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ANTI-UROLITHIATIC ACTIVITY OF SINAPIC ACID AGAINST ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN ALBINO WISTAR RATS

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ABSTRACT

Background: Urolithiasis is a prevalent urological disorder characterized by the formation of calcium oxalate (CaOx) crystals in the urinary tract, often resulting in renal dysfunction and recurrence. Despite the availability of conventional therapies, their associated limitations have prompted the exploration of safer and more effective natural therapeutic agents.**Objective:**The present study was designed to investigate the antiurolithiatic effects of Sinapic Acid in an ethylene glycol-induced calcium oxalate urolithiasis in male Albino Wistar rats. **Methods:**Thirty male Albino Wistar rats (150-250 g) were selected and divided into five groups (n=6). Urolithiasis was induced in Groups II–V by administering 0.75% ethylene glycol in drinking water for 14 days. Group I was served as the Normal control, Group II disease control, Group III animals were treated with standard drug Cystone (750mg/kg, p.o.) and Groups IV and V were treated with Sinapic Acid 20mg/kg and 40mg/kg p.o. respectively for 14 days. After 14 days urinary parameters, renal profile, body weight, water intake, and histopathological changes in kidney were evaluated. Statistical analysis was performed by using Two-way ANOVA.**Results:**Ethylene glycol administration significantly reduced body weight and urine volume while increasing water intake and serum urea, creatinine, blood urea nitrogen, uric acid, and calcium levels compared with the normal control ($p < 0.001$). Sinapic acid significantly attenuated these changes in a dose-dependent manner. At 40 mg/kg, serum urea, creatinine, blood urea nitrogen, uric acid, and calcium were reduced ($p < 0.001$ vs. disease control), with effects comparable to Cystone. Sinapic acid also restored urine volume, normalized water intake, markedly reduced calcium oxalate crystal deposition, and preserved normal renal histoarchitecture in a dose-dependent manner.**Conclusion:**The protective effects observed with Sinapic Acid at 40 mg/kg were comparable to those of the standard drug. Cystone and Sinapic Acid have a strong dose-dependent anti-urolithiatic and Reno protective effect, which is likely to be mediated by its antioxidant, anti-inflammatory, and diuretic effects. The study findings are strong to support Sinapic Acid as a future therapeutic agent for urolithiasis prevention and management as a natural compound.

Keywords: Sinapic Acid, Urolithiasis, Ethylene glycol, Kidney stones.

1. INTRODUCTION

Urolithiasis, also referred to as nephrolithiasis or renal stone disease, is a common urological disorder characterized by the formation of calculi within the urinary tract. It is a significant global health problem, affecting approximately 10% of men and 6% of women during their lifetime, with the highest incidence occurring between 20 and 60 years of age. In addition to its high prevalence, urolithiasis is associated with considerable morbidity, frequent recurrence, and an increasing economic burden on healthcare systems [1, 2].Calcium oxalate (CaOx) stones constitute more than 80% of all urinary calculi and remain the predominant type of kidney stone [3]. Their formation is a complex, multifactorial process involving urinary supersaturation, crystal nucleation, growth, aggregation, and retention within the renal tubules. Several metabolic abnormalities, including hyperoxaluria,

hypercalciuria, and hypocitraturia, contribute to calcium oxalate stone formation [4]. Furthermore, oxidative stress and renal tubular epithelial injury have been recognized as critical factors that facilitate crystal adhesion and promote stone development [5].

The pathogenesis of urolithiasis is influenced by a combination of endogenous and exogenous factors. Endogenous factors include disturbances in the metabolism of calcium, oxalate, phosphorus, uric acid, and other nitrogenous waste products, whereas exogenous factors such as inadequate fluid intake, dehydration, hot climatic conditions, dietary habits, and consumption of hard water further increase the risk of stone formation[6]. Recent evidence also indicates that systemic disorders, including diabetes mellitus, obesity, hypertension, chronic kidney disease, and metabolic syndrome, substantially increase the susceptibility to urinary stone disease through alterations in urinary composition and renal function.Despite advances in medical management, the prevention and treatment of recurrent urolithiasis remain challenging. Currently available pharmacological therapies, including thiazide diuretics, potassium citrate, and allopurinol, are effective only in selected patients and are often associated with limited efficacy, adverse effects, high treatment costs, and poor long-term compliance [7]. Consequently, there is growing interest in identifying naturally occurring bioactive compounds that can safely inhibit stone formation by targeting multiple pathogenic mechanisms, particularly oxidative stress, inflammation, crystal aggregation, and renal epithelial injury [8].

