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Evaluation of Anti-Urolithiatic Activity of Glucosinolates in Rats

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ABSTRACT

Background : Urolithiasis, commonly known as kidney stones, is a recurrent and painful urological disorder that significantly impacts patient health and quality of life. The majority of urinary calculi are composed of calcium oxalate, with other types including uric acid, strive, and cystine stones. Glucosinolates, naturally occurring phytochemical found in cruciferous vegetables, have demonstrated antioxidant, anti-inflammatory, and nephroprotective properties, suggesting potential efficacy in preventing or reducing stone formation. This study was designed to evaluate the anti-urolithiatic activity of glucosinolates in experimental rat models.**Methodology :** Wistar rats were divided into four groups: normal, control, standard (cystone 750 mg/kg), and test (glucosinolates 200 mg/kg). The induction of urolithiasis was performed with ethylene glycol, then the blood and urine was collected after 28 days to determine the biochemical parameters such as urinary calcium, oxalate, phosphate, uric acid, and creatinine, along with histopathological examination of renal tissues.**Results :** Glucosinolates (200~mg/kg) significantly reduced the urinary excretion of calcium (2.07±0.10~mg/dl), phosphate (4.91±0.37~mg/dl), and oxalate (0.59±0.04~mg/dl). In serum analysis, Glucosinolates significantly lowered uric acid to 1.75±0.13~mg/dl, serum creatinine to 1.08±0.10~mg/dl and urea nitrogen to 21.25±1.10~mg/dl. In histopathological study, Glucosinolates suggesting improved urinary flow and promoting stone disintegration.

Keywords : Urolithiasis, Ethylene Glycol, Glucosinolates, Cystone, Stone formation, Urine, Histopathology, Phytochemical, Rat models

INTRODUCTION

Urolithiasis is a condition in which a stone formed in the kidney moves down the urinary tract to the ureter, bladder, or urethra. The underlying cause of renal calculi is an imbalance between lithogenic mechanisms and stone-forming processes, which gradually leads to their accumulation in the kidney. Recurrence occurs in half of patients within five years and in as many as 80% within 10 years[1]. Approximately 70-80% of kidney stones are composed of calcium oxalate and calcium phosphate. Of the rest, 10% are struvite, 10% of uric acid; and less than 1% are composed of cystine or are diagnosed as drug-related stones[2]. Globally, urolithiasis accounts for about 3 million visits each year by patients to healthcare providers, while about half a million patients are admitted to the emergency room as a result of casualties. Further research in epidemiological data suggests numerous factors, such as age, diet, environment, industrialisation, sex, and socioeconomic status, as the most important factors influencing the prevalence of urolith formation and development[3].

