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Research Article

Evaluation of a Polyherbal Formulation for Antiurolithitic Activity: A Biochemical and Histopathological Analysis in Ethylene Glycol Induced Rat Model

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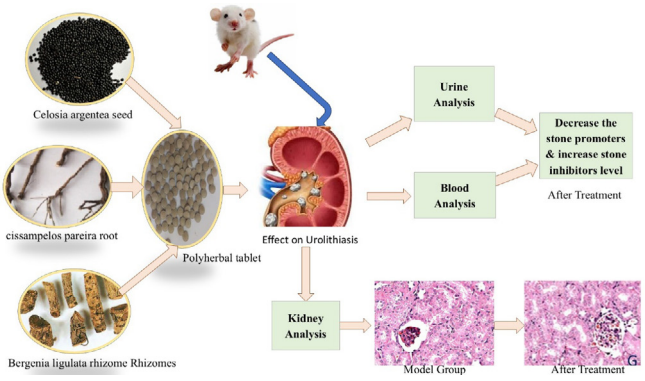
ABSTRACT

Urolithiasis (kidney stones) is a prevalent urinary system disorder affecting populations worldwide and has been recognized as a long-standing human ailment, contributing to renal failure. While various conventional therapies exist for urolithiasis, they are often associated with undesirable side effects. In India, numerous herbal formulations are utilized for treating urolithiasis, known for their efficacy and safety. Specifically, extracts from *Celosia argentea* seeds, *Cissampelos pareira* roots, and *Bergenia ligulata* rhizomes exhibit anti-urolithitic activity. However, combining these herbs in a particular ratio often yields greater therapeutic effects and reduces toxicity compared to using single extracts. Therefore, this research was designed to scientifically evaluate the anti-urolithic efficacy of a polyherbal formulation against urolithiasis using an ethylene glycol (EG)-induced model in rats. Rats were administered EG (0.75 v/v) with water for 28 days to cause renal stone formation, and simultaneously received a single dose of the herbal formulation orally once daily. On day 28, 24-hour urine and serum samples were collected, along with kidney homogenate, for biochemical analysis and histological examination. Cystone was used as a standard herbal formulation for comparison. The co-administration of the polyherbal formulation resulted in a substantial (p <0.001) decrease in urinary and serum levels of renal stone promoters such as calcium, oxalate, phosphate, uric acid, and urea, as well as a significant increase in magnesium and citrate. Histological analysis demonstrated reduced renal cell damage in the treatment group. These results indicate that the polyherbal formulation possesses anti-urolithic activity and holds promise for the treatment of renal calculi.

Introduction

Urolithiasis, the pathological process of urinary tract stone formation, has significantly impacted civic health over the past 20 years. There are numerous kinds of stones that exist, with calcium oxalate stones accounting for above 80% of cases and 5 to 10% of cases caused by uric acid cases in globally. A study by the National Health and Nutrition Examination found that 10.6% of men and 7.1% of women in the United States have renal stone disease.[1] In India, approximately 15% of individuals suffer from kidney stones annually, with Rajasthan, Maharashtra, Gujarat, Punjab, Haryana, and Delhi experiencing the highest prevalence.[2]



Graphical Abstract

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Renal stone formation involves a complex interplay of factors, both internal and external. These include impaired metabolism of substances like oxalic acid, phosphorus, calcium, and uric acid, in addition to urea and other nitrogen-containing waste products. External factors contributing to kidney stone development include hard water, high temperatures, dehydration, and dietary habits.[3] Urine supersaturation with substances such as oxalate, calcium, and phosphate promotes kidney stone formation. This process involves crystallization, crystal development, and crystal process of accumulation process. Urine contains a variety of stone inhibitors, such as citrate and magnesium, which form a dissolved complex through Ca^{2+} ions and lower CaOx ions supersaturation; however, each person's inhibition capacity is different.[4]