Among naturally occurring phenolic compounds, sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) has attracted considerable attention because of its broad spectrum of pharmacological activities [9]. It is abundantly present in cereals, oilseeds, berries, spices, and several medicinal plants. Experimental studies have demonstrated that sinapic acid possesses potent antioxidant, anti-inflammatory, antihyperglycemic, antimicrobial, and neuroprotective properties [10]. In addition, its strong free radical-scavenging and peroxynitrite-neutralizing activities have been shown to protect against oxidative tissue damage in various experimental models. Emerging evidence further suggests that sinapic acid exerts significant nephroprotective effects by attenuating oxidative stress, suppressing inflammatory responses, and reducing renal injury, including tacrolimus-induced nephrotoxicity [11].

Although several naturally occurring phenolic acids and flavonoids have demonstrated promising antiurolithiatic activity in experimental models, the potential role of sinapic acid in preventing or treating calcium oxalate urolithiasis has not yet been investigated. Considering its potent antioxidant and nephroprotective properties, sinapic acid may represent a promising therapeutic candidate for reducing calcium oxalate crystal formation and minimizing renal tissue damage [12]. Therefore, the present study was designed to evaluate the antiurolithiatic efficacy of sinapic acid in an ethylene glycol-induced calcium oxalate urolithiasis model in male Wistar rats using biochemical, renal functional and histopathological examination.

2. MATERIALS AND METHODS

Drugs and Chemicals

Sinapic acid (purity ≥98%) was obtained from Yucca Enterprises, Mumbai, India. Ethylene glycol and all other reagents and chemicals used in the study were of analytical grade and were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Cystone® tablets, manufactured by The Himalaya Wellness Company, Bengaluru, India, served as the standard reference drug in the experimental investigation.

Experimental Animals

Healthy adult male Wistar albino rats weighing 150–250 g were used in the present study. The animals were housed in polypropylene cages under standard laboratory conditions, maintained at a temperature of 22 ± 2 °C with a relative humidity of 55–65% and a 12 h light/12 h dark photoperiod. Standard laboratory pellet diet and filtered drinking water were provided ad libitum throughout the acclimatization and experimental periods [13].

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

Induction of Urolithiasis and Experimental Design

The experiment was carried out for 14 days. Urolithiasis was induced by administering ethylene glycol in the drinking water throughout the study. Sinapic acid and Cystone® were administered orally once daily from Day 1 to Day 14.The treatment regimen was initiated simultaneously with ethylene glycol administration to evaluate the preventive effect of sinapic acid against ethylene glycol-induced calcium oxalate urolithiasis [14].

Thirty rats were randomly divided into five groups (n = 6) as follows

- Group I: Normal control– Vehicle (normal saline) only
- Group II: Disease control– 0.75% v/v ethylene glycol in drinking water for 14 days
- Group III: Standard– Ethylene glycol + Cystone 750 mg/kg body weight, p o.
- Group IV: Sinapic acid low dose– Ethylene glycol + Sinapic acid 20 mg/kg, p o.
- Group V: Sinapic acid high dose– Ethylene glycol + Sinapic acid 40 mg/kg, p o.

Urine Analysis

Twenty-four-hour urine samples were collected on Days 0, 7, and 14 by placing the animals individually in metabolic cages. Urine volume and pH were recorded immediately after collection. The urine samples were analyzed for calcium, oxalate, phosphate, uric acid, and creatinine. Body weight and daily water intake by the animals were also monitored throughout the experimental period [15].

