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ANTI UROLITHIATIC ACTIVITY OF AQUEOUS ALCOHOLIC EXTRACT OF *PHASEOLUS VULGARIS* LINN SEEDS IN *WISTAR RATS*

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ABSTRACT

Phaseolus vulgaris linn (Fabaceae) is commonly known as French beans also known as kidney beans, is a common Indian dish. The effect of oral administration of aqueous and alcoholic extract of *Phaseolus vulgaris* seeds on calcium oxalate urolithiasis has been studied in Wistar albino rats. On administration of ethylene glycol and ammonium chloride increase in the deposition of calcium and oxalate level in the kidney and increase urinary excretion of calcium, oxalate and phosphate were observed. Treated with aqueous and alcoholic *Phaseolus vulgaris* linn seeds extract significantly reduced the elevated urinary calcium and oxalate levels. The increased deposition of stone forming constituents in the kidneys of rats was also significantly lowered by being treated with aqueous and alcoholic extracts. The typical medication used was cystone 750mg/kg. Urine, serum, and kidney histopathology analyses were used to gauge the anti-lithiatic activity. According to the findings, *Phaseolus vulgaris* linn seeds possess antiurolithiatic action.

Keywords: *Phaseolus vulgaris*, Urolithiasis, Ethylene glycol, Ammonium chloride, Cystone

INTRODUCTION:

In Indian cuisine, kidney beans, or *Phaseolus vulgaris*, are frequently used. Due to its abundance of phytochemicals, including proteins, amino acids, complex carbohydrates, dietary fibre, oligosaccharides, phenols, saponins, flavonoids, alkaloids, and tannins with potential health benefits, the seed of *Phaseolus vulgaris* is gaining more and more attention as a functional or nutraceutical food. A common therapy for water retention in pregnant women, the seeds were said to have diuretic effect. The results of studies suggest that *Phaseolus vulgaris* seeds have properties that improve the bifidogenic, antioxidant, anti-mutagenic, anti-carcinogenic, and anti-hyperglycemic effects **[1]**.

Beans are grown all throughout the world, and numerous cultivars and varieties of beans have been created. America is the plant's native continent. *Phaseolus vulgaris* Linn, an easily accessible plant in India, has been investigated for its potential to possess analgesic and anti-inflammatory, antioxidant, anti-obesity, trypsin and -amylase inhibitory, hyperglycaemic, low nitrate stress, antitubular, atypical anti-psychotic, and anti-fungal activity **[2, 12]**.

Kidney stones are solid deposits of calcium-containing minerals that can form in the kidneys (nephrolithiasis) or the urinary tract (urolithiasis) **[13]**. The care of urolithiasis requires a combined surgical and medicinal approach involving percutaneous nephron-lithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL), and antibiotics, in addition to lots of fluid intake and urine retention. Surgery is a somewhat expensive, uncomfortable procedure that needs a skilled surgeon and the right tools. The hunt for natural materials with anti-urolithiatic activity has been sparked as a result **[14]**. For centuries, several treatments have been used to treat renal stones. With the exception of a few plants and certain proprietary composite herbal medications, most cures were derived from plants and proved to be beneficial, however the science behind their usage is not well established. Hence the search for anti-urolithiatic drugs from natural sources has assumed greater importance **[15]**.

The present study is aimed at evaluating the anti-urolithiatic activity of the ethanolic extract and aqueous extracts of the seeds of *Phaseolus vulgaris* was investigated in *Wistar rats* against Calcium oxalate urolithiasis induced by ethylene glycol (EG) and ammonium chloride (AC) in drinking water.

2. MATERIALS AND METHODS:

2.1 Materials and tools

The tests' chemicals and biochemical testing kits were purchased from SD Fine Chemicals in India. High-performance liquid chromatography (HPLC) analytical-grade chemicals and solvents were used. A 0.25-m filter membrane was employed to filter and double distil the water used in the analytical processes.

