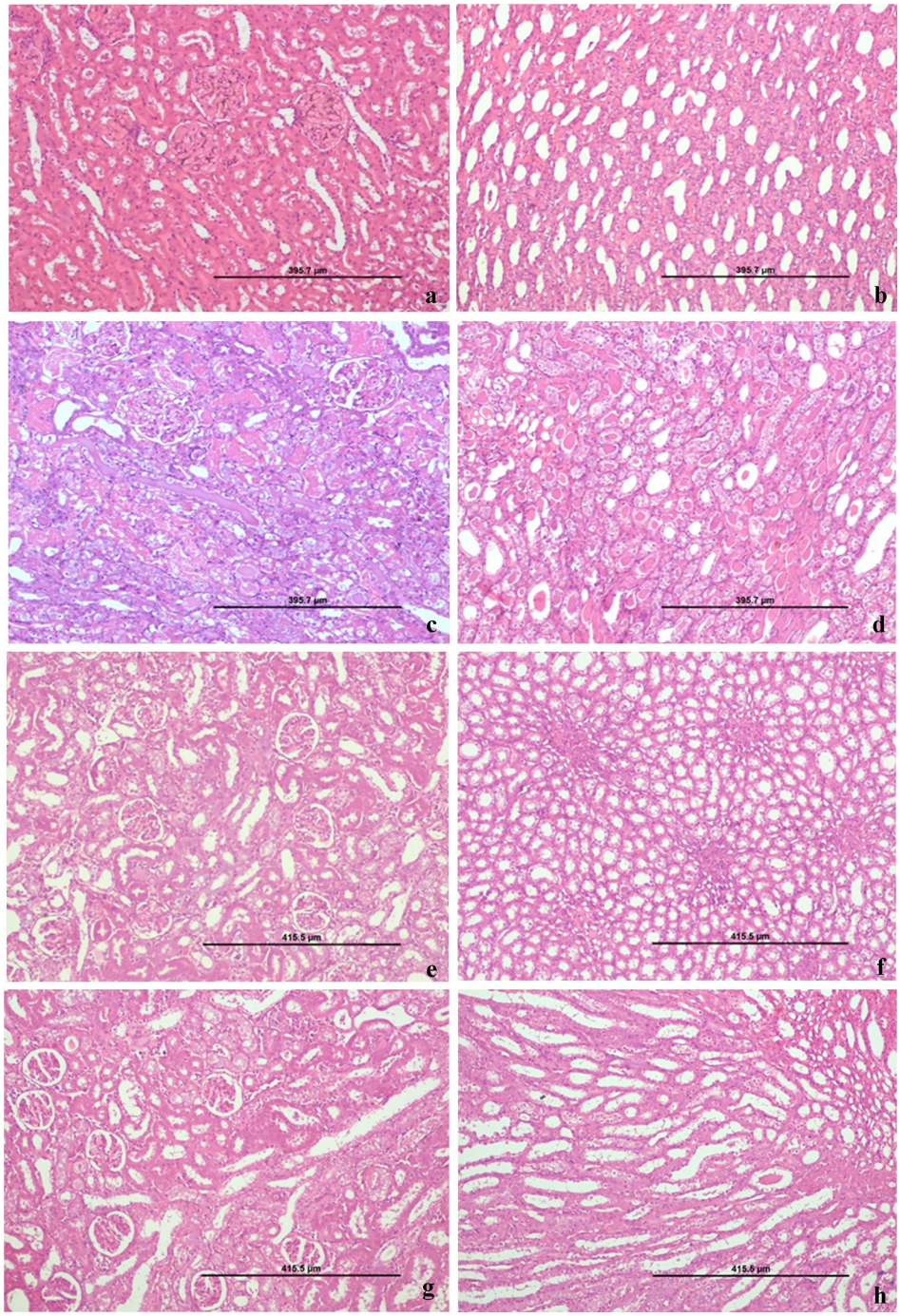

Figure 6. Micrographs (a–h) showing kidney sections of rats stained with hematoxylin and eosin (H&E) (×100): (a) kidney cortex and (b) medulla of healthy control showing normal architect; (c) kidney section of cortex in disease control showing proximal convoluted tubule (PCT) with severe tubular degeneration, epithelial vacuolation, and presence of eosinophilic proteinaceous material; (d) distal convoluted tubule (DCT) of kidney medulla having tubular degeneration, epithelial vacuolation, and tubular dilatation; (e) kidney cortex in the cystone-treated group showing PCT and (f) DCT of medulla with mild tubular degeneration; and (g) kidney cortex showing PCT and (h) medulla showing DCT in *O. Tenuiflorum* depicting mild tubular dilatation.

damage. Gentamicin can primarily accumulate in the kidney cortex, which can lead to oxidative stress and lipid peroxidation. It has a tendency to mediate the production of ROS such as hydroxyl radical, H_2O_2, and superoxide anion in mitochondria leading to kidney damage. Gentamicin leads to reduced adenosine triphosphate levels in renal tubular cells by inhibiting oxidative phosphorylation and mitochondrial