Biochemical Analysis

On Day 14, blood samples were collected from the retro-orbital plexus under light ketamine anesthesia. Serum was separated by centrifugation at 3000 rpm for 10 min and analyzed for creatinine, blood urea nitrogen (BUN), urea, uric acid, and calcium using commercially available diagnostic kits on a semi-automated biochemical analyzer [16].

Histopathological Examination

At the end of the study (Day 14), the animals were euthanized by using Euthanesia chamber. Both kidneys were excised, rinsed with ice-cold normal saline, blotted dry, and weighed. Kidney tissues were fixed in 10% neutral buffered formalin. Tissue was sectioned by using semiautomatic Cryostat and the Sections (5 µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope at ×400 magnification to evaluate calcium oxalate crystal deposition and renal tubular histopathological changes [17-19].

Statistical Analysis

The calculated results were shown as the Mean ± Standard Deviation. To identify significant differences, the collected data was analyzed using a one-way analysis of variance (ANOVA). Using Dunnett's t-test, significant differences between groups were evaluated. A significant p-value of less than 0.01 was found. For statistical analysis and data visualization, Graph Pad in Stat Version 3.06 (Graph Pad Software, Inc., La Jolla, CA, USA) and Microsoft Excel 2013 Standard (Microsoft Corp., Redmond, WA, USA) were utilized.

3. RESULTS

Effect of Sinapic acid on Body Weight and Water Intake

Administration of 0.75% ethylene glycol for 14 days caused significant reduction in body weight gain (Table 1) and marked increase in water intake in disease control group (Group II) compared to normal control ($p < 0.001$). Simultaneous treatment with Sinapic acid 20 mg/kg and 40 mg/kg significantly restored the body weight and normalised water intake in a dose-dependent manner. The higher dose (40 mg/kg) showed effects comparable to Cystone 750 mg/kg (Table 2)

Table 1: Effect of Sinapic acid on body weight of rats during 14-day treatment period

GROUP	0^{th} day (Mean ± SEM)	7^{th}day(Mean ± SEM)	14^{th}day (Mean ± SEM)
Normal	259.00 ± 2.65	275.00 ± 9.64	299.33 ± 12.13
Diseased	339.67 ± 12.72	299.67 ± 8.65	278.00 ± 16.56
Standard	325.00 ± 10.15	260.00 ± 43.41	309.67 ± 3.93
Test Low dose	314.33 ± 15.96	305.67 ± 22.70	296.67 ± 19.34
Test High dose	288.00 ± 11.79	289.33 ± 15.07	271.00 ± 12.49

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; †p<0.05, ††p<0.01, †††p<0.001 vs disease control.

Table 2: Effect of Sinapic acid on daily water intake in rats

Group	Day 0 (ml)	Day 7 (ml)	Day 14 (ml)
Normal control	45 ± 2.1	47 ± 2.3	46 ± 2.4
Disease control (EG)	44 ± 2.2	68 ± 3.5***	82 ± 4.1***
EG + Cystone 750 mg/kg	46 ± 2.0	49 ± 2.4†††	48 ± 2.5†††
EG + SA 20 mg/kg	45 ± 2.3	58 ± 3.1††	55 ± 3.2†††

EG + SA 40 mg/kg	44 ± 2.1	50 ± 2.6†††	47 ± 2.4†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; †p<0.05, ††p<0.01, †††p<0.001 vs disease control.

Effect on Urine Volume and pH

Ethylene glycol administration significantly reduced 24-h urine volume and urinary pH (acidic urine) in Group II ($p < 0.001$). Sinapic acid treatment (20 and 40 mg/kg) produced marked diuresis and significantly increased urinary pH toward normal values ($p < 0.01$ and $p < 0.001$, respectively). The 40 mg/kg dose was almost equipotent to Cystone (Table 3).

