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Ethanolic Leaf Extract of *Chenopodium album Linn.* Attenuates Calcium Oxalate-Associated Urolithiasis in Rats: An Integrated In Vitro and In Vivo Evaluation

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Abstract:

Background: The primary stone component in urolithiasis, a recurrent urinary tract disease characterized by crystal nucleation, development, aggregation, and retention, is calcium oxalate (CaOx). *Chenopodium album Linn.* has a long history of ethnomedical use for urinary and renal issues and has demonstrated an initial anti-crystallization effect. However, little information is known about the relationship between in vivo renal and urinary outcomes and in vitro CaOx crystallization. Objective: This study evaluated the anti-urolithiatic potential of an ethanolic leaf extract of *C. album* (EECA) using complementary in vitro and in vivo techniques. **Methods:** The leaves were subjected to Soxhlet extraction using ethanol to obtain a 6.0% w/w extract, and preliminary phytochemical screening was carried out. The nucleation of CaOx was measured at 10–100 mg/mL, and the inhibition of crystal growth was also examined. The standard treatment was cystone (750 mg/kg). EECA was given at doses of 50 and 200 mg/kg to rats with urolithiasis associated with calcium oxalate. Urinary uric acid, urine volume, and renal histology were determined. **Results:** EECA inhibited CaOx nucleation in a concentration-dependent manner by 18.75% at 10 mg/mL and 77.50% at 100 mg/mL. Inhibition at 100 mg/mL exceeded the observed Cystone response of 72.50%. EECA also raised urine production and reduced urinary uric acid, particularly at 200 mg/kg. The results of the histopathology showed improved kidney structure. **Conclusion:** EECA demonstrated anti-crystallization and protective qualities in experimental urolithiasis, encouraging further mechanistic, phytochemical-standardizing, and translational research.

Keywords: *Chenopodium album*; calcium oxalate; urolithiasis; nucleation; crystallization; Cystone; urinary uric acid; renal histopathology; plant extract.

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1. Introduction

Crystalline material forms and remains in the kidney or urinary system in urolithiasis, also known as renal stone disease, a common and long-term disorder of the urinary tract. Geographical, climatic, nutritional, metabolic, genetic, and lifestyle factors all influence its occurrence. According to available data, kidney stone disease is a significant global health concern. Although there is considerable methodological and regional variation, a recent meta-analysis estimated the incidence of urolithiasis to be approximately 10.85% worldwide [1]. Because patients may need repeated medical or surgical interventions and are at risk for future stone recurrence, the fact that stone disease is chronic is especially relevant. Recurrence is a significant treatment issue, although current clinical guidelines concentrate on appropriate fluid intake, dietary modification, a metabolic evaluation, and particular pharmaceutical prophylaxis [2, 3].

Calcium oxalate (CaOx) is the most common crystalline molecule found in most urinary calculi, which are calcium-containing stones. Urinary supersaturation, nucleation, normal crystalline development, aggregate formation, adherence to renal epithelial surfaces, and retention in the kidney are all steps in the multistage process of CaOx stone formation. Urinary supersaturation may be increased by hypercalciuria, hyperoxaluria, hypocitraturia, hyperuricosuria, and decreased urine volume, favoring CaOx crystallization [2,3]. According to new research, the intestinal and urinary microbiome may influence how oxalate is processed, which could lead to a particular vulnerability to the development of CaOx stones [4]. Therefore, therapies that protect renal tissue, promote urine dilution, or disrupt early crystallization processes may provide additional strategies to conventional stone avoidance. Despite improvements in endogenous and pharmaceutical treatment, prevention remains an essential component of urolithiasis management. The applicability, adherence, side-effect profile, and efficacy of dietary and medication-based strategies to lower recognized urinary risk factors may vary among patients. Therefore, recent systematic reviews have highlighted the increasing demand for plant-derived products and phytochemical compounds as potential alternative approaches to CaOx stone preventive measures [5,6]. Proposed mechanisms include diuresis promotion, inhibition of crystal nucleation and aggregation, regulation of urinary biochemical parameters, antioxidant activity, and reduction of renal inflammation and oxidative injury. However, many plant-based interventions continue to be largely supported by preclinical evidence, and there is a need for systematic evaluation of standard extracts, mechanisms, dose-response, and clinical safety [6,7].

