e-ISSN: 2349-2678, p-ISSN:2961-6050

available online on www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

NLM (National Library of Medicine ID: 101732687)

Index Copernicus Value 2019: 71.05

Volume 11 Issue 4; July-August; 2023; Page No. 23-34

ResearchArticle

Evaluation of Anti-Urolithiatic Activity of *Rumex hastatus* D. Don in Wistar Rats

Rashmi*, Tirath Kumar, Virendra Kaur, Komal Chandra

Department of Pharmaceutical Sciences, Sir J.C. Bose Technical Campus Bhimtal, Kumaun University Nainital. Uttarakhand 263136.

Received: 29-05-2023 / Revised: 24-06-2023 / Accepted: 15-07-2023

Corresponding author: Rashmi

Conflict of interest: Nil

Abstract

Despite advancements in modern medical remedies, the persistence and prevalence of kidney stone remain a significant concern for humanity as there is no effective remedy available. In the present study, we aimed to evaluate the potential anti-lithiatic effect of *Rumex hastatus* D. Don, an herbal extract towards ethylene glycol-induced lithiasis in rats. To conduct the experiment rats were administered ethylene glycol in their using water for 28 days, along with co-administration of ammonium chloride for the initial three days; anti-urolithiatic action of methanolic extract of *Rumex hastatus* (MERH) was studied. The rats were orally given MERH regularly for 28 days at doses of 200 and 400 mg/kg, aiming to assess its effectiveness against hyperoxaluria triggered lithiasis, Cystone, an establishing treatment was used as a standard reference at a dose of 750mg/kg administered orally. The impact of both doses of MERH on urine output, urinary excretion of Na, K^+, Ca^{2+}, and Mg^{2+}, as well as various serum parameters, kidney and liver characteristic markers and histopathology were observed. The results and subsequent discussion revealed that the oral administration of MERH at doses of 200, and 400 mg/kg to rats with ethylene glycol and ammonium chloride- prompted renal calculi demonstrated a dose-dependent anti-urolithiatic activity. This activity was evident through significant improvements observed in urine and serum parameters, as well as histopathological studies, indicating a reversal of the pathological changes associated with kidney stones.

Keywords: Urolithiasis, Anti-oxidant, Calcium oxalate, Methanolic extract of *Rumex hastatus* (MERH), Cystone

Introduction

Urolithiasis appertains the process of renal stone formation, which is considered a frequent metabolic a painful urological ailment with a high-risk rate of prevalence and recurrence. It affects 15% of the population worldwide [1]. The recurrence rate is higher in males, ranging from 70-80%, compared to females, where it ranges from 47-60% [2]. In most cases eighty percent of renal calculi are made up of calcium oxalate, while the remaining 20% consist of struvite, uric acid, and drug-associated renal stones.

Urolithiasis was described for the 1st time in the vintage Sanskrit literature of India, in the Vedas, Puranas, and Samhita. The anatomy, physiology, pathology, diagnosis and treatment of urolithiasis were briefly described by Charka Samhita in Mutravahaasmari, and the types of surgery in urolithiasis were described in Sushruta Samhita as well.

The most commonly used terms in the epidemiological studies of urolithiasis are incidence, prevalence, and lifetime prevalence. The approximate annual prevalence and lifetime prevalence recorded were 3-5% and 15-25% respectively. The incidence rate of urolithiasis in adults in the western region is comparatively higher than that of adults in the eastern region of the world. In the late seventies, much less than 4% of the populace had stone-forming ailment. By means of the early 1990s, the part of the population with the disease had increased to greater than 5% [5]. In 1990, 77.78 million incidence cases of urolithiasis were recorded and till 2019, incidence cases were increased by upto 115.55 million. So, the incidence rate was increased by 48.57% [6]. Compared to Saudi Arabia, the United States, Canada and Europe were reported to have a high-risk prevalence rate of 20.1%, 13-15%, 12%, and 5-9% respectively [7][8]. In India, approximately 2 million cases of renal calculi are recorded annually, with a maximum reported in the states of Gujrat, Maharashtra, Punjab, Delhi and the Northeast. India is also recognized as being located in a region highly prone to kidney stone formation, commonly known as the stone-prone belt. Kidney stones are prevalent across all age groups in India, and they often reoccur at a significant rate [2]. Recurrence rates with renal calculi are approximately 10% over 1, 33% over 5, 50% over 10, and 75% over 20 years. In epidemiological studies of the last few decades, paediatric urolithiasis has also been observed [9].

Urolithiasis is a multifactorial disorder, the main causes attributed to stone formation are hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia and hypomagnesuria. Alteration in the concentration of minerals triggers the formation of renal calculi. Supersaturation alters the morphology of the kidney, causes metabolic abnormalities, changes urine flow and causes UTIs. Interaction of oxalate ions with tubular renal cells accelerates mitochondria to initiate lipid signalling and start producing reactive oxygen species (ROS), which causes damage to kidney cells, alters the biochemical reactions and defensive antioxidant system. Stone formation is a multiple step procedure that consists of nucleation (association of free ions into microparticles after urine supersaturation), crystal growth and aggregation.

Surgical tactics like percutaneous nephrolithotomy, extracorporeal shock wave lithotripsy are the same old tactics employed to fragment renal calculi. However, their widespread utilization had been hindered by their considerable expenses and potential for traumatic effects associated with shock waves. Furthermore, these approaches may raise the likelihood of recurrence [7][9] .

Medication used to treat stones covers analgesics, uric acid inhibitors, alpha-blockers, calcium channel blockers, corticosteroids, thiazide diuretics and antibiotics. Sometimes probiotics are also suggested in the treatment of renal calculi. Other drugs which are used to prevent stone formation are potassium citrate and magnesium. [7][8] They seem to reduce the rate of recurrence of calculi formation however do no longer appear to be clinically effective.

Plants have been utilized for centuries to effectively address renal calculi in various forms such as decoction, infusion, dried powder, fresh leaves, or in the form of juice. These plants contain a diverse range of medicinal bioactive compounds or secondary metabolites, including flavonoids, alkaloids, polyphenols and steroids. These compounds play a significant role in exerting anti-urolithiatic effects by demonstrating anti-inflammatory, analgesic, anti-oxidant and diuretic activities. The World Health Organization recognizes herbal treatments as effective due to their abundant phytochemical content, wide availability, affordability and minimal side effects compared to conventional treatments. Among the herbal remedies, Cystone, a polyherbal formulation, has been widely used over an extended period to treat renal calculi [5].

