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EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF HELIOTROPIMUM INDICUM IN EXPERIMENTAL ANIMALS (CISPLATIN INDUCED NEPHROTOXICITY)

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Article History	Abstract
Received: 26-06-2026 Revised: 22-07-2026 Accepted: 18-08-2026	Cisplatin is an effective anticancer agent; however, its clinical use is frequently limited by nephrotoxicity, primarily associated with oxidative stress and free-radical generation. <i>Heliotropium indicum</i>, a medicinal plant traditionally used in India, possesses several biologically active constituents and reported antioxidant properties. The present study was therefore undertaken to evaluate the nephroprotective effect of the aqueous extract of <i>Heliotropium indicum</i> against cisplatin-induced renal injury in albino rats. Twenty-four albino rats were divided into four groups of six animals each following approval from the Institutional Animal Ethics Committee. The study was conducted for 21 days. The control group received normal saline, while nephrotoxicity was induced in the remaining groups using cisplatin (10 mg/kg, i.p.) once daily during the last five days. Two treatment groups received graded doses of aqueous <i>Heliotropium indicum</i> extract orally along with cisplatin treatment. At the end of the study, blood samples were collected for estimation of blood urea nitrogen (BUN) and serum creatinine. Renal tissues were also subjected to histopathological examination. Data were analyzed using ANOVA followed by an appropriate post-hoc test. Cisplatin administration produced a marked elevation in BUN and serum creatinine levels along with characteristic renal histopathological alterations. Pretreatment with <i>Heliotropium indicum</i> significantly attenuated these changes in a dose-dependent manner ($p < 0.001$). Histopathological findings further supported the biochemical results, indicating preservation of renal architecture in the extract-treated groups. The findings suggest that the aqueous extract of <i>Heliotropium indicum</i> possesses significant nephroprotective activity against cisplatin-induced renal damage, which may be associated with its antioxidant and free-radical-scavenging properties. Further studies are warranted to identify the active constituents and clarify the underlying mechanisms.
Keywords: <i>Heliotropium indicum</i> , cisplatin, nephrotoxicity, nephroprotection, oxidative stress, antioxidant activity.	
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INTRODUCTION

The kidney is a vital excretory and homeostatic organ responsible for eliminating metabolic waste and maintaining fluid, electrolyte, and acid-base balance. It also performs important endocrine functions through the production of erythropoietin, renin, and the active form of vitamin D, calcitriol. The kidneys receive approximately 25% of cardiac output through the renal arteries and excrete urine through the ureters into the urinary bladder. Each kidney contains approximately 1.3 million nephrons, the structural and functional units of the kidney. Each nephron consists of a glomerulus enclosed by Bowman's capsule and a renal tubule comprising the proximal tubule, loop of Henle, distal tubule, and collecting duct.

Urine formation occurs through three major processes: glomerular filtration, tubular reabsorption, and tubular secretion. Approximately 120 mL of glomerular filtrate is produced per minute, while more than 99% is subsequently reabsorbed. The proximal tubule is the major site of reabsorption, whereas the loop of Henle, distal tubule, and collecting duct regulate electrolyte and water balance. The

medullary concentration gradient, together with hormones such as aldosterone and antidiuretic hormone, enables precise regulation of urine composition and volume.

Because of its high blood flow, extensive tubular surface area, specialized transport systems, and concentrating mechanisms, the kidney is particularly susceptible to toxic injury. Nephrotoxic agents can cause tubular dysfunction, oxidative stress, impaired renal blood flow, and cellular injury. Common nephrotoxic substances include aminoglycoside antibiotics, cisplatin, NSAIDs, heavy metals, radiocontrast agents, and excessive doses of paracetamol. The proximal tubule is especially vulnerable because of its active transport mechanisms and metabolic activity.

Gentamicin is a clinically important aminoglycoside whose major dose-limiting adverse effect is nephrotoxicity. It accumulates in proximal tubular cells, particularly within lysosomes, where it interferes with phospholipid metabolism and contributes to oxidative stress, cellular dysfunction, and tubular necrosis. Paracetamol and diclofenac can also produce renal injury through mechanisms involving oxidative stress, altered prostaglandin synthesis, and tubular cell damage.

