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PHARMACOLOGICAL EVALUATION AND NEPHROPROTECTIVE POTENTIAL OF THUNBERGIA ERECTA FLOWER EXTRACT IN A RAT MODEL

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ABSTRACT

This study evaluates the nephroprotective activity of the methanolic extract of *Thunbergia erecta* in Gentamycin-induced nephrotoxicity in rats. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, tannins, and phenols. Gentamycin administration led to marked renal damage, characterized by apoptosis, necrosis, and increased serum creatinine levels, mimicking acute renal failure. Treatment with *Thunbergia erecta* extract (100 mg/kg and 200 mg/kg) significantly improved renal function, as evidenced by restored body weight, increased urine output, and reduced serum creatinine. Histopathological examination confirmed that the extract reversed kidney damage and restored normal kidney architecture. These findings suggest that *Thunbergia erecta* possesses potent nephroprotective activity, likely due to its antioxidant properties and flavonoid content, making it a promising candidate for future research in nephrotoxicity management.

KEYWORDS: Thunbergia erecta, nephroprotective activity, Gentamycin-induced nephrotoxicity, serum creatinine, histopathology.

INTRODUCTION

Chronic kidney disease (CKD) and acute kidney injury (AKI) are major global health concerns, often resulting from oxidative stress, inflammation, and nephrotoxic agents (Lameire et al., 2013). The kidneys play a vital role in maintaining homeostasis by filtering waste, balancing electrolytes, and regulating blood pressure (Basile et al., 2012). However, exposure to nephrotoxic drugs, heavy metals, and metabolic disorders such as diabetes mellitus contribute to renal damage, leading to progressive loss of kidney function (Tervaert et al., 2010).

Medicinal plants have gained significant attention in nephroprotective research due to their antioxidant, anti-inflammatory, and cytoprotective properties (Afsar et al., 2020). *Thunbergia erecta* (Acanthaceae), commonly known as Bush Clock Vine, is traditionally used for its hepatoprotective, antimicrobial, and anti-inflammatory effects (Vijayalakshmi et al., 2017). Phytochemical investigations have revealed the presence of flavonoids, tannins, saponins, and alkaloids, which are known to exert protective effects against oxidative stress-induced organ damage (Akinmoladun et al., 2019). However, its potential nephroprotective activity remains largely unexplored.

Nephrotoxicity is a major health issue associated with drug-induced toxicity, metabolic disorders, and environmental pollutants. Nephrotoxic agents, including cisplatin, gentamicin, and nonsteroidal anti-inflammatory drugs (NSAIDs), cause oxidative stress and inflammation, leading to renal dysfunction (Pabla & Dong, 2008). Chronic kidney disease (CKD) and acute kidney injury (AKI) are increasing globally, necessitating effective therapeutic interventions to prevent renal damage (Lameire et al., 2013).

Oxidative stress plays a crucial role in kidney injury, with reactive oxygen species (ROS) triggering lipid peroxidation, protein oxidation, and DNA damage in renal tissues (Nath & Norby, 2000). Several studies highlight the importance of antioxidants in mitigating oxidative stress-induced nephrotoxicity (Ratliff et al., 2016). Herbal medicines have been explored as nephroprotective agents due to their bioactive compounds that counteract oxidative and inflammatory damage in the kidneys (Afsar et al., 2020).

Several medicinal plants have shown promise in nephroprotection. *Phyllanthus niruri*, *Curcuma longa*, *Moringa oleifera*, and *Withania somnifera* exhibit antioxidant, anti-inflammatory, and diuretic properties, contributing to kidney protection (Basu et al., 2019). Extracts from these plants have been reported to reduce elevated levels of serum creatinine, blood urea nitrogen (BUN), and uric acid—key markers of renal function (Yadav et al., 2017).

Studies on *Thunbergia laurifolia*, a close relative of *Thunbergia erecta*, have demonstrated hepatoprotective and nephroprotective properties, suggesting that plants of this genus may have therapeutic effects on renal disorders (Vijayalakshmi et al., 2017). However, the nephroprotective potential of *Thunbergia erecta* remains underexplored.

Thunbergia erecta (Acanthaceae), commonly known as Bush Clock Vine, has been traditionally used for treating inflammation, infections, and oxidative stress-related disorders (Sivanesan et al., 2015). Phytochemical studies reveal that the plant contains flavonoids, tannins, alkaloids, phenolics, and saponins, which are known for their antioxidant and anti-inflammatory properties (Akinmoladun et al., 2019).

Flavonoids, particularly quercetin and kaempferol, play a crucial role in renal protection by reducing ROS levels and enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Kumar et al., 2020). Additionally, phenolic compounds have been shown to inhibit lipid peroxidation and improve renal histopathology in nephrotoxic models (Abdel Moneim, 2016).

While studies on *Thunbergia erecta* are limited, preliminary reports suggest its potential therapeutic benefits. Vijayalakshmi et al. (2017) reported the hepatoprotective activity of *Thunbergia erecta* flower extract, highlighting its ability to reduce liver toxicity markers. Additionally, its antimicrobial and anti-inflammatory activities have been documented, suggesting its possible application in oxidative stress-related conditions (Kumar et al., 2020).

Considering the plant's phytochemical profile and pharmacological properties, investigating its nephroprotective potential is warranted. This study aims to evaluate the protective effects of *Thunbergia erecta* flower extract on renal function and oxidative stress markers in a rat model of nephrotoxicity.

This study aims to evaluate the nephroprotective effects of Thunbergia erecta flower extract in a rat model, assessing renal function biomarkers, oxidative stress markers, and histopathological alterations. By investigating the therapeutic potential of this plant, the study may provide insights into the development of alternative treatments for nephrotoxicity and kidney disorders.

