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Reckoning of antiurolithiatic effect of *Flemingia Strobilifera* R. BR using ethylene glycol-induced urolithiatic animal model: demystifying traditional medicine

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Abstract

Background Urolithiasis is a painful condition and current treatment doesn't assure the prevention of recurrence. This research aims to demonstrate the scientific reliability of Chloroform leaf extract of *Flemingia Strobilifera* R.Br. (CEFS) for antiurolithiatic activity using ethylene glycol-induced urolithiasis model.

Results Ethylene glycol (EG) was used to induce hyperoxaluria in male Wistar rats. The rats were grouped into 7-groups containing six each. Group I and II served as negative and positive control, group III received standard treatment, whereas Group IV to VII served as testing groups. CEFS of 30 mg/kg body-weight and 60 mg/kg body-weight was used as a preventive and curative regimen. The urine biochemistry was analysed for the presence of calcium, magnesium, phosphate, and oxalate. The rats were sacrificed for histopathological examination and LDH detection. The 24-hours urine volume was increased in both EG-treated groups as well as CEFS-treated groups, indicating the diuretic activity of plant. CEFS dose-dependently inhibited urine excretion of phosphate, calcium, and oxalate compared to the positive-control group. The histopathological examination of CEFS-treated rats' kidneys had reduced loss of renal structure and lessened deposition of calcium oxalate crystals.

Conclusion CEFS exhibited significant antiurolithiatic activity by reducing supersaturation of urine and excretion of stone forming components.

Keywords *Flemingia Strobilifera* R. Br, Antiurolithiatic, Kidney stones, Ethylene Glycol, Histopathology

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Background

The incidence of urolithiasis has been increasingly widespread with a projected relapse rate of 15/100 person years [1]. The most common presentation of kidney stones involves calcium oxalate (CaOx) crystals. A sequence of physicochemical events, including oversaturation, nucleation, growth, amalgamation, and accumulation within the renal tubules, result in the intricate process of forming kidney stones [2]. Urinary calculi can lead to excruciating renal haemorrhage, hydronephrosis, blockage, and infection. While there exist several surgical methods for treating urolithiasis, such as extracorporeal shockwave lithotripsy and percutaneous nephrolithotomy, but they do not guarantee the prevention of calculi recurrence [3]. Additionally, they have a number of negative consequences, including bleeding, high blood pressure, tubular necrosis, and renal fibrosis. Even though several randomised clinical studies have demonstrated the viability of synthetic drug therapy, complete remission is not always achieved without unpleasant side effects [4, 5]. Throughout history, medicinal plants have been an integral part of many traditional medicinal systems. To this day, plants provide an affordable substitute to pharmaceutical medications for a variety of disease conditions. Particularly, a number of pharmacological studies on the conventional antiurolithic medicinal plants have demonstrated their therapeutic efficacy in both in vitro and in vivo settings [6, 7].

Flemingia Strobilifera R. Br is a medicinally significant plant found in Sindh, Rajputana, Bengal, South India, and the Andaman Islands. Previous phytochemical researches revealed that the primary bio-constituents identified in this genus include flavonoids, flavonoid glycosides, chalcones, epoxychromenes, and pterocarpanes [8]. The conventional medical literature of the *F. Strobilifera* R.Br. plant purports that it is beneficial against an array of ailments, notably for renal and other urinary disorders [9, 10]. In line with this, the current work aims to demonstrate the scientific reliability of *F. Strobilifera* R.Br. leaf extract for its antiurolithiatic activity using an ethylene glycol-induced urolithiasis model.

Methods

Reagents

Ethylene glycol and chloroform were obtained from SD fine Chem., Mumbai, India. Kits used in this study for the determination of calcium, magnesium, phosphate and LDH were purchased from Span diagnostics Ltd., India. Cystone was a gift sample from Himalaya drugs company, Ahmedabad, India. All the chemicals and reagents used were of analytical grade.

