International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 16, Issue 5, 2024

Original Article

TO EVALUATE THE ANTIUROLITHIATIC ACTIVITY OF AQUEOUS EXTRACT OF *CATHARANTHUS ROSEUS* IN ETHYLENE-GLYCOL-INDUCED UROLITHIASIS IN RATS

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Received: 22 Jun 2024, Revised and Accepted: 07 Aug 2024

ABSTRACT

Objective: This study aimed to evaluate the potential antiurolithiatic activity of the aqueous extract of **Catharanthus roseus** in a rat model of ethylene-glycol-induced urolithiasis.

Methods: Thirty male Wistar rats were divided into five groups: Group I served as the control, Group II received ethylene glycol (0.75% v/v) in drinking water for the induction of urolithiasis, Group III received standard treatment (cystone), and Group IV was treated with the aqueous extract of *Catharanthus roseus* (200 mg/kg). Throughout the experimental period, various parameters such as urinary calcium, phosphate, oxalate levels, and kidney histopathology were assessed to evaluate the antiurolithiatic potential of *Catharanthus roseus*.

Results: The results demonstrated a significant reduction in urinary calculi formation, lowered calcium and oxalate excretion, and ameliorated histological changes in the kidneys of rats treated with the aqueous extract of *Catharanthus roseus*.

Conclusion: Our findings suggest that the aqueous extract of **Catharanthus roseus** possesses promising antiurolithiatic activity, potentially attributed to its ability to modulate urinary stone-forming parameters. Further studies are warranted to elucidate the underlying mechanisms and explore the therapeutic potential of **Catharanthus roseus** in managing urolithiasis.

Keywords: *Catharanthus roseus*, Urolithiasis, Ethylene-glycol, Antiurolithiatic activity and herbal medicine

DOI: https://dx.doi.org/10.22159/ijcpr.2024v16i5.5027 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Urolithiasis, the formation of urinary calculi or stones within the urinary tract, is a prevalent and debilitating condition affecting a substantial portion of the global population [1]. The incidence of urolithiasis has been steadily rising, necessitating an exploration of novel therapeutic approaches, particularly from natural sources[2]. *Catharanthus roseus*, commonly known as Madagascar periwinkle or Vinca rosea, is a plant with a rich ethnobotanical history and is traditionally employed in various medicinal formulations [3].

Catharanthus roseus is renowned for its diverse pharmacological properties, including anti-inflammatory, antioxidant[4], and diuretic effects, suggesting its potential utility in managing urolithiasis. Ethylene-glycol-induced urolithiasis in experimental animal models simulates key aspects of human stone formation, making it a relevant platform to assess the antiurolithiatic efficacy of herbal extracts.

This study aims to investigate the antiurolithiatic activity of the aqueous extract of *Catharanthus roseus* in a rat model of ethylene-glycol-induced urolithiasis. The choice of *Catharanthus roseus* is grounded in its ethnomedicinal significance and the need for novel therapeutic agents to address the multifaceted nature of urolithiasis.

The escalating economic and health burdens associated with urolithiasis underscore the urgency for innovative and efficacious treatment modalities. By exploring the potential benefits of *Catharanthus roseus*, we aim to contribute to the growing body of research on herbal interventions for urolithiasis and provide a basis for further investigations into the mechanisms underlying its antiurolithiatic properties.

This research has the potential to unveil a natural and accessible remedy for urolithiasis, offering a complementary approach to conventional treatments. As we delve into the experimental findings, we seek to unravel the therapeutic potential of *Catharanthus roseus* in mitigating the complexities of urolithiasis, ultimately paving the way for future clinical applications.

MATERIALS AND METHODS

Chemicals and equipment requirement

Ethylene glycol was purchased from Merck. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers. Apparatus, such as the metabolic cages homogenizer, were used in the study.

Plant material

The plants Flowers of *Catharanthus roseus*, were purchased from local vendors and were identified and authenticated from CSIR-National Institute of Science Communication and Policy Research (NISCAIR and NISTADS). Authentication No: NIScPR/RHMD/ Consult/2022/4092-93-3.

Standard drug

Cystone tablet 500 mg/kg is used as a standard drug.

Extraction of flowers of *Catharanthus roseus* (Vinca) [5]

Flowers of vinca were shade-dried, finely ground with motor pastle. Then boiled for 10 min (5 gm/100 ml distilled water). The mixture was filter with 4 layers of surgical gauge. The conc. of resultant filtrate was found to be 0.4 mg/ml.

