



Research Article

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## **Anti-Urolithiatic Potential of Methanolic Extract of *Hygrophila salicifolia* on Ethylene Glycol Induced Urolithiasis in Rats**

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### **ABSTRACT**

The anti-urolithiatic action of *Hygrophila salicifolia* (Acanthaceae) was determined by ethylene glycol induced lithiasis. Ethylene glycolated water (0.75% v/v) was given to experimental animals for 28 days to produce stone in kidney. Methanolic extracts of *Hygrophila salicifolia* were given from the 1<sup>st</sup> day as inhibitory agent. Urinary parameters such as calcium, oxalate and inorganic phosphate level were raised by ethylene glycol where as test extract lowered down the raised level of these ions in urine. Methanolic extract of *Hygrophila salicifolia* also increased urinary magnesium concentration while raised concentration of serum creatinine was found to be cut down during the experiment. Methanolic extract of test drug was found impressively better from histological study. These results obtained from the study confirmed the anti-urolithiatic potential of *Hygrophila salicifolia*.

**Keywords:** *Hygrophila salicifolia*, Ethylene glycol, Anti-urolithiatic action.

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### **INTRODUCTION**

Kidney stone formation is a universal, wide spread disease to mankind from several centuries. Production of stone in urinary tract system is known as urolithiasis. [1-2] Urolithiasis is the most common ailments in females & males but it is more frequent in male, ratio 2:11. It is because of testosterone increases potency of stone formation while oestrogen decreases stone formation. [3] The repetition rate is reached 50% and raised to 70% within 12 years. Its repetition rate is high because of imbalance between promoter and inhibitor. [4] Nephrolithiasis occurs more frequently after urinary tract diseases. In Nephrolithiasis, crystal formation, crystal aggregation, crystal deposition occurs in renal tract. So it is very painful condition to the kidney stone patients. Most of the doctors prefer surgery to remove

stone but there is chance of reoccurrence of kidney stone if it is not completely removed from the body. More over there is no significant drugs for urolithiasis without any side effect and no low cost treatment. Hence there is great demand to find novel anti-urolithiatic herbal drug with high potency without any side effect and low cost. In Ayurveda there are many magical herbs to treat diseases. So to remove kidney stone completely from the body with herbal treatment is better choice as far as side effects and low cost concerns. *Hygrophila salicifolia* has been advocated for the therapy of various diseases including jaundice, urinary infection, gout, hepatic disorder, rheumatism, impotence, anti-bacterial and inflammation. [5] It is an erect or ascending herb belongs to the family Acanthaceae. It is bestowed with many medicinal uses in traditional systems of medicine including ayurveda. Seeds and leaves used as poultice on inflammatory swellings. Leaves are strongly diuretic. The plant is discovered in moist and marshy places throughout the

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greater part of India. Stems up to 3 ft. long, more or less quadrangular, rooting at the lower nodes, leaves subsessile, linear-lanceolate; flowers pale purple in dense axillary whorls, capsule oblong, seeds many, ovoid, compressed, mucilaginous, hairy. The leaves are eaten as pot-herb. They contain 8% ash rich in potassium and are strongly diuretic. In Malaya, the leaves are used as poulticing swellings. The seeds swell into a gelatinous shining mass with water and used in Java, in poultices for headaches and fevers. They yield 25% of a fatty oil and contain traces of unidentified alkaloid, a bitter substance and 4% ash consisting chiefly of calcium phosphate and potassium chloride. [5-6] The plant has been reported to contain chlorophyll, pigments, gums, glucose, starch, fat, various minerals, alkaloids, flavanoids, tannins and sterols. [7-9] The present research was aimed to determine the anti urolithiatic action of methanolic extract of *Hygrophila salicifolia* whole herb.

## MATERIAL AND METHODS

### Procurement of chemicals

Cystone was obtained from Himalaya herbal health care, Bangalore. The solvents used were of laboratory grade obtained from Emerck Ltd. Mumbai.

### Plant material & extract

Whole herb was obtained from the local areas in and around Gujarat. The herb was identified and authenticated as *H. salicifolia* in the Bioscience department, Vallabh Vidyanagar, Gujarat. The plant was shade dried and ground to a fine powder in grinder to produce a coarse powder. Plant extracts were then obtained using Soxhlet's apparatus with methanol. The extract was concentrated using vacuum evaporator. It was dried in desiccators. [10-12]

### Animals

Wistar albino rats weighing between (150-200 g) were used for the work. They were held in standard environmental conditions and fed with standard rat food and water *ad libitum*. All animal experimental procedures were conducted accordingly CPCSEA guidelines and approved by the IAEC. (Reg. No.APC/2016-IAEC/1616).