Plant material

The fresh leaves of the plant *F. Strobilifera* R.Br. belonging to the family Fabaceae was collected from the Western Ghats of Maharashtra in the month of July. The plant was authenticated by Dr. Jawahar Raveendran, Conservative Research & Action group, Foundation for Revitalization of Local Health Traditions (FRLHT) and preserved a specimen sample of the same in the herbarium section of the FRLHT, Bangalore-64, with the voucher No. 100,154 for future reference.

Preparation of plant extract

The collected leaves were subjected to soxhlet extraction using petroleum ether to remove the chlorophyll pigment. Afterwards, they were air dried, pulverised, and then macerated in chloroform. Chloroform was selected as the extraction solvent because of its non-polar characteristics, which enables it to extract various groups of bioactive compounds like bioflavonoids, terpenoids, tannins, alkaloids, and saponins. The resulting extract was then strained. The filtrate was dried by evaporating it until no remnant remained. The leftover was then transferred to a China dish, allowed to evaporate at 40°C over a thermostat-controlled water bath, and preserved in a refrigerator till requisite anew. The dried powdered leaves of *F. Strobilifera* R.Br. yielded 40 g W/W of extract.

Animals

42 male Wistar albino rats, weighing between 150 and 200 g apiece were procured from the Indian Institute of Science, Bengaluru, India for experimentation. They were housed in cages made of polypropylene, with a 12-hour light/dark cycle at a temperature of 27 ± 2 °C and a relative humidity of $65 \pm 10\%$. The animals had unlimited accessibility to water and were fed the standard diet provided by Amrut Laboratory, Bangalore, India. They were separated into seven groups of six animals each post-acclimatization for two weeks. The study protocol was approved by the Institutional Animal Ethics Committee (Ref. No.:152/1999/CPCSEA).

Acute toxicity study

To determine the Maximum Tolerated Dosage (MTD) of the plant extract, acute oral toxicity tests were conducted in accordance with the relevant OECD 420 guidelines. Groups of three animals were utilised for the toxicity assessments. A suspension of the plant extract in distilled water was prepared using Tween 80 (10%) for all dosage levels. Following a fasting period of three to four hours, the investigational compounds were administered to the animal subjects in a single dose via an alimentary intubation tube.

The initial dose of the experimental components (plant extract) was set at 2000 mg/kg body weight, adhering to

OECD 420 recommendations. For antiurolithiatic action, therapeutic dosages were considered at 1/5th and 1/10th of the MTD.

Ethylene Glycol Induced Urolithiasis Model

The antilithiatic action in albino rats was evaluated using a model of hyperoxaluria induced by ethylene glycol. Group I served as the negative control group; they were given access to unlimited water and food on a regular basis. Groups II–VII were administered drinking water containing ethylene glycol (0.75%V/V) to induce renal calculi until the 28th day. While Group III received Cystone of 750 mg/kg body weight, a standard antiurolithiatic medication, from the 15th to the 28th day, Group II functioned as a positive control group without receiving any therapy or intervention. Groups IV and V acted as the curative regimen groups. Group IV was administered 30 mg/kg body weight of Chloroform Leaf Extract of *F. Strobilifera* (CEFS), whereas Group V was given 60 mg/kg body weight of CEFS from day 15 to day 28. As a preventative regimen, Group VI received CEFS at a dose of 30 mg/kg body weight, while Group VII received CEFS at a dose of 60 mg/kg body weight from the first to the 28th day. The CEFS extract was administered orally once a day.

Assessment of antiurolithiatic activity

Collection and analysis of urine Each animal was housed in a separate metabolic cage, and on the 28th day, the urine samples were collected throughout a 24-hour period. Throughout the urine collection time, drinking water was available to animals without encumbrance. The collected urine volume was determined. The presence of calcium, magnesium, phosphate, and oxalate components in urine was examined. The presence of calcium and phosphate was analysed even in serum and the renal clearance was calculated using the following formula,

$$\text{Renal Clearance} = \frac{U \times V}{P}$$

Where, U=concentration of substance in urine; V=volume of urine produced; P=Plasma concentration of substance.

