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EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF AERIAL PARTS OF *HIBISCUS VITIFOLIUS* LINN

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ABSTRACT

The present study indicating the presence of antiurolithiatic effect in *Hibiscus vitifolius* Linn. Aerial parts against EG induced urolithiasis mediated possibly through a combination of CaOx crystal inhibition, diuretic, antioxidant effects. Its medicinal use for urinary pathologies in the Indian folk medicine has been included as an antiurolithiatic drug. The results indicate that the administration of ethanolic extract of *Hibiscus vitifolius* Linn. significantly reduced the growth of urinary stones.the underlying mechanism could be due to its diuretic effect, antioxidant effect, nephroprotective property and lowering the concentration of urinary stone forming constituents and it may prove to be effective for the treatment of kidney stones.

KEYWORDS: Antiurolithiatic effect, *Hibiscus vitifolius* Linn., nephroprotective, Kidney stones.

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INTRODUCTION

Urolithiasis is the third most common urinary tract disorder responsible for serious human suffering and economic cost to society. It is estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females. Occurrence of urolithiasis requires formation of a nidus, its retention and growth in the urinary tract which may cause obstruction of the ureter. Urolithiasis is a disease caused by the presence and effects of uroliths (stones) or claculi or excessive amounts of crystals in the urinary tract. The disease is referred to by many names, including cystitis, urethritis, urinary calculi, bladder stones, or kidney stones. (1-5) Some common causes are inadequate urinary damage, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates & calcium, vitamin abnormalities viz. vitamin A deficiencies, vitamin D excess, metabolic diseases like hyperparathyroidism, cystinuria, gout & intestinal dysfunction. The current treatment of choice for urolithiasis include Surgery, Extra corporeal shock wave lithotripsy, and percutaneous nephrolithotomy

techniques which cause undesirable side effects such as tubular necrosis, hypertension, haemorrhage and subsequent fibrosis leading to cell injury and reoccurrence of renal stone formation. Several plant extracts have been used to treat urolithiasis with promising effects, both in its prevention and treatment. Thus the objective of the present study is to evaluate the Anti-urolithiatic activities of *Hibiscus vitifolius* Linn. (6-10).

MATERIALS AND METHODS

Plant Extraction

The aerial part of *Hibiscus vitifolius* Linn were collected from the farmlands of West Godavari district, Andhrapradesh. Care was taken to collect only the healthy parts. The collected parts were authenticated at the Department of Botany, Acharya Nagarjuna University, India. The parts were then shade dried, coarsely powdered in such a way that it passed through sieve no. 20 and was retained on sieve no. 40. About 500g of the dry powder was extracted continuously in soxhlet apparatus with 99% ethanol for 72 h. After 72 h, the solvent was evaporated to obtain the crude extract. The extract was then dried under vacuum and suspended in water before use. The preliminary phytochemical screening gave positive results for carbohydrates, alkaloids, flavanoids, steroids, glycosides, saponins, tannins and phenolic compounds (11).

Animal Experimentation

Animal facility of this institute is approved by CPCSEA, NewDelhi. The experimental protocols for the antiurolithiatic activity have been approved by the Insititutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. The animals were maintained at a well ventilated, temperature controlled $30^{o}C \pm 1^{o}C$ animal room for 7days prior to the experimental period and provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once (12).

Ethylene glycol induced urolithiasis

Experimental Animals

Adult Wistar albino rats weighing 200-220 g were used for the study. In the laboratory, rats were fed with standard rat pellet diet (Lipton India Ltd, Bangalore) and water *ad libitum*. They were housed in Tarson's polypropylene cages with metal grill tops and acclimated to the laboratory conditions. (13)

Drugs

The ethanolic extract of *Hibiscus vitifolius* was administered at doses of 200 mg/kg and 400 mg/kg, p.o. Cystone was used as standard drug at a dose of 750 mg/kg, p.o. The agent used for inducing lithiasis was 0.75% ethylene glycolated water.

Experimental Design

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats following procedures as under.Animals were divided into 5 groups containing 6 animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water *ad libitum*. All the remaining groups (Groups II-V) received calculi inducing treatment, comprised of ethylene glycol (0.75% v/v) in drinking water *ad libitum* for 15 days to accelerate lithiasis. Groups III, IV and V were administered cystone (750 mg/kg body wt.) and extract at doses of 200 and 400 mg/kg body wt. from day 1 to day 15 of calculi induction, respectively. Extract and standard drug were suspended in distilled water and given by gastric intubation once daily. Urine was collected on the 15^{th} day for 24 h by keeping the animals in polypropylene metabolic cages. The collected urine was analyzed for calcium, oxalates and inorganic phosphates using standard methods. The volume of urine collected from all groups was recorded. The rats were sacrificed by cervical dislocation after 24 h of above treatment. The blood was collected by cardiac puncture and the serum uric acid and creatinine levels were estimated. Finally, the prevalence of lithiasis was confirmed by histopathological studies of the kidneys isolated from the sacrificed animals. (14-16).

