

PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF ANTIUROLITHIC ACTIVITY OF *WEDELIA CHINENSIS* EXTRACT IN EXPERIMENTAL RATS

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ABSTRACT

The present study, titled “*Phytochemical Investigation and Pharmacological Evaluation of Antiurolithic Activity of Wedelia chinensis Extract in Experimental Rats*,” aimed to assess the therapeutic efficacy of *Wedelia chinensis* against urolithiasis, a disorder characterized by the formation of urinary calculi. Methanolic extraction of the plant material yielded 6.03%, signifying good efficiency for polar phytoconstituents. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, carbohydrates, proteins, tannins, phenolic compounds, and saponins, while steroids and triterpenoids were absent. The extract exhibited high total phenolic (66.3 mg/g gallic acid equivalent) and flavonoid (26.65 mg/g rutin equivalent) contents, reflecting strong antioxidant potential, which was further supported by DPPH radical scavenging activity ($IC_{50} = 48.46 \mu\text{g/ml}$) compared to ascorbic acid ($IC_{50} = 22.64 \mu\text{g/ml}$). In vivo studies on ethylene glycol-induced urolithiasis in rats demonstrated that *W. chinensis* extract at 200 mg/kg and 400 mg/kg significantly reduced serum creatinine, urea, calcium, phosphorus, and BUN levels in a dose-dependent manner. The higher dose (400 mg/kg) exhibited effects comparable to the standard formulation, Cystone (750 mg/kg). Histopathological evaluation corroborated these findings, showing marked renal protection with reduced crystal deposition and tubular damage. The study concludes that the methanolic extract of *W. chinensis* exhibits potent antiurolithiatic activity, likely mediated by its antioxidant and nephroprotective mechanisms. These findings substantiate its traditional use in urinary tract disorders and suggest its potential as a natural therapeutic agent for kidney stone management. Further studies focusing on bioactive compound isolation, toxicity profiling, and clinical validation are recommended.

Keywords: *Wedelia chinensis*, antiurolithiatic activity, phytochemical analysis, antioxidant potential, nephroprotection, urolithiasis, renal histopathology, Cystone.

1. INTRODUCTION

Urolithiasis, commonly known as kidney stone disease, is a major global health problem characterized by the formation of crystalline deposits, primarily calcium oxalate, within the urinary tract. It affects a significant portion of the population, with high recurrence rates even after medical or surgical intervention (Allam, 2024). The condition arises due to supersaturation of urine with lithogenic substances such as calcium, oxalate, phosphate, and uric acid, which leads to nucleation, crystal growth, aggregation, and retention within the

renal tubules. These processes are often associated with oxidative stress, reduced antioxidant defense mechanisms, and renal tubular injury, all of which contribute to stone formation and progression (Ying *et al.*, 2025).

Despite advances in modern medicine, the currently available therapeutic approaches for urolithiasis—such as lithotripsy, surgical removal, and administration of synthetic drugs—are often limited by high costs, side effects, and the possibility of stone recurrence (Raheem *et al.*, 2017). Moreover, many of the synthetic drugs used to manage kidney stones may cause adverse effects such as gastrointestinal disturbances, hypotension, and electrolyte imbalance. Hence, there is a growing interest in exploring herbal remedies as safer, cost-effective, and long-term alternatives for the prevention and treatment of urolithiasis (York *et al.*, 2015).

Medicinal plants have been an integral part of traditional healthcare systems like Ayurveda, Siddha, and Unani for centuries. They contain a variety of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, and saponins that exhibit diuretic, antioxidant, anti-inflammatory, and nephroprotective properties—all of which are beneficial in the management of urolithiasis (Negi and Mirza 2020). The antioxidant property, in particular, plays a crucial role by mitigating oxidative stress and lipid peroxidation in renal tissues, which are considered key contributors to stone formation. Therefore, phytochemical investigation and pharmacological evaluation of medicinal plants provide a scientific basis for their traditional use and therapeutic potential (Ying *et al.*, 2025).