MATERIAL AND METHOD

List of reagent and chemicals used

The study utilized reagents from Merck (glacial acetic acid, nitroprusside, sodium hydroxide, ammonia), Researchlab (petroleum ether), Fizmerck (H_2SO_4), Molychem (ethanol), Clorofiltind (95% alcohol, HCl, chloroform), Himedia (magnesium), and Rankem (1% $CuSO_4$ solution). Glassware, including beakers, rods, flasks, pipettes, and test tubes, was sourced from Borosilicate.

Plant collection

Thunbergia erecta flowers (300 g) were collected, shade-dried for three days, and oven-dried at 45°C until completely dry. The dried flowers were stored in airtight glass containers in a cool, dry place. The plant was authenticated by Dr. Jagrati Tripathi (Govt. Botanist) with Authentication No. AC/037/24.

Extraction

The plant material was extracted using the Soxhlet apparatus via continuous hot percolation. Thunbergia erecta powder was first extracted with petroleum ether at 60°C, followed by methanol re-extraction of the dried marc. Extraction continued until no color change was observed in the siphon tube. The extracts were concentrated using a rotary vacuum evaporator at 40°C, and the yield percentage was calculated:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}}$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al.*, 2002)**.

Phytochemical investigation

The qualitative phytochemical analysis of Thunbergia erecta was performed to identify various phytoconstituents using standard procedures (Kokate et al., 2000).

1.1.1 Test for Carbohydrates

- **Molisch's Test**: Purple ring formation with sulfuric acid indicates carbohydrates.
- **Fehling's Test**: Reddish-brown precipitate shows reducing sugars.
- **Benedict's Test**: Green, yellow, or red color formation indicates reducing sugars.
- **Barfoed's Test**: Red color indicates monosaccharides.

1.1.2 Tests for Alkaloids

- **Dragendorff's Test**: Orange-red precipitate indicates alkaloids.
- **Wagner's Test**: Reddish-brown precipitate confirms alkaloids.

- **Mayer's Test**: White precipitate indicates alkaloids.
- **Hager's Test**: Yellow precipitate confirms alkaloids.

1.1.3 Test for Saponins

- **Froth Test**: Stable froth formation indicates saponins.

1.1.4 Test for Triterpenoids and Steroids

- **Libermann-Burchard Test**: Bluish-green color indicates steroids.
- **Salkowski Test**: Bluish-red color in chloroform and green fluorescence in the acid layer confirms steroids.

1.1.5 Test for Tannin and Phenolic Compounds

- **Ferric Chloride Test**: Dark blue color indicates tannins.
- **Gelatin Test**: White precipitate confirms phenolic compounds.
- **Lead Acetate Test**: White precipitate indicates phenolic compounds.

1.1.6 Test for Flavonoids

- **Shinoda's Test**: Red to pink color indicates flavonoids.

1.1.7 Test for Glycosides

- **Borntrager's Test**: Pink to red color in the ammonia layer indicates anthraquinone glycosides.
- **Keller Killiani Test**: Blue color in the acetic acid layer confirms cardiac glycosides.

1.1.8 Test for Fats and Oils

- **Solubility Test**: Solubility in chloroform or ethanol indicates fats and oils

Quantitative Phytochemical Estimation

TPC

The total phenolic content of Thunbergia erecta extract was determined using the Folin-Ciocalteu Assay. For the assay, 0.2 mL of Thunbergia erecta extract was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution, then diluted to a total volume of 7 mL with distilled water. The mixture was allowed to stand at room temperature for 2 hours before measuring the absorbance at 760 nm using a spectrophotometer. A calibration curve was created using standard solutions of Gallic Acid Equivalent (GAE) at concentrations of 20, 40, 60, 80, and 100 µg/mL. The Folin-Ciocalteu reagent reacts with reducing compounds, including polyphenols, to form a blue color, which was measured spectrophotometrically (Tangco et al., 2015).

TFC

Flavonoid content in Thunbergia erecta extract was determined using the Aluminium chloride method. 0.5 mL of the extract was mixed with 2 mL of distilled water, followed by 0.15 mL of 5% sodium nitrite and 0.15 mL of 10% Aluminium chloride. After 6 minutes, 2 mL of 4% sodium hydroxide was added, and the absorbance was measured at 510 nm using a UV spectrophotometer. A calibration curve using Rutin Equivalent (RE) standards (20-100 µg/mL) was used to determine the flavonoid content, expressed as mg Rutin equivalent per gram of dry extract weight (Parthasarathy S et al., 2009).

DPPH

The antioxidant activity of Thunbergia erecta extract was evaluated using the DPPH free radical scavenging assay. Different concentrations (20-100 µg/mL) of the extract/standard were mixed with 2 mL of 0.1 mM DPPH solution and incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm using a UV spectrophotometer (Shimadzu 1700). The control consisted of 3 mL of DPPH solution incubated under the same conditions, with absorbance taken against methanol as blank (Athavale et al., 2012).

Percentage antioxidant activity of sample/standard was calculated by using formula.

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

FT-IR

FT-IR spectroscopy was used to identify functional groups in the methanolic extract. The sample was dried, ground with KBr pellets, and analyzed using a Perkin Spectrum BX spectrophotometer on a Thermo Nicolet model 6700. A 100 mg KBr disk with 2% sample was examined in the 400-4000 cm^{-1} range (Luciene et al., 2008).

Acute Toxicity Study

The acute toxic class method follows a stepwise procedure, using 3 animals of the same sex per step. The substance is administered orally, and the next step is based on the observed mortality or moribund status of the animals. The process may require 2-4 steps. The starting dose is selected from fixed levels: 5, 50, 300, and 2000 mg/kg body weight (Guideline Document on 1996).

Experimental work Animals Protocol

Group	Treatment	Dose	Administration
Group I	Normal control	1 mL/kg normal saline	Oral
Group II	Nephrotoxic control (Gentamycin)	40 mg/kg Gentamycin	Intraperitoneal
Group III	Standard nephroprotective drug (Silymarin)	200 mg/kg Silymarin	Oral
Group IV	Gentamycin + Thunbergia erecta extract	100 mg/kg	Oral
Group V	Gentamycin + Thunbergia erecta extract	200 mg/kg	Oral

Animals were housed under controlled conditions and administered treatments for 14 days (Rad et al., 2017).