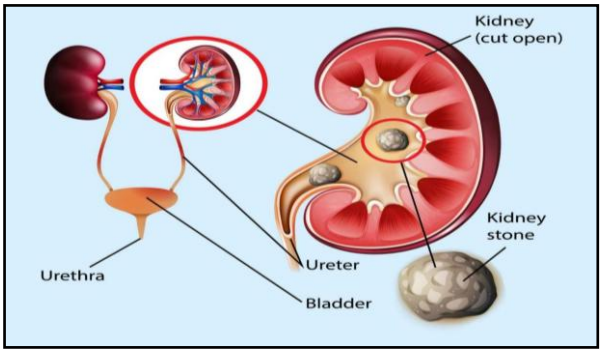

Figure.01 Structure of kidney stones

The kidney stone formation occurs in various stages and are: super saturation, nucleation, crystal growth, crystal aggregation, crystal cell interaction, stone formation[4]. Approximately 15 to 16% of affected children develop chronic kidney failure, and a subset even require kidney transplantation. The etiology of paediatric urolithiasis is complex, often involving a combination of metabolic disorders, genetic factors, anatomical abnormalities of the urinary tract, diet, and environmental influences. Approximately 71% of affected children require surgical intervention, with over 12% undergoing open surgery. In addition, the recurrence rate of paediatric urolithiasis is alarmingly high, ranging from 16% to 44%[5]. Ethylene glycol–induced urolithiasis is a commonly used experimental model which induces urolithiasis primarily by metabolising into oxalate, causing hyperoxaluria (excessive urinary oxalate). This results in urinary supersaturation, where oxalate binds with calcium to form calcium oxalate crystals. These crystals deposit in renal tubules, causing oxidative stress, tissue damage, and eventually stone formation, typically used in animal models to simulate kidney stone development[6,7,8]. Glucosinolates are part of a group of secondary metabolites that contain sulphur and are primarily found in vegetables belonging to the crucifer family, such as broccoli, cabbage, and radishes. When they undergo hydrolysis, they yield active substances like isothiocyanate and in-doles, which are associated with numerous beneficial effects including, but not confined to, antimicrobial, anti-inflammatory, antioxidant, anticancer, and gastroprotective properties. The extensive range of biological actions suggests that glucosinolates could be valuable in the management of gastrointestinal issues characterized by inflammation and increased motility. Their prevalence in nature and established safety profiles further enhance their potential as alternative treatments for urolithiasis[9,10,11]. Thus, the study intended to investigate the anti-urolithiatic effect of glucosinolates in Wistar rats using the ethylene glycol-induced urolithiasis and methods like serum and urine analysis.

AIM: To evaluate the anti-urolithiatic activity of glucosinolates in rats.

OBJECTIVES:

- To compare the anti-urolithiatic effect of glucosinolates with that of standard agents.
- To determine if Glucosinolates can prevent the initial formation of stones when administered alongside a lithogenic agent (like Ethylene Glycol).
- To evaluate if the compounds can reduce the size or number of pre-existing stones in the renal system.
- To observe if Glucosinolates treatment prevents the crystal-induced damage to the renal tubules.

DRUG PROFILE

Glucosinolates consists of a sulfonated aldoxime domain linked to a D-thioglucose group together with a side chain (a-glycone) derived from one or several amino acids. GLs contain an oxidised sulphur atom of 3-phosphoadenosine, 5-phosphosulfate, a reduced glutathione atom and in case of methionine derived aliphatic GLs , a third sulphur atom.

GLS are classified as

- Aliphatic glucosinolates
- Aromatic glucosinolates
- Indole glucosinolates

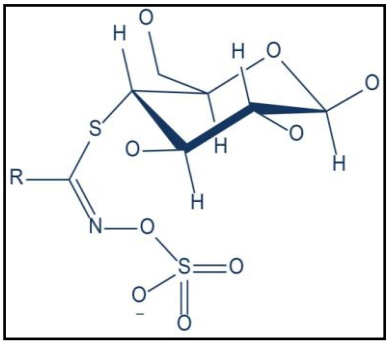

Figure.02 Structure of glucosinolate

MATERIALS AND METHODS

Experimental animals: Healthy Wistar rats (150–200 g) were used. Animals were housed at a temperature of 24±2ºC and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. Approval from Institutional Animal Ethics Committee (IAEC) was obtained in accordance with CPCSEA guidelines.

Drugs and chemicals

- Glucosinolates
- Cystone
- Ethylene glycol
- Formalin
- Ketone

Animal Grouping

Animals were divided into five groups:

Table. 1. Experimental procedure

ANIMAL GROUP	TESTING METHOD
GROUP 1	0.1% carboxy methyl cellulose + normal water
GROUP 2	0.75%v/v of Ethylene glycol + Drinking water
GROUP 3	0.75%v/v of Ethylene glycol + 750mg/kg of cystone + Drinking water
GROUP 4	0.75%v/v of Ethylene glycol + 200mg/kg of glucosinolate + Drinking water

Ethylene glycol-induced urolithiasis

Ethylene glycol metabolise into oxalate which bind with calcium to form crystals and it deposits in renal tubules causing stone formation. Rats were fed with 0.75% ethylene glycol in drinking water for the induction of urolithiasis for 28 days.

Histopathological study

Histopathological study evaluates the composition and structure of stones as well as tissue damage and crystal deposition in kidney. After administration of ethylene glycol for 28 days, the rats were euthanised with excess anaesthesia and dissected the kidney. Kidney was examined macroscopically to study the histopathological changes.

