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Original Article

Tamarindus indica L. Seed Hydroalcoholic Extract as a Natural Inhibitor for Urolithiasis: Evidence from *in vitro* and *in vivo* Studies

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ABSTRACT

Background: Urolithiasis remains a recurrent condition that affects patients' quality of life and causes significant mortality. Once common in Afro-Asian regions, its prevalence is rising in India and other tropical countries. **Objectives:** This study explores the therapeutic potential of *Tamarindus indica* L. Seed Hydroalcoholic Extract (TISHE) against urolithiasis. **Materials and Methods:** *In vitro* dissolution assays demonstrated TISHE's capacity to dissolve crystals of calcium oxalate and calcium phosphate. An *in vivo* rat model of urolithiasis was induced by administering 0.75% Ethylene Glycol (EG) over 28 days, resulting in calcium oxalate crystal formation and altered urine parameters. From day 15 to 28, rats received TISHE at doses of 200 and 400 mg/kg, and treatment with a standard Cystone® tablet (150 mg/kg). **Results:** TISHE treatment significantly improved urinary biochemical markers, increasing creatinine and magnesium while reducing calcium, calcium oxalate, phosphorus, urea, and uric acid levels ($p<0.001$). Plasma analysis revealed normalized electrolytes and nitrogenous waste. Oxidative stress markers showed decreased malondialdehyde and increased glutathione and catalase in kidneys, indicating amelioration of oxidative damage ($p<0.001$). **Conclusion:** TISHE demonstrated potential in dissolving kidney stones, restoring biochemical balance, and protecting renal tissue from oxidative injury, suggesting its promise as a therapeutic agent in urolithiasis management.

Keywords: Bio-chemical Analysis, Kidney Stone, Lithogenic Compounds, Oxidative Stress, Urological Disorder.

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INTRODUCTION

Urolithiasis refers to stones formation (calculi) within kidneys, bladder, or urethra, and is recognized as a multifarious urological disorder. The term originates from the Greek words ouron (urine), oros (flow), and lithos (stone). This condition affects individuals across all age groups children, adolescents, and adults and is eminent for its high incidence and prevalence globally. The occurrence of urolithiasis differs across regions and populations. In North America, it affected about 8.8% of people between 2007 and 2010, while in Europe it was about 5 -10% before 2011, and in Asia about 1-5%. Worldwide, the rate is estimated to be between 1% and 5%, but it is higher in wealthy countries (2-13%) compared to developing ones like (0.5-1%) (Sorokin *et al*., 2017). These differences are likely due to multiple contributing reasons such as climate, diet, water intake, water composition, genetic predisposition, occupational risks, urinary tract infections, gender and age. Although kidney stones have traditionally been more common in Afro-Asian countries, they are now becoming more frequent in tropical and sub-Saharan African regions (Kassaw *et al*., 2024). This rise is linked to increasing global temperatures and improving socioeconomic conditions, which have led to more Western-style diets and lifestyles (Namdeo *et al*., 2022; Watson *et al*., 2022).

Kidney stone formation often results from disturbances in renal acid-base handling, abnormal metabolite excretion, or excessive gastrointestinal absorption of stone-forming substances. These disruptions lead to the accumulation of lithogenic compounds. The process initiates when urinary crystals precipitate in a supersaturated environment, adhering to the urothelial lining, which acts as a nucleation site for further crystal growth (Dawson and Tomson, 2012; Tamborino *et al*., 2024). Factors such as organic molecules (e.g., albumin and phospholipids) and increased expression of cell-surface adhesion proteins enhance the propensity for crystal attachment and stone maturation (Asselman *et al*., 2005). The composition of uroliths is variable and may include Calcium Oxalate (CaOx), magnesium ammonium phosphate, calcium hydrogen phosphate, urates,

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or uric acid (Allam, 2024; Shastri *et al*., 2023), with CaOx being the predominant constituent in approximately 75-90% of all cases (Allam, 2024). Upon adhesion, crystals can induce cellular damage through mechanisms such as oxidative stress, mitochondrial dysfunction, inflammation, and disruption of cellular membranes. These events can similarly contribute to the development of urinary casts, which may hamper urinary flow and supports development of stone (Sun *et al*., 2024).

The most common symptom of urolithiasis is an episode of sudden, intense visceral pain, often accompanied by hematuria. This pain is typically colicky in nature, fluctuating in intensity, and can shift in location as the stone moves through the urinary tract (Shastri *et al*., 2023). The condition can substantially disrupt daily functioning and overall quality of life. If left unmanaged, it may progress to complications such as urinary tract infections or acute kidney injury (Shastri *et al*., 2023). The treatment approach is largely influenced by the size and position of the calculi. Stones larger than 5 mm or those that are unable to pass naturally may require interventional procedures like lithotripsy or laparoscopic surgery (Jiang *et al*., 2021). However, these interventions can be costly, carry a risk of renal damage, and are associated with a high recurrence rate, often within five to ten years (Scales *et al*., 2012; Pearle *et al*., 2014; Konnopka *et al*., 2022).