Fig. 1: Extraction of flowers of *Catharanthus roseus* (Vinca)

Procurement of animals

Healthy Male Wistar albino rats, weighing between-140 to 200 g procured from Punjab University, Chandigarh and kept in Animal House Facility, St. Soldier institute of Pharmacy, Jalandhar, Punjab, India. The animals will keep in polypropylene cages and maintained under standardized conditions. Standard laboratory conditions: 12 h light and dark cycle, maintain temperature: 25±2 °C Relative humidity: 60±5%. Standard pellet diet and water ad libitum is given to animals [6].

Induction of disease

Ethylene-glycol (EG) induce hyperoxaluria model is used for the study. EG is a typical lithogenic agent in the rodent animal model. EG in the concentration of 0.75%, added to drinking water and given to group II to group VIII rats for 28 d. Crystalluria develops within 12 d and renal crystal deposits within 3 w. Administration of EG may result in induction of hyperoxaluria, crystalluria and calcium oxalate nephrolithiasis [7].

Experimental design

"Male Wistar albino rats" will used for the study. Weighing between-140 to 200 g. The animals will keep in polypropylene cages and maintained under standardized conditions. Standard laboratory conditions: 12 h light and dark cycle, maintain temperature: 25±2 °C Relative humidity: 60±5%. Standard pellet diet and water ad libitum is given to animals [6]. Animals were divided into the 8 groups, each group contain 6 rats. Group I is taken as 'Control group' and receive regular food and drinking water for 28 d. Group II is taken as 'Negative control group' and treated with Ethylene glycol only for 28 d (for the study of disease). Group III is taken as 'Standard group' and treated with EG for 28 d and antiurolithiatic drug, cystone (500 mg/kg), from the 15th d till 28th d. Group IV is taken as 'Treatment group' and treated with EG for 28 d. Group IV is treated with '*Catharanthus roseus* extract', from the 15th d till 28th d.

Various test and procedures to be performed in *in vivo* studies in detail

Behaviour parameters

- **Body weight analysis (%):** Body weight analysis (%) should be done on regular basis in each group of animals. After the end of study, changes in the body weight should be recorded in each group of animals [8].
- **Water intake analysis:** Water intake capacity (24h) of each group of animals should be recorded regularly. Changes in water intake capacity of animals should be determined at the end of the study for each group of animals [8].

Table 1: Grouping of animals

S. No.	Groups	Study design	Treatment	Dose (mg/kg/P. O.)	Animal required	Days
1.	I	Control group	Vehicle only	-	6	0-28
2.	II	Disease group	Ethylene glycol (EG)	0.75%	6	0-20
3.	III	Standard group	Cystone+EG	500+0.75%	6	15-28
4.	IV	*Catharanthus roseus* extract	*Catharanthus roseus* extract+EG	200+0.75%	6	15-28

Biochemical parameters

- **Urine analysis:** Urine sample will collect after the dosing of animals by keeping the animals into metabolic cages where animals having free access to drink water. The urine sample will collect and analyse the values of calcium, phosphorous, magnesium, urine volume and urine pH. At the end of the study determine the changes occur in each group of animals [6, 9].

Purpose

To evaluate the comparison of decreased and increased level of calcium, phosphorus, magnesium, urine volume and urine pH of animals in control, disease and treatment group.

- **Serum analysis:** Blood sample of each group of animals will collect from the retro orbital puncture. After that blood sample was centrifuged at 3000 rpm, 20 °C for 15 min and analyse the serum calcium, phosphorus, blood urea nitrogen, uric acid, urea and creatinine. At the end of the study determine the difference in each group of animals [6].

Purpose

To evaluate the comparison of decreased and increased level of calcium, phosphorus, magnesium, and urine pH of animals in control, disease and treatment group.

- **Histopathology:** For histopathological examination rats were sacrifice and kidneys were removed. Each kidney will be weighed. The kidneys were washing with 0.15 M KCl and stored in buffered formalin (10%), fixed in bouin liquid, soaked in paraffin, cut at 2–3 µm intervals, and the slices were stained using hematoxylin and eosin. Tissue slices were photographed using optical microscopy under polarized light [8,10].

Purpose

Histopathology examination reveals the changes in renal structure like renal injury, shrinkage in glomerulus and necrosis in cells of cortex and medulla in diseases and treated group of animals [11].