Blood collection techniques used in the present study

On the 15th day, animals were sacrificed under ether anesthesia. Blood was collected via retro-orbital vein puncture, allowed to stand for 30 min at 37°C, and then centrifuged to separate the serum for biochemical marker evaluation.

Preparation of kidney homogenate

The kidney was removed, perfused with ice-cold saline (0.9% NaCl), and a portion was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4). The homogenate was centrifuged at 5000 rpm for 10 minutes, and the supernatant was used for biochemical assays.

Analysis of general parameters

Estimation of urine volume

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume are taken after 24 hours. The food wastes and fecel matters are removed from the urine. And the volume of urine is measured by using measuring cylinder.

Estimation of Body weight

At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal are individually weighed and the weight were recorded.

Analysis of serum biochemical parameters

Estimation of Serum Creatinine (Slot C *et al.*, 1965)

Five test tubes (A, B, C, D, and E) were prepared as follows:

- E (blank): 2 mL distilled water
- C & D (test): 0.5 mL serum + 1.5 mL water
- A & B (standard): 1.5 mL water + 0.5 mL creatinine standard (3 mg/dL)

Then, 6 mL of picric acid and 0.4 mL of sodium hydroxide (2.5M) were added to all tubes.

Estimation of Serum Blood urea nitrogen (BUN) (Fawcett, J.K *et al.*, 1960)

Blood urea was estimated using the Berthelot method (Fawcett and Scott, 1960).

- 1000 µL of reagent-I (urease, salicylate, hypochlorite, nitroprusside) was added to 10 µL serum, 10 µL standard urea, and 10 µL water (blank).
- Incubated at 37°C for 5 min, then 1000 µL of reagent-II (alkaline buffer) was added and incubated for another 5 min.
- Ammonia released reacted with the reagent to form a blue-green color, measured at 578 nm.
- Urea concentration was calculated spectrophotometrically

$$\text{Blood urea (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times 100$$

RESULTS

Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Thunbergia erecta* is shown in Table: 3.

Table 3: Percentage Yield of crude extracts of *Thunbergia erecta* extract.

S. no	Plant name	Solvent	Theoretical weight	Yield (gm)	% yield
1	*Thunbergia erecta*	Pet. ether	282.99	1.71	0.60%
2		Methanol	298.32	6.16	2.06%

Preliminary Phytochemical study

Table 4: Phytochemical testing of extract.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	**Methanolic extract**
1.	**Alkaloids**		
1.1	Mayer's reagent test	Present	Absent
1.2	Wagner's reagent test	Present	Absent
1.3	Hager's reagent test	Present	Absent
2.	**Glycoside**		
2.1	Borntrager test	Present	Absent
2.2	Killer-Killiani test	Absent	Absent
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Present	Absent
4.	**Flavonoids**		

4.1	Shinoda's Test	Present	Absent
4.2	Alkaline's Test	Present	Absent
4.3	Lead Acetate's Test	Present	Absent
5.	**Tannin and Phenolic Compounds**		
5.1	Ferric Chloride test	Absent	Absent
5.2	Gelatin Test	Present	Present
5.3	Lead Acetate Test	Present	Present
6.	**Saponin**		
6.1	Froth Test	Present	Absent
7.	**Test for Triterpenoids and Steroids**		
7.1	Salkowski's test	Present	Absent
8	**Test for Protein and Amino Acid**		
8.1	Biuret		Absent
8.2	Ninhydrin		Absent

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation.

Table 5 Standard table for Gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.147
2.	40	0.176
3.	60	0.189
4.	80	0.235
5.	100	0.271

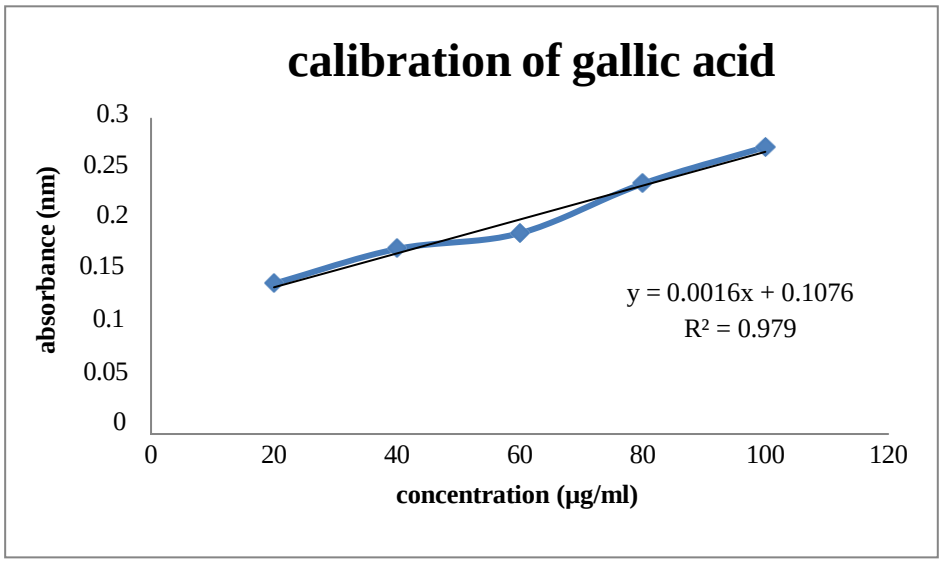


Figure 9: Represent standard curve of Gallic acid.

