



Research Article

Antiuro lithiatic Potential of *Ficus racemosa* L. Leaf Gall Extract: In Vivo, In Silico, and Histopathological Insights into Calcium Oxalate Nephrolithiasis

Krushna B Deshmukh¹, Rajashree D Ghogare^{2*}, Sanjay B Bhawar³, Santosh B Dighe⁴, Nikita G Dighe⁵

1. Pravara Rural College of Pharmacy, Pravaranagar, Loni (Bk), Ahmednagar, Maharashtra, India. <https://orcid.org/0009-0005-2958-7022>.
2. Pravara Rural College of Pharmacy, Pravaranagar, Loni (Bk), Ahmednagar, Maharashtra, India. <https://orcid.org/0000-0002-7364-4929>.
3. Pravara Rural College of Pharmacy, Pravaranagar, Loni (Bk), Ahmednagar, Maharashtra, India. <https://orcid.org/0000-0002-5345-0799>.
4. Pravara Rural College of Pharmacy, Pravaranagar, Loni (Bk), Ahmednagar, Maharashtra, India. <https://orcid.org/0000-0003-3260-2981>.
5. Pravara Rural College of Pharmacy, Pravaranagar, Loni (Bk), Ahmednagar, Maharashtra, India. <https://orcid.org/0009-0003-6118-3197>

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Abstract

Background: Urolithiasis, primarily caused by calcium oxalate crystal deposition, remains a recurrent clinical challenge despite available surgical and pharmacological interventions. Traditional Ayurvedic medicine highlights *Ficus racemosa* L (gular) for renal disorders, yet its leaf galls remain underexplored for antiuro lithiatic potential. **Objective:** To evaluate the preventive and curative efficacy of *Ficus racemosa* L. leaf gall extract (FRGE) in an ethylene glycol-induced urolithiasis model in Wistar rats, and to explore its mechanistic basis through biochemical, histopathological, and in silico approaches. **Methods:** Methanolic extracts of authenticated leaf galls were administered orally at graded doses (100, 250, 500 mg/kg) in both preventive (days 1–28) and curative (days 15–28) regimens. Urinary and serum biochemical markers, renal oxidative stress parameters, and histopathological changes were assessed. Molecular docking of major phytoconstituents (quercetin, rutin, catechin, epicatechin) with lithogenic proteins (osteopontin, NF-κB, NADPH oxidase) was performed, alongside ADMET profiling. **Results:** FRGE significantly normalized urinary calcium, oxalate, phosphate, and uric acid levels, while reducing serum creatinine, uric acid, and blood urea nitrogen. Antioxidant enzyme activities (SOD, CAT, and GSH) were restored, and lipid peroxidation was attenuated. Histopathology confirmed preserved renal architecture and reduced calcium oxalate deposition, particularly at 500 mg/kg. Docking studies revealed strong binding affinities of quercetin and rutin to lithogenic proteins, supporting inhibition of crystal adhesion and oxidative pathways. ADMET analysis indicated favorable pharmacokinetic and safety profiles. **Conclusion:** FRGE demonstrated significant preventive and curative antiuro lithiatic activity, mediated through modulation of urinary biochemistry, oxidative stress, and renal protection. These findings validate its ethnomedicinal use and highlight *Ficus racemosa* L. leaf galls as a promising natural therapeutic candidate for urolithiasis management.

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Introduction

Urolithiasis, commonly referred to as urinary stone disease, represents a global health concern characterized by the formation and persistence of calculi within the urinary tract. Its prevalence is influenced by dietary habits, sedentary lifestyles, and environmental factors, contributing to substantial clinical and socioeconomic burdens (1). Although current therapeutic options such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and

pharmacological agents like potassium citrate and thiazide diuretics are available, they are often invasive, expensive, and associated with high recurrence rates, with nearly half of patients experiencing relapse within five years. These limitations have prompted increased interest in plant-based interventions, particularly those with a history of ethnomedicinal use and favorable safety Profiles (2).

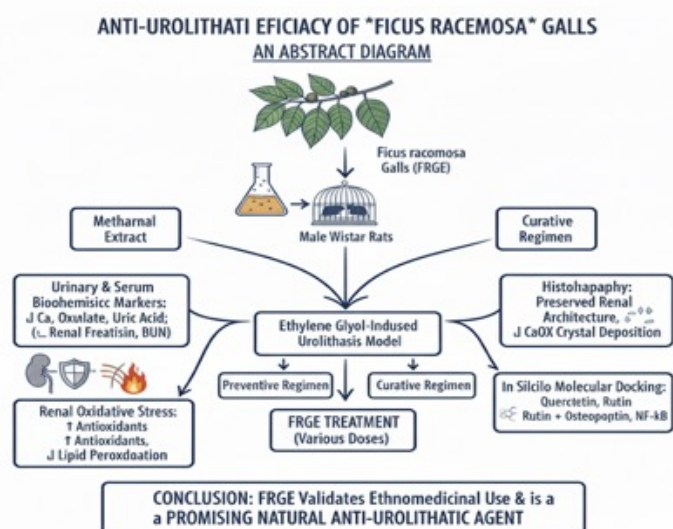
Ficus racemosa L. (commonly known as gular), a member of the Moraceae family, has long been recognized in traditional medicine for its renal protective properties. While its bark, fruit, and leaves have been pharmacologically studied, the leaf galls—formed in response to insect-induced stress—remain underexplored despite being rich in bioactive compounds such as flavonoids, tannins, phenolic acids, and triterpenoids. These constituents exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities, suggesting potential utility in managing urolithiasis (3–5).

