Research Article

Pharmacological Evaluation of Antiurolithiatic Activity of Aqueous Leaves Extract of *Bauhinia Variegate* Against Ethylene Glycol Induced Urolithiasis in Wistar Rats

Sonu[1], Mr. Rakesh Sharma[2], Dr. Ram Garg[3], Mrs. Mamta Sharma[4]

1 Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan.

2 Professor, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan.

3 Professor, Department of Pharmacology, Arya College of Pharmacy, Jaipur, Rajasthan.

4 Assistant Professor, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan.

Corresponding Author*: Sonu, Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, India,
Email ID: skcoolsonu116@gmail.com

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Abstract:

Urolithiasis, commonly known as kidney stones, is a prevalent urological disorder affecting millions of people worldwide. The present study aims to investigate the potential Antiurolithiatic activity of the aqueous leaves extract of Bauhinia variegata against ethylene glycol-induced urolithiasis in Wistar rats. This research involves a comprehensive pharmacological evaluation, including various biochemical, histological, and molecular analyses, to elucidate the mechanism of action and therapeutic potential of the plant extract. When ethylene glycol (EG) is given to rats in their drinking water at a concentration of 0.75 percent (v/v), oxalate is immediately and considerably excreted in the urine. Thirty inbred Wistar rats (weighing between 180 and 200g each) were utilized. There were five groups created for this 28-day research. The first group (the controls) received merely water. Group 2 was the uroolithiatic-induced group and received Ethylene glycol (EG) in drinking water at a concentration of 0.75 percent (v/v). Ethylene glycol (EG) drinking water (0.75% v/v) and 50 mg/kg body weight (i.p.) were administered to Group 3. Test-1 consisted of an aqueous Bauhinia variegate leaf extract. Group 4 received 100 mg/kg of body weight i.p. and ethylene glycol (EG) in water at a concentration of 0.75 v/v. Test-2 consisted of an aqueous Bauhinia variegate leaf extract. Group 5 received the gold-standard treatment, 5 ml/kg body weight p.o. of Cystone (Himalaya health care Pvt. Ltd) functioned as a benchmark sample for comparison. Biochemical and histological analysis indicated that Bauhinia variegate leaf extract prevented oxidative stress and harm to renal cells brought on by calcium oxalate crystals.

Keywords: Kidney stones, calcium oxalate, and the Bauhinia variegata

Introduction:

Urolithiasis, characterized by the formation of urinary stones primarily composed of calcium oxalate, is a prevalent urological disorder. Ethylene glycol (EG) is a commonly used agent to induce urolithiasis in animal models. This study investigates the potential protective effects of an aqueous Bauhinia variegate leaf extract against EG-induced urolithiasis in Wistar rats. Thirty rats were divided into five groups, including control, urolithiatic-induced, treatment with Bauhinia variegate leaf extract (Test-1 and Test-2), and a positive control group treated with Cystone. Biochemical and histological analyses were performed to assess the impact of the extract on oxidative stress and renal tissue damage induced by calcium oxalate crystals. The results suggest that Bauhinia variegate leaf extract may have protective properties against urolithiasis.

Urolithiasis is a common urinary disorder characterized by the formation of solid crystalline deposits, known as urinary stones, in the kidneys, bladder, or urethra. These stones predominantly consist of calcium oxalate and are associated with excruciating pain, urinary tract obstruction, and various complications. The prevalence of urolithiasis has been steadily increasing globally, posing a significant burden on healthcare systems.

Bauhinia variegata, a medicinal plant commonly found in tropical and subtropical regions, has a long history of traditional use for the treatment of urinary disorders. The plant is known for its diverse pharmacological properties, including anti-inflammatory, antioxidant, and diuretic effects. This study aims to evaluate the Antiurolithiatic potential of the aqueous leaves extract of Bauhinia variegata in an experimental model of ethylene glycol-induced urolithiasis in Wistar rats. Although kidney stones have been documented ever since human civilization began, current estimates suggest that only around 5–10% of American women and 10–15% of American men will get one over their lifetimes. If stones from the whole urinary system were included, these numbers would be significantly higher. Many people are impacted during their prime working years, which means that in addition to the pain and suffering of an acute stone incident, the treatment incurs large expenditures, and extra costs occur from time lost from work.