Since ancient times, herbal therapies have been utilized in the treatment of renal calculi, and several plant-based formulations continue to show potential benefits in this domain (Movaliya and Gohel, 2024). Many of these antiurolithiatic herbs function by promoting diuresis, thereby facilitating the elimination of stone-forming substances through increased urinary flow. This elevated urine output reduces crystal nucleation by lowering solute supersaturation and maintaining an optimal physiological pH. In addition to promoting urinary excretion, certain natural compounds can inhibit crystal formation or exhibit lithotriptic properties, helping to break down or prevent the aggregation of existing stones (Charde *et al*., 2011; Shukla *et al*., 2017). Furthermore, some herbs are known to support renal function and modulate oxalate metabolism, thereby reducing the likelihood of stone recurrence (Pareta *et al*., 2011). Experimental studies in both human and animal models have highlighted the role of natural antioxidants such as phenolics and flavonoids in neutralizing free radicals, thus preventing oxidative stress-induced stone pathogenesis (Raj *et al*., 2024). Certain medicinal plants and their polyherbal formulations present promising, less invasive alternatives to surgical interventions for both the treatment and prevention of kidney stones (Allam and Sabra, 2024).

Tamarindus indica L. (*T. indica*), commonly referred to as the tamarind tree, is a tall fruit-bearing plant that can grow up to 24 M in height and reach a girth of 7 M. It belongs to the Fabaceae (Leguminosae) family and is widely spread across Africa, the Indian subcontinent, and various tropical regions (Bhadoriya *et al*., 2011). The fruits of *T. indica* L. are commonly used as a laxative and febrifuge across the Sahel and Sudan ecological zones in Africa. In central West Africa, tamarind bark and leaves are frequently applied in the treatment of wounds. The bark serves as a remedy for diarrhoea in West Africa, whereas in East Africa, the leaves are used for the same purpose (Havinga *et al*., 2010). This evergreen species features small leaves, pale yellow to pink blossoms, and sour fruit pods. Various plant parts including its flowers, leaves, fruits, roots, and seeds are traditionally used for their medicinal properties and nutritional benefits (Kuru, 2014). Phytochemical investigations have identified multiple bioactive compounds in *T. indica*, including phenolics, cardiac glycosides, tartaric acid, malic acid, uronic acids, mucilage, pectin, and sugars such as arabinose, xylose, galactose, and glucose. Notably, the *Tamarindus indica* L. Seed Hydroalcoholic Extract (TISHE) has demonstrated protective effects against γ-radiation-induced kidney toxicity in rat models by mitigating oxidative stress and suppressing the radiation-induced impression of pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α (Tawfik *et al*., 2020). Moreover, the plant of *T. indica* L. is reported for nephroprotective activity (Shrivastav *et al*., 2023). Based on this evidence, the current study explores the efficacy of TISHE in urolithiatic rats induced by Ethylene Glycol (EG)-intake. Both *in vitro* and *in vivo* techniques were employed to evaluate the extract's activity in dissolving kidney stones and mitigating urolithiasis.

MATERIALS AND METHODS

Collection and identification of plant

Seeds of *T. indica* L. were collected from the district Nanded in Maharashtra, India. The plant species was identified and taxonomically verified using Flora of Marathwada authored by Naik, V. N. (1998). Official authentication was conducted by the Department of Botany, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India and documented under reference number SLS/2021-22/903.

Hydroalcoholic extract preparation

Seeds of *T. indica* L. were collected from mature, ripened fruits. The seeds were shade-dried for seven days at a controlled temperature of around 25±2°C. After thorough washing with absolute ethanol, the dried seeds were crushed into a fine powder. The powdered material was then macerated in a 1:1 mixture of ethanol and distilled water for a period of 5 to 7 days. Following maceration, the mixture was filtered to remove solid residues, and the filtrate was concentrated through evaporation to obtain the Hydroalcoholic Seed Extract (TISHE).

Preliminary phytochemical screening

The TISHE was evaluated using standard qualitative phytochemical tests to detect the presence of various bioactive constituents. These assays detected carbohydrates, alkaloids,

glycosides, steroids, saponins, tannins, amino acids and proteins (Balamurugan *et al*., 2019).

In vitro anti-urolithiatic activity

Chemicals and preparation

Sodium oxalate, Tris buffer, sodium metabisulfite, calcium chloride dihydrate, p-phenylenediamine, and potassium permanganate (Loba Chemicals Ltd., India) were used for dissolution assay. Cystone® tablets (Himalaya Drug Company, India) were obtained from a local pharmacy. To prepare the positive control, the tablets were decolorized using absolute ethanol, powdered, suspended in distilled water, and filtered to achieve a clear solution with a concentration of 400 mg/mL. Dialysis membranes with semi-permeable properties were acquired from Yash Enterprises, Pune, India and kept under refrigeration at 2-8°C in a moist environment with a neutral pH range of 7.0-7.4. The test solution, referred to as TISHE (5 mL), was also prepared for use in the assay.

Preparation of crystals and reagents

Calcium Oxalate (CaOx) crystals, Calcium Phosphate (CaPh) crystals, 0.02 M Potassium Permanganate ($KMnO_4$) solution, 1N Sulphuric acid (H_2SO_4), Molybdate-sulfuric acid reagent, and Reducing reagent solution were prepared in accordance with a previously published protocol (Yelne and Shinde, 2025).

For the solutions of CaOx and CaPh used in assays, the respective crystals (10 mg) were suspended in distilled water (10 mL) to achieve a 1 mg/mL concentration.