Table 3: Effect of Sinapic acid on 24-hour urine volume on day 0, day 7 and day 14

Group	Day 0 (ml)	Day 7 (ml)	Day 14 (ml)
Normal control	12.5 ± 0.8	13.2 ± 0.9	13.0 ± 1.0
Disease control (EG)	12.8 ± 0.7	8.5 ± 0.6***	6.2 ± 0.5***
EG + Cystone 750 mg/kg	12.6 ± 0.9	12.8 ± 0.8†††	13.1 ± 1.0†††
EG + SA 20 mg/kg	12.7 ± 0.8	10.8 ± 0.7†	11.9 ± 0.9††
EG + SA 40 mg/kg	12.4 ± 0.7	12.5 ± 0.8†††	12.9 ± 1.0†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; †p<0.05, ††p<0.01, †††p<0.001 vs disease control.

Effect of Sinapic acid on Urinary and Serum Biochemical Parameters

Disease control rats exhibited significant elevation in urinary calcium, oxalate, phosphate and uric acid levels along with increased serum creatinine, blood urea nitrogen (BUN), urea and uric acid compared to normal control ($p < 0.001$). Co-administration of Sinapic acid (20 and 40 mg/kg) dose-dependently and significantly reduced the excretion of lithogenic promoters in urine and normalised serum renal function markers ($p < 0.05$ to $p < 0.001$). The higher dose (40 mg/kg) restored all parameters close to normal and showed activity comparable to standard drug Cystone (Tables 4–8).

Table 4: Effect of Sinapic acid on serum urea level in rats

Group	Serum Urea (mg/dl)
Normal control	28.5 ± 1.2
Disease control (EG)	58.2 ± 2.8***
EG + Cystone 750 mg/kg	31.4 ± 1.5†††
EG + SA 20 mg/kg	42.6 ± 2.1††
EG + SA 40 mg/kg	30.8 ± 1.4†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; ††p<0.01, †††p<0.001 vs disease control.

Table 5: Effect of Sinapic acid on serum uric acid level

Group	Serum Uric Acid (mg/dl)
Normal control	2.1 ± 0.1
Disease control (EG)	5.8 ± 0.3***

EG + Cystone 750 mg/kg	2.3 ± 0.1†††
EG + SA 20 mg/kg	3.9 ± 0.2††
EG + SA 40 mg/kg	2.4 ± 0.1†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; ††p<0.01, †††p<0.001 vs disease control.

Table 6: Effect of Sinapic acid on serum calcium level in rats

Group	Serum Calcium (mg/dl)
Normal control	9.2 ± 0.4
Disease control (EG)	12.8 ± 0.6***
EG + Cystone 750 mg/kg	9.5 ± 0.4†††
EG + SA 20 mg/kg	10.9 ± 0.5††
EG + SA 40 mg/kg	9.4 ± 0.4†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; ††p<0.01, †††p<0.001 vs disease control.

Table 7: Effect of Sinapic acid on blood urea nitrogen (BUN) level in ethylene glycol-induced urolithiatic rats

Group	BUN (mg/dl)
Normal control	13.2 ± 0.6
Disease control (EG)	27.5 ± 1.3***
EG + Cystone 750 mg/kg	14.1 ± 0.7†††
EG + SA 20 mg/kg	19.8 ± 0.9††
EG + SA 40 mg/kg	14.3 ± 0.7†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; ††p<0.01, †††p<0.001 vs disease control.

Table 8: Effect of Sinapic acid on serum creatinine level in rats

Group	Serum Creatinine (mg/dl)
Normal control	0.65 ± 0.03
Disease control (EG)	1.42 ± 0.07***
EG + Cystone 750 mg/kg	0.68 ± 0.03†††
EG + SA 20 mg/kg	0.98 ± 0.05††
EG + SA 40 mg/kg	0.70 ± 0.03†††

Values are mean ± SEM (n=6); ***p<0.001 vs normal control; ††p<0.01, †††p<0.001 vs disease control.