Rumex hastatus D. Don, also called *Rumex dissectus*, is a member of the Polygonaceae family and goes by the common referred to by various names in different languages and regions. In Hindi, it is called Churki, while in Punjab, it is known as Khatimal and Katambal. In Kumaun, it goes by the names Amlora and Chulmora, and in Nepali, it is called Kapu. This plant species is most prevalent in India and its

subcontinental countries, particularly at an elevation of 2600 meters. It is far a bushy shrub or undershrub, 30-90 cm in height, taking place chiefly on dry rocks and hillsides of the Western Himalayas, from Kashmir to Kumaun, at an altitude among 300 and a couple of 2,400m. Its rootstock is woody and plant leaves are 2.5-6.5 cm lengthy, hastate, thick and fleshy. Plant flowers are green-tinged with pink or pink, polygamous, small and numerous. The fruits are pinkish and consist of one-seeded nutlets [10]. The existence of quinones (Chrysophanol, Emodin, Aloe-emodin, Physcion, Rhein, Przewaoskinone B, 1,3,7-Trihydroxy-6-methylanth-raquinone), flavonoids (kaempferol, Quercetin, Rutin), stilbenoids (Resveratrol), naphthalene's (rumexoside, nepodin, rumexneposides A and B, torachrysone-8-yl β-D-glucopyranoside, 8,6-Hydroxymusizin-8-O-β-D-glucopyranoside, hastatuside A and B), terpenes (Taraxasterol acetate), phenolic compounds (gallic acid and methylate gallate) , and other compounds are orientaloside nepalin, rumicin attributes to the medicinal property. The root contains naphthalene acyl glucosides [11]. *Rumex hastatus* is utilized in the treatment of various health condition like bloody dysentery, rheumatism, blood pressure, skin disease, piles, bilious complaints, hyperthermia, neoplasm, wounds and throat pain, jaundice, in some kind of pulmonary disease and intestinal disorders, also used for its diuretic, carminative, laxative, tonic and astringent property. [11][12][13]

The rationale for using *Rumex hastatus* D. Don for its anti-stone forming activity in ethylene glycol prompted urolithiasis. *Rumex* species, belongs to the Polygonaceae family, encompass about two hundred species appreciably scattered around the world. The term *Rumex* have rewritten from Latin word indicating dart, referring to the shape of the leaves. Ethnobotanicals and ethnopharmacological literature reviews have extensively covered the occurrence and primitive indications of *Rumex* species [41]. Numerous anti-urolithiatic drugs available on the market are not highly effective and are associated with undesirable effects and the risk of recurrence. *Rumex hastatus* D. Don is a wealthy source of phytochemicals such as saponins, alkaloids, flavonoids, polyphenols, sterols and more. These constituents are believed to exert anti-urolithiatic activity by demonstrating diuretic, anti-oxidant, anti-inflammatory, and analgesic properties. Therefore, we conducted a study to evaluate the antilithiatic activity of *Rumex hastatus* against ethylene glycol -precipitate urolithiasis in rats.

Materials and Methods

Plant collection and extract preparation: The shoot part of *Rumex hastatus* D. Don were picked from the Bhimtal region of Uttarakhand in October and authenticated through the Botanical Survey of India, Dehradun.

Collected aerial parts were thoroughly cleaned using distilled water, dusk dried for twenty days. Once dried, they were ground into a coarse powder and subjected to maceration using 80% methanol for a period of 15 days. On the 15th day, the mixture was filtered through a muslin cloth, resulting in strained liquid. The strained liquid was further processed using a rotatory evaporator to get a concentrated extract. The concentrated extract was then dried using a waterbath at 40℃. Finally, the dried extract was kept in a desiccator for future use.

Chemical and apparatus:

All chemicals employed in this study were of standard grade, acquired from an approved supplier. Apparatus including metabolic cages, U.V spectrophotometer, autoanalyzer, centrifuge and flame photometer have been employed in this study.

Animals:

Male Wistar rats in youthful stage, having weight between 150-250grams, were utilized in this study. These rats were procured from the departmental animal house and placed into polypropylene cages underneath standard environmental conditions, having a 12:12 light-dark cycle, a relative humidity 55±10%, and a temperature of 25±2ºC. The rats had been nourished with a standard pellet diet and water *ad libitum.* Prior to the start of the experiment, the rats were acclimated to laboratory ambience for one week to adapt to their new surroundings. The protocol for this experiment was authorized

by the Institutional Animal Ethic Committee of Department. Protocol no: KUDOPS/175.

Induction of urolithiasis:

To induce urolithiasis, a solution of 0.75% v/v of ethylene glycol in water was administered to the rats for a duration of 28 days. Additionally, for the first 3 days, the rats were co-administered with 1% w/v ammonium chloride. The study consisted of five groups, and each comprising six rats. Group I was designated as vehicle control group and was treated with normal saline (2.5ml/kg intraperitoneally). Group II referred to as lithiatic control, received ethylene glycol along with ammonium chloride for entire 28-days duration. Group III, the positive control group, given the standard drug Cystone orally at a dose of 750mg/kg, along with lithiasis inducing agents. Group IV and V were labelled as the low and high dose test groups, respectively. These groups received ethylene glycol and ammonium chloride along with oral administration of MERH at doses 200mg/kg and 400mg/kg. Treatment duration for all groups was 28 days.

Urine collection and analysis:

Afterwards housing the rats in metabolic cages, 24-h urine samples were collected from each rat at two time points: 0 and 28 days after administering the calculus precipitation remedy. All urine samples were analysed for various urine parameters. Urine output was determined. Concentrations of Sodium and Potassium ion were analysed with the aid of flame photometry. Calcium and magnesium content were tested in an autoanalyzer using diagnostic kits.

Serum analysis:

Blood samples had been collected separately from retro-orbital plexus route using capillary. This procedure is performed under anaesthesia. The serum was separated from the blood samples through centrifugation at 3000rpm for ten minutes. The serum samples were then analysed for various parameters such as urea, uric acid, creatinine, SGOT, SGPT and ALP.