Kidney histopathology and homogenate analysis On day 28, the rats were euthanized and their kidneys were excised. The separated kidneys' superfluous tissue was removed, and the kidneys were then washed in ice-cold physiological saline solution. The left kidney was weighed after being cleaned with normal saline and dried in a hot air oven at 80°C. The dry weight was then recorded. The half of the right kidney was fixed in 10% neutral buffered formalin, processed through a succession of grades of alcohol and xylene, encased in paraffin wax, sectioned at 5 µm, and stained with Hematoxylin and Eosin (H & E) for histological analysis. Both polarised and light microscopes were used to evaluate the kidney's anatomy and calcium oxalate deposits on the slides. The other half of the right kidney was utilised to determine LDH. In order to accomplish this, 10% of the tissue was homogenised in 0.1 M Tris HCl buffer (pH 7.4) using a homogenizer, and the homogenate was centrifuged for 30 min at 12,000 X g. The LDH estimate was performed using the supernatant that was collected following centrifugation.

Statistical analysis

All the statistical testing was carried out using SPSS software. The results are expressed as Mean±Standard Error of Mean (SEM). The statistical significance was assessed using one-way Analysis of Variance (ANOVA) and Tukey's Kramer post hoc analysis. *P*value<0.05 was considered as statistically significant.

Results

There were no immediate detectable toxicities in the immediate 24-hours of administration of the chloroform plant leaves extract. No animals died of acute toxicity. Acute toxicity findings showed that 300 mg/kg body weight was the maximum tolerable dose of CEFS. In view of this, 30 and 60 mg/kg of body weight were taken as study's therapeutic doses, respectively. Table 1 displays the urine production of the experimental and vehicle-treated rats. The rats treated with ethylene glycol (EG) had a considerably (*P*<0.05) higher urine volume than the vehicle-treated groups. Urine volume was considerably greater in CEFS-treated groups compared to EG-treated groups and vehicle-treated groups.

in the current work, hyperoxaluria was caused due to the development of renal stone leveraging ethylene glycol. Rats treated with EG had far higher excretions of calcium, oxalate, and phosphorus in their 24-hour urine

Table 1 Effect of chloroform extract of *F. Strobilifera* R. Br. leaves on urinary output in urolithiasis induced rats

Treatment	Volume of Urine (mL/24hr) Mean ± SEM
Vehicle control (1 ml Vehicle)	8.21 ± 0.419
Positive control (EG-treated 0.75% v/v)	11.45 ± 0.843 [§]
Cystone treated (750 mg/kg)	16.33 ± 0.869***
Curative regimen (CEFS: 30 mg/kg)	10.90 ± 0.396
Curative regimen (CEFS: 60 mg/kg)	14.60 ± 0.734 [†]
Preventive regimen (CEFS: 30 mg/kg)	12.96 ± 0.706
Preventive regimen (CEFS: 60 mg/kg)	15.38 ± 0.753**

[§]*p* < 0.001 vs. vehicle control (Group I); ****p* < 0.001; ***p* < 0.01 and **p* < 0.05 vs. Lithiatic Control (Group II)

Table 2 Effect of chloroform extract of *F. Strobilifera* R.Br. leaves on urinary calcium, phosphate, oxalate, and magnesium levels in urolithiasis induced rats