Wedelia chinensis (Family: Asteraceae), commonly known as “Bhringraj” or “Marathi Jangli,” is a well-known medicinal herb distributed widely across tropical and subtropical regions. Traditionally, *W. chinensis* has been used in the treatment of various ailments such as jaundice, hepatic disorders, cough, wounds, and urinary tract infections (Qin *et al.*, 2022). Previous pharmacological studies have reported that the plant possesses hepatoprotective, antioxidant, anti-inflammatory, and antimicrobial properties, which may contribute to its potential nephroprotective and antiurolithiatic effects (Tienda-Vázquez *et al.*, 2022). The phytochemical constituents of *W. chinensis*—including flavonoids, alkaloids, phenolic compounds, and saponins—are known for their free radical scavenging and cytoprotective activities, suggesting possible therapeutic action against urolithiasis (Singh *et al.*, 2021).

Given this background, the present study was designed to investigate the phytochemical profile and evaluate the pharmacological efficacy of the methanolic extract of *Wedelia chinensis* against experimentally induced urolithiasis in rats.

2. MATERIAL AND METHODS

2.1 Chemicals

Sodium Hydroxide, Glacial Acetic Acid, Ammonia and Nitroprusside was acquired from Merck. Himedia gave the Magnesium. Chloroform, Conc. HCl, 95% Alcohol was supplied by Clorofiltind. Fizmerck supplied Conc. H₂SO₄. Rankem supplied the Methanol. Research lab gave the Ethyl acetate whereas Join Hub Pharma supplied Normal saline. Ethanol obtained from Molychem and Orion Chem supplied Ethylene glycol.

2.2 Procurement of plant material

For this study, *Wedelia chinensis* (254 grams) was collected from a local region in Bhopal. The plant was identified and verified by a qualified botanist. After thorough washing, the leaves were shade-dried at room temperature for three days, then further dried in a hot air

oven at 45°C until completely moisture-free. The dried leaves were then ground into a fine powder and stored in airtight containers for further use.

2.3 Extraction of Plant Material by Soxhlet Process

The Soxhlet extraction method is commonly employed to isolate bioactive compounds from natural sources. In this process, dried *Wedelia chinensis* plant material is placed inside a thimble, which is then inserted into the Soxhlet apparatus. A distillation flask containing methanol acts as the solvent. As the solvent heats and evaporates, it condenses and gradually fills the chamber holding the plant material. Once it reaches a certain level, the siphon mechanism automatically drains the solvent back into the distillation flask. During this process, the solvent carries the extracted compounds into the flask, while the solvent itself cycles back to the plant material. This continuous cycle of heating, condensation, and siphoning repeats multiple times until the extraction is complete (Panigrahi *et al.*, 2017). Last but not least, use the formula to ascertain the extracts' yield:

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

2.4 Quantitative Estimation of Phytoconstituents

The plant material was obtained for the purpose of quantitative estimate after initial phytochemical tests confirmed the existence of flavonoids, alkaloids, phenols, saponins, and tannins.

2.4.1 Determination of total phenols content by spectrophotometric method:

To evaluate the total phenolic content in the methanolic leaves extract of *Wedelia chinensis*, a colorimetric method using the Folin–Ciocalteu reagent was employed. In a test tube, 200 µL of Folin–Ciocalteu reagent, 3.16 ml of distilled water, and 40 µL of the seed extract (prepared at 1 mg/mL concentration in methanol) or standard gallic acid solution were mixed thoroughly. The mixture was allowed to stand for 8 minutes at room temperature. Subsequently, 600 µL of a sodium carbonate solution was added, and the contents were mixed gently. The reaction mixture was then incubated at 40 °C for 30 minutes. After incubation, the absorbance was recorded at 760 nm against a blank using a spectrophotometer. A calibration curve was generated using gallic acid standard solutions at concentrations of 15, 30, 45, 60, and 75 µg/mL (Islam *et al.*, 2021).

2.4.2 Using spectrophotometry to determine the total flavonoid content

The total flavonoid content of *Wedelia chinensis* was determined using the aluminum chloride colorimetric method. Briefly, 0.2 g of the seed extract was dissolved in 1 ml of deionized water. To 0.5 mL of this extract solution, 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride hexahydrate (AlCl₃), 0.1 ml of 1 M potassium acetate (CH₃COOK), and 2.8 ml of deionized water were added. The mixture was thoroughly mixed and incubated at room temperature for 40 minutes. Following incubation, the absorbance was measured at 510 nm using a spectrophotometer, with deionized water serving as the blank. Rutin was used as the reference standard to generate a calibration curve, prepared at concentrations of 15, 30, 45, 60, and 75 µg/ml. The total flavonoid content was calculated from the calibration curve and expressed as milligrams of rutin equivalents (mg RE) per gram of dry extract weight (Velavan, 2015).