Histopathological studies

The isolated kidneys were weighed and transferred to 10% neutralized formalin (pH 7.4). Pathological changes were observed in the sections of kidney fixed

in paraffin that were stained with hematoxylin and eosin.

Statistical analysis

Data are presented as Mean ± SEM. The data was analysed using one way analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnett's multiple comparison test.

RESULTS AND DISCUSSION

Anti –urolithialitic activity of Ethanolic Extract of *Hibiscus vitifolius* (EEHv)

Ethylene glycol increases the risk of urolithiasis by increasing urinary levels of stone constituents (calcium, phosphates) and facilitating an optimal environment for stone growth. The Glomerular Filteration rate decreases in urolithiasis due to obstruction to the outflow of urine by stones in the urinary system and the waste products such as uric acid get accumulated in blood which indicates marked damage of kidneys.Results of anti-urolithiatic activity are presented in table1 & 2. The results showed significant increase in uric acid, calcium, phosphate levels in serum and urine in the EG control group compared to normal control. The levels decreased after treatment with EEHv and cystone there by hastening the process of dissolving the performed stones and prevention of new stone formation in the urinary system (Fig 1-4)

Table-1 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* on Urinary excretion

Group	Treatment	Dose	Urine	
			Calcium (mg/dl)	Phosphate (mg/dl)
I	Control (Normal saline)	2 ml/kg, p.o.	1.450 ± 0.089	3.683±0.079
II	Urolithiatic Control (Ethylene glycolated water)	0.75% v/v, p.o.	6.450 ± 0.043***	1.300 ±0.037***
III	Standard (Cystone)	750 mg/kg, p.o.	2.033± 0.033***	4.433±0.049***
IV	Ethylene glycolated water + EEHv	0.75% v/v + 200 mg/kg, p.o.	1.517 ±0.070ns	1.533±0.042***
V	Ethylene glycolated water + EEHv	0.75% v/v + 400mg/kg, p.o.	3.283 ± 0.087***	3.267± 0.067***

n = 6. Values are expressed as ± S.E.M.

***P < 0.001, ns P > 0.05 Vs Control (One way ANOVA followed by Dunnett's test).

Table-2 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* on Serological parameters

Group	Treatment	Dose	Serum	
			Uric Acid (mg/dl)	Creatinine (mg/dl)
I	Control (Normal saline)	2 ml/kg, p.o.	5.917±0.060	0.417±0.105
II	Urolithiatic Control (Ethylene glycolated water)	0.75% v/v, p.o.	7.700±0.037***	0.517±0.048ns
III	Standard (Cystone)	750 mg/kg, p.o.	6.950±0.056***	2.150±0.056***
IV	Ethylene glycolated water +EEHv	0.75% v/v +200 mg/kg, p.o.	3.017±0.040***	0.183±0.048*
V	Ethylene glycolated water +EEHv	0.75% v/v +400mg/kg, p.o.	6.333±0.033***	0.417±0.031ns

n = 6. Values are expressed as ± S.E.M.

