PHARMACOLOGICAL EVALUATION, ANTIOXIDANT AND NEPHROPROTECTIVE ACTIVITY OF *HERBAL PLANT EXTRACT* IN EXPERIMENTAL RATS

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ABSTRACT

This study investigated the phytochemical composition, antioxidant potential, and nephroprotective activity of *Ipomoea triloba* leaf extracts in paracetamol-induced nephrotoxicity in rats. Petroleum ether extraction yielded 5.03% compared to 2.6% with methanol, indicating a higher recovery of non-polar compounds. Phytochemical screening revealed diverse bioactive constituents, with the methanolic extract particularly rich in alkaloids, flavonoids, saponins, and phenolics. Quantitative analysis confirmed substantial levels of total phenolics (75.3 mg/g GAE) and flavonoids (48.3 mg/g RE), correlating with moderate antioxidant activity observed in the DPPH assay (IC_{50} = 48.26 µg/ml). In vivo evaluation demonstrated that *I. triloba* extract improved renal function in a dose-dependent manner, as evidenced by reductions in serum creatinine and blood urea nitrogen, along with restoration of urine volume and body weight. Histopathological findings supported these results, showing preserved renal architecture and reduced tissue injury, particularly at 400 mg/kg, with effects comparable to the standard drug. Acute toxicity studies revealed no mortality or major adverse effects, confirming safety at the tested doses. Overall, the methanolic extract of *I. triloba* exhibited significant antioxidant and nephroprotective effects, likely mediated by its phenolic and flavonoid content. These findings highlight its potential as a safe, plant-derived therapeutic for managing nephrotoxicity and oxidative stress-related renal disorders, warranting further studies including chronic toxicity, active compound isolation, and clinical validation.

Keywords: *Ipomoea triloba*, nephroprotective activity, antioxidant, phytochemicals, paracetamol-induced nephrotoxicity, flavonoids, phenolics

1. INTRODUCTION

Kidney diseases represent a global health burden, with oxidative stress playing a central role in renal damage. Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a medicine or chemicals **(Kwiatkowska *et al.*, 2021).** Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances **(Shamna and Shijikumar 2020).** The present review is about the some of the medicinal plants possessing nephroprotective activity on Gentamicin induced nephrotoxicity1 . Nephrotoxicity can be authentic as renal disease or dysfunction that arises

as absolute or aberrant aftereffect of exposure to medicines, and environmental or industrial chemicals. Several factors accept been articular which accomplish the kidney accessible to toxic injury due to indigenous medicines. These drugs were producing produced nephrotoxicity because they are most frequently causing of renal injury inchildren. In addition, their nephrotoxicity is acquired by altered mechanisms **(Baudoux and Nortier 2017).**

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Use of herbs and traditional systems of medicine is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of diseases **(Wang *et al.,* 2023).** The current world scenario of utilization of plant derived natural remedies has created a dire need for accurate and up to date information on the characteristic properties and therapeutic uses, efficacy, safety and quality of medicinal plant products **(Chaachouay and Zidane 2024).**

Ipomoea triloba L., a member of the Convolvulaceae family, has attracted scientific interest not only for its ecological significance but also for its potential pharmacological properties. Traditionally, various *Ipomoea* species have been employed in folk medicine to treat ailments such as inflammation, infections, digestive disorders, and skin conditions **(Srivastava and Rauniyar 2020).** Although *I. triloba* is less extensively studied compared to related species like *Ipomoea batatas* (sweet potato) or *Ipomoea aquatica*, emerging evidence suggests that it possesses a variety of bioactive compounds, including alkaloids, flavonoids, tannins, phenols, and glycosides **(Srivastava and Rauniyar 2020).** Preliminary phytochemical investigations have indicated that extracts of *I. triloba* exhibit promising biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and cytotoxic effects. These properties point to its potential role in the development of plant-based therapeutic agents. Furthermore, the presence of phenolic compounds and other secondary metabolites may contribute to its free radical scavenging ability, which is relevant in the prevention of oxidative stress–related diseases **(Ahmadinejad *et al.,* 2017).** Further studies, including in vivo and in vitro analyses, are essential to validate its traditional uses, identify active constituents, and determine its safety profile. A systematic exploration of this plant may contribute significantly to the discovery of novel bioactive compounds with therapeutic applications **(Dar *et al.,* 2023).**

The present study aims to evaluate the antioxidant potential of *I. triloba* leaf extracts and investigate their nephroprotective efficacy in experimental rats.