2.5 Determination of antioxidant activity by the DPPH test

The DPPH[•] test is based on ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. The DPPH[•] radical displays an intense UV-VIS absorption spectrum. This test uses the following technique to decolorize a radical solution following reduction with either an antioxidant (AH) or a negative energy (R[•]): $\text{DPPH}^{\bullet} + \text{R}^{\bullet} \rightarrow \text{DPPH}^{\bullet-}\text{R}$, $\text{DPPH}^{\bullet} + \text{AH} \rightarrow \text{DPPH}^{\bullet-}\text{H} + \text{A}^{\bullet}$. This test for spectrophotometry makes use of the stable radical 2, 2'-diphenylpicrylhydrazyl (DPPH) as a reagent. 5 ml of a 0.004% methanol solution of DPPH were mixed with fifty microliters of extracts from *Wedelia chinensis* leaves in methanol at different doses (20–100 µg/ml). Following a half-hour incubation time at room temperature, the absorbance at 517 nm was measured against a blank. The following formula was used to determine the percentage (I %) of inhibition of free radical DPPH (Lin *et al.*, 2007).

% Scavenging Activity = 100[(Ac – As)/Ac] Where the absorbances of the sample and negative control are denoted by as and Ac, respectively.

2.6 Acute Oral Toxicity Study

The acute oral toxicity study was conducted in accordance with OECD guideline 423, using a stepwise procedure involving three animals per dose level. Ethical approval for the study was granted by the Institutional Animal Ethics Committee. Experimental animals were randomly assigned into groups, each consisting of three rats (n=3), with unrestricted access to standard feed and water. The treatment groups received a single oral dose of *Wedelia chinensis* methanolic seed extract at concentrations of 5, 50, 300, and 2000 mg/kg body weight. Meanwhile, the control group was administered an equivalent volume of the vehicle, distilled water (Tepe *et al.*, 2005).

2.7 In vivo study of Anti-Urolithic activity in rats

2.7.1 Animals

Experimental animals were randomly selected from the animal facility at Pinnacle Biomedical Research Institute (PBRI), Bhopal, India. They were then randomly assigned to different treatment groups and housed in polypropylene cages containing sterile husk bedding. The animal room was maintained at a controlled temperature of $22 \pm 2^{\circ}\text{C}$, with a relative humidity of 30.7% and a 12-hour light/dark cycle. Animals were provided with standard pellet feed (Golden Feeds, New Delhi, India) and had free access to drinking water throughout the study. Prior to experimentation, the rats were acclimatized to laboratory conditions for a period of seven days. Each experimental study was carried out using a separate group of six rats (n = 6). The Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal, approved all animal procedures.

Animals used

- Strain - Rats
- Sex - Male
- Body weight - $200 \pm 80\text{g}$

2.7.2 Ethylene glycol-induced hyperoxaluria model

An ethylene glycol-induced hyperoxaluria paradigm was employed to assess the anti-urolithic effect in albino rats. Five groups of six animals each were formed from the animals (Green *et al.*, 2005).

Experimental procedure

Group	Group Description	Treatment Details
Group I	Normal Control	Standard rat diet and water only
Group II	Inducer	0.75% ethylene glycol in drinking water for 28 days
Group III	Test Group – Low Dose	0.75% ethylene glycol + 200 mg/kg <i>Wedelia chinensis</i> extract orally for 28 days
Group IV	Test Group – High Dose	0.75% ethylene glycol + 400 mg/kg <i>Wedelia chinensis</i> extract orally for 28 days
Group V	Standard Drug Control	0.75% ethylene glycol + 750 mg/kg Cyst one orally for 28 days

2.7.3 Analysis for Anti-Urolithic activity

• Serum analysis

One hour after administering the final treatment dose, blood samples were collected from the retro-orbital sinus of the animals. The blood was then centrifuged at $10,000 \times g$ for 10 minutes to separate the serum. Biochemical parameters, including creatinine, serum phosphorus, and blood urea nitrogen (BUN), were analyzed using standard diagnostic kits, with BUN levels assessed spectrophotometrically (P'ng *et al.*, 2012).

• Urine analysis

After blood collection, each animal was placed in an individual metabolic cage for urine collection at five-hour intervals. Urine samples were collected and analyzed for volume, creatinine concentration, and total protein content. Both creatinine and total protein levels were measured using standard diagnostic kits (Pawar and Vyawahare 2015).

