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PHARMACOLOGICAL EVALUATION AND ANTI-UROLITHIC ACTIVITY OF *RAIN LILY* PLANT EXTRACT ON RATS

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ABSTRACT

Aim: This study aimed to pharmacologically evaluate the potential anti-urolithic activity of *rain lily (Zephyranthes spp.)* plant extract in an experimental rat model. **Method:** The rain lily plant extract was obtained and subjected to phytochemical screening to identify its constituents. Male Wistar rats were divided into groups and induced with urolithiasis using a standard protocol. The rats were then administered varying doses of the rain lily extract orally for a specified duration. Parameters such as urinary pH, urinary volume, calcium oxalate deposition, and serum biochemical markers were assessed to evaluate the anti-urolithic activity of the extract. **Result:** Phytochemical screening of the rain lily extract revealed the presence of alkaloids, flavonoids, and glycosides, among other constituents. Treatment with the rain lily extract significantly increased urinary pH and volume while reducing calcium oxalate crystal deposition in the kidneys of urolithiasis-induced rats. Moreover, serum levels of biochemical markers associated with kidney function were restored towards normal levels in the treated groups compared to the control. **Conclusion:** The findings of this study indicate that the rain

lily plant extract possesses notable anti-urolithic activity, as evidenced by its ability to mitigate urolithiasis-induced alterations in urinary parameters and serum biochemistry in rats. These results support the traditional use of rain lily in folk medicine and suggest its potential as a natural remedy for urolithiasis. Further investigations are warranted to elucidate the underlying mechanisms of action and evaluate its safety and efficacy for clinical use.

Keyword: *Rain lily*, Urolithic activity, Evaluation, Phytochemical screening, Natural remedy.

INTRODUCTION

Urolithiasis has become a widespread clinical illness, with increasing cases of kidney stones worldwide. Calcium-containing kidney stones, particularly calcium oxalate, account for over 80% of occurrences, occurring in either monohydrate or dihydrate form **(Finkielstein and Goldgarb, 2006)**. Crystal formation involves three difficult stages: nucleation, growth, and aggregation. Stone formation starts with the production of calculi in the urinary tract, caused by supersaturated urine **(Alelign and Petros, 2018).** Urine supersaturation is influenced by a variety of parameters, including ionic strength, pH, glycoprotein content, complexation, and pathogenic factors **(Ratkalkar and Kleinman, 2011).** Urinary stone disease affects 12% of the population in industrialized countries, with males having a higher recurrence rate (70-80%). than females (47-60%) **(Ingale et al., 2012; Tiwari et al., 2012).** Urolithiasis is a multifactorial disease characterized by the formation of stones throughout the urinary tract. Kidney stones can injure the urinary tract and block the urine stream. Urinary tract infections can emerge from problems before or after urolithiasis treatment **(Zanetti et al., 2008).** Although the disease is rarely life-threatening, patients may experience burning sensations and discomfort. Calcium oxalate (CaOx), also known as non-infectious crystals, is commonly seen in the urinary tract and can cause irritation and cell damage. Injury and inflammation can increase phosphatidylserine levels in the cell membrane, leading to increased CaOx crystal binding by kidney epithelial cells **(Srivastava et al., 2014).** Herbs are increasingly being used as supplements and alternative medications for treating human ailments globally. Plants and herbal formulations can reduce crystallization and recurrence of stones, as well as provide anti-inflammatory and antibacterial benefits. In this series we used rain lily extract to evaluate the urolithiasis activity. Rain lily (*Zephyranthes* spp.), belonging to the Amaryllidaceae family, is a perennial herb known for its attractive, lily-like flowers that bloom in response to rainfall. Native to tropical and subtropical regions, rain lilies are widely cultivated for ornamental purposes **(Ptak and Zolnierczyk, 2010).** Traditionally, various species of *Zephyranthes* have

been used in folk medicine for their purported therapeutic benefits. They have been utilized to treat a range of ailments including fever, pain, and inflammatory conditions **(Chang and Chen, 2007).** The bulbs, in particular, are rich in bioactive compounds such as alkaloids, flavonoids, and glycosides, which contribute to their medicinal properties. Pharmacological studies have demonstrated that extracts from rain lily possess significant biological activities. These include antioxidant **(Kumar and Sharma, 2014)**, anti-inflammatory **(Rivera and Castano, 2012),** antimicrobial **(Ahmed and Ahamed, 2005)**, and anticancer properties **(Hassan and Ahamed, 2015).** The alkaloid lycorine, found in rain lily bulbs, has shown promising anticancer activity by inducing apoptosis in various cancer cell lines **(Jeong and Kim, 2016; Wang and Zhang, 2013)**. Additionally, other studies have highlighted the potential of rain lily extracts in combating microbial infections and reducing inflammation, making them valuable in the development of new therapeutic agents.

