



PHARMACOLOGICAL EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF *DELONIX REGIA* SEEDS EXTRACT IN WISTAR RATS

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ABSTRACT

The present study aimed to evaluate the phytochemical composition, antioxidant activity, and antiuro lithiatic potential of the methanolic seed extract of *Delonix regia* in ethylene glycol- and ammonium chloride-induced urolithiasis in Wistar rats. The seeds of *Delonix regia* were successively extracted using petroleum ether and methanol. Preliminary phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging assay were performed. Acute oral toxicity was evaluated according to standard guidelines. Antiuro lithiatic activity was assessed in Wistar rats using ethylene glycol and ammonium chloride-induced urolithiasis. Animals were treated with methanolic seed extract at doses of **100 mg/kg** and **200 mg/kg**, while **Cystone** served as the standard drug. Serum biochemical parameters including creatinine, calcium, urea, and phosphorus were estimated, followed by histopathological examination of kidney tissues. The methanolic extract showed a higher percentage yield (**2.20%**) than the petroleum ether extract (**0.53%**). Phytochemical screening confirmed the presence of alkaloids, flavonoids, carbohydrates, tannins, phenolic compounds, and saponins in the methanolic extract. The extract exhibited a total phenolic content of **48 mg GAE/g**, a total flavonoid content of **18.16 mg RE/g**, and significant antioxidant activity with a DPPH **IC₅₀ value of 53.53 µg/mL**, compared with **21.59 µg/mL** for ascorbic acid. In the antiuro lithiatic study, the methanolic extract significantly reduced elevated serum creatinine, urea, calcium, and phosphorus levels in a dose-dependent manner. The **200 mg/kg** dose produced greater protection than the **100 mg/kg** dose and showed results comparable to the standard treatment. Histopathological studies further demonstrated reduced calcium oxalate crystal deposition and improved renal architecture in treated groups. The methanolic seed extract of *Delonix regia* exhibited significant antioxidant and antiuro lithiatic activities, which may be attributed to its phenolic and flavonoid constituents. The findings suggest that *Delonix regia* seed extract possesses nephroprotective potential and may serve as a promising natural therapeutic agent for the prevention and management of urolithiasis.

KEYWORDS: *Delonix regia*, urolithiasis, calcium oxalate, antioxidant activity, DPPH assay, phytochemicals, nephroprotection, Wistar rats, ethylene glycol, methanolic seed extract.

1. INTRODUCTION

Urolithiasis, commonly referred to as kidney stone disease, is one of the most prevalent disorders of the urinary tract and represents a significant global health concern due to its high incidence, recurrence, and economic burden. It is characterized by the formation of calculi within the kidneys, ureters, bladder, or urethra as a result of supersaturation, nucleation, crystal growth, aggregation, and retention of insoluble urinary

constituents (Samantha *et al.*, 2021). Calcium-containing stones, particularly calcium oxalate (CaOx), account for nearly 70–80% of all urinary calculi, followed by calcium phosphate, uric acid, struvite, and cystine stones. The prevalence of urolithiasis has increased steadily over recent decades owing to changes in dietary habits, sedentary lifestyle, obesity, metabolic disorders, dehydration, and genetic susceptibility. Furthermore, the disease is associated with a high

recurrence rate, with approximately 50% of patients experiencing recurrence within 5–10 years after the first episode (Wagner, 2021).

The pathogenesis of urolithiasis is a multifactorial process involving urinary supersaturation with stone-forming constituents, oxidative stress, renal tubular epithelial injury, inflammation, and impaired urinary inhibitors of crystallization. Hyperoxaluria is considered one of the major risk factors for calcium oxalate stone formation (Alelign and Petros, 2018). Excess oxalate promotes crystal nucleation and aggregation, resulting in crystal retention within the renal tubules and subsequent renal tissue damage. Reactive oxygen species generated during hyperoxaluria further aggravate renal injury by inducing lipid peroxidation, inflammation, and apoptosis of renal epithelial cells, thereby facilitating crystal adherence and stone formation (Wu *et al.*, 2024).

Medicinal plants have long been used in traditional medicine for the treatment of urinary disorders because of their diuretic, antioxidant, anti-inflammatory, and nephroprotective properties. Phytochemicals such as flavonoids, phenolic compounds, tannins, alkaloids, and saponins have been reported to inhibit calcium oxalate crystal nucleation, growth, and aggregation while protecting renal tissues from oxidative damage. These properties make plant-derived extracts promising candidates for the prevention and management of urolithiasis (Ferdosh, 2023).