Chenopodium album Linn. (Bathua) is an edible medicinal plant, used traditionally for a long time in different Asian regions for nutritional and medicinal purposes. Recent phytochemical reviews have shown that *C. album* is a source of various bioactive constituents like flavonoids, phenolic compounds such as saponins, alkaloids, vitamins, minerals, and other secondary metabolites [8,9]. These substances have been related to anti-inflammatory, antioxidant, antimicrobial, metabolic, and other pharmacological effects. Previous experimental studies have offered clear evidence for the potential role in urolithiasis. An aqueous leaf extract of *C. album* impacted crystal development and prevented the production and aggregation of CaOx nuclei in vitro. In rats with ethylene-glycol-induced urolithiasis, methanolic and aqueous leaf extracts decreased biochemical abnormalities and renal crystal formation [10,11]. The observations provide a significant scientific basis for the study of *C. album* as a source of anti-crystallization activity.

First, earlier studies have mainly examined aqueous or methanolic preparations, with limited information on an ethanolic leaf extract evaluated with a defined concentration-response approach. Second, CaOx stone formation is a multi-step process, and the evaluation of one crystallization endpoint does not adequately indicate biological relevance. Thirdly, the combination of findings of in vitro crystallization with functional indicators of urine and histological examination may provide better evidence of the potential protective effect of the extract. Finally, the phytochemical complexity of *C. album* suggests the need for further standardization and mechanistic studies before the findings can be translated toward medicinal development.

Therefore, the current study was designed to assess an ethanolic leaf extract of *C. album* (EECA) with complementary experimental methods. The work included preliminary phytochemical analysis and quantitative characterization of CaOx nucleation and crystal-growth assays before being tested in an artificially generated rat model of calcium oxalate-associated urolithiasis. Kidney histology was utilized to investigate tissue-level alterations, and urine volume and urinary uric acid were evaluated as functional and biochemical markers. The provided study assessed EECA at 50 and 200 mg/kg after assessing acute oral toxicity, using Cystone as a reference therapy.

Therefore, rather than declaring therapeutic efficacy, the study's goal was to establish preliminary evidence for EECA's anti-crystallization and renal protective potential. The main goal was to find out if EECA could reduce specific urinary and renal changes linked to experimentally produced urolithiasis and prevent CaOx nucleation and crystal formation. A more thorough preclinical evaluation of *C. album* leaves is anticipated to clear the way for further studies involving quantitative phytochemical standardization, identification of active constituents, mechanistic studies of crystal–cell interaction, extensive urinary biochemical profiling, and appropriately powered animal and clinical studies. Determining whether *C. album* has real potential as a standardized plant-derived supplement for preventing recurrent CaOx stone illness could be aided by such research.



Figure 1. Morphological characteristics of *Chenopodium album Linn.*

2. Materials and Methods

2.1 Plant collection and authentication

In November and December of 2025, fresh leaves of *C. album* Linn. were gathered from Bhilai, Durg district, Chhattisgarh, India, and validated at the Department of Botany, Government V.Y.T.P.G. Autonomous College, Durg, Chhattisgarh.

2.2 Preparation of ethanolic extract

After being washed, the leaves were left to dry at room temperature for a week before being milled into a powder. The powder was filtered via Sieve No. 24. A 50-g sample was placed on filter paper and extracted for 16 cycles using ethanol in a Soxhlet device. The extract was obtained and utilized in additional studies. The percentage extraction yield was determined [12].

2.3 Preliminary phytochemical screening

Standard assays were used to qualitatively screen EECA for alkaloids, tannins, anthraquinones, saponins, phenols, flavonoids, and carbohydrates. Dragendorff, Wagner, Mayer, and Hager's reagents were used to assess alkaloids; ferric chloride was used to assess tannins and phenols; Borntrück's test was used to assess anthraquinones; foam formation was used to assess saponins; the described qualitative test was used to assess flavonoids; and Fehling and Benedict's tests were used to assess carbohydrates [13].

2.4 Experimental animals and ethical approval

Male and female albino rats weighing between 150 and 200 grams were kept at 25 ± 2 °C and 60 ± 5% relative humidity, and they had unrestricted access to a commercial pellet diet. The Institutional Animal Ethics Committee accepted the study under protocol number SSPU/KIPS/IACE/2024/009 and registration number 2312/PO/ReBi/S/2024/CCSEA. (n = 4/group; total efficacy-study animals = 20).

2.5 Acute oral toxicity

The acute oral toxicity of EECA was evaluated using the approved method. Changes in locomotor activity, attentiveness, posture, respiration, eating and drinking intake, convulsions, tremors, salivation, diarrhea, and mortality were all observed in the animals. 50 and 200 mg/kg were chosen for the efficacy investigation after allowing tolerance up to 2000 mg/kg with no mortality [14].