Total Phenolic Content in extract Table 6: Total Phenolic Content

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.136	56.33 mg/gm
2	0.177	
3	0.189	

Table 7: Total Phenolic Content of extract *Thunbergia erecta.*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	61.33

Total Flavonoids content (TFC) estimation Table 8 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.173
2.	40	0.203
3.	60	0.277
4.	80	0.316
5.	100	0.331

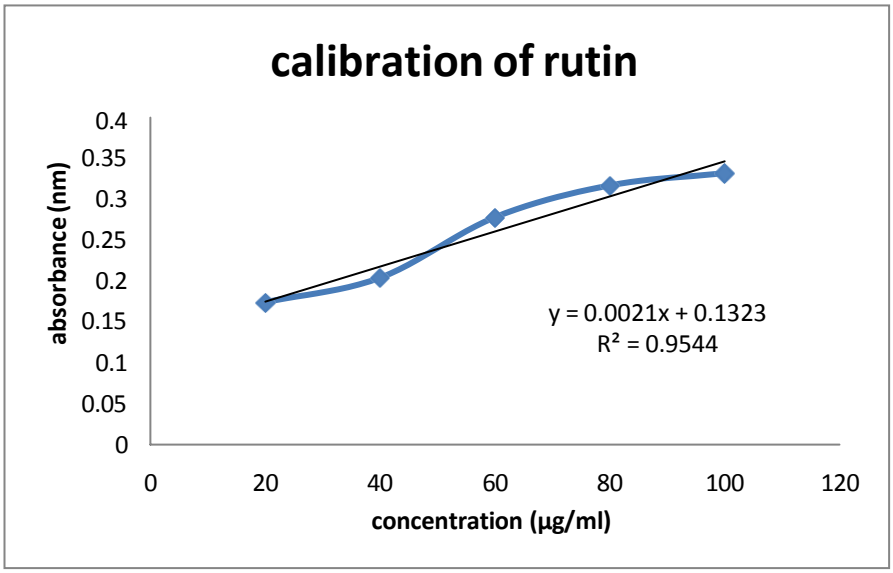


Figure 10: represent standard curve of Rutin.

Total Flavonoid Content in extract

Table 9: **Total Flavonoid Content.**

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.147	17.83 mg/gm
2	0.161	
3	0.192	

Table 10: **Total Flavonoid Content of extract *Thunbergiaerecta.***

Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Methanol	17.83

***In vitro* Antioxidant Assays**

In the present investigation, the *in vitro* anti-oxidant activity of extracts of *Thunbergia erecta was* evaluated by DPPH radical scavenging activity. The results are summarized in Tables.

DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 11: DPPH radical scavenging activity of Std. Ascorbic acid.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	51.209
40	0.435	56.149
60	0.344	65.322
80	0.285	71.270
100	0.145	85.383
Control	0.992	
IC50		

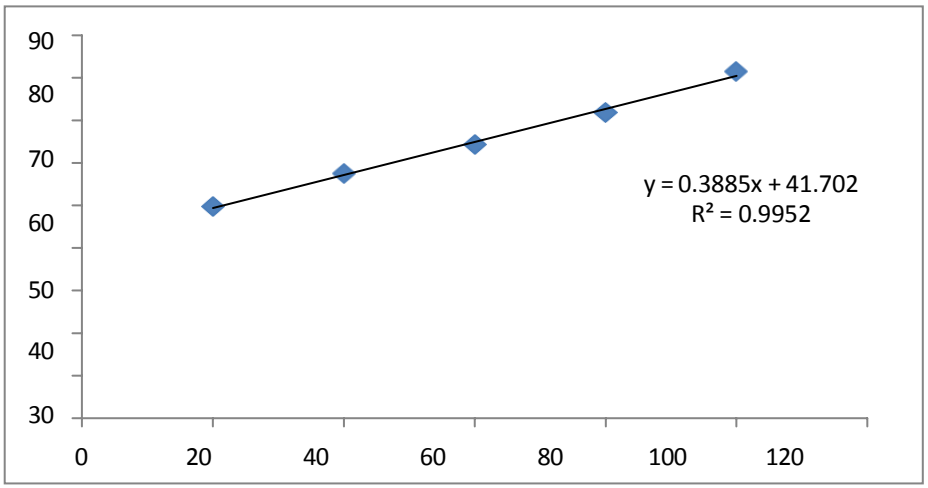


Figure 12: DPPH radical scavenging activity of Std. Ascorbic acid.

Table 12: DPPH radical scavenging activity of methanol extract of *Thunbergia erecta.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.519	43.831
40	0.465	49.675
60	0.454	50.865
80	0.413	55.303
100	0.367	60.281
Control	0.924	
IC50		

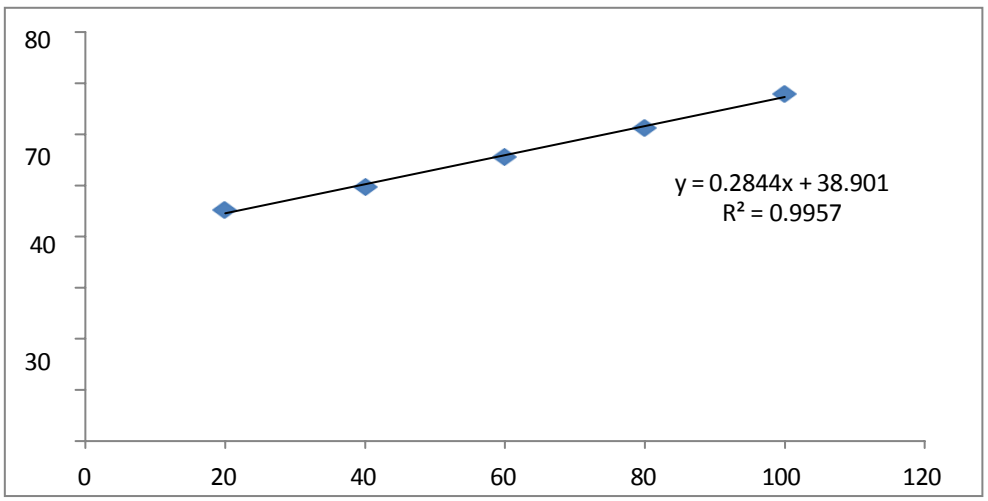


Figure 13: represents the Percentage Inhibition *Vs* Concentration of extract of.