Delonix regia (Family: *Fabaceae*), commonly known as Gulmohar or Flame Tree, is a medicinally important ornamental tree widely distributed in tropical and subtropical regions. Various parts of the plant, including leaves, flowers, bark, roots, and seeds, have been used traditionally for the treatment of inflammation, microbial infections, diabetes, rheumatism, constipation, and urinary disorders (Modi *et al.*, 2016). Phytochemical investigations have revealed that the seeds are rich in flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides, carbohydrates, and other bioactive constituents possessing potent antioxidant and free radical scavenging activities. Previous studies have also demonstrated anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and nephroprotective properties of *Delonix regia*, suggesting its potential therapeutic value in oxidative stress-mediated disorders (Jain *et al.*, 2025).

Considering the role of oxidative stress in calcium oxalate nephrolithiasis, the antioxidant and nephroprotective properties of *Delonix regia* seeds may contribute to the inhibition of crystal formation and protection against renal injury. However, scientific evidence regarding the antiurolithiatic efficacy of the seed extract remains limited. Therefore, further pharmacological evaluation is required to validate its traditional use and establish its therapeutic potential (Nirumand *et al.*, 2018).

The ethylene glycol and ammonium chloride-induced urolithiasis model in Wistar rats is a well-established and reproducible experimental model that closely mimics human calcium oxalate nephrolithiasis (Pawar and Vyawahare, 2015). Administration of ethylene glycol induces hyperoxaluria, while ammonium chloride acidifies the urine and accelerates calcium oxalate crystal deposition within the renal tubules. This model enables the evaluation of urinary biochemical parameters, renal function markers, oxidative stress, crystal deposition, and histopathological alterations associated with kidney stone formation (Shamlan and Alansari, 2024).

Therefore, the present study was designed to investigate the pharmacological evaluation of the methanolic seed extract of *Delonix regia* against ethylene glycol and ammonium chloride-induced urolithiasis in Wistar rats. The antiurolithiatic potential of the extract was assessed by evaluating urinary and serum biochemical parameters, kidney function markers, antioxidant activity, and histopathological changes. The findings of this study are expected to provide scientific evidence supporting the traditional use of *Delonix regia* seeds and their potential as a safe, effective, and economical natural therapeutic agent for the prevention and management of urolithiasis.

2. MATERIAL AND METHOD

2.1 Chemical

Conc. H₂SO₄, Glacial Acetic Acid, and Ammonia was acquired from Merck. Himedia gave the Sodium Hydroxide and 1% Copper Sulphate Solution. Ethanol was supplied by Clorofiltind. Fizmerck supplied Nitroprusside. Rankem supplied the Magnesium, 95% Alcohol, and Conc. HCl. Research lab gave the Petroleum ether.

2.2 Plant collection

The medicinal plant *Delonix regia* (300g) was harvested from localized area near by Pinnacle Biomedical Research Institue. After cleaning, the plant parts (seeds) were dried in the shade at room temperature for three days before being oven dried at 45°C until completely dry. To prevent contamination and deterioration, dried plant parts (seeds) were stored in airtight glass containers in a dry and cool environment. Identification of chosen traditional plants - A plant taxonomist verified the medicinal herb *Delonix regia* to ensure its identification and quality.

2.3 Extraction

In this work, material from plants was extracted utilizing the continuous hot percolation method and the Soxhlet equipment. *Delonix regia* powder was inserted in a thimble of the soxhlet device. Soxhlation was carried out at 60°C with petroleum ether as a nonpolar 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 using a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and the percentage yield for each extract was calculated using the following formula.

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

The prepared extracts were examined for organoleptic characteristics (percentage yield, color, and odor) and then packaged in an airtight container and labeled for future use. (Baidya *et al.*, 2002).

2.4 Quantitative Phytochemical Estimation

2.4.1 TPC

The total phenolic content of the *Delonix regia* extract was measured using the Folin-Ciocalteu Assay. The *Delonix regia* extracts (0.2 mL from the stock solution) were combined with 2.5mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted with distilled water until it reached 7mL. The resultant solutions were then allowed to rest at room temperature for two hours before being measured spectrophotometrically at 760 nm. Calibration curves were created using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic acid was concentrated to 20, 40, 60, 80, and 100µg/mL. The Folin-Ciocalteu reagent is sensitive to reducing substances like polyphenols. They react to generate a blue color. The blue color was assessed spectrophotometrically (Tangco *et al.*, 2015).