* Corresponding Author:

Rajashree D Ghogare

Pravara Rural College of Pharmacy,
Pravaranagar, Loni (Bk),
Ahmednagar, Maharashtra, India.

Email Id: rajshrighogare9@gmail.com

Figure 1 Graphical Abstract

Given the established link between oxidative stress and inflammation in stone pathogenesis, this study investigates the antiurolithiatic efficacy of *Ficus racemosa* L. leaf gall extract (FRGE) using a well-established ethylene glycol-induced rat model (5). The research integrates phytochemical screening, in vivo biochemical and histopathological assessments, and in silico molecular docking and ADMET profiling to elucidate the therapeutic potential and mechanistic pathways of FRGE. The findings aim to validate its traditional use and support the development of safe, natural alternatives for urolithiasis management (6).

Materials and Methods

Plant Collection and Authentication

Leaf galls of *Ficus racemosa* L. were collected from the botanical garden based on ethnobotanical evidence of their use in renal disorders. The plant material was authenticated by a qualified botanist, and voucher specimens were deposited for future reference (7).

Preparation of Plant Extract

The collected galls were washed thoroughly under running tap water, surface sterilized with 1% hydrogen peroxide, and rinsed with distilled water. The material was dried in a hot air oven at 40°C for five days until complete moisture removal, then powdered using a mechanical grinder. A 100 g portion of the powder was subjected to successive maceration with polar solvents (methanol, water) and non-polar solvents (hexane, chloroform) for 72 hours each. Extracts were filtered through Whatman No. 1 paper, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C. The methanolic extract, which yielded the highest phytochemical content, was selected for pharmacological evaluation (8,9).

Experimental Animals

Healthy male Wistar rats (150–200 g) were procured from a CPCSEA-registered breeder and housed at the Pharmacology-II Laboratory, Pravara Rural College of Pharmacy, Maharashtra, India (Registration No. 1942/PO/Re/S/17/CPCSEA). Ethical clearance was obtained from the Institutional Animal Ethics Committee (PRCOP/CCSEA/IAEC/2024/01/03). Animals were acclimatized for seven days under controlled conditions (22 ±

2°C, 50 ± 10% humidity, 12-hour light/dark cycle) and provided with a standard pellet diet and water ad libitum (10).

Induction of Urolithiasis

Urolithiasis was induced by administering 0.75% v/v ethylene glycol (EG) in drinking water ad libitum for 28 days. This model is widely recognized for simulating calcium oxalate nephrolithiasis through hyperoxaluria and crystal deposition in renal tubules (11).

Acute Toxicity Study

Acute oral toxicity of the extract was assessed following OECD guidelines. A limit dose of 2000 mg/kg was administered, and animals were observed continuously for 4 hours, periodically for 24 hours, and daily for 14 days. No mortality or adverse effects were noted (12).

Experimental Design

Fifty-four rats were randomly divided into nine groups (n = 6 per group):

Table 1: Experimental Design of Animal Groups

Group	Treatment Type	Intervention	Dose (mg/kg/day)	Duration (Days)
I	Normal Control	Distilled water only	—	1–28
II	Disease Control	Ethylene glycol	(0.75% v/v)	1–28
III	Standard Control	EG + Cystone (p.o.)	750	15–28
IV (T1CR)	Curative regimen	EG + FRGE	100	15–28
V (T2CR)	Curative regimen	EG + FRGE	250	15–28
VI (T3CR)	Curative regimen	EG + FRGE	500	15–28
VII (T1PR)	Preventive regimen	EG + FRGE	100	1–28
VIII (T2PR)	Preventive regimen	EG + FRGE	250	1–28
IX (T3PR)	Preventive regimen	EG + FRGE	500	1–28

EG = Ethylene glycol (0.75% v/v in drinking water), FRGE = *Ficus racemosa* L. leaf gall extract.

All treatments were administered once daily by oral gavages. The doses of FRGE (100, 250, and 500 mg/kg/day, p.o.) were selected based on the results of the acute oral toxicity study conducted according to OECD Guideline 423, in which no mortality or significant toxic effects were observed up to 2000 mg/kg. Therefore, 100, 250, and 500 mg/kg, representing approximately 1/20th, 1/8th, and 1/4th of the maximum non-toxic dose, respectively, were selected to evaluate the dose-dependent antiurolithiatic activity. These doses were also consistent with previous pharmacological studies on *Ficus racemosa* extracts (12).

Curative regimen: In the curative protocol, ethylene glycol was administered from Day 1 to induce urolithiasis. FRGE treatment was initiated on Day 15 and continued until Day 28, when calcium oxalate crystal deposition had already begun. This regimen was designed to evaluate the therapeutic efficacy of

FRGE in reducing established renal calculi and associated renal injury (13). Preventive regimen: In the preventive protocol, FRGE was administered concurrently with ethylene glycol from Day 1 to Day 28. This regimen assessed the prophylactic potential of FRGE by determining its ability to inhibit the initiation, nucleation, growth, aggregation, and retention of calcium oxalate crystals during stone formation(14).

Sample Collection and Biochemical Analysis: On day 28, animals were individually housed in metabolic cages for 24-hour urine collection. A few drops of 0.1 N hydrochloric acid (HCl) were added to the urine collection vessel before the collection period to preserve the urine, prevent microbial growth, and minimize precipitation of urinary constituents (15). At the end of the collection period, the total urinary volume and pH were recorded. Urine aliquots were then analyzed for calcium, oxalate, phosphate, and uric acid using commercial diagnostic kits and standard methods(16).