2.6 Calcium oxalate nucleation assay

EECA was evaluated at 10, 25, 50, 75, and 100 mg/mL while calcium chloride and sodium oxalate interacted at 37 °C under the specified assay conditions. At 620 nm, optical density was observed. In the provided dataset, 100 mg/mL of cystine was utilized as the reference treatment. The percentage inhibition was computed as follows: [15]

$$\% \text{ inhibition} = [(A_control - A_test) / A_control] \times 100$$

where A_control is the absorbance of the untreated crystallization system, and A_test is the absorbance in the presence of EECA or reference treatment.

2.7 Calcium oxalate crystal-growth assay

Sodium oxalate solution (10 mL) was combined with calcium chloride solution (2 mM; 10 mL) and calcium oxalate monohydrate crystal slurry (1 mL). Absorbance at 214 nm was measured while the reaction was agitated at 800 rpm for 15 minutes while EECA was present [16].

2.8 Induction of urolithiasis and treatment groups

Twenty rats were divided into five experimental groups, each consisting of four animals: Group I, normal control; Group II, disease control induced by calcium oxalate; Group III, Cystone 750 mg/kg; Group IV, EECA 50 mg/kg; and Group V, EECA 200 mg/kg. Days 1–9 were spent

inducing calcium oxalate, while Days 10–21 were spent receiving either conventional or EECA therapy. On Days 1, 10, and 21, urine samples were taken. One inconsistent remark stating there were six animals per group in the provided paper has been revised throughout to the explicit group table that shows n = 4/group [17].

Table 1. Using experimental grouping and treatment regimen, the anti-urolithiatic activity of ethanolic leaf extract of *Chenopodium album Linn.* was evaluated. (EECA) in urolithiasis caused by calcium oxalate.

Group	n	Treatment
I	4	Normal control
II	4	Calcium oxalate-induced disease control
III	4	Cystone 750 mg/kg
IV	4	EECA 50 mg/kg
V	4	EECA 200 mg/kg

2.9 Urine analysis

Urine samples were collected from the experimental animals on the first, tenth, and 21st days of the study to evaluate urinary parameters. Each experimental group's urine volume was noted, and urinary uric acid was calculated using a suitable biochemical estimation technique. Animals in the normal control, illness control, standard-treated, and EECA-treated groups were compared using the measured values, which were represented as mean ± standard deviation. The impact of EECA on urinary changes linked to calcium oxalate-induced urolithiasis was evaluated using the measured urine-volume values and urinary uric acid levels [18].

2.10 Histopathology

Standard histological procedures, such as fixation, tissue processing, paraffin embedding, sectioning, and hematoxylin and eosin (H&E) staining, were applied to the kidney tissues. Under a microscope, the stained sections were examined for structural alterations associated with calcium oxalate-induced urolithiasis, such as alterations in renal tubules, glomeruli, interstitial tissue, inflammatory alterations, and crystal-related lesions. The protective impact of the extract on renal tissue was evaluated by comparing the histopathological results of the EECA-treated groups with those of the normal control, disease control, and conventional Cystone-treated groups [19].

3. Results

3.1 Extract yield and phytochemical profile

A reported yield of 6.0% w/w was obtained by Soxhlet extraction of 50 g of powdered *C. album* leaves with ethanol for 16 cycles (Table 2). Ethanol may be used to extract a wide range of slightly polar phytoconstituents, which might assist in clarifying the biological activity observed in subsequent studies.

Table 2. Extraction yield of the ethanolic extract of *Chenopodium album Linn.* leaves obtained by Soxhlet extraction.

Parameter	Value
Starting powdered material	50 g
Extraction method	Soxhlet
Solvent	Ethanol

Extraction cycles	16
Extract yield	6.0% w/w

3.2 Preliminary phytochemical profile

The first phytochemical screening of EECA revealed the presence of alkaloids, tannins, saponins, phenols, flavonoids, and sugars. Table 3 shows that the extract did not include anthraquinones. All things considered, the findings show that a variety of secondary metabolite types may be implicated in the biological activity of EECA.

Table 3. *Chenopodium album Linn.* ethanolic extract's initial phytochemical profile.

Phytochemical class	Test(s) reported	Result
Alkaloids	Dragendorff, Wagner, Mayer, Hager	+ (all reported)
Tannins	Ferric chloride	+
Anthraquinones	Bornträger	−
Saponins	Foam test	+
Phenols	Ferric chloride	+
Flavonoids	Flavonoid test	+
Carbohydrates	Fehling and Benedict	+ (both reported)

3.3 Calcium oxalate nucleation inhibition

EECA clearly inhibited CaOx nucleation in a concentration-dependent manner (Table 4; Figure 2). As inhibition increased from 18.75% to 77.50%, optical density gradually dropped from 0.650 ± 0.010 at 10 mg/mL to 0.180 ± 0.010 at 100 mg/mL. As a result, the reaction increased by 58.75 percentage points within the measured dose range. The Cystone value (72.50%) in the provided dataset was 5.00 percentage points lower than the reported 100 mg/mL EECA inhibition (77.50%). An approximate 50% inhibitory value of 54.2 mg/mL is obtained by linear interpolation between 50 mg/mL (47.50% inhibition) and 75 mg/mL (62.50% inhibition).