Treatment	Calcium (mg/24hr)	Phosphate (mg/24hr)	Oxalate (mg/24hr)	Magnesium (mg/24hr)
Vehicle control	0.5 ± 0.0723	3.15 ± 0.256	0.185 ± 0.034	3.92 ± 0.309
Positive control (EG-treated 0.75% v/v)	2.59 ± 0.243 ^{SSS}	12.5 ± 0.437 ^{SSS}	1.99 ± 0.304 ^{SSS}	1.52 ± 0.190 ^{SSS}
Cystone treated (750 mg/kg)	0.951 ± 0.064 ^{***}	5.23 ± 0.465 ^{***}	0.37 ± 0.148 ^{***}	3.74 ± 0.268 ^{***}
Curative regimen (CEFS: 30 mg/kg)	2.14 ± 0.254	9.91 ± 0.624 [*]	1.29 ± 0.148 [*]	1.68 ± 0.288
Curative regimen (CEFS: 60 mg/kg)	1.66 ± 0.173 [*]	9.25 ± 0.592 ^{**}	1.15 ± 0.069 ^{**}	2.26 ± 0.317
Preventive regimen (CEFS: 30 mg/kg)	1.36 ± 0.163 ^{***}	8.35 ± 0.618 ^{***}	0.92 ± 0.083 ^{***}	2.36 ± 0.258 ^{ns}
Preventive regimen (CEFS: 60 mg/kg)	1.18 ± 0.231 ^{***}	6.11 ± 0.549 ^{***}	0.58 ± 0.068 ^{***}	3.22 ± 0.344 ^{**}

All values are expressed as mean ± S.E.M.

^{SSS}*p* < 0.001 vs. vehicle control (Group I); ^{***}*p* < 0.001; ^{**}*p* < 0.01 and ^{*}*p* < 0.05 vs. Lithiatic Control (Group II)

Table 3 Effect of chloroform extract of *F. Strobilifera* R.Br. leaves on serum calcium and phosphate in urolithiasis induced rats

Treatment	Calcium (mg)	Phosphate (mg)
Vehicle control	7.9 ± 1.52	6.7 ± 0.93
Positive control (EG-treated 0.75% v/v)	10.3 ± 1.64 ^{SSS}	8.10 ± 1.786 ^{SSS}
Cystone treated (750 mg/kg)	8.2 ± 0.81 ^{***}	7.62 ± 0.103 [*]
Curative regimen (CEFS: 30 mg/kg)	9.14 ± 0.93	7.89 ± 0.112 [*]
Curative regimen (CEFS: 60 mg/kg)	8.96 ± 0.459 ^{**}	7.96 ± 0.753 [*]
Preventive regimen (CEFS: 30 mg/kg)	8.6 ± 0.52 ^{***}	7.5 ± 0.713 ^{**}
Preventive regimen (CEFS: 60 mg/kg)	8.3 ± 0.821 ^{***}	7.71 ± 0.952 ^{***}

All values are expressed as mean ± S.E.M.

^{SSS}*p* < 0.001 vs. vehicle control (Group I); ^{***}*p* < 0.001; ^{**}*p* < 0.01 and ^{*}*p* < 0.05 vs. Lithiatic Control (Group II)

as compared to vehicle administered rats. However, notably in groups IV–VII, treatment with CEFS substantially and dose-dependently inhibited urine excretion of phosphate, calcium, and oxalate (table 2). CEFS significantly decreased even serum biochemical markers such as calcium and phosphate (table 3). The average renal clearance of calcium was found to be 0.51 ml/24 hours in vehicle control animals, 2.87 ml/24 hours in positive control group, 1.89 ml/24 hours in cystone treated group, 2.95 ml/24 hours in curative treatment (30 mg/kg CEFS group), 2.70 ml/24 hours in curative treatment (60 mg/kg CEFS group), 2.04 ml/24 hours in preventive group (30 mg/Kg CEFS group) and 2.18 ml/24 hours in preventive group (60 mg/Kg CEFS group). Similarly, 3.85 ml/24 hours, 17.66 ml/24 hours, 11.20 ml/24 hours, 13.69 ml/24 hours, 16.96 ml/24 hours, 14.4 ml/24 hours and 12.18 ml/24 hours are the renal clearances of phosphate