Renal histopathology:

A histopathology examination was conducted following the sacrifice of the animals. The abdominal area was incised using a sharp tool and each kidney were extracted from individual animal. The remoted kidneys were wiped clean of any extraneous tissue and preserved in 10 % formalin solution. Subsequently, the kidneys were examined to determine the existence of calcium oxalate deposition, necrosis and inflammation in the kidney cells.

Statistical analysis:

Results were presented as mean (±SEM) and analysed using one-way ANOVA followed. Post -hoc multiple comparison tests, specially Dunnett's and Tukey's tests, were performed using Graph Pad Prism Software for statistical analysis. Statistical significance was determined with the thresholds of $P<0.05$, $P< 0.01$ and $P<0.001$.

Results and Discussion

Table 1: Effect of MERH against ethylene glycol induced urolithiasis in rats – urine output

S. No.	Groups	Treatment	Urine output day 0	Urine output Day 28
	Normal control	Normal saline (2.5ml/kg i.p)	10.77±0.48	10.92±0.37
	Lithiatic control	EG (0.75%) in drinking water	10.37±0.49	8.26±0.53
	Standard	EG (0.75%) + Oral administration of Cystone (750mg/kg)	$10.84±0.35^{ns}$	11.38±0.83**
	MERH $_1$	EG (0.75%) + MERH (200 mg/kg body weight)	$10.95±0.74^{ns}$	11.27±81**
	MERH $_2$	EG (0.75%) + MERH (400 mg/kg body weight)	$11.57±0.74^{ns}$	12.62±0.38***

****P < 0.01, ***P<0.001, The results are presented as mean ± SEM, with a sample size n = 6. Statistical comparisons were performed using one-way analysis of variance (ANOVA), comparing the treatment group to lithiatic control group.**

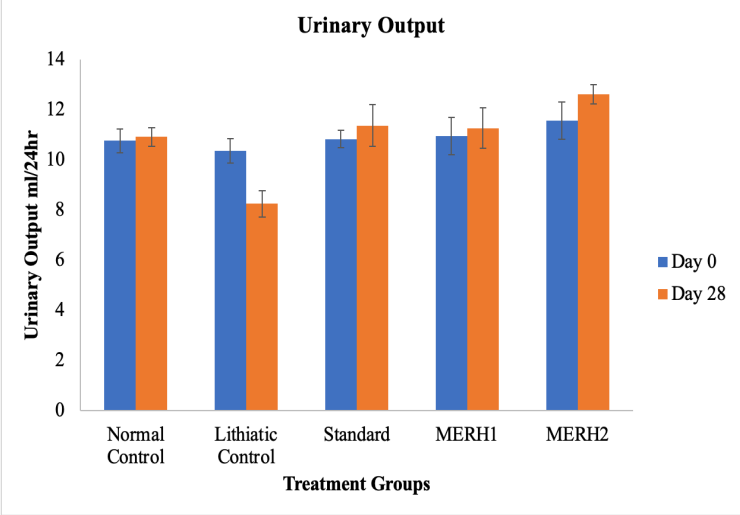

Figure 1. Urinary Output following treatment with Ethylene glycol, ammonium chloride, $MERH_1$: (200mg/kg), $MERH_2$: (400mg/kg) in rats was analysed using a one-way ANOVA with Dunnett's multiple comparison test.

Table 2: Impact of MERH on urinary sodium and potassium excretion in rats with ethylene glycol triggered lithiasis.

S. No.	Groups	Treatment	Na^+ Concentration (mmol/L)	K^+ Concentration (mmol/L)
1.	Normal control	Normal saline (2.5ml/kg i.p)	44.3±6.2	29.57±1.27
2.	Lithiatic control	EG (0.75%) in drinking water	82.26±5.4	52.89±1.90
3.	Standard	EG (0.75%) + Oral administration of Cystone(750mg/kg)	53.31±3.1***	35.95±2.35***
4.	MERH 1	EG (0.75%) + MERH (200 mg/kg body weight)	69.66±1.3*	45.10±2.64
5.	MERH 2	EG (0.75%) + MERH (400 mg/kg body weight)	64.08±2.4**	42.22±2.86**

***P < 0.05, **P < 0.01, ***P<0.001, all values are presented as mean ± SEM, with a sample size n = 6. Statistical comparisons were performed using one-way ANOVA, comparing the treatment group to lithiatic control group.**

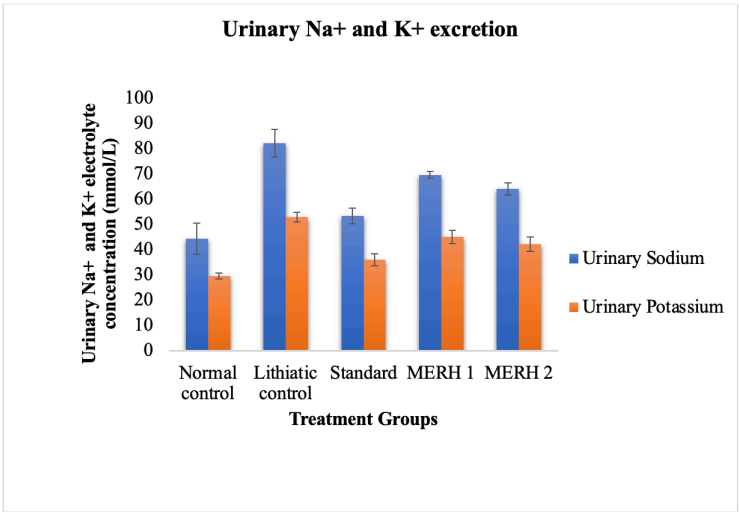

Figure 2. Urinary of Sodium and Potassium excretion following urolithiasis induction and treatment with $MERH_1$: (200mg/kg), $MERH_2$: (400mg/kg) in rats. The data is presented as mean ± SEM (n = 6), analysed using one-way ANOVA following Dunnett's multiple comparison test.

Table 3: Effect of MERH on ethylene glycol triggered urolithiasis in rats – Urinary excretion of calcium.