The location and size of renal stones dictate the appropriate treatment approach. While numerous treatments are available, including chelating drugs, probiotics, citrate, and diuretics, each has its own pharmacological limitations and potential side effects. Surgery is commonly employed for stone removal. Stone recurrence rates reach approximately 50%, and complications such as hypertension, renal fibrosis, bleeding, and tubular necrosis can arise.[5]

Ayurveda has long utilized medicinal herbs to treat urolithiasis.[6] The Sarangdhar Samhita emphasizes the use of polyherbal formulations for enhanced therapeutic efficacy. The active phytochemical components of individual plants may not provide the desired therapeutic effect; combining various herbs in specific ratios can result in a more potent outcome.[7]

Therefore, a herbal tablet was developed, assessed, and researched for its antiurolithiatic action, utilizing *Celosia argentea* seeds (Amaranthaceae), *Cissampelos pareira* roots (Menispermaceae), and *Bergenia ligulata* rhizomes (Saxifragaceae). *C. argentea* seeds, flowers, young shoots, and leaves are edible and rich in iron, calcium, phosphorus, vitamin A, and vitamin C.[8] While all parts of *C. argentea* have been used to treat snakebites, the seeds are considered most effective, acting as a poultice, astringent, hemostatic, and parasiticide. It exhibits antiurolithiatic activity and has been reported to suppress urinary calculi formation.[9,10] *C. pareira*, also known as Patha, is mentioned in the ancient Charaka Samhita as a remedy for various ailments, including fever, vomiting, asthma, diarrhea, itching, burning sensation, leprosy, heart problems, worm infections, poisoning, and stomach tumors.[11] *B. ligulata* rhizomes (Pashanbhed) are used to treat vesicular calculi, urinary discharge, excessive uterine bleeding, bladder disorders, dysentery, splenomegaly, menorrhagia, and heart problems.[12] Studies have shown that extracts from these three plants are effective individually; however, combination extracts often demonstrate superior activity in shorter periods compared to monotherapy. Many commercially available herbal formulations contain combinations of 8 to 10 herbs. Therefore, this study's objective was to prepare a polyherbal formulation using the extracts of these three selected plants and evaluate its antiurolithiatic action.

Material And Methods

Plant Materials

The *C. argentea* seeds (CAS), *C. pareira* roots (CPR), and rhizomes of Bergenia ligulata (BLR) were collected locally in November 2022 and authenticated by Dr. S.K. Patel, an ethanobotanist from the Botany Department, Government Science College, Gandhinagar, Gujarat, India. Specimen voucher numbers are SSO/05-07/2023/224 for *C. argentea*, SSO/01-03/2023/223 for *C. pareira*, and SSO/10-09/2023 for *B. ligulata*. The dried seeds, roots, and rhizomes were powdered for subsequent use and stored in a closed container at room temperature.

Preparation of Herbal Tablet

The powdered plant materials *C. argentea* [CAS], *C. pareira* [CPR], and Bergenia ligulata [BLR] were extracted with methanol to obtain their respective extracts. These extracts were then combined in varying ratios: 55% CAS, 44% CPR, and 1% BLR. Isopropyl alcohol was used as the solvent for tablet granule preparation, and talc was employed as a lubricant, sodium starch glycolate (SSG) as a disintegrator, lactose as a filler, and PVPK30 as a binder. The formulations were coded as F1, F2, and F3 (Table 1).[13] The effective doses for urolithiasis treatment were determined based on previous studies of the selected plant extracts. These studies also indicated that none of the extracts exhibited oral toxicity.[14-16]

Chemicals and Reagents

Ethylene glycol (EG) was acquired from Merck Ltd., Mumbai, India. All the chemicals used for present work are of AR. Cystone tablets were purchased from the market in Gandhinagar. Diagnostic kits used to measure creatinine, phosphorus, calcium, and blood urea nitrogen (BUN) were obtained from Lab Care Diagnostics (India) Pvt. Ltd. and Erba Mannheim.