Urine analysis

All rats were housed in metabolic cages. On 28th day, 24 hour urine samples were collected from all the animals. Calcium, Phosphate and Oxalate contents were was estimated by the method mention by Lorentz, 1982 using standard diagnostic kit.

Serum analysis

Blood was collected and serum was separated by centrifugation.Then, the blood urea nitrogen and creatinine level was estimated by Bert-helot method (Fawcett and Scott, 1960) using standard kit and the uric acid content was estimated by the method of Caraway, 1963.

RESULTS

HISTOPATHOLOGICAL STUDY

1. Urine sample

a) Group –I : Normal Control.

MICROSCOPIC APPEARANCE:

Under light microscopy, urine samples obtained from the control group exhibited normal morphological characteristics. No birefringent calcium oxalate crystals—either in the monohydrate or dihydrate forms—were detected, indicating that there is no any pathological changes.

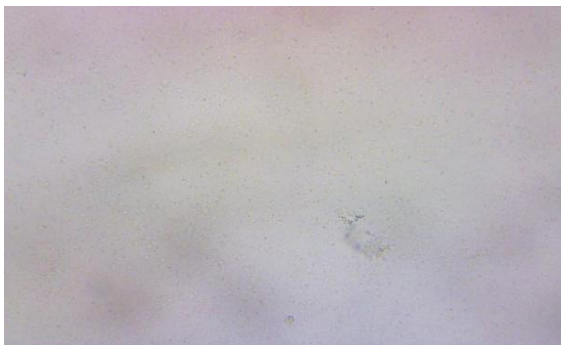

Figure.03 Histopathological study of urine sample in normal control under microscope

b) Group –II : Ethylene Glycol Control.

MICROSCOPIC APPEARANCE:

Microscopic analysis of urine sediment from the group II revealed abundant, birefringent calcium oxalate crystals**,** characterized by the presence of both envelope-shaped dihydrate and needle- or dumbbell-shaped monohydrate morphologies.

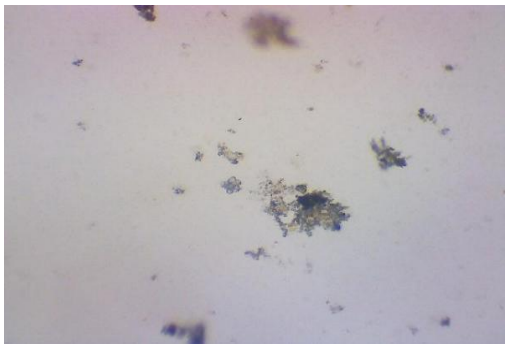

Figure.04 Histopathological study of urine sample in Ethylene Glycol control under microscope

c) Group –III : Cystone

MICROSCOPIC APPEARANCE:

Microscopic examination of the urine sediment in the group III revealed no evidence of any abnormality and calcium oxalate (CaOx) crystals. Mild tiny needle like crystals which appears to be get disintegrated.

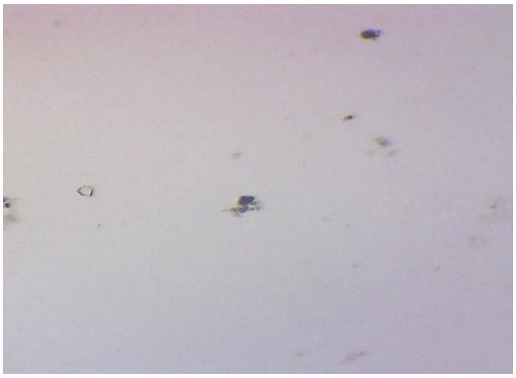

Figure.05 Histopathological study of urine sample in Cystone under microscope

d) Group –IV : Glucosinolates

MICROSCOPIC APPEARANCE:

Urine analysis showed that partial crystallogenesis**,** as evidenced by a substantial reduction in the urinary oxalate crystal and the presence of occasionally fragmented or broken crystals, indicating its potential role in both preventing crystal growth and promoting stone disintegration.

