



## EFFECT OF ETHANOLIC LEAF EXTRACT OF *PISTIA STRATIOTES* ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN LABORATORY ANIMALS

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### ABSTRACT

*Pistia stratiotes* is a plant commonly used as a traditional herbal medicine and possesses the wide range of pharmacological applications. The present study investigated the antiurolithiatic activities of an ethanolic leaf extract of *Pistia stratiotes* (Family: Araceae). Ethylene glycol induced urolithiasis (0.75% v/v in drinking water for 28 days) was used to study the antiurolithiatic effect of ethanolic leaf extract of *Pistia stratiotes* at the oral dose of 200 mg/kg in Wistar albino rats. Cystone (750 mg/kg, p.o.) was used as a standard reference drug. After completion of the 28-days respective treatments, the level of various urolithiatic promoters in the urine for assessing the antiurolithiatic effect. Results indicate that, in the animals treated with *Pistia stratiotes* significantly decreased the calcium, Oxalate and phosphate to  $2.46 \pm 0.19$  ( $P < 0.05$ ),  $1.42 \pm 0.12$  ( $P < 0.01$ ) and  $4.85 \pm 0.36$  ( $P < 0.001$ ) respectively compared to control. From the results it was concluded that ethanolic leaf extract of *Pistia stratiotes* exhibits antiurolithiatic activity in experimental animals.

**Key words:** *Pistia stratiotes*, Urolithiasis and Cystone.

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### INTRODUCTION

Kidney stones, one of the most painful of the urologic disorders, are not a product of modern life. Kidney stones are one of the most common disorders of the urinary tract, the others being frequently occurring urinary tract infections (Hiatt *et al.*, 1983). Men tend to be affected more frequently than women. Kidney stones may contain various combinations of chemicals. Urinary stone disease has

afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of renal stone is quite high and in spite of tremendous advances in the field of medicine, there is no truly satisfactory allopathic drug for the treatment of renal calculi (Anderson *et al.*, 1967). Although some drugs used to prevent the disease have some positive effects, they are not effective in all patients and often have adverse effects. Alternative treatment using phytotherapy has been required. In recent years there has been a resurgence of interest in medicinal plants that are effective, safe and culturally acceptable.

*Pistia* is a genus of aquatic plant in the arum family, Araceae. It comprises, *Pistia stratiotes*, is often called water cabbage, water lettuce, Jalakumbhi. Its native distribution is uncertain, but

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probably pantropical, it was first discovered from the Nile near Lake Victoria in Africa. It is now present, either naturally or through human introduction, in nearly all tropical and subtropical fresh waterways. In Ayurveda it is used in many ways, which includes Antifungal, Cytotoxic, antioxidant, Diuretics, Use over different heavy metals & Phosphorous, Detoxification of metals, Reduction of Arsenic etc (Pal and Kundu, 2014). The plant was reported to have Anthelmintic activity (Sundeeep Kumar *et al.*, 2010) Antidermatophytic & Antifungal activity (Premkumar and Shymsundar, 2005) antioxidant activity (Megha Jha *et al.*, 2010), antihypertensive and bronchodilating activity (Achola *et al.*, 1997). The leaves of *Pistia stratiotes* exhibits diuretic activity, hence the present study is planned to evaluate the antiurolithiatic activity of ethanolic leaf extract of *Pistia stratiotes* in rats.

## MATERIALS & METHODS

### Plant Collection

The leaves of *Pistia stratiotes* was collected from the pond near Pondicherry. The plant was identified as *Pistia stratiotes* and authenticated by by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai. The voucher specimen was deposited in the herbarium for further reference.

### Preparation of Extract

The collected leaves were washed in running tap water and shade dried. The dried *Pistia stratiotes* leaves were ground into coarse powder. The powder was then subjected to exhaustive extraction by a maceration process using 70% ethanol as a solvent at room temperature for 7 days. The ethanolic extract was concentrated by vacuum distillation to dry. The collected extract was stored in desiccators and used for further pharmacological study.

