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Research Article

ANTI-NEPHROLITHIASIS ACTIVITY OF ETHANOL EXTRACT OF *IPOMOEA CARNEA* JACQ. AGAINST ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN RATS

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ABSTRACT

Objective: Urolithiasis, one of the significant urological conditions, develops due to excessive calcium oxalate crystal accumulation, paving the way for various renal disorders. The use of conventional drug therapies often leads to numerous health consequences as far as their adverse effects are concerned, herbal interventions on the other hand have always been a ray of hope in that case. The present research investigated the therapeutic efficacy of ethanolic extract of *Ipomoea carnea* Jacq. (EEIC), in ethylene glycol (EG)-induced urolithiasis in rat animal models, diuretic potential, and antiurolithiatic activity were evaluated through the assessment of various biochemical and histopathological parameters.

Methods: Initially, key bioactive compounds were identified through phytochemical screening, qualitative analysis, and quantitative estimation of EEIC. Thirty, male Wistar albino rats were used in the study, divided into five groups, each group containing six animals. Group 1 (normal control), Group 2 (negative control: EG 0.75% v/v), Group 3 (positive control: Lumasiran 3 mg/kg), and Groups 4 and 5 (EEIC treated test groups: 250 mg/kg and 500 mg/kg., p.o., respectively). Different biochemical parameters were evaluated in urine, serum, and kidney homogenate after the 28th day. Pathological examinations of renal tissues were also performed.

Results: EEIC treatment demonstrated significant dose-dependent improvements in urinary and serum biomarkers linked to kidney stones and renal health. At doses of 250 and 500 mg/kg, urine volume significantly increased (2.17 mL, $p<0.01$; 2.35 mL, $p<0.005$), with enhanced Na+ and K+ excretion. After 28 days, urine output reached 4.31±0.38 mL ($p<0.005$), urine pH shifted from acidic (5.27±0.36) to alkaline (6.26±0.19, $p<0.05$), and levels of calcium, oxalate, and uric acid decreased significantly. EEIC also improved serum creatinine levels, renal tissue integrity, and supported magnesium and citrate increases, aligning with prior research.

Conclusion: The present study highlights that EEIC treatment improved serum creatinine levels, renal tissue integrity, and increased magnesium and citrate levels and has promising potential as a natural therapeutic alternative against urolithiasis, warranting further clinical investigation.

Keywords: *Ipomoea carnea* Jacq., Urolithiasis, Ethylene glycol, Diuretic properties, Phytochemicals.

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INTRODUCTION

Urolithiasis, also known as King's disease, is classified as the most common urological disorder possessing, a significant global public health concern. Urolithiasis creates pathological conditions due to improper regulation of stone-forming factors and obstructs normal urinary biochemical processes where hard deposits build up within renal parenchyma and calyces and urinary ducts ending at the bladder which results in painful conditions with bleeding risks and possible renal failure consequences [1]. Hyperoxaluria is a major cause of idiopathic calcium oxalate (CaOx) stone formation, as it increases urinary oxalate levels, promoting CaOx crystal formation and stone development. Factors contributing to urinary oxalate levels include hepatic oxalate synthesis, gastrointestinal absorption and bioavailability, as well as oxalate handling processes. The process of lipid peroxidation seems to play a role in renal cellular membrane disruption thus worsening the condition [2].

Extreme evolutionary change occurred concerning calculi prevalence patterns and incidence statistics during past periods. The prevalence rates of stone diseases in North America range between 7% and 13%, whereas European regions have 5% to 9% and Asian regions report rates from 1% to 5% [3]. Stone recurrence happens at a rate of up to 80% to 90% for 10 years and about 50% during 5 years. Even after surgical treatment, urolithiasis often occurs. Such recurrence can impair kidney function, escalate treatment costs, diminish quality of life, thereby increasing both public and private health expenses [4]. Urolithiasis treatment includes analgesics with anti-inflammatory drugs, diuretics and alkalizing agents as well as smooth muscle relaxants such as calcium channel blockers, alpha-blockers, and hyoscine butyl bromide, whose use leads to adverse effects such as dizziness and postural hypotension and flushing and ankle swelling [5,6]. Surgical procedures such as shock wave lithotripsy, ureteroscopy, and percutaneous nephrolithotomy have also been employed but are associated with multiple complications such as urinary tract infection, bleeding, ureteral injury, urinary retention, flank pain, and injury to nearby organs which may become the cause for relapse of the disease in future [7,8].

To overcome such complications, it is demanded to work with herbal options rather than conventional drug therapies with severe kinds of adverse responses and surgical procedures that may cause physical damage to kidneys and further cause relapses of the disease. India has a diverse history of utilizing ethnobotanical remedies for nephrolithiasis. For instance, *Boerhavia diffusa* is well known for its diuretic potential and is used for the management of kidney stones [9]. Likewise, *Tribulus terrestris* (Gokhru) has long been used for its diuretic and antiurolithiatic properties [10]. Another prominent medicinal plant used to cure kidney stones in Northeast India is *Bergenia ciliata* [11]. *Rotula aquatica* has also been used for the management of nephrolithiasis and bladder

stones [12]. Moreover, *Rubia cordifolia*, a traditional medicinal plant, has also been used in urolithiasis management [13].

The herbal extract of *Ipomoea carnea* Jacq., a morning glory species from the Convolvulaceae family, with major active phytoconstituents such as polyphenols and flavonoids, has been investigated in the present study for mitigation of urolithiasis. Historically, different parts of this plant have been used for their various medicinal properties. The latex part of the plant shows anti-inflammatory properties which are beneficial for wound treatment. In addition, the plant also exhibits aphrodisiac, purgative, and cathartic properties [14]. The present study was undertaken after a review of all the peculiar features of this plant concerning ethnobotanicals claims, which reported pharmacological study [15], and phytoconstituent profiling [16] which indicates its potential for the mitigation of urolithiasis that has still not been scientifically so far reported [17,18].