Table 1: Polyherbal tablets preparation

Ingredient	*Low dose*	*Mild dose*	*Higher dose*
	F1	*F2*	*F3*
Celosia argentea	25	37.5	50
Cissampelos pariera	20	30	40
Bergenia ligulata	0.5	0.75	1
Pvpk30	5%	5%	5%
SSG	4 mg (2%)	4 mg (2%)	4 mg (2%)
Lactose	148 mg	125.75 mg	103 mg
Talc	2 mg (1%)	2 mg (1%)	2 mg (1%)
Total wt	200 mg	200 mg	200 mg

Animal

The Institutional Animal Ethical Committee (IAEC) of S S R College of Pharmacy, Sayli, Silvasa 396230 UT of Dadra & Nagar Haveli, India, gave its approval to the experimental protocol, approval no SSR/IAEC/2024/001. For this study, 150–200 g male albino Wistar rats in good health were selected. Throughout the study period, they were housed in standard laboratory conditions, including a 12-hour light/dark cycle, a temperature of 25 ± 2°C, relative humidity of 60 ± 5%, and provided with a standard pellet diet and water, in addition to ethylene glycol.

Antiurolithiatic Action of Polyherbal Tablet

To induce calcium oxalate stone formation, ethylene glycol-induced hyperoxaluria was utilized. For this study, six groups of six male Wistar rats each were selected. To cause renal calculi, groups II through VI were administered ethylene glycol (EG) at 0.75% in 28 days of drinking water. The groups were allocated as follows:

Group I: Normal Group

Group II: Control Group - EG

Group III: Standard Group - EG + 750 mg/kg Cystone During 28 days, orally

Group IV: Treatment Group I - EG + Low dose of formulation during 28 days, orally

Group V: Treatment Group II - EG + Mid dose of formulation, during 28 days, orally

Group VI: Treatment Group III - EG + High dose of formulation during 28 days, orally

Urine Collection and Examination

On 28th day, urine samples were taken after 24 hours; each animal was kept separately in metabolic cages. Crystalluria, pH, and urine volume were monitored as key factors. Urine was acidified with concentrated HCl (a few drops) and stored at 20°C before analysis using standard kits to determine uric acid, calcium, urea, oxalate, phosphate and magnesium levels. Oxalate and citrate levels were also determined. [17-19]

Collection and Examination of Serum

On 28th using the capillary technique, blood was drawn from the retro-orbital plexus while under mild anesthesia. Serum was isolated for examination after blood was centrifuged at 10,000 g for 10 minutes for calcium, magnesium, uric acid, creatinine, and BUN and AST and ALT liver markers with diagnostic kits.

Kidney Homogenate and Histopathology Analysis

Both kidneys were removed for additional research on the 28th day following the collection of urine and serum. The isolated kidneys' extraneous tissue was taken out, washed with regular saline, and weighed. After being stored in a 10% v/v neutral formalin solution, the left kidney was used for the histology investigation and sent to Accupath Diagnostic Laboratory in Ahmedabad, Gujarat, India, for hematoxylin and eosin staining. The kidney sections were examined under a microscope to check for tubular casts, glomerular congestion, interstitial edema, blood vessel congestion, epithelial adhesion, inflammatory cells, and CaOx crystal depositions. After the right kidney was finely chopped, 20% of the homogenate was made in a pH 7.4 tris-HCl buffer. To determine calcium, phosphate, oxalate, uric acid, urea, and lactate dehydrogenase (LDH), a prepared homogenate was utilized and catalase by using kits.

Statistical Analysis

The results were presented as mean ± SEM. One-way ANOVA was employed to analyze group data, then Sidak's multiple comparison tests with the version of GraphPad Prism 6. A *p-value* of < 0.001 was considered statistically significant.

Results

The traditional herbal formulation was evaluated for its antiurolithiatic potential through *in-vivo* research conducted on male albino Wistar rats over a 28-day period.