The current resurgence of interest in plant treatments can be attributed to a number of causes, including the efficacy of plant medicines and the lower risk of adverse effects compared to contemporary pharmaceuticals. Over the centuries, many different treatments have been tried for urinary stones. Most effective treatments used in old medical systems were derived from plants. Except for a select few herbal medicine and plant composites, the rationale for their usage is not well established by comprehensive pharmacological and clinical investigations.

Urolithiasis is a major public health issue. In addition to being out of reach for most people, the costs associated with more modern methods of treating renal stones, such as surgical removal,

percutaneous techniques, and extracorporeal shock wave lithotripsy (ESWL), often result in the need for a patient to undergo careful follow-up over the course of several years. However herbal therapies have been demonstrated to be useful in renal stone illness. In comparison to conventional pharmaceuticals, herbal remedies are more effective and have less adverse side effects.

Materials and Method:

The process of extraction

500 grams of powdered Bauhinia variegata leaves were combined with distilled water in a soxhlet device and subjected to a 35-40^{0}C aqueous extraction. After being dissolved in water, the material was filtered through a Buchner funnel and then dried in an oven. The yield % was calculated afterwards[30].

Antiurolithiatic Activity

Bauhinia variegata leaf aqueous extracts were prepared in two dosages, 50 mg/kg and 100 mg/kg p.o. a solution of your body weight in distilled water. Protocols for Treatment: After 28 days of treatment:

The first group (the controls) received merely water. Group 2 was the nephrolithiasis-inducing group, and they were administered Ethylene glycol (EG) in drinking water at a concentration of 0.75 percent (v/v).

Ethylene glycol (EG) drinking water (0.75% v/v) and 50 mg/kg body weight (i.p.) were administered to Group 3. as Test-1 (50 mg/kg p.o.) was a group given an aqueous extract of Bauhinia variegata leaves.

Group 4 received 100 mg/kg of body weight i.p. and ethylene glycol (EG) in water at a concentration of 0.75 v/v. of Bauhinia variegata leaf extract (100 mg/kg p.o.) and used as Test-2 group.

Cystone (Himalaya health care Pvt. Ltd), a conventional medicine, was administered orally at a dose of 5 ml/kg in Group 5. Functioned as a benchmark sample for comparison.

Results:

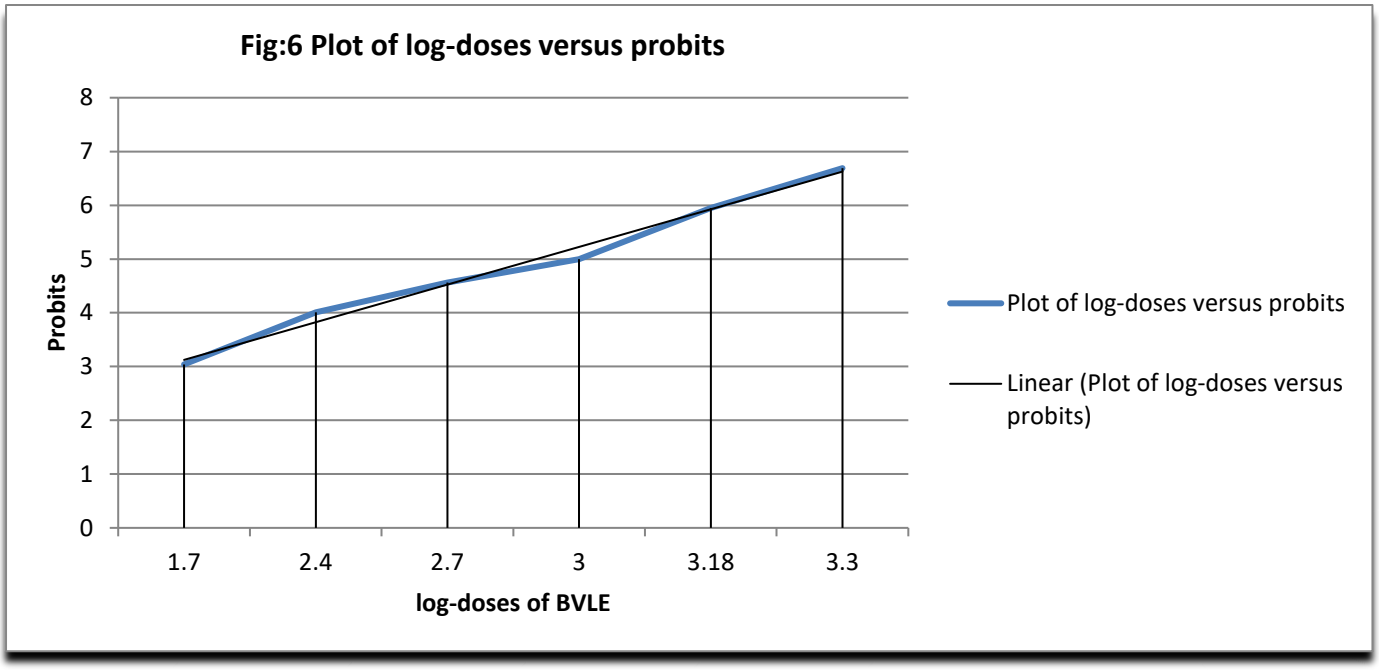
Testing for Acute Toxicity

Table 13: Lethal Doses of BVLE (LD50) After Oral Administration in Rats

Group No.	Dose (mg/kg) of BVLE	Log Dose of BVLE	No. of Deaths	% Deaths	Corrected %	Probits
1	50	1.7	0/6	0	4.16	3.04
2	250	2.4	1/6	16.66	16.66	4.01
3	500	2.7	2/6	33.33	33.33	4.56
4	1000	3.0	3/6	66.66	66.66	5.00
5	1500	3.18	5/6	83.33	83.33	5.95
6	2000	3.3	6/6	100	95.83	6.69

*Corrected % Formula: For 0 and 100 % deaths,

For 0% dead: 100(0.25/n), for 100% dead: 100(n-0.25/n)