MATERIALS AND METHODS

Plant material

Whole plants of *Ipomoea carnea* Jacq. were collected locally from Kangra district, Himachal Pradesh, and the authentication verification was done by Dr. K.C. Bhatt, who serves as the Principal Scientist at the National Bureau of Plant Genetic Resources (ICAR), New Delhi, under reference number AC-84/2022.

Chemicals and glassware

Lumasiran, a marketed formulation of Oxlumo™, was utilized as the reference standard drug for this study. Ethylene glycol (EG) was administered to induce urolithiasis in an animal model. Diagnostic kits facilitated the biochemical analysis of urine and serum samples. In addition, laboratory-grade chemicals and reagents were employed for the phytochemical screening and the quantitative determination of phytoconstituents in the plant extract.

Experimental animals

Male Wistar albino rats weighing 200–250 g were employed for this study and housed inside polypropylene cages with husk bedding in standard environmental conditions, with a controlled temperature 25±2°C and relative humidity of 55±5%. The rodent subjects received standard pellet food and free water access during the study. The rats were acclimatized to standard laboratory conditions for one week prior to the start of the experiment. The study received approval from the Institutional Animal Ethical Committee under proposal number CCSEA/LIPH/2023/35, in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA) and the Organization for Economic Cooperation and Development (OECD).

Methods

Preparation of plant extract

Plant materials were cleaned, shadow-dried, and coarsely minced with a mechanical grinder. A 100 g sample of plant material was defatted with petroleum ether (60–80°C) and plant material was dried below 40°C and again extracted with 500 mL of 95% ethanol using a Soxhlet apparatus. The extract obtained was then filtered and subsequently concentrated by evaporating the solvent under reduced pressure with a rotary evaporator, resulting in the crude ethanolic extract. The extract (ethanolic extract of Ipomoea carnea Jacq. [EEIC]), obtained in this study was kept in a sealed container at 4°C until use.

Phytochemical screening and quantitative estimation of phytoconstituents

Qualitative analysis of phytochemicals

The prepared extract (EEIC) of the plant was analyzed to determine the presence or absence of the various phytochemical compounds, such as alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins, and polyphenols using the standard suggested methods followed by their quantitative analysis [19,20].

Standardization of extract

Determination of total polyphenolic content [21,22]

The phenolic content in EEIC was measured through the spectrophotometric Folin-Ciocalteu method, with gallic acid as the standard. Plant extract (0.5 mL) was combined with 3 ml of demineralized water, adding 0.25 ml of Folin-Ciocalteu reagent, and then vigorously shaken. The solution spent 5 min dark time before sodium carbonate (Na_2CO_3) solution concentration of 1 ml at 7.5% was incorporated. The tubes were then kept for incubation at room temperature for 90 min. Similarly, reagent blanks were prepared. Absorbance was measured at a λ max of 760 nm using a Shimadzu UV-1900i spectrophotometer, with reagent blanks as reference. The proportion of total phenolic compounds in the extract was expressed in milligrams of gallic acid equivalent (GAE) per 100 g of sample (mg GAE/100g), calculated using a linear calibration curve.

Determination of total flavonoid content [21,23]

The flavonoid content of the EEIC was measured using an aluminum chloride colorimetric assay. The test solution contained a mixture of 0.5 ml extract volume with 2 ml distilled water inside a 10 ml test tube. Each test tube was initially treated with 0.15 mL of a 5% sodium nitrite ($NaNO_2$) solution and incubated at room temperature for 5 minutes. Following incubation, 0.15 mL of a 10% aluminum chloride ($AlCl_3$) solution was added to each tube. Subsequently, 1 mL of 1M sodium hydroxide (NaOH) solution was introduced. After 1 minute, the total volume in each tube was adjusted to 5 mL by adding distilled water. The Shimadzu UV-1900i spectrophotometer was utilized to identify the maximum absorbance of the solution at a wavelength, λ max of 510 nm. The flavonoid content in the sample was measured using Quercetin equivalents, calculated from a linear calibration curve, and milligrams per 100 g of sample (mg QE/100 g sample).

Determination of total terpenoid content [24]

The total terpenoid content in the EEIC was quantified through a modification using a vanillin-H_2SO_4 assay. To prepare a 2% vanillin-H_2SO_4 reagent, 2 grams of vanillin dissolved in 100 ml 5% of H_2SO_4. The analytical procedure required mixing 1 ml of the reagent with 0.5 ml of the diluted extracts obtained through the methanol solution. Vigorous mixing of the solution occurred in an ice bath before it was transferred to a water bath and heated at 60°C for 20 min. The terpenoid content was then quantified using a Shimadzu UV-1900i spectrophotometer, at λ max = 608 nm which measured the total content in milligrams of linalool equivalent per 100 grams of sample (mg LU/100 g sample) through the assessment of calibration curve.

Determination of diuretic activity

The evaluation of diuretic effects from EEIC employed the procedure outlined by Lipschitz *et al.* [25,26]. The study of animal subjects included 24 rats weighing between 200 and 250 g which were distributed into four equal groups of six rats in each group. The rats underwent an 18-h fasting period before the experiment while they received only water for drinking. Group I (control) received an oral dose of 0.5% w/v CMC at 10ml/kg while Group II received 15 mg/kg furosemide orally. A single oral administration of 250 mg/kg and 500 mg/kg EEIC serving as treatments were granted to Groups III and IV, respectively [27]. Seven hours post-treatment, experimental animals were kept in a separate metabolic cage for urine sample collection, and the urine volume of each rat was measured after manually emptying the bladder by tail pulling from the tail base. The diuretic potential of EEIC was further analyzed by measuring urinary excretion and the concentrations of Na^+ and K^+ using an AVL 9180 Electrolyte Analyzer from Roche.