Histopathological Study

After 28 days, histopathological examination was performed on the kidneys of every animal. Calcium oxalate microcrystals were found in the cortical area of kidney sections from rats exposed to ethylene glycol. Conversely, kidney sections from the treated groups showed a marked reduction in crystal accumulation (Fig. 1). Furthermore, there was no notable tubular damage, bleeding, disruption of the brush border, or cortical tubular congestion in the kidneys of rats administered the polyherbal formulation – a contrast to the renal tissues of animals with the disease.

Urine Examination

The disease control group exhibited a marked decrease in urine output (4.07 ± 0.07 mL/24h) compared to the normal control group (4.6 ± 0.06 mL/24h). This reduction is consistent with oliguria, a common symptom in urolithiasis patients due to compromised kidney function. Notably, both standard treatment and test formulations at varying doses resulted in significant increases in urine volume. The high-dose group (4.75 ± 0.05 mL/24h) showed the greatest increase, even exceeding the standard treatment volume. Throughout the 28-day study, animal body weight was monitored; disease-induced animals experienced weight gain, while those treated with the polyherbal formulation showed weight loss.

Urine Biochemistry

Urine analysis in animals with induced disease revealed increased concentrations of phosphate, calcium, uric acid, oxalate, creatinine, and urea. Conversely, polyherbal treatment significantly reduced these ion levels in the urine (Table 2). Importantly, treated rats demonstrated a marked increase in magnesium and citrate levels. The

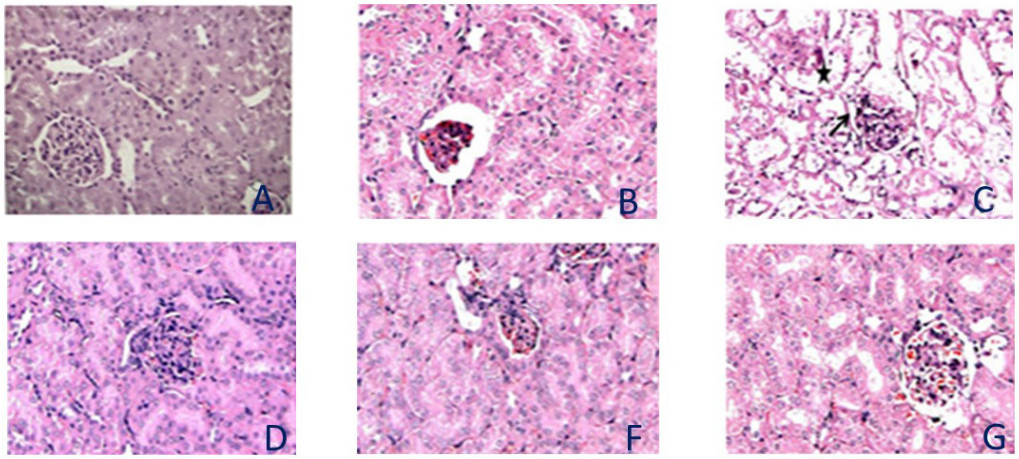

Fig. 1: Histopathology in (A) normal group, (B) disease group, (C) standard group, (D) low dose, (E) mid dose, (F) high dose

Disease Control group showed a notable acidification of urine (pH 5.3 ± 0.03) compared to the normal control (pH 6.0 ± 0.06). The high-dose group (pH 6.1 ± 0.05) not only restored the urine pH to normal but also slightly elevated it above the normal range, potentially offering enhanced protection against stone formation (Fig. 2).

Serum Biochemistry

On the 28th day, serum analysis was performed to assess the concentrations of calcium, creatinine, uric acid, BUN, and magnesium. The findings revealed that in animals with induced disease, levels of uric acid, calcium, BUN, and creatinine were higher than those in the normal control group. However, these significant amounts were considerably reduced in animals treated with the herbal formulation. Conversely, magnesium levels were lower in infected rats compared to the normal control group, but were considerably increased in the treated group (Table 3 and Fig. 3).