Spectrophotometric analysis of CaOx and CaPh using dissolution model

A dialysis-based dissolution model was recently established in laboratory for the spectrophotometric evaluation of CaOx and CaPh crystals, adapting methodologies from previously published protocols (Yelne and Shinde, 2025; Phatak and Hendre, 2015). In this model, semi-permeable dialysis membranes were filled with the respective test solutions for each experimental group and securely tied at one end using thread. Experimental design for this experiment as shown in (Table 1).

In conical flask, each dialysis membrane pouch was immersed in 150 mL of 0.1 m Tris buffer. The thread used to seal the membrane was affixed to the neck of the flask and secured using aluminum foil. All experimental groups were incubated at 37°C for 4 hr daily over a period of three days to simulate physiological conditions. Following incubation, the contents of each dialysis membrane were carefully retrieved and transferred into test tubes for analysis. To evaluate CaOx dissolution, 0.02 M $KMnO_4$ (60-80 µL) and of 1N H_2SO_4 (4 mL) were added to each sample, followed by an incubation period of 2 hr. For CaPh analysis, Molybdate-sulfuric acid reagent (3 mL), 1N H_2SO_4 (4 mL) then reducing reagent solution (1 mL) were added and also left to react for 2 hr. In the case of CaOx, the solution's color shifted from dark pink to colorless, indicating the extent of dissolution. Spectrophotometric analysis was conducted at 620 nm to measure the color intensity, which corresponded to the concentration of dissolved calcium. Final calcium levels were calculated based on absorbance values compared to a standard calibration curve prepared for both CaOx and CaPh.

In vivo anti-urolithiatic activity

All the procedures involving animals were executed in strict accordance with ethical guidelines and received prior approval from the Institutional Animal Ethics Committee (IAEC) at Scitesla Private Ltd, Navi Mumbai, India (Postal Code: 400710). The study was conducted under registration number 2166/PO/RcBi/S/22/CPCSEA and was granted Form B approval (No. SCI/IAEC/2023-24/63). We followed guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for animal experiments. Animal welfare and humane treatment were maintained consistently throughout the study.

For the *in vivo* studies, Wistar rats (male) aged 12 to 16 weeks and weighing between 250 and 280 g were selected. The animals were housed in polypropylene cages lined with autoclaved corn cob bedding to maintain hygiene and minimize contamination. They had free access to standard laboratory chow and water throughout the duration of the experiment. Animals housed in well-maintained animal facility (23±1°C temperature and 70% relative humidity) with a 12-hr light/dark cycle.

The study was conducted in alignment with the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines, which are designed to enhance the transparency, reproducibility, and scientific integrity of animal research. Adherence to these guidelines ensures that experimental methods and outcomes are reported with clarity and precision, supporting the reliability and ethical standards of the research (Du *et al*., 2020).

Induction of urolithiasis in experimental rats

To induce urolithiasis, rats were provided with 0.75% EG in their drinking water continuously for 28 days, based on an established experimental protocol (Yelne and Shinde, 2025; Patel and Acharya, 2020). The EG solution was freshly prepared in potable water each day. For treatment administration, the TISHE was suspended with 0.5% Carboxymethyl Cellulose (CMC) solution, ensuring uniform dosing. Cystone® tablet was used as a reference standard, prepared by dissolving two tablets in distilled water (12 mL) prior to oral administration.

In vivo experimental design

The *in vivo* study was structured in accordance with previously published study (Yelne and Shinde, 2025; Dalvi *et al*., 2026). A total of 30 adult Wistar rats (male) were randomly divided into

five experimental groups (n=6 per group). The normal control group was provided with standard drinking water throughout the 28-day period. The disease control group received 0.75% EG in place of regular water for the entire period to induce urolithiasis. For the standard treatment group, rats were exposed to 0.75% EG for 14 days, after which they were administered Cystone® tablet at a dose of 150 mg/kg body weight from day 15 to 28, while continuing EG intake. The remaining two groups were used to evaluate the dose-dependent effects of the test extracts. These groups received TISHE orally at 200 mg/kg (Low dose) and 400 mg/kg (High dose), respectively, each day from day 15 to day 28, along with continued EG intake.

The experimental design included the following groups as per Table 2.

Bio-chemical analysis of urine and blood

The Samples of urine and blood were collected from each experimental group at three key time points: day 0 (to establish baseline values), day 14 (to confirm the induction of urolithiasis), and day 18 (to evaluate the effects of treatment interventions) (Yelne and Shinde, 2025; Panigrahi *et al*., 2016).

Urine samples were obtained by housing rats individually in metabolic cages for a period of 24 hr. The collected urine was centrifuged at 1500 rpm for 10 min to remove any particulate matter. The resulting pellet was preserved for microscopic examination of urinary crystals, while the supernatant was used for bio-chemical analysis. The clear supernatant was split into two separate portions. One portion was acidified by adding 3N hydrochloric acid (1-2 drops), which was then used to assess electrolytes such as magnesium, calcium and phosphorus using a commercial diagnostic kit (Tulip Diagnostics (P) Ltd., Goa, India). The non-acidified portion was analysed for nitrogenous waste products, as well as urea, creatinine, and uric acid (Panigrahi *et al*., 2016). Urinary oxalate was measured using a rat-specific ELISA kit, with a colorimetric readout at 550 nm.

To collect blood samples, rats were lightly anesthetized using isoflurane, after which blood was collected from the retro-orbital venous plexus. The samples were placed into sterile vials pre-coated with heparin and were promptly centrifuged at 3000 rpm for 20 min. The resulting plasma was isolated and utilized for bio-chemical assessments, which involved the estimation of levels of calcium, magnesium, potassium, urea, creatinine and uric acid, in accordance with manufacturer's instructions (Tulip Diagnostics Pvt. Ltd., Goa, India).