Determination of antiurolithiatic activity

The EG model of urolithiasis is a well-established method for inducing kidney stones in rats. In this model, rats were administered 0.75% v/v EG solution, in drinking water throughout the screening period, which causes hyperoxaluria and leads to the development of calcium oxalate

crystals in their kidneys, ultimately resulting in urolithiasis.

The study included 30 male Wistar albino rats weighing 200–250 gm, divided into five groups (n=6). Group I (normal control) received standard rat pellet food and water. Urolithiasis was induced in Groups II to V by administering 0.75% v/v EG in drinking water for 28 days of the experimental protocol, where the initial duration from day one to fourteen considered as lithiasis induction and subsequent next 14 days from day 15-28 as treatment period. Group II (negative control) served as the disease control. Group III (positive control) received a single subcutaneous dose of Lumasiran (3 mg/kg) on day 15 as the standard treatment. Groups IV and V were treated with EEIC at 250 and 500 mg/kg orally, respectively, from day 15 to 28 [28,29].

Laboratory investigation of various urinary diagnostic biomarkers (oxalate, calcium, phosphate, uric acid, urea, magnesium, and citrate) and creatinine clearance was done on fourteen and twenty-eight day through analysis of urine samples collected from all research subjects. Investigations also include the measurement of urine volume and pH values. Blood samples were collected from anesthetized animals via the retro-orbital plexus on days 14 and 28. The research analyzed blood samples to determine systemic metabolic alterations of lithiatic key biomarkers such as calcium, creatinine, phosphate, uric acid, urea, citrate, and magnesium concentrations within serum. In addition, at the end of the screening protocol on day 28, each animal was euthanized, and both kidneys were extracted for histopathological examination to assess histological changes including detection of crystal precipitation within the renal architectural organization. The kidney homogenate was subsequently examined for calcium, oxalate, uric acid, phosphate, and catalase activity to validate the findings from the urine and serum analysis [30,31].

Estimation of biochemical parameters [32]

Urine collection and analysis

Urine samples were collected on days 14 and 28 by individually housing the animals in metabolic cages, following the experimental protocol. The samples were collected and received thymol drops as a preservative. The diagnostic kit analyzed the oxalate and calcium content with essential urolithiasis markers by following the manufacturer's guidelines. The pH measurement of fresh urine samples within each group took place utilizing a well-calibrated digital pH meter.

Serum collection and analysis

Blood specimens were collected from the rats through the retro-orbital plexus using a capillary under mild anesthesia. The samples were centrifuged at standard condition 4000 rpm for 10 min to minimize hematocyte damage. The serum was analyzed for urolith markers like calcium, phosphate, uric acid, urea, creatinine, and magnesium, which have a key role in urolithiasis. All parameters were examined using a diagnostic kit as per the prescribed instructions.

Kidney histopathology and homogenate analysis

On the 28th day, all animals were euthanized, and their kidneys were harvested. The kidney tissue underwent ice-cold saline rinsing for total removal of any surrounding tissue. After tissue harvesting, kidney specimens were preserved in 10% v/v neutral formalin solution before embedding in paraffin. Each thin vertical slice of the organ measured 5 µm before subjecting them to hematoxylin-Eosin staining for analysis. Light microscopic analysis of a kidney section revealed the extent of crystal deposits with all recorded pathological changes. A 20% kidney homogenate was prepared using finely minced kidney tissue in Tris-HCl buffer at pH 7.4. The homogenate was centrifuged at 2000 rpm for 10 min, after which the supernatant was collected. An analysis using biochemical kits determined the concentrations of oxalate, calcium, phosphate, uric acid, and catalase in the supernatant extracted from the kidney homogenate preparation.

Data analysis

Data on various parameters were expressed as the mean±standard deviation and subjected to ANOVA to evaluate statistical significance among different groups. P-value below 0.05 was considered as statistically significant. Overall, this comprehensive analysis evaluated the efficacy of the whole plant EEIC in preventing and treating EG-induced urolithiasis in a rat model.

RESULTS

Extraction of EEIC

The extract was successfully obtained with a yield of 9.23% w/w. and underwent both qualitative and quantitative evaluation.

Qualitative analysis of phytochemicals

Phytochemical screening of EEIC revealed an abundance of diverse bioactive compounds such as alkaloids, terpenoids, steroids, glycosides, tannins, and polyphenols (Table 1).

Quantitative analysis of phytochemicals

Quantitative estimation of phytochemicals for EEIC revealed the presence of various phytoconstituents with concentrations of polyphenols (208.45±0.06 mg/g), flavonoids 1(219.8±0.07 mg/g), and terpenoids (106.5±0.06 mg/g) (Table 2).

Diuretic efficacy of EEIC

At 7 h post-administration of 15 mg/kg of the reference drug (furosemide) demonstrated a significant rise in urine volume, having a diuretic index of 4.18 and marked elevations in sodium and potassium concentrations compared to various doses of EEIC at 250 and 500 mg/kg, resulting in diuretic indices of 1.53 and 1.64, respectively, significantly increases urine volume of 2.17 mL (250 mg/kg), 2.35 ml (500 mg/kg), sodium concentration of 64.16 mEq/L (250 mg/kg), 64.37 mEq/L (500 mg/kg), and potassium concentration of 10.39 mEq/L (250 mg/kg), 10.62 mEq/L (500 mg/kg). Result findings indicated a dose-dependent increase in the diuretic activity of EEIC, comparable to the standard drug, as depicted in Fig. 1.