in vehicle-control, positive-control, cystone-treated, curative treatment (30 mg/Kg), curative treatment (60 mg/Kg), preventive treatment (30 mg/Kg) and preventive treatment (60 mg/Kg) groups respectively. Comparing EG-treated animals to vehicle-treated rats, the magnesium levels in the 24-hour urine sample dropped considerably. On the other hand, groups IV–VII treated with CEFS showed a dose-dependent increase in urine magnesium excretion and a considerable prevention of these shifts (table 2). There was a substantially higher levels of LDH in EG-treated rat kidneys as opposed to vehicle administered rats. The LDH levels were brought down to near normal with CEFS treatment. Rats treated with EG had higher kidney weight (both wet and dry) than rats in the vehicle control group; in contrast, rats treated with Cystone had lower kidney weight (both wet and dry) than rats treated with EG. Similarly, kidney weight (both wet and dry) reduction was noticed in accordance with dose following CEFS administration (table 4)

The histopathological examination of the kidney uncovered typical structure of constituent features such as tubules and glomeruli in vehicle-treated rats, while the H&E-stained renal article highlighted the loss of natural architecture with a detectable presence of crystalline structure in several tubules and glomeruli in EG-treated rats (Fig. 1). The afflicted tubules displayed a total loss of lining epithelium, while certain tubules demonstrated vacuolar epithelial cell degeneration. Under a polarising microscope, the same slice discovered an abundance of white chalky coloured calcium oxalate crystals in many tubules and glomeruli (Fig. 2). The current investigation observed that the CEFS-treated groups specifically maintained normal renal architecture with infrequent calcium oxalate accumulation.

Table 4 Effect of chloroform extract of *F. Strobilifera* R.Br. leaves on LDH and kidney weight (wet and dry) of in urolithiasis induced rats

Treatment	LDH (IU/L)	Wet kidney Weight (g/200 g body weight)	Dry kidney Weight (g/200 g body weight)
Vehicle control	300.8 ± 25.46	0.552 ± 0.030	0.240 ± 0.023
Positive control (EG-treated 0.75%V/V)	554.3 ± 38.68 ^{SSS}	0.969 ± 0.044 ^{SSS}	0.358 ± 0.016 ^{SSS}
Cystone treated (750 mg/kg)	313.5 ± 31.89 ^{***}	0.601 ± 0.065 ^{***}	0.251 ± 0.029 ^{***}
Preventive regimen (CEFS: 30 mg/kg)	426.6 ± 12.88 ^{***}	0.662 ± 0.059 ^{**}	0.277 ± 0.024 ^{**}
Preventive regimen (CEFS: 60 mg/kg)	320.8 ± 17.67 ^{***}	0.620 ± 0.049 ^{***}	0.261 ± 0.017 ^{***}
Curative regimen (CEFS: 30 mg/kg)	431.6 ± 12.42 [*]	0.684 ± 0.057 ^{**}	0.292 ± 0.023 [*]
Curative regimen (CEFS: 60 mg/kg)	416.6 ± 6.80 ^{**}	0.638 ± 0.054 ^{**}	0.280 ± 0.019 ^{**}

All values are expressed as mean ± S.E.M.

^{SSS}*p* < 0.001 vs. vehicle control (Group I); ^{***}*p* < 0.001; ^{**}*p* < 0.01 and ^{*}*p* < 0.05 vs. Lithiatic Control (Group II).

Discussion

The present endeavour is an animal model study which employed male rats to induce urolithiasis using ethylene glycol as their urinary system is recognized to mimic that of humans [11], and prior research indicated that female rats exhibited considerably less development of stones [12]. Urinary supersaturation in relation to stone-forming particles is thought to be one of the causal variables in calculogenesis. Studies in the past has demonstrated that young male albino rats develop renal calculi primarily constituted of calcium oxalate following ethylene glycol (0.75% V/V) ingestion for 14 days. The biochemical process beneath this formation is thought to entail a spike in oxalate particulate in the urine [13]. One of the key elements in defining the particular type of crystal and the kind of macromolecules that are present on the crystals' surface is the urine biochemistry. As a result, this study incorporated the testing of the urine composition related to the elements that contribute to renal calculi.