Assessment of oxidative stress biomarkers

At the end of the treatment, animals were ethically sacrificed using isoflurane and oxygen to minimize discomfort. Under sterile conditions, kidneys were excised with care. The collected organs were immediately rinsed with ice-cold physiological saline solution to remove blood and contaminants. Following rinsing, the kidney tissues were precisely weighed and homogenized in a suitable buffer with a tissue homogenizer to produce a uniform mixture. The homogenates were then centrifuged at high speed to eliminate cellular debris, and the resulting supernatant was collected for bio-chemical evaluation. Oxidative stress was evaluated by measuring key markers Glutathione (GSH), catalase, and Malondialdehyde (MDA) within the kidney homogenates to determine levels of oxidative injury and antioxidant activity. The analysis protocols followed methodologies previously described in the literature (Choudhary *et al*., 2023).

Histopathological evaluation

To investigate structural and pathological changes associated with urolithiasis and treatment response, histopathological analysis was performed on the residual kidney tissue samples. The specimens were processed using an automated tissue processor to ensure thorough dehydration, clearing, and infiltration with embedding medium, thereby preserving cellular architecture. Tissues were then embedded using a precision embedding station (MYR EC350, Germany) to facilitate consistent sectioning. The 15 µm thin sections were obtained using a microtome, enabling uniform and detailed microscopic examination. Hematoxylin and Eosin (H&E) staining method was employed to enhance visualization of cellular structures and tissue organization, providing insight into both normal histology and pathological alterations (Panigrahi *et al*., 2016). The stained sections were examined microscopically for signs of tissue degeneration, inflammation, and fibrosis. Particular attention was given to identifying Calcium Oxalate (CaOx) crystal deposits, a hallmark of urolithiasis-induced renal damage. The comprehensive histopathological assessment enabled evaluation of the extent of renal injury and the efficacy of the therapeutic intervention in ameliorating tissue damage caused by stone formation (Choudhary *et al*., 2023). These observations were pivotal in elucidating the nephroprotective potential of the treatment and its role in maintaining kidney function.

Table 1: Experimental design for *in vitro* experiment.

Group	Crystal solution of (1 mL)	Treated with (1 mL)
Group I	CaOx	Distilled water
Group II	CaOx	Cystone® tablet solution (400 mg/mL)
Group III	CaOx	TISHE (at five concentrations 10, 20, 30, 40 and 50 mg/mL)
Group IV	CaPh	Distilled water
Group V	CaPh	Cystone® tablet solution (400 mg/mL)
Group VI	CaPh	TISHE (at five concentrations 10, 20, 30, 40 and 50 mg/mL)

Statistical analysis

Statistical evaluation of the effects of various drug treatment was performed with GraphPad Prism software (version 8.3.0) and the results were stated as mean±Standard Deviation (SD) for each experimental group (n=6). Group comparisons were conducted using one-way Analysis of Variance (ANOVA) and followed by Dunnett's *post hoc* test for multiple comparisons. Statistical significance was set at p<0.05.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical Screening of the TISHE revealed the presence of several bioactives, including carbohydrates, alkaloids, tannins, steroids, glycosides, and saponins.

In vitro dissolution assay

The *in vitro* assessment of TISHE's calcium crystal dissolution activity is depicted in (Figure 1). The assay quantified the dissolution of CaOx and CaPh crystals by various concentrations of TISHE (10-50 mg/mL) in a 1 mg/mL crystal suspension (Figure 1A). Illustrates the effect on CaOx crystals, while (Figure 1B) shows results for CaPh crystals. These outcomes were benchmarked compared to the reference standard drug, Cystone® tablet. The concentration dependent increase in dissolution activity was observed across all extract concentrations. The 50 mg/mL dose of TISHE exhibited the highest efficacy, dissolving 77.8% of CaOx crystals compared to 81.3% by Cystone® tablet, and 67.3% of CaPh crystals relative to 69.6% by Cystone® tablet.

In vivo anti-urolithiatic activity

Effect of TISHE on urinary biochemical parameters

All experimental groups exhibited comparable urinary biochemical profiles at day 0 (baseline). Rats consuming EG with drinking water exhibited elevated urinary calcium, urea and uric acid levels at day 14 as compared to the normal control group maintained on standard drinking water (p<0.001) as shown in (Figure 2). In contrast, these EG-treated rats showed marked reductions in urine volume, magnesium levels and creatinine clearance (p<0.001), indicating impaired renal function. By day 28, the lithiatic control group continued to display elevated concentrations of urinary calcium, calcium oxalate, phosphorus and uric acid, and urea, along with reduced urine output, creatinine clearance, and magnesium, relative to normal controls (p<0.001). These findings suggest progressive renal injury in the absence of therapeutic intervention. Treatment with TISHE or the reference drug Cystone® tablet produced significant improvements in these parameters. Both treatments enhanced urine volume (Cystone® tablet and TISHE 400 mg/kg, p<0.001), creatinine clearance (Cystone® tablet, p<0.001; TISHE 200 mg/kg, p<0.05; TISHE 400 mg/kg, p<0.001), magnesium levels (Cystone® tablet and TISHE 400 mg/kg, p<0.001), calcium oxalate (Cystone® tablet and TISHE 200 and 400 mg/kg, p<0.001) and phosphorus (Cystone® tablet and TISHE 200 and 400 mg/kg, p<0.001). Additionally, treatment normalized the increased calcium, uric acid, and urea levels. Specifically, TISHE (400 mg/kg) and Cystone® tablet significantly reduced calcium (p<0.001), uric acid (p<0.001), and urea levels (Cystone® tablet and TISHE 400 mg/kg, p<0.05) compared to untreated lithiatic controls.