2. MATERIAL AND METHODS

2.1 Chemicals

Phosphate Buffered Saline was obtained from Merk. Chloroform was acquired from SRF Limited. Conc. HCl was procured from Chemie Organic chemicals. Ammonia was received from CF Industries Holiding. Magnesium was acquired from Otto chemie Pvt Ltd, while Conc. H_2SO_4 was obtained from TATA Chemicals. Ethanol was received from Bajaj Hindusthan Suga. Sodium Chloride was obtained from Vinipul Chemicals Pvt. Ltd. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from JoinHub pharma**,** Micro Labs Limited, Volo sol, Tata chemical limited and Modi Chemicals.

2.2 Procedure of plant material

Ipomoea triloba plant material was collected from its natural environment, with careful attention given to accurate identification and authentication. The collection site was selected from a geographic region where the plant is traditionally known for its medicinal value. After collection, the plant specimen was verified by a certified taxonomist or botanist at an accredited institution. An herbarium sample was prepared and deposited in the institution's herbarium for future reference. Once authenticated, the plant material was thoroughly washed to remove any dirt or impurities, then shade-dried under controlled conditions to preserve its phytochemical content. After drying, it was coarsely ground using a mechanical grinder and stored in airtight containers at room temperature until required for extraction and phytochemical analysis.

2.3 Extraction of Plant Material by Soxhlation Process

Plant material was extracted utilizing the continuous hot percolation method with Soxhlet equipment. *Ipomoea trilobal* powder was placed in the thimble of a soxhlet apparatus. Soxhlation was performed at 60°C with petroleum ether as the non-polar solvent. The exhausted plant material (marc) was dried and then re-extracted with methanol solvent. Soxhlation was maintained for each solvent until no visible color change was noticed in the siphon tube, and extraction was confirmed by the absence of any residual solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type) **(Ansari *et al.*, 2024).** The dried extract was weighed, and each extract's % yield was calculated. The percentage yield of the *Ipomoea triloba*extract is determined using the following formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

2.4 Quatitative Estimation of Phytoconstituents

After the confirmation of presence of phenols, alkaloids, flavonoids, saponins and tannins preliminary phytochemical tests, the plant material was taken for quantitative estimation.

2.4.1 Quantitative Phytochemical Estimation of TPC

The Folin–Ciocalteu Assay was used to assess the total phenolic content of *Ipomoea triloba* extract. In a test tube, 40 µL (1 mg per 1 mL of methanol) of the plant extract (or standard gallic acid solution), and 200 µL Folin-Ciocalteu reagent were put and mixed by shaking gently. This mixture was diluted up to 3.16 mL with distilled water. After an incubation of 8 min, 600 µL sodium carbonate solutions were added and mixed. The mixture was incubated at 40 °C for 30 min before recording its absorbance in a spectrophotometer at 760 nm against a blank. Calibration curves were created with standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic Acid was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically **(Polori, 2015).**

2.4.2 Quantitative Phytochemical Estimation of TPC

The flavonoid content was measured using the aluminum chloride technique. 0.2 gm of *Ipomoea triloba*combined with 1 mL of distilled water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the mixture was shaken and mixed thoroughly. The mixture's absorbance at 510 nm was measured using a UV spectrophotometer. Calibration curves were created using standard solutions containing Rutin Equivalent (RE) mg/g. Rutin concentrations were prepared at 20, 40, 60, 80, and 100 µg/mL. The calibration curve was used to calculate the total flavonoid concentration, which was then expressed as mg Rutin equivalent per gram dry extract weight (**Suchiang *et al.*, 2024).**