• Kidney homogenate analysis

The abdomen of each animal was carefully opened to extract both kidneys. After removing any excess tissue, the kidneys were preserved in a 10% neutral formalin solution. The kidneys were then dried in a hot air oven set at 80°C. Following this, 100 milligrams of the dried kidney tissue were homogenized after being boiled for 30 minutes in 10 milliliters of 1N hydrochloric acid.

3. RESULT AND DISCUSSION

3.1 Procurement of plant material

Table 2: Percentage Yield of plant material

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
<i>Wedelia chinensis</i>	Methanol	254	15.08	6.032

3.2 Phytochemical Test

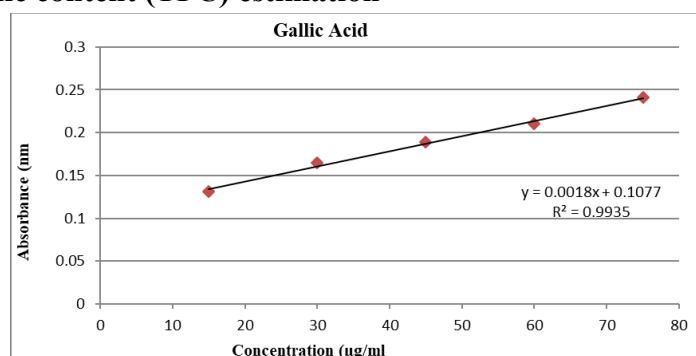
Table 3: Phytochemical test of *Wedelia chinensis* extract of methanol

Experiment	Presence or absence of phytochemical test
	Methanol extract
Alkaloids	
Dragendorff’s test	Present (+ ve)
Mayer’s reagent test	Present (+ ve)
Wagner’s reagent test	Present (+ ve)
Hager’s reagent test	Present (+ ve)
Glycoside	
Borntrager test	Present (+ ve)
Legal’s test	Present (+ ve)
Killer-Killiani test	Present (+ ve)
Carbohydrates	

Molish's test	Present (+ ve)
Fehling's test	Present (+ ve)
Benedict's test	Present (+ ve)
Barfoed's test	Present (+ ve)
Proteins and Amino Acids	
Biuret test	Present (+ ve)
Ninhydrin test	Present (+ ve)
Flavonoids	
Alkaline reagent test	Present (+ ve)
Lead Acetate test	Present (+ ve)
Tannin and Phenolic Compounds	
Ferric Chloride test	Present (+ ve)
Saponin	
Foam test	Present (+ ve)
Test for Triterpenoids and Steroids	
Salkowski's test	Absent (- ve)
Libbermann-Burchard's test	Absent (- ve)

3.3 Qualitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation



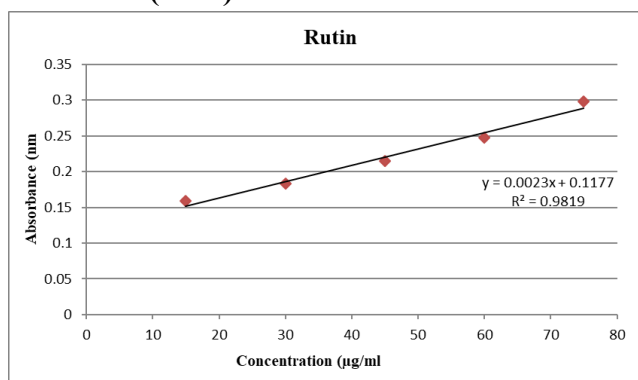
Graph 1: Represent standard curve of Gallic acid