MATERIAL AND METHOD

Plant collection

The medicinal plant *Zephyranthes citrina* (300 gm) was collected. After cleaning, plant part (flower) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant part (flower) was stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Zephyranthes citrina* was authenticated by a plant taxonomist in order to confirm its identity and purity.

Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Zephyranthes citrina* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethanol solvent. For each solvent, soxhlation was continued till no visual colour change will observed in siphon tube and completion of extraction were confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

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

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

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used **(Kokate *et al*., 2000**)**.**

Quantitative Phytochemical Estimation

TPC

The total phenolic content of *Zephyranthes citrina* extract was determined using the Folin-Ciocalteu Assay. The *Zephyranthes citrina* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepa

The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically (**Tangco *et al*., 2015)**

TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Zephyranthes citrina* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. 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).**

DPPH

The antioxidant activity of *Zephyranthes citrina* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared. Different concentration of *Zephyranthes citrina* extracts /standard (20 – 100µg/ml) were prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm **(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

To establish the presence of the functional groups in the isolated fraction (A) of *PI* methanolic extract, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The sample was dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 100 mg of KBr was prepared with a mixture of 2% finely dried

sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm-1 **(Luciene *et al.*, 2008).**

Acute Toxicity Study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight (**Guideline Document on 1996**).

Experimental work

- **Animals Protocol**

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

- **Housing Condition-**

Male rat aged 2-3 months and weighing 200-250 were taken for the study. The animals were maintained in standard animal house conditions with ad libitum access to food and water under a 12 hour light dark cycle prior to treatment.

- **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 *Zephyranthes citrina* extract at a dose of 100mg/kg orally, for 28 days.

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

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

Assessment of Antiurolithiatic activity

Collection of urine analysis all animals were kept in individual metabolic cages and urine samples of 24 hr were collected on 28th day. Animals had free access to drink water during the urine collection period. A drop of concentrated hydrochloride acid is added to the urine before being stored at 4ºC. Urine was analysed for calcium, phosphate and oxalate content.

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000×g for 10 min and analysed for creatinine, urea test and phosphorus test.

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80ºC in a hot air oven. A sample of 100mg of the dried kidney was boiled in 10ml of 1N hydrochloric acid for 30min and homogenized.

RESULTS

Percentage Yield

Table 1: Percentage Yield of crude extracts of *Zephyranthes citrina* extract

S.No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Zephyranthes citrina*	Pet ether	298	1.51	0.50%
2		Methanol	299	6.51	2.17%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Present	Present
1.2	Mayer's reagent test	Present	Present
1.3	Wagner's reagent test	Present	Present
1.3	Hager's reagent test	Present	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Absent
2.2	Legal's test	Present	Absent

2.3	Killer-Killiani test	Present	Absent
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Present
8.2	Libbermann-Burchard's test	Absent	Present

Quantitative Analysis

Total Phenolic content (TPC) & Total Flavonoids content (TFC) estimation

Table 3 Standard table for Gallic acid and Rutin

S. No.	Concentration (µg/ml)	Absorbance (Gallic acid)	Absorbance (Rutin)
1.	20	0.155	0.182
2.	40	0.180	0.205
3.	60	0.198	0.283
4.	80	0.226	0.325
5.	100	0.275	0.337