2.4.2 TFC

The flavonoid content was measured using the aluminium chloride technique. 0.5 mL of *Delonix regia* extract solution was combined with 2 mL of distilled water. Then 0.15 mL of sodium nitrite (5%) was added and thoroughly mixed. After that, wait 6 minutes before adding 0.15ml of 10% aluminum chloride and allowing standing for 6 minutes. Then 2 mL of 4% sodium hydroxide was added. The substance had been vigorously shook and blended. The mixture's absorbance at 510nm was determined using a UV spectrophotometer. Calibration curves were created using standard Rutin Equivalent (RE) mg/gm solutions. Rutin was produced at concentrations of 20, 40, 60, 80, and 100µg/mL. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight (Parthasarathy S *et al.*, 2009).

2.5 DPPH

The antimicrobial capacity of *Delonix regia* extract was evaluated using the DPPH free radical scavenging test. A 1mg/ml methanol extract/standard solution was produced. *Delonix regia* extracts/standard were produced at various concentrations (20-100µg/ml) using a 1mg/mL stock solution and a 0.1mM DPPH solution (2mL). The resultant mixture was vortexed, incubated for 30 minutes

at room temperature in a relatively dark environment, and then measured at 517 nm using a UV spectrophotometer (Shimadzu 1700). For the control, add 3 ml of 0.1mM DPPH solution and incubate at room temperature in the dark for 30 minutes. The absorbance of the control was recorded against methanol (as blank) at 517 nm (Athavale *et al.*, 2012).

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

$$\% \text{ Inhibition} = [(Ab \text{ of control} - Ab \text{ of sample}) / Ab \text{ of control} \times 100]$$

2.6 Acute Toxicity Study

The acute toxic class approach described in the guideline is a step-by-step procedure that uses three animals of the same sex per phase. Depending on the animal's mortality and/or moribund stage, 2-4 steps may be required to determine the acute toxicity of the test drug. A set of experimental animals are given the drug orally at one of the prescribed doses. The chemical is evaluated in stages, with three animals of the same sex used in each. The absence or presence of compound-related mortality in animals dosed at one stage determines the next phase, i.e., no further testing is required, dosing of three further animals with the same dose, and dosing of three other animals at the next higher or lower dose level. Each phase requires three animals. The beginning dose is set from one of four fixed levels: 5, 50, 300, and 2000 mg/kg body weight (Daniyan *et al.*, 2024).

2.7 Experimental work

Animals Protocol

IAEC Approval All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).

Animal used

Weight 200±250 gm

Strain Rat

Sex Male

□ Housing Condition

Male rats aged 2-3 months and weighing 200-250 g were used in the investigation. Animals were taken from animal house of Pinnacle Biomedical Research Institute. Prior to treatment, the animals were kept in regular animal house settings, with ad libitum access to food and water and a 12-hour light-dark cycle.

□ Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model was used to assess the antiurolithic activity in rat (Nishanthi *et al.*, 2016; Ashmi *et al.*, 2017; Azadbakht *et al.*, 2020).

Animals divided into five groups containing six animals in each group.

Group I- serves as control and received regular rat food and drinking water ad libitum

Group II - Lithiatic control: The animals were given normal diet and 1% Ethylene glycol in drinking water, for 28 days.

Group III - Received 1% ethylene glycol in drinking water and then treated with *Delonix regia* extract at a dose of 100mg/kg orally, for 28 days.

Group IV - Received 1% Ethylene glycol in drinking water and then treated with *Delonix regia* extract at a dose of 200mg/kg orally, for 28 days.

Group V-Received standard anti-urolithiatic drug, cystone 600mg/kg

2.8 Assessment of Antiurolithiatic activity

Urine sample collection and analysis all animals were kept in individual metabolic cages, and urine samples from the previous 24 hours were obtained on the 28th day. During the urine collection period, animals were able to drink water. Urine is treated with a drop of concentrated HCl acid and stored at 4°C. Calcium, phosphate, and oxalate concentrations in urine were determined.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 1: Percentage Yield of crude extracts of *Delonix regia* extract.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
<i>Delonix regia</i>	Pet ether	297	1.59	0.53%
	Methanol	299.23	6.61	2.20%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract.