Kidney Homogenate Biochemistry

On 28th day, kidney homogenate analysis was performed to assess the concentrations of calcium, phosphate, uric acid and oxalate. The findings revealed that in animals with induced disease, levels of calcium, phosphate, uric acid and oxalate were higher than those in the normal control group. However, these significant amounts were considerably reduced in animals treated with the herbal formulation (Table 4 and Fig. 4).

Discussion

Urolithiasis has long been a health concern and is

Table 2: Impact of formulation on urine parameters in EG-induced urolithiasis in rats.

Biochemical parameter	*Group-I (Normal control)*	*Group-II (Disease control)*	*Group-III (Standard)*	*Group-IV (Low dose)*	*Group-V (Mid dose)*	*Group-VI (High dose)*
Urine volume (ml/24h)	4.6 ± 0.06	4.07 ± 0.07##	4.58 ± 0.06***	4.45 ± 0.04***	4.6 ± 0.06***	4.75 ± 0.05***
Urinary pH	6.0 ± 0.03	5.7 ± 0.03##	6.5 ± 0.04***	5.95 ± 0.04*	5.96 ± 0.06**	6.1 ± 0.05***
Calcium (mg/24h)	7.5 ± 0.27	9.45 ± 0.06##	7.4 ± 0.13***	8.9 ± 0.04	8.5. ± 0.05**	7.8 ± 0.06***
Oxalate (mg/24h)	1.25 ± 0.04	8.05 ± 0.78##	3.28 ± 0.06***	5.53 ± 0.04***	4.25 ± 00.4***	3.75 ± 0.04***
Phosphate (mg/24h)	22.83 ± 0.47	51.4 ± 0.58##	25.4 ± 0.40***	39.2 ± 0.03***	31.4 ± 0.53***	24.4 ± 0.31***
Uric acid (mg/24h)	4.5 ± 0.08	10.1 ± 0.30##	7.1 ± 0.10***	8.0 ± 0.13***	6.1 ± 0.10***	4.75 ± 0.07***
Urea (mg/24h)	28.2 ± 0.23	55.8 ± 0.66##	42.9 ± 0.38***	47.8 ± 0.40***	35.4 ± 0.31***	30.4 ± 0.19***
Citrate (mg/24h)	15.2 ± 00.7	13.7 ± 0.13##	18.78 ± 0.09***	17.3 ± 0.05***	19.1 ± 0.04***	20.55 ± 0.07***
Magnesium (mg/24h)	2.9 ± 0.06	0.57 ± 0.01##	1.76 ± 0.02***	1.1 ± 0.01***	1.8 ± 0.04***	2.2 ± 0.01***
Creatinine Clearance (mg/24h)	0.6 ± 0.01	2.9 ± 0.04##	1.09 ± 0.01***	2.18 ± 0.02***	1.79 ± 0.01***	1.3 ± 0.01***

All values are mean _ SEM (n ¼ 6), one-way ANOVA followed by Sidak's test.
##p < 0.001 versus Normal group.
***p < 0.001, **p < 0.05 and *p < 0.01 versus disease control group.