2.5 Determination of antioxidant activity by the DPPH test

The DPPH free radical scavenging assay was used to determine *Ipomoea trilobal* extract's antioxidant properties. 1mg/ml methanol solution of extracts/standard was prepared. To prepare different concentrations of *Ipomoea trilobal* extracts/standards (20-100µg/ml), a 1mg/mL stock solution was mixed with 2mL of 0.1mM DPPH solution. The mixture was vortexed, incubated for 30 minutes at room temperature in a dark place, and then read using a UV spectrophotometer (Shimadzu 1700) at 517 nm. For the control, add 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. The absorbance of the control was measured against methanol (as a blank) at 517 nm **(Sultan *et al.*, 2023).**

$$\% \text{ Scavenging Activity} = 100[(Ac - As)/Ac]$$

Where, Ac and As are absorbances of negative control and sample, respectively

2.6 Acute Toxicity Study

An acute toxicity study was performed in accordance with OECD guideline 423, utilizing a stepwise procedure involving three animals in each phase. The study was approved by the Faculty Ethical Committee. Oral administration of the methanol extract of *Ipomoea triloba* at doses of 5, 50, 300, and 2000 mg/kg body weight showed no mortality or significant adverse effects during the 14-day observation period. The dose may be administered in divided portions within a 24-hour period. Furthermore, no unusual clinical symptoms were observed in the treated animals. Body weight and food intake remained consistent, with no significant differences compared to the untreated control group **(Bedi and Krishan 2020).**

2.7 *In vivo* study of Nephroprotective activity in rats

2.7.1 Experimental work

The study received approval from the Institutional Animal Ethics Committee (IAEC) and was carried out in compliance with its guidelines. A total of 30 adult Wistar albino rats, each weighing 200 ± 20 g, were procured from the Pinnacle Biomedical Research Institute (PBRI). All animals were housed in accordance with approved institutional standards.

2.7.1.1 Experimental model

Throughout the acclimatization period, the rats were provided with a Golden Feed diet (Bhopal) and fresh water add libitum. They were kept under consistent environmental conditions—temperature at 22 ± 1 °C, humidity between 40–60%, and a controlled 14-hour light and 10-hour dark cycle—for one week prior to the experiment. Sixteen hours before the

study commenced, the animals had access to water only. All surgical procedures were carried out under sterile conditions.

2.7.1.2 Drugs

- **Paracetamol**

Paracetamol suspension was formulated using 1% carboxymethyl cellulose in saline phosphate buffer, and administered orally to the animals via gastric tube at a dose of 750 mg/kg. The suspension was prepared such that 1 mL contained 750 mg/kg of paracetamol.

- **N-Acetylcysteine (NAC)**

A 40 mg dose of NAC was dissolved in 0.9% saline solution adjusted to pH 7.0. The animals received this NAC solution orally via gastric tube at a dosage of 40 mg/kg.

2.7.1.3 Experimental protocol

The experimental protocol was designed for 14 days. Rats of either sex were divided into five groups, and each group was comprised of six rats.

Group I (Control group) served as untreated control

Group II received an oral administration of paracetamol (750 mg/kg bwt)

Group III received a daily oral administration of 40 mg/kg bwt N-acetylcysteine

Group IV received a daily oral administration of 250 mg/kg bwt *Ipomoea triloba* extracts

Group V received a daily oral administration of 500 mg/kg bwt *Ipomoea triloba* extracts

All the treatments were carried out for 14 days, starting at 4 days before the oral administration of N-acetylcysteine. Weekly measurement of body weight of all the animals was done during the experimental period.

2.7.2 Blood samples for biochemical estimation

Blood samples were collected from retro orbital venous plexus in non-heparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were collected and stored at 4 ºC prior immediate determination of serum biochemical constituents which was performed by using ready-made kits from Erba.