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

2.9 Serum analysis

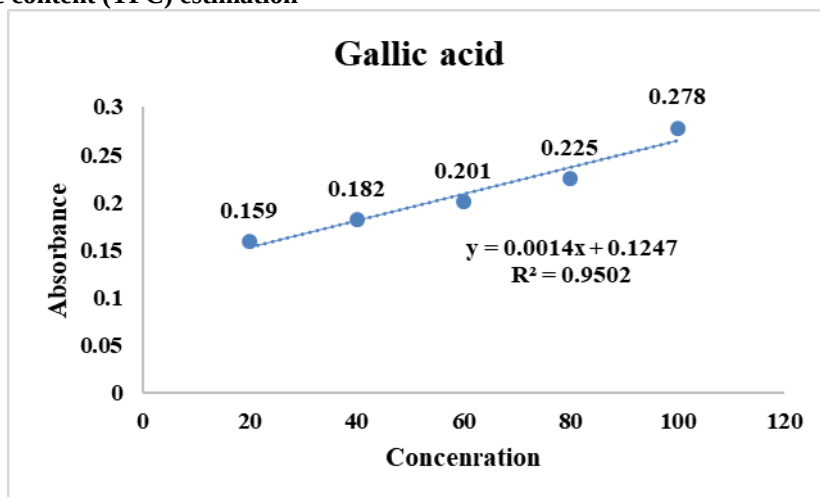
Following the experiment, blood was obtained from the retro-orbital under anaesthesia, and animals were sacrificed via cervical decapitation. Serum was centrifuged at 10,000×g for 10 minutes and analyzed for creatinine, urea, and phosphorus levels.

2.10 Kidney homogenate analysis

Each animal's abdomen was sliced open in order to remove both kidneys. Extraneous tissue was removed from isolated kidneys before preserving them in 10% neutral formalin. The kidneys were dried using a hot air oven at 80°C. A sample of 100mg of dried kidney was cooked in 10ml of 1N hydrochloric acid for 30 minutes and homogenised.

3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) estimation



Graph 1: Represent standard curve of Gallic acid.

3.3.1.1 Total Phenolic Content in extract.

Table 3: Total Phenolic Content.

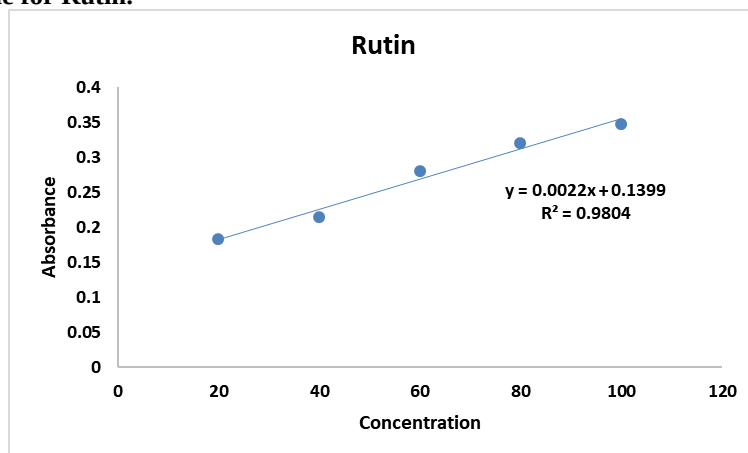
Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.132	48 mg/gm
0.185	
0.199	

Table 4-Total Phenolic Content of extract *Delonix regia*.

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	48

3.3.2 Total Flavonoids content (TFC) estimation

Table 5- Standard table for Rutin.



Graph 2: Represent standard curve of Rutin.

3.3.2.1 Total Flavonoid Content in extract

Table 6: Total Flavonoid Content.

Absorbance	TFC in mg/gm equivalent of Rutin
0.158	18.16mg/gm
0.169	
0.199	

Table 7: Total Flavonoid Content of extract *Delonix regia*.