2.2 Collection and identification of plants:

Farmers in Ooty, Tamil Nadu, India provided the *Phaseolus vulgaris* Linn seeds for collection. Dr. Stephen Ph.D., Professor, Department of Botany, American College, Madurai, verified their authenticity.

2.3. Preparation of extracts of *Phaseolus vulgaris linn:*

a. Aqueous extract

Phaseolus vulgaris Linn seeds were cold macerated in water for 15 days to obtain a 300 gramme coarse powder. Surface evaporation was used to concentrate the extract, and Hoover drying came next. For additional phytochemical and pharmacological investigations, the dry powder was weighed and kept in airtight containers.

(b) Ethanolic extract

Phaseolus vulgaris Linn seeds were ground into a coarse powder and extracted with 250 ml of ethanol using the hot percolation method and a Soxhlet apparatus. The extraction took 72 hours to complete. To get a concentrated extract, the solvent was removed after extraction. For additional phytochemical and pharmacological research, the concentrated extract was vacuum dried and the dry extract was stored in an airtight container.

2.4 Experimental Animals:

In this study, either sexed *Wistar rats* weighed 150–200g and were housed in an animal facility. The chosen animals were kept together in polypropylene cages at a temperature of 23 20 with a 12-hour cycle of darkness and light. The animal was given unlimited access to food and water. One week before testing, all animals were housed in regular, sanitary laboratory settings. The Institutional Animal Ethical Committee's (IAEC) proper approval was obtained before the experimental protocols were carried out on animals.

2.5 Induction of lithiasis:

Lithiasis was induced in rats by administering 0.75% v/v of Ethelene Glycol and 1 % w/v of Ammonium Chloride in drinking water for 14 days. Ethylene glycol and ammonium chloride induce Calcium oxalate crystalluria without severe renal damage in rats and they mimic the etiology of stone formation in humans **[16]**.

2.6 Acute Toxicity Studies:

Oral acute toxicity studies were carried out in accordance with the OECD guidelines 423 **[17]**.

2.7 Experimental Design:

Rats were divided into seven groups of three animals each (n = 3). Cystone (750 mg/kg) was used as a standard drug, Bothaqueous and alcoholic extract of *Phaseolus vulgaris* was administered at doses of 200 and 400 mg/kg. All groups were maintained on commercial pellet diet for 28 days.

The treatment schedule was planned as follows:

Group I: Normal (untreated)

Group II: control (EG + AC from day 1 to 14, Vehicle from day 15 to 28)

Group III: Standard (EG + AC from day 1 to 14, Cystone 750 mg/kg, orally from day 15 to 28)

Group IV: Low dose (EG + ACfrom day 1 to 14, AEPV 200 mg/kg, orally from day 15 to 28)

Group V: High dose (EG + AC from day 1 to 14, AEPV 400 mg/kg, orally from day 15 to 28)

Group VI: Low dose (EG +AC from day 1 to 14, EEPV 200 mg/kg, orally from day15 to 28)

Group VII: High dose (EG +AC from day 1 to 14, EEPV 400 mg/kg, orally from day15 to 28)

ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY: [18, 19]

a. Urine collection and analysis:

The animals were kept apart in metabolic cages for 24 hours at the end of the 28th day in order to collect their urine. Water was available to animals at all times when pee was being collected. Urine volume, creatinine, calcium, magnesium, oxalate, and phosphate were assessed in the urine samples that were collected. Using the measuring cylinder, the amount of urine was measured and expressed in milliliters. The colorimetric analysis is used to determine calcium and magnesium. Using the Hodgkinson and Williams technique, oxalate was determined. Using the Fiske and Subbarow technique, phosphorus.

b. Serum Analysis:

The retro-orbital venous plexus was used to collect blood samples, which were then centrifuged at 1500 rpm for 15 minutes to separate the serum. The serum was then used to estimate uric acid, creatinine, and blood urea nitrogen (BUN) using ERBA diagnostic kits in accordance with the manufacturer's instructions. The estimations were made using a fully automated auto analyzer (Erba EM-200, Transasia Biomedicals Ltd, Mumbai).