***P< 0.001, *P< 0.05, ns P > 0.05 Vs Control (One way ANOVA followed by Dunnett's test).

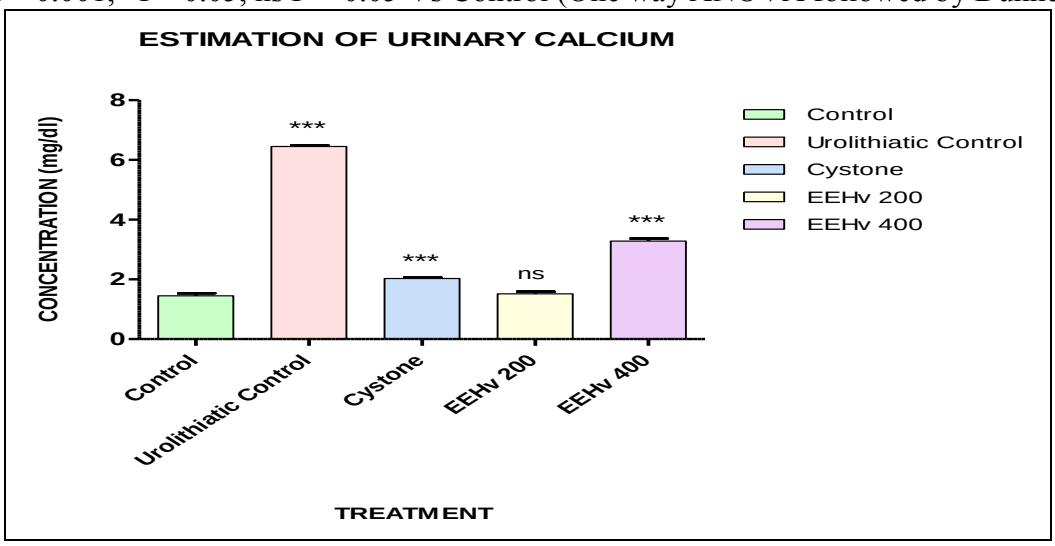

Fig-1 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* Linn on Urinary Calcium excretion

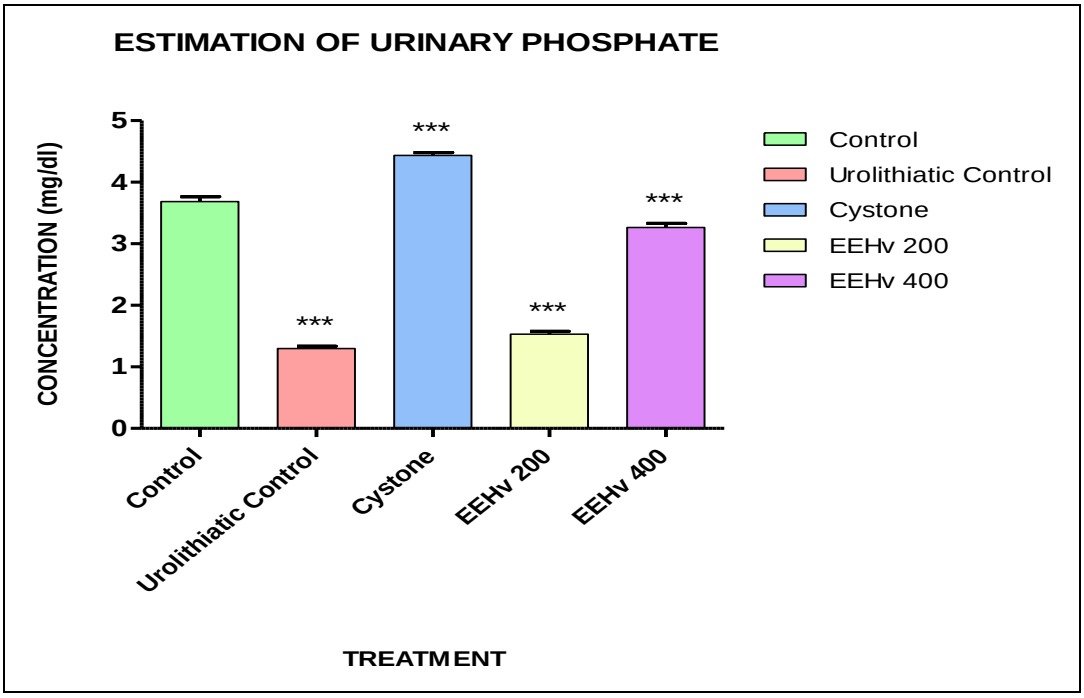

Fig-2 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* Linn on Urinary Phosphate excretions