### Evaluation for anti-urolithiatic activity

There were five groups each consisted of six albino rats and treated for 28 days. Group-I was given drinking water and rat food and acted as control group. 1% tween 80 solution was given to group-II and acted as lithiatic group. Standard drug, Cystone (750 mg/kg) was given to group-III from 15 day to 28<sup>th</sup> day. Two test doses of methanolic extract (300 mg/kg & 500 mg/kg) were given to group-IV & V respectively. All groups received 0.75% ethylene glycol in water for production of kidney stone except group-I. Rats were kept in separate metabolic cages. Urine samples were obtained on 28<sup>th</sup> day and urine volume was measured. It was stored at 4°C after adding a drop of conc. hydrochloric acid. Urine sample was determined for various biological parameters like calcium, creatinine, phosphate, magnesium and oxalate content. [13-22]

## Histopathology

Kidneys were dissected out of each group after scarification. They were washed with saline and fixed in 8-10% phosphate buffered formalin. Sections were taken using paraffin method and stained with eosin & haematoxylin. Light microscope was used to detect any histopathological changes and photographs were taken.

## Statistical Analysis

The results were expressed as Mean ± SEM. Statistical calculation was done by one way ANOVA followed by Tukey test.  $P < 0.05$  was considered as statistical significant.

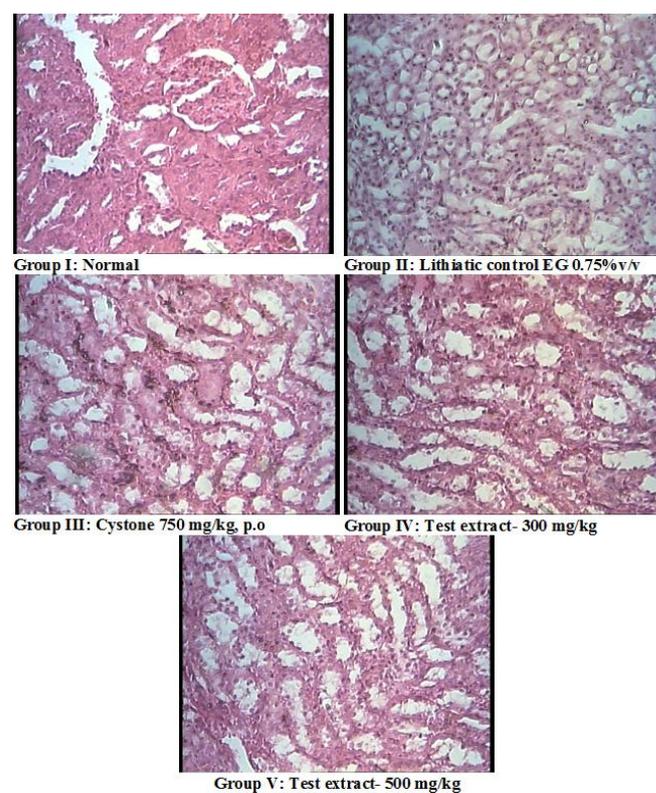


Fig. 1: Histopathology study

## RESULT AND DISCUSSION

Weight of kidney was observed to be raised in group II model control compared to normal Group I, but therapy with herbal drug and cystone as a standard resulted in remarkable ( $P < 0.05$ ) reduction in dry kidney weight, when compared with Group II. Body weight of the model group was discovered to be notably reduced compared to the normal group, while herbal extract therapy groups exhibited remarkable raise in the body weight of the animals, as analyzed to the model group. Therapy with standard and herbal extract 500 mg/kg resulted remarkable raise in urine volume at the end of the study, however 300 mg/kg dose of herbal extract produced in notably raise in volume of urine collected as analyzed to the model group. ethylene glycol (0.75%) in drinking water demonstrated statistically significant ( $P < 0.05$ ) raise in urinary calcium levels of Group II as analyzed to normal control animals of Group I (Table 1).

**Table 1: Effect on physiological parameters**

Groups	Group-I	Group-II	Group-III	Group-IV	Group-V
Wet kidney Weight (g)	0.91±0.06	1.12±0.20	0.86±0.10	0.93±0.04	0.98±0.05
Dry kidney Weight (g)	0.78±0.04	1.34±0.16	0.74±0.07	0.73±0.04	0.74±0.04
% change Body weight	8.03±2.11	-18.14±6.19	10.36±2.34	-6.621±2.51	-4.88±2.71
Urine volume	11.23±0.69	3.76±0.49	9.1±0.66	3.96±0.37	6.5±0.15

Values expressed are mean ± S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test &  $P < 0.05$  was considered as significant.