Table 4. The ethanolic extract of *Chenopodium album Linn.* leaves (EECA) inhibits calcium oxalate nucleation in a concentration-dependent manner.

Treatment	Concentration (mg/mL)	OD, 620 nm	Inhibition (%)
Control	—	0.800 ± 0.010	0.00
Cystone	100	0.220 ± 0.010	72.50
EECA	10	0.650 ± 0.010	18.75
EECA	25	0.540 ± 0.010	32.50
EECA	50	0.420 ± 0.010	47.50
EECA	75	0.300 ± 0.010	62.50
EECA	100	0.180 ± 0.010	77.50

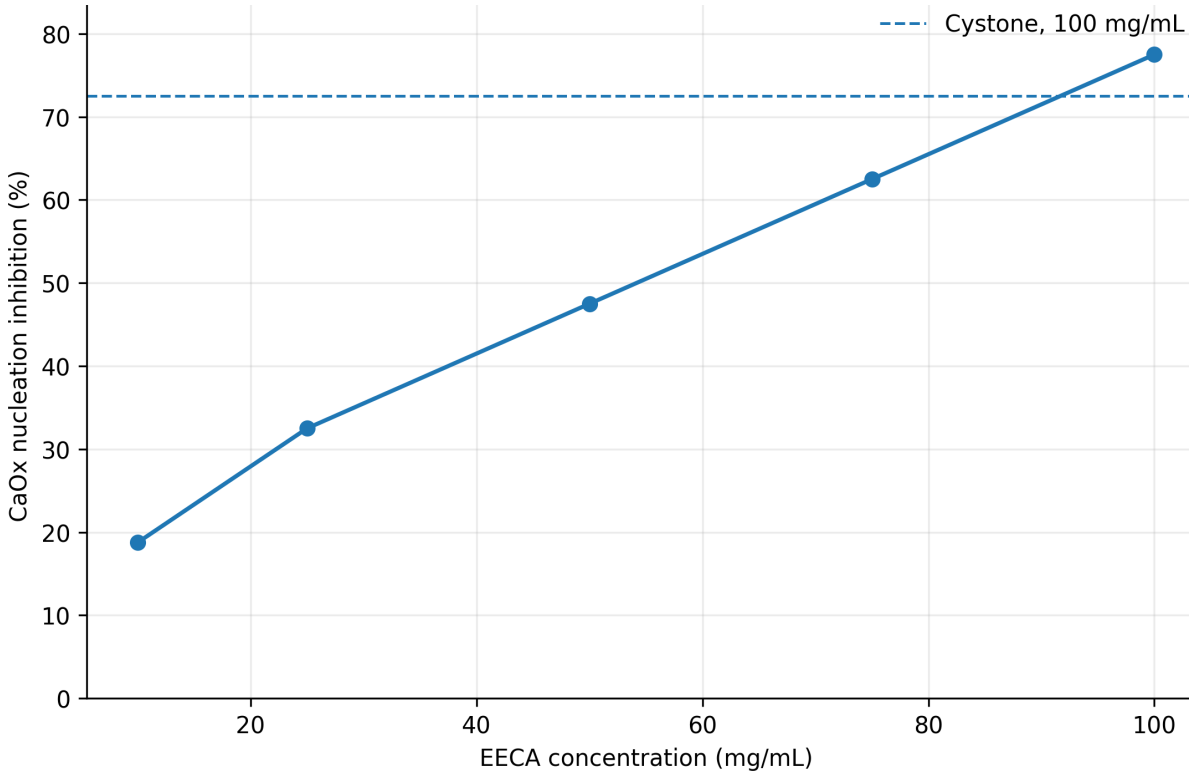


Figure 2. EECA inhibits calcium oxalate nucleation in a concentration-dependent manner. Cystone (100 mg/mL) is shown as the reported reference value.

3.4 Calcium oxalate crystal-growth inhibition

Calcium oxalate crystal growth was inhibited by the EECA in a concentration-dependent manner, suggesting that it may obstruct crystal formation. The inhibitory effect gradually increased with concentration, and the activity was noted at 100 mg/mL (Table 5, Figure 3).