Thunbergia erecta

Functional group identified by FTIR Study

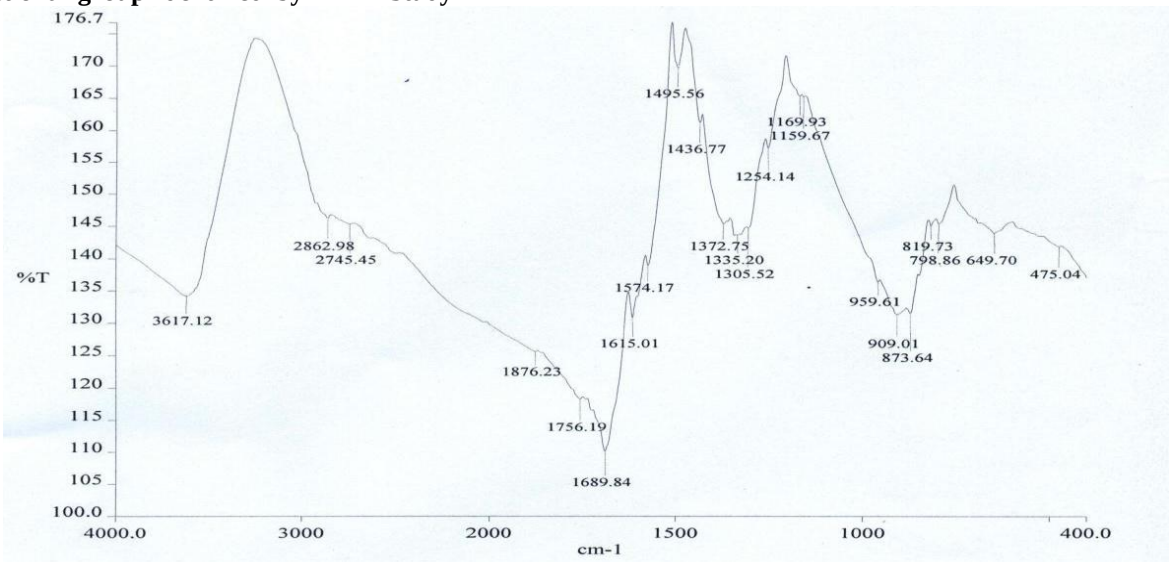
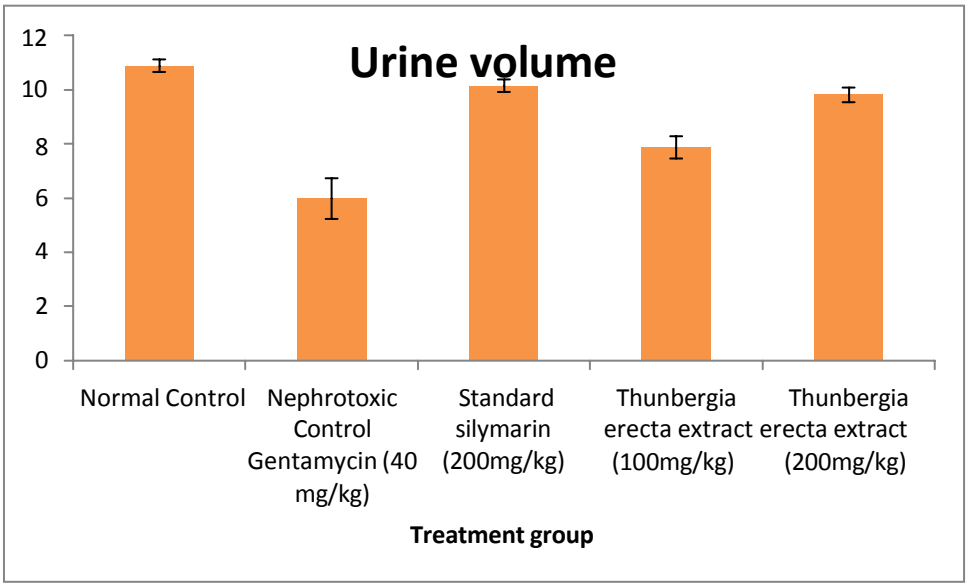


Figure 14: FTIR of *Thunbergia erecta.*

Analysis of general parameters Estimation of urine volume

Table 13: Urine volume.

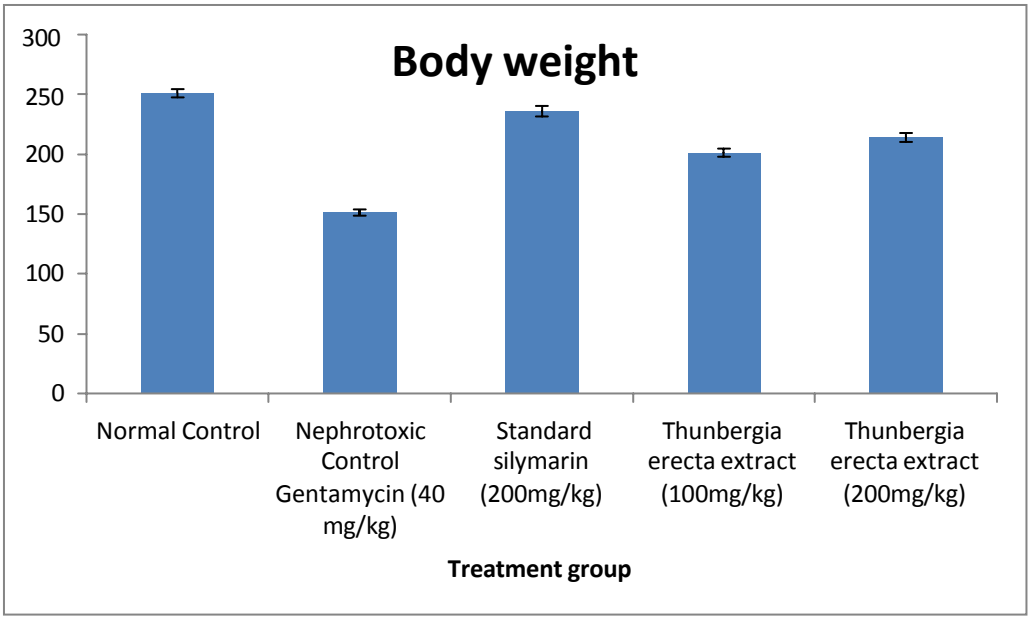
Groups	Urine volume
Normal Control	10.88±0.23
Nephrotoxic Control Gentamycin (40 mg/kg)	5.98±0.75
Standard silymarin (200mg/kg)	10.14±0.23
***Thunbergia erecta* extract (100mg/kg)**	7.87±0.41
***Thunbergia erecta* extract (200mg/kg)**	9.80±0.27



Graph 1: Urine volume.