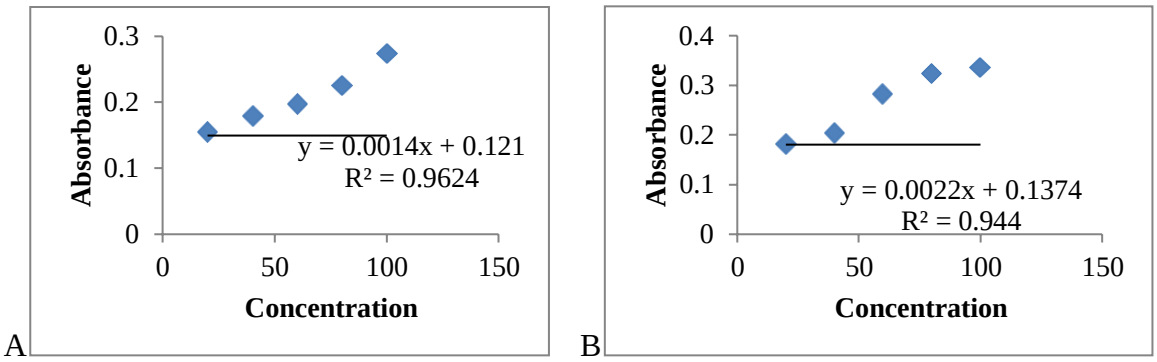


Figure 1: Represent standard curve of Gallic acid (A) and Rutin (B)

Total Phenolic Content & Total Flavonoid Content in extract

Table 4: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.135	49.33 mg/gm
2	0.182	
3	0.194	

Table 5: Total Flavonoid Content

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.155	18.16 mg/gm
2	0.168	
3	0.197	

In vitro Antioxidant Assays

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

Table 6: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.483	51.457
40	0.435	56.281
60	0.339	65.929
80	0.287	71.155
100	0.144	85.527
Control	0.995	
IC50	21.30	

Table 7: DPPH radical scavenging activity of methanol extract of *Zephyranthes citrina*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.521	43.060
40	0.468	48.852
60	0.458	49.945
80	0.418	54.316
100	0.369	59.672
Control	0.915	
IC50	54.09	

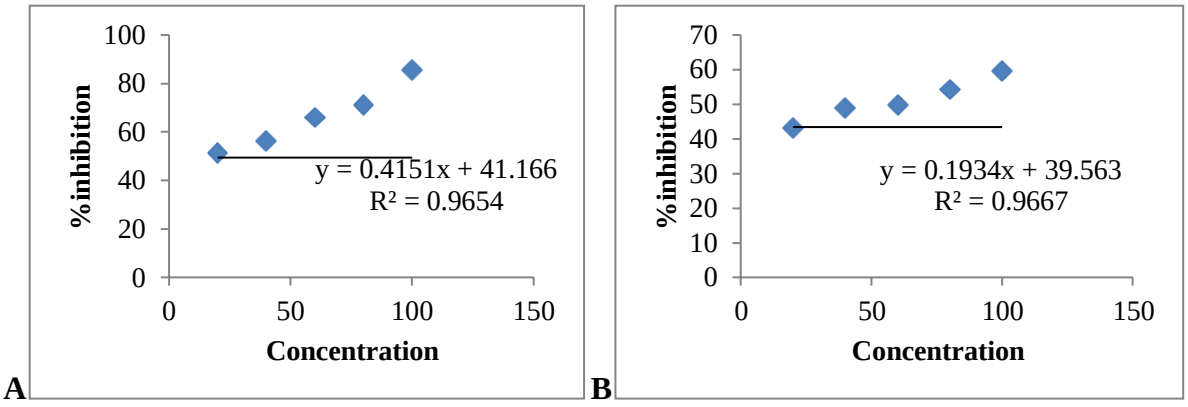


Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid (A) and extract of *Zephyranthes citrine* (B)