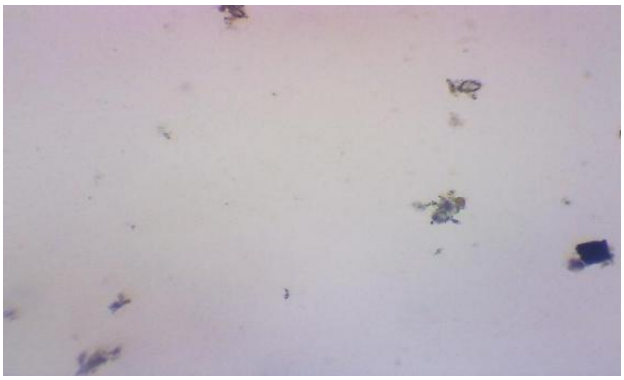

Figure.06 Histopathological study of urine sample in Glucosinolate under microscope

2. Kidney

a) **Group –I** : Normal Control.

MICROSCOPIC APPEARANCE:

Histological examination of the kidney sections from the vehicle control group revealed a well preserved renal architecture with no observable pathological lesions or abnormalities.

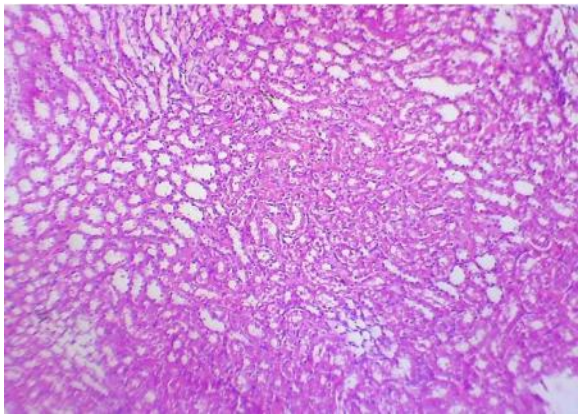

Figure.07 Histopathological study of kidney in normal control under microscope

b) Group –II : Ethylene Glycol Control.

MICROSCOPIC APPEARANCE:

Microscopic examination of renal sections reveals a significant accumulation of calcium oxalate crystals, which appear as translucent, pale-yellowish, and strongly birefringent structures under polarised light. Crystals are predominantly localised within the lumens of the proximal tubules and the collecting ducts, often forming large, polymorphic aggregates that obstruct the tubular space.

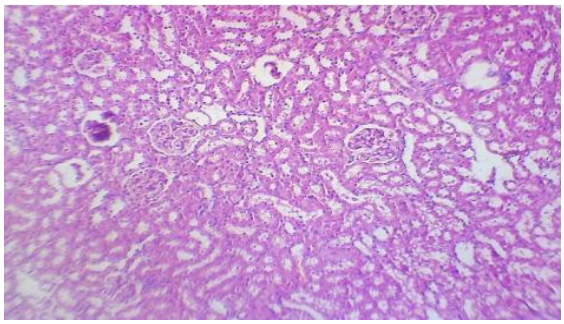

Figure.08 Histopathological study of kidney in Ethylene Glycol control under microscope

c) Group –III : Cystone

MICROSCOPIC APPEARANCE:

Histopathological examination of renal sections from the Cystone-treated group reveals a marked reduction in the quantity and size of birefringent calcium oxalate (CaOx) crystals compared to the ethylene glycol-only group. Only sparse, small crystal deposits are visible within the tubular lumens, indicating that Cystone effectively prevents the aggregation and retention of lithogenic particles.

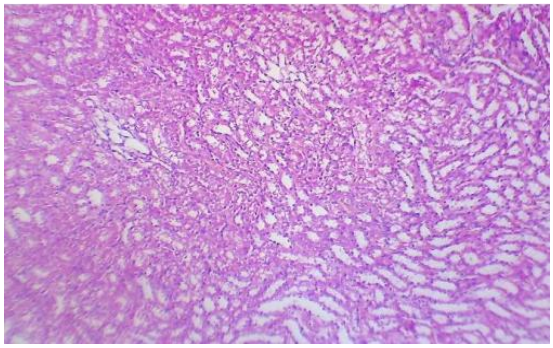

Figure.09 Histopathological study of kidney in Cystone under microscope

d) Group –IV : Glucosinolates

MICROSCOPIC APPEARANCE:

The renal tubules show significant amelioration of the necrotic changes and epithelial desquamation induced by ethylene glycol. The tubular lining appears more intact, with less evidence of brush border loss or cytoplasmic vacuolation. here is a notable decrease in the severity of tubular dilation and the presence of intratubular casts, suggesting improved urinary flow and reduced obstruction.

