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EVALUATION OF THE ANTI-UROLITHIASIS POTENTIAL OF AYURVEDIC MARKETING FORMULATION IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT: Urolithiasis is a common urinary tract disorder characterized by the formation and retention of urinary calculi, predominantly composed of calcium oxalate. The present study evaluated the anti-urolithiatic potential of Go-Pathri, an Ayurvedic marketed formulation, in experimental rats. Male Wistar albino rats were divided into five groups (n=6). Urolithiasis was induced using 0.75% ethylene glycol, followed by treatment with Cystone (750 mg/kg) or Go-Pathri (50 and 100 mg/kg) for 14 days. Urine volume, urinary calcium, oxalate, phosphorus and magnesium, and serum uric acid, creatinine and BUN were evaluated. Histopathological examination of renal tissues was also performed. Ethylene glycol produced marked alterations in urinary and serum biochemical parameters and promoted renal calcium oxalate deposition. Go-Pathri treatment improved urine output, reduced urinary stone-forming constituents and serum uric acid, creatinine and BUN levels, and reduced renal crystal deposition. The 100 mg/kg dose showed greater improvement than 50 mg/kg. These findings demonstrated the potential anti-urolithiatic and renoprotective effects of Go-Pathri.

INTRODUCTION: Urolithiasis is a common disorder of the urinary tract characterized by the formation of calculi in the kidneys, ureters, urinary bladder, or urethra. It develops through urinary supersaturation followed by crystal nucleation, growth, aggregation, adhesion, and retention within the urinary tract¹. Calcium oxalate and calcium phosphate are the predominant constituents of urinary stones, while uric acid, struvite, and cystine stones may also occur.

Inadequate fluid intake, dietary factors, metabolic abnormalities, urinary tract infections, genetic predisposition, and alterations in urinary pH are important contributing factors^{2, 3, 4}. Oxidative stress, inflammation, and renal tubular injury may further facilitate crystal retention and progression of stone formation. Clinically, urolithiasis may cause colicky pain, hematuria, dysuria, nausea, vomiting, and urinary obstruction⁵.

Current management includes adequate fluid intake, analgesics, antispasmodics, urinary alkalizers, and medications that facilitate stone passage, whereas larger or complicated calculi may require surgical or endoscopic intervention. The high recurrence rate associated with urolithiasis has

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increased interest in preventive and complementary therapeutic approaches^{6, 7, 8}.

Ayurveda has traditionally utilized medicinal plants and polyherbal formulations for the management of Mutrashmari and other urinary disorders⁹. Go-Pathri, an Ayurvedic marketed formulation, is traditionally used for urinary calculi, dysuria, burning micturition, and urinary tract discomfort¹⁰. The formulation contains several herbal and alkaline ingredients, including Punarnava (*Boerhavia diffusa*), Gokshura (*Tribulus terrestris*), Pippali (*Piper longum*), Giloy (*Tinospora cordifolia*), Yavashar derived from *Hordeum vulgare*, Tulsi (*Ocimum sanctum*), Lajwanti

(*Mimosa pudica*), and SajjiKshar (SwarjikaKshara)^{11, 12, 13, 14}.

These ingredients are traditionally associated with diuretic, anti-inflammatory, antioxidant, nephroprotective, and ashmari-hara properties. Phytoconstituents such as flavonoids, alkaloids, saponins, tannins, terpenoids, essential oils, and other bioactive compounds may contribute to the overall pharmacological effects of the formulation¹⁵. The combined action of these ingredients may support urinary flow, reduce crystal formation and aggregation, alleviate urinary tract inflammation, and facilitate the elimination of urinary calculi^{16, 17}.



FIG. 1: AYURVEDIC FORMULATION GO-PATHRI

Despite its traditional use, systematic scientific evaluation of the anti-urolithiatic efficacy of Go-Pathri is necessary to establish its therapeutic potential using validated experimental models. Therefore, the present study is proposed to evaluate the anti-urolithiatic potential of Go-Pathri in experimental animal models by assessing relevant urinary and serum biochemical parameters and associated renal tissue changes. The findings may provide scientific evidence regarding the traditional use of Go-Pathri and contribute to understanding its potential role in the management of urolithiasis.