Effects of EEIC on various urine parameters against EG-induced urolithiasis

Following the induction of lithiatic condition with EG on the 14th day, the disease control group exhibited a significant decrease in urine volume and altered levels of urinary biochemical markers such as oxalate, calcium, uric acid, phosphate, urea, magnesium, and creatinine

Table 1: Phytochemical screening of EEIC (whole plant)

S. No.	Group of compounds	Observation EEIC
1.	Carbohydrate	-
2.	Alkaloid	+
3.	Flavonoid	+
4.	Saponin	-
5.	Steroid	+
6.	Terpenoid	+
7.	Glycoside	+
8.	Tannin	+
9.	Polyphenols	+
10.	Protein	-

(+: Indicates presence, -: Indicates absence). EEIC: Ethanolic extract of *Ipomoea carnea* Jacq.

Table 2: Quantitative phytochemical estimation of EEIC

S. No.	Ethanolic extract	Polyphenols (mg GAE/ 100 g)	Flavonoids (mg QE/ 100 g)	Terpenoids (mg LU/ 100 g)
1.	*Ipomoea carnea* Jacq.	208.45±0.06	219.8±0.07	106.5±0.06

(GAE: Gallic acid, QE: Quercetin, LU: Linalool). EEIC: Ethanolic extract of *Ipomoea carnea* Jacq. Data is represented as Mean±standard deviation (n=3)

as compared to that of the normal control group. At the end of protocol on the 28th day, on treatment with the standard drug and EEIC (250 and 500 mg/kg), the standard group demonstrated significant restoration of urine volume and near normalization of urinary biochemical markers. Treatment with the EEIC showed dose-dependent improvements in these urinary parameters, the higher dose (500 mg/kg) produced more desirable outcomes than the lower dose (250 mg/kg) for managing urolithiasis. Furthermore, notable improvements in urinary pH, calcium, and citrate concentrations toward normal levels were observed (Fig. 2).

Effects of EEIC on various serum parameters against EG-induced urolithiasis

Initially, the disease control group showed significantly raised serum levels of phosphate, calcium, uric acid, creatinine, and urea on day 14 (Induction), confirming the establishment of a pathological state. By the 28th day, treatment with the standard drug had effectively normalized the levels of abnormal concentrations. Treatment with the test drug at doses of 250 and 500 mg/kg resulted in a dose-dependent improvement of renal calculus serum biomarkers. Notably, a higher dose was more effective, dropping the calcium levels to 13.58 mg/dL compared to 16.52 mg/dL found in the lower dose. Phosphate and uric acid levels similarly improved, with the higher dose yielding phosphate levels of 7.35 mg/dL and uric acid levels of 7.43 mg/dL. Creatinine levels also significantly reduced, particularly with the 500 mg/kg dose reaching 2.67 mg/dL (Fig. 3).

Effects of EEIC on various kidney homogenate parameters against EG-induced urolithiasis

The diseased group exhibited significantly raised levels of nephrolithiasis biomarkers phosphate, calcium, uric acid, and

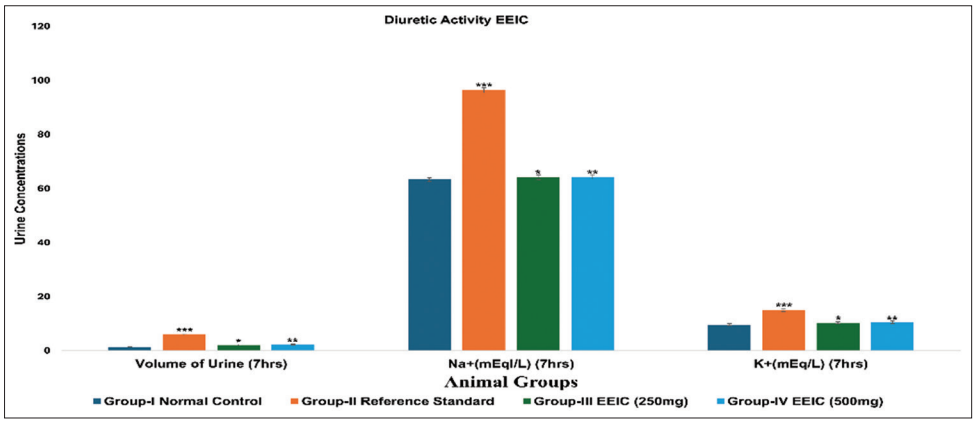

Fig. 1: The urinary parameters including the urine volume with sodium and potassium contents underwent evaluation following ethanolic extract of *Ipomoea carnea* Jacq. treatment: All statistical analyses were performed through ANOVA along with Dunnett's test for evaluation. Significance thresholds are indicated as p<0.001(***), and p<0.005 (**), and p<0.01 (*)

