

Original Research Article

Antiuro lithiatic Effect of Ethanolic Leaf Extract of *Ipomoea reniformis* Against Ethylene Glycol Induced Urolithiasis in Experimental Animals

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Abstract: Kidney stone is a common painful condition of multifactorial etiopathogenesis, and calcium oxalate is the commonest component of stone. Traditionally various medicinal plants were used in the treatment of urolithiasis globally due to its least side effects. The present study was undertaken with an aim of evaluating the antiuro lithiatic effect of ethanolic leaf extract of *Ipomoea reniformis* against, ethylene glycol induced urolithiasis in rats. The animals were divided in to 5 groups of 6 animals each. Group I served as normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of renal calculi till 28th day. Group II served as uro lithiatic control, received drinking water and group III received standard antiuro lithiatic drug, Cystone (750mg/kg weight) from 15th day till 28th day. Groups IV and V served as curative regimen received ethanolic leaf extract of *Ipomoea reniformis* (200 and 400 mg/kg, respectively) from 15th day till 28th day once daily by oral route. On 28th day, urine samples were collected from all the groups and analysed Calcium, Phosphate and Oxalate content. Blood was also withdrawn through retro-orbital puncture under phentobarbitone anesthesia; serum was separated and subjected to Creatinine, Uric acid and Urea Nitrogen.

Keywords: *Ipomoea reniformis*, Urolithiasis, Cystone and Kidney Stone.

INTRODUCTION

Urolithiasis is a common problem in populations around the world, and contributes significantly to the development of end stage renal disease. It is a matter of debate whether the metabolic factors responsible for renal stone formation are similar or variable in different populations around the globe. The incidence peaked in the third and fourth decades, and prevalence increased with age until approximately the age of 70 years. Importantly, kidney stones were a recurrent disorder, with lifetime recurrence risks reported to be as high as 50%. Mankind has been afflicted by urinary or kidney stones since centuries and it are proven to be an important cause of renal failure. It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by renal disease. Among those, kidney stones are common in industrialized nations with an annual incidence of 0.5-1.9%. About 12% of the population of India is expected to have kidney stones and out of that about 50% of cases encounter loss of one or both kidneys with or without renal damage up to some extent [1]. A large number of plants have been used in India since ancient times, which claim the efficient cure of urinary stone [2].

Herbs and herbal drugs have created interest among the people by its clinically proven effects. The overuse of synthetic drugs, results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies. One such distinguished herb is *Ipomoea reniformis* traditionally used for treating the kidney stones. *Ipomoea reniformis* belonging to the family Convolvulaceae is a perennial, much branched herb (creeper). It is widely distributed all over the India, especially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western-Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa [3]. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums [4], *Ipomoea reniformis* reported to possess Antioxidant [5], Antihypertensive [6], Analgesic, Anti-inflammatory, Antipyretic [7],

Antidiabetic [8], Antibacterial [9], Anticancer [10] and Nephro protective [11] activities. The traditionally important, *Ipomoea reniformis* was reported to possess various pharmacological activities. However, there is no scientific evidence to mark the effect of *Ipomoea reniformis* against urolithiasis. Hence, the present study was conducted with an aim to investigate the protective effect of *Ipomoea reniformis* in kidney stone.

MATERIAL AND METHODS

Plant material

The leaves of *Ipomoea reniformis* were collected from outskirts of Tirunelveli District, in the month of December. It was identified and authenticated as *Ipomoea reniformis* by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, and Coimbatore. The voucher specimen (BSI/SRC/12/42/2015-16/Sci/1555) has been deposited in department for further references.

Preparation of Extract

The leaves of *Ipomoea reniformis* were, shade dried and then 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 150–180 gm were used for the study. The animals were obtained from animal house of Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India. 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 $24 \pm 2^\circ\text{C}$ and relative humidity of 30–70 %. A 12:12 light: dark cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (932/a/06/CPCSEA) and were in accordance with the guidelines of the IAEC.