c. Histopathological examination:

Animals were immediately slaughtered by cervical dislocation while being given ether

anaesthesia following blood sample. Prior to this experiment's end point, no animals perished. Each rat's two kidneys were promptly removed, removed any extra blood, and cleaned with phosphate buffered saline (PBS). All groups' kidneys underwent at least a 72-hour fixation in 10% neutral buffered formalin before being cleaned, dehydrated, and paraffin-embedded. Hematoxylin and Eosin (H&E) staining was applied to sections that were 5 m thick.

d. Statistical Analysis:

Graph Pad 7.0 software was used to assess data for all the parameters. Analysis of Variance (ANOVA); a single ANOVA was conducted, followed by a Dunnett's t-test. Mean SEM was used to express the values. P value 0.05 and p value 0.01 were regarded as significant.

RESULTS:

Effect of AEPV and EEPV on Urine Volume and Urine biochemical parameters

1. Urinary volume in Lithiatic group is decreased, positive control group is increased. Urinary volume in AEPV in high dose is almost equal to the low dose.
2. Calcium, Oxalate, Phosphate level in Lithiatic group is increased, positive control and the extracts of AEPV in low dose and high dose may be decreased.
3. Magnesium level in Lithiatic group is decreased, positive control and the extracts of AEPV in low dose and high may be increased.

Effect of AEPV/EEPV on Serum biochemical parameters

Serum Creatinine, uric acid and blood urea nitrogen level (BUN) in Lithiatic control is increased, whereas in AEPV/EEPV group is decreased when compare to lithiatic control group. Positive control decreases when compare to normal control group **(Figure 1, Table 2)**.

Table 1: Effect of AEPV on Urine Volume and Urine biochemical parameters on 28th day

Group	Urine analysis				
	Urinary volume (ml/24h)	Calcium mg/dl	Oxalate mg/dl	Phosphate mg/dl	Magnesium mg/dl
Normal	5.95±0.13	4.14±0.3796	0.34±0.02	5.85±0.42	0.94±0.01
Lithiatic control (Untreated)	3.61±0.14	7.26±0.515	2.47±0.11	8.57±0.419	0.69±0.012
Positive control (Cystone treated)	5.08±0.1***	7.24±0.53	2.53±0.1	8.56±0.52	0.71±0.01
AEPV Low dose (200mg/kg)	4.16±0.17***	7.08±0.38	2.4±0.09	8.51±0.57	0.68±0.01
AEPV High dose (400mg/kg)	4.91±0.13***	5.07±0.43*	0.8±0.04 *	6.29±0.61*	1.09±0.01*

Values are expressed as Mean ± SEM. *, **, *, ^{ns} - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively.**