Urinary biochemical parameters were estimated using commercially available diagnostic kits according to the manufacturer's instructions. Urinary calcium was determined by the **o-cresolphthalein complexone (OCPC) colorimetric method**, urinary phosphate by the **ammonium molybdate UV/colorimetric method**, urinary uric acid by the **uricase-peroxidase enzymatic method**, and urinary oxalate by an **enzymatic/colorimetric oxalate assay** (or the specific method recommended by the kit manufacturer). Absorbance was measured using a UV-Visible spectrophotometer, and concentrations were expressed as mg/dL.

On the final day, blood samples were collected under anesthesia via retro-orbital puncture. Serum was separated by centrifugation (10,000 × g, 10 min) and analyzed for creatinine, uric acid, and blood urea nitrogen (BUN) (17).

Kidney Harvest and Homogenate Preparation: After blood collection, animals were sacrificed by cervical dislocation. Kidneys were excised, washed in ice-cold saline, blotted dry, and weighed. One kidney from each animal was fixed in 10% neutral buffered formalin for histopathology, while the other was homogenized in phosphate-buffered saline for biochemical assays (18).

Assessment of Oxidative Stress Markers: Renal homogenates were analyzed for lipid peroxidation (malondialdehyde; MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) using established spectrophotometric methods. These parameters were chosen to evaluate oxidative stress and the nephroprotective effect of FRGE (19).

Histopathological Evaluation: Formalin-fixed kidneys were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) (20). Sections were examined under a light microscope for pathological features including tubular atrophy, epithelial desquamation, calcium oxalate crystal deposition, interstitial inflammation, and glomerular damage (21).

In Silico Analysis: To complement in vivo findings, molecular docking studies were performed using Schrodinger Glide Suite v14.0. Phytoconstituents identified by based on published phytochemical investigations of *Ficus racemosa* leaf galls quercetin, rutin, epicatechin, and catechin were selected as representative bioactive phytoconstituents for molecular docking studies, (22) it is docked with target proteins implicated in urolithiasis, including osteopontin (OPN), NADPH oxidase, and NF-κB. Binding affinities and interaction profiles were assessed,

and receptor structures were validated using Ramachandran plots (23).

ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of lead compounds were predicted using Swiss ADME and pkCSM tools to evaluate drug-likeness and pharmacokinetic safety (24,25).

Statistical Analysis: Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way ANOVA followed by Bonferroni's post hoc test in GraphPad Prism v10.4. A p-value < 0.05 was considered statistically significant (26).

Observations and results

The antiurolithiatic efficacy of *Ficus racemosa* L. leaf gall extract (FRGE) was investigated using both **curative** and **preventive** regimens in a validated ethylene glycol-induced urolithiasis model in male Wistar rats. The results are as follows:

Characterization of Extract

Table 2: Quantitative Determination of Primary Phytochemical

Phytochemical	Test/Reagent	Observation (Colour Change)	Result
Alkaloids	Dragendorff's test	Orange–reddish brown precipitate formed	+
	Mayer's test	Cream or pale yellow precipitate formed	+
	Hager's test	Yellow crystalline precipitate formed	+
	Wagner's test	Reddish-brown precipitate formed	+
Carbohydrates	Molisch's test	Violet/purple ring formed at the interface	+
	Fehling's test	Brick-red precipitate formed	+
	Benedict's test	Brick-red precipitate formed	+
Glycosides	Legal's test	Pink to red colour developed	+
	Keller–Killiani test	Brown ring at the interface with bluish-green upper layer	+
Steroids	Liebermann–Burchard test	No green or bluish-green colour developed	–
	Salkowski test	No reddish-brown ring observed	–
Flavonoids	Shinoda's test	Pink to crimson-red colour developed	+
Saponins	Foam test	Stable persistent froth (≈1 cm or more) formed	+
Tannins & Phenolics	Lead acetate test	White/yellowish precipitate formed	+
	Ferric chloride test	Blue-black or dark green colour developed	+
	Potassium dichromate test	Yellow-brown precipitate formed	+
Triterpenes	Sulphuric acid test	Reddish-brown colour developed	+

Urinary Biochemical Parameters

Acute oral toxicity of the methanolic extract of *Ficus racemosa* L. leaf gall (FRGE) was evaluated according to OECD Guideline

423. A single oral dose of 2000 mg/kg was administered, and the animals were observed continuously during the first 4 hours, periodically during the first 24 hours, and daily for 14 days. The parameters assessed included mortality, clinical signs of toxicity (changes in skin, fur, eyes, and mucous membranes), behavioral changes (locomotor activity, alertness, tremors, convulsions, salivation, lethargy, sleep, and coma), food and water intake, body weight, and any gross pathological abnormalities at necropsy. No mortality or significant treatment-related toxic effects were observed up to 2000 mg/kg, indicating that FRGE is safe at this dose.

The calculi-induced control (CIC) group demonstrated significant elevations in urinary concentrations of uric acid, phosphate, calcium, and oxalate compared to the normal control (NC) group ($p < 0.001$), indicating successful induction of hyperoxaluric urolithiasis. These alterations represent a conducive urinary environment for the nucleation, aggregation, and growth of calcium oxalate (CaOx) crystals (27,28).