As previously observed, urinary volume is significantly increased in EG-treated rats. The higher urine volume was also noted in CEFS-treated groups, and this is attributable to the drug's diuretic conduct [14]. In the present study, all curative and preventive dosages of plant extract caused diuresis in the test subjects. Studies in both clinical and experimental settings have demonstrated that diuresis and the afflux of calcium and oxalate into the urine are critical components in the production of calcium stones [15]. Diuresis hinders the development of emerging crystals and accelerates the breakdown of existing ones. Diuresis renders the process simpler to remove

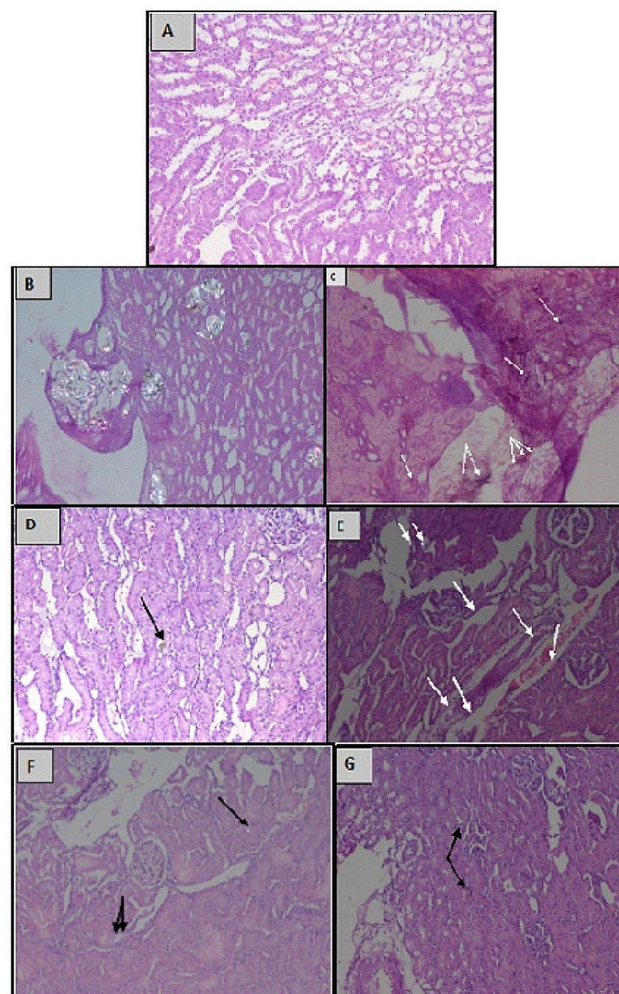


Fig. 1 Light microscopic views of architecture and calcium oxalate deposits in the kidney sections. Kidney sections of **A** – Vehicle Control; **B** – Urolithiatic; **C** – Cystone treated; **D** – Prophylactic treatment with CEFS at the dose of 60 mg/kg; **E** – Prophylactic treatment with CEFS at the dose of 30 mg/kg; **F** – Curative treatment with CEFS at the dose of 60 mg/kg; **G** – Curative treatment with CEFS at the dose of 30 mg/kg, (**H** and **E** × 200 X)

tiny crystals and less likely for them to develop and aggregate, which assists in preventing new stones formation in the renal system. Additionally, diuresis result in the dilution of the urine, which lowers the chance of stone development by dropping the saturation level of the calcium oxalate [15].