- **Kidney homogenate:** Renal tissue will excise, weight and cut into small pieces and 10% (w/v) homogenate prepared in 0.1 M PBS buffer (pH 7.4) and Magnesium, phosphate, calcium level, ACP, Alkaline phosphate (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) were estimated [8, 9].

RESULTS AND DISCUSSION

Changes in body weight and water intake capacity

The weight (in grams) of the animals was noted on the first and last day of treatment and the percentage change in body weight was calculated. Initial and final amount of water observed for 24 h to calculate the water intake values are expressed in g/24 h or g/d.

Group I show no change in body weight and water intake capacity whereas group II shows loss in body weight and high water intake capacity. Other Groups, including group III-VI showed significant gain in body weight. Water intake was not significantly different among the all groups except Group II, which was significantly high as compare to control group as shown in table 14.

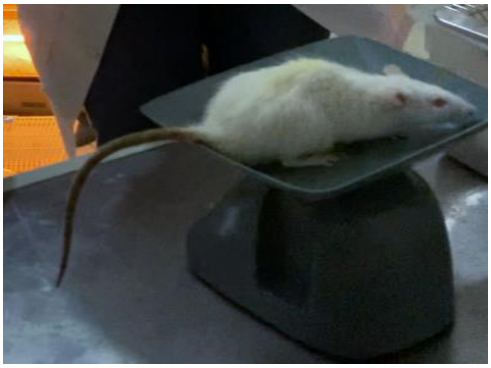
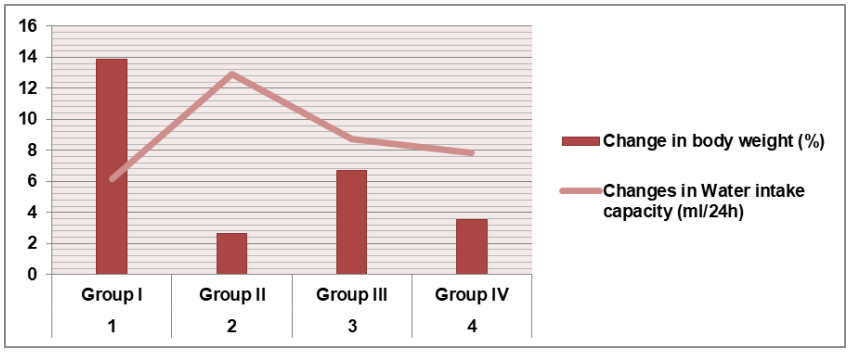


Fig. 2: Rat placed on weighing balance



Graph 1: Changes in body weight and water intake capacity, values for urine parameters are measured in 24 h urine sample. All values are stated as mean±SEM (n=6). a Comparisons are made with Group I, b Comparisons are made with Group II, *P. The mean difference is significant at the 0.05 level

Biochemical parameters

Urine analysis

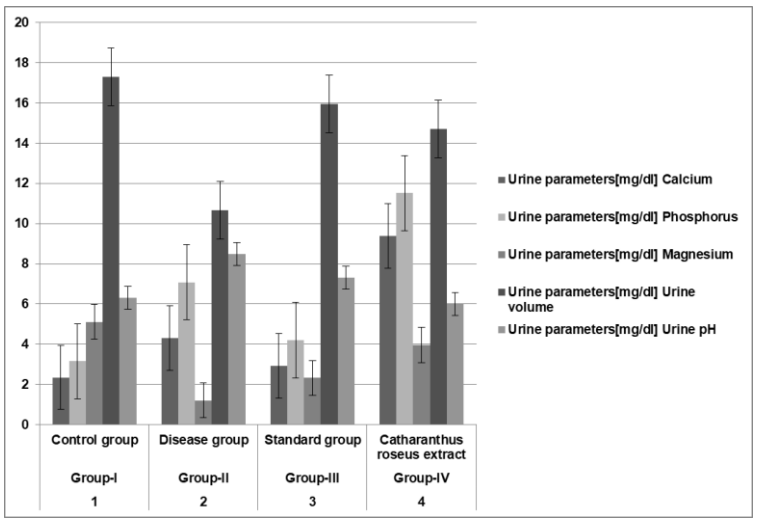
All animals are kept in individual metabolic cages and urine samples of 24 h were collected on 28th d. Animals had free access to drinking water during the urine collection period. A drop of concentrated HCL was added to the urine before being stored at 40 °C. The following urine contents were analysed.