MATERIALS & METHODS:

Procurement of Go-Pathri Capsule: Ayurvedic Marketed Formulation (Go-Pathri) capsules were procured from a Spine Health Care, Haryana. After

procurement, the capsules were stored under standard recommended storage conditions, ensuring protection from moisture, heat, and direct sunlight, in order to maintain their stability and integrity. The samples were preserved appropriately until they were required for further analysis.

In-vivo Anti-Urolithiasis Activity:

Selection of Animal Model: The present study was conducted as per CPCSEA no: CAH/BIU/2026/016, Date: 21/02/2026. The Male Wistar albino rats [150-200g] used for this study were produced from Central Animal House, BIU College of Pharmacy, Bareilly International University, Bareilly, U.P., India. Recently acquired Wistar albino rats were quarantined and

acclimatized for one week before experimentation. The animals were housed in well-ventilated, climate-controlled polypropylene cages under controlled environmental conditions with 55–60% relative humidity and paddy husk bedding. They were provided a standard pellet diet and clean drinking water *ad libitum*, except during fasting. Bedding was changed twice weekly, and animals

were identified using appropriate cage labels and picric acid markings. Urolithiasis was induced by administering 0.75% ethylene glycol in drinking water for the specified experimental period^{18, 19}.

Treatment Groups: The animals were divided into five groups, with six animals in each group, as described below²⁰:

TABLE 1: TREATMENT GROUPS

Group	Description	Route & Dose Schedule
Group 1	Animals were given normal food and drinking water <i>ad libitum</i> .	Oral, daily for 28 days
Group 2	Animals received 0.75% (v/v) ethylene glycol (EG).	Oral, daily for 14 days
Group 3	Animals were administered the standard drug, Cystone (750 mg/kg).	Oral, daily for 14 days for disease induction, followed by Cystone (750 mg/kg) orally for the next 14 days
Group 4	Animals were administered Go-Pathri Capsule at a dose of 50 mg/kg (low dose; standard dose = 500 mg).	Oral, daily for 14 days for disease induction, followed by Go-Pathri Capsule (50 mg/kg) orally for the next 14 days
Group 5	Animals were administered Go-Pathri Capsule at a dose of 100 mg/kg (high dose; standard dose = 500 mg).	Oral, daily for 14 days for disease induction, followed by Go-Pathri Capsule (100 mg/kg) orally for the next 14 days

Groups 2, 3, 4, and 5 were provided with regular food *ad libitum* and drinking water containing 0.75% (v/v) ethylene glycol (EG) *ad libitum* to induce hyperoxaluria and promote calcium oxalate (CaOx) deposition in the kidneys. Groups 3, 4, and 5 received their respective treatments from the 15th day to the 28th day. Group 3 received the standard drug Cystone (750 mg/kg), while Groups 4 and 5 received Go-Pathri Capsule at doses of 50 mg/kg and 100 mg/kg, respectively, as curative treatment regimens²¹.

Analysis of Urine and Serum: Urine samples were collected from individually housed rats in metabolic cages over 24 h on days 0, 14, and 28. Urine volume was recorded, and samples were preserved at 4°C after appropriate acidification. Urinary calcium, magnesium, phosphorus, and oxalate levels were estimated using standard

analytical methods. Urinary sediments were examined microscopically for crystal size and morphology. Blood samples were collected on days 0, 14, and 28 under mild anesthesia, allowed to clot, and centrifuged to obtain serum. Serum uric acid, creatinine, and blood urea nitrogen (BUN) were estimated using standard diagnostic kits^{22, 23}.

Histopathology: After the completion of experimental period, the rats were anesthetized using ether and then sacrificed. An incision was made over the abdomen and the kidneys were removed, cleaned and preserved using 10% neutral formalin. One kidney was fixed using formalin and embedded in paraffin. Later, 5µm thick sections were prepared and stained with hematoxylin-eosin solution. These sections were examined under plain and polarized microscope to capture the histopathological changes^{24, 25}.





FIG. 2: DISSECTION OF RAT

Data Analysis: Differences among data were determined using one-way ANOVA followed by Dunnett's multiple comparison tests (Graph pad Prism software for Windows, Version 2.03.1998). $P < 0.05$ was considered to be statistically significant^{26, 27}.