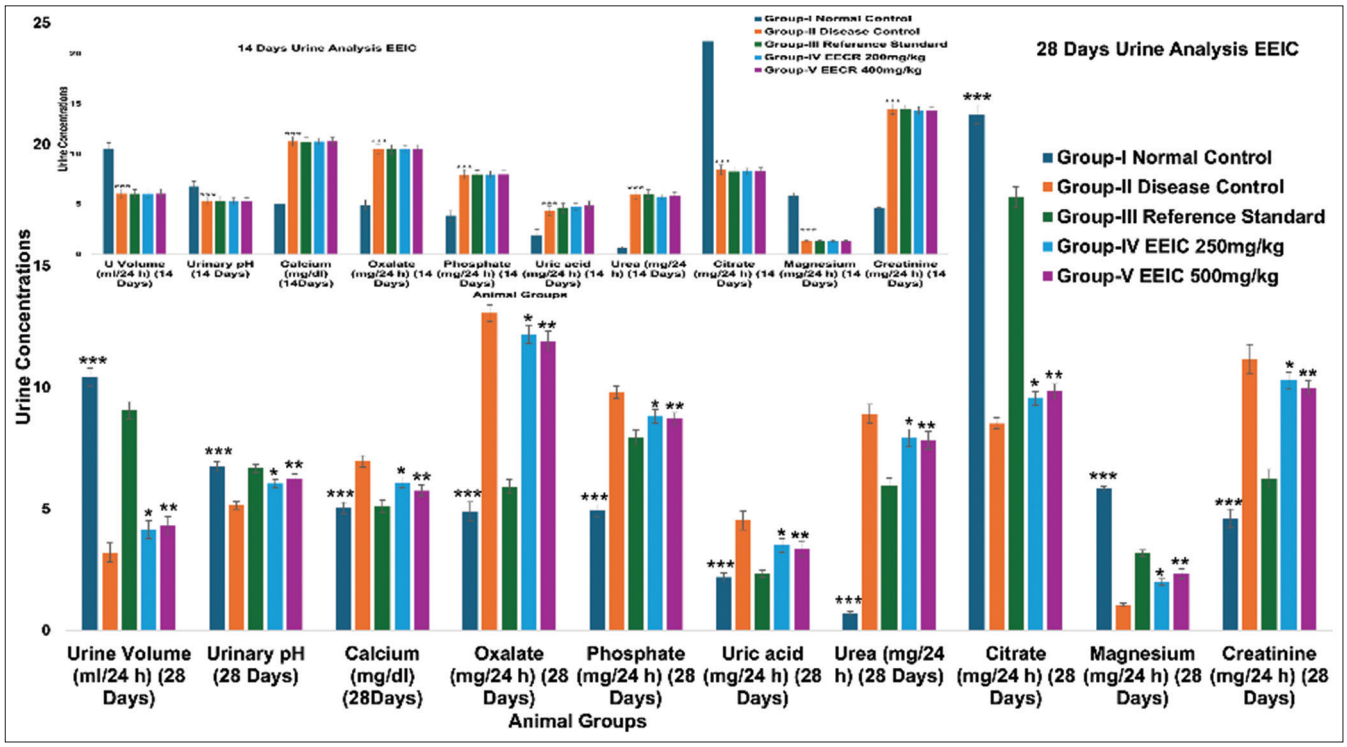

Fig. 2: Analysis of effects of ethanolic extract of *Ipomoea carnea* Jacq. on urine parameters in EG-induced urolithiasis: All values are stated as mean±SD (n=6). A two-way ANOVA was monitored by Dunnett's test for multiple comparisons. Significance levels are indicated as ###p<0.001 and #p<0.05 for comparisons between normal and disease groups; ***p<0.001, *p<0.05, and **p<0.005 for comparisons between the disease and all other groups

oxalate, markedly reduced catalase activity in kidney homogenate, confirming the induction of renal dysfunction. Treatment with the standard drug significantly ameliorated the concentrations of these biomarkers and improved catalase activity. The test drug-treated groups, administered at doses of 250 mg/kg and 500 mg/kg, exhibited dose-dependent improvements in biochemical corrections. Notably at higher doses, calcium levels decreased to 7.79 mg/gm tissue, while phosphate levels dropped to 5.16 mg/gm tissue. Uric acid and oxalate levels similarly improved, and catalase activity increased to 1.11 nmoles of H_2O_2 utilized/min/mg protein, approaching normal levels (Fig. 4).

Histopathology of harvested kidneys for the investigation of EEIC effects against EG-induced urolithiasis

The induction of renal urolithiasis by EG resulted in significant histological alterations. Microscopic examination of kidney histological sections revealed normal architecture in Group I normal control (Fig. 5a). In Group II disease control (negative control), histological analysis detected the existence of calcium oxalate crystals and extensive impairment of the medulla, glomeruli, tubules, and interstitial spaces. The study also revealed intrusion of interstitial mononuclear cells and inflammatory cells, along with significant histological alterations, including tubular dilatation and atrophy, as depicted in Fig. 5b. In Group III the standard group (Positive Control) treated with Lumasiran showed no crystal deposits and renal damage from tubular atrophy was restored to normal levels (Fig. 5c). In Group IV treatment Group I (250 mg/kg/p.o.), and Group V treatment Group II (500 mg/kg/p.o.) treated with EEIC, microscopic examination revealed significant amelioration of kidney damage. This improvement was accompanied by a decreased presence of calcium oxalate crystals in intratubular space and restoration of tubular structures proportionally to the dose, compared with Group II (disease control) (Fig. 5d and e).