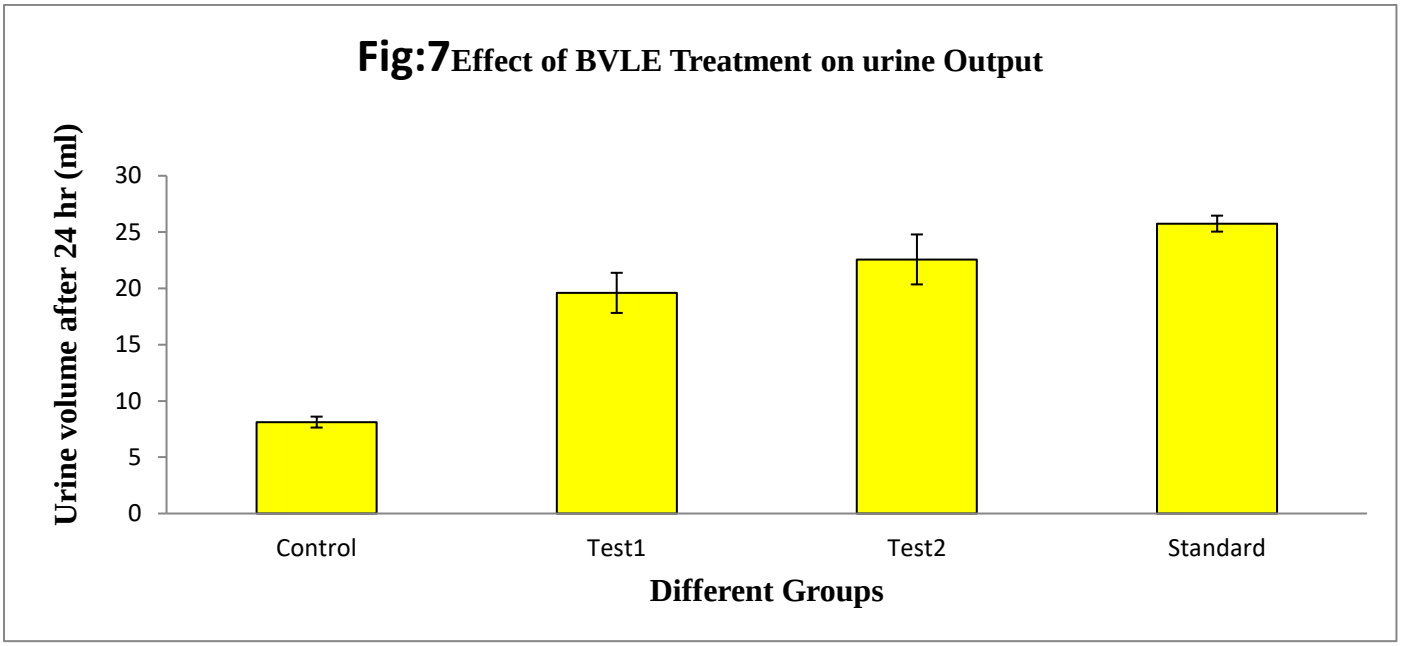
Urine volume

Urine Output

Groups	Doses (Per Kg Body weight)	Urine Vol. after 5 hr/100 g body weight	Urine Vol. after 24 hr/100 g body wt	pH
Control (normal saline)	10 ml	1.5 ± 0.29	8.12 ± 0.486	7.42
Reference (urea)	960 mg	2.95 ± 0.87*	25.75 ± 1.782**	7.40*
Bauhinia variegate Extract 1	50 mg	1.45 ± 0.66	19.60 ± 2.219**	7.14*
Bauhinia variegate Extract 2	100 mg	1.32 ± 0.23	22.57 ± 0.709**	7.23**

Table 15: Lipschitz Values for Bauhinia variegate Leaves Extract

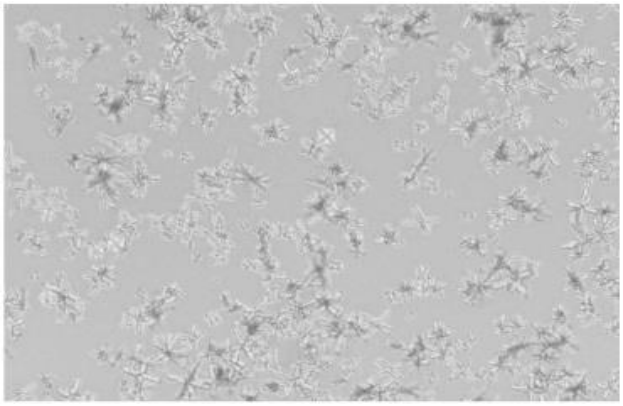
Treatment	T/U at 5 hr	T/U at 24 hr
Bauhinia variegate Leaves Extract (50 mg/kg)	0.491	0.761
Bauhinia variegate Leaves Extract (100 mg/kg)	0.447	0.876



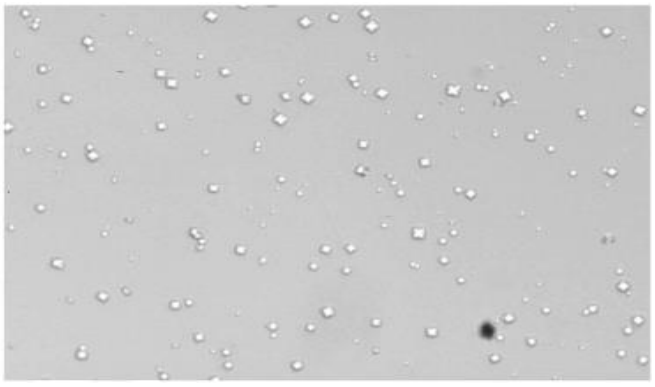
Microscopic Urinalysis:

Fig 11: Presence of Calcium oxalate crystals in the urine of different treatment groups seen under microscope (Leica DME) at 40x10X magnification

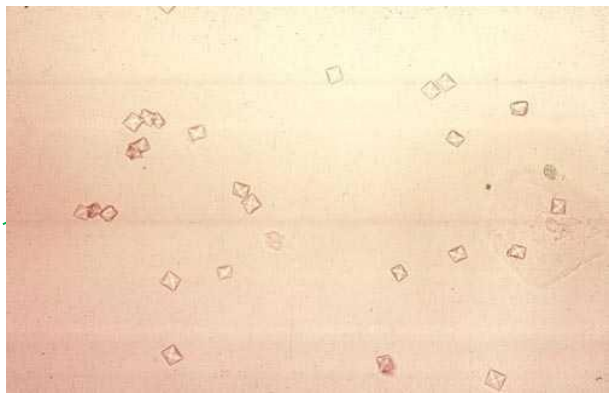
1. Normal Control



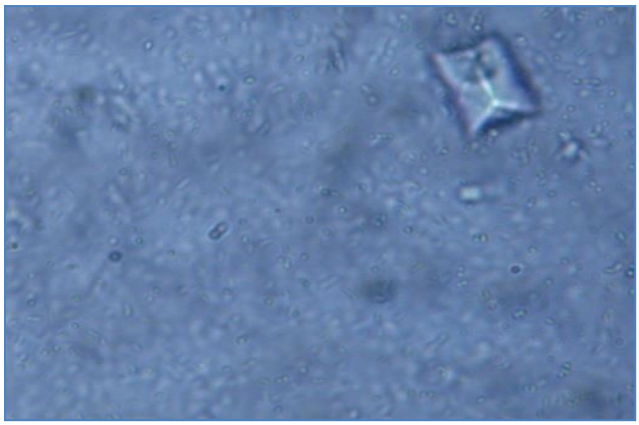
2. EG control



3. BVLE 50 mg/kg Treatment



4. BVLE 100 mg/kg Treatment



5. Standard

Serum Analysis:

Table 19: Effect of Treatments on Serum Parameters

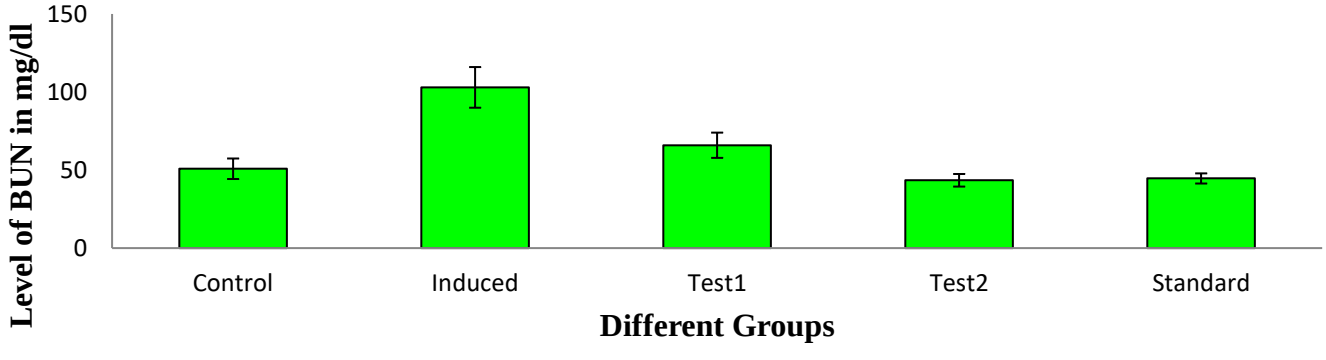
Observational Parameter	Group 1 (Control)	Group 2 (Induced)	Group 3 (Test 1)	Group 4 (Test 2)	Group 5 (Standard)
Blood Urea Nitrogen (BUN)	50.831 ± 6.575	102.952 ± 13.028 b	65.881 ± 8.094d	43.431 ± 4.034c,d	44.621 ± 3.245 d
Creatinine (mg/dl)	0.713 ± 0.047	0.952 ± 0.027 b	0.603 ± 0.031c	0.507 ± 0.083 c,d	0.571 ± 0.055 d
Phosphate (mg/dl)	25.245 ± 0.368	39.015 ± 1.025 c	31.133 ± 0.443 c	29.699 ± 0.236c	29.494 ± 0.469 b
Oxalate (mg/dl)	0.337 ± 0.022	1.957 ± 0.067 b	1.202 ± 0.039 d	0.750 ± 0.030 b, d	0.720 ± 0.025 b, d
Calcium (mg/dl)	1.738 ± 0.045	3.493 ± 0.177 b	2.768 ± 0.088 d	2.122 ± 0.116 b, d	2.115 ± 0.147 b, d

All values are expressed as mean ± S.E.M. for six animals in each group.

a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;

c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Fig 14: Effect of Treatments on Blood Urea Nitrogen Level in Serum