Table 16 illustrates the amounts of phosphate, calcium, and magnesium in group I to v urine samples. In the current study, male Wistar rats were given 1% ethylene glycol in drinking water, which significantly (P 0.001 vs. Group I) increased the content of phosphorus and calcium and decreased the concentration of magnesium in their urine (Group II). On the other hand, therapy significantly (P 0.01 vs. Group II) decreased phosphorus and calcium excretion and increased magnesium excretion in urine in both the preventive and curative groups (Group IV-VIII, respectively), and was equivalent to the control group (Group III, cystone treated).

Serum analysis

Blood sample of each group of animals will collect from the retro-orbital puncture. After that blood sample was centrifuged at 3000 rpm, 20 °C for 15 min and analyze the serum calcium, phosphorus, urea and creatinine. At the end of the study determine the difference in each group of animals [6].

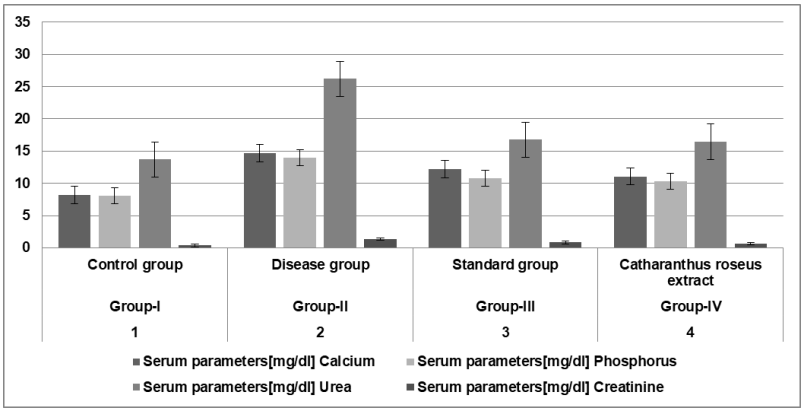
Fig. 3: Rat placed in metabolic cage for urine sample



Graph 2: Changes in urinary parameters in different groups of animals, values for urine parameters are measured in 24 h urine sample. All values are stated as mean±SEM (n=6). [a]Comparisons are made with Group I, [b]Comparisons are made with Group II, *P. The mean difference is significant at the 0.05 level

Fig. 4: Blood collection by retro-orbital puncture

Serum calcium, phosphorus, urea, and creatinine levels in Group I–VIII were measured to assess the function of the kidneys. As a symptom of renal damage, Group II (the disease group) had considerably higher concentrations of calcium, phosphate, urea, and creatinine. Nevertheless, treatment groups exhibit a decline in calcium, phosphate, urea, and creatinine levels equivalent to the Standard group (Group III).



Graph 3: Changes in serum parameters in different groups of animals; the mean difference is significant at the 0.05 level

Analysis of Kidney Histopathology

According to renal histopathology investigation, the control group (Group I) kidneys did not have any CaOx crystal deposits or other abnormalities, as shown in fig. 4(A). As demonstrated in figure, the disease-induced group (Group II) renal's tissue, however, had many CaOx crystal deposits in the renal tubules as well as congestion and dilated parenchymal blood vessels. In the control group (Group III), the kidney had typical architecture with dilatation of tubules in the corticomedullary junction, little interstitial inflammation, and sporadic renal tubules with CaOx crystal deposits. In the group (Group IV–VII), the kidney had normal architecture and only a small number of renal tubules that displayed vacuolar degeneration but no CaOx crystal deposits.