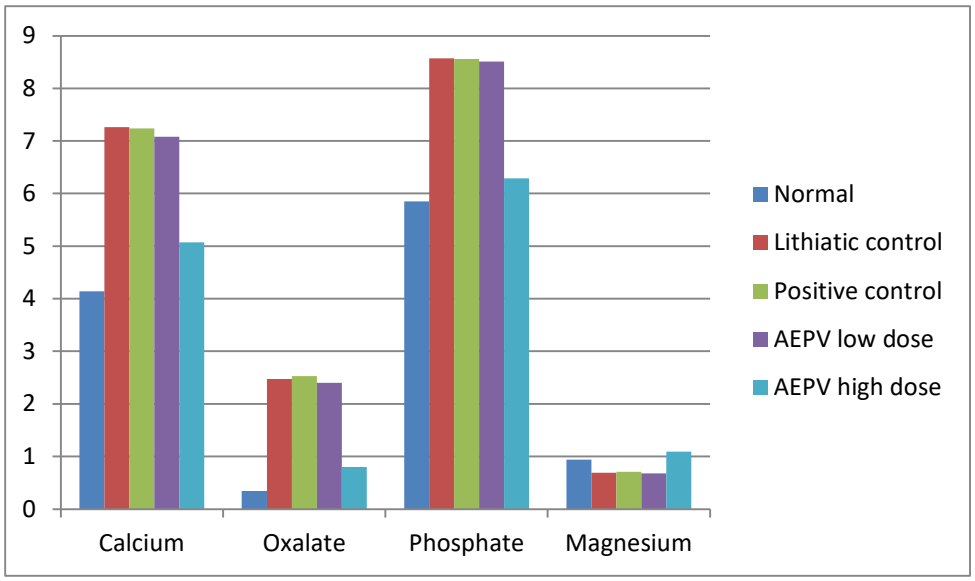


Figure 1: Effect of AEPV on Urine biochemical parameters on 28th day

Table 2: Effect of EEPV on Urine Volume and Urine biochemical parameters on 28th day

Group	Urine analysis				
	Urinary volume (ml/24h)	Calcium mg/dl	Oxalate mg/dl	Phosphate mg/dl	Magnesium mg/dl
Normal	7.89±0.12	4.40±0.59	0.34±0.013	5.83±0.39	0.96±0.59
Lithiatic control (Untreated)	4.82±0.17	7.98±0.64	3.07±0.02	8.85±0.61	0.51±0.64
Positive control (Cystone treated)	6.8±0.04	6.55±0.32**	1.58±0.04**	6.9±0.59**	1.31±0.32*
EEPV Low dose (200mg/kg)	7.23±0.11	6.15±0.51**	1.78±0.03**	7.83±0.56**	1.29±0.51*
EEPV High dose (400mg/kg)	7.21±0.20	6.13±0.46**	1.68±0.03*	7.31±0.217**	1.54±0.46*

Values are expressed as Mean ± SEM. *, **, *, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and nonsignificant respectively.**

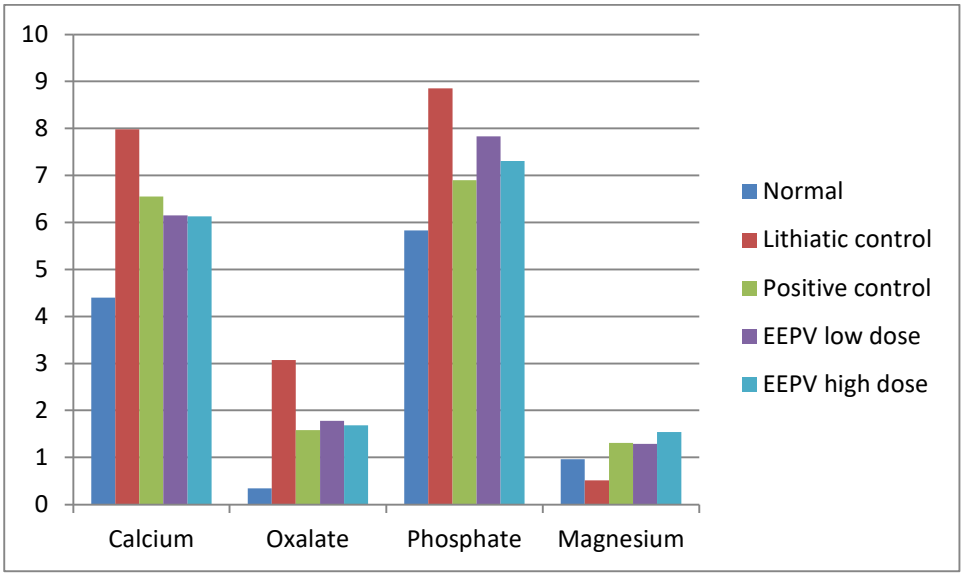


Figure 2: Effect of EEPV on Urine biochemical parameters on 28th day

Table 3: Effect of AEPV on Serum biochemical parameters on 28th day

Group	Serum analysis (mg/dl)		
	Creatinine	Uric acid	Blood Urea Nitrogen (BUN)
Normal	0.54±0.02	2.49±0.19	40.13±0.71
Lithiatic control (Untreated)	0.99±0.06	4.11±0.25	61.60±0.66
Positive control (Cystone treated)	0.39±0.002*	1.80±0.13*	37.41±0.78*
AEPV Low dose (200mg/kg)	0.75±0.05**	2.08±0.026*	42.70±0.94*
AEPV High dose (400mg/kg)	0.73±0.08**	1.04±0.16*	39.10±0.98*