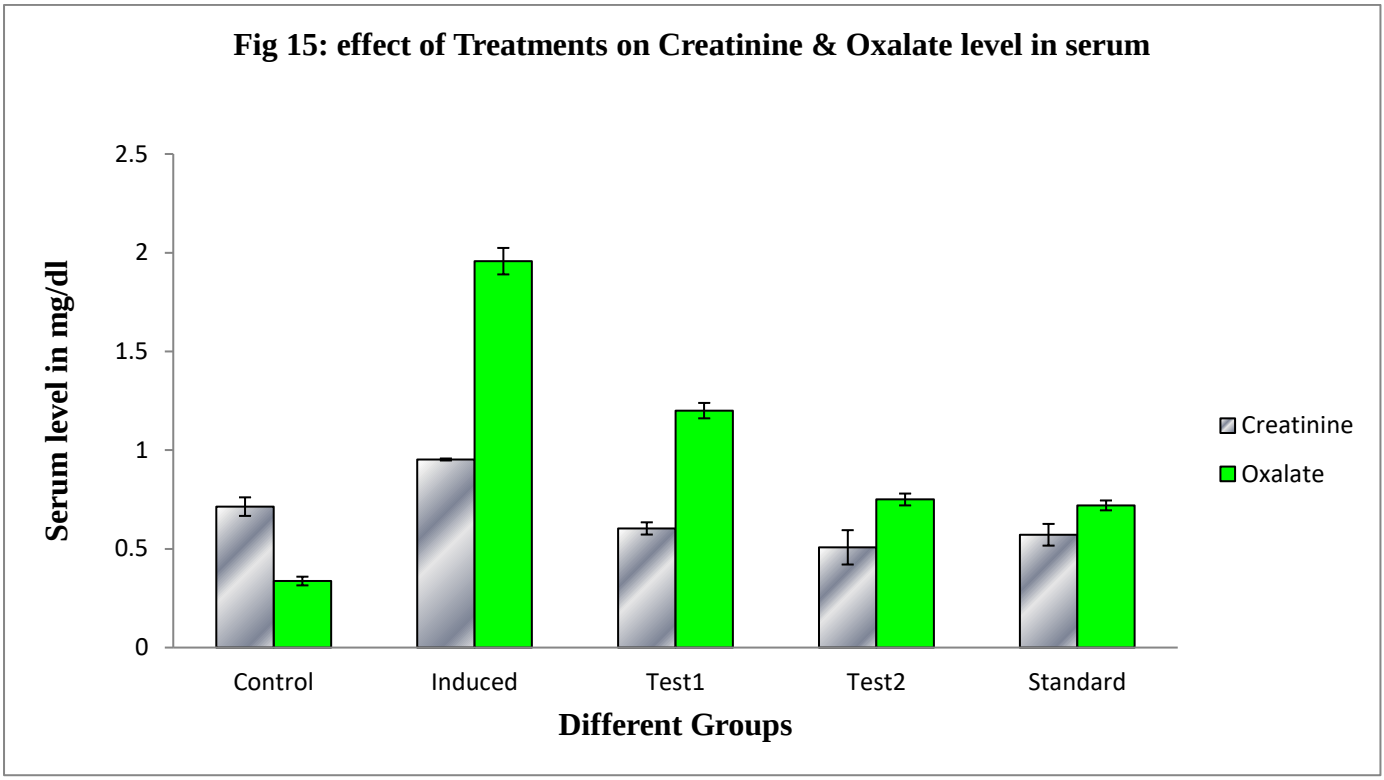
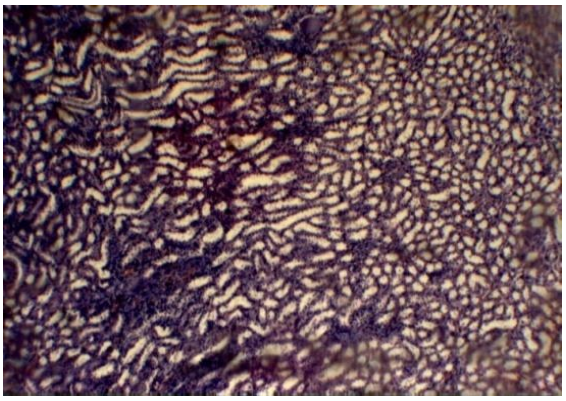


Table 23: Histopathological Features of Kidneys in Different Treatment Groups

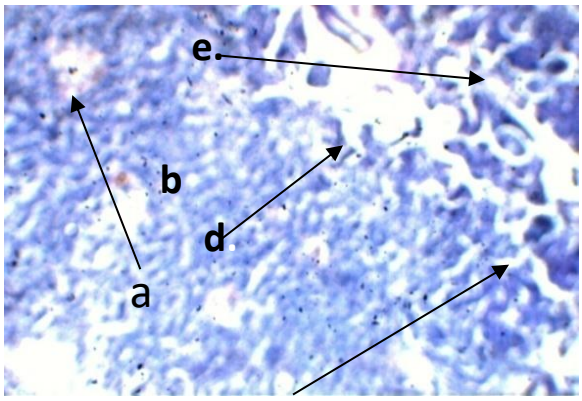
Histopathological Feature	Group I (Normal Control)	Group II (EG treated)	Group III (EG treated and BVLE treated at 50mg/kg p.o.)	Group IV (EG treated and BVLE treated at 100mg/kg p.o.)	Group V (EG & Std)
Glomerular Congestion	_	++	+	_	_
Peritubular Congestion	_	++	+	+	+
Epithelial Desquamations	_	+	_	_	_
Blood Vessel Congestion	_	++	+	_	_
Interstitial Edema	_	+	_	_	_
Inflammatory Cells	_	++	+	_	_
Tubular Casts	_	+	_	_	_