Effect of TISHE on serum minerals

Base levels of serum mineral were comparable at day 0 across the groups. The changes in the blood parameters calcium level

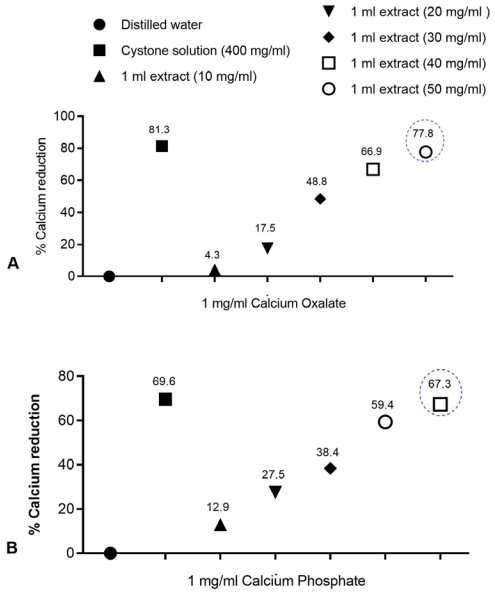
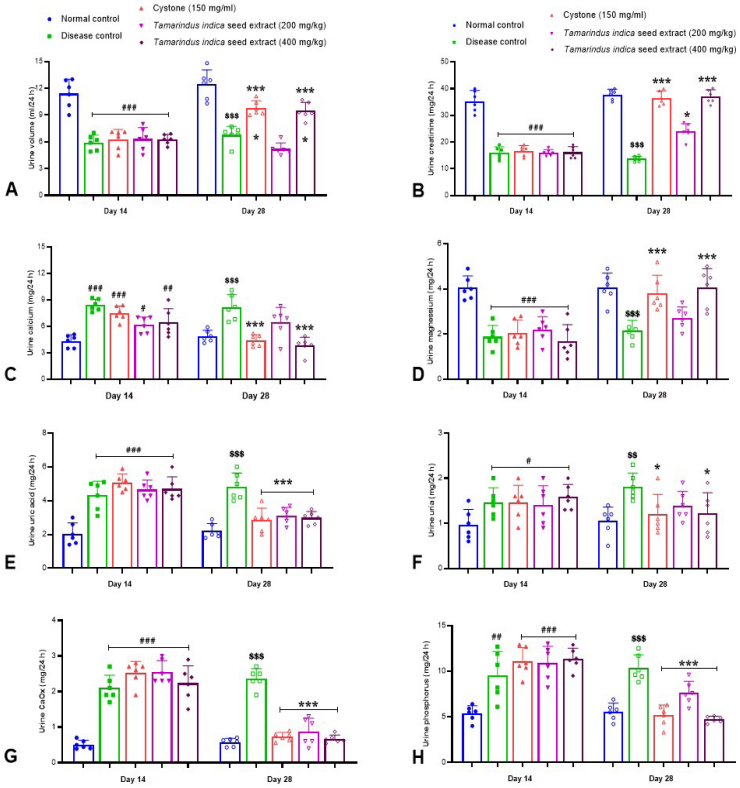


Figure 1: Evaluation of the dissolution capacity of TISHE on (A) calcium oxalate and (B) calcium phosphate crystals at various concentrations. The efficacy of the extract was compared with that of the standard reference solution, Cystone® tablet.

Table 2: Experimental design for *in vivo* experiment.

Group	Group name	No. of Animals	Treatment	Treatment period
I	Normal control	6	Drinking water	Day 0-28
II	Disease control	6	No treatment	Day 0-28
III	Standard	6	Cystone® tablet (150 mg/kg)	Day 15-28
IV	Test (Low dose)	6	TISHE (200 mg/kg)	Day 15-28
V	Test (High dose)	6	TISHE (400 mg/kg)	Day 15-28



Statistical significance: One-way ANOVA followed by post-hoc Dunnett's test
#p<0.05 and ###p<0.001 vs normal control rats on day 14 (onset of urolithiasis)
$$p<0.01 and $$$p<0.001 vs normal control rats on day 28
*p<0.05, **p<0.01 and ***p<0.001 vs disease control group on day 28

Figure 2: Urine collection data: urine volume (A), Creatinine clearance (B), and Levels of calcium (C), Magnesium (D), Uric acid (E), urea (F), Calcium oxalate (G) and Phosphorus (H) Across normal, urolithiasis, and treated rats.

(1-A), potassium level (1-B), and magnesium (1-C) level for the various groups at day 14 (when the illness first appeared) and day 28 (when the 14-day treatment period ended) are summarized in (Figure 3-I). On day 14, drinking EG in water considerably raised the levels of calcium, potassium, and magnesium of all groups in comparison to the group of normal control (p<0.001). Treatment with TISHE and Cystone® tablet (150 mg/kg) showed significant drop in the levels of calcium (all p<0.001), potassium (Cystone® tablet, p<0.001; TISHE 200 mg/kg (Low dose), p<0.05; TISHE 400 mg/kg (High dose), p<0.001) and magnesium (all p<0.001) on day 28 as compared to disease control groups.