Estimation of Body weight Table 14: Body weight

Groups	Average body weight
Normal control	250 ± 3.404
Nephrotoxic control Gentamycin (40 mg/kg)	150.12 ± 2.650
Standard silymarin (200 mg/kg)	235.32 ± 4.354
Thunbergia erecta (flower) extract (100 mg/kg)	200.32 ± 3.425
Thunbergia erecta (flower) extract (200 mg/kg)	210 ± 3.671

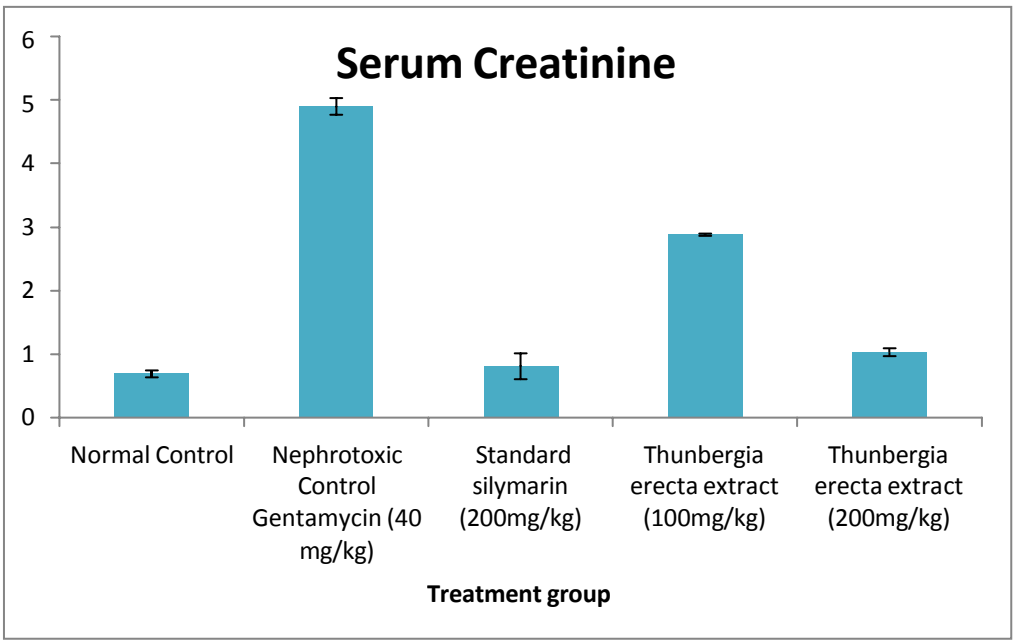


Graph 2: Body weight.

Analysis of serum biochemical parameters

Estimation of Serum Creatinine Table 15: Serum Creatinine.

Groups	Serum Creatinine
Normal Control	0.69±0.055
Nephrotoxic Control Gentamycin (40 mg/kg)	4.90±0.131
Standard silymarin (200mg/kg)	0.81±0.203
***Thunbergia erecta* extract (100mg/kg)**	2.88±0.018
***Thunbergia erecta* extract (200mg/kg)**	1.03±0.062

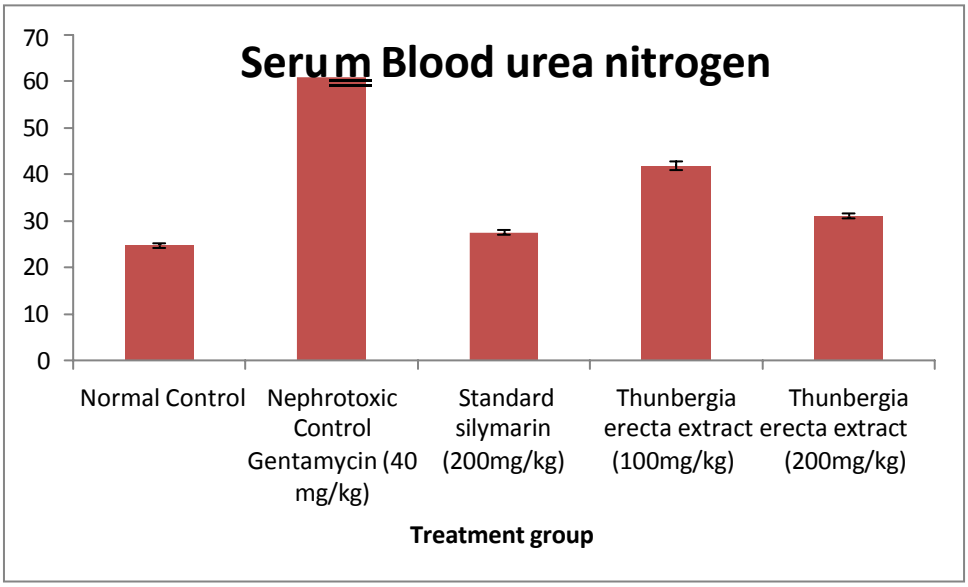


Graph 3: Serum Creatinine.

Estimation of Serum Blood urea nitrogen (BUN)

Table 16: Serum Blood urea nitrogen.