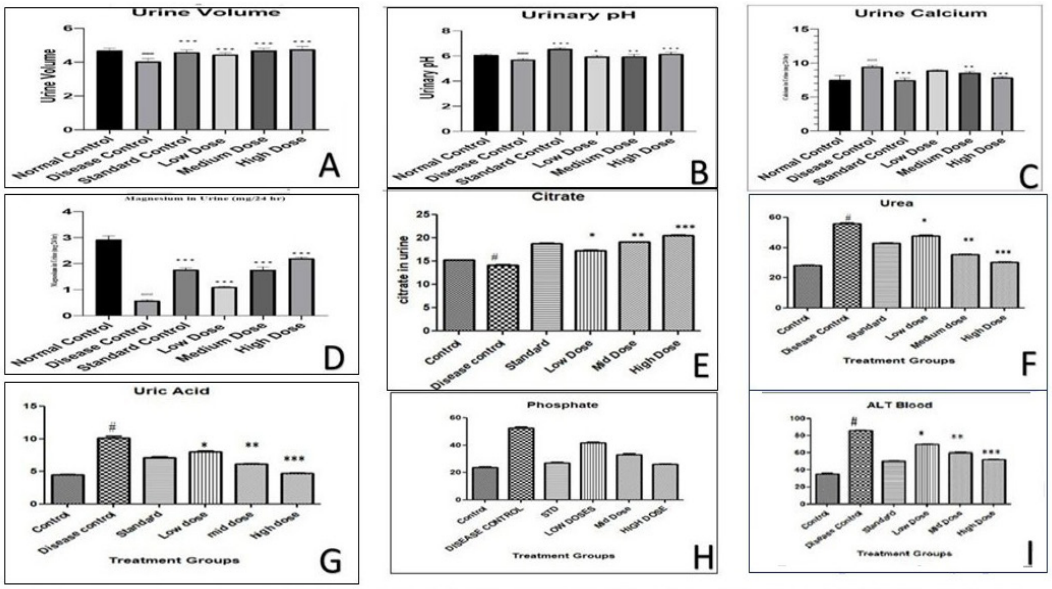

Fig. 2: (A) Urine Volume, (B) Urine Ph, (C) Urine Calcium, (D) Magnesium in Urine, (E) Citrate in Urine, (F) Urea in Urine, (G) Uric Acid Level in Urine, (H) Phosphate in Urine, (I) Oxalate in Urine

Table 3: Effect of formulation on serum parameters in EG-induced urolithiasis in rats.

Biochemical parameter	*Group-I (Normal control)*	*Group-II (Disease control)*	*Group-III (Standard)*	*Group-IV (Low dose)*	*Group-V (Mid dose)*	*Group-VI (High dose)*
Calcium (mg/dl)	9.4 ± 0.06	14.1 ± 0.92##	11.2 ± 0.43**	10.5 ± 0.36***	10.4 ± 0.25***	7.9 ± 0.47***
Uric acid (mg/dl)	1.8 ± 0.04	3.8 ± 0.04##	2.15 ± 0.04***	3.6 ± 0.04**	3.0 ± 0.03***	2.6 ± 0.04**
BUN (mg/dl)	18.3 ± 0.21	45.9 ± 0.38##	28.3 ± 0.2***	34.18 ± 0.19***	34.18 ± 0.17***	30.35 ± 0.19***
Magnesium (mg/dl)	2.9 ± 0.04	0.6 ± 0.01##	1.7 ± 0.01***	1.0 ± 0.02***	1.6 ± 0.03***	2.1 ± 0.02***

All values are mean _ SEM (n ¼ 6), one-way ANOVA followed by Sidak's test.
##$p < 0.001$ versus Normal group.
***$p < 0.001$, **$p < 0.05$ and *$p < 0.01$ versus disease control group.

recognized as a contributing factor to renal failure [20]. While no single pharmacological treatment is universally effective, surgical procedures are often necessary when other treatments fail. However, the cost of surgery can be prohibitive for many patients, highlighting the need for natural remedies. Pashanbhed plants, a group of therapeutic herbs used in Indian Ayurvedic medicine, are valued for their antiurolithiatic properties. [21,22] *Celosia argentea*, *Cissampelos pareira*, and *Bergenia ligulata* are traditionally used as both antiurolithic and diuretic agents. Individual plant extracts may lack the necessary phytochemical constituents for optimal therapeutic effects. Combining multiple herbs in a specific ratio often yields better therapeutic results and reduces toxicity.[23,24] This study investigated the antiurolithic efficacy of a polyherbal formulation.[25] Research suggests that urinary supersaturation is a key factor in stone formation, with elevated oxalate levels observed in animals treated with ethylene glycol (EG). This may be due to increased oxalate excretion and urine retention.[26,27] In this study,