3.3.1.1 Total Phenolic Content in *Wedelia chinensis* extract

Table 4: Total Phenolic Content

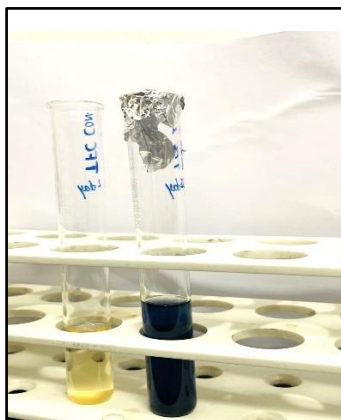
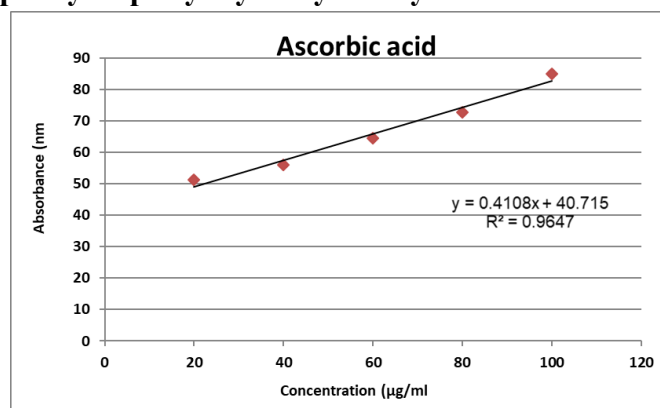
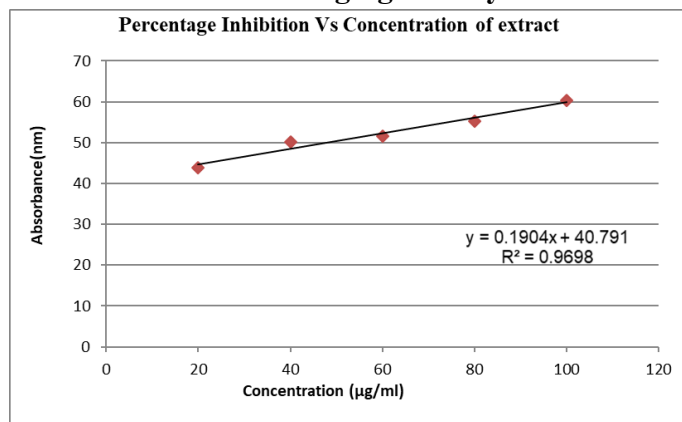
Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.131	66.3mg/gm
0.187	
0.205	

3.3.2 Total Flavonoids content (TFC) estimation



Graph 2: Represent standard curve of Rutin**3.3.2.1 Total Flavonoid Content in *Wedelia chinensis* extract****Table 5: Total Flavonoid Content**

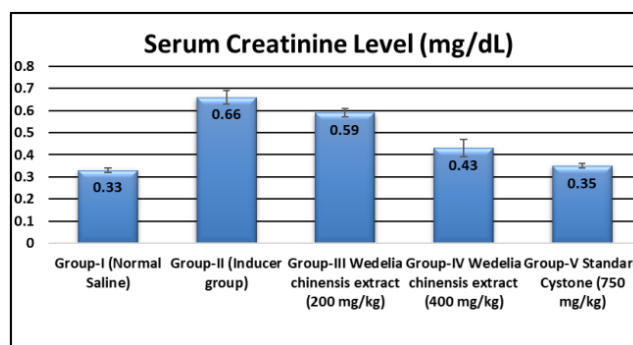
Absorbance	TFC in mg/gm equivalent of Rutin
0.156	26.65mg/gm
0.172	
0.187	

**Figure 1: Flavonoids content (TFC) and Total Phenolic content (TPC) estimation****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 extract**

3.5 The activity of ethylene glycol-induced antiurolithiatic in rats

3.5.1 Table 6: Creatinine test

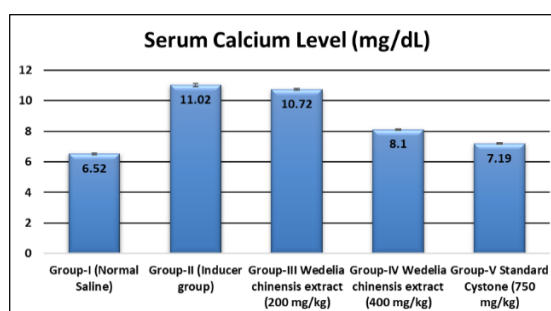
Study Groups	Serum Creatinine Level (mg/dL)
Group-I (Normal Saline)	0.33±0.01
Group-II (Inducer group)	0.66±0.03
Group-III <i>Wedelia chinensis</i> extract (200 mg/kg)	0.59±0.02
Group-IV <i>Wedelia chinensis</i> extract (400 mg/kg)	0.43±0.04
Group-V Standard Cystone (750 mg/kg)	0.35±0.01