S. No.	Groups	Treatment	Urinary Calcium Conc. (mg/24hrs)
1.	Normal control	Normal saline (2.5ml/kg i.p)	5.3±0.15
2.	Lithiatic control	EG (0.75%) in drinking water	9.55±0.14
3.	Standard	EG (0.75%) + Oral administration of Cystone (750mg/kg)	6.50±0.14***
4.	MERH $_1$	EG (0.75%) + MERH (200 mg/kg body weight)	8.00±0.30**
5.	MERH $_2$	EG (0.75%) + MERH (400 mg/kg body weight)	7.72±0.40***

****P < 0.01, ***P<0.001, the results are presented as mean ± SEM, n = 6, data were analysed using one-way ANOVA following Tukey's test to compared lithiatic control group with the treatments groups.**

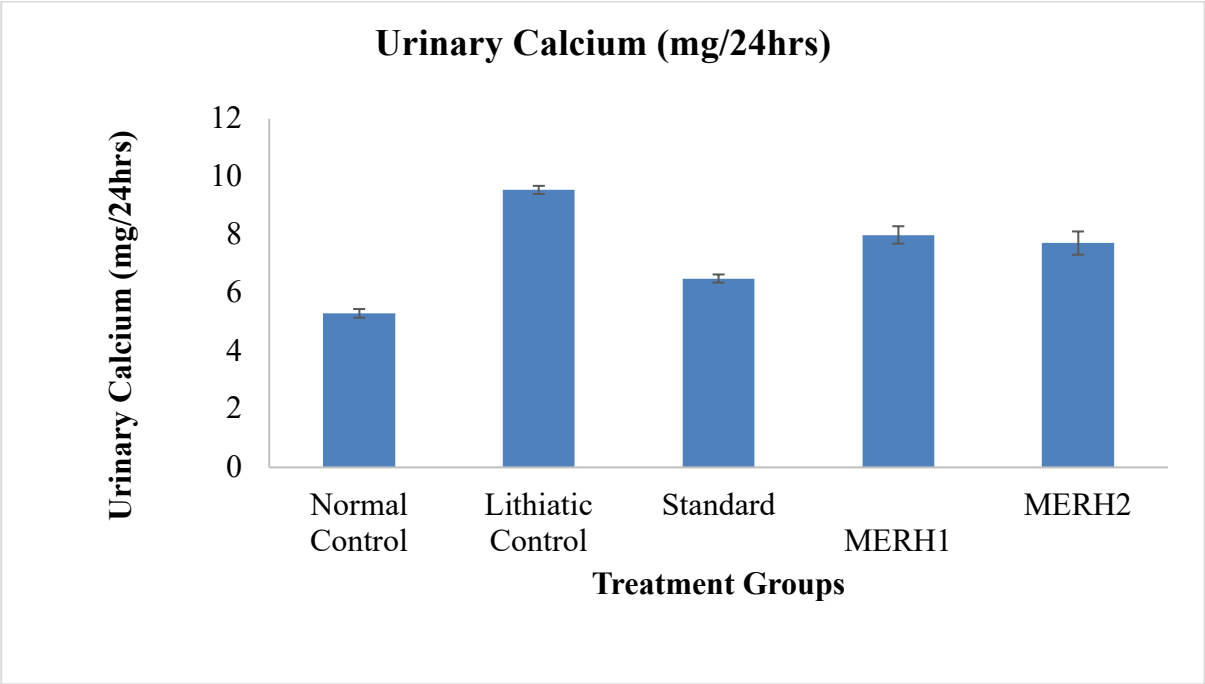

Figure 3. Urinary excretion of Calcium afterwards urolithiasis induction and treatment with MERH$_1$: (200mg/kg), MERH$_2$: (400mg/kg) in rats. The results are presented as the mean ± SEM (n = 6), and were analysed using one-way ANOVA following Tukey's multiple comparison test.

Table 4: Effect of MERH on ethylene glycol prompted urolithiasis in rats – Urinary excretion of magnesium.

S. No.	Groups	Treatment	Magnesium Conc. (mg/24hrs)
	Normal control	Normal saline (2.5ml/kg i.p)	0.83±0.01
	Lithiatic control	EG (0.75%) in drinking water	0.33±0.01
	Standard	EG (0.75%) + Oral administration of Cystone (750mg/kg)	0.63±0.04***
	MERH $_1$	EG (0.75%) + MERH (200 mg/kg body weight)	0.47±0.02*
	MERH $_2$	EG (0.75%) + MERH (400 mg/kg body weight)	0.54±0.05**

***P< 0.05, **P < 0.01, ***P<0.001, values are presented as mean ± SEM, n = 6, when compared to lithiatic control by using one-way ANOVA following Tukey's multiple comparison test.**

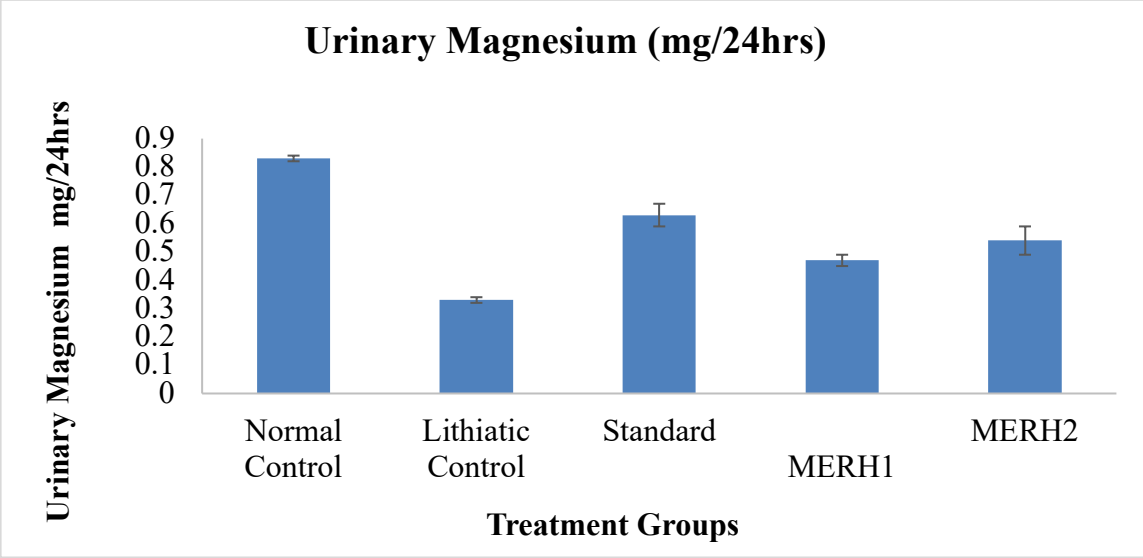

Figure 4. Urinary excretion of magnesium afterwards urolithiasis induction and treatment with $MERH_1$: (200mg/kg), $MERH_2$: (400mg/kg) in rats. The data is presented as Mean ± SEM (n = 6), and analysed using one-way ANOVA followed by Tukey's multiple comparison test.