Values are expressed as Mean ± SEM. *, **, *, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively.**

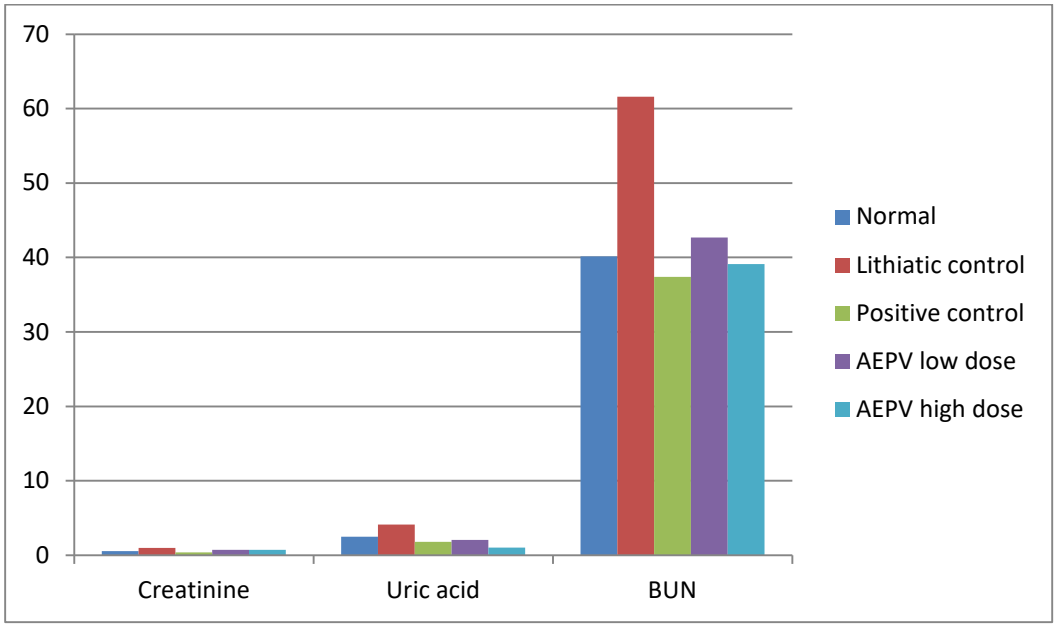


Figure 3: Effect of AEPV on Serum biochemical parameters on 28th day

Table 4: Effect of EEPV on Serum biochemical parameters on 28th day

Group	Serum analysis (mg/dl)		
	Creatinine	Uric acid	Blood Urea Nitrogen (BUN)
Normal	1.54±0.15	1.18±0.08	37.62±0.15
Lithiatic control (Untreated)	2.31±0.05	2.56±0.03	41.20±1.45
Positive control (Cystone treated)	1.82±0.05*	1.49±0.05*	26.70±1.48*
EEPV Low dose (200mg/kg)	1.85±0.04*	1.65±0.08*	35.21±1.22*
EEPV High dose (400mg/kg)	2.05±0.07*	2.10±0.16*	32.20±1.41*

Values are expressed as Mean ± SEM. *, **, *, ns - Mean values are significantly different when compared with lithiatic control mean values at P <0.05, P<0.01, P<0.001 and non-significant respectively.**