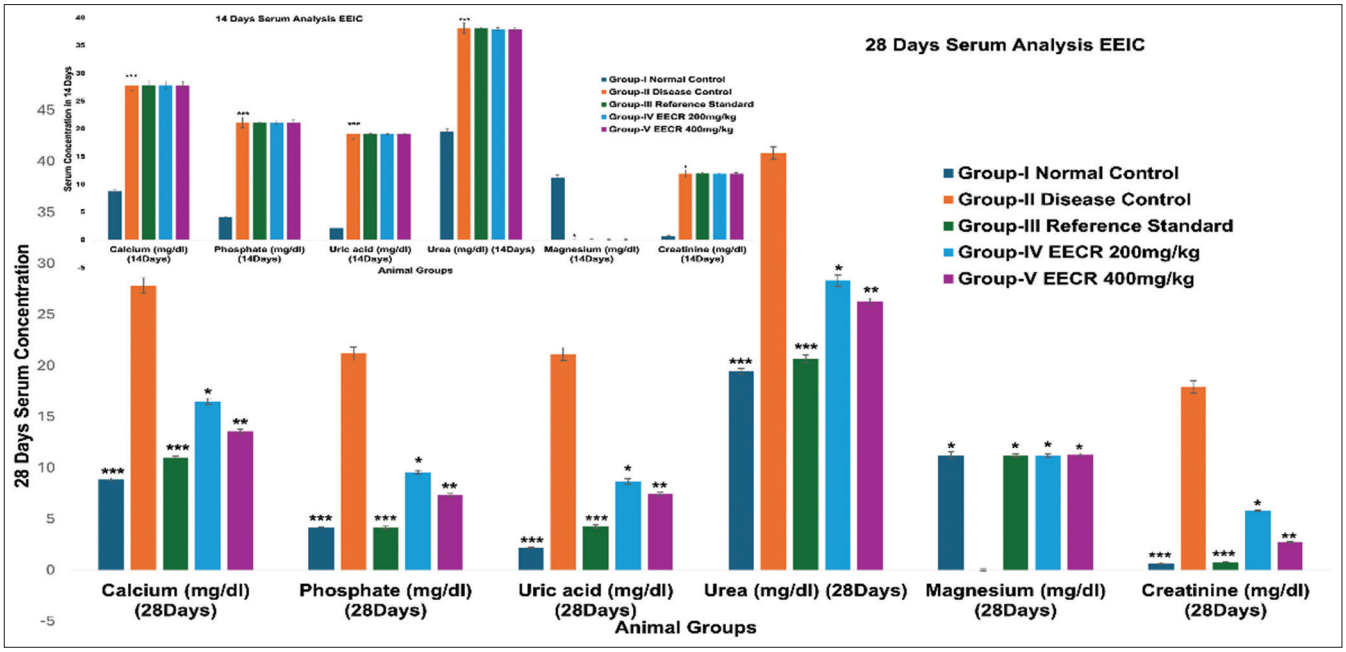

Fig. 3: Analysis of effects of ethanolic extract of *Ipomoea carnea* Jacq. on various serum markers in ethylene glycol-induced urolithiasis; all values are reported as mean±SD (n=6). A two-way ANOVA proposed by Dunnett's test was used for data analysis and interpretation. Significant differences are indicated as; $^{###}$p<0.001 and $^{#}$p<0.05 for comparisons between normal and disease groups, ***p<0.001, *p<0.05, and **p<0.005 for comparisons between disease and other groups

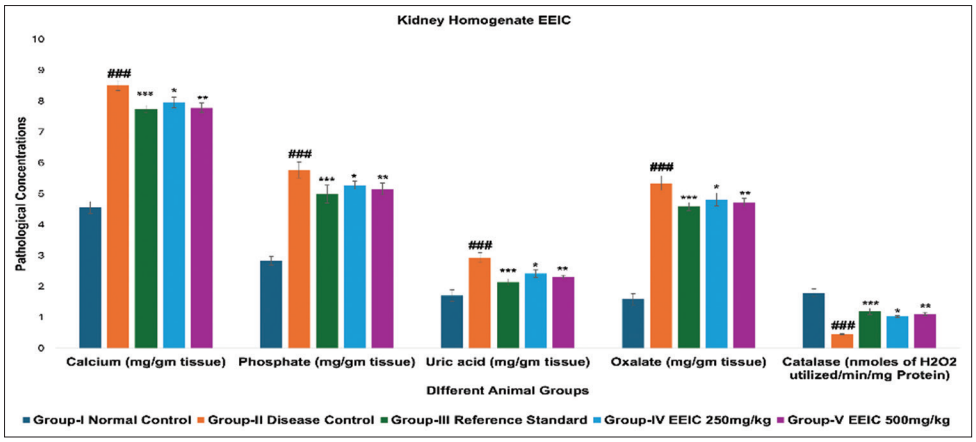

Fig. 4: Analysis of effects of ethanolic extract of *Ipomoea carnea* Jacq. on kidney homogenate parameters in the context of EG-induced urolithiasis: All values are presented as mean±SD (n=6). A two-way ANOVA followed by Dunnett's test was performed to analyze various kidney homogenate parameters. Significant levels are indicated as; $^{###}$p<0.001 and $^{#}$p<0.05 for comparison between normal and disease groups, ***p<0.001, *p<0.05, and **p<0.005 for comparison between disease and other groups

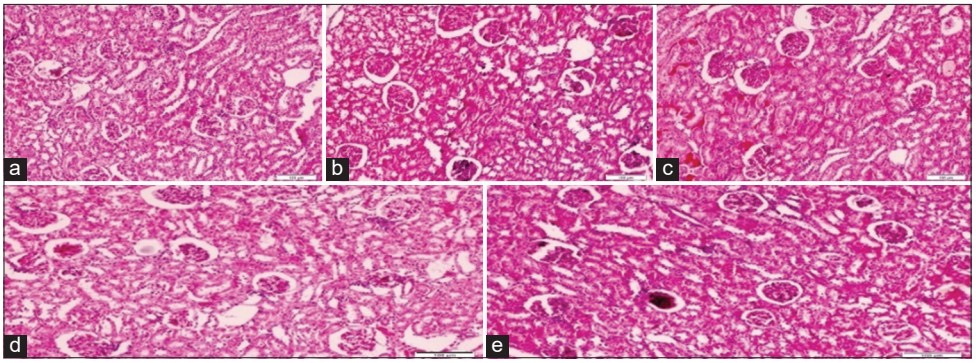

Fig. 5: Histology of the kidney: (a) Group I-Normal control; (b) Group II-disease Control (Negative control); (c) Group III-Standard group (positive control), treated with Lumasiran (3 mg/Kg/S.C.); (d) Group IV-treatment Group I, treated with EEIC 250 mg/kg orally; (e) Group V-treatment Group II, treated with ethanolic extract of *Ipomoea carnea* Jacq. 500 mg/kg/orally