pathway-dependent oxidative damage in renovascular defects. Lysosomal phospholipases are inhibited by the interaction of aminoglycosides with negatively charged phospholipids, and their buildup in the lysosomes of tubular cells leads to phospholipidosis resulting in necrosis. Additional organs may also experience damage due to side effects of gentamicin-induced AKI[55]. Cardiovascular toxicity may also result from

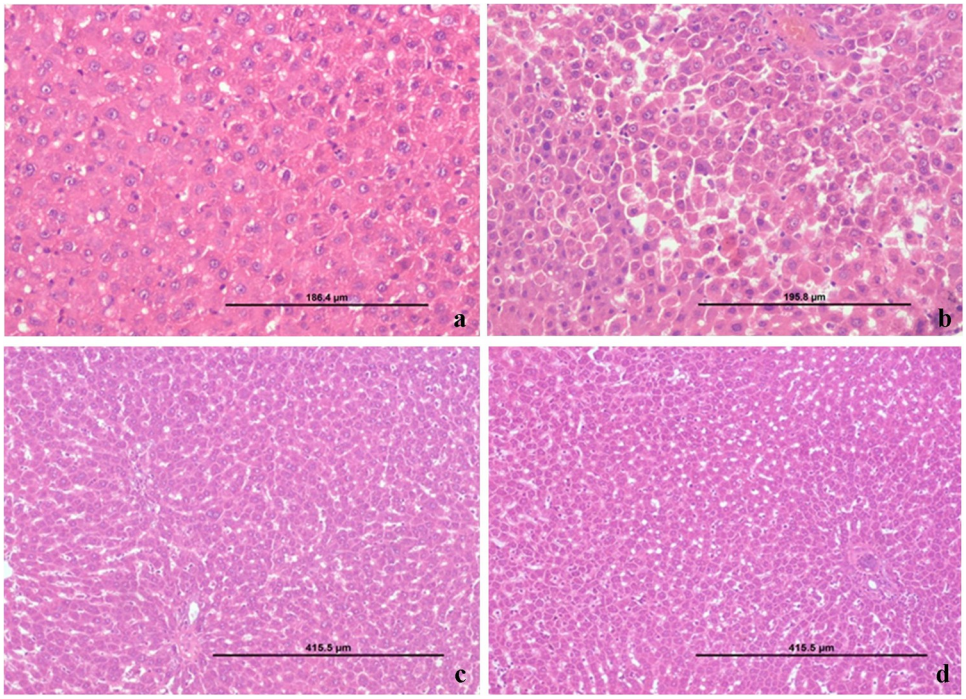

Figure 7. Micrographs (a–d) showing liver sections of rats stained with H&E (×100): (a) hepatocytes in healthy control with normal architect; (b) liver of disease control showing swollen hepatocytes, hypertrophied Kupffer cells, and necrosis; (c) cystone treatment group; and (d) *O. tenuiflorum* treatment group showed normal liver parenchyma with mild dilatation of sinusoidal spaces. H&E, hematoxylin and eosin.

assemblage of uremic toxins in case of AKI. Treatment with *O. tenuiflorum* L. prevented the renal damage along with a reduction in the effects of gentamicin on liver tissue and myocardium, which was evident from mild tubular degeneration, mild dilatation of sinusoidal spaces, and mild degeneration of cardiac myocytes.