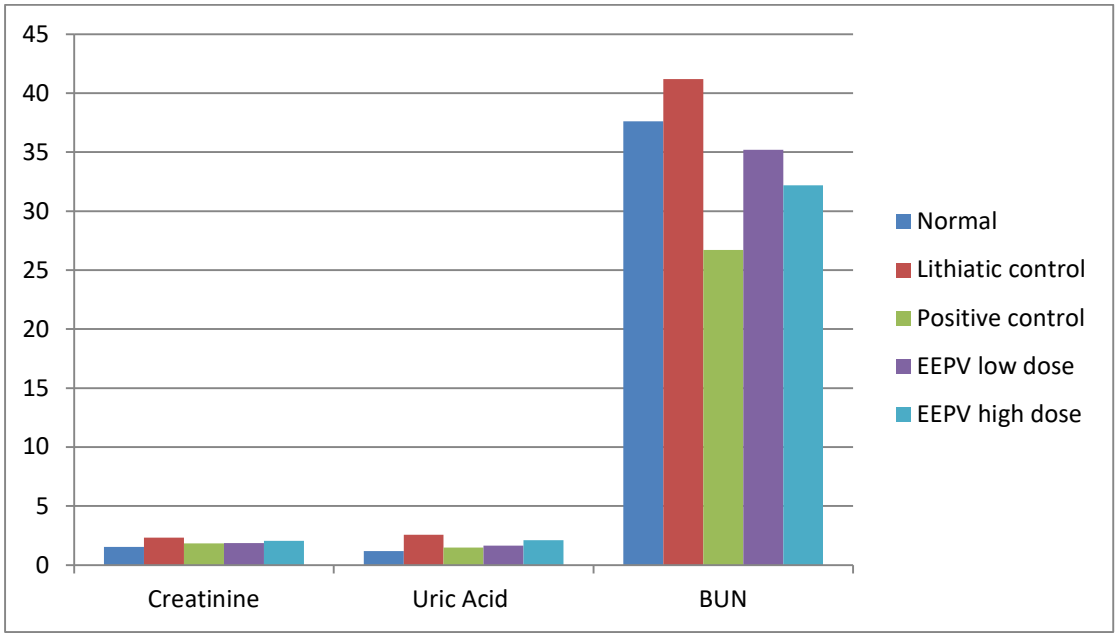


Figure 4: Effect of EEPV on Serum biochemical parameters on 28th day

Histopathological examination:

Histopathological findings of kidney under a light microscope (×40)

A. Normal: Renal parenchyma with normal tubules, and glomeruli

B. Lithiatic control: Oxalate renal stone, tubular dilation and renal tubular damage, severe damage to the medulla, glomeruli, tubules.

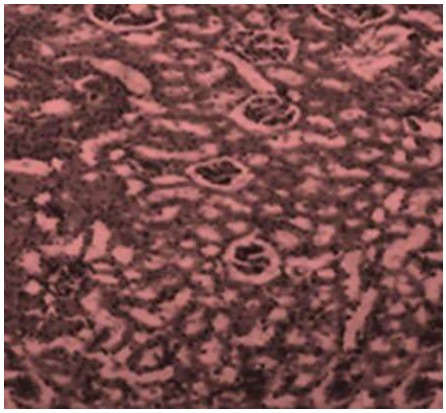
C.Positive Control: Mild vascular degeneration of renal tubular epithelial cells

D. Low dose (AEPV 200 mg/kg): Presence of CaOx crystals in the lumen of dilated renal tubules.

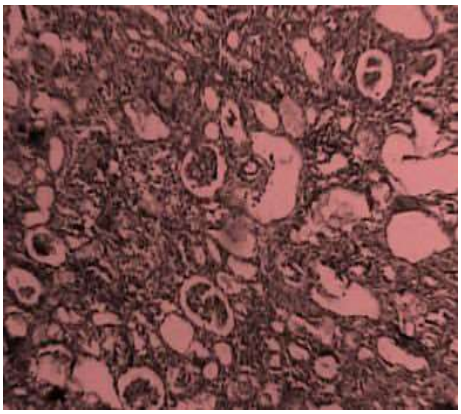
E. High dose (AEPV 400 mg/kg): Mild vacuolar degeneration of renal tubular epithelial cells