**Table 2: Effect of methanolic extracts of *Hygrophila salicifolia* on Urinary biological parameter**

Therapy groups	Urinary excretion parameter (mg/24 hrs)			
	Calcium	Oxalate	Phosphate	Magnesium
Group-I (Normal)	2.71±0.29	6.84±0.15	3.17±0.04	5.96±0.53
Group-II (Lithiatic control)	18.38±0.22	12.41±0.39	6.94±0.19	1.88±0.07
Group-III (Standard)	13.56±0.31	4.23±0.09	3.41±0.17	5.89±0.22
Group-IV (Test-300 mg/kg)	16.38±0.19	7.3±0.37	6.19±0.20	13.49±1.24
Group-V (Test-500 mg/kg)	13.75±0.67	5.9±0.44	2.82±0.26	6.24±0.69

Values expressed are mean ± S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test &  $P < 0.05$  was considered as significant.

**Table 3: Effect of methanolic extracts of *Hygrophila salicifolia* on serum & urinary creatinine level**

Biological parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
Serum Creatinine (mg/dl)	1.38 ± 0.39	5.19±0.19	0.39±0.08	0.58±0.54	0.57±0.14
Urine Creatinine (mg/24hrs)	0.59±0.05	0.08±0.01	0.59±0.10	0.75±0.09	0.72±0.10

Values expressed are mean ± S.E.M. Statistical analysis was done by one way ANOVA test for multiple comparisons followed by Tukey test &  $P < 0.05$  was considered as significant.

Therapy with standard drug Cystone exhibited remarkable ( $P < 0.05$ ) reduction in urinary calcium levels. Therapy with herbal drug extract, 500 mg/kg, in Group V depicted remarkably ( $P < 0.05$ ) lower urinary calcium levels compared to lithiatic control. Remarkable decrease in urinary calcium levels were observed in Group V which was treated with herbal drug extract dose 500 mg/kg. significant ( $P < 0.05$ ) higher urinary oxalate levels were reported in model control Group II animals as analyzed to the normal control Group I. Pre-therapy with standard drug and herbal drug extracts for 28 days remarkably ( $P < 0.05$ ) reduced urinary oxalate levels (Table 2). Ethylene glycol (0.75%) in drinking water demonstrated statistically significantly ( $P < 0.05$ ) decrease in urinary creatinine levels as analyzed to normal control animals as shown in figure 3. Creatinine levels in urine were discovered to be remarkably ( $P < 0.05$ ) more in Groups III-V. Serum creatinine were observed to be remarkably ( $P < 0.05$ ) higher in lithiatic group compared to the group I control animals. Pretherapy with Cystone and herbal drug extract demonstrated notably ( $P < 0.05$ ) reduction in the serum creatinine levels when compared with model group (Table 3). Significant raise was observed in urinary inorganic phosphate levels in Group II as analyzed to the normal control Group I. Standard drug Cystone and herbal drug extract (500 mg/kg) treated groups exhibited significant ( $P < 0.05$ ) reduction in phosphorous levels, while in notably decline was observed in group IV treated with herbal drug extract (300 mg/kg), compared to the model group II. Remarkable ( $P < 0.05$ ) decline was observed in urinary magnesium levels in Group II as analyzed to the normal control Group I (table 2). Significant ( $P < 0.05$ ) raise was observed in all therapy groups. In histopathology study, ethylene glycol treated model

group exhibited marked tubules dilation and crystal deposition. However, therapy with cystone and herbal drug extracts notably reduced tubular dilation and crystal deposition (figure 1). 0.75% ethylene glycol in drinking water induced urolithiasis in experimental animals as evident from the results. Standard drug Cystone 750 mg/kg therapy decreased dry kidney weight, urinary calcium, oxalate, inorganic phosphate and serum creatinine levels ( $P < 0.05$ ), raised urinary magnesium and creatinine. Raised urine volume proves its remarkable diuretic activity. Herbal extract dose 500 mg/kg decreased notably ( $P < 0.05$ ) urinary calcium, oxalate, inorganic phosphate and serum creatinine. Remarkable raise was observed in urinary magnesium and creatinine levels. 500 mg/kg dose produced notably fall in dry kidney weight and raise in urine volume demonstrating its anti-urolithiatic activity. Herbal extract dose 300 mg/kg decreased significantly ( $P < 0.05$ ) urinary oxalate, and serum creatinine levels, while it raised remarkably urinary creatinine levels. With insignificant effect on rest of the parameters, it may be concluded that high dose of the herbal drug extract produce anti-urolithiatic effect.

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