Groups	Serum Blood urea nitrogen
Normal Control	24.68±0.503
Nephrotoxic Control Gentamycin (40 mg/kg)	61±0.790
Standard silymarin (200mg/kg)	27.54±0.52
***Thunbergia erecta* extract (100mg/kg)**	41.87±0.94
***Thunbergia erecta* extract (200mg/kg)**	31.06±0.55



Graph 4: Serum Blood urea nitrogen.

Acute Oral Toxicity

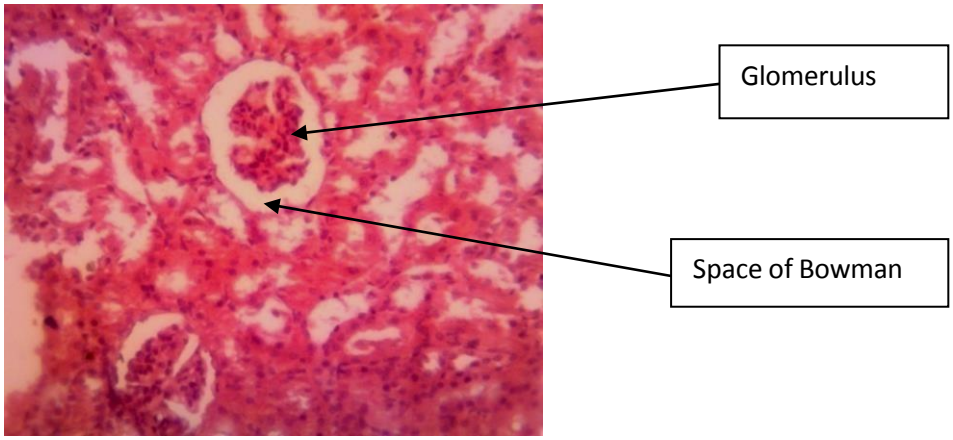
Acute toxicity refers to the immediate adverse effects after a single drug dose. Two plant extract solutions were prepared: 100 mg and 200 mg of extract dissolved in 10 mL distilled water.

- 0.2 mL of the 100 mg extract solution was administered to group 4 animals, and 0.2 mL of the 200 mg solution was administered to group 5.
- After observation, group 4 animals lost weight, while group 5 gained weight, indicating toxicity, and treatment was provided.

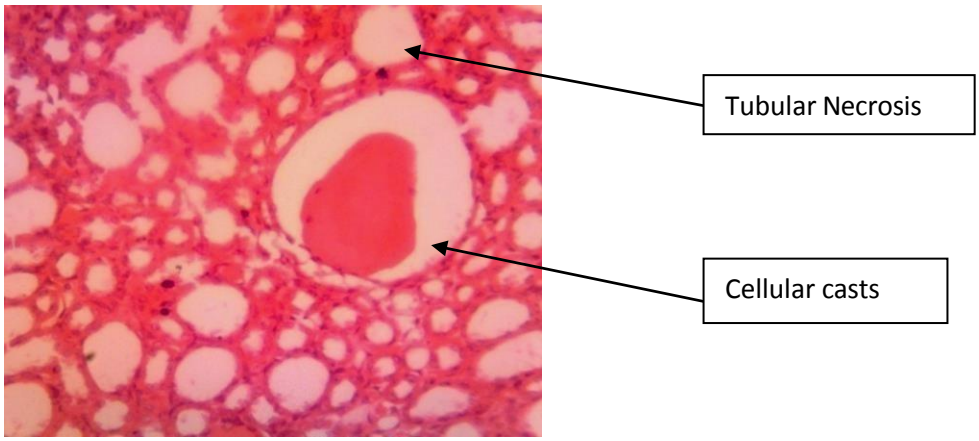
Figure 15: (a) Oral dose, (b) IP dose.