3.3 Functional group identified by FTIR Study

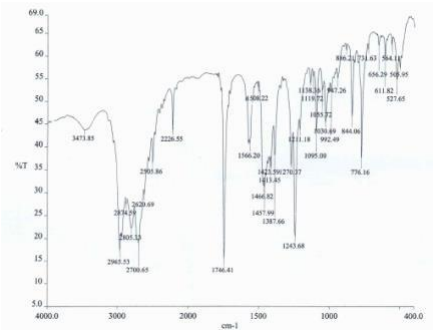


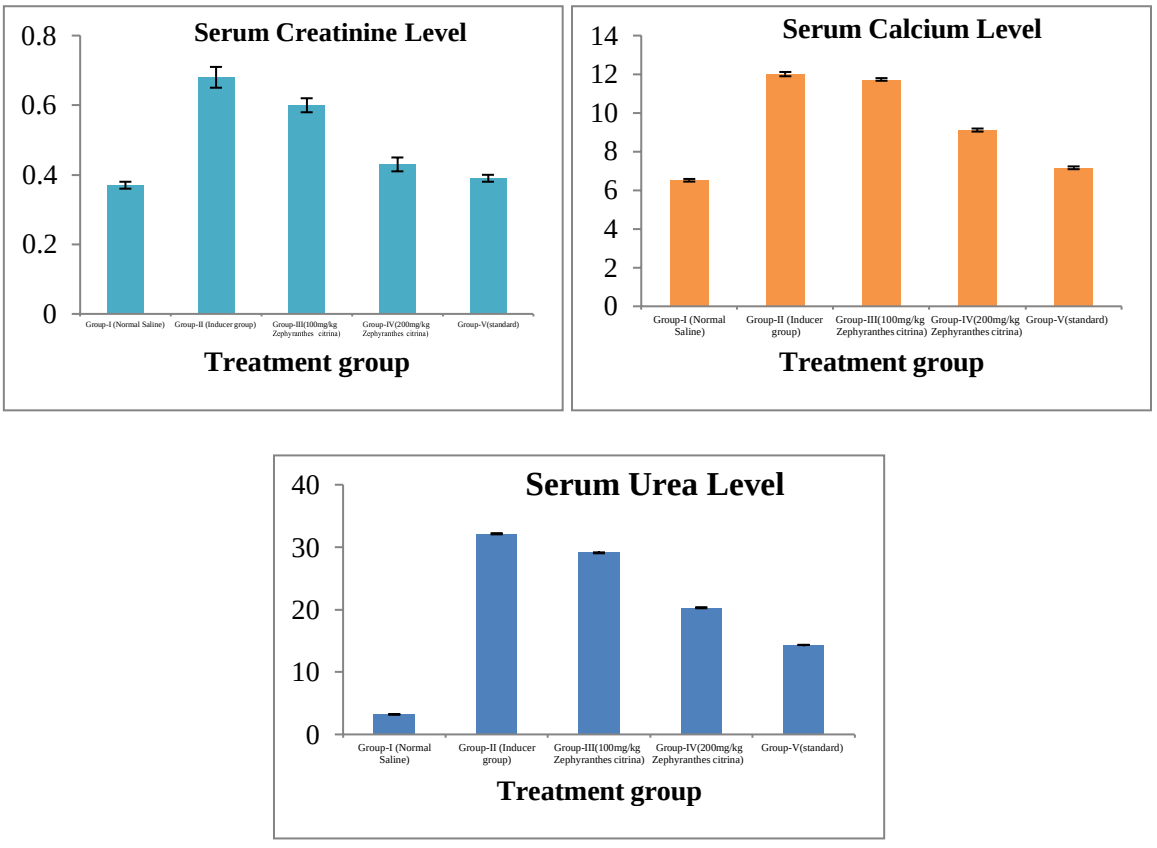
Figure 3: FTIR of *Zephyranthes citrina*

Ethylene glycol induced antiurolithiasis activity

Creatinine test, Calcium test and Urea test

Table 8: Creatinine test Calcium test and Urea test

Study Groups	Serum Creatinine Level	Serum Calcium Level	Serum Urea Level
Group-I (Normal Saline)	0.37±0.01	6.52±0.07	3.21±0.05
Group-II (Inducer group)	0.68±0.03	12.01±0.11	32.15±0.10
Group-III(100mg/kg *Zephyranthes citrina)*	0.60±0.02	11.73±0.07	29.11±0.07
Group-IV(200mg/kg *Zephyranthes citrina)*	0.43±0.02	9.12±0.08	20.32±0.08
Group-V(standard)	0.39±0.01	7.17±0.07	14.34 ±0.03