Effect on Microscopical Examination of Urine and Kidney Histopathology

Microscopic examination of urine from disease control group revealed abundant calcium oxalate crystals (Figure 1A). Treatment with Cystone (Figure 1B), Sinapic acid 20 mg/kg (Figure 1C) and 40 mg/kg (Figure 1D) markedly reduced crystal count. Histopathological sections of kidney from disease control rats showed heavy calcium oxalate

crystal deposition, tubular dilatation, and severe tubular epithelial damage. Sinapic acid treatment dose-dependently reduced crystal deposition, preserved tubular architecture and prevented renal damage.

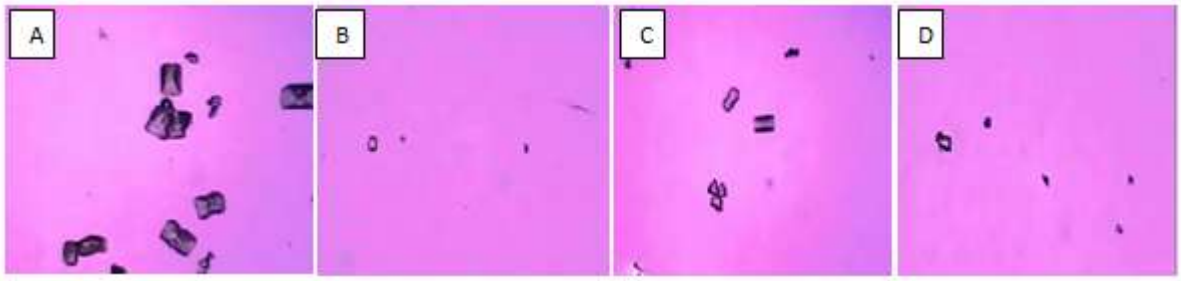

Fig. 1: Microphotographs of urine sediment showing calcium oxalate crystals (400×). A – Disease control, B – Standard (Cystone), C – Sinapic acid 20 mg/kg, D – Sinapic acid 40 mg/kg

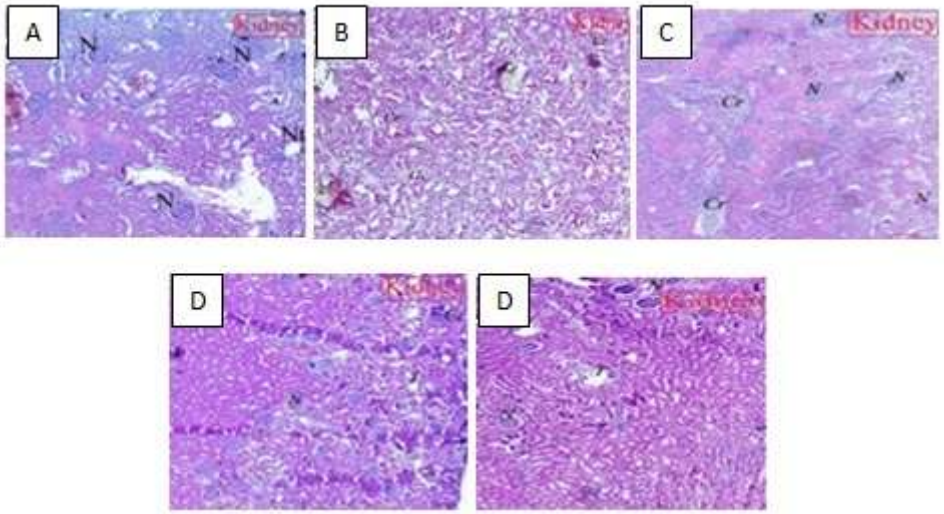

Fig. 2: Effect of Sinapic acid on Histopathological examination of kidney
A = Normalcontrol, B = Lithiaticcontrol, C = Cystone750mg/kg, D = Sinapic acid 20 mg/kg, D = Sinapic acid 40 mg/kg.
Cr – CaOx crystals; D – Dilatation of tubules; G – Glomerular damage; Gs – Glomerularsclerosis;I – Inflammation;N–Normalglomeruli;Nt –Normaltubules; R – Regenerative changes in glomeruli; T – Tubular necrosis.