DISCUSSION

The EEIC demonstrates a robust profile of phytochemicals with significant antioxidant and hepatoprotective properties, as evidenced through qualitative and quantitative analysis. The presence of critical bioactive compounds such as terpenoids, alkaloids, steroids, polyphenols, glycosides, and tannins is significant, with quantified levels showing 208.45 mg GAE/100 g of polyphenols, 219.8 mg QE/100 g of flavonoids, and 106.5 mg LU/100 g of terpenoids. These findings are consistent with recent studies that outline similar pharmacological potentials of plant extracts. For example, a study published in *Heliyon A Cell Press Journal* (2024) illustrates the antioxidant capabilities of flavonoids from other plant sources, showing their efficacy in mitigating oxidative stress [33]. Another research article in the Journal of Ethnopharmacology (2022) highlights the hepatoprotective properties of polyphenols found in different botanical extracts, reinforcing the therapeutic potential seen in EEIC [34]. Further, a study in Biomedicine and Pharmacotherapy (2023) explores how terpenoids exert hepatoprotective effects, offering insights that parallel the protective mechanisms observed with EEIC's terpenoid content [35]. Moreover, the presence of terpenoids in EEIC, which possesses anti-inflammatory activity, also highlights the plants' potential to protect the renal tissue. As per the previous studies, it has been seen that terpenoids inhibit the proinflammatory cytokines, thereby reducing renal tissue inflammation [36]. This suggests that the dual role of EEIC is not limited to mitigating oxidative stress but also to reducing inflammation, which could be a good approach to managing urolithiasis. A comprehensive review in Phytotherapy Research (2021) discusses the systemic roles of glycosides and tannins in reducing liver fibrosis, which aligns with the hepatoprotective findings related to EEIC [37]. These studies collectively support the potential health benefits of EEIC, highlighting its relevance in therapeutic applications against oxidative stress-induced cellular damage and hepatic dysfunction, possibly by modulating oxalate levels through hepatic peroxisomal glycolate metabolism.

Recent studies have demonstrated how well a wide range of medicinal plants can treat urolithiasis through their diuretic properties. A thorough study published in the *Journal of Nephrology* highlighted that plant-based therapies lower the risk of nephrolithiasis by increasing urine production and modulating urine pH [38]. Likewise, research in the *Journal of Pharmacology and Toxicology* revealed that plant extracts such as *Zea mays* and *Amni visnaga* have strong diuretic effects which add to their antiurolithiatic activity [39]. The diuretic efficacy of the EEIC was evaluated and compared with standard diuretic furosemide. Significant dose-dependent diuretic effects from EEIC were examined at dosages of 250 and 500 mg/kg, with EEIC increasing urine volume to 2.17 mL ($p<0.01$) and 2.35 mL ($p<0.005$), respectively, alongside moderate increases in urinary Na^+ and K^+ excretion, indicating its potential as a natural diuretic agent. These results are consistent with recent research emphasizing the benefits of plant-based diuretics. For instance, a study from the *Journal of Ethnopharmacology* (*2024*) demonstrated the diuretic properties of *Hedyotis scandens extract*, highlighting the role of natural phytochemicals in enhancing renal function and fluid excretion [40]. Furthermore, research published in Frontiers in Pharmacology (2020) explored the renal protective effects and diuretic action of phytochemicals, showing their efficacy in promoting urine production and electrolyte excretion with insignificant side effects compared to synthetic diuretics [41]. Another study from the Journal of Natural Products (2023) reported the clinical efficacy of traditional and innovative plant-based diuretics in managing electrolyte balance and facilitating natriuresis, supporting their therapeutic potential [42]. Furthermore, a review article in Journal Nutrients (2022) discussed the long-term safety and clinical applicability of natural extracts as diuretic alternatives, promoting their use in medical practice for conditions requiring diuresis [43]. These studies collectively underscore the promising role of EEIC and similar phytochemical-rich plant extracts in therapeutic applications, particularly in scenarios where safe and effective diuresis is needed, advocating for their expanded research and development in clinical settings.

Recent studies have proven the efficacy of certain medicinal plants in treating urolithiasis by altering urine parameters and improving diuretic activity. A study demonstrated that extracts from various medicinal plants have a significant impact on reducing crystalluria [44]. Like this, a thorough analysis emphasized how herbal drugs can cure urolithiasis by changing urinary ion composition, offering diuretic effects, and providing antioxidant advantages [45]. In this study, the anti-urolithiatic impacts of EEIC were estimated. Significant effects on urinary parameters suggest their potential to mitigate stone formation by modulating solute excretion and stabilizing pH. EEIC treatment led to a moderate increase in urine volume and a shift toward a more alkaline urinary pH, both critical factors in preventing urine supersaturation and subsequent crystal formation. Specifically, EEIC enhanced urine volume to 4.31±0.38 mL ($p<0.005$) at 28 days and adjusted urinary pH from a more acidic 5.27±0.36 to a less conducive stone-forming environment at 6.26±0.19 ($p<0.05$). These results are aligned with recent emphasizing the crucial role of urine volume and pH in managing urolithiasis. A study from the *Journal of Nephrology* (2010) mentions the diuretic and pH-modulating properties of herbal extracts [46].