Note: "+" indicates the degree of presence, "_" indicates absence,

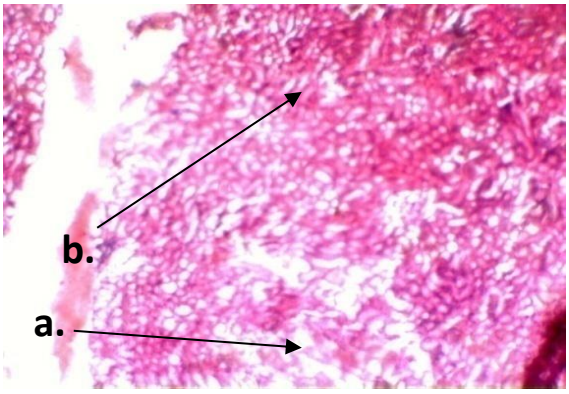
Fig 19: Histopathological examination of Kidney tissue of different groups



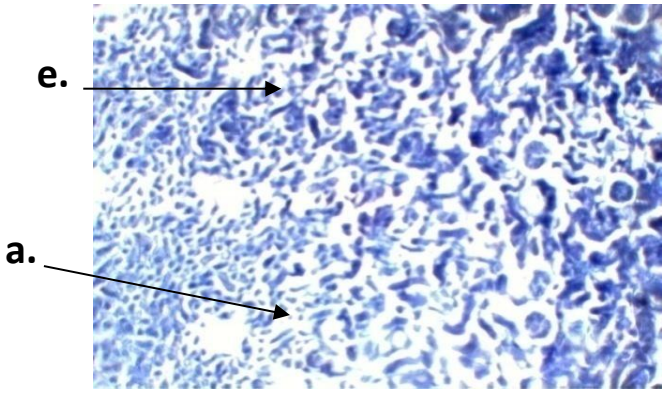
1. Normal



2. Induced



3. Test1



4. Test2

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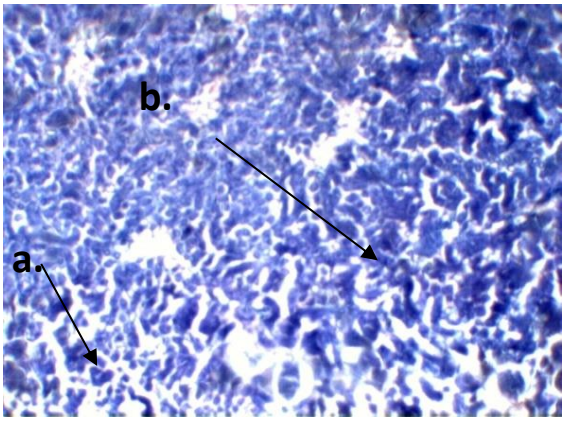

5. Standard. Blood vessel congestion

a. Congestion of Glomerular and peritubular

b. Inflammatory cell infiltration

c. Epithelial desquamation

d. Necrosis

Discussions:

Diuretic property is the preliminary need of the Antiurolithiatic action. According to the reviewed literature, Bauhinia variegate leaves extract (BVLE) has a diuretic effect that increases urine output according to dosage (50-100 mg/kg body weight, p.o.). Increases in water and sodium and potassium excretion point to the diuretic activity of Bauhinia variegate leaves extract. Preliminary phytochemical examination of the extract indicated the presence of polar chemicals including flavonoids and steroids, which may be responsible