Table 5: Impact of MERH on ethylene glycol triggered urolithiasis in rats –serum parameters (uric acid, creatinine).

S. No.	Groups	Treatment	Uric acid Conc. (mg/dl)	Creatinine Conc. (mg/dl)
1.	Normal control	Normal saline (2.5ml/kg i.p)	0.93±0.04	0.66±0.03
2.	Lithiatic control	EG (0.75%) in drinking water	2.18±0.11	3.29±0.13
3.	Standard	EG (0.75%) + Oral administration of Cystone(750mg/kg)	1.41±0.10***	1.87±0.08***
4.	MERH 1	EG (0.75%) + MERH (200 mg/kg body weight)	1.80±0.06*	2.66±0.12*
5.	MERH 2	EG (0.75%) + MERH (400 mg/kg body weight)	1.69±0.05**	2.42±0.19***

P < 0.05, **P < 0.01, ***P<0.001, all values are presented as mean ± SEM, with a sample size n = 6. Statistical comparisons were performed using one-way ANOVA, comparing the treatment group to lithiatic control group.

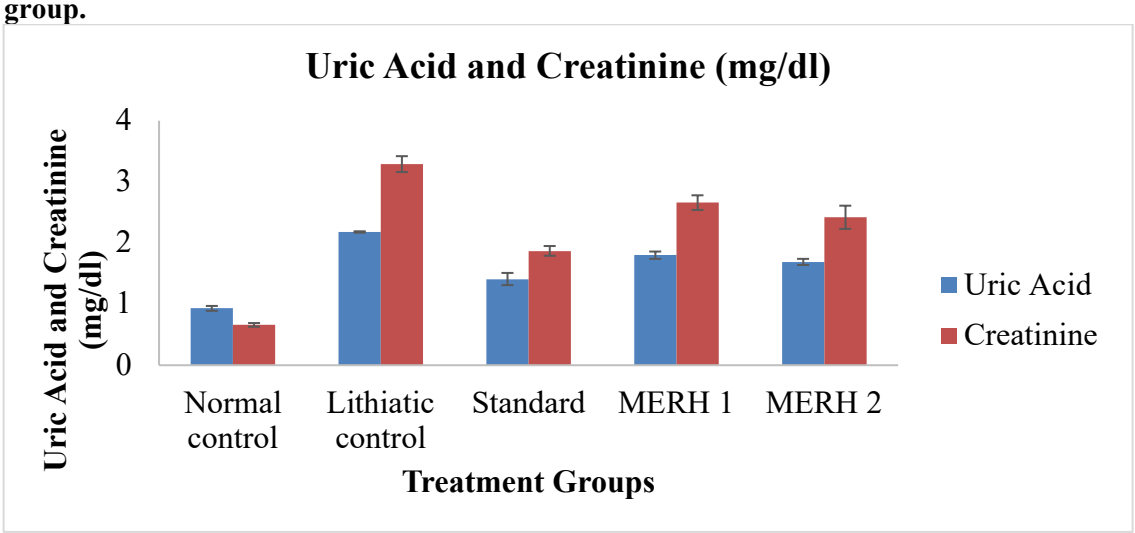

Figure 5. serum parameters afterwards urolithiasis induction and treatment with $MERH_1$: (200mg/kg), $MERH_2$: (400mg/kg) in rats. The results are presented as mean ± SEM (n = 6), data was analysed using one-way ANOVA following Tukey's test.

Table 6: Effect of MERH against ethylene glycol precipitated urolithiasis in rat – serum parameters (Blood urea).

S. No.	Groups	Treatment	Blood Urea (mg/dl)
1.	Normal control	Normal saline (2.5ml/kg i.p)	17.11±0.50
2.	Lithiatic control	EG (0.75%) in drinking water	29.06±0.66
3.	Standard	EG (0.75%) + Oral administration of Cystone (750mg/kg)	21.22±2.07***
4.	MERH $_1$	EG (0.75%) + MERH (200 mg/kg body weight)	24.70±0.55*
5.	MERH $_2$	EG (0.75%) + MERH (400 mg/kg body weight)	23.45±0.99**

***P <0.05, **P<0.01, ***P<0.001, results are presented as mean ± SEM, n = 6. Statistical comparisons were performed using one-way ANOVA followed by Dunnett's multiple comparison test, when comparing the treatment group to lithiatic control group.**