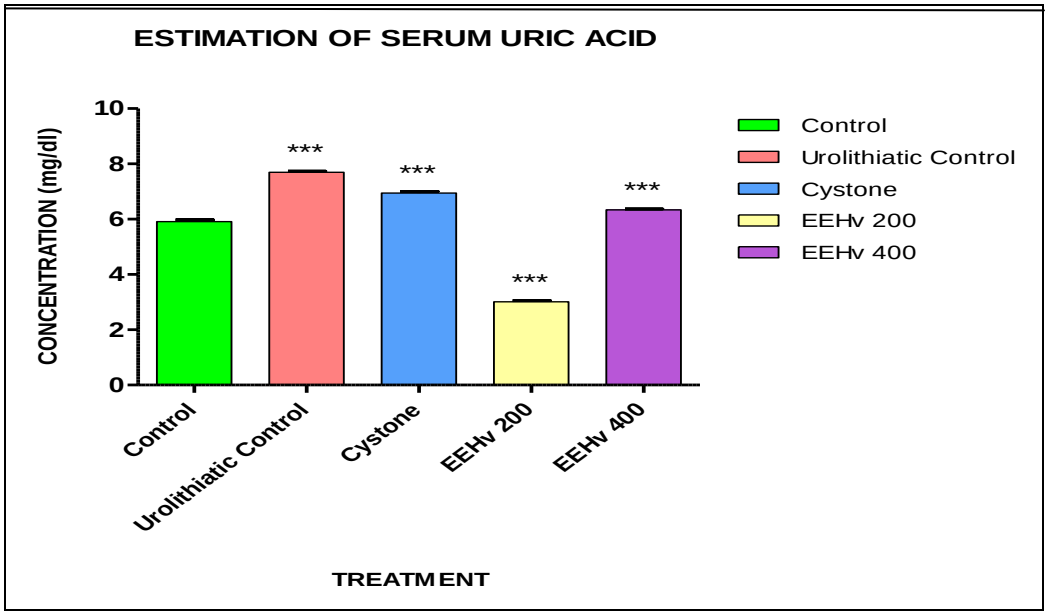

Fig-3 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* Linn on Serum Uric acid excretion

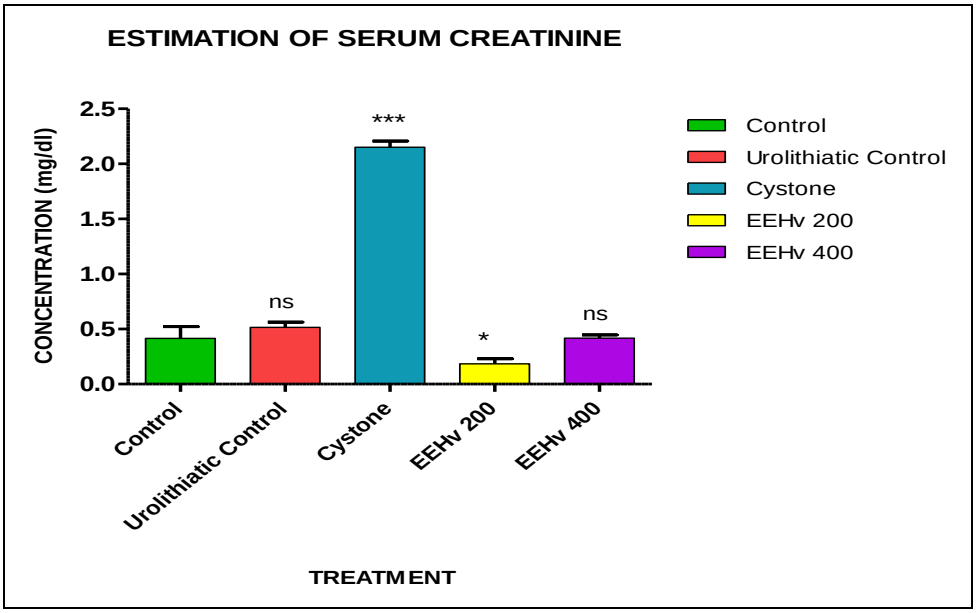

Fig-4 Effect of ethanolic extract of aerial parts of *Hibiscus vitifolius* Linn on Serum Creatinine excretion

Histopathology

EEHv (200 mg/kg) treated group shows glomerular tufts show mild congestion. Pelvis and few blood vessels are dilated. EEHv (400 mg/kg) treated group shows kidney tissues with normal histology (Fig-5-9).