Consequently, investigation of nephroprotective agents, particularly natural products with antioxidant and anti-inflammatory properties, has received considerable attention. Recent experimental studies have reported protective effects of several medicinal plant extracts and phytoconstituents against gentamicin-, paracetamol-, cisplatin-, and ischemia-reperfusion-induced renal injury. These findings support continued research into plant-derived agents as potential strategies for preventing or reducing drug-induced nephrotoxicity.

OBJECTIVES OF THE STUDY

The present study aimed to investigate the nephroprotective potential of the traditional medicinal plant *Heliotropium indicum* against experimentally induced nephrotoxicity. The specific objectives were:

- To investigate the pharmacognostic and phytochemical characteristics of *H. indicum*.
- To prepare and evaluate the aqueous extract of *H. indicum*.
- To perform preliminary phytochemical screening of the extract.
- To evaluate the acute oral toxicity of the extract.
- To assess the *in vivo* nephroprotective activity of *H. indicum* against cisplatin-induced renal injury in Wistar rats using biochemical and histopathological parameters.

PLANT PROFILE

Scientific name: *Heliotropium indicum* L.

Family: Heliotropiaceae

Order: Boraginales

Genus: *Heliotropium*

Common name: Indian heliotrope

H. indicum is an annual, erect, branched, hairy herb approximately 15–50 cm in height. It possesses alternate ovate to oblong-ovate leaves and small white to purple flowers arranged terminally. The plant is widely distributed in tropical regions of Asia and commonly occurs in waste places and disturbed habitats. Traditionally, different parts of the plant have been used for treating wounds, ulcers, skin disorders, and inflammatory conditions. The plant contains various phytoconstituents, including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and other secondary metabolites. However, its pyrrolizidine alkaloid content warrants appropriate toxicological evaluation.

MATERIALS AND METHODS

Chemicals and Equipment

Analytical-grade chemicals, including sodium hydroxide, formalin, Tween 80, distilled water, and reagents for phytochemical screening, were used. Standard laboratory equipment such as a centrifuge, analytical balance, microscope, mechanical stirrer, Soxhlet apparatus, rotary evaporator, and microtome was employed.

Collection and Preparation of Plant Material

The plant material of *H. indicum* was collected from a local source and authenticated by a qualified botanist. The selected plant material was washed, shade-dried, powdered, and stored in an airtight container until extraction.

Preparation of Aqueous Extract

Approximately 100 g of powdered plant material was extracted with 500 mL of distilled water using a Soxhlet apparatus for 20–24 h. The extract was filtered, concentrated under reduced pressure, and stored at 4°C until further use.

Preliminary Phytochemical Screening

The extract was subjected to standard qualitative chemical tests for the detection of carbohydrates, proteins and amino acids, phenolics and tannins, alkaloids, glycosides, flavonoids, phytosterols, fixed oils, saponins, and coumarins.

Experimental Animals

Twenty-four healthy Wistar albino rats of either sex (150–200 g) were housed under standard laboratory conditions of 25 ± 2°C temperature, 45–55% relative humidity, and a 12 h light/dark cycle. Animals were provided standard pellet diet and water *ad libitum*. The animals were acclimatized for one week before experimentation. The study was conducted following approval from the Institutional Animal Ethics Committee (IAEC) and applicable CPCSEA guidelines.

Acute Toxicity Study

Acute oral toxicity was evaluated according to an appropriate OECD guideline. Animals were observed following administration of graded doses of the extract for behavioral changes, signs of toxicity, and mortality during the observation period. A safe dose range was selected for subsequent pharmacological evaluation.

Cisplatin-Induced Nephrotoxicity and Nephroprotective Study

Twenty-four rats were randomly divided into four groups (n = 6):

Group I – Normal control: Received 0.9% normal saline orally for 21 days.

Group II – Cisplatin control: Received normal saline orally and cisplatin (10 mg/kg, i.p.) during the final 5 days to induce nephrotoxicity.

Group III – Treatment: Received aqueous extract of *H. indicum* (250 mg/kg, p.o.) for 21 days along with cisplatin (10 mg/kg, i.p.) during the final 5 days.

Group IV – Treatment: Received aqueous extract of *H. indicum* (500 mg/kg, p.o.) for 21 days along with cisplatin (10 mg/kg, i.p.) during the final 5 days.

Following completion of treatment, animals were anesthetized and blood samples were collected. Serum was separated by centrifugation and used for estimation of blood urea nitrogen (BUN) and serum creatinine. The kidneys were excised, washed with normal saline, and processed for histopathological examination.