2.8 Analysis of genial parameters

2.8.1 Analysis of urine

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume is taken after 24 hours. The food wastes and fecal matters are removed from the urine. And the volume of urine is measured by using measuring cylinder **(Li, 2023).**

2.8.2 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 **(Casiraghi *et al.*, 2016).**

2.8.3 Serum Creatinine and blood urea nitrogen (BUN) analysis

Plasma samples were collected for the analysis of creatinine and blood urea nitrogen (BUN) levels. The concentrations of creatinine and BUN were measured in the plasma. For accuracy, samples were spiked with 10 µL of a creatinine standard stock solution in 0.2 N HCl, while control samples received 10 µL of 0.2 N HCl. Serum creatinine (SCR) and blood urea nitrogen (BUN) are used to evaluate renal function, though BUN alone is a limited indicator of glomerular filtration rate (GFR) in cases of mild to moderate kidney impairment. BUN levels were measured using a biochemical analyzer. Briefly, urea was enzymatically broken

down into ammonia and carbon dioxide by urease. In a subsequent indicator reaction, glutamate dehydrogenase catalyzed the formation of glutamate and NAD from ammonia and alpha-ketoglutarate. The resulting oxidation of NADH to NAD was monitored by a decrease in absorbance at 340 nm **(Kamal, 2014).**

3. RESULTS

3.1 Procurement of plant material

Table: 1 Percentage Yield of plant material

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Ipomoea triloba	Methanol	400	10.40	2.6
	Pet. Ether	386	19.45	5.03

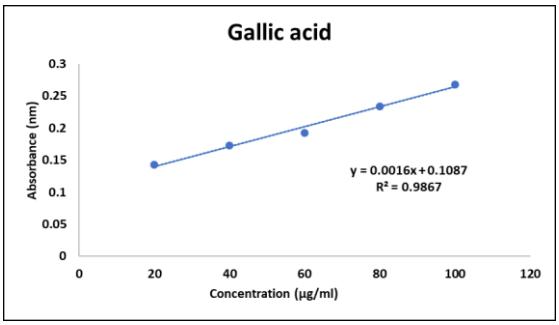
3.2 Phytochemical Test

Table 2: Phytochemical test of *Ipomoea triloba* extract of Petroleum ether and Methanolic extract

Experiment	Presence or absence of phytochemical test	
	Petroleum ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	- ve	+ ve
Mayer's reagent test	- ve	+ ve
Wagner's reagent test	- ve	+ ve
Hager's reagent test	- ve	+ ve
Glycoside		
Borntrager test	- ve	- ve
Legal's test	- ve	- ve
Killer-Killiani test	- ve	- ve
Carbohydrates		
Molish's test	+ ve	+ ve
Fehling's test	+ ve	+ ve
Benedict's test	+ ve	+ ve
Barfoed's test	+ ve	+ ve
Proteins and Amino Acids		
Biuret test	+ ve	- ve
Ninhydrin test	+ ve	- ve
Flavonoids		
Alkaline reagent test	+ ve	+ ve
Lead Acetate test	+ ve	+ ve
Tannin and Phenolic Compounds		
Ferric Chloride test	- ve	+ ve
Saponin		
Foam test	+ ve	+ ve
Test for Triterpenoids and Steroids		
Salkowski's test	- ve	- ve
Libbermann-Burchard's test	- ve	- ve

3.3 Quatitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation



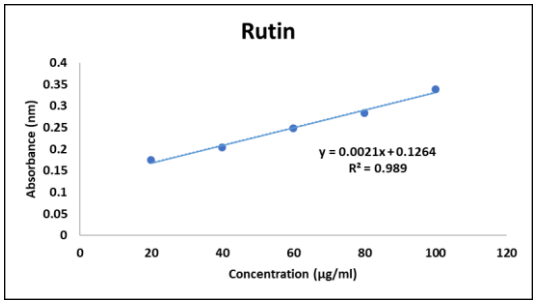
Graph 1: Represent standard curve of Gallic acid

3.3.1.1 Total Phenolic Content

Table 3: Total Phenolic Content in *Ipomoea triloba* extract

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.147	75.3 mg/gm
0.188	
0.218	