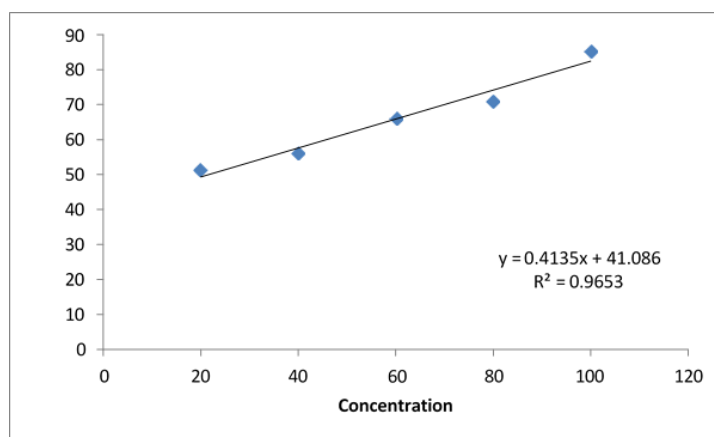
Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Methanol	18.16

3.4 *In vitro* Antioxidant Assays.

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

Table 8: DPPH radical scavenging activity of Std. Ascorbic acid.

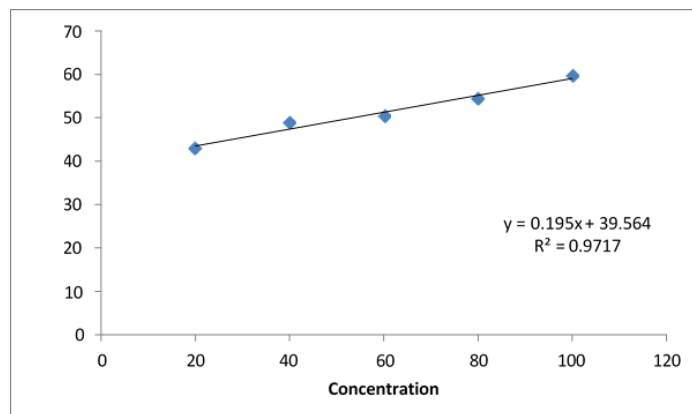
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	51.307
40	0.437	56.036
60	0.338	65.995
80	0.289	70.925
100	0.147	85.211
Control	0.994	
IC50		



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

Table 9: DPPH radical scavenging activity of methanol extract of *Delonix regia*.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.524	42.919
40	0.469	48.910
60	0.455	50.435
80	0.419	54.357
100	0.370	59.694
Control	0.918	
IC50		53.53

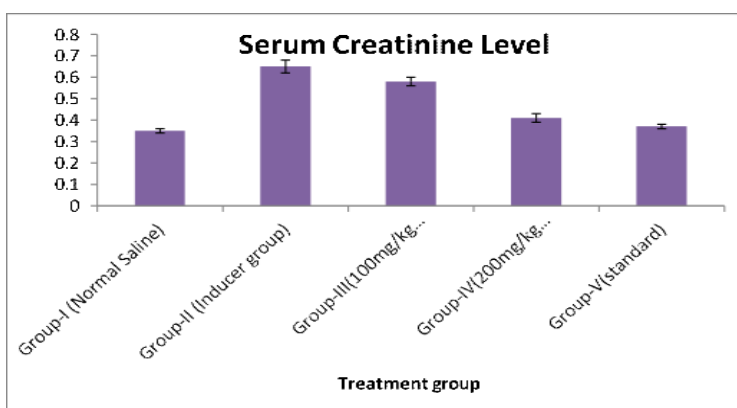
Graph 4: Represents the Percentage Inhibition Vs Concentration of extract of *Delonix regia*.

3.5 Ethylene glycol induced antiurolithiasis activity.

3.5.1 Creatinine test

Table 10: Creatinine test.

Study Groups	Serum Creatinine Level
Group-I (Normal Saline)	0.35±0.01
Group-II (Inducer group)	0.65±0.03
Group-III(100mg/kg <i>Delonix regia</i>)	0.58±0.02
Group-IV(200mg/kg <i>Delonix regia</i>)	0.41±0.02
Group-V(standard)	0.37±0.01

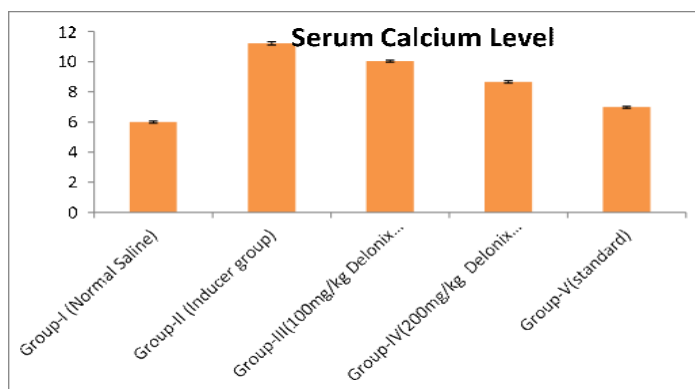


Graph 5: Bar graph represents the serum creatinine level.