Table 5. In vitro development of calcium oxalate crystals is influenced by the ethanolic extract of *Chenopodium album Linn.* leaves (EECA).

Treatment	Concentration	Absorbance at 214 nm	% inhibition
Control	—	0.800 ± 0.010	0.00
Cystone	100 mg/mL	0.220 ± 0.010	72.50
EECA	10 mg/mL	0.650 ± 0.010	18.75
EECA	25 mg/mL	0.540 ± 0.010	32.50
EECA	50 mg/mL	0.420 ± 0.010	47.50
EECA	75 mg/mL	0.300 ± 0.010	62.50
EECA	100 mg/mL	0.180 ± 0.010	77.50

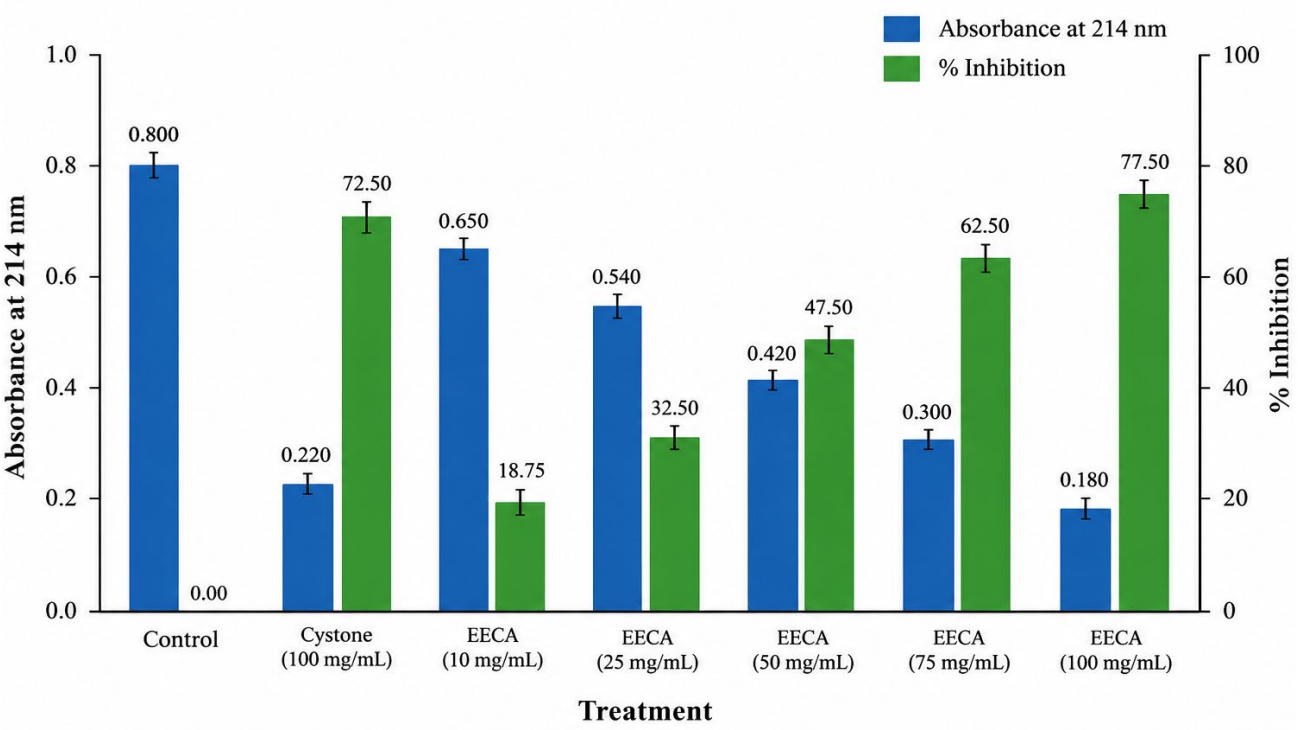


Figure 3. Impact of *Chenopodium album Linn*'s ethanolic extract. EECA on the in vitro development of calcium oxalate crystals. Mean SD (n=3) is used to express the data.

3.5 Acute oral toxicity

Oral dosage of EECA up to 2000 mg/kg failed to result in any death or significant toxicity. As a result, the extract appeared to be well tolerated in the testing circumstances. 50 and 200 mg/kg dosages were chosen for the ensuing efficacy investigation based on the acute toxicity results.