F. Low dose (EEPV 200 mg/kg): Renal tubular dilation and glomerular atrophy.

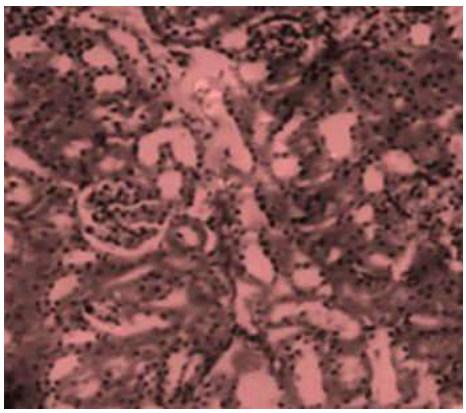
G. High dose (EEPV 400 mg/kg): Regenerated to normal glomerular structure.



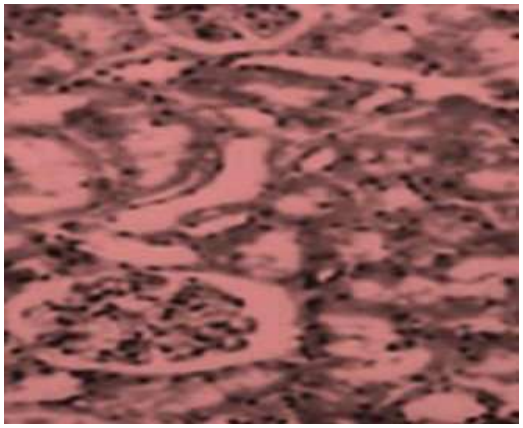
A. NORMAL



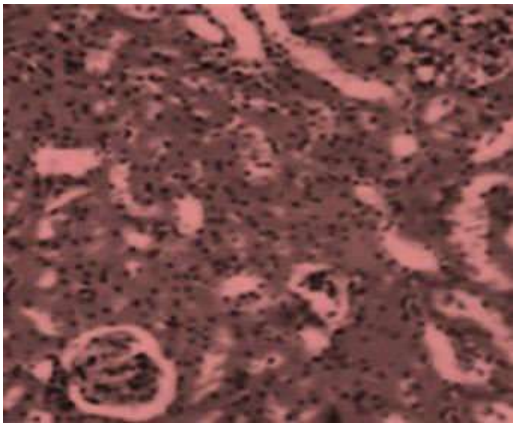
B. LITHIATIC CONTROL



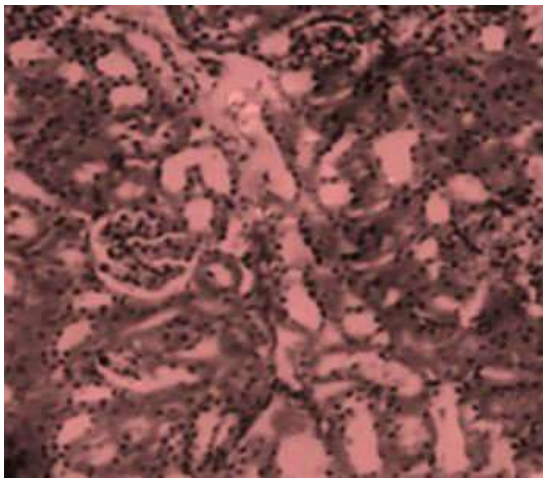
C. POSITIVE CONTROL



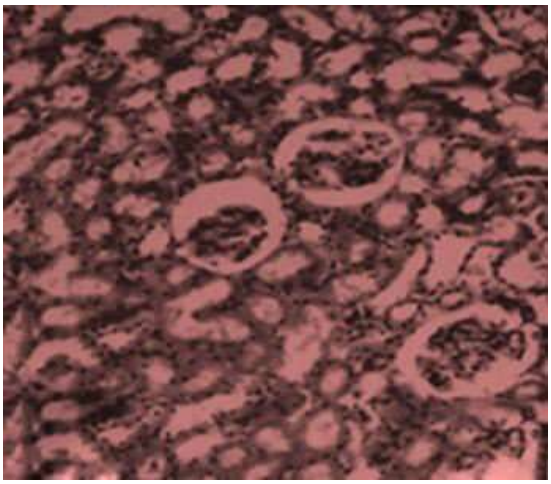
D. LOW DOSE (AEPV 200mg/kg)