3.5.2 Calcium test

Table 11: Calcium test.

Study Groups	Serum Calcium Level
Group-I (Normal Saline)	5.99±0.07
Group-II (Inducer group)	11.21±0.11
Group-III(100mg/kg <i>Delonix regia</i>)	10.03±0.07
Group-IV(200mg/kg <i>Delonix regia</i>)	8.66±0.08
Group-V(standard)	6.97±0.07

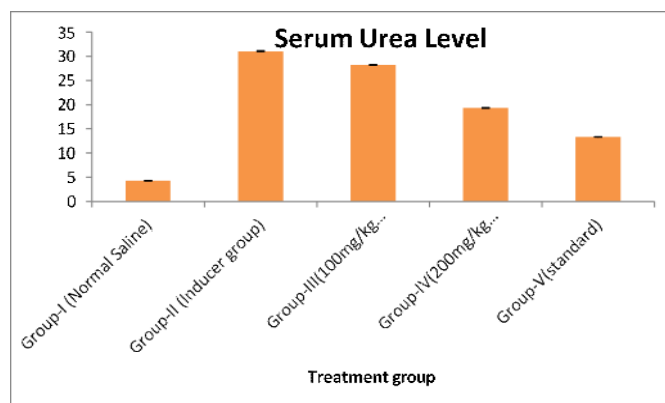


Graph 6: Bar graph represents the serum calcium level.

3.5.3 Urea test

Table 12: Urea test.

Study Groups	Serum Urea Level
Group-I (Normal Saline)	4.29±0.05
Group-II (Inducer group)	31.05±0.10
Group-III(100mg/kg <i>Delonix regia</i>)	28.21±0.07
Group-IV(200mg/kg <i>Delonix regia</i>)	19.32±0.08
Group-V(standard)	13.34 ±0.03

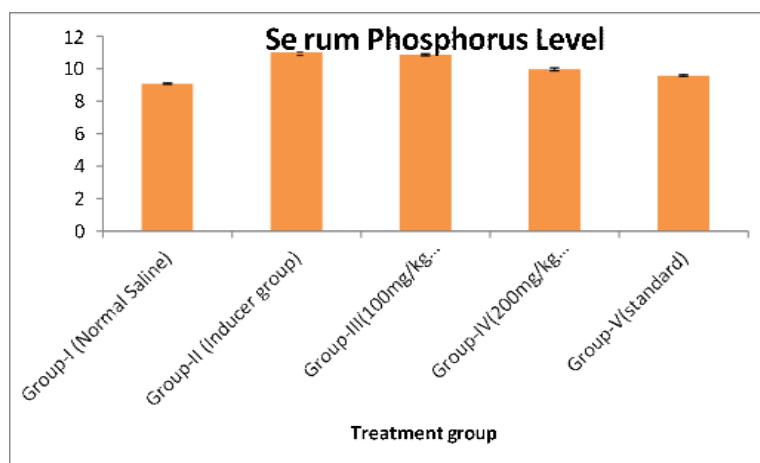


Graph 7: Bar graph represents the serum urea level.