Histopathology

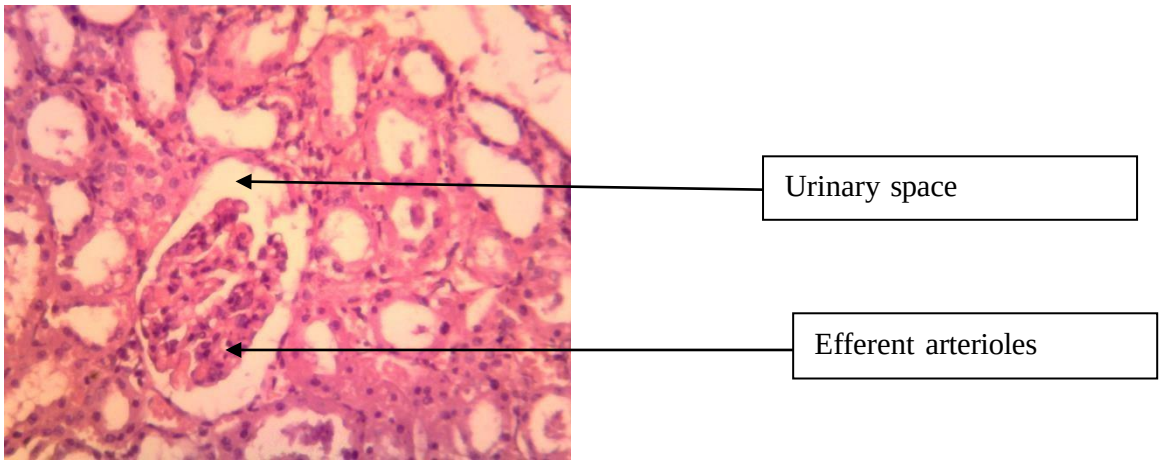
Group 1: normal Control



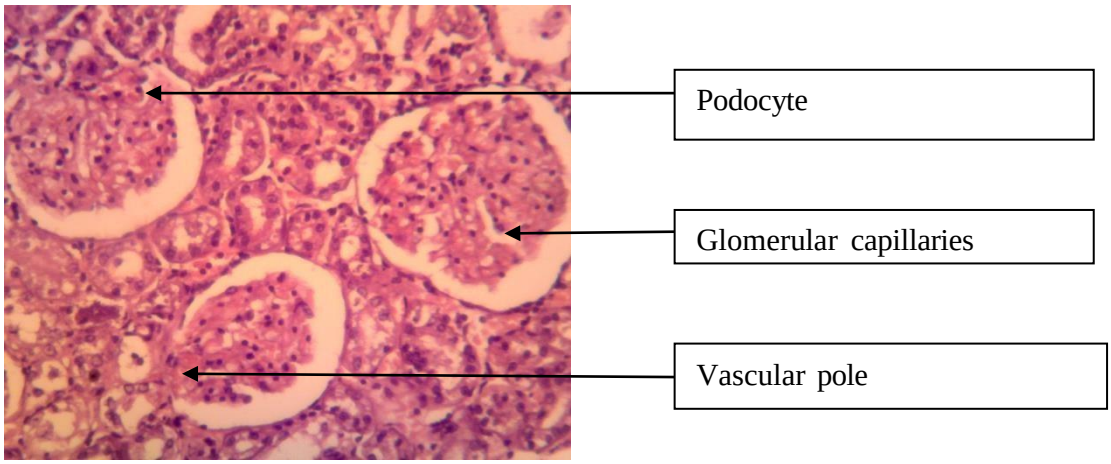
Group I: Normal.



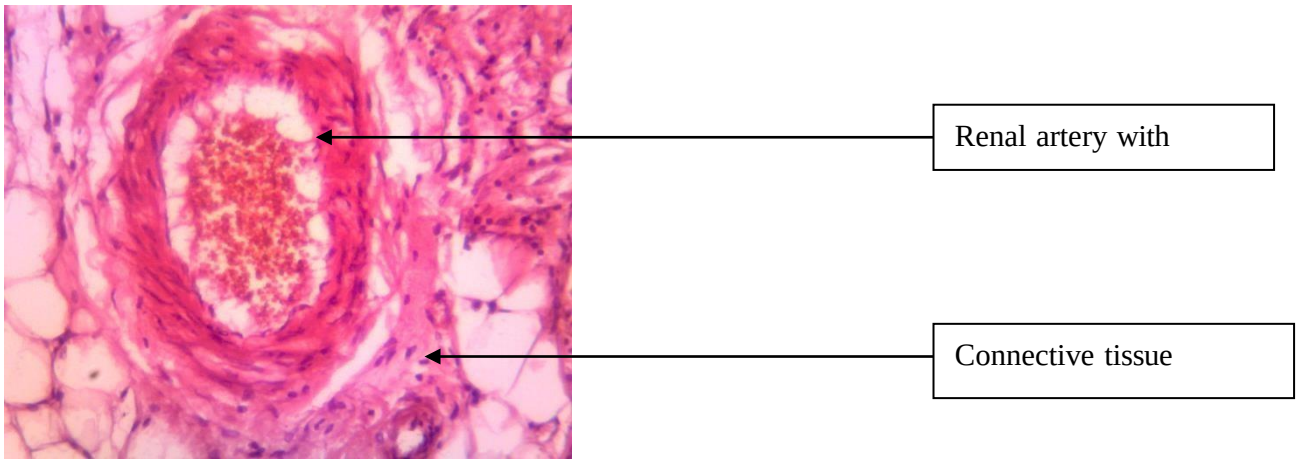
Group II: Induced.



Group 3: Standard treats with silymarin.



Group 4: 100 mg extract.



Group 5: 200 mg extract.

Histopathological analysis showed severe kidney damage in rats treated with GM, including renal tubule dilation, necrosis, glomerular sclerosis, cellular swelling, and edema. Normal kidney structure was restored in NC rats, while GM-treated rats showed extensive renal tubule necrosis. Gentamicin induced severe nephrotoxicity with tubular necrosis and inflammation. Silymarin and the 200 mg plant extract dose offered protection, maintaining renal structure and function, while the 100 mg dose showed no protective effects. These findings suggest the 200 mg plant extract could be further explored for its nephroprotective potential.

DISCUSSION

Phytochemical analysis of Thunbergia erecta's methanolic extract revealed alkaloids, phenolics, flavonoids, saponins, glycosides, and tannins. Quantitative assays showed total phenolic content (TPC) and total flavonoid content (TFC), with DPPH radical scavenging activity showing 60.28% inhibition and an IC50 of 49.84 µg/ml, compared to ascorbic acid's 85.38% inhibition and IC50 of 22.01 µg/ml. In acute toxicity studies, no toxicity was observed up to 2000 mg/kg, so 100 mg/kg and 200 mg/kg were used for further studies.

In the study, gentamicin induced nephrotoxicity, shown by weight loss, reduced glomerular filtration rate, and elevated creatinine. Thunbergia erecta (100 mg/kg and 200 mg/kg) significantly reduced creatinine levels, improved weight gain, and increased urine output. Gentamicin also caused a marked increase in blood urea nitrogen (BUN), which was lowered by Thunbergia extract. Histopathological analysis showed tubular necrosis and inflammation in gentamicin-treated rats, while the 200 mg dose of Thunbergia protected kidney structure, suggesting its potential as a nephroprotective agent. The 100 mg dose showed no protection.

CONCLUSION

In conclusion, the methanolic extract of Thunbergia erecta demonstrated significant nephroprotective activity in rats induced with nephrotoxicity by Gentamycin. Phytochemical analysis confirmed the presence of bioactive compounds, including alkaloids, flavonoids, glycosides, tannins, and phenols, which may contribute to its protective effects. The extract significantly improved body weight, urine volume, and serum creatinine levels in nephrotoxic rats. Histopathological findings showed reversal of kidney damage and restoration of normal kidney architecture. The nephroprotective potential of Thunbergia erecta is likely due to its flavonoid content and antioxidant properties, making it a promising candidate for further nephrotoxicity research.

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Conflict of Interest

The authors declare no conflict of interest related to this study.

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