3.3.2 Total flavonoid content estimation



Graph 2: Represent standard curve of Rutin

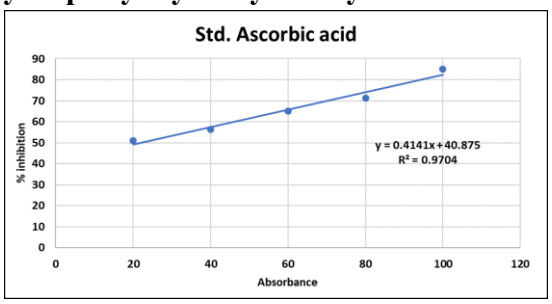
3.3.2.1 Total Flavonoid Content

Table 4: Total Flavonoid Content in *Ipomoea triloba* extract

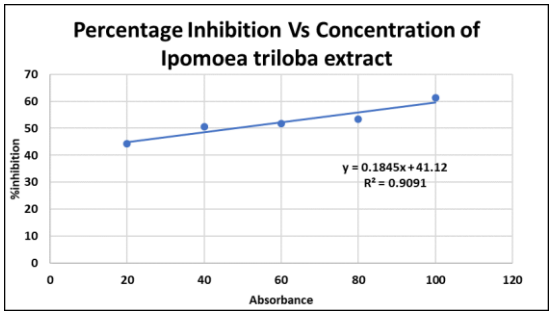
Absorbance	TFC in mg/gm equivalent of Rutin
0.168	48.3 mg/gm
0.201	
0.300	

3.4 Anti-Oxidant Activity

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



Graph 3: DPPH radical scavenging activity of Std. Ascorbic acid



Graph 4: Represents the Percentage Inhibition *Vs* Concentration of *Ipomoea triloba* extract

3.5 Paracetamol induced Nephrotoxicity Model

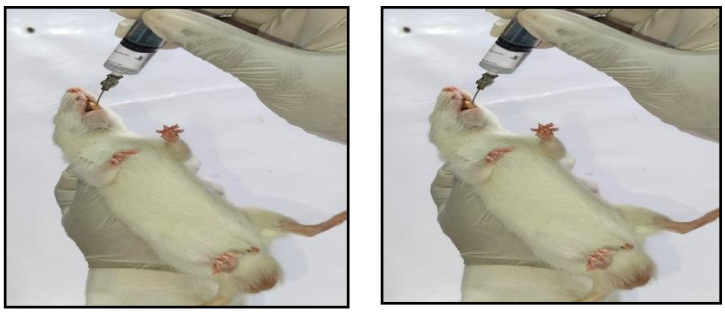


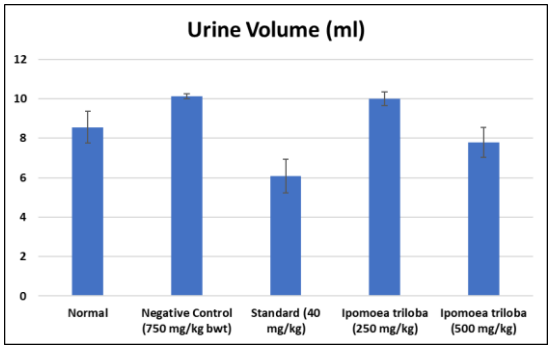
Figure 1: Paracetamol induced Nephrotoxicity Model

3.6 Analysis of general parameters

3.6.1 Estimation of urine volume

Table 5: Urine volume

Groups	Urine Volume (ml)
Normal	8.56±0.82
Negative Control (750 mg/kg bwt)	10.13±0.12
Standard (40 mg/kg)	6.08±0.85
Ipomoea triloba (250 mg/kg)	9.99±0.35
Ipomoea triloba (500 mg/kg)	7.80±1.31