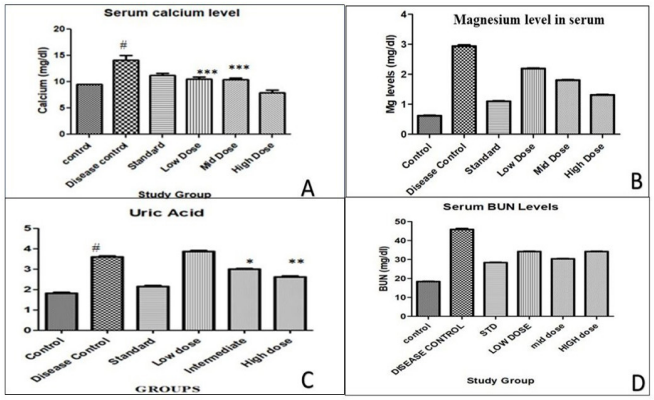

Fig. 3: (A) Serum Calcium, (B) Magnesium in Serum, (C) Uric Acid in Serum, (D) Blood Urea Nitrogen in Serum

EG-induced urolithiasis rats exhibited elevated oxalate levels in urine, while those treated with the polyherbal formulation showed a reduction in oxalate excretion. This reduction may result from the inhibition of oxalate

Table 4: Effect of formulation on Kidney homogenate parameters in ethylene glycol-induced urolithiasis in rats

Biochemical parameter	*Group-I (Normal control)*	*Group-II (Disease control)*	*Group-III (Standard)*	*Group-IV (Low dose)*	*Group-V (Mid dose)*	*Group-VI (High dose)*
Calcium (mg/gm tissue)	0.88 ± 0.09	4.7 ± 0.14##	3.18 ± 0.09***	4.1 ± 0.10**	2.8 ± 0.05***	1.95 ± 0.07***
Phosphate (mg/gm tissue)	23.8 ± 0.47	52.5 ± 1.05##	27.2 ± 0.60***	41.8 ± 0.60***	33.2 ± 0.70***	26.0 ± 0.36***
Uric acid (mg/gm tissue)	0.6 ± 0.06	4.2 ± 0.12##	2.8 ± 0.09***	3.6 ± 0.07***	2.5 ± 0.07***	1.5 ± 0.06***
Oxalate (mg/gm tissue)	1.35 ± 0.05	8.2 ± 0.13##	3.75 ± 0.14***	5.3 ± 0.05***	4.35 ± 0.07***	3.51 ± 0.04**

All values are mean _ SEM (n ¼ 6), one-way ANOVA followed by Sidak's test.
##$p < 0.001$ versus Normal group.
***$p < 0.001$, **$p < 0.05$ and *$p < 0.01$ versus disease control group.

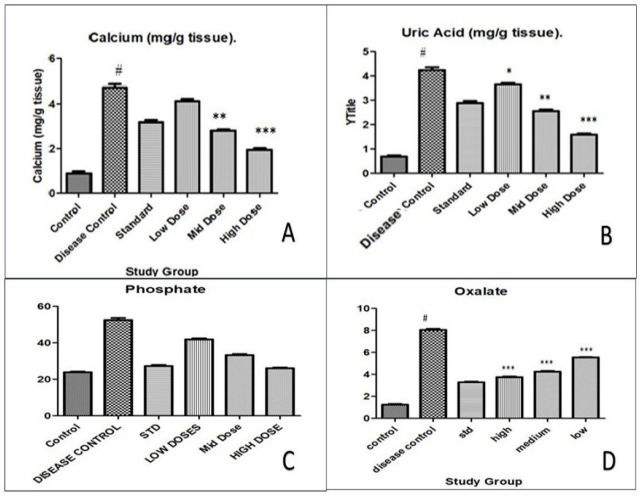

Fig. 4: (A) Calcium in Kidney, (B) Uric Acid in Kidney, (C) Phosphate in Kidney, (D) Oxalate in Kidney

formation by plant constituents, possibly through the suppression of activity of the enzyme oxalate oxidase, which is thought to contribute to the development of stones.