for the plant's diuretic properties, but this has not yet been confirmed. Loss of fluid might account for the rats' dramatic weight loss; in the treated groups, urine volume rose. Changes in urine chemistry, such as hypercalciuria and hyperoxaluria, cause urinary supersaturation, which then causes crystallization, aggregation, and, ultimately, stone formation in the urinary tract. Ethylene glycol-induced renal CaOx deposition in rats is commonly used to model the development of urinary stones in people. So, using this model, we tested the curative potential of Bauhinia variegate leaves extract for CaOx urolithiasis. After 28 days of treatment with CaOx stone inducing chemicals, the animals were found to have crystalluria, and the induced animals were found to have excreted more and bigger crystals. Although people who produce stones are more likely to excrete bigger, more aggregated particles, crystalluria might happen to both groups. Renal stones form when clumps of CaOx crystals are trapped in the kidney's tubules. Extract of Bauhinia variegate leaves considerably mitigated crystalluria caused by lithogenic therapy. Compared to both the control and induced groups, BVLE considerably increased the amount of urine produced by the animals. It is possible that the diuretic activity of BVLE is responsible for the increased urine volume seen in the induced groups as a result of the lithogenic therapy. Increased oxalate excretion following stone production by hyperoxaluria was inhibited by BVLE in a dose-dependent manner.

Treatment with the aqueous leaves extract of Bauhinia variegate protected the rats from the effects of ethylene glycol, while treatment with ethylene glycol alone resulted in significantly increased (p0.01) urine creatinine, serum creatinine, blood urea, blood urea nitrogen, and kidney weights compared to normal rats.

A dosage related decrease in the size of the stones was found with 50 and 100 mg/kg body weight p.o. doses. Bauhinia variegate leaves extract at a dosage of 100 mg/kg body weight p.o. significantly reduced stone deposition compared to the induced control group. When compared to the induced group II, stone deposition was reduced by 65% in animals given an aqueous extract of Bauhinia variegate's leaves at a dosage of 100 mg/kg body weight p.o. As stones formed on the foreign body in this investigation, the urine pH of the rats rose from its initial 6.5-7.0 to 7.0-8.0. The extract brought the pH levels down to a more manageable range of 6.5-7.2.

The formation of kidney stones is associated with tissue damage and compromised renal function. Raised BUN and serum creatinine and decreased creatinine clearance are markers of glomerular and tubular damage that were observed in untreated rats after lithogenic treatment; these changes were prevented in a dose-dependent manner in animals receiving a simultaneous treatment with BVLE.

Histopathological analysis revealed damage to the glomeruli and tubules, indicative of oxidative stress, in the kidneys of untreated rats after exposure to a therapy designed to induce stones. In addition, it worsened renal functioning and raised indicators of oxidative stress, including malondialdehyde (MDA) and protein content. All

of the induced rats' kidneys showed significant dilation, which may have been due to distal blockage of renal tubular flow by massive crystals. Antiurolithic effects and antioxidant capacity in vivo were confirmed in groups treated with an extract of Bauhinia variegate leaves, which considerably reduced all these consequences of lithogenic therapy.

Histological Examination

To gain insight into the effects of Bauhinia variegate leaf extract on renal tissue, kidney samples were preserved in neutral buffered formalin (10% formaldehyde in Phosphate buffered saline) for histopathological analysis. The tissue sections were stained and meticulously examined for crystal deposition, inflammatory reactions, and tissue damage. This histological examination provided valuable information about the protective effects of the extract on the kidneys. The histological examination of kidney tissues provided further evidence of the potential protective effects of Bauhinia variegate leaf extract. Kidney sections from Group 2 (Urolithiatic-Induced) exhibited extensive crystal deposition within the renal tubules, interstitial inflammation, and tubular damage. These histological findings were consistent with EG-induced urolithiasis, demonstrating the development of crystal-related renal pathology.

In contrast, kidney tissues from Group 3, Group 4, and Group 5 displayed reduced crystal deposition, milder inflammation, and preserved renal architecture. These histological observations strongly suggest that treatment with Bauhinia variegate leaf extract (Test-1 and Test-2) and Cystone effectively protected against EG-induced renal tissue damage.

Conclusion:

According to the results of the current study, Bauhinia variegata seed extract has an Antiurolithiatic action against calcium oxalate stones. CaOx crystal development is inhibited, and the kidney is protected from oxidative stress and renal cell damage, probably by a combination of diuretic and antioxidant activities. The research supports its potential as a treatment for urinary stone illness. This lends credence to the plant's use in traditional medicine for the treatment of urolithiasis. Histopathological tests corroborated the results, showing that the extract of Bauhinia variegata seeds reduced oxidative stress and damage to the kidneys caused by calcium oxalate crystals.

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