E. HIGH DOSE (AEPV 400mg/kg)



F. LOW DOSE (EEPV 200mg/kg)



G. HIGH DOSE (EEPV 400mg/kg)

DISCUSSION:

The following conclusions can be drawn from the findings of the current study on the assessment of anti-urolithiatic activity of aqueous and ethanolic extracts in rat models of lithiasis. Significant decreases in the levels of calcium, oxalate, and phosphate in urine were seen with AEPV and EEPV, as well as an increase in urinary magnesium and a return to normal urine volume. The extracts also decreased serum levels of creatinine, uric acid, and BUN to normal levels. Additionally, histopathological findings support this. These results suggest that the extracts may have the ability to prevent kidney stone formation and exert a lithotriptic effect on kidney stones that have already developed. In the rat models for urolithiasis, aqueous extract is more effective than ethanolic extract.

When compared to the control group (Lithiatic), animals given Cystone, AEPV, and EEPV demonstrated a significant (p0.05, p0.01) reduction in calcium and oxalate accumulation in the kidney. The amount of urine also significantly contributed to the development of calcium oxalate stones. In this study, a decrease in urine output was seen in the control group (Lithiatic), indicating that calcium oxalate stones were obstructing the urinary flow. On treatment with EEPV and AEPV, an increase in urine output was seen, demonstrating its diuretic activity. Additionally, it dilutes the concentration of urine electrolytes, which may lessen the likelihood of stone development **[20]**.

When compared to the norm, a rise in urine phosphate excretion was seen in the control (Lithiatic) groups. By producing calcium phosphate crystals, which lead to calcium oxalate deposition, elevated urine phosphate excretion and oxalate-induced stress appear to create an environment that is conducive to stone development. The probability of stone formation was decreased by treatment with both the standard (cystone) and the plant extract treated (EEPV and AEPV).

Following the reduction in urine volume and the concentration of chemicals that prevent stone formation, a process known as super saturation takes place when there are high concentrations of compounds that cause kidney stones in the urine. When ethylene glycol was administered in our investigation, the magnesium level in the urine significantly decreased in the urolithiatic group. When compared to the control group, animals treated with starndard (cystone) and plant extracts (EEPV and AEPV) restored the increase in urine magnesium level.

CONCLUSION

Due to the restriction of urine flow by urinary system stones in urolithiasis, there is a

decrease in glomerular filtration. This results in decreased excretion of waste products, especially nitrogenous ones such uric acid, creatinine, and BUN, as well as buildup in the blood. It also impairs renal function. When compared to the normal group, the control (Lithiatic) groups in the current study administration of ethylene glycol with ammonium chloride exhibited a significantly higher level of blood creatinine, uric acid, and BUN excretion, which highlights the kidney injury. However, treatment of rats with the plant extract therapy (AEPV and EEPV) and the conventional treatment (cystone) resulted in lower serum levels of creatinine, uric acid, and BUN due to enhanced glomerular filtration rate.

In tests using histopathology, the kidneys of healthy rats displayed normal renal glomeruli and tubules with no signs of tubular degeneration, dilatation, or inflammatory response. While this was going on, the Lithiatic control group showed typical histological abnormalities, including severe vacuolar degeneration of the renal tubular epithelium and the presence of calcium oxalate crystals in the lumen of enlarged renal tubules. The group that received plant extract treatment (AEPV and EEPV) had a modest improvement as evidenced by renal tubule vacuolar degeneration and the development of calcium oxalate crystals in the lumina of some renal tubules.

Acknowledgments: The authors wish to thank those who contributed to this research.

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