### Animals

Male Wistar albino rats weighing between 150 – 200 gm were used for this study. The animals were obtained from animal house, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

### Experimental design (Mitra *et al.*, 1988)

Ethylene glycol-induced hyperoxaluria method was used. Healthy male Wistar rats were divided into four groups of six animals each. Group-1 served as normal control, received 0.1% Carboxy Methyl Cellulose solution. Ethylene glycol (0.75%) in drinking water was fed to Group-2 to Group-4 for induction of renal calculi till 28<sup>th</sup> day. Group-3 received standard antiurolithiatic drug, Cystone (750 mg/kg, b.wt.) from 15<sup>th</sup> day to 28<sup>th</sup> day. Group-4 received ethanolic leaf extract of *Pistia stratiotes* (200 mg/kg b.wt.) from 15<sup>th</sup> day till 28<sup>th</sup> day. All doses were given once daily by oral route by suspending in 0.1% Carboxy Methyl Cellulose solution.

### Assessment of antiurolithiatic activity

Collection and analysis of urine: Urine samples (24 h) were collected on 28<sup>th</sup> day by keeping the animals in metabolic polypropylene cages. Animals had free access to drinking water during urine collection period. The volume of urine from each group of animal was measured. A drop of concentrated hydrochloric acid was added to the collected urine before being stored at 4°C. Urine was analyzed for calcium (Moorehead *et al.*, 1974), phosphate (Fiske and Subbarow, 1925) and oxalate (Hodgkinson and Williams, 1972).

### Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' – test using graph pad. P values <0.05 were considered significant.

Antiurolithiatic activity of *Pistia stratiotes* was studied against ethylene glycol induced urolithiasis in rats and the results were showed in table 1. The urine was collected on 28<sup>th</sup> day and the stone forming constituents namely calcium, phosphorous and oxalate were estimated. In the present study, chronic administration of 0.75 % ethylene glycol to rats increases the calcium, Oxalate and phosphate to 4.68±0.26, 3.53±0.26 and 9.26±0.64 respectively. In the animals treated with standard drug Cystone significantly (P<0.001) reduced the calcium, Oxalate and phosphate to 1.86±0.17, 1.06±0.16 and 3.67±0.21 respectively. In the animals treated with *Pistia stratiotes* significantly reversed the calcium, Oxalate and phosphate to 2.46±0.19 (P<0.05), 1.42±0.12 (P<0.01) and 4.85±0.36 (P<0.001) respectively compared to control.

## RESULTS

**Table: 1.** The table shows the effect of *Pistia stratiotes* leaf extract on ethylene glycol induced urolithiasis in rats.

Drug Treatment	Urine (mg/dl)		
	Calcium	Oxalate	Phosphate
Group I Normal Control 0.1% CMC	1.65±0.12	0.54±0.03	3.45±0.22
Group II Ethylene Glycol (0.75%)	4.68±0.26	3.53±0.26	9.26±0.64
Group III Ethylene Glycol (0.75%) + Cystone (750mg/kg)	1.86±0.17***	1.06±0.16***	3.67±0.21***
Group IV Ethylene Glycol (0.75%) + <i>Pistia stratiotes</i> (200mg/kg)	2.46±0.19*	1.42±0.12**	4.85±0.36***

Values are in mean ± SEM (n=6), \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 Vs Control

## CONCLUSION

From the study it was concluded that, *Pistia stratiotes* showed protection from ethylene glycol induced urolithiasis by suppressing urine urolithiatic promoters such as calcium, oxalate and phosphate. Thus, the present finding emphasizes that the

ethanolic leaf extract of *Pistia stratiotes* possess potential medicinal value and beneficial in the prevention of renal stone. Further studies need to be carried to isolate and characterize the active constituent liable for antiurolithiatic activity of *Pistia stratiotes*.

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