RESULTS AND DISCUSSION:

In-vivo Anti-Urolithiatic Activity:

Urine Volume: The effect of Go-Pathri capsule on urine volume was evaluated over 28 days, with measurements recorded on days 0, 14, and 28 and expressed as mean $\pm$ SEM. Baseline urine volumes were comparable among all groups.

The normal control group showed a gradual increase in urine volume, whereas the disease control group receiving 0.75% ethylene glycol showed a progressive reduction, which was significant on day 28 ($p < 0.05$). Cystone (750 mg/kg) significantly improved urinary output compared with the disease control group. Go-Pathri at 50 and 100 mg/kg partially restored urine volume, with the 100 mg/kg dose producing a greater numerical improvement. However, its effect remained less pronounced than that of Cystone, suggesting a potential diuretic and urinary tract protective effect.

TABLE 2: EFFECT OF GO-PATHRI CAPSULE ON URINE VOLUME IN RATS

Group	Treatment	Urine volume (mL/24 h)		
		Day 0 th	Day 14 th	Day 28 th
Group 1	Normal control	13.8 $\pm$ 0.76	14.6 $\pm$ 0.77	15.8 $\pm$ 0.65
Group 2	Disease control (0.75% EG)	14.4 $\pm$ 0.65	11.7 $\pm$ 0.54	10.5 $\pm$ 0.87*
Group 3	Standard (Cystone 750 mg/kg)	15.9 $\pm$ 0.54	14.2 $\pm$ 0.31	14.3 $\pm$ 0.54**
Group 4	Go-Pathri 50 mg/kg	16.5 $\pm$ 0.79	13.5 $\pm$ 0.45	11.8 $\pm$ 0.77*
Group 5	Go-Pathri 100 mg/kg	15.4 $\pm$ 0.45	14.9 $\pm$ 0.88	12.3 $\pm$ 0.55**

Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $P < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

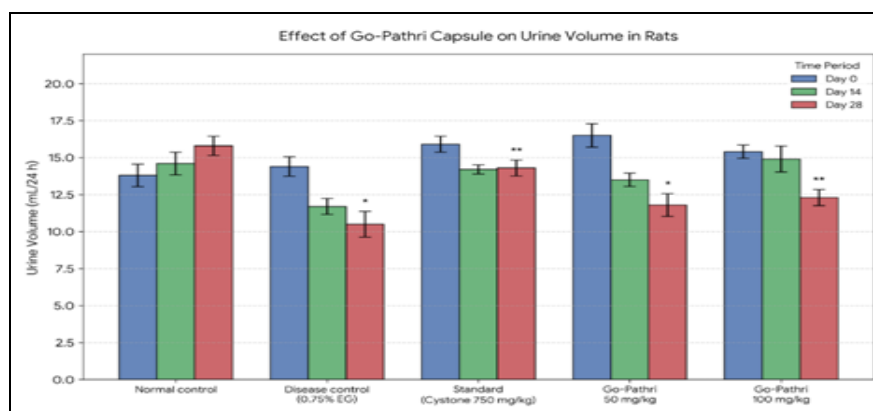


FIG. 3: EFFECT OF GO-PATHRI CAPSULE ON URINE VOLUME IN RATS. Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $P < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

Urine Constituents: The effect of Go-Pathri Capsule on urinary stone-forming constituents, including calcium, oxalate, phosphorus, and magnesium, was evaluated and expressed as mean $\pm$ SEM.

The disease control group receiving 0.75% ethylene glycol showed significant alterations compared with the normal control group, with increased calcium (4.651 ± 0.02), oxalate (5.310 ± 0.02), and phosphorus (5.264 ± 0.18), along with

decreased magnesium (1.555 ± 0.14 ; $p < 0.05$). Cystone markedly restored these parameters toward normal values. Go-Pathri at 50 and 100 mg/kg produced dose-dependent improvements, reducing calcium, oxalate, and phosphorus while increasing magnesium levels, with the 100 mg/kg dose showing greater activity than 50 mg/kg. Overall, Go-Pathri demonstrated a favorable corrective effect on altered urinary biochemical parameters associated with urolithiasis.