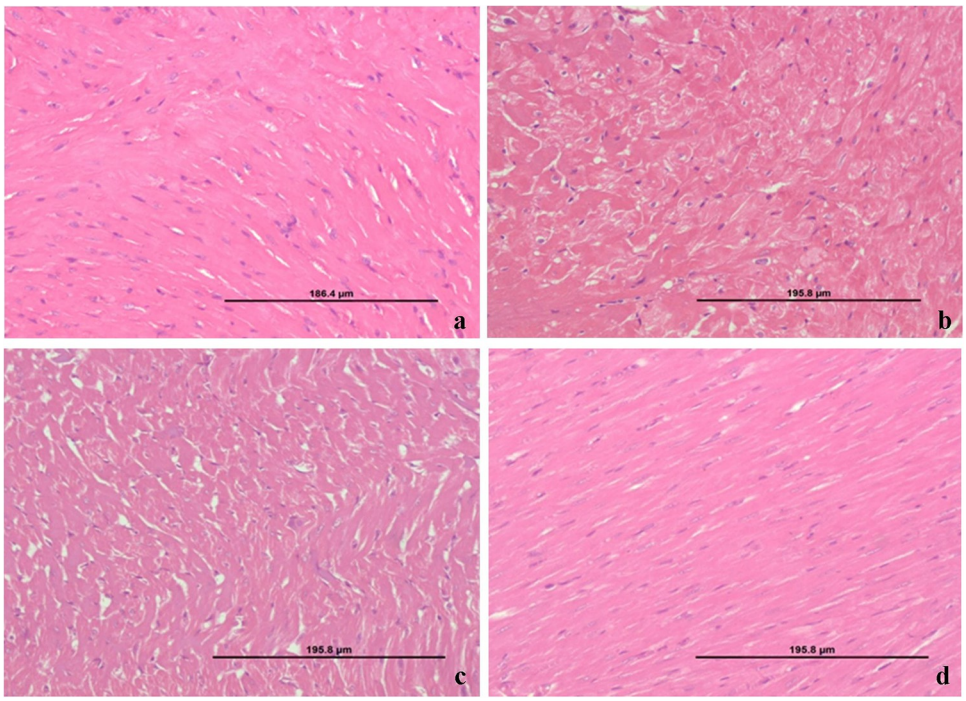

Figure 8. Micrographs (a–d) showing myocardium sections of rats stained with H&E (×200): (a) branching fibers of cardiac myocytes in healthy animals; (b) degeneration of cardiac myocytes in disease control; (c) mild degeneration of cardiac myocytes in the cystone treatment group; (d) branching fibers of cardiac myocytes similar to healthy control. H&E, hematoxylin and eosin.