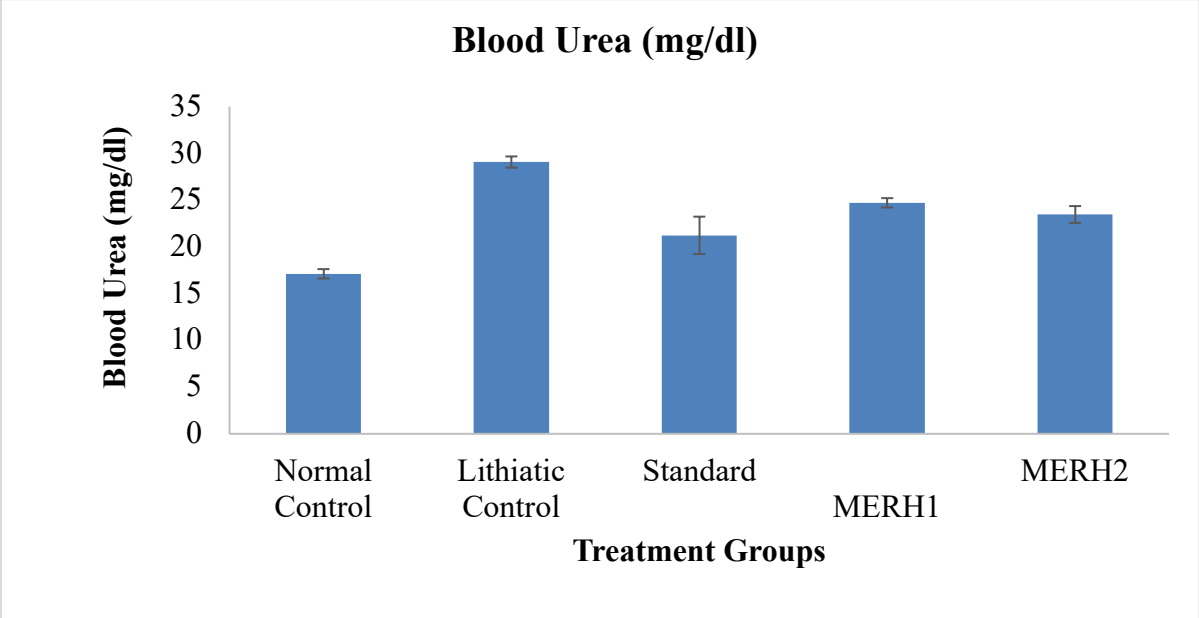

Figure 6: Blood Urea afterwards urolithiasis induction and treatment with MERH$_1$: (200mg/kg), MERH$_2$: (400mg/kg) in rats. The data is presented as ean ± SEM (n = 6), analysed using one-way ANOVA following Dunnett's multiple comparison test.

Table 7: Effect of MERH towards ethylene glycol triggered urolithiasis in rats –serum parameters (SGOT, SGPT and ALP).

S. No.	Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
	Normal control	Normal saline (2.5ml/kg i.p)	76.36±1.90	43.36±1.42	46.02±0.90
	Lithiatic control	EG (0.75%) in drinking water	128.6±2.24	86.82±3.06	79.33±2.79
	Standard	EG (0.75%) + Oral administration of Cystone (750mg/kg)	94.10±2.76***	58.53±3.34***	61.11±2.25***
	MERH $_1$	EG (0.75%) + MERH (200 mg/kg body weight)	112.3±5.63*	73.26±3.96*	70.81±3.65
	MERH $_2$	EG (0.75%) + MERH (400 mg/kg body weight)	106±4.54***	65.60±4.95**	66.79±2.77**

***P <0.05, **P <0.01, ***P<0.001. Results are presented as mean ± SEM, n = 6, when compared to lithiatic group by using one-way ANOVA following Dunnett's multiple comparison test.**

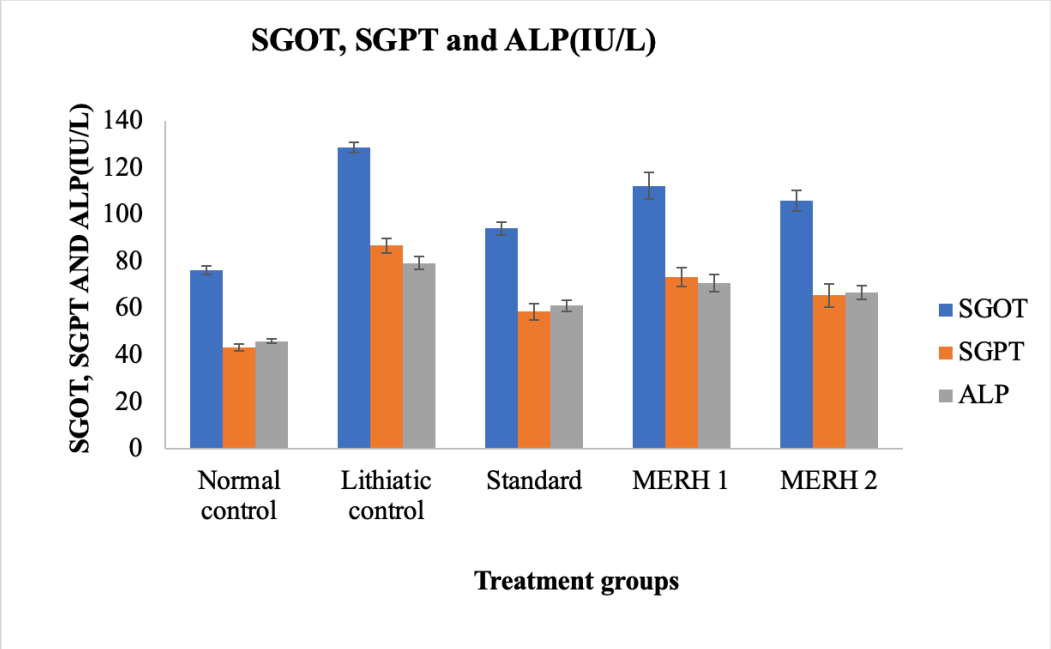

Figure 7: Serum SGOT, SGPT and ALP afterwards urolithiasis induction and treatment with $MERH_1$: (200mg/kg), $MERH_2$: (400mg/kg) in rats. The data is expressed as mean values ± SEM (n = 6), analysed using one-way ANOVA following Dunnett's multiple comparison test.