TABLE 3: EFFECT OF GO-PATHRI CAPSULE ON STONE-FORMING CONSTITUENTS

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
	Normal control	Disease control (0.75% EG)	Cystone 750 mg/kg	Go-Pathri 50 mg/kg	Go-Pathri 100 mg/kg
Calcium	1.723 ± 0.05	$4.651 \pm 0.02^*$	2.091 ± 0.09	3.955 ± 0.08	$3.344 \pm 0.15^{**}$
Oxalate	0.935 ± 0.035	$5.310 \pm 0.02^*$	1.354 ± 0.12	4.038 ± 0.38	3.282 ± 0.23
Phosphorus	1.325 ± 0.096	5.264 ± 0.18	$1.753 \pm 0.08^{**}$	4.344 ± 0.30	$3.521 \pm 0.24^*$
Magnesium	5.638 ± 0.185	$1.555 \pm 0.14^*$	$5.132 \pm 0.31^{**}$	2.691 ± 0.09	$3.378 \pm 0.06^*$

Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $p < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

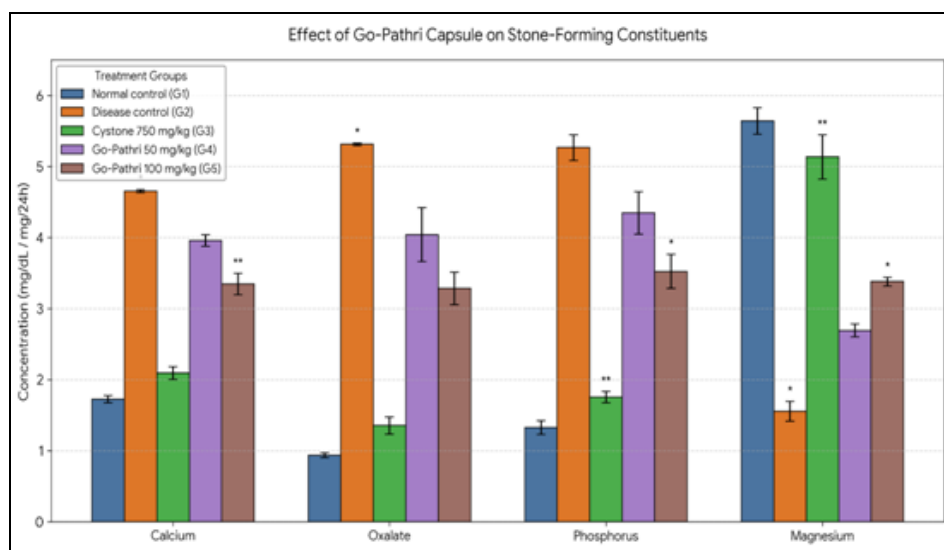


FIG. 4: EFFECT OF GO-PATHRI CAPSULE ON STONE-FORMING CONSTITUENTS. Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $p < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

Serum Uric Acid: The effect of Go-Pathri Capsule on serum uric acid levels was evaluated over a 28-day treatment period, with results expressed as mean $\pm$ SEM. Baseline uric acid levels were comparable among all experimental groups (1.81 ± 0.07 – 1.84 ± 0.08 mg/dL).

On day 14, the disease control group receiving 0.75% ethylene glycol (EG) showed an increase in serum uric acid to 2.76 ± 0.12 mg/dL compared with 1.85 ± 0.08 mg/dL in the normal control group. On day 28, EG administration further

increased uric acid to 3.18 ± 0.14 mg/dL ($p < 0.05$), whereas Cystone, Go-Pathri 50 mg/kg, and Go-Pathri 100 mg/kg reduced the levels to 2.05 ± 0.09 , 2.28 ± 0.10 , and 2.12 ± 0.08 mg/dL, respectively ($p < 0.05$ vs. disease control).

Thus, Go-Pathri significantly attenuated EG-induced elevation of serum uric acid, with the 100 mg/kg dose producing a greater effect than the 50 mg/kg dose and approaching the response observed with Cystone.