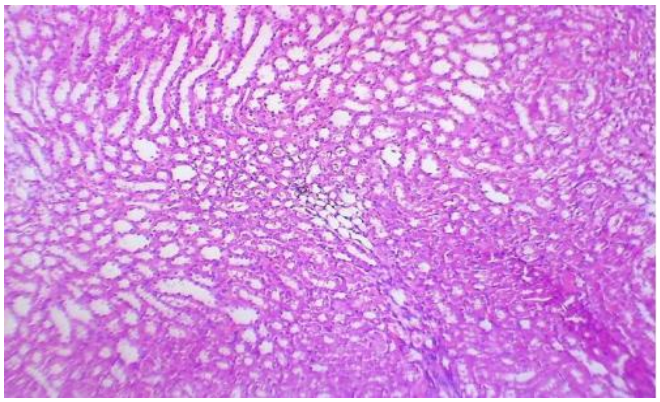

Figure.10 Histopathological study of kidney in Glucosinolate under microscope

In histopathological study, Glucosinolates significantly reduced crystal formation and protected renal tissue, showing results comparable to the standard drug Cystone.

URINE ANALYSIS

Table. 2. Effect of Glucosinolates on urine analysis in ethylene glycol induced urolithiasis in rats

Group	Drug Treatment	Urine Analysis		
		Calcium (mg/dl)	Phosphate (mg/dl)	Oxalate (mg/dl)
I	Normal Control (0.1% CMC)	1.37±0.11	4.87±0.28	0.53±0.02
II	Urolithiasis Control (0.75% Ethylene Glycol)	6.77±0.40	12.60±1.14	4.27±0.29
III	Positive Control (Cystone 750mg/kg)	2.55±0.13***	5.08±0.35***	0.62±0.04***
IV	Test Control (Glucosinolate 200mg/kg)	2.07±0.10***	4.91±0.37***	0.59±0.04***

Values are in mean ± SEM (n=6),

*P<0.05 , **P<0.01, ***P<0.001 compared to Group II

This table summarise the levels of calcium, phosphate and oxalate in urine. Glucosinolates (200~mg/kg) significantly reduced the urinary excretion of calcium (2.07±0.10~mg/dl), phosphate (4.91±0.37~mg/dl), and oxalate (0.59±0.04~mg/dl).