Furthermore, EEIC demonstrated the capacity to reduce concentrations of calcium (5.76±0.23 mg/dL, $p<0.05$), oxalate (11.50±0.43 mg/24 h), and uric acid (3.36±0.29 mg/24 h, $p<0.05$), which are principal components in kidney stone formation. This aligns with the mechanisms reported in recent publications like those by Patel *et al.* (2022) in their exploration of natural inhibitors of oxalate synthesis [28]. EEIC also improved levels of magnesium (2.35±0.19 mg/24 h, $p<0.05$) and citrate (9.84±0.31 mg/24 h, $p<0.05$), both known inhibitors of calcium stone formation, as discussed in various studies that revealed the roles of citrate in preventing calcium oxalate aggregation [47,48]. The findings of this research suggest that significant restoration of normal oxalate levels indicates hepatocellular function restoration efficiency of EEIC.

In case of hepatic dysfunction hepatic peroxisomes fail to metabolize glyoxylate to glycine efficiently and consequently glyoxylate gets metabolized to oxalate a key lithiatic biomarker, this enhanced oxalate level is responsible for the condition considered as hyperoxaluria. Thus, the hepatoprotective potential of EEIC counteracts this hepatic metabolism and demonstrates its efficient therapeutic role in the management of nephrolithiasis in a dose-dependent manner [49].

Moreover, the observed reductions in urea (7.82±0.37 mg/24 h, $p<0.05$) and creatinine levels (9.99±0.29 mg/24 h, $p<0.05$) suggest a protective effect of EEIC on renal function, which is supported by findings from various studies evaluating the renal protective effects of herbal compounds in urolithiasis models [50]. These multifaceted effects of EEIC highlight its capability as a holistic approach to the prevention and management of urolithiasis, advocating for further clinical investigation to fully understand its mechanisms and optimize its use in urological practice.

The findings of this study are consistent with recent research on the role of natural compounds in regulating serum parameters associated with kidney stone formation. The reduction in calcium and phosphate levels was observed in this study, where EEIC decreased calcium to 13.58±0.11 mg/dL and phosphate to 7.35±0.14 mg/dL at protocol of 28 days, which is consistent with the outcomes of research conducted in various studies, which reported that certain plant-based therapies are beneficial to regulate calcium-phosphate metabolism to mitigate hypercalcemia and its role in stone formation [50,51]. Similarly, the observed reductions in uric acid levels to 7.43±0.08 mg/dL align with findings in a 2012 study in the Journal of Functional Foods that documented the ability of polyphenol-rich plant extracts to reduce serum uric acid through inhibition of xanthine oxidase, a critical enzyme in uric acid synthesis [52]. The decrease in urea levels in our study, suggesting improved kidney function, mirrors the results from a study in the *Journal of Young Pharacists* (2011), where herbal extracts demonstrated nephroprotective effects and enhanced renal clearance [53]. The restoration of magnesium levels to 11.26±0.13 mg/dL in our study supports the findings of research work in the *Journal of Ayurveda and Integrative Medicine* (2017), which emphasized the role of magnesium in binding urinary oxalates, thus reducing calcium oxalate stone formation [29]. Furthermore, a significant reduction in creatinine levels to 2.76±0.05 mg/dL was observed by EEIC [54]. These comparative insights validate the ability of EEIC to promise a holistic approach for urolithiasis, with effects consistent with other plant-based interventions reported in the literature. This underscores the growing recognition of phytochemical-rich therapies in nephrology and their translational potential in clinical applications.

The histological assay carried out in the present study with EEIC is consistent with findings in this literature that highlight the anti-inflammatory and renal tissue-protective effects of plant-based therapeutics. The significant reduction in calcium oxalate crystal deposition and amelioration of tubular abnormalities by EEIC in a dose-dependent manner mirrors findings by the various studies, where phytochemicals in plant extracts were reported to dissolve intratubular crystal deposits and improve histological integrity through anti-inflammatory mechanisms [55,56]. Furthermore, the anti-inflammatory properties of EEIC, as evidenced by diminished interstitial mononuclear and inflammatory cell infiltration, align with the observations made by different studies. These studies demonstrated that secondary metabolites in botanical extracts reduced oxidative stress and inflammation, crucial factors in preventing tubular damage and crystal retention [57,58].

The tubular restoration and reduced atrophy seen in the present study are comparable to findings in a 2022 study in Phytomedicine by Sharma *et al.,* which highlighted the ability of flavonoids and polyphenols to restore tubular architecture and reduce inflammatory markers in EG-induced urolithiasis models [59-61]. The consistency of our findings with existing literature strengthens its potential for further exploration in preclinical and clinical studies, particularly in its role as a natural anti-inflammatory and renal tissue restorative agent.

CONCLUSION

The EEIC demonstrates significant anti-urolithiatic, antioxidant, nephroprotective, and diuretic properties. It effectively reduces calcium oxalate crystal deposition, mitigates oxidative stress, restores renal and hepatic function, and enhances urine output, thereby contributing to pH stabilization and improved solute excretion. Its rich phytochemical profile, including polyphenols, flavonoids, and terpenoids, supports these therapeutic effects, aligning EEIC as a potential natural alternative to standard therapies. Future research should focus on clinical validation, bioavailability studies, and exploring synergistic applications with other phytotherapeutics to optimize its role in managing urolithiasis and associated renal and hepatic disorders.

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AUTHORS' CONTRIBUTIONS

Dr. Madan L. Kaushik conceptualized and supervised the study, guided the experimental design and data analysis, and reviewed the manuscript. Nishant Goutam performed the study, conducted experiments, analyzed data, interpreted results, and prepared the manuscript. All authors were involved in the critical revision of the work for intellectual content and have approved the final version of the manuscript for submission. Dr. Mahendra Singh Ashawat reviewed the manuscript.

CONFLICTS OF INTEREST

Not applicable.

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