Magnesium is one of numerous calculi inhibitors found in normal urine. Rats prone to stone formation had low magnesium levels, which normalized after drug action. Magnesium creates complexes with oxalate, reducing calcium oxalate supersaturation and subsequently decreasing calcium oxalate crystal growth and nucleation rate.[28-33] Additionally, magnesium inhibits the absorption and elimination of oxalate, thereby preventing its supersaturation. A high dosage of the polyherbal formulation significantly increased urinary magnesium levels, reducing the possibility of developing calcium oxalate stones.

Calculi in the urinary system impair glomerular filtration rate (GFR) in urolithiasis, leading to decreased urine outflow. This blockage results in the accumulation of waste materials, especially nitrogenous substances such as creatinine and BUN, in the bloodstream. Treatment with a high dose of the polyherbal formulation has been shown to significantly lower serum concentrations of creatinine and BUN, thereby reducing the possibility of urinary flow block by waste products in the urinary tract. Raised levels of nitrogen-bearing metabolites in the serum also indicate potential renal dysfunction, which is notably minimized in rats given the polyherbal formulation.[34-36]

Urine samples from both control and treated groups were collected on day 28, and relative analyses assessed body weight, kidney weight, and urine volume. This analysis demonstrated a notable increase in urine volume in animals treated with the polyherbal formulation, indicating a diuretic effect of the herbs. The treated animals weighed more than the control group, likely due to the reduction in stone and urine deposition. Kidney weights in treated animals were higher than in the control group, likely due to stone deposition, while a significant reduction was observed in animals given the polyherbal formulation. This reduction may be attributed to decreased stone deposition and excretion facilitated by the polyherbal formulation. However, the diuretic effect of the polyherbal formulation warrants further systematic investigation.[37,38]

Scanning electron microscopy (SEM) of the kidney cortex revealed notable differences between both the test and control groups in tubular epithelial cells (Fig. 4). Kidney glomeruli within each group receiving the reference medication and polyherbal formulation showed no significant structural alterations, with organelles in epithelial cells remaining intact.[39,40] Microscopic analysis of kidney sections showed microcrystal deposits in the tubules, which could lead to inflammation in disease-induced rats, a condition significantly improved by administering a high dose of the polyherbal formulation. Calculi may hinder solute reabsorption from the tubular lumen, decreasing passive water reabsorption at the proximal convoluted tubule (PCT). Nonetheless, a high dose of the polyherbal formulation was similarly active in lowering the number of calculi and promoting their evacuation through diuretic action. Additionally, the polyherbal formulation induced an effect of urine alkalinization. Serum analysis revealed increased levels of BUN and creatinine in disease-induced animals compared

to controls, while these levels were significantly lower in animals given a high dose of the polyherbal formulation (Table 3). This indicates that BUN and creatinine secretion were elevated in animals with ailments because of stone development, suggesting nitrogenous substance deposit and potential renal damage.[35] A high dose of the polyherbal formulation considerably reduced the excretion of BUN and creatinine by minimizing the size and preventing the development of stones.

Conclusion

Based on the provided information, it is possible to conclude that the formulation demonstrates therapeutic efficacy against urolithiasis induced by ethylene glycol administration. This is evidenced by the reduction of various stone promoters (such as oxalate, calcium, and phosphate) in urine, kidney tissue, and serum, alongside an increase in stone inhibitors (like citrate and magnesium). The formulation holds potential medical importance for both preventing and treating kidney stone expansion, as highlighted by the study's findings. The final high-dose formulation exhibited strong anti-urolithiatic activity and demonstrated stability and excellent results. While effective in the study, further research is required before clinical use in humans. This includes conducting clinical trials according to government guidelines, alongside pharmacokinetics, pharmacodynamics, and toxicity studies on a larger animal population.

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