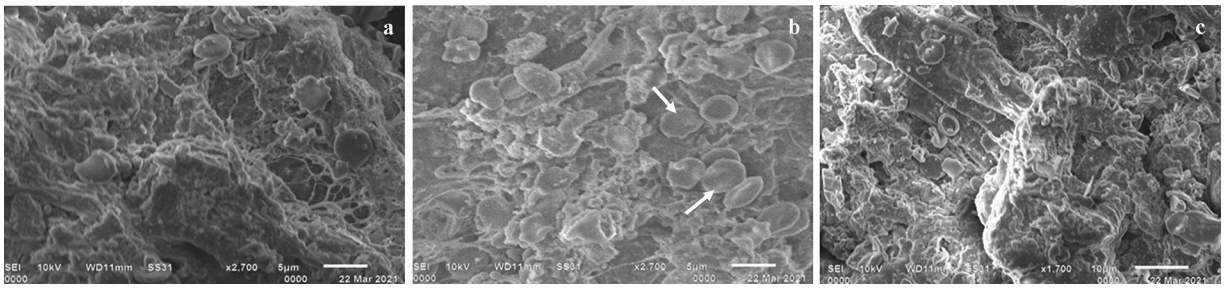

Figure 9. Scanning electron micrograph of kidney tissue samples depicting (a) tuft of microvilli from squamous cell lining the tubule in healthy control; (b) proximal convoluted tubule basal lamina filled with debris and erythrocytes (arrow) in disease control; (c) tuft of microvilli from squamous cells and few erythrocytes noticed in the *O. tenuiflorum* treatment group.

Early detection of kidney injury would help in the identification of patients requiring more intensive treatment. Different kidney injury markers have been employed for the early detection of kidney damage. CysC and KIM-1 values in urine and serum, and urine GGT levels were higher in disease control compared to healthy control. CysC estimation in serum and urine has been found to be superior to SCr for diagnosing and detecting kidney dysfunction[56]. Increased concentration of CysC in body fluids or tissues may be indicative of pathology as it is involved in extracellular proteolysis. KIM-1 has been reported to be expressed in the apical aspect of PCT cells in higher amounts during the regeneration phase after an injury, and urine KIM-1 levels rise more rapidly compared to SCr[57]. Prior to the appearance of casts in urine, levels of urinary KIM-1 increase and can help in estimating the severity of disease. Urine levels of GGT are due to tubular rather than glomerular injury pertaining to its high molecular weight and inability to pass the glomerular barrier[58]. *Ocimum tenuiflorum* L.-treated animals showed a significant reduction in levels of these biomarkers as compared to disease control, which may be attributed to renoprotective, anti-inflammatory, and antioxidant activities of rosmarinic acid, eugenol, and carvacrol[47,59]. The statistical significance would have been higher if the study had been conducted in a larger population of rats with gentamicin-induced AKI. Despite this drawback, it appears that the multivariate strategy aimed at understanding the pathophysiology of gentamicin-induced AKI did not significantly impact the reliability of the findings.

Conclusion

The findings of the present study demonstrate the curative efficacy of *O. tenuiflorum* L. in the gentamicin-induced AKI rat model, which might be in the form of various properties like antioxidant, anti-inflammatory, organoprotective, and potential to inhibit biochemical parameters involved in renal impairment. In short, *O. tenuiflorum* L. extract decreased LPO, TNF-α, and kidney injury biomarker (KIM-1, CysC, and GGT) levels and increased antioxidant enzymes (GSH and CAT) and IL-10 levels in urine, serum, and tissue homogenates. Due to the limited availability of nephroprotective remedies, alternative therapeutic measures such as ethnoveterinary medicine play a significant role in preventing kidney damage. Further detailed studies in larger populations may be established to evaluate the efficacy and safety of *O. tenuiflorum* L. in human clinical trials.

Ethical approval

Ethical approval was obtained from IAEC wide letter number IAEC/CVSc/VMD/341. The present study followed international, national, and/or institutional guidelines for humane animal treatment.

Consent

This study does not involve human subjects, and therefore, informed consent was not required.

Source of funding

None.

Author's contribution

N.T.: Conceptualization, data curation, investigation, methodology, visualization, and writing – original draft. S.K.S.: Conceptualization and supervision. M.K.: Conceptualization, resources, and supervision. C.G.E.: Formal analysis, methodology, and writing – review and editing. A.S.: Investigation. R.: Conceptualization and methodology. A.K.S.: Investigation. K. D.M.: Formal analysis. M.S.: Investigation and formal analysis. P.C.: Methodology and writing – review and editing. U.K.D.: Methodology and writing – review and editing. K.S.: Methodology and writing – review and editing.