Observation

Table 3: The Effect of *Ficus racemosa* L. leaf gall extracts On Urine Parameters

Groups and Dose	Urine Parameters Mg/dl±SEM			
	Uric acid	Phosphate	Calcium	Oxalate
Normal control	5.33 ±0.44	27.17± 2.35	4.197± 0.52	0.4833± 0.06
Disease Control	12.70± 0.66***	61.17± 1.75***	12.67± 0.71***	1.400± 0.05***
STD 750 mg/kg	6.417± 0.45####	35.17± 2.46####	6.000± 0.53####	0.5167 ±0.04####
T1CR 100mg/kg	9.900± 0.35@@@	49.33± 1.38@@@	9.800± 0.28@@	1.033± 0.06@@@
T2CR 250mg/kg	8.517± 0.47@@@	40.00± 1.29@@@	8.383± 0.37@@@	0.900± 0.02 @@@
T3CR 500mg/kg	5.483± 0.44@@@	31.33± 1.83@@@	6.250± 0.38@@@	0.500± 0.05@@@
T1PR 100mg/kg	9.917± 0.28+ +	48.67± 0.88+ ++	9.867± 0.36+ +	0.9467± 0.04+++
T2PR 250mg/kg	9.083± 0.37+ ++	39.50± 1.17+ ++	9.017± 0.37+ ++	0.8483± 0.02+++
T3PR 500mg/kg	6.083± 0.53+ ++	30.00± 2.12+ ++	6.917± 0.63+ ++	0.5167± 0.07+++

Data are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 versus Normal Control. #P < 0.05, ##P < 0.01, ###P < 0.001 (Standard Control vs Disease Control); @P < 0.05, @@P < 0.01, @@@P < 0.001 (Curative groups vs Disease Control); +P < 0.05, ++P < 0.01, +++P < 0.001 (Preventive groups vs Disease Control).

Medium-dose groups (T2CR/PR) showed moderate reductions, while the low-dose groups (T1CR/PR) exhibited only marginal improvement over CIC. This clear dose-response trend confirms the extract's concentration-dependent therapeutic action.

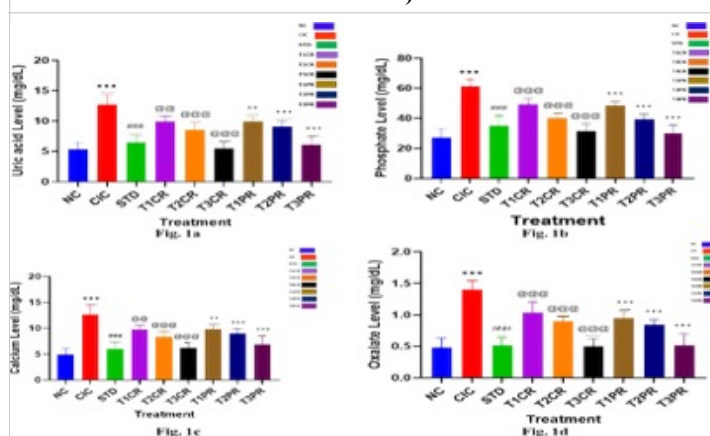
Renal Oxidative Stress Parameters

The CIC group exhibited marked oxidative stress, as indicated by elevated renal malondialdehyde (MDA) levels (11.50±0.76 nmol/mg protein) and reduced antioxidant enzymes: superoxide dismutase (SOD 3.50± 0.42 U/mg protein), catalase (CAT: 0.020 ± 0.003 µmol/min/mg), and glutathione (GSH: 0.85 ± 0.14 µmol/mg).

FRGE significantly restored antioxidant defense in treated groups. In the high-dose curative group (T3CR), MDA was reduced to 6.33 ± 0.49 nmol/mg, while SOD, CAT, and GSH were elevated to 10.17 ± 0.33U/mg, 0.078 ± 0.004 µmol/min/mg, and 3.91 ± 0.32

µmol/mg, respectively. A comparable antioxidant profile was observed in T3PR (preventive regimen).

Figure 2: Urinary Biochemical Parameters (2a. Uric Acid levels in Urine of grouped animals; 2b. Phosphate levels in Urine of grouped animals; 2c. Calcium levels in Urine of grouped animals; 2d. Oxalate levels in Urine of grouped animals)



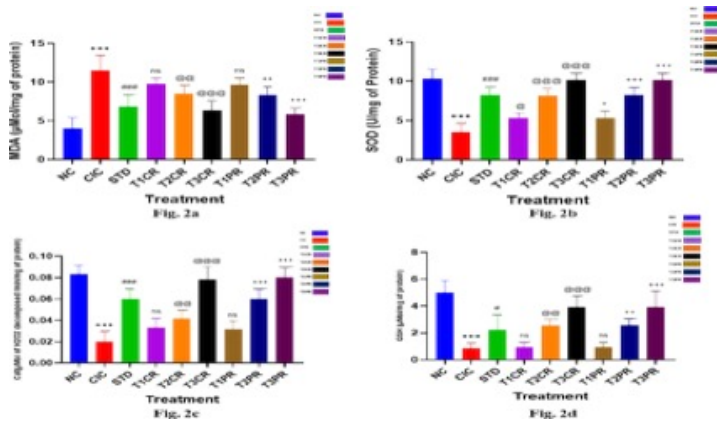
Data are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 versus Normal Control. #P < 0.05, ##P < 0.01, ###P < 0.001 (Standard Control vs Disease Control); @P < 0.05, @@P < 0.01, @@@P < 0.001 (Curative groups vs Disease Control); +P < 0.05, ++P < 0.01, +++P < 0.001 (Preventive groups vs Disease Control).