It was observed that the oxalate and the calcium excretion rose gradually in group II rats (positive control). Earlier studies that treated rats with ethylene glycol also showed similar outcomes [16, 17]. One of the factors that promotes the nucleation and crystallisation of calcium oxalate or phosphate in urine is an increase in the concentration of calcium in the urine and the lateral crystal development phenomenon [18]. The administration of CEFS substantially decreased the calcium and oxalate levels in the urine. Reduced oxalate excretion

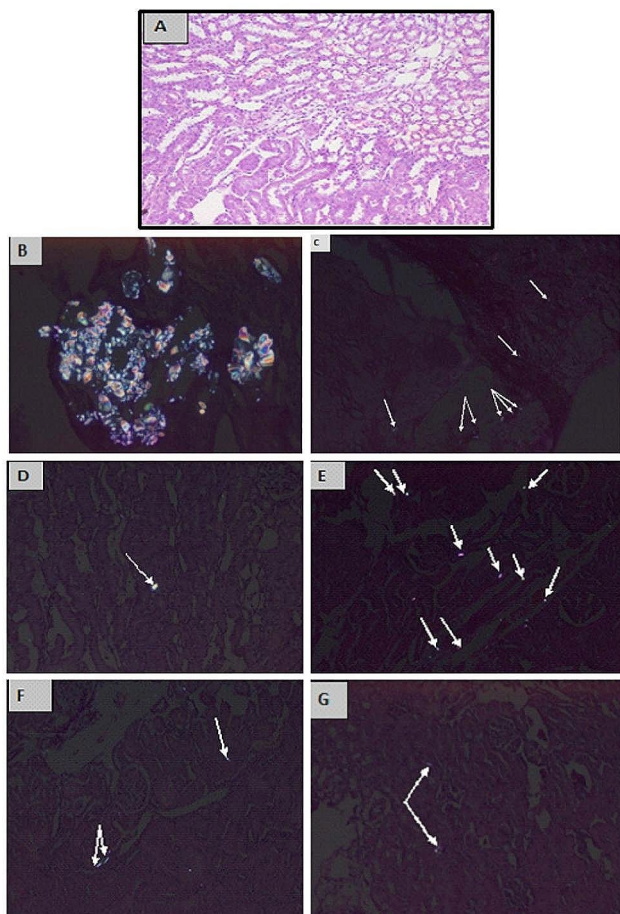


Fig. 2 Polarizing microscopic views of architecture and calcium oxalate deposits in the kidney sections. Kidney sections of **A** – Vehicle Control; **B** – Urolithiatic; **C** – Cystone treated; **D** – Prophylactic treatment with CEFS at the dose of 60 mg/kg; **E** – Prophylactic treatment with CEFS at the dose of 30 mg/kg; **F** – Curative treatment with CEFS at the dose of 60 mg/kg; **G** – Curative treatment with CEFS at the dose of 30 mg/kg. (**H** and **E** ×200 X)

may be a result of oxalate production inhibition, which validates the effectiveness of herbal drugs as antilithiatic. There was a significant increase in renal clearance of calcium and phosphate in CEFS treated animals. Compared to cystone treated groups, the difference in renal clearance of calcium was found to be -1.06 ml/24 hours was observed in curative 30 mg/Kg CEFS treatment, -0.81 ml/24 hours in curative 60 mg/Kg CEFS treatment, -0.15 ml/24 hours in preventive 30 mg/Kg CEFS treatment and -0.29 ml/24 hours in preventive 60 mg/Kg CEFS treatment groups. The similarity of the results with the cystone, the CEFS was found to be effective as a prophylaxis. Thus, the CEFS inhibits the development of renal calculi by decreasing the calcium excretion as well as degree of supersaturation of the urine. Magnesium is one of the several well-known inorganic and organic crystallisation inhibitors encountered in normal urine [19]. Decreased amounts of magnesium were found in both stone formers and stone forming rats, but

CEFS therapy restored the magnesium to normal levels, thus reducing the formation of calcium oxalate crystals. It has been discovered that diets rich in magnesium preserve the rats' kidneys from calcium oxalate buildup [20]. EG-treated rats exhibited a progressive rise in urine phosphorus. By producing calcium phosphate crystals, which epitaxially drive calcium oxalate accumulation, elevated urinary phosphorus outflow combined with oxalate appears to foster an environment conducive to stone formation [21]. On the other hand, CEFS therapy returns the phosphorus level practically to normal, and thus typically lowering the chance of stone formation.