TABLE 4: EFFECT OF GO-PATHRI CAPSULE ON SERUM (URIC ACID) IN RATS

Group	Treatment	Uric acid (mg/dL)		
		Day 0 th	Day 14 th	Day 28 th
Group 1	Normal control	1.82 ± 0.09	1.85 ± 0.08	1.80 ± 0.07
Group 2	Disease control (0.75% EG)	1.84 ± 0.08	2.76 ± 0.12	3.18 ± 0.14*
Group 3	Cystone 750 mg/kg	1.81 ± 0.07	2.70 ± 0.11	2.05 ± 0.09*
Group 4	Go-Pathri 50 mg/kg	1.83 ± 0.08	2.74 ± 0.12	2.28 ± 0.10**
Group 5	Go-Pathri 100 mg/kg	1.82 ± 0.09	2.72 ± 0.11	2.12 ± 0.08**

Results were expressed as mean ± SEM. *p<0.05 compared to the normal control group, **P<0.05 compared to the disease control using one way ANOVA followed by Dunnett's test.

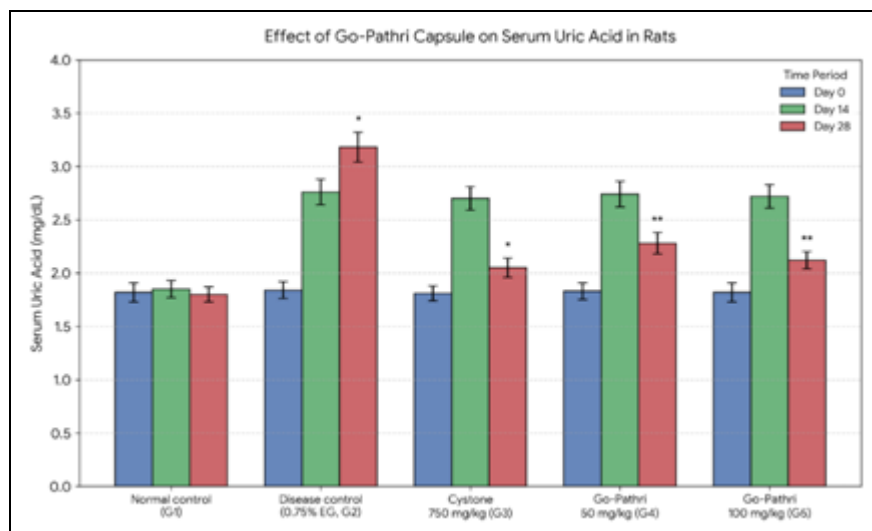


FIG. 5: EFFECT OF GO-PATHRI CAPSULE ON SERUM (URIC ACID) IN RATS. Results were expressed as mean ± SEM. *p<0.05 compared to the normal control group, **P<0.05 compared to the disease control using one way ANOVA followed by Dunnett's test.

Serum Creatinine Levels: The effect of Go-Pathri Capsule on serum creatinine levels in rats was evaluated over a 28-day treatment period, and the results were expressed as mean ± SEM. On Day 0, serum creatinine levels were comparable among all experimental groups, ranging from 0.71 ± 0.04 to 0.73 ± 0.04 mg/dL, indicating similar baseline renal function. On Day 14, the normal control group maintained a nearly constant creatinine level of 0.74 ± 0.03 mg/dL, whereas the disease control group receiving 0.75% EG showed an increase to 1.08 ± 0.05 mg/dL. The Cystone, Go-Pathri 50 mg/kg, and Go-Pathri 100 mg/kg groups showed levels of 1.05 ± 0.04 , 1.07 ± 0.05 , and 1.06 ± 0.04

mg/dL, respectively. On Day 28, the disease control group exhibited a marked increase in serum creatinine to 1.32 ± 0.06 mg/dL ($p < 0.05$ vs. normal control), while Cystone significantly reduced the level to 0.79 ± 0.03 mg/dL ($p < 0.05$ vs. disease control). Go-Pathri at 50 and 100 mg/kg reduced serum creatinine to 0.88 ± 0.04 and 0.82 ± 0.03 mg/dL, respectively ($p < 0.05$ vs. disease control). Thus, Go-Pathri Capsule significantly attenuated EG-induced elevation of serum creatinine, with the 100 mg/kg dose showing a greater reduction than the 50 mg/kg dose and approaching the response observed with Cystone.