3.6 Effect on Urine volume

On Day 21, urine volume was significantly greater in the groups treated with EECA and Cystone than in the disease control (Table 6; Figure 4). The amount of disease-control urine dropped from 7.6 ± 0.9 on Day 1 to 6.8 ± 0.6 on Day 21. EECA 50 and 200 mg/kg, on the other hand, resulted in Day 21 values of 11.2 ± 1.0 and 12.5 ± 1.1, respectively. These represent increases of approximately 64.7% and 83.8% in relation to the disease control. Indicating a partial return to the reference treatment, the Cystone value (12.5 versus 13.6) and the high-dose response were 91.2%.

Table 6. Impact of *Chenopodium album Linn.* leaf ethanolic extract (EECA) on urine volume in rats with urolithiasis caused by calcium oxalate.

Group	Day 1	Day 10	Day 21
Disease control	7.6 ± 0.9	7.2 ± 0.8	6.8 ± 0.6
Cystone 750 mg/kg	13.9 ± 1.2	11.8 ± 1.0	13.6 ± 1.2
EECA 50 mg/kg	10.8 ± 1.0	9.4 ± 0.9	11.2 ± 1.0
EECA 200 mg/kg	12.4 ± 1.1	10.1 ± 0.9	12.5 ± 1.1

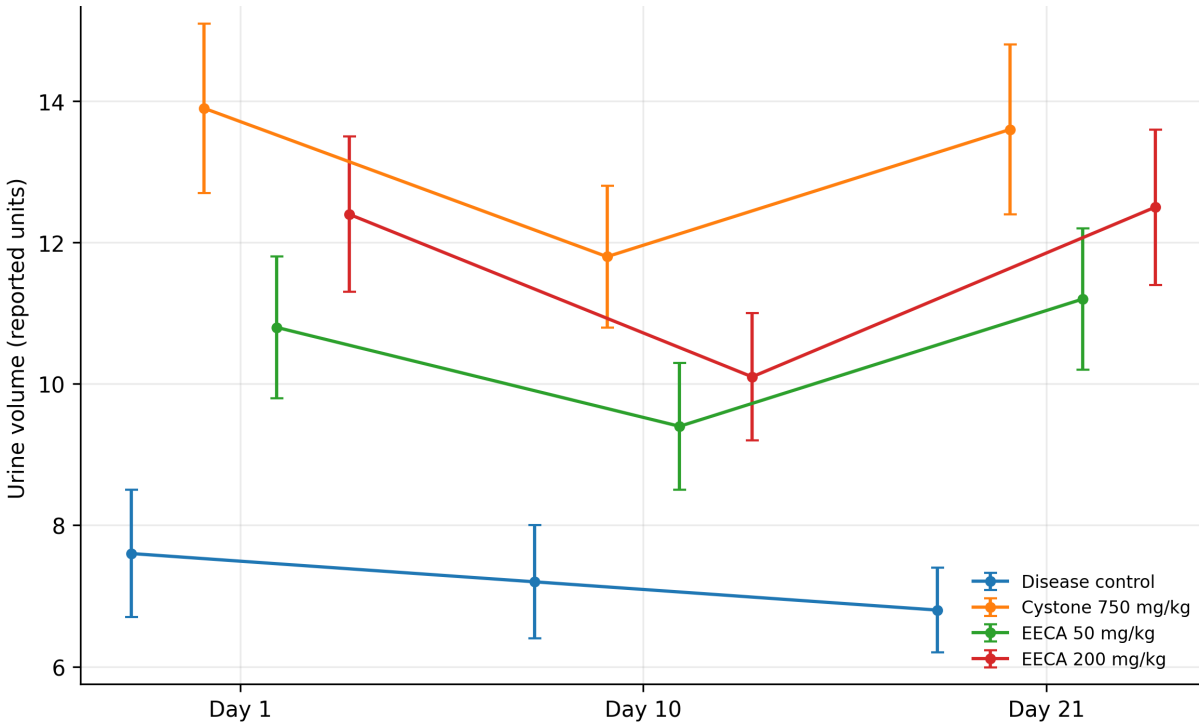


Figure 4. Effect of EECA on urine volume at Days 1, 10, and 21.

Data are expressed as mean SD (n=3)

3.7 Effect on Urinary uric acid

In the disease-control group, urine uric acid levels rose significantly from 4.26 ± 0.05 on Day 1 to 6.43 ± 0.09 on Day 21. With Day-21 values of 5.34 ± 0.05 at 50 mg/kg and 4.56 ± 0.07 at 200 mg/kg, EECA treatment reduced this rise in a dose-dependent manner (Table 7; Figure 5). These indicate decreases of about 16.9% and 29.1%, respectively, in terms of disease control. Only 0.03 reported units separated the 200 mg/kg value from the Cystone value of 4.53 ± 0.09. Although statistical significance cannot be asserted without the raw observations and specific analysis, these data bolster evidence for a treatment-associated improvement in a urine biochemical outcome.