3.5.4 Phosphorus test.

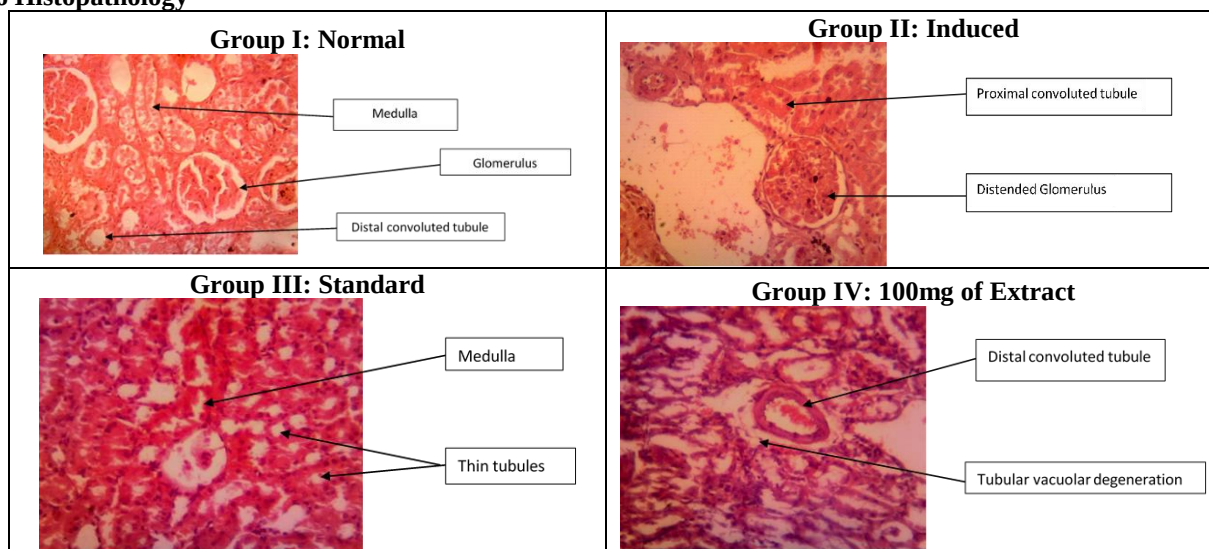
Table 13: Phosphorus test.

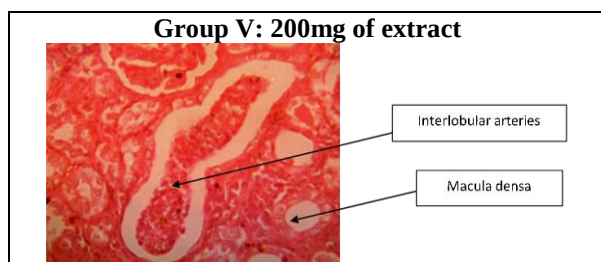
Study Groups	Serum Phosphorus Level
Group-I (Normal Saline)	9.08±0.04
Group-II (Inducer group)	11.01±0.11
Group-III(100mg/kg <i>Delonix regia</i>)	10.85±0.06
Group-IV(200mg/kg <i>Delonix regia</i>)	9.96±0.09
Group-V(standard)	9.58±0.05



Graph 8: Bar graph represents the serum phosphorous level.

3.6 Histopathology





DISCUSSION

Phytochemical analysis of methanolic extract of *Delonix regia* showed the presence of alkaloids, phenolics, flavonoid, saponins, carbohydrates, saponin, tannin and phenolic. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. Results shown in Table 7 & table10. DPPH radical scavenging activity of *Delonix regia* extract exhibited percent inhibition 59.69% and its IC₅₀ value was found to be 53.53µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 85.21% and showed IC₅₀ value of 21.59µg/ml.

In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study.

Administration of EG and AC in drinking water in disease control groups, increased serum levels of uric acid, creatinine, calcium test and phosphorus when compared to the normal group (I). These levels were significantly decreased in treatment groups on treatment with cystone and different doses. In this study, enhanced deposition of calcium and oxalate in the kidney and their urinary excretion was observed in the disease control group animals indicating that administration of EG and AC induced CaOx urolithiasis. On administration of *Delonix regia* the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinary excretion in both the preventive and curative group animals reveals the potential of *Delonix regia* in both preventing the formation and dissolving the preformed CaOx stones.

4. CONCLUSION

The present study demonstrated that the methanolic seed extract of *Delonix regia* possesses significant antiurolithiatic activity against ethylene glycol and ammonium chloride-induced urolithiasis in Wistar rats. The extract effectively reduced calcium oxalate crystal deposition, improved renal biochemical parameters, and exhibited nephroprotective effects. These therapeutic benefits are likely attributed to the presence of bioactive phytoconstituents such as flavonoids, polyphenols, and antioxidants. Overall, the findings suggest that *Delonix regia* seed extract has promising potential as a safe,

natural, and cost-effective agent for the prevention and treatment of urolithiasis.

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