Ethylene Glycol Induced Urolithiasis Model

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Animals were divided into four groups containing six animals in each. Group I served as normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Groups II–V for induction of renal calculi till 28th day. Group II served as uro lithiatic control, received drinking water and group III received standard

antiurolithiatic drug, Cystone (750mg/kg weight) from 15th day till 28th day [12]. Groups IV and V served as curative regimen received ethanolic leaf extract of *Ipomoea reniformis* (200 and 400 mg/kg, respectively) from 15th day till 28th day once daily by oral route.

Assessment of Antiurolithiatic activity

Urine Analysis

All animals were kept in individual metabolic cages and urine samples of 24h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium [13], phosphate [14] and oxalate [15] contents.

Serum Analysis

After the experimental period, blood was collected from the retro-orbital under Phentobarbitone (45mg/kg) anesthetic conditions. Serum was separated by centrifugation at 10,000x g for 10 min and analyzed for creatinine [16], uric acid [17] and urea nitrogen [18].

Statistical Analysis

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

RESULT

The effect of *Ipomoea reniformis* on ethylene glycol induced urolithiasis in rats was studied and the urine (Calcium, Phosphate and Oxalate) and serum (Creatinine, Uric Acid and Urea Nitrogen) parameters were shown on Table 1. In uro lithiatic control, Ethylene glycol enhanced the urinary calcium, phosphate and oxalate compared to control. The reference control, Cystone significantly ($P < 0.001$) reduced urinary calcium, phosphate and oxalate content compared to uro lithiatic control. Ethanolic leaf extract of *Ipomoea reniformis* 200 and 400mg/kg, significantly ($P < 0.001$ and $P < 0.01$, respectively) reduced the elevated urinary calcium, phosphate and oxalate content compared to uro lithiatic control.

In serum parameters, Ethylene glycol significantly elevated the levels of Creatinine ($P < 0.001$), Uric acid, and urea nitrogen ($P < 0.01$) compared to control animals. Treatment of ethanolic leaf extract of *Ipomoea reniformis* at 200mg/kg showed significant decrease in the levels of Creatinine ($P < 0.01$), Uric acid, and urea nitrogen ($P < 0.05$) compared to uro lithiatic control. Ethanolic leaf extract of *Ipomoea reniformis* at 400mg/kg showed significant decrease in the levels of Creatinine ($P < 0.001$), Uric acid, and urea nitrogen ($P < 0.01$) compared to uro lithiatic control and the effect

produced were similar to that of the positive control Cystone.

Table 1: Shows the effect of ethanolic leaf extract of *Ipomoea reniformis* on urine and serum analysis against ethylene glycol induced urolithiasis in rats

Groups & Treatment	Drug	Urine (mg/dl)			Serum (mg/dl)		
		Calcium	Phosphate	Oxalate	Creatinine	Uric Acid	Urea Nitrogen
Group I Normal Control		1.22±0.09	3.62±0.02	0.36±0.01	0.81±0.03	1.56±0.02	37.62±1.33
Group II Uro lithiatic Control Ethylene Glycol (0.75%)		5.63±0.24	7.38±0.27	3.55±0.09	1.53±0.09	3.27±0.12	51.54±2.52
Group III Reference Control Cystone (750mg/kg)		1.46±0.02***	3.95±0.21***	0.64±0.05***	0.94±0.08***	1.88±0.06**	39.62±2.66**
Group IV <i>Ipomoea reniformis</i> (200mg/kg)		2.35±0.01**	4.55±0.33**	1.64±0.04**	1.07±0.02**	2.35±0.04*	43.77±1.42*
Group V <i>Ipomoea reniformis</i> (400mg/kg)		1.76±0.03***	4.03±0.31***	0.75±0.06***	0.98±0.06***	1.98±0.01**	40.56±2.89**

Values are in mean ± SEM (n=6),

*P<0.05, **P<0.01, ***P<0.001 Vs Uro lithiatic Control

CONCLUSION

The ethanolic leaf extract of *Ipomoea reniformis* studied for its antiurolithiatic activity against ethylene glycol induced urolithiasis in rats. From the results it was concluded that, *Ipomoea reniformis* leaf extract exhibited antiurolithiatic activity, and it may be due to the presence of flavonoids. Further study may be requiring in order proving its mechanism of action.

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