The Lactate Dehydrogenase enzyme catalyses the conjugation of glyoxylate oxidation with reduction amid the presence of pyridine nucleotide coenzyme, resulting in the concurrent synthesis of glycollate and oxalate, whilst Ethylene glycol disrupts oxalate metabolism through expanding substrate flexibility, which increases the level of activity of oxalate synthesising enzymes in rats [22]. In this particular inquiry, the kidneys of rats with EG-induced urolithiatic kidneys showed a markedly elevated level of LDH activity. When CEFS was administered, the kidneys of urolithiatic rats showed an enormous drop in LDH activity. Thus, elevated LDH activity substantiated their causal relationship with intrinsic oxalate precipitation in EG induced urolithiasis. The EG-treated rats' renal weights, both wet and dry, arose as a result of calcium oxalate crystals depositing in the glomerulus and renal tubules. Rats receiving CEFS did not exhibit this elevated kidney weight.

The microscopic examination of kidney sections derived from EG-induced urolithiatic rats evidenced a collapse of regularity alongside the infestation of crystalline structure in tubules and glomeruli under bright-field microscope. The lining epithelium of the afflicted tubules had completely disappeared, and in certain tubules, there was vacuolar atrophy of the epithelial cells. A polarising microscope examination of the same section demonstrated an array of chalky calcium oxalate crystals in tubules and glomeruli. The crystal deposits were highly pretentious, polycrystalline, and structured in clasp, which is a classic hallmark of calcium oxalate crystals. The existence of these aggregates indicates adherence and pentide persistence inside the renal tubules. The recuperation benefit was seen in rats treated with CEFS and this could be attributed to the flavonoids and flavonoid glycosides present in the leaves of *F. Strobilifera R.Br.* These rats showed greater dissolution of calcium oxalate crystal deposits and regained normal renal architecture. The treatment of CEFS to EG-induced urolithiasis rat models decreased and circumvented the formation of urinary stones. Although the exact mechanism underlying this phenomenon is uncertain, this may be facilitated by decreasing the concentration of components that

result in stones in the urine and diuresis. Additionally, it appears that CEFS's preventative benefits outweighed its therapeutic ones.

Conclusion

In summary, the chloroform leaf extract of *F. Strobilifera* R. Br. has demonstrated notable antiurolithiatic effects in-vivo. The propensity of *F. Strobilifera* R. Br to preserve renal function, minimise renal damage, and lower crystal elimination in urine has been shown to indicate its anti-lithiatic potential. Therefore, the current study suggests the capaciousness of *F. Strobilifera* R. Br to avert the precipitation of calcium oxalate and serves as a foundation for the development of an antiurolithiatic agent.

Abbreviations

CEFS	Chloroform leaf extract of <i>Flemingia Strobilifera</i> R.Br
EG	Ethylene glycol
LDH	Lactate Dehydrogenase
CaOx	Calcium Oxalate
FRLHT	Foundation for Revitalization of Local Health Traditions
H & E	Hematoxylin and Eosin
ANOVA	Analysis of Variance

Acknowledgements

The authors would like to express a sincere appreciation to the management of Vokkaligara Sangha, Bengaluru for providing the facilities to carry out this research.

Author contributions

1-2: Conceptualization, Data Acquisition, Resources, Original Draft 3: Formal Statistical Analysis 4: Analysis, Data Acquisition, Data Curation 5-6: Writing Original Article, Reviewing and Formatting the Article.

Funding

The authors did not receive funding from any of the sources or organization.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

Animal ethical approval was approved by the Institutional Animal Ethics Committee.

Consent for publication

None.

Competing interests

The authors declare no competing interests.

Received: 13 January 2024 / Accepted: 24 July 2024

Published online: 08 August 2024

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