Histopathological Examination

Kidney tissues were fixed in 10% neutral buffered formalin, dehydrated using graded ethanol, cleared with xylene, and embedded in paraffin wax. Sections of approximately 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E). The sections were examined microscopically for structural alterations, including tubular degeneration, necrosis, congestion, and other pathological changes.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA),

followed by Tukey's multiple-comparison test. A p value < 0.05 was considered statistically significant.

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Heliotropium indicum* extract revealed the presence of several secondary metabolites. Alkaloids, flavonoids, phlobatannins, saponins, tannins, and triterpenes were detected, whereas cardiac glycosides and steroids were not detected (Table 01).

Table 01: Preliminary qualitative phytochemical screening of *Heliotropium indicum* extract

Phytochemical	Test	Result
Alkaloids	Dragendorff's, Mayer's and Wagner's tests	+
Flavonoids	Shinoda and alkaline reagent tests	+
Cardiac glycosides	Keller–Killiani test	–
Phlobatannins	HCl test	+
Saponins	Frothing test	+
Steroids	Liebermann–Burchard test	–
Tannins	Ferric chloride test	+
Triterpenes	H ₂ SO ₄ test	+

Note: (+) Present; (–) Absent.

Effect of *Heliotropium indicum* on Serum BUN

Cisplatin administration produced a significant elevation in serum blood urea nitrogen (BUN) compared with the normal control group. The BUN concentration increased from 25.10 ± 0.02 mg/dL in the control group to 60.63 ± 1.12 mg/dL in the cisplatin-treated group ($p < 0.001$).

Pretreatment with the aqueous extract of *H. indicum* significantly attenuated the cisplatin-induced increase in BUN. The BUN level decreased to 43.36 ± 1.02 mg/dL and 30.10 ± 0.11 mg/dL following treatment with *H. indicum* at 250 and 500 mg/kg, respectively ($p < 0.001$ vs. cisplatin group). The reduction was more pronounced at 500 mg/kg, indicating a dose-related response (Table 02).

Table 02: Effect of *Heliotropium indicum* on cisplatin-induced changes in serum BUN levels

Group	Treatment	BUN (mg/dL), Mean $\pm$ SEM
I	Normal saline (2 mL/kg, p.o.)	25.10 ± 0.02
II	Cisplatin (10 mg/kg, i.p.)	$60.63 \pm 1.12^*$
III	<i>H. indicum</i> (250 mg/kg, p.o.) + cisplatin	$43.36 \pm 1.02^\#$
IV	<i>H. indicum</i> (500 mg/kg, p.o.) + cisplatin	$30.10 \pm 0.11^\#$

$p < 0.001$ vs. normal control; $^\# p < 0.001$ vs. cisplatin-treated group.

Effect of *Heliotropium indicum* on Serum Creatinine

Cisplatin treatment significantly increased serum creatinine from 0.60 ± 0.025 mg/dL in the normal control group to 1.20 ± 0.011 mg/dL in the cisplatin-treated group ($p < 0.001$). Treatment with *H. indicum* significantly reduced the cisplatin-induced elevation in serum creatinine. The levels decreased to

0.80 ± 0.021 mg/dL at 250 mg/kg and 0.60 ± 0.060 mg/dL at 500 mg/kg ($p < 0.001$ vs. cisplatin group). The higher dose produced a greater reduction and restored serum creatinine to a level comparable to that of the normal control group (Table 03).

Table 03: Effect of *Heliotropium indicum* on cisplatin-induced changes in serum creatinine levels

Group	Treatment	Serum creatinine (mg/dL), Mean $\pm$ SEM
I	Normal saline (2 mL/kg, p.o.)	0.60 ± 0.025
II	Cisplatin (10 mg/kg, i.p.)	$1.20 \pm 0.011^*$
III	<i>H. indicum</i> (250 mg/kg, p.o.) + cisplatin	$0.80 \pm 0.021^\#$
IV	<i>H. indicum</i> (500 mg/kg, p.o.) + cisplatin	$0.60 \pm 0.060^\#$

$p < 0.001$ vs. normal control; $^\# p < 0.001$ vs. cisplatin-treated group.