Conflicts of interest disclosure

The authors disclose no conflict of interest.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Neeraj Thakur.

Provenance and peer review

The article was invited for a special issue.

Data availability statement

The data will be made available from the corresponding author on reasonable request.

Acknowledgements

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar, Uttarakhand, for providing all necessary facilities during the experiment.

Assistance with the study

None.

Presentation

None.

Statement of novelty

The curative efficacy of O. tenuiflorum L. hydroethanolic extract has been studied with various properties like antioxidant, organoprotective, anti-inflammatory, and potential to inhibit biochemical parameters involved in renal impairment.

References

[1] Wans EM, Ahmed MM, Mousa AA, *et al*. Ameliorative effects of corn silk extract on acetaminophen-induced renal toxicity in rats. Environ Sci Pollut Res 2021;28:1762–74.

[2] Konda VR, Arunachalam R, Eerike M, *et al*. Nephroprotective effect of hydroethanolic extract of *Azima tetracantha* root in glycerol induced acute renal failure in Wistar albino rats. J Ttradit Complement Med 2016;6:347–54.

[3] vanDoorn KJ. Acute kidney injury in the critically ill still remains a challenge. EMJ Nephrol 2017;5:93–101.

[4] Kellum JA, Ronco C, Bellomo R. Conceptual advances and evolving terminology in acute kidney disease. Nat Rev Nephrol 2021;17:493–502.

[5] Packialakshmi B, Stewart IJ, Burmeister DM, *et al*. Large animal models for translational research in acute kidney injury. Ren Fail 2020;42:1042–58.

[6] Rashwan AM, Abumandour MM, Kandyel R, *et al*. Implications of endoplasmic reticulum stress and beta-cell loss in immunodeficient diabetic NRG-Akita mice for understanding monogenic diabetes. Int J Surg 2024;110:6231–42.

[7] Ramesh G, Reeves WB. Inflammatory cytokines in acute renal failure. Kidney Int 2004;66:S56–61.

[8] Wang Y, Zhang H, Chen Q, *et al*. TNF-α/HMGB1 inflammation signalling pathway regulates pyroptosis during liver failure and acute kidney injury. Cell Prolif 2020;53:e12829.

[9] Yin C, Wang N. Kidney injury molecule-1 in kidney disease. Ren Fail 2016;38:1567–73.

[10] Waring WS, Moonie A. Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. Clin Toxicol 2011;49:720–28.

[11] Thakur N, Shukla SK, Ahmad AH, *et al*. Ameliorative, antioxidant and immunomodulatory potential of vitamin D on aminoglycoside induced acute kidney injury in Wistar rats. Indian J Anim Res 2022;56:655–61.

[12] Das D, Sarma K, Eregowda CG, *et al*. Naturally occurring *Anaplasma marginale* infection in cattle: molecular prevalence and associated risk factors, haemato-biochemical alterations, oxidant/antioxidant status and serum trace mineral levels. Microb Pathog 2022;167:105575.

[13] Thakur N, Choudhary SS, Chethan GE, *et al*. Urokinase alteration and its correlation with disease severity and oxidative/nitrosative stress in buffalo calves with obstructive urolithiasis. Res Vet Sci 2019;127:18–26.

[14] Case J, Khan S, Khalid R, *et al*. Epidemiology of acute kidney injury in the intensive care unit. Crit Care Res Pract 2013;2013:479730.

[15] Rao SK, Sharma A, Jain D. A review medicinal and traditional uses on Tulsi plant (*Ocimum sanctum* L.). World J Biol Pharm Health Sci 2023;13:450–56.

[16] Lahiri D, Nag M, Dutta B, *et al*. Antibiofilm and anti-quorum sensing activities of eugenol and linalool from *Ocimum tenuiflorum* against *Pseudomonas aeruginosa* biofilm. J Appl Microbiol 2021;131:2821–37.

[17] Ngai HH, Sit WH, Wan JM. The nephroprotective effects of the herbal medicine preparation, WH30^{+}, on the chemical-induced acute and chronic renal failure in rats. Am J Chin Med 2005;33:491–500.