Table 7. Impact of *Chenopodium album Linn.* leaf ethanolic extract (EECA) on urine uric acid levels in rats with urolithiasis caused by calcium oxalate.

Group	Day 1	Day 10	Day 21
Disease control	4.26 ± 0.05	6.12 ± 0.07	6.43 ± 0.09
Cystone 750 mg/kg	4.25 ± 0.06	5.95 ± 0.04	4.53 ± 0.09
EECA 50 mg/kg	4.26 ± 0.06	5.87 ± 0.04	5.34 ± 0.05
EECA 200 mg/kg	4.26 ± 0.07	5.12 ± 0.05	4.56 ± 0.07

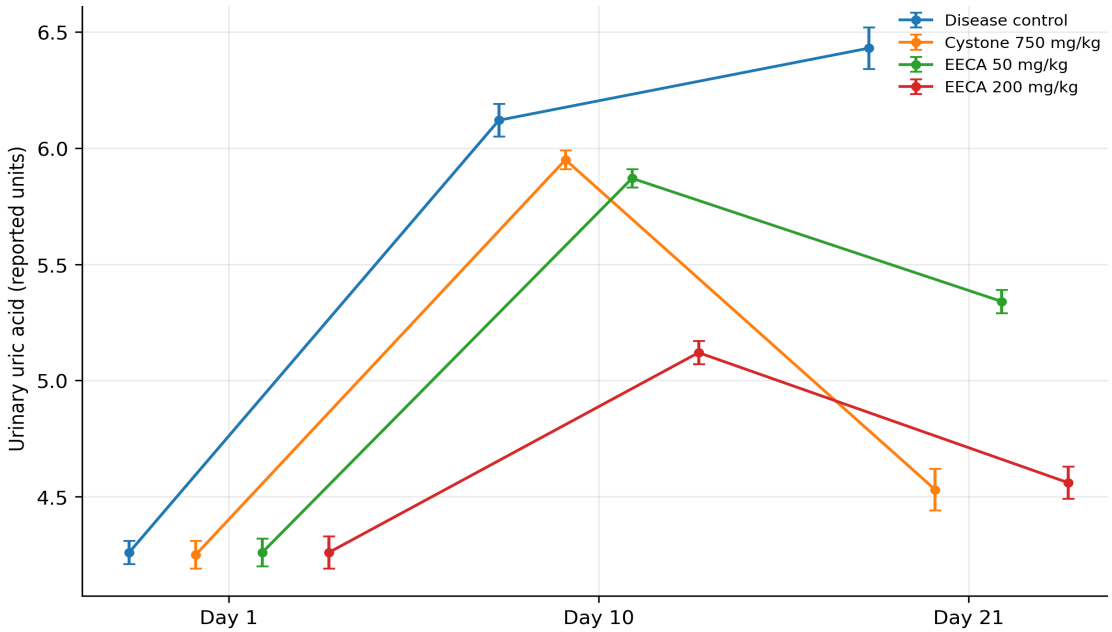


Figure 5. Effect of EECA on urinary uric acid at Days 1, 10, and 21.

Mean SD (n=3) is used to express the data.

3.8 Histopathology of the kidneys

According to histopathological investigation, the control group (D) had relatively maintained kidney structure, but the low-dose group (A) exhibited much greater tissue alterations. The high-dose (B) and standard-dose (C) groups had better renal structure and fewer obvious tubular abnormalities than the low-dose group. EECA has a dose-dependent protective effect against calcium oxalate-induced renal damage (Figure 6).