TABLE 5: EFFECT OF GO-PATHRI CAPSULE ON SERUM (CREATINE) IN RATS

Group	Treatment	Creatinine (mg/dL)		
		Day 0 th	Day 14 th	Day 28 th
Group 1	Normal control	0.72 ± 0.04	0.74 ± 0.03	0.71 ± 0.04
Group 2	Disease control (0.75% EG)	0.73 ± 0.03	1.08 ± 0.05	1.32 ± 0.06*
Group 3	Cystone 750 mg/kg	0.71 ± 0.04	1.05 ± 0.04	0.79 ± 0.03**
Group 4	Go-Pathri 50 mg/kg	0.72 ± 0.03	1.07 ± 0.05	0.88 ± 0.04**
Group 5	Go-Pathri 100 mg/kg	0.73 ± 0.04	1.06 ± 0.04	0.82 ± 0.03**

Results were expressed as mean ± SEM. *p<0.05 compared to the normal control group, **P<0.05 compared to the disease control using one way ANOVA followed by Dunnett's test.

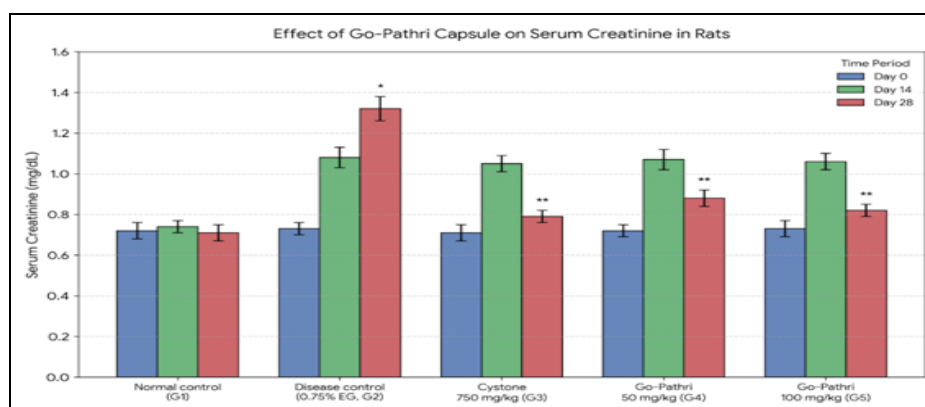


FIG. 6: EFFECT OF GO-PATHRI CAPSULE ON SERUM (CREATINE) IN RATS. Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $P < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test

Serum Blood Urea Nitrogen (BUN): The effect of Go-Pathri Capsule on serum blood urea nitrogen (BUN) levels in rats was evaluated over a 28-day treatment period, and the results were expressed as mean $\pm$ SEM. On Day 0, BUN levels were comparable among all experimental groups, ranging from 18.3 ± 0.71 to 18.6 ± 0.69 mg/dL, indicating similar baseline values. On Day 14, the normal control group maintained a BUN level of 18.7 ± 0.68 mg/dL, whereas the disease control group receiving 0.75% EG showed an increase to 27.8 ± 0.91 mg/dL. The Cystone, Go-Pathri 50 mg/kg, and Go-Pathri 100 mg/kg groups showed BUN levels of 27.1 ± 0.88 , 27.4 ± 0.90 , and $27.2 \pm$

0.87 mg/dL, respectively. On Day 28, the disease control group exhibited a marked elevation in BUN to 32.5 ± 1.02 mg/dL ($p < 0.05$ vs. normal control), whereas Cystone significantly reduced the level to 20.4 ± 0.74 mg/dL ($p < 0.05$ vs. disease control). Go-Pathri at 50 and 100 mg/kg reduced BUN levels to 22.8 ± 0.81 and 21.1 ± 0.76 mg/dL, respectively ($p < 0.05$ vs. disease control). Overall, Go-Pathri Capsule significantly attenuated the EG-induced elevation of serum BUN, with the 100 mg/kg dose showing greater improvement than the 50 mg/kg dose and approaching the response observed with Cystone.