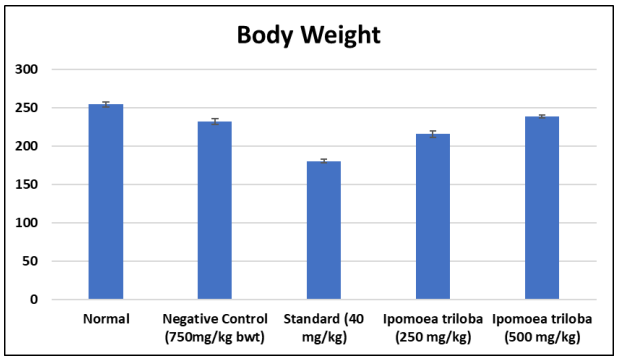


Graph 5: Urine volume

3.6.2 Estimation of Body weight

Table 6: Body weight

Groups	Body Weight
Normal	254.03±3.28
Negative Control (750mg/kg bwt)	232.15±3.85
Standard (40 mg/kg)	180.15±2.56
Ipomoea triloba (250 mg/kg)	215.30±4.23
Ipomoea triloba (500 mg/kg)	238.2±2.04

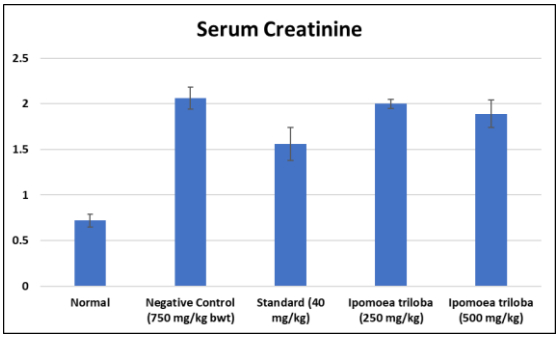


Graph 6: Body weight

3.6.3 Estimation of Serum Creatinine

Table 7: Serum Creatinine

Groups	Serum Creatinine
Normal	0.72±0.07
Negative Control (750 mg/kg bwt)	2.06±0.12
Standard (40 mg/kg)	1.56±0.18
Ipomoea triloba (250 mg/kg)	2.00±0.05
Ipomoea triloba (500 mg/kg)	1.89±0.15

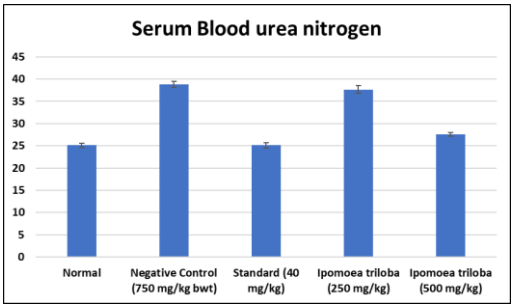


Graph 7: Serum Creatinine

3.6.4 Estimation of Serum Blood urea nitrogen (BUN)

Table 8: Serum Blood urea nitrogen

Groups	Serum Blood Urea Nitrogen
Normal	25.12±0.48
Negative Control (750 mg/kg bwt)	38.81±0.72
Standard (40 mg/kg)	25.11±0.62
Ipomoea triloba (250 mg/kg)	37.65±0.88
Ipomoea triloba (500 mg/kg)	27.56±0.45