Impact of TISHE on kidney function markers

Initial serum levels of nephrological biomarkers were uniform across all groups at day 0. (Figure 3-II) presents the creatinine level (II-A), urea level (II-B) and uric acid level (II-C) for each group at day 14 (disease onset) and day 28 (post-treatment). By day 14, the rats given EG with drinking water exhibited significantly elevated levels of all three markers comparing to the group of normal control (p<0.001). A 14-day treatment with either TISHE or Cystone® tablet (150 mg/kg) resulted to a marked drop in serum creatinine (p<0.001), urea (Cystone® tablet and TISHE 400 mg/kg (High dose), p<0.001; TISHE 200 mg/kg (Low dose), p<0.01, and uric acid levels (p<0.001) compared to the untreated urolithiatic group.

Impact of TISHE on oxidative stress biomarkers

The development of kidney stones was associated with a marked reduction in antioxidant enzyme activity. On day 28, rats exposed to EG shown a major reduction in glutathione levels (p<0.001), as illustrated in (Figure 3-III). However, administration of Cystone® tablet and TISHE from days 14 to 28 led to a significant increase in glutathione levels compared to the untreated disease group (p<0.001).

Catalase activity, which was notably reduced in the disease control rats, was significantly restored by Cystone® tablet and the higher dose of TISHE (400 mg/kg) (p<0.001). The lower dose of TISHE (200 mg/kg), however, did not significantly improve catalase activity.

Furthermore, the disease control group exhibited a marked increase in Malondialdehyde (MDA) levels, indicating elevated lipid peroxidation. Treatment with Cystone® tablet and both doses of TISHE significantly reduced MDA concentrations compared to the disease control group (p<0.001), demonstrating their potential antioxidant effect.

Histopathological assessment of renal tissue

The histopathological findings are illustrated in (Figure 4). Normal control rats (A) showed well-preserved kidney architecture with no evidence of Calcium Oxalate (CaOx) crystal deposits. In contrast, rats exposed to EG for 14 days (B) exhibited significant pathological changes, including glomerular swelling, tubular dilation, degeneration of epithelial cells, blood vessel congestion, inflammatory cell infiltration, epithelial shedding, necrosis, and CaOx crystals accumulation at the corticomedullary junction.

Treatment with standard Cystone® tablet (C) or TISHE (D and E) provided protective effects, notably preserving the structural integrity of renal tissues and minimizing damage induced by EG.

Black arrow shows- Renal tubular lumen showing deposited urinary stone, Red arrow shows- MNC infiltration in the renal interstitial space with degenerated tubules, Green arrow shows- Dilated renal tubules, Yellow arrow shoes- Hypertrophic glomeruli.

DISCUSSION

The accumulation of Calcium Oxalate (CaOx) crystals in urinary tract may cause painful symptoms and severely impact overall quality of life. Modern treatments for urolithiasis focus on both stone removal and prevention of recurrence along with surgical removal. However, recurrence remains a major challenge for both practitioners and patients in either of the cases. Traditionally, medicinal plants have been widely employed to prevent stone formation and dissolve existing stones (Butterweck and Khan, 2009; Nirum and *et al*., 2018). In this study, we studied the therapeutic efficacy of TISHE in an experimental rat model of kidney stones. While previous research has reported the anti-urolithiatic activity of TISHE in rats (Shrivastav *et al*., 2023),