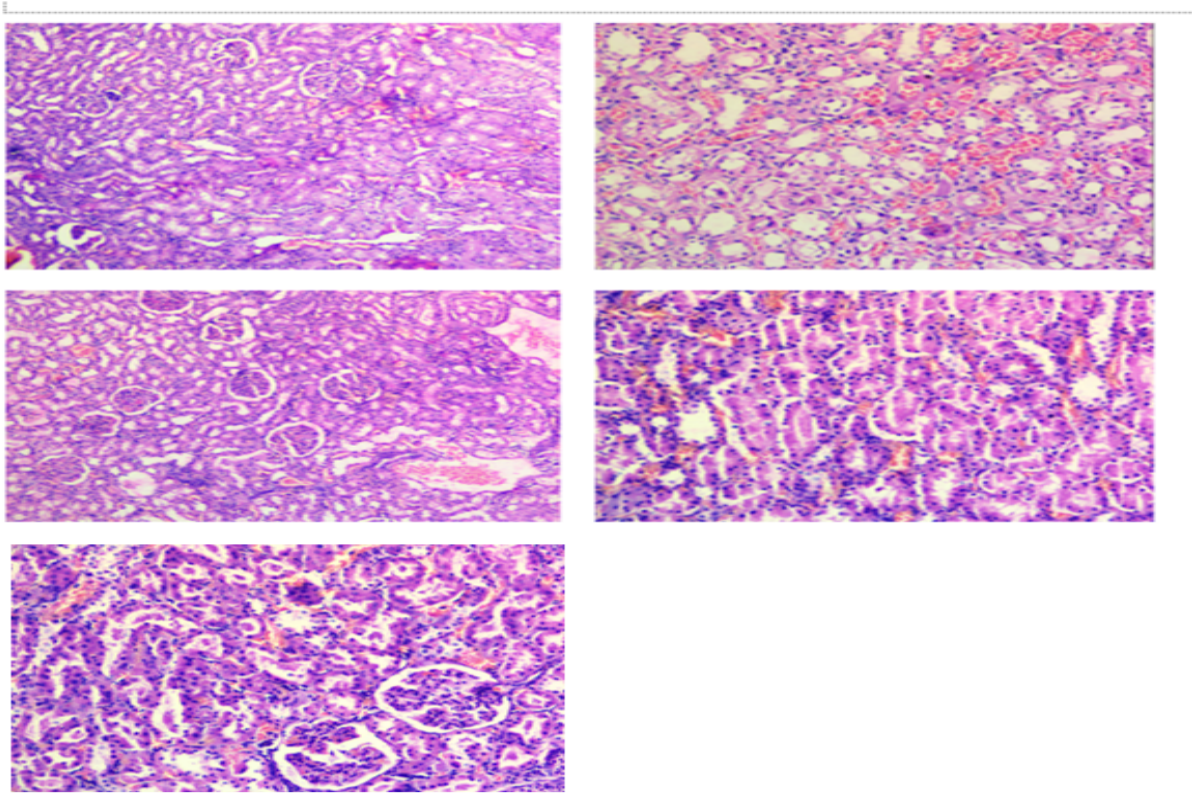

Figure 8: Histopathology of kidney tissue of (A) Normal control, the kidney tissue exhibits a healthy structure (B) Lithiatic group showing significant changes includes tubular dilatation, tubular atrophy, calculi deposition followed by cell infiltration (C) Standard Group, Cystone administered suggesting the restoration of renal cells and (D and E) preventive treatment with MERH at both doses demonstrates significant improvements in renal cells.

Urine Analysis:
Normal control group of rats didn't show any sizable variation in the urinary output level throughout the course of the test duration. In contrast, the lithiatic rats displayed a significant decrease in urine volume compared to normal rats. Administration with Cystone resulted in a substantial elevation in urine volume ($p < 0.01$) compared to the lithiatic group. Rats treated with MERH at doses of 200 and 400 mg/kg body weight also showed a significant ($p < 0.01$ to 0.001) elevation in urine level when compared to Lithiatic control rats.
To induce renal stone formation a hyperoxaluria model triggered by ethylene glycol and ammonium chloride was employed in this study. This model is characterized by a low excretion of magnesium and increased excretion of sodium, potassium, and calcium. However, treatment with standard (Cystone) 750mg/kg body weight and MERH at doses 200, 400mg/kg for 28 days effectively mitigated these imbalances. The administration of these treatment reduced the elevated excretion of sodium, potassium, and calcium while elevate the reduced excretion of magnesium in a dose-based manner whilst in comparison to the lithiatic control groups.
Serum Analysis: The induction of urolithiasis using the hyperoxaluria model resulted in impaired renal and liver function, which is characterized by increased levels of urea, creatinine, uric acid, SGOT, SGPT, and ALP. However, treatment with Cystone (750mg/kg), MERH at doses of 200 and 400mg/kg body weight for the duration of 28 days efficiently restored normal renal and liver function parameters while as compared to lithiatic control rats.

Renal histopathology: In various regions of the kidneys of rats, the administration of ethylene glycol therapy resulted in significant deposition of calcium oxalate crystal and cell harm, as well as oxidative harm. Calcium oxalate crystals were observed in tubular and interstitial regions of the kidneys in rats with lithiasis, along with glomerular congestion, lymphoplasmacytic infiltration, and tubular necrosis. However, treatment with Cystone (750mg/kg) and MERH at doses of 200 and 400mg/kg body weight for 28 days inhibited the production of calcium oxalate calculi in different parts of the renal tubules. It also reduced glomerular congestion, lymphoplasmacytic infiltration, and tubular necrosis, potentially by accelerating the breakdown of preformed stones and/or minimized the formation of new calculi.

Currently available drug regimens for the management of renal calculi have certain limitations. Therefore, there is a need for safer and extra effective anti-urolithic drugs. The use of conventional medicine and medicinal plants in most developing nations as a normative basis for the protection of excellent health has been broadly observed *Rumex hastatus* D. Don is a multifunctional medicinal plant with numerous potential benefits. The present study aimed to evaluate the pharmacological properties of MERH for anti-urolithiatic activity.

Extraction of aerial parts of the plant *Rumex hastatus* D. Don was carried out using 80% methanol through a cold maceration method. Phytochemical investigation revealed the presence of flavonoids, saponins, alkaloids, polyphenols, sterols, etc.

Various epidemiological studies demonstrates that calcium oxalate (CaOx) usually forms approximately 90% of calculi. Several animal models using rats have been employed to induce calcium oxalate urolithiasis. Among these models, the rat model by ethylene glycol and ammonium chloride triggers hyperoxaluria, allows for rapid precipitation of calcium oxalate stones in the renal tubules of experimental rats. This model was utilized for the rapid screening of anti-urolithiatic regimens. [3]

Male Wistar rats has been selected to accelerate urolithiasis because they accurately reflect human physiology, mimic the pathology of renal calculi formation in humans, and exhibit a good response to this activity. Male rats were chosen because the rate and amount of stone deposition in male rats is extensively higher compared to female rats. [15]

Urinary supersaturation with respect to stone-precipitating constituents is considered one of the causative factors in calculogenesis. Prior researches indicates that after being treated with ethylene glycol for 28 days in adult male wistar rats leads to the formation of renal calculi

primarily composed of calcium oxalate. The formation of calculi in ethylene glycol-fed rats is attributed to hyperoxaluria, which triggers renal retention and elevated excretion of oxalate. Ammonium chloride has been shown to accelerate lithiasis [16]. Therefore, this animal model was used to evaluate the protecting action of *Rumex hastatus* D. Don towards urolithiasis.