Table 4: *Ficus Racemosa* leaf gall extract on Renal Oxidative Stress Parameters

Groups and Dose	Kidney Parameters			
	MDA nmol/mg protein	SOD U/mg protein	CAT µmol/min/mg	GSH µmol/mg
Normal Control	4.0± 0.57	10.33± 0.49	0.833± 0.003	5.00 ±0.36
Calculi Induced Control	11.50± 0.76***	3.50 ±0.42***	0.020± 0.003***	0.85± 0.14***
STD750 mg/kg	6.83 ±0.60####	8.25 ± 0.38####	0.060± 0.003####	2.25 ± 0.44#
T1CR100 mg/Kg	9.75 ±0.30	5.33 ±0.24@	0.033± 0.003	0.96 ±0.11
T2CR250 mg/kg	8.50 ±0.42@@	8.15 ±0.32@@@	0.041± 0.003@@	2.56 ± 0.18@@
T3CR500 mg/kg	6.33 ± 0.49@@@	10.17± 0.33@@@	0.078± 0.004@@@	3.91± 0.32@@@
T1PR100mg /kg	9.65 ±0.34	5.33 ±0.35+	0.031± 0.003	0.96 ±0.11
T2PR250mg /kg	8.33 ±0.42++	8.25 ±0.38++ +	0.060± 0.003+++	2.58± 0.18++
T3PR500mg /kg	5.83 ± 0.30+ ++	10.17± 0.33+++	0.080± 0.003+++	3.91± 0.49+++

Data are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 versus Normal Control. #P < 0.05, ##P < 0.01, ###P < 0.001 (Standard Control vs Disease Control); @P < 0.05, @@P < 0.01, @@@P < 0.001 (Curative groups vs Disease Control); +P < 0.05, ++P < 0.01, +++P < 0.001 (Preventive groups vs Disease Control).

Figure 3 Renal Oxidative Stress Parameters (3a. MDA in Kidney Tissue of grouped animals; 3b. SOD in Kidney Tissue of grouped animals; 3c. CAT in Kidney Tissue of grouped animals; 3d. GSH in Kidney Tissue of grouped animals)



These improvements suggest that the extract's protective effect against CaOx nephrolithiasis may be mediated, in part, through mitigation of oxidative damage—a key contributor to crystal retention and renal epithelial injury.

Serum Biochemical Parameters

Serum analysis was confirmed renal damage in the CIC group, which showed significantly elevated levels of uric acid (9.92 ± 0.89 mg/dL), creatinine (1.20 ± 0.08 mg/dL), and blood urea nitrogen (BUN: 29.50 ± 0.76 mg/dL).

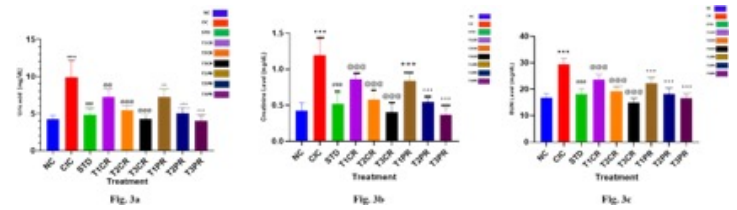
Ethylene glycol administration significantly elevated serum uric acid, creatinine, and BUN levels in the Disease control group compared with the normal control ($P < 0.001$), indicating renal impairment. FRGE treatment significantly and dose-dependently reduced these serum biochemical markers in both curative and preventive regimens. The 500 mg/kg dose (T3CR and T3PR) showed the greatest renoprotective effect, with values comparable to those of the standard treatment (Cystone 750 mg/kg).

Table 5: The Effect of *Ficus racemosa* L. leaf gall extract on Serum Parameters

Groups and Dose	Serum Parameters Mg/dl $\pm$ SEM		
	Uric acid	Creatinine	BUN
Normal Control	4.233 $\pm$ 0.20	0.4250 $\pm$ 0.04	16.75 $\pm$ 0.62
Calculi Induced Control	9.917 $\pm$ 0.89***	1.200 $\pm$ 0.08***	29.50 $\pm$ 0.76***
STD750mg/kg	4.817 $\pm$ 0.35####	0.5167 $\pm$ 0.06####	18.17 $\pm$ 0.70####
T1CR100mg/kg	7.250 $\pm$ 0.38@@	0.8633 $\pm$ 0.02@@@	23.67 $\pm$ 0.66@@@
T2CR250 mg/kg	5.450 $\pm$ 0.25@@@	0.5833 $\pm$ 0.04@@@	19.17 $\pm$ 0.60@@@
T3CR500 mg/kg	4.283 $\pm$ 0.23@@@	0.4083 $\pm$ 0.04@@@	15.00 $\pm$ 0.57@@@
T1PR100mg/kg	7.250 $\pm$ 0.38+ +	0.8400 $\pm$ 0.03+++	22.33 $\pm$ 0.76+ +
T2PR250mg/kg	5.050 $\pm$ 0.28+ ++	0.5467 $\pm$ 0.02+++	18.33 $\pm$ 0.80+ ++
T3PR500mg/kg	4.033 $\pm$ 0.27+ ++	0.3667 $\pm$ 0.04+++	16.67 $\pm$ 0.55+ ++

Data are expressed as mean $\pm$ SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus Normal Control. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ (Standard Control vs Disease Control); @ $P < 0.05$, @@ $P < 0.01$, @@@ $P < 0.001$ (Curative groups vs Disease Control); + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ (Preventive groups vs Disease Control).

Figure 4 Serum Biochemical Parameters 4a. Uric Acid in Serum of grouped animals; 4b. Creatinine in Serum of grouped animals; 4c. BUN in Serum of grouped animals.



Medium and low-dose groups showed moderate to minimal changes. These results align with the urinary Parameters and oxidative stress findings, indicating comprehensive renoprotection and restoration of nephron integrity by the extract.