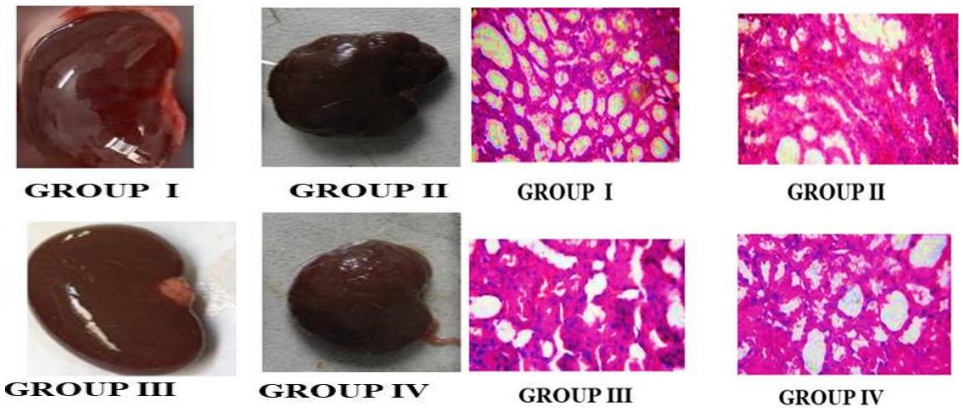
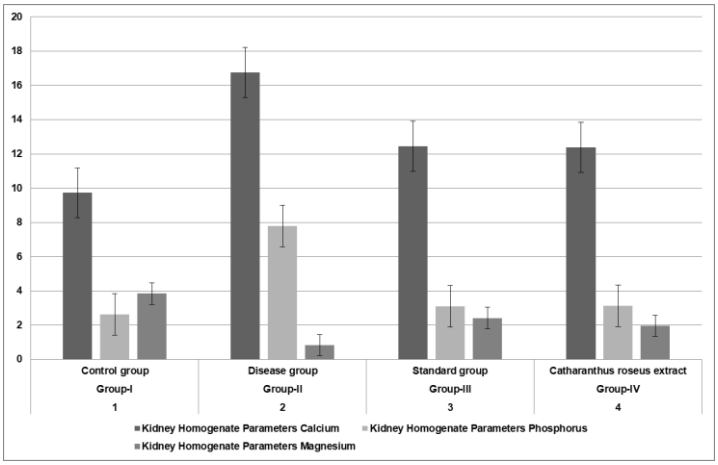


Fig. 5: Histopathology of kidney

Analysis of kidney homogenate analysis

In the stone-induced group, phosphate and calcium deposition in the renal tissue was considerably increased, but magnesium level was lowered. In both the preventive and curative groups, treatment with the herbal combination considerably lowered the amounts of phosphorus and calcium and raised the magnesium to a level that was almost normal compared to the standard group.



Graph 4: Changes in Kidney homogenate parameters in different groups of animals, all values are stated as mean±SEM (n=6). a Comparisons are made with group I, b Comparisons are made with Group II, *P. The mean difference is significant at the 0.05 level

The outcomes of the histological investigations revealed that there was no kidney injury and no microcrystalline deposition in the treatment groups (Group III-VIII). All of these findings allowed researchers to establish the herbal combinations' ability to treat and prevent urolithiasis caused by ethylene glycol.

As a result of the herbal combination's success in preventing the early phases of stone growth, urolithiasis may be prevented from returning. Although the exact mechanism behind this action is yet unknown, it enhanced diuresis and decreased urine concentrations of components that cause stones.

CONCLUSION

In conclusion, our study provides valuable insights into the antiurolithiatic potential of the aqueous extract of *Catharanthus roseus* in a rat model of ethylene-glycol-induced urolithiasis. The administration of *Catharanthus roseus* demonstrated a significant reduction in urinary calculi formation, indicating its ability to modulate crucial parameters involved in the pathogenesis of urolithiasis.

The observed decrease in urinary calcium and oxalate levels in rats treated with *Catharanthus roseus* suggests a potential role in preventing the precipitation of these stone-forming components. Furthermore, the amelioration of histological changes in the kidneys of treated rats highlights the protective effect of *Catharanthus roseus* against renal damage associated with urolithiasis.

These findings underscore the promising therapeutic potential of *Catharanthus roseus* as a natural remedy for urolithiasis. However, further studies are essential to unravel the precise mechanisms responsible for its antiurolithiatic activity and to determine the optimal dosage and treatment duration.

In summary, the results of this study contribute to the growing body of evidence supporting the use of herbal remedies, specifically *Catharanthus roseus*, as a potential adjunctive therapy for urolithiasis. The development of alternative treatments for urolithiasis is crucial, and *Catharanthus roseus* holds promise as a natural and effective option in this regard.

AUTHORS CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

ACKNOWLEDGMENT

It's our privilege to express profound sense of gratitude and cordial thanks to our respected chairman Mr. Anil Chopra; Vice Chairperson, Ms. Sangeeta Chopra, St. Soldier Educational Society, Jalandhar for providing the necessary facilities to complete this work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declared none

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