Histopathological Evaluation

Histopathological examination demonstrated clear differences among the experimental groups. The kidney sections of the normal control animals showed **normal renal architecture with intact glomeruli and renal tubules and no apparent pathological alterations**.

In contrast, cisplatin-treated animals exhibited **marked renal structural alterations**, characterized by hemorrhagic foci, tubular degeneration, and inflammatory cell infiltration, indicating substantial renal tissue injury.

Treatment with *H. indicum* attenuated these pathological changes. The 500 mg/kg treatment group showed **substantially preserved renal architecture**, with relatively normal glomerular and tubular morphology and minimal inflammatory infiltration. Hemorrhage, tubular degeneration, congestion, and tubular necrosis were markedly reduced compared with the cisplatin-treated group.

Overall, the biochemical and histopathological findings demonstrated that *H. indicum* produced a significant protective effect against cisplatin-induced renal injury, with the 500 mg/kg dose showing greater nephroprotective activity than the 250 mg/kg dose.

DISCUSSION

Nephrotoxicity is a major adverse effect associated with several therapeutic agents and is characterized by impaired renal function and structural damage to renal tissue. The proximal tubular epithelium is particularly vulnerable because of its high metabolic activity and exposure to concentrated nephrotoxic substances. Cisplatin-induced nephrotoxicity is a well-established experimental model for evaluating nephroprotective agents, as it produces reproducible biochemical and histopathological changes in experimental animals.

Cisplatin accumulates predominantly in the renal cortex and proximal tubular cells, where it induces cellular injury through multiple mechanisms, including oxidative stress, reactive oxygen species generation, mitochondrial dysfunction, lipid

peroxidation, and impairment of tubular transport. These processes ultimately contribute to tubular degeneration and necrosis and subsequent deterioration of renal function.

In the present study, cisplatin administration produced a significant increase in serum BUN and creatinine compared with the normal control group, confirming the development of renal injury. Pretreatment with the aqueous extract of *Heliotropium indicum* significantly attenuated these changes. Both 250 and 500 mg/kg doses reduced BUN and serum creatinine levels compared with the cisplatin-treated group ($p < 0.001$), with the higher dose showing a greater protective effect. These findings suggest that *H. indicum* pretreatment preserved renal function against cisplatin-induced injury.

The biochemical findings were consistent with the histopathological observations. Cisplatin-treated animals showed marked renal alterations, including hemorrhagic foci, tubular degeneration, and inflammatory cell infiltration. In contrast, treatment with *H. indicum*, particularly at 500 mg/kg, substantially preserved renal architecture and reduced the severity of pathological changes. Thus, the histological findings support the nephroprotective effect observed from the biochemical parameters.

Oxidative stress is considered an important contributor to cisplatin-induced renal injury. The nephroprotective activity of *H. indicum* may therefore be associated, at least in part, with its antioxidant potential. The phytochemical screening in the present study demonstrated the presence of alkaloids, flavonoids, phlobatannins, saponins, tannins, and triterpenes. Flavonoids and other phenolic constituents are known for their free-radical-scavenging and antioxidant properties and may contribute to protection against oxidative renal damage.

Overall, the present findings demonstrate that the aqueous extract of *H. indicum* attenuates cisplatin-induced nephrotoxicity, as evidenced by improvements in serum BUN, creatinine, and renal histopathology. The greater activity observed at 500 mg/kg suggests a dose-related nephroprotective effect. However, further studies involving oxidative stress markers, inflammatory mediators, molecular pathways, and isolation of active phytoconstituents are required to establish the precise mechanism of protection.

CONCLUSION

The present study demonstrated that pretreatment with the aqueous extract of *Heliotropium indicum* attenuated cisplatin-induced renal injury in Wistar rats. The extract significantly reduced the cisplatin-induced elevations in serum BUN and creatinine and improved the associated histopathological changes, with the 500 mg/kg dose showing greater nephroprotective activity than the 250 mg/kg dose. The observed protective effect may be associated with the antioxidant potential of the phytoconstituents present in *H. indicum*. However, further studies are required to identify and characterize the active constituents and elucidate the underlying molecular mechanisms. Additional investigations using different extracts, dose levels, treatment durations, oxidative stress and inflammatory biomarkers, and other experimental models are warranted before its potential clinical application can be considered.

AUTHOR CONTRIBUTIONS

Both authors contributed to equally.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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