Graph 8: Serum Blood urea nitrogen

3.7 Histopathological studies

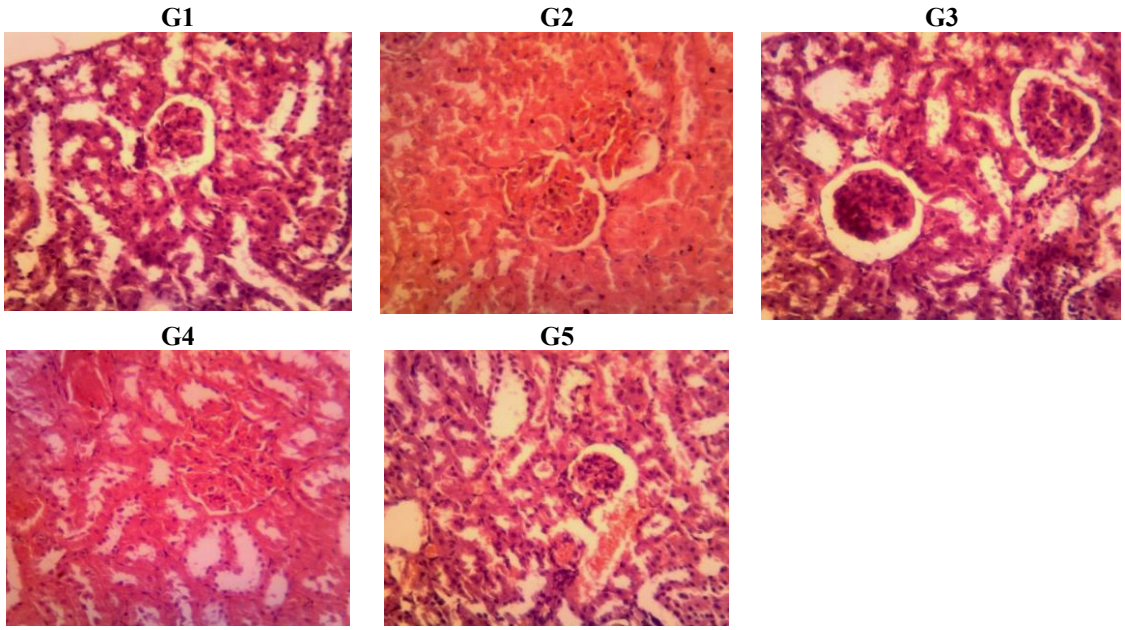


Figure 2: Histopathological studies

3.8 Acute Toxicity Study

Table 9: Acute toxicity study

Toxicological parameter	1day	2day	3day	4day	5day	6day	7day
Skin	Present	Present	Absent	Absent	Present	Absent	Present
Eye	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mucous membrane	Absent	Absent	Absent	Absent	Absent	Absent	Present
Salivation	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Stool	Absent	Absent	Absent	Absent	Present	Absent	Absent
Urine	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Sleep	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Behaviour	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Toxicological parameter	**8day**	**9day**	**10day**	**11day**	**12day**	**13day**	14day
Skin	Present	Present	Present	Present	Absent	Present	Present
Eye	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mucous membrane	Absent	Absent	Absent	Present	Absent	Absent	Present
Salivation	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Stool	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Urine	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Sleep	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Behaviour	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Mortality	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Discussion

The present study evaluated the pharmacological, antioxidant, and nephroprotective effects of *Ipomoea triloba* leaf extracts. Petroleum ether showed a higher extraction yield (5.03%) than methanol (2.6%), indicating more non-polar constituents. Phytochemical screening confirmed that the methanolic extract contained diverse bioactive compounds such as alkaloids, flavonoids, saponins, carbohydrates, and phenolics. Quantitative analysis demonstrated appreciable phenolic (75.3 mg/g GAE) and flavonoid (48.3 mg/g RE) content, supporting its

antioxidant activity. The methanol extract exhibited moderate DPPH radical scavenging ability (IC_{50} 48.26 µg/mL), though weaker than ascorbic acid.

In the paracetamol-induced nephrotoxicity model, the extract showed dose-dependent nephroprotective effects, with notable improvements in serum creatinine, blood urea nitrogen, urine output, and body weight—especially at 500 mg/kg. Histopathology confirmed preservation of renal architecture with minimal damage. Acute toxicity testing over 14 days showed no mortality and no major toxic effects, indicating a favorable safety profile.

Overall, *Ipomoea triloba* demonstrated significant nephroprotective and antioxidant potential, likely due to its phenolic and flavonoid constituents, supporting its therapeutic relevance in renal disorders.

4. CONCLUSION

The present study demonstrates that *Ipomoea triloba* leaves possess notable nephroprotective and antioxidant potential. The methanolic extract, rich in phenolics and flavonoids, showed moderate free radical scavenging activity and effectively protected against paracetamol-induced renal damage in a dose-dependent manner, with the 400 mg/kg dose producing effects comparable to the standard drug. No signs of acute toxicity or behavioural changes were observed, confirming its safety. Histopathology further supported its protective action by showing preserved renal structure and minimal tissue injury. Overall, *Ipomoea triloba* appears to be a promising and safe plant-based candidate for managing nephrotoxicity and oxidative stress-related kidney disorders. Further studies are needed to isolate active compounds and validate its efficacy through long-term toxicity assessments and clinical trials.

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