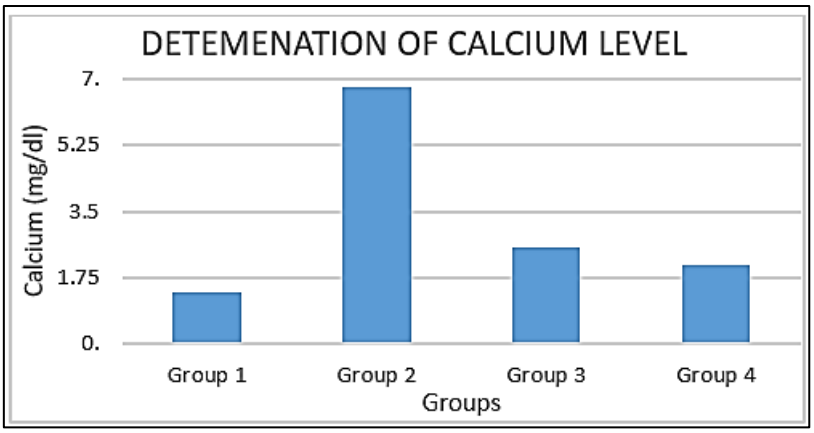

Figure.11 Determination of Calcium Level in Urine Analysis

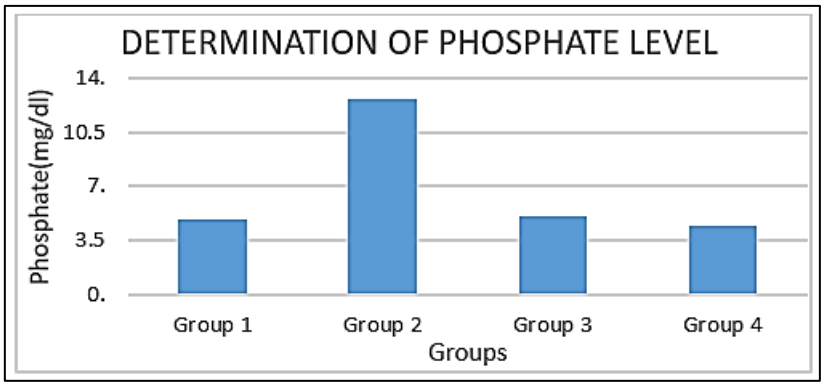

Figure.12 Determination of Phosphate Level in Urine Analysis

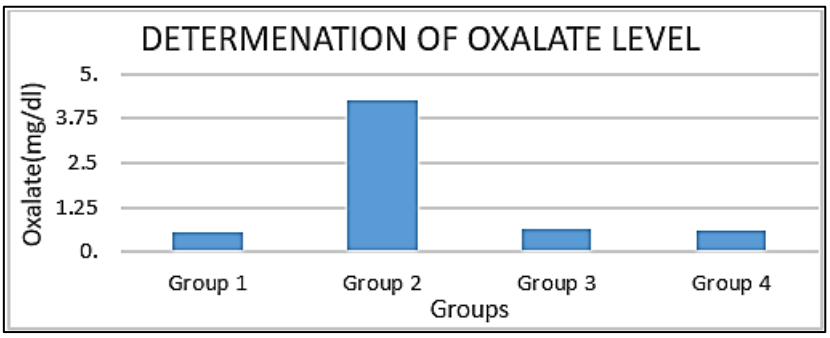

Figure.13 Determination of Oxalate Level in Urine Analysis

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SERUM ANALYSIS

Table. 3. Effect of Glucosinolates on serum analysis in ethylene glycol induced urolithiasis in rats

Group	Drug Treatment	Serum Analysis		
		Urea Nitrogen (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
I	Normal Control (0.1% CMC)	20.37±1.27	0.62±0.03	1.27±0.17
II	Urolithiasis Control (0.75% Ethylene Glycol)	37.42±2.74	2.89±0.18	3.62±0.20
III	Positive Control (Cystone 750mg/kg)	21.86±1.52***	0.93±0.07**	1.67±0.12***
IV	Test Control (Glucosinolate 200mg/kg)	21.25±1.10***	1.08±0.10**	1.75±0.13***

Values are in mean ± SEM (n=6),

*P<0.05 , **P<0.01, ***P<0.001 compared to Group II

This table summarise the levels of urea nitrogen, creatinine and uric acid in serum. In serum analysis, Glucosinolates significantly lowered uric acid to 1.75±0.13~mg/dl, serum creatinine to 1.08±0.10~mg/dl and urea nitrogen to 21.25±1.10~mg/dl.