TABLE 6: EFFECT OF GO-PATHRI CAPSULE ON SERUM (BUN) IN RATS

Group	Treatment	BUN (mg/dL)		
		Day 0	Day 14	Day 28
Group 1	Normal control	18.4 ± 0.72	18.7 ± 0.68	18.2 ± 0.65
Group 2	Disease control (0.75% EG)	18.6 ± 0.69	27.8 ± 0.91	$32.5 \pm 1.02^*$
Group 3	Cystone 750 mg/kg	18.3 ± 0.71	27.1 ± 0.88	$20.4 \pm 0.74^*$
Group 4	Go-Pathri 50 mg/kg	18.5 ± 0.70	27.4 ± 0.90	$22.8 \pm 0.81^*$
Group 5	Go-Pathri 100 mg/kg	18.4 ± 0.73	27.2 ± 0.87	$21.1 \pm 0.76^{**}$

Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $P < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

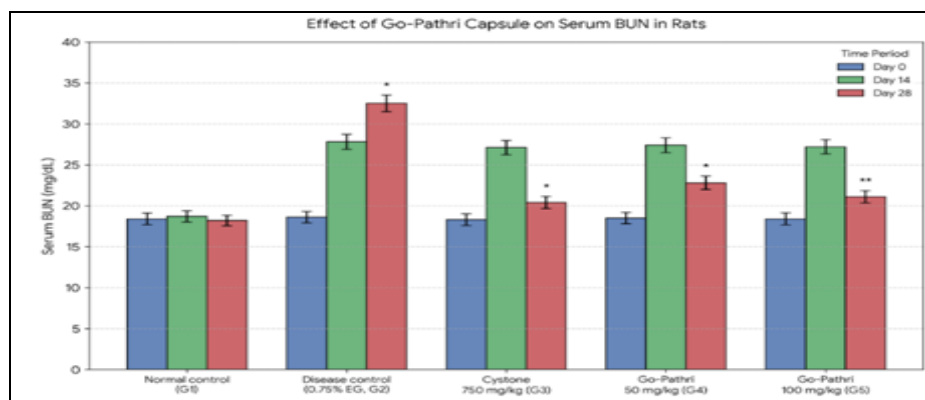


FIG. 7: EFFECT OF GO-PATHRI CAPSULE ON SERUM (BUN) IN RATS. Results were expressed as mean $\pm$ SEM. * $p < 0.05$ compared to the normal control group, ** $P < 0.05$ compared to the disease control using one way ANOVA followed by Dunnett's test.

Histopathology: Histopathological examination of the renal tissues revealed marked differences among the experimental groups. The vehicle-treated control group showed normal renal architecture, with intact glomeruli and tubules and no evidence of calcium oxalate crystal deposition or other pathological alterations in the nephron segments. In contrast, the urolithiatic disease control group exhibited several prominent calcium oxalate deposits within the renal tubules, accompanied by dilation of the proximal tubules and mild-to-moderate interstitial inflammation. These alterations indicated substantial renal injury associated with experimentally induced urolithiasis. Treatment with Go-Pathri in groups IV–V resulted in a noticeable reduction in the number of intratubular calcium oxalate deposits, with comparatively preserved renal tubular architecture. Similarly, rats treated with the standard drug Cystone (group III) showed fewer calcium oxalate deposits than the disease control group. Overall, the findings suggest that Go-Pathri and Cystone provided protective effects against crystal deposition and associated renal tissue damage.

CONCLUSION: The present study demonstrated that Go-Pathri Capsule possessed promising anti-urolithiatic activity in ethylene glycol-induced experimental urolithiasis in rats. Treatment with Go-Pathri improved urine volume and favorably modulated urinary calcium, oxalate, phosphorus and magnesium levels. It also attenuated the elevation of serum uric acid, creatinine and BUN associated with ethylene glycol-induced renal dysfunction. Histopathological findings further supported these biochemical observations by showing a reduction in intratubular calcium oxalate deposition and preservation of renal tubular architecture.

The 100 mg/kg dose generally produced greater improvement than the 50 mg/kg dose and showed responses approaching those of the standard drug Cystone. The observed effects may be attributed to the combined diuretic, antioxidant, anti-inflammatory and nephroprotective properties of the formulation's herbal and alkaline constituents. Overall, Go-Pathri showed potential as an anti-urolithiatic formulation, although further studies are required to establish its mechanisms and clinical efficacy.

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