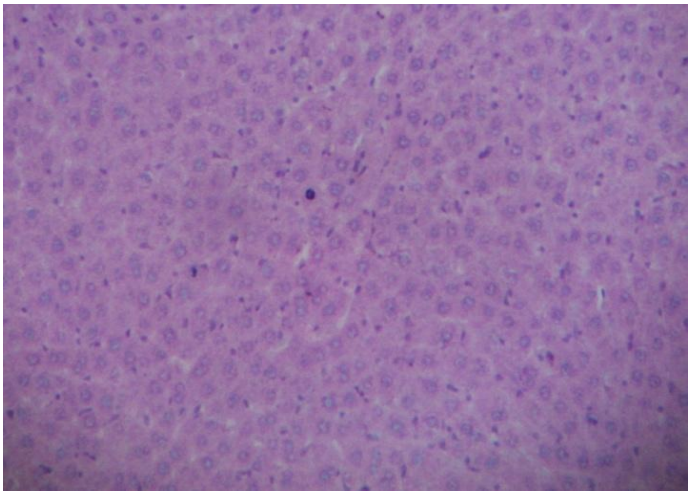

Fig-5 T.S. of Kidney - Control Group

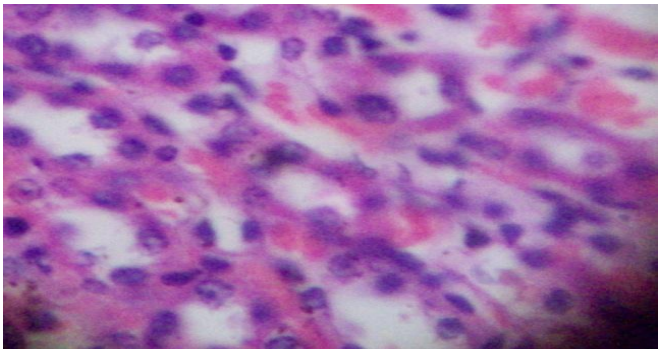

Fig-6 T.S. of Kidney Urolithiatic Control Group

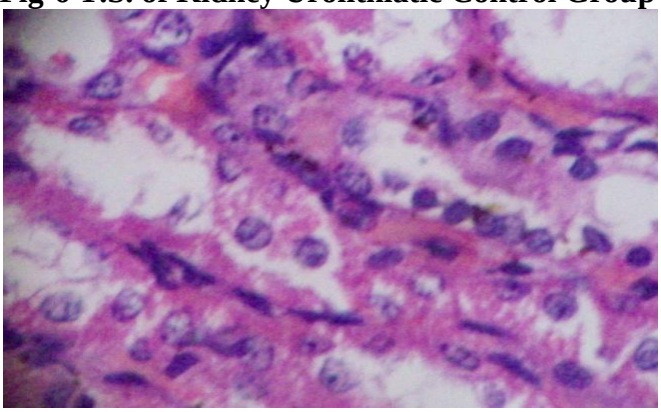

Fig-7 T.S. of Kidney Standard Group

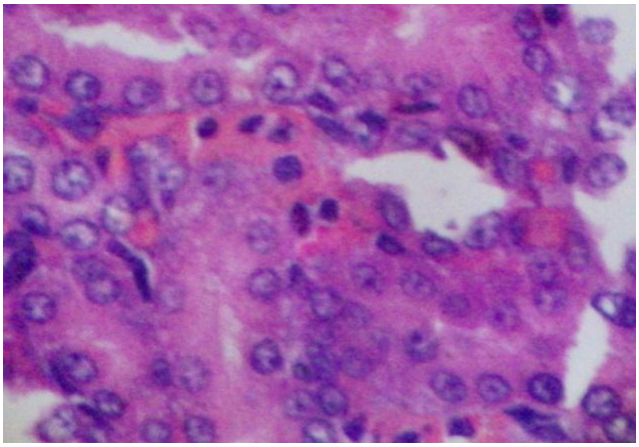

Fig-8 T.S. of Kidney EEHv (200 mg/kg)

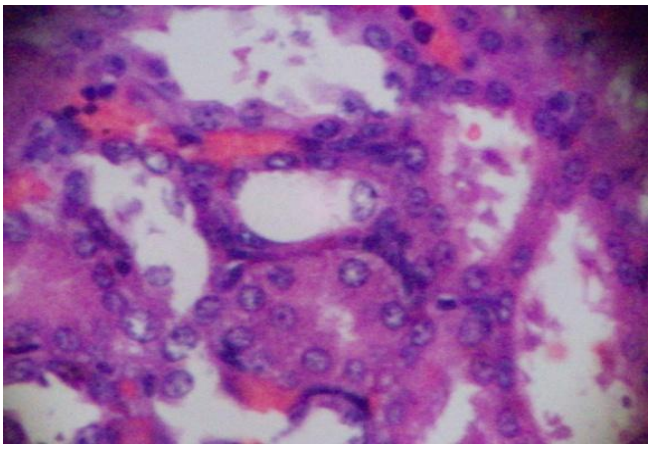

Fig-9 T.S. of Kidney EEHv (400 mg/kg)

CONCLUSION

From the results it is indicated that the ethanolic extract of aerial parts of *Hibiscus vitifolius* Linn possess significant diuretic activity by increasing urine volume and excretion of sodium, potassium and chloride ions. However the activity was not comparable in terms of quantitative activity elicited by standard drug. This could be due to use of crude extracts. In future, further analysis might provide an insight to identify and characterize the exact active phytoconstituents responsible for the antiurolithiatic, antioxidant and diuretic action and to elucidate the exact mechanism of action, which is responsible for the observed significant activity with low toxicity and better therapeutic index.

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