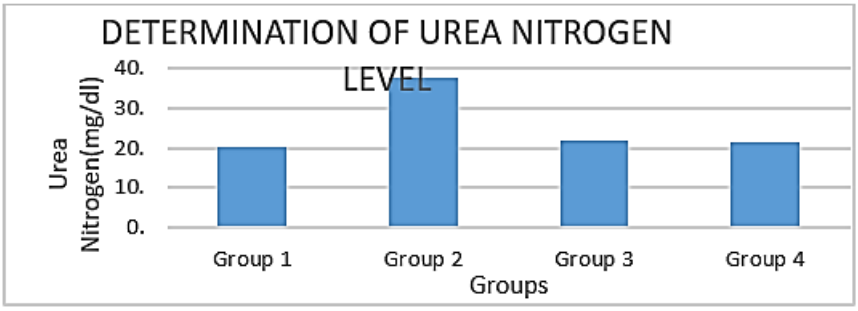

Figure.14 Determination of Urea Nitrogen Level in Serum Analysis

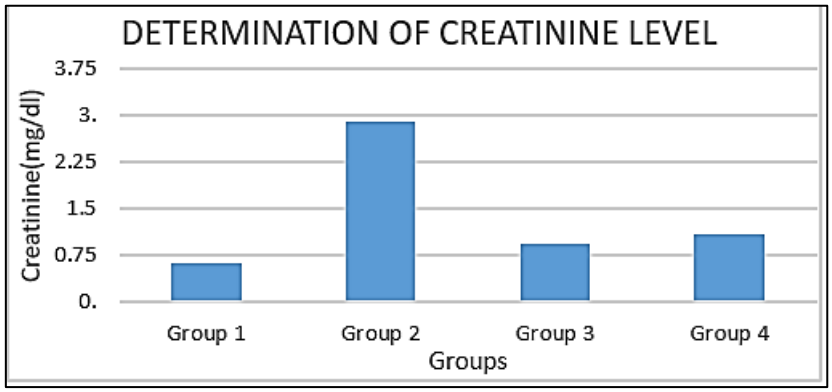

Figure.15 Determination of Creatinine Level of Serum Analysis

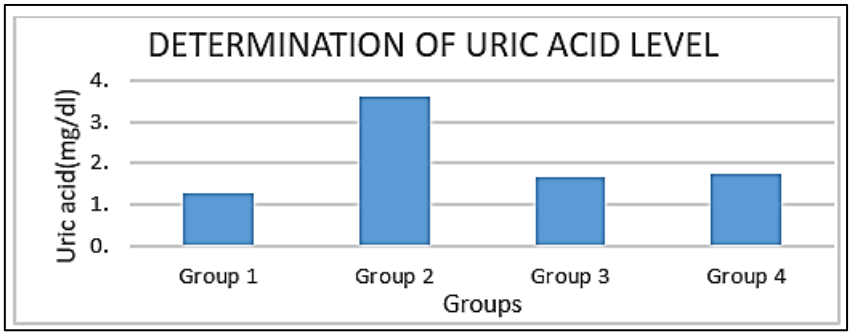

Figure.16 Determination of Uric Acid Level in Serum Analysis

DISCUSSION

The present study successfully evaluated the anti-urolithiatic activity of Glucosinolates using an ethylene glycol-induced urolithiasis model in Wistar albino rats. The administration of ethylene glycol (0.75%) resulted in a significant increase in stone-forming constituents, including calcium, phosphate, and oxalate, in the urine of the urolithiasis control group. In histopathological study, Glucosinolates notably decreased crystal formation and reduced the damage of renal tissue, demonstrating outcomes similar to the standard medication Cystone.

Treatment with Glucosinolates (200~mg/kg) significantly reduced the urinary excretion of calcium (2.07±0.10~mg/dl), phosphate (4.91±0.37~mg/dl), and oxalate (0.59±0.04~mg/dl). These reductions suggest that Glucosinolates effectively inhibit the supersaturation of urine, thereby preventing the nucleation and growth of renal calculi. Serum analysis revealed that the urolithiasis control group exhibited elevated levels of urea nitrogen, creatinine, and uric acid, indicating renal impairment caused by stone formation. The administration of Glucosinolates significantly lowered uric acid to 1.75±0.13~mg/dl, serum creatinine to 1.08±0.10~mg/dl and urea nitrogen to 21.25±1.10~mg/dl, demonstrating a protective effect on renal function and the ability to reduce kidney damage associated with urolithiasis. The anti-urolithiatic activity of Glucosinolates was found to be comparable to that of the standard drug, Cystone (750~mg/kg). The test group showed similar statistical significance ($p<0.001$ and $p<0.01$) in reducing both urinary and serum analysis when compared to the positive control group.