Graph 5: The serum creatinine level is displayed as a bar graph

3.5.2 Table 7: Calcium test

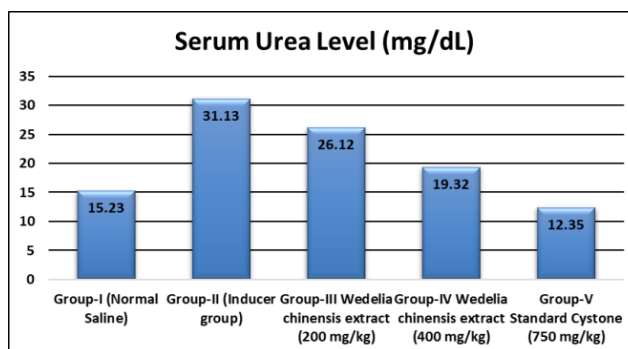
Study Groups	Serum Calcium Level(mg/dL)
Group-I (Normal Saline)	6.52±0.06
Group-II (Inducer group)	11.02±0.11
Group-III <i>Wedelia chinensis</i> extract (200mg/kg)	10.72±0.07
Group-IV <i>Wedelia chinensis</i> extract (400 mg/kg)	8.10±0.05
Group-V Standard Cystone (750 mg/kg)	7.19±0.04



Graph 6: A bar graph shows the amount of calcium in the serum.

3.5.3 Table 8: Urea test

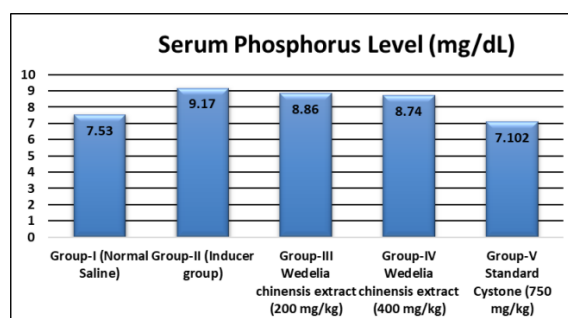
Study Groups	Serum Urea Level(mg/dL)
Group-I (Normal Saline)	15.23±0.06
Group-II (Inducer group)	31.13±0.10
Group-III <i>Wedelia chinensis</i> extract (200mg/kg)	26.12±0.08
Group-IV <i>Wedelia chinensis</i> extract (400 mg/kg)	19.32±0.05
Group-V Standard Cystone (750 mg/kg)	12.35 ±0.02



Graph 7: A bar graph illustrates the level of serum urea

3.5.4 Table 9: Phosphorus test

Study Groups	Serum Phosphorus Level(mg/dL)
Group-I (Normal Saline)	7.53±0.06
Group-II (Inducer group)	9.17±0.12
Group-III <i>Wedelia chinensis</i> extract (200mg/kg)	8.86±0.07
Group-IV <i>Wedelia chinensis</i> extract (400 mg/kg)	8.74±0.010
Group-V Standard Cystone (750 mg/kg)	7.102±0.08