Histological examination of kidney sections is presented in Figure 2. The normal control group (A) showed normal renal architecture with intact normal glomeruli (N) and normal tubules (Nt). In contrast, the lithiatic control group (B) exhibited marked calcium oxalate crystal deposition (Cr), tubular dilatation (D), tubular necrosis (T), glomerular damage (G), glomerulosclerosis (Gs), and inflammatory cell infiltration (I). Treatment with Cystone (750 mg/kg) (C) reduced crystal deposition and improved renal architecture, with evidence of regenerative changes in glomeruli (R). Sinapic acid at 20 mg/kg (D) showed moderate improvement with reduced crystal deposition, inflammation, and tubular injury. The 40 mg/kg dose (E) produced greater protection, with minimal crystal deposition, reduced inflammation, regenerative changes, and near-normal glomerular and tubular architecture, indicating dose-dependent renoprotective activity against ethylene glycol-induced urolithiasis.
The 40 mg/kg dose exhibited near-normal renal histology likeCystone-treated group (Figure 17A–E).All the protective effects of Sinapic acid were statistically significant and dose-dependent, with 40 mg/kg showing maximum efficacy.

4. DISCUSSION

The present study demonstrated that sinapic acid effectively protected against ethylene glycol-induced calcium oxalate urolithiasis in rats. Ethylene glycol administration produced typical features of nephrolithiasis, including reduced body weight gain, increased water intake, decreased urine volume, impaired renal function and extensive calcium oxalate crystal deposition [20-22]. Sinapic acid treatment significantly improved these alterations in a dose-dependent manner, with the 40 mg/kg dose showing efficacy comparable to Cystone.
The restoration of body weight and normalization of water intake indicate improved renal function and reduced metabolic disturbances. Sinapic acid also increased urine volume, which may reduce urinary supersaturation and facilitate the elimination of stone-forming crystals [23].

Disease control animals exhibited significant elevations in serum urea, creatinine, blood urea nitrogen, uric acid and calcium, reflecting renal dysfunction caused by crystal-induced tubular injury. Treatment with sinapic acid significantly restored these parameters toward normal values, suggesting preservation of renal function [24]. Microscopic examination of urine and kidney histopathology further confirmed its protective effect by demonstrating reduced calcium oxalate crystal deposition and preservation of normal renal architecture.
The antiurolithiatic activity of sinapic acid may be attributed to its antioxidant and anti-inflammatory properties, which reduce oxidative stress and renal tubular damage, thereby limiting crystal formation and retention. The superior efficacy of the 40 mg/kg dose indicates a dose-dependent protective effect [25-27].
Overall, the findings suggest that sinapic acid exerts significant nephroprotective and antiurolithiatic effects by improving urinary parameters, preserving renal function and reducing calcium oxalate crystal deposition. These results support its potential as a natural therapeutic agent for the prevention and management of urolithiasis.

5. CONCLUSION

The present study demonstrated that sinapic acid possesses significant antiurolithiatic activity against ethylene glycol-induced calcium oxalate nephrolithiasis in rats. Sinapic acid improved body weight, normalized water intake, increased urine output, restored renal biochemical parameters and markedly reduced calcium oxalate crystal deposition and renal tissue damage. The protective effects were dose-dependent, with the 40 mg/kg dose showing efficacy comparable to standard compound Cystone. These findings suggest that the antiurolithiatic effect of sinapic acid is mediated through nephroprotective, antioxidant and anti-inflammatory mechanisms. Overall, sinapic acid shows promise as a potential natural therapeutic agent for the prevention and management of urolithiasis. Further studies are needed to elucidate its molecular mechanisms and establish its clinical applicability.

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AUTHORS CONTRIBUTIONS

All the authors contributed in Designing the study, Planning the research, carrying out the project, and writing the manuscript & Statistical analysis: Dr. P. Rajyalakshmi Devi, Vinod Kumar Nelson& N Amali, Biochemical evaluation and Manuscript drafting: Dr. Mote Srinath.Sri Bhavadharani and S. VimalManuscript Data analysis and Result Interpretation, all authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

There are no conflicts of interest to disclose in this research.

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