CONCLUSION

Glucosinolates treatment significantly lowered urinary calcium, oxalate, phosphate, and uric acid levels compared to the standard group (cystone). Glucosinolates exhibited significant improvement in kidney histology with reduced crystal deposition and reduced the number and size of crystals and preserved the normal structure of renal tissues. Glucosinolates improved renal function by normalising uric acid, creatinine and urea levels. Kidney tissue analysis revealed reduced crystal deposition in treated groups. The findings suggest that glucosinolates could serve as a preventive and therapeutic agent for urolithiasis, especially in populations with high recurrence risk. Incorporating glucosinolate-rich foods (e.g., broccoli, cabbage, mustard greens) may provide a natural prophylactic approach. The study highlights that glucosinolates exhibit significant anti-urolithiatic activity by protecting renal tissues, and reducing stone recurrence risk. Their natural origin, safety profile, and multifunctional properties make them a promising agent for the management of kidney stones.

REFERENCES

1.Wróblewski K, Wróblewska P, Szukalska S, Karczewska M, Lichwala K, Samborska A, Balajewicz B, Siwek L. Current Perspectives on Urolithiasis: Pathogenesis, Clinical Management, and Treatment. Cureus. 2026 Jan 9;18(1).
2.Han H, Segal AM, Seifter JL, Dwyer JT. Nutritional management of kidney stones (nephrolithiasis). Clin Nutr Res. 2015;4(3):137–152. doi:10.7762/cnr.2015.4.3.137.
3.Oswal M, Varghese R, Zagade T, Dhatrak C, Sharma R, Kumar D. Dietary supplements and medicinal plants in urolithiasis: diet, prevention, and cure. Journal of Pharmacy and Pharmacology. 2023 Jun 1;75(6):719-45.
4.Tamborino F, Cicchetti R, Mascitti M, Litterio G, Orsini A, Ferretti S, et al. Pathophysiology and main molecular mechanisms of urinary stone formation and recurrence. Int J Mol Sci. 2024;25:3075. doi:10.3390/ijms25053075.
5.Hu J, Zhang J, Wang J, Tan Y, Zhou L, Chan W, et al. Global, regional, and national epidemiology of pediatric urolithiasis (1990–2021) and 2040 forecast. J Urol. 2025 Oct;214(4):435-445.
6.Jarald EE, Kushwah P, Edwin S, Asghar S, Patni SA. Effect of Unex on ethylene glycol-induced urolithiasis in rats. Indian Journal of Pharmacology. 2011 Jul 1;43(4):466-8.

7.Partovi N, Fatemi SJ, Ebadzadeh MR. Antiurolithiatic effects of Cassia fistula Lin. fruit extracts on ethylene glycol-induced nephrolithiasis in rats. Microscopy Research and Technique. 2024 Jul;87(7):1494-506.
8.Kumar RR, Janadri S, Mudagal MP, Sharma UR, Vada S, Babu HT, Gangireddy AB. In vivo and in vitro experimental models for urolithiasis pathophysiology research. Asian Journal of Urology. 2025 Oct 1;12(4):486-95.
9.Baldelli S, Lombardo M, D'Amato A, Karav S, Tripodi G, Aiello G. Glucosinolates in Human Health: Metabolic Pathways, Bioavailability, and Potential in Chronic Disease Prevention. Foods. 2025 Mar 7;14(6):912.
10. Connolly EL, Sim M, Travica N, Marx W, Beasy G, Lynch GS, Bondonno CP, Lewis JR, Hodgson JM, Blekkenhorst LC. Glucosinolates From Cruciferous Vegetables and Their Potential Role in Chronic Disease: Investigating the Preclinical and Clinical Evidence. Front Pharmacol. 2021 Oct 26;12:767975.
11. Murugan S, Purusothaman D, Richard EJ, Chalichem NSS, Bethapudi B, Chandrasekaran PR, Velusami CC, D'Souza P, Mundkinajeddu D. Anti-diarrhoeal activity of a polyherbal formulation in rats and elucidation of its cellular mechanisms. Avicenna J Phytomed. 2020 Jul-Aug;10(4):417-427.

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Conflict of Interest Statement: All authors have nothing else to disclose.