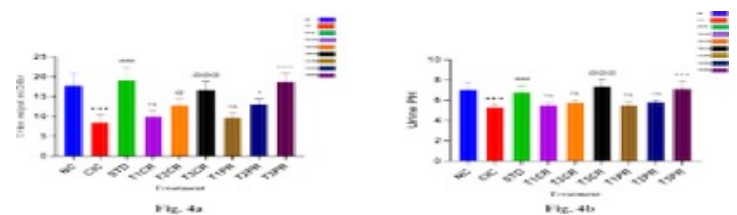
Urine Parameters

Table 6: The Effect of *Ficus racemosa* L. leaf gall extract on Urine Parameters

Groups and dose	Urine output	Urine PH
Normal Control	17.83 $\pm$ 1.24	7.033 $\pm$ 0.29
Calculi Induced control	8.50 $\pm$ 0.76***	5.317 $\pm$ 0.10***
STD750 mg/kg	19.17 $\pm$ 1.30####	6.783 $\pm$ 0.23 ###
T1CR100 mg/kg	10 $\pm$ 0.57	5.483 $\pm$ 0.09
T2CR250 mg/kg	12.83 $\pm$ 0.60@	5.733 $\pm$ 0.09
T3CR500 mg/kg	16.67 $\pm$ 0.88@@@	7.350 $\pm$ 0.29@@@
T1PR100 mg/kg	9.667 $\pm$ 0.49	5.500 $\pm$ 0.13
T2PR250 mg/kg	13 $\pm$ 0.57+	5.800 $\pm$ 0.07
T3PR500 mg/kg	18.67 $\pm$ 0.88+++	7.117 $\pm$ 0.31+++

Data are expressed as mean $\pm$ SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus Normal Control. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ (Standard Control vs Disease Control); @ $P < 0.05$, @@ $P < 0.01$, @@@ $P < 0.001$ (Curative groups vs Disease Control); + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ (Preventive groups vs Disease Control).

Figure 5: Urine parameter within Groups of Study animals (5a. Urine Output within Groups of Study animals; 5b. Urine pH within Groups of Study animals)



Data are expressed as mean $\pm$ SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus Normal Control. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ (Standard Control vs Disease Control); @ $P < 0.05$, @@ $P < 0.01$, @@@ $P < 0.001$ (Curative groups vs Disease Control); + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ (Preventive groups vs Disease Control).

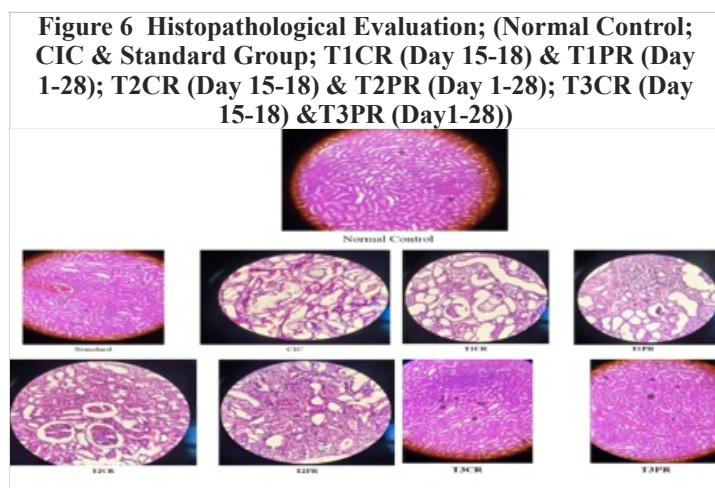
Histopathological Evaluation

Kidneys from the CIC group revealed extensive histopathological changes: epithelial desquamation, tubular dilation, interstitial inflammation, and abundant CaOx crystal deposits. These findings were absent in the NC group, where renal architecture remained normal.

In the high-dose curative group (T3CR), tissue sections showed nearly intact tubular and glomerular structures, minimal inflammation, and reduced crystal deposition. Preventive high-dose treatment (T3PR) further preserved renal morphology, with negligible epithelial damage, sparse inflammatory infiltration, and absence of CaOx crystals.

Medium-dose groups showed moderate improvements, while low-dose groups retained moderate histopathological damage.

Figure 5 Illustrates representative kidney sections from each group, confirming the protective effect of FRGE in a dose-dependent manner.



The histopathological evaluation of kidney sections from various experimental groups revealed significant insights into renal health and the effects of urolithiasis and herbal treatments. The control group, which received no ethylene glycol or treatment, exhibited intact renal architecture, characterized by well-preserved tubular structures, clear lumens, and normal glomerular morphology, serving as a baseline for assessing pathological changes.

In contrast, the ethylene glycol group, used to induce urolithiasis, displayed severe histopathological alterations indicative of calcium oxalate nephropathy. Notable findings included significant tubular atrophy, epithelial cell injury, and extensive inflammatory cell infiltration, alongside abundant calcium oxalate crystals obstructing tubular lumens. This group effectively represents a model of stone-induced renal damage.

Rats treated with herbal extracts in both curative and preventive regimens (Group A) showed partial renal protection, with near-normal tubular morphology and reduced interstitial inflammation. The most significant improvement was observed in the reduction of calcium oxalate crystal deposition, suggesting the therapeutic potential of the herbal formulation.