[18] Biglari F, AlKarkhi AF, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem 2008;107:1636–41.

[19] Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. J Agric Food Chem 2004;52:5032–39.

[20] Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Sci Technol 2007;40:344–52.

[21] Asnaashari S, Dadizadeh E, Talebpour AH, *et al*. Free radical scavenging potential and essential oil composition of the *Dorema glabrum* Fisch. CA mey roots from Iran. BioImpacts: BI 2011;1:241.

[22] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337–41.

[23] Erdem A, Gündogan NÜ, Usubütün A, *et al*. The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. Nephrol Dial Transplant 2000;15:1175–82.

[24] Thakur N Studies on ameliorative efficacy of *Ocimum tenuiflorum* and *Zanthoxylum armatum* in gentamicin induced acute renal toxicity rat model. Doctoral dissertation, GB Pant University of Agriculture and Technology, Pantnagar. 2021.

[25] Prins HK, Loos JA. Glutathione. In: Biochemical Methods in Red Cell Genetics. Academic Press, New York; 1969. 115–37.

[26] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal Biochem 1966;16:359–64.

[27] Bergmayer HU. UV method of catalase assay. In: Methods of Enzymatic Analysis, 3, 1983, 273.

[28] Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 1998;35:184–88.

[29] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192–205.

[30] Rehman SU. Lead induced regional lipid peroxidation in brain. Toxicol Lett 1984;21:333–37.

[31] Green LC, Wagner DA, Glogowski J, *et al*. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131–38.

[32] O'Sullivan ME, Song Y, Greenhouse R, *et al*. Dissociating antibacterial from ototoxic effects of gentamicin C-subtypes. Proc Natl Acad Sci 2020;117:32423–32.

[33] Sodimbaku V, Pujari L, Mullangi R, *et al*. Carrot (*Daucus carota* L.): nephroprotective against gentamicin-induced nephrotoxicity in rats. Indian J Pharmacol 2016;48:122–27.

[34] Ahmad M, Tahir M, Hong Z, *et al*. Plant and marine-derived natural products: sustainable pathways for future drug discovery and therapeutic development. Front Pharmacol 2025;15:1497668.

[35] Venuprasad MP, Kandikattu HK, Razack S, *et al*. Phytochemical analysis of *Ocimum gratissimum* by LC-ESI–MS/MS and its antioxidant and anxiolytic effects. S Afr J Bot 2014;92:151–58.

[36] Thyagaraj VD, Koshy R, Kachroo M, *et al*. A validated RP-HPLC-UV/DAD method for simultaneous quantitative determination of rosmarinic acid and eugenol in *Ocimum sanctum* L. Pharm Methods 2013;4:1–5.

[37] Mousavi L, Salleh RM, Murugaiyah V. Phytochemical and bioactive compounds identification of *Ocimum tenuiflorum* leaves of methanol extract and its fraction with an anti-diabetic potential. Int J Food Prop 2018;21:2390–99.

[38] Agarwal K, Singh DK, Jyotshna J, *et al*. Antioxidative potential of two chemically characterized *Ocimum* (Tulsi) species extracts. Biomed Res Ther 2017;4:1574–90.

[39] Liu YD, He J, Luan W, *et al*. A new autophagy-driven protective pathway of *Haematococcus astaxanthin* against chemical damage in Wistar rats with gentamicin-induced acute kidney injury. Algal Res 2023;72:103107.

[40] Sarma K, Eregowda CG, Roychoudhury P, *et al*. A 5-year prospective study on incidence and clinico-pathological changes associated with naturally occurring trypanosomosis in dogs of Mizoram, India. Acta Parasitol 2022;67:61–71.