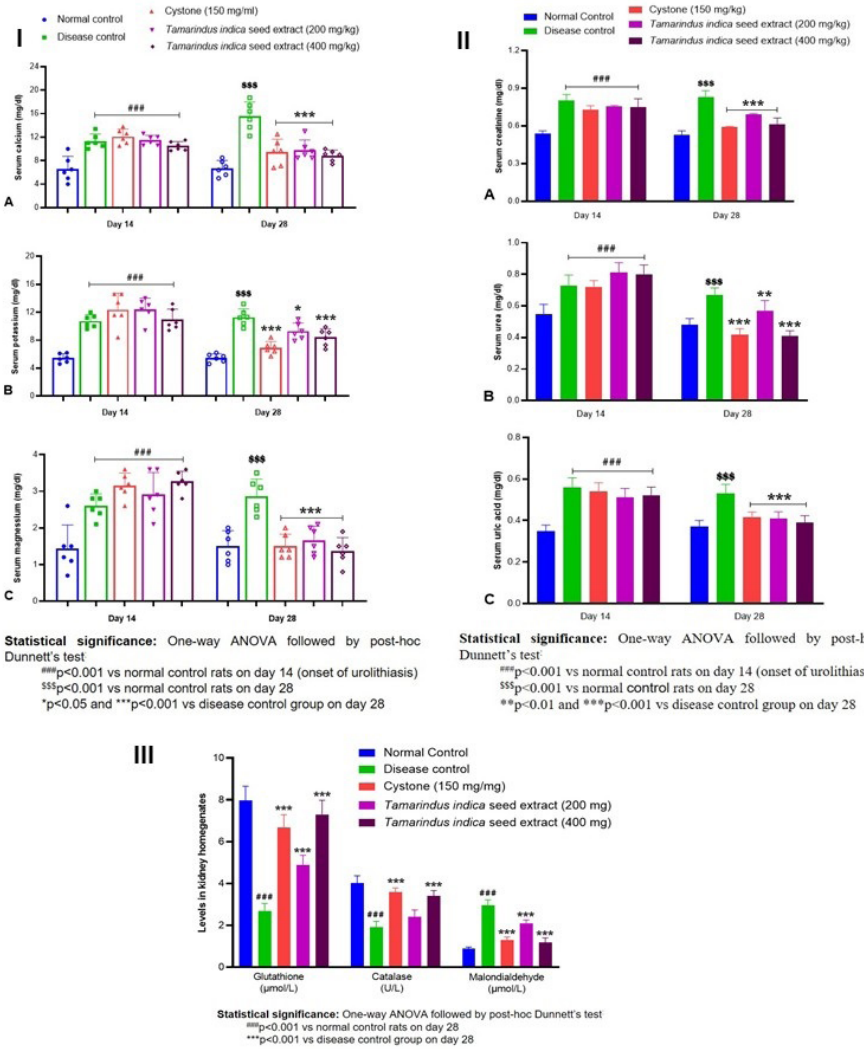


Figure 3: Biochemical and antioxidant profile in experimental groups. (I) Blood sample analysis showing levels of calcium (I-A), potassium (I-B), and magnesium (I-C). (II) Serum sample analysis showing levels of serum creatinine (II-A), urea (II-B), and uric acid (II-C). (III) Levels of antioxidant enzymes-Glutathione (GSH) and Catalase (CAT) and oxidative stress marker Malondialdehyde (MDA) in control, urolithiasis-induced, and treated rat groups.

our study provides novel insights into the potential of the seed extract as a curative agent for urolithiasis.

The present study first assessed the TISHE's effect on *in vitro* urolithiatic activity. In a dissolution model with a semi-permeable dialysis membrane, TISHE showed concentration-dependent increase in activity against CaOx and CaPh solutions. Maximum dissolution was observed at 50 mg/mL for both CaOx and CaPh solutions. This is comparable to that of the Cystone® tablet; a polyherbal formulation used as standard drug whose efficacy stems from the synergistic activity of its various herbal constituents. It contains tannins, flavonoids, and saponins, which exhibit diuretic, stone-dissolving, anti-inflammatory, antioxidant, astringent, and demulcent properties (Palaniyamma and Jeyaraman, 2017; Luengthanaphol *et al*., 2004).

For the *in vivo* assessment, an EG-induced urolithiasis model was employed, which mimics human kidney stone formation (Satapathy *et al*., 2025; Sathish *et al*., 2010; Jammalamadaka and Raissi, 2010; Kumar *et al*., 2025). Male rats received 0.75% EG in drinking water for 14 days, leading to hyperoxaluria and oxalate crystal deposition by the second week as we standardised the same in laboratory (Yelne and Shinde, 2025). By the second week, EG administration induced hyperoxaluria, causing oxalate crystal accumulation in inorganic urinary sediments. Microscopic analysis confirmed crystal formation, while biochemical assays revealed increased renal retention and oxalate excretion.

Extracts of various parts of *T. indica* L. plant also exhibited the nephroprotective activity (Lingam, 2024). Ethanol extract of tamarind seed coat demonstrated antioxidant activity which may underlie the antiurolothiatic activity (Luengthanaphol *et al*., 2004). TISHE was administered from days 14 to 28 as a curative treatment in urolithiatic rats, with Cystone® tablet as the reference standard (Kumar *et al*., 2025; Erickson *et al*., 2011). Urine volume plays a crucial role in the pathophysiology of urolithiasis because decreased urine volume increases the concentration of substances that cause stones, which encourages crystallization and the production of stones (Siener and Hesse, 2003). Factors such as dehydration, certain medications, or underlying medical conditions that disrupt fluid balance or kidney function can reduce urine output, thereby increasing the risk of stone formation (Sohgaura and Bigoniya, 2017).

By the second week, TISHE reduced urinary oxalate levels. Elevated urinary oxalate is a well-established risk factor for CaOx stone formation, and its reduction is indicative of a favorable therapeutic response. EG exposure caused elevated calcium, uric acid, and urea excretion, all significantly lowered by TISHE, similar to Cystone® tablet. TISHE also dissolved preformed CaOx stones, reducing renal retention and excretion. Hyperoxaluric rats showed increased uric acid levels, promoting CaOx crystallization, but TISHE countered this, aiding stone solubilization. Additionally, TISHE restored creatinine and magnesium levels, improving renal function. These findings align with previous studies (Pandhare *et al*., 2021). The TISHE-treated group showed a statistically significant decrease in both urinary calcium and oxalate levels compared to the untreated urolithiasis group, suggesting a reduction in stone-forming propensity. In our study, we focused on evaluating the therapeutic efficacy of TISHE in modulating CaOx crystal levels rather than delineating the specific phase of stone formation affected by the treatment. While these findings support the efficacy of TISHE in reducing lithogenic markers, future studies are warranted to elucidate the precise phase of stone formation that is targeted by the treatment. We acknowledge that the mechanistic insights into which phase is inhibited remain unexplored.

After 14 days of EG administration, serum analysis revealed elevated calcium, magnesium, potassium, uric acid, creatinine, and urea levels. Reduced creatinine and magnesium in urine suggest retention in the blood (Pandhare *et al*., 2021). Kidney stone formation impaired renal function, leading to nitrogenous waste accumulation such as creatinine, urea, and uric acid in blood indicating considerable renal damage following urolithiasis.