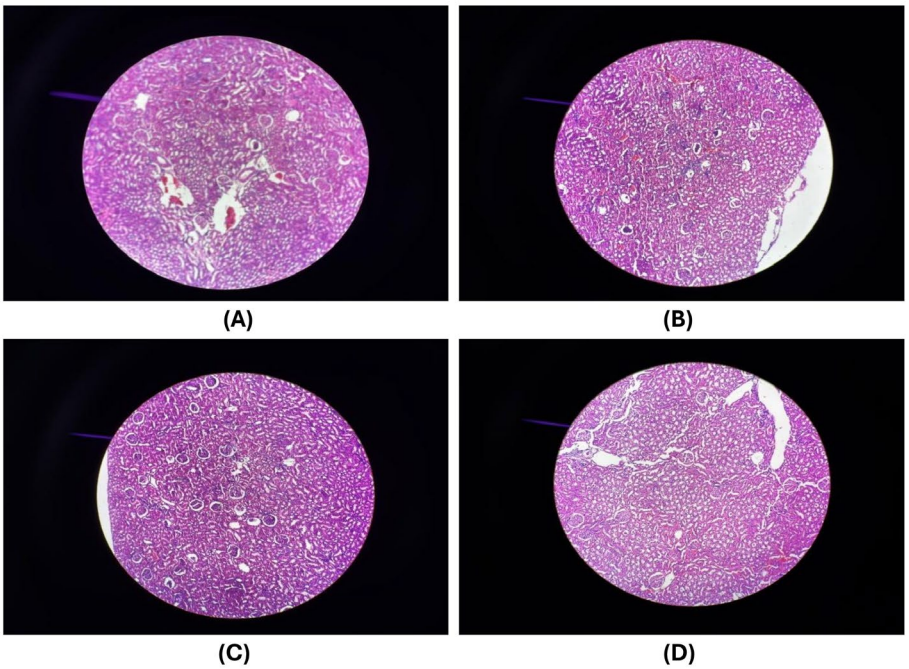


Figure 6. Histopathological examination of kidney sections from the experimental groups in the

calcium oxalate-induced urolithiasis model. **(A)** Low dose, **(B)** high dose, **(C)** Standard dose, **(D)** control

4. Discussion

The current study provides convergent preliminary evidence that EECA can improve some urine parameters and avoid CaOx crystallization in a rat model of experimentally induced urolithiasis. Concentration-dependent reduction of CaOx nucleation, which increases from 18.75% at 10 mg/mL to 77.50% at 100 mg/Ml is the quantitative result. The monotonic response and the drop in optical density from 0.650 to 0.180 imply a direct effect of increasing extract concentrations on the early crystallization process. Sharma et al. discovered that an aqueous leaf extract prevented CaOx crystallization, including effects on nucleation and aggregation, which is biologically consistent with previous studies on *C. album* . However, in an ethylene-glycol-induced rat model, Sikarwar et al. showed the preventive benefits of *C. album* leaf extracts and revealed decreases in renal oxalate deposition as well as biochemical abnormalities linked to urine and plasma stones [20,21]. Therefore, by analyzing an ethanolic leaf preparation and clearly measuring a concentration-response pattern for nucleation, the current study expands on the prior evidence.

In the nucleation assay, the reported 100 mg/mL EECA response (77.50%) was five percentage points higher than the reported Cystone response (72.50%). A second line of evidence is provided by the in vivo urine data. By Day 21, both EECA doses increased urine volume, with the 200 mg/kg group exhibiting an 83.8% higher value than disease control, while disease controls showed low and gradually decreasing urine volume. Because higher urine dilution may lower the likelihood of crystal supersaturation and retention, increased urine flow may be related to lithiasis. Urinary calcium, oxalate, citrate and creatinine, pH, renal oxalate content, and direct measurement of renal crystal deposition would provide greater mechanistic evidence, but urine volume alone cannot demonstrate a decreased stone burden.

A concurrent treatment-associated improvement was observed in urinary uric acid. By Day 21, EECA 50 and 200 mg/kg decreased the corresponding values to 5.34 ± 0.05 and 4.56 ± 0.07, while the disease-control value increased to 6.43 ± 0.09. Significant normalization of this endpoint was suggested by the high-dose value's proximity to the Cystone value. Sikarwar et al. showed that *C. album* leaf extracts reduced urine biochemical abnormalities in urolithiasis caused by ethylene glycol.
[21].

The phytochemical profile included phenols, alkaloids, flavonoids, and saponins. The hypothesis is that multiple secondary metabolites contribute to the observed activity. In order to standardize EECA, quantify important marker constituents, and then connect constituent abundance with in vivo efficacy and nucleation/growth inhibition, a more thorough high-impact-journal study would employ chromatographic identification.

Conclusion

Early integrated evidence given in this study suggests that the ethanolic leaf extract of *Chenopodium album Linn.* possesses anti-urolithiatic potential. EECA demonstrated concentration-dependent reduction of CaOx nucleation, reaching 77.50% at 100 mg/mL, and induced positive alterations in urine volume and urinary uric acid in rats with calcium oxalate-associated urolithiasis. The high-dose extract's histopathological examination also showed

comparatively improved kidney structure. These results validate the traditional significance of *C. album* and call for additional research. Future research should standardize phytochemicals, elucidate molecular pathways, identify active ingredients, incorporate thorough biomarkers for urinary stone risk, and verify safety and efficacy in studies.

Conflict of Interest

The author(s) declared no conflict of interest.

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Ethical Approval

The experimental animal study was conducted at Shri Shankaracharya Professional University in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and relevant guidelines for the care and use of laboratory animals. The study was conducted after obtaining approval from the Institutional Animal Ethics Committee.

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