Urinary chemistry plays a vital role to determine of type and character of crystal formed. Therefore, studying the urinary chemistry associated with stone forming constituent provides a better understanding of the extent of stone induction. [16]

A decrease in urine output is associated with lithiasis induction, but treatment with MERH for 28 days causes an increase in urine volume.

By looking some previous reviews stones precipitation with the aid of ethylene glycol triggers an increase in excretion of Ca^{2+} K^{+}, and Na^{+}, in the lithiatic control group. MERH treatment for 28 days significantly decreased urinary excretion of Na^{+}, K^{+}, and Ca^{2+} [3].

Normal urine contains many organic and inorganic inhibitors of crystallization, with magnesium being a promising inhibitor. Low tiers of magnesium are often found in stone formations. Magnesium is recommended as a remedy to treat calcium oxalate stones as it reduces the recurrence rate by promoting the oxalate complexation, destabilizing calcium oxalate ions, and thus reducing the size of the aggregate [17]. Treatment with MERH for 28 days appreciably restored the urinary magnesium level, thereby decreasing the risk of calculi formation.

In this study, an elevation in the levels of serum parameters (blood urea, uric acid, creatinine, SGOT, SGPT, and ALP) was observed in lithiatic control rats compared to normal control rats. However, treatment with MERH at both doses for 28 days reduced the levels of these serum parameters.

The histopathology of the kidney cells did not show any toxic effects of MERH. Treatment with MERH at both doses diminishes the precipitation of calcium oxalate stones in renal tubules, glomerular congestion, lymphoplasmacytic infiltration and tubular necrosis in renal cells.

These researches supported the folk knowledge regarding the antilithiatic action of the plant.

Conclusion

The anti-urolithiatic activity of MERH was confirmed through measurements of urine parameters (urine output, urinary excretion of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}), serum parameters (uric acid, creatinine, blood urea, SGOT, SGPT and ALP) and histopathological study of kidney. The presence of saponins, alkaloids, flavonoids, polyphenols, and sterols in MERH is proposed to contribute to its anti-urolithiatic activity by exhibiting diuretic, anti-oxidant, anti-inflammatory, and analgesic activities. Further research and development may be conducted to explore its potential for the management of urolithiasis.

References

1. Salem, P.P., Vieira, N.B., Garcia, D.A., Nicácio, K.J., Dias, D.F., & de Paula, A.C. (2020). Anti-urolithiatic and anti-inflammatory activities through a different mechanism of actions of *Cissus gongylodes* corroborated its ethnopharmacological historic. *Journal of Ethnopharmacology*.
2. Sohgaura, A., Bigoniya, P. (2017). A review on epidemiology and etiology of renal stone. *American Journal of Drug Discovery and Development*, 7(2), 54-62.
3. Pandhare, R.B., Shende, R.R., Avhad, M.S., Deshmukh, V.K., Mohite, P,B., Sangameswaran, B., et al. (2021). Anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. *Journal of Traditional and Complementary Medicine*,11(6), 545-551.
4. Kasote, D.M., Jagtap, S.D., Thapa, D., Khyade, M.S., Russell, W.R. (2017). Herbal remedies for urinary stones used in India and China: A review. *Journal of Ethnopharmacology*, 203, 55-68.
5. Shah, J., Whitfield, H.N. (2002). Urolithiasis through the ages. *British Journal of Urology International*, 89(8), 801-10.

6. Qian, X., Wan, J., Xu, J., Liu, C., Zhong, M., Zhang, J. et al. (2022). Epidemiological Trends of Urolithiasis at the Global, Regional, and National Levels: A Population-Based Study. *International Journal of Clinical Practice*, 1-12.
7. Ramello, A., Vitale, C., Marangella, M. (2001). Epidemiology of nephrolithiasis. *Journal of Nephrology*, 13, S45-50.
8. Robertson, W.G., & Hughes, H. (1994). Epidemiology of Urinary Stone Disease in Saudi Arabia. Urolithiasis, *Plenum Press*, *New York*, 2, 453-455.
9. Sharma, A.P., Filler, G. (2010). Epidemiology of pediatric urolithiasis. *Indian Journal of Urology: IJU: Journal of the Urological Society of India*, 26(4), 516-522.
10. Sahreen, S., Khan, M.R., Khan, R.A. (2011). Phenolic compounds and antioxidant activities of *Rumex hastatus* D. Don. Leaves. *Journal of Medicinal Plants Research*, 5(13), 55-65.
11. Sultana, S., Ali, M., Mir, S.R. (2017). Chemical Constituents from the Aerial Parts of *Rumex hastatus* D. Don. *Journal of Pharmaceutical and Biological Sciences*, 5(5),179-187.
12. Vasas, A., Orbán-Gyapai, O., Hohmann, J. (2015). The Genus *Rumex*: Review of traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 175, 198-228.
13. Li, J.J., Li, Y.X., Li, N., Zhu, H.T., Wang, D., Zhang, Y.J. (2022). The genus Rumex (Polygonaceae): an ethnobotanical, phytochemical and pharmacological review. *Natural Products and Bioprospecting*, 12(1),1-29.
14. Tonny, T.S., Sultana, S., Siddika, F. (2017). Study on medicinal uses of Persicaria and Rumex species of polygonaceae family. *Journal of Pharmacognosy and Phytochemistry*, 6(6), 587-600.
15. Rao, G.S., Jayakumari, S. (2007). Effect of *Dichrostachys cinerea* (Linn.) root extract on ethylene glycol induced urolithiasis in rats. *Natural Product Sciences*, 13(3), 180-185.
16. Makasana, A., Ranpariya, V., Desai, D., Mendpara, J., Parekh, V. (2014). Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats. *Toxicology Reports*, 1, 46-52.
17. Massey, L. (2005). Magnesium therapy for nephrolithiasis. *Magnesium research*, 18(2), 123-6.