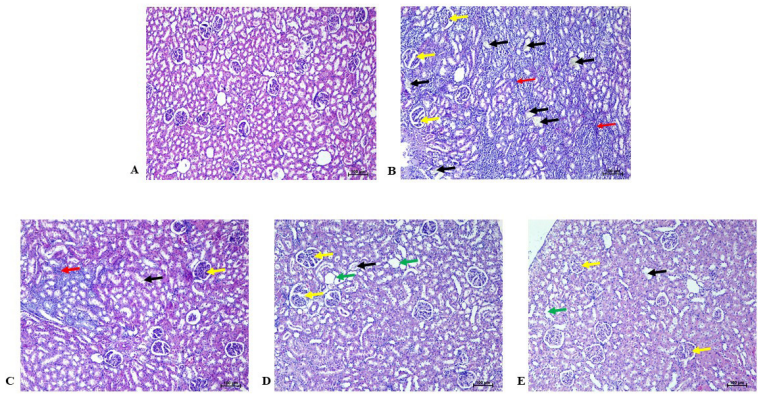


Figure 4: Histological examination of kidney tissues (A) Normal control group, (B) Disease control group, (C) Standard group Cystone® tablet at 150 mg/kg, (D) Treatment group TISHE at 200 mg/kg (Low dose), and (E) Treatment group TISHE at 400 mg/kg (High dose).

Increased level of uric acid may also be the plausible reason for alterations in the pH of urine towards acidic range, a common reason for burning sensation in urine. Treatment with TISHE and Cystone® tablet (days 14-28) significantly lowered serum calcium and improved renal health by promoting diuresis, reducing kidney weight, and decreasing urea, uric acid, and creatinine levels.

Renal crystal formation is closely linked to oxidative stress, which damages renal tubular cells and promotes crystal adhesion and aggregation (Davalos *et al*., 2010; Kaur *et al*., 2025). Reactive oxygen species increase the expression of crystal-binding molecules like osteopontin and hyaluronan, facilitating stone retention. Different phytoconstituents control oxidative stress by free radical scavenging, inhibition of lipid peroxidation, enhancing antioxidant enzyme activity and maintaining the redox balance. This way these phytoconstituents showed effects in inhibiting crystal nucleation, preventing crystal aggregation and reducing renal epithelial injury (Hong and Qin, 2023). Urolithiatic rats showed reduced glutathione and catalase levels but elevated malondialdehyde. Levels of GSH and catalase indicate the antioxidant capacity of the kidney tissue and its ability to counteract oxidative stress induced by urolithiasis and elevated MDA levels specify suggested oxidative stress and cellular damage within the kidney tissues. Treatment with Cystone® tablet and TISHE increased glutathione and catalase activity, and lowered malondialdehyde. TISHE's antioxidant effects stem from its phytoconstituents particularly flavonoids, isovanillic acid, fatty acids and phenolic compounds, which exhibit potent free radical scavenging properties (Tavanappanavar *et al*., 2024). The hydroxyl radical scavenging ability may be linked to the hydrogen-donating capacity of phenolic compounds.

Histopathological analysis showed that EG administration caused severe renal damage, including edema, tubular dilatation, epithelial degeneration, inflammation, congestion, necrosis, and CaOx crystal deposition. TISHE and Cystone® tablet treatment prevented these alterations, highlighting their protective role against calculi formation.

CONCLUSION

This study provides preliminary evidence of TISHE's anti-urolithiatic efficacy in a rat model. However, the small sample size and short treatment duration are limitations. Future research should explore preventive treatment regimen to assess the applicability of *T. indica* L. as supplement in diet. Our findings suggest that TISHE's anti-urolithiatic properties stem from its antioxidant properties and ability to reduce stone-forming constituents. Future studies should focus on identifying active phytoconstituents responsible for stone dissolution and clarifying the molecular mechanisms of *T. indica*'s anti-urolithiatic activity.

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ABBREVIATIONS

ANOVA: Analysis of Variance; **ARRIVE:** Animal Research: Reporting of *in vivo* Experiments; **CaOx:** Calcium Oxalate; **CaPh:** Calcium Phosphate; **CMC:** Carboxymethyl Cellulose; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **EG:** Ethylene Glycol; **GSH:** Glutathione; **H&E:** Hematoxylin and Eosin; **H_2SO_4:** Sulphuric Acid; **IAEC:** Institutional Animal Ethics Committee; **IL-1β:** Interleukin 1 Beta; **IL-6:** Interleukin 6; **$KMnO_4$:** Potassium Permanganate; **MDA:** Malondialdehyde; **SD:** Standard Deviation; **T. indica:** *Tamarindus indica*; **TISHE:** *Tamarindus indica* L. Seed Hydroalcoholic Extract; **TNF-α:** Tumor Necrosis Factor-Alpha.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUMMARY

This study investigates the anti-urolithiatic potential of *Tamarindus indica* L. seed extract through both laboratory and animal models. The extract demonstrated a significant ability to dissolve calcium-based crystals *in vitro*. In a 28-day rat model, TISHE treatment successfully reversed kidney damage, reduced stone-forming minerals in the urine, and boosted the body's natural antioxidant defenses. These findings suggest that tamarind seeds could be a valuable natural resource for managing and preventing kidney stones.

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