[41] Al-Gheffari HK, Aljahdali SM, Albalawi M, *et al*. Mycogenic zinc nanoparticles with antimicrobial, antioxidant, antiviral, anticancer and anti-Alzheimer activities mitigate the aluminium toxicity in mice: effects on liver, kidney, and brain health and growth performance. Pak Vet J 2024;44:763–75.
[42] Choudhary SS, Panigrahi PN, Dhara SK, *et al*. *Cucumis callosus* (Rottl.) Cogn. fruit extract ameliorates calcium oxalate urolithiasis in ethylene glycol induced hyperoxaluric rat model. Heliyon 2023;9:e14043.
[43] Satyamitra M, Mantena S, Nair CKK, *et al*. The antioxidant flavonoids, orientin and vicenin enhance repair of radiation-induced damage. SAJ Pharm Pharmacol 2014;1:1.
[44] Udupa V, Prakash V. Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol Rep 2019;6:91–99.
[45] Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovasc Ther 2012;30:49–59.
[46] Sadeghi A, Bastin AR, Ghahremani H, *et al*. The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Mol Biol Rep 2020;47:3557–66.
[47] Domitrović R, Potočnjak I, Crnčević-Orlić Ž, *et al*. Nephroprotective activities of rosmarinic acid against cisplatin-induced kidney injury in mice. Food Chem Toxicol 2014;66:321–28.
[48] Alvi K, Ijaz MU, Hamza A, *et al*. Nephroprotective effects of delphinidin against bisphenol a induced kidney damage in rats. Pak Vet J 2022;43:189–93.
[49] Parasuraman S, Balamurugan S, Christapher PV, *et al*. Evaluation of antidiabetic and antihyperlipidemic effects of hydroalcoholic extract of leaves of *Ocimum tenuiflorum* (Lamiaceae) and prediction of biological activity of its phytoconstituents. Pharmacogn Res 2015;7:156.
[50] Barhoma RA. The role of eugenol in the prevention of chromium-induced acute kidney injury in male albino rats. Alexandria J Med 2018;54:711–15.
[51] Meligy FY, El-Deen Mohammed HS, Mostafa TM, *et al*. Therapeutic potential of mesenchymal stem cells versus omega n-3 polyunsaturated fatty acids on gentamicin-induced cardiac degeneration. Pharm 2022;14:1322.
[52] Samsu N, Soeharto S, Rifai M, *et al*. Rosmarinic acid monotherapy is better than the combination of rosmarinic acid and telmisartan in preventing podocyte detachment and inhibiting the progression of diabetic nephropathy in rats. Biol Targets Ther 2019;13:179–90.
[53] Ahmad M, Ehsan N, Khan HA, *et al*. Ameliorative effects of rhamnetin against polystyrene microplastics-induced nephrotoxicity in rats. Pak Vet J 2023;43:623–27.
[54] Ali FAZ, Abdellah N, Hafez L, *et al*. Sesame oil ameliorates gentamicin-induced cardiotoxicity in Wistar albino rats. J Adv Vet Res 2020;10:81–87.
[55] Yarijani ZM, Najafi H, Shackebaei D, *et al*. Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by *Malva sylvestris* extract in gentamicin induced renal toxicity. Biomed Pharmacother 2019;112:108635.
[56] Bagshaw SM, Bellomo R. Cystatin C in acute kidney injury. Curr Opin Crit Care 2010;16:533–39.
[57] Tanase DM, Gosav EM, Radu S, *et al*. The predictive role of the biomarker kidney molecule-1 (KIM-1) in acute kidney injury (AKI) cisplatin-induced nephrotoxicity. Int J Mol Sci 2019;20:5238.
[58] Lippi I, Perondi F, Meucci V, *et al*. Clinical utility of urine kidney injury molecule-1 (KIM-1) and gamma-glutamyltransferase (GGT) in the diagnosis of canine acute kidney injury. Vet Res Commun 2018;42:95–100.
[59] Kadir A, Sher S, Siddiqui RA, *et al*. Nephroprotective role of eugenol against cisplatin-induced acute kidney injury in mice. Pak J Pharm Sci 2020;33:1281–87.