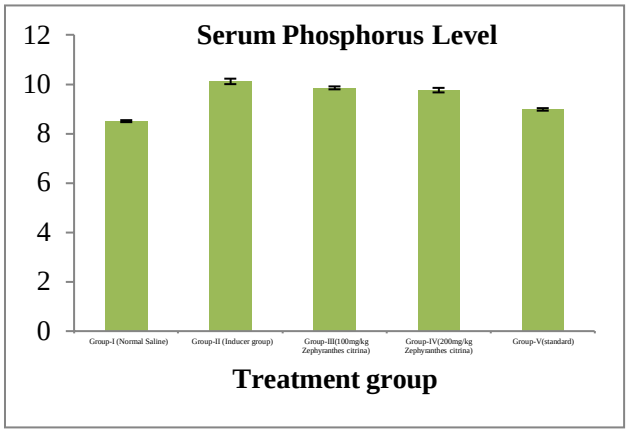


Graph 1: Bar graph represents the serum creatinine, serum calcium and serum urea level

Phosphorus test

Table 9: Phosphorus test

S. No	Study Groups	Serum Phosphorus Level
1.	Group-I (Normal Saline)	8.50±0.04
2.	Group-II (Inducer group)	10.11±0.11
3	Group-III(100mg/kg *Zephyranthes citrina)*	9.85±0.06
4.	Group-IV(200mg/kg *Zephyranthes citrina)*	9.76±0.09
5.	Group-V(standard)	8.98±0.05



Graph 2: Bar graph represents the serum phosphorous level

Histology

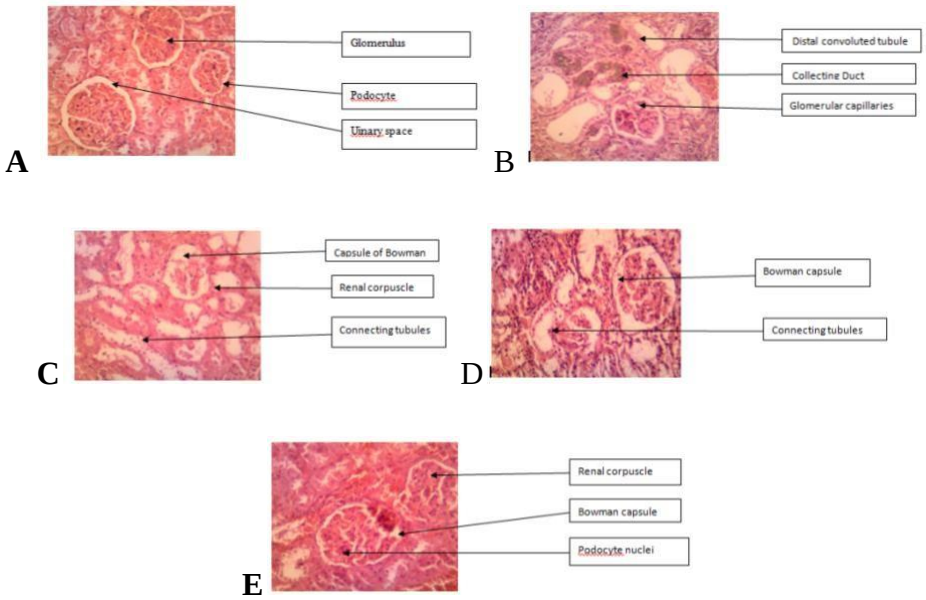


Figure 4: Histology of all five groups

DISCUSSION

Phytochemical analysis of methanolic extract of *Zephyranthes citrina* showed the presence of alkaloids, phenolics, flavonoid, saponins, carbohydrates, saponin, steroids, 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. DPPH radical scavenging activity of *Zephyranthes citrina* extract exhibited percent inhibition 59.67% and its IC 50value was found to be 54.09µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 85.52% and showed IC 50 value of 21.30µg/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 uria 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 *Zephyranthes citrina* the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinaryexcretion in both the preventive and curative group animals reveals the potential of *Zephyranthes citrina* in both preventing the formation and dissolving the preformed CaOx stones.

CONCLUSION

Urinary lithiasis is generally the result of an imbalance between inhibitors and promoters in the kidneys. Human kidney stones are usually composed of CaOx1, and several studies have examined the effect of the citrus juices on calcium salt crystallization. However, the conclusions from those studies were not consistent. Many *in vivo* models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease. Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans.

This study concluded that the administration of methanolic extract of *Zephyranthes citrina* showed significant anti-urolithiatic activity against ethylene glycol and ammonium chloride–induced urolithiasis model in rats.

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