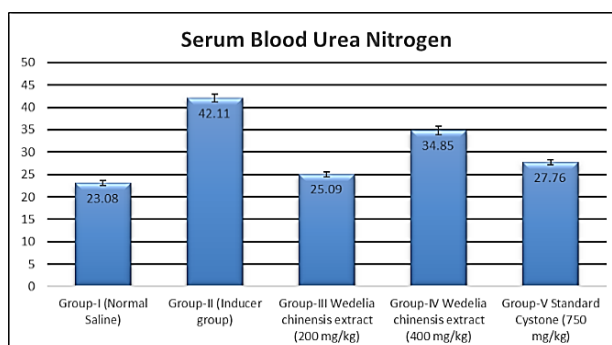


Graph 8: The serum phosphorus level is shown as a bar graph

3.5.1 Estimation of Serum Blood urea nitrogen (BUN)

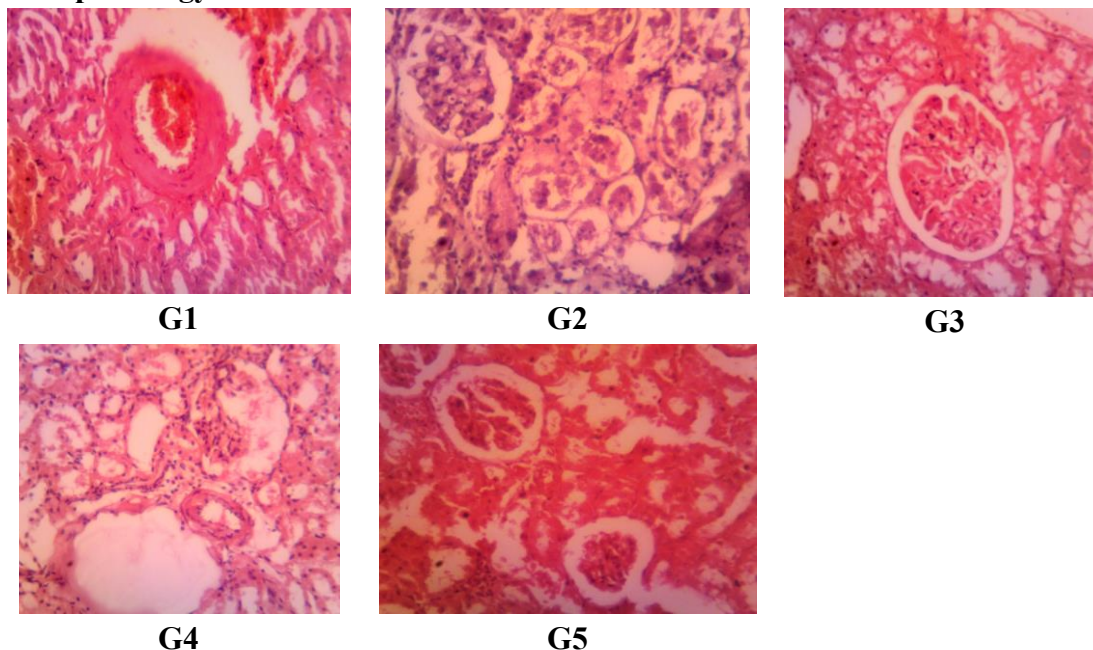
Table 10: Serum Blood urea nitrogen

Groups	Serum Blood Urea Nitrogen
Group-I (Normal Saline)	23.08±0.53
Group-II (Inducer group)	42.11±0.85
Group-III <i>Wedelia chinensis</i> extract (200 mg/kg)	25.09±0.56
Group-IV <i>Wedelia chinensis</i> extract (400 mg/kg)	34.85±0.99
Group-V Standard Cystone (750 mg/kg)	27.76±0.56



Graph 9: The Serum Blood urea nitrogen shown as a bar graph

3.6 Histopathology



Discussion

The present study investigated the phytochemical profile and antiurolithiatic potential of *Wedelia chinensis* methanolic extract in an ethylene glycol-induced rat model. The extraction yielded 6.03%, reflecting efficient recovery of polar phytochemicals. Phytochemical analysis demonstrated the presence of key bioactive constituents including alkaloids, flavonoids, glycosides, carbohydrates, proteins, tannins, phenolics, and saponins—compounds well known for their pharmacological and antioxidant properties. The absence of steroids and triterpenoids indicates that the extract's biological activity is primarily associated with these phenolic and flavonoid-rich constituents.

The high total phenolic (66.3 mg/g GAE) and flavonoid (26.65 mg/g RE) contents highlight the extract's strong antioxidant capacity, further validated by its DPPH radical scavenging activity ($IC_{50} = 48.46 \mu\text{g/ml}$), suggesting that oxidative stress modulation may play a key role in its protective mechanism. Although its antioxidant potency was moderate compared to the standard ascorbic acid, the extract still demonstrated significant free radical neutralization ability, which is crucial in preventing renal oxidative damage associated with urolithiasis. In vivo, *W. chinensis* extract showed notable protective effects against ethylene glycol-induced renal calculi. Both treatment doses (200 mg/kg and 400 mg/kg) significantly restored serum levels of creatinine, urea, calcium, phosphorus, and BUN towards normal, indicating improved renal function. The response was dose-dependent, with the higher dose showing comparable efficacy to the standard formulation, Cystone (750 mg/kg). Histopathological examination confirmed the biochemical findings, showing preserved renal architecture and reduced crystal deposition in treated groups. These outcomes imply that the extract mitigates stone formation by inhibiting lithogenic ion aggregation and oxidative injury, thereby protecting kidney tissues.

4. CONCLUSION

Wedelia chinensis extract exhibits significant antiurolithiatic and nephroprotective effects, supporting its traditional use in urinary disorders. Its efficacy, especially at higher doses, indicates potential as a natural alternative for kidney stone management.

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