Group Receiving a second herbal formulation, demonstrated enhanced histological recovery, with restored tubular integrity and minimal epithelial damage. Interstitial inflammation was

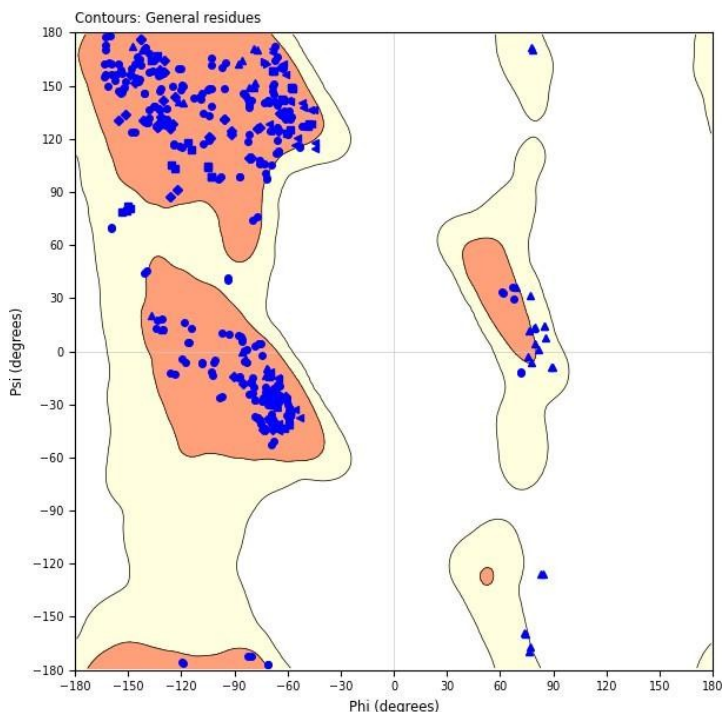
significantly reduced, and crystal deposition was either absent or limited, indicating a strong antiurolithiatic effect.

Finally, Group C, treated with a third herbal formulation, exhibited the most favorable histopathological profile, with preserved tubular architecture and negligible inflammation. The absence of calcium oxalate crystals further underscores the formulation's efficacy in preventing urolithiasis and maintaining renal structure and function.

In Silico Molecular Docking and ADMET Profiling

Molecular docking revealed strong binding affinities of FRGE phytoconstituents—particularly quercetin, rutin, epicatechin, and catechin—to key proteins involved in stone pathogenesis, such as osteopontin (OPN), NF- κ B, and NADPH oxidase.

Figure 7 Ramachandran Plot of 2ETE



Quercetin exhibited the most favorable docking score (-5.505 kcal/mol), forming multiple hydrogen bonds with residues THR50, ASN52, VAL56, and hydrophobic interactions with ALA55. Rutin also showed significant interactions with GLU58 and ASP60. These interactions may disrupt crystal-binding, inflammation, and oxidative cascades integral to stone formation. Molecular docking was performed using Schrödinger Glide Suite v14.0. Ligands were obtained from PubChem, and target proteins were retrieved from the Protein Data Bank (PDB) and prepared using the Protein Preparation Wizard. Docking was carried out using Glide XP, and binding interactions were visualized using Discovery Studio Visualizer.

ADMET predictions by using Swiss ADME confirmed favorable drug-likeness for the major compounds, with high gastrointestinal absorption, non-carcinogenicity, and no major CYP450 inhibition. These pharmacokinetic insights support their therapeutic potential and bioavailability in vivo.

Comparative Efficacy: Preventive vs. Curative

A comparative analysis of the two treatment modalities (preventive and curative) revealed that both regimens exerted significant antiurolithiatic effects across all evaluated parameters.

However, the preventive regimen (PR), particularly at the high dose (T3PR), demonstrated slightly superior efficacy in urinary phosphate reduction and antioxidant enzyme restoration.

This suggests that early intervention with FRGE may offer enhanced prophylaxis against stone development and associated renal injury, aligning with the principle of interrupting lithogenic events at an earlier stage of pathogenesis.

Discussion

The present investigation offers compelling evidence supporting the antiurolithiatic potential of *Ficus racemosa* L. leaf gall extract (FRGE) in an ethylene glycol-induced rat model (29) of urolithiasis. By employing both preventive and curative treatment regimens alongside comprehensive biochemical, histopathological, and in silico analysis, this study provides mechanistic insights into FRGE's therapeutic efficacy against urinary stone formation.

The preliminary phytochemical screening revealed that FRGE is rich in flavonoids, tannins, phenolic compounds, and triterpenoids—bioactive constituents frequently associated with antioxidant, anti-inflammatory, and crystallization-inhibitory properties (30). These phytoconstituents, particularly flavonoids like quercetin and rutin identified through in silico analysis, may be responsible for the observed therapeutic effects. Flavonoids have been documented to inhibit calcium oxalate crystallization through multiple mechanisms, including chelation of calcium ions, prevention of crystal aggregation, and modification of crystal morphology. The presence of polyphenols further enhances FRGE's protective capacity through antioxidant activity, crucial for mitigating oxidative stress—a recognized contributor to stone formation.

The significant alteration in urinary biochemical parameters observed in ethylene glycol-treated rats represents a lithogenic environment conducive to crystal formation. Increased urinary calcium in the calculi-induced control (CIC) group indicates hypercalciuria, a primary risk factor for calcium oxalate crystallization (31). Similarly, elevated urinary phosphate, oxalate, and uric acid levels further facilitate stone formation through supersaturation, epithelial injury, and heterogeneous nucleation.

FRGE administration in both curative and preventive regimens effectively normalized these urinary parameters in a dose-dependent manner. The high-dose treatments (T3CR and T3PR) demonstrated remarkable efficacy, with restoration of calcium, oxalate, phosphate, and uric acid levels comparable to those achieved with the standard treatment (Cystone). This normalization reflects FRGE's capacity to interfere with multiple lithogenic processes, potentially through:

- Inhibition of calcium oxalate crystal aggregation and growth
- Reduction of urinary supersaturation
- Modulation of crystal-retaining particle formation
- Interference with crystal-cell adherence mechanisms (32)

The comparable effectiveness of both preventive and curative regimens suggests that FRGE can both prevent crystal formation and potentially dissolve preformed crystals. However, the slightly superior results observed in preventive treatment (T3PR) underscore the value of early intervention in urolithiasis management.

Oxidative stress represents a critical pathogenic mechanism in urolithiasis, facilitating crystal retention through renal epithelial injury and inflammatory responses. The significant elevation in malondialdehyde (MDA) levels alongside depleted antioxidant reserves (SOD, CAT, and GSH) in untreated urolithic rats confirms substantial oxidative damage to renal tissues (33). FRGE treatment significantly restored antioxidant homeostasis, with high-dose regimens demonstrating near-complete normalization of oxidative stress parameters. This antioxidant activity likely stems from the phenolic and flavonoid constituents, which possess free radical scavenging abilities and can enhance endogenous antioxidant defenses. The restoration of antioxidant enzymes may protect renal epithelium from peroxidative damage, thereby reducing crystal adhesion sites and preventing subsequent stone formation (34). The antioxidant mechanism appears particularly significant in the preventive regimen, where early administration of FRGE (T3PR) yielded more pronounced improvements in SOD and CAT activities compared to corresponding curative treatment. This suggests that preemptive antioxidant intervention may offer enhanced protection against ethylene glycol-induced oxidative nephrotoxicity (35).

Serum biochemical parameters serve as reliable indicators of renal function, with elevated levels of creatinine, uric acid, and BUN signifying compromised filtration capacity. The marked elevation of these parameters in the CIC group confirms substantial renal dysfunction secondary to crystal-induced tubular obstruction and epithelial injury (36).

FRGE treatment significantly attenuated these markers of renal impairment, particularly at the high dose (500 mg/kg), where values approximated those of normal controls. This normalization reflects preserved renal architecture and function, corroborating the extract's nephroprotective potential. The mechanism likely involves multiple pathways: Reduction of crystal-induced mechanical injury through inhibition of crystal formation and retention, Mitigation of oxidative stress-mediated tubular damage, suppression of inflammatory cascades that exacerbate renal injury, enhancement of renal tissue repair mechanisms (37).

The significant improvement in serum biochemistry with both preventive and curative regimens demonstrate FRGE's versatility as both a prophylactic and therapeutic agent. However, the marginally superior results in preventive treatment groups highlight the importance of early intervention before substantial renal damage occurs.

Histopathological examination provides definitive evidence of FRGE's antiurolithiatic efficacy. The extensive renal damage observed in untreated urolithic rats characterized by tubular atrophy, epithelial desquamation, interstitial inflammation, and abundant calcium oxalate crystal deposits—aligns with established pathological features of nephrolithiasis.

Treatment with FRGE resulted in remarkable histological restoration, particularly at the high dose (T3CR and T3PR). The substantially reduced crystal deposition, preserved tubular architecture, minimal inflammatory infiltration, and maintenance of glomerular integrity collectively validate the extract's comprehensive renoprotective effect. These histopathological improvements were consistent with the biochemical findings, confirm FRGE's ability to attenuate multiple pathological processes involved in stone formation.

The in-silico investigations provide valuable mechanistic insights into FRGE's antiurolithiatic activity at the molecular level. The favorable binding affinities of major phytoconstituents,

particularly quercetin and rutin, to key proteins involved in stone pathogenesis (osteopontin, NF- κ B, NADPH oxidase) suggest potential molecular targets for FRGE's therapeutic action.

Osteopontin, an important crystal-binding protein that promotes calcium oxalate retention, showed strong interaction with quercetin through multiple hydrogen bonds and hydrophobic interactions. This binding may disrupt osteopontin's crystal adherence function, thereby reducing crystal retention in renal tissues. Similarly, interactions with NF- κ B may suppress inflammatory cascades, while NADPH oxidase inhibition would attenuate oxidative stress both critical factors in stone pathogenesis.

A critical aspect of this study was the comparative evaluation of preventive versus curative treatment paradigms. Both regimens demonstrated significant antiurolithiatic efficacy, with dose-dependent improvements across all parameters. However, subtle differences were observed that merit consideration for clinical translation.

The preventive regimen (particularly T3PR) demonstrated marginally superior results in urinary phosphate reduction, antioxidant enzyme restoration, and histopathological preservation. This enhanced efficacy likely stems from early intervention in the lithogenic process, preventing crystal nucleation, growth, and epithelial injury before substantial damage occurs. Conversely, the curative approach addresses established pathology, requiring reversal of existing crystal deposits and repair of damaged tissue inherently more challenging processes.

These findings carry important clinical implications, suggesting that prophylactic administration of FRGE may offer optimal benefits for high-risk individuals, including recurrent stone formers, those with metabolic predispositions, or patients with family history of urolithiasis. In contrast, the curative approach remains valuable for managing established stone disease, particularly given the comparable efficacy of high-dose treatment (T3CR) to standard therapy.

Conclusion

Ficus racemosa L. leaf gall extract (FRGE) demonstrated significant antiurolithiatic effects in both curative and preventive regimens in an ethylene glycol-induced rat model. The extract normalized urinary biochemical markers, reduced renal oxidative stress, and improved serum indicators of kidney function in a clear dose-dependent manner. High-dose FRGE treatment notably restored renal architecture and minimized crystal deposition, as confirmed by histopathological analysis. In silico studies supported these findings, revealing strong binding affinities of FRGE phytoconstituents to key proteins involved in stone formation. The results validate the traditional use of *F. racemosa* galls for urolithiasis and highlight their potential as a safe, natural therapeutic option. Further clinical studies are warranted to confirm these promising preclinical outcomes.

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