

Scientific Validation of Antiurolithiatic Activity of *Luffa acutangula* Root Extract against Ethylene Glycol Induced Urolithiasis in Wistar Albino Rats

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Original Research Article

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Article History

Received: 01.12.2017

Accepted: 14.12.2017

Published: 30.12.2017

DOI:

10.36347/sjams.2017.v05i12.031



Abstract: Nephrolithiasis or renal stone disease remains a significant health problem in the adult population, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of urolithiasis is quite high. The present-day medical management of nephrolithiasis is either costly or not without side-effects. In contrast, traditional medicines have offered a substitute for many diseases due to its least side effects. The present study was undertaken with an aim of evaluating the antiurolithiasis effect of ethanolic root extract of *Luffa acutangula* against, ethylene glycol induced urolithiasis in rats. The animals were divided into 5 groups of 6 animals each. Group I served as normal control and received regular diet 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 urolithiatic control, received drinking water and group III received standard antiurolithiatic drug, Cystone (750mg/kg weight) from 15th day till 28th day. Groups IV and V served as curative regimen received ethanolic root extract of *Luffa acutangula* (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 Blood was also withdrawn through retro-orbital puncture under anesthesia; serum was separated and subjected to Creatinine, Uric acid and Urea Nitrogen. Treatment with cystone and *Luffa acutangula* reversed the elevated levels of Calcium, Phosphate and Oxalate in urine and Creatinine, Uric acid and Urea Nitrogen in blood induced by ethylene glycol. From the result it was concluded that the ethanolic root extract of *Luffa acutangula* exhibited antiurolithiatic activity against ethylene glycol induced urolithiasis in wistar albino rats.

Keywords: *Luffa acutangula*, Urolithiasis, Cystone and Kidney Stone.

INTRODUCTION

Urolithiasis has afflicted humankind since antiquity and can persist with serious consequences throughout a patient's lifetimes. The management of urolithiasis remains a problem. Etiopathogenesis of this disorder is yet uncertain, and recurrence cannot be prevented by surgery or lithotripsy. A large number of plants have been used in India since ancient times, which claim the efficient cure of urinary stone [1]. One of such reputed plants is *Luffa acutangula* L., belongs to the family Cucurbitaceae, is commonly known as ridge gourd and it is used as vegetable in Asian countries. The ethnobotanical survey revealed that very fine powder of fruits of *L. acutangula* is used as a snuff to protect jaundice [2]. A powder of the fruit is used for rubbing on the swollen hemorrhoids. Kernel of the seeds is soft smooth and an efficient remedy for dysentery while the juice of roasted young fruit is applied to cure headache. Traditionally the plant has diuretic properties; used as an expectorant, laxative and

purgative; hypoglycemic agent, bitter tonic; used in the enlargement of spleen. The roots of ridge gourd added to milk or water is helpful in the removal of kidney stones. The roots of ridge gourd are added to cooled water, boiled and applied on skin in the swelling of the lymph glands. The leaves of the ridge gourd are useful in the treatment of dysentery. The leaves or juice of the ridge gourd are used as dressing in the diseases such as inflammation of spleen, ringworms, and piles and even in leprosy. Pounded leaves mixed with garlic are applied locally for a relief in leprosy. Oil is extracted from the seeds of ridge gourd and used in the treatment of skin diseases. Ridge gourd is also an effective home remedy for the prevention of premature greying of hair. Ridge gourd is chopped in small pieces along with the ribbed skin and completely dried in the sun. Once the ridge gourd is fully dried, made into a powder and used to prevent the premature greying of hair [3]. Various parts of *Luffa acutangula* was reported to have different activities like hepatoprotective [4], antidiabetic [5],

antiulcer [6], antiproliferative and anticancer [7], antioxidant [8], analgesic [9], fungistatic [10], antimicrobial [11] activity. The study has been planned to establish the scientific validity of the antiurolithiatic activity of the ethanolic root extract of *Luffa acutangula* using ethylene-glycol-induced urolithiasis in the male albino Wistar rats.

MATERIALS AND METHODS

Plant material

The roots of *Luffa acutangula* were collected from the local farm of Pondicherry. It was identified and authenticated as *Luffa acutangula* by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The voucher specimen (BSI/SRC/12/42/2017-18/Sci/01203) has been deposited in department for further references.

Preparation of Extract

The roots of *Luffa acutangula* were washed, dried and cut into small pieces. The roots were shade dried and subjected to steam distillation. The distillate collected was subjected to extraction with ethanol. The resulted ethanolic extract was dried, and stored in airtight container, protected from light.

Animals

Healthy male Wistar albino rats weighing between 180–200 g were used for the study. The animals were obtained from King's Institute, Guindy and was housed in animal house, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kancheepuram. 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 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 five 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 urolithiatic 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 root extract of

Luffa acutangula (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.

RESULTS

The effect of *Luffa acutangula* root extract 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 urolithiatic 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 urolithiatic control. Ethanolic root extract of *Luffa acutangula* at 200 mg/kg significantly reduced the elevated calcium ($P < 0.05$) phosphate ($P < 0.01$) and oxalate ($P < 0.05$) levels in urine and 400mg/kg, significantly showed marked significance ($P < 0.001$) decrease in calcium, phosphate, and oxalate content compared to urolithiatic control.

In serum parameters, Ethylene glycol significantly elevated the levels of Creatinine, Uric acid, and urea nitrogen compared to control animals. Treatment of ethanolic root extract of *Luffa acutangula* at 200mg/kg showed significant ($P < 0.05$) decrease in the levels of Creatinine, urea nitrogen compared to urolithiatic control and no change in uric acid level. Ethanolic root extract of *Luffa acutangula* at 400mg/kg showed significant decrease in the levels of Creatinine ($P < 0.001$), Uric acid, and urea nitrogen ($P < 0.01$) compared to urolithiatic control. The effect produced by ethanolic root extract of *Luffa acutangula* at 400mg/kg was equipotent as that of the reference control Cystone.

Table-1: Shows the effect of ethanolic root extract of *Luffa acutangula* on urine and serum analysis against ethylene glycol induced urolithiasis in rats

Groups & Drug Treatment	Urine (mg/dl)			Serum (mg/dl)		
	Calcium	Phosphate	Oxalate	Creatinine	Uric Acid	Urea Nitrogen
Group I Normal Control	1.25±0.08	3.55±0.03	0.54±0.27	0.80±0.02	1.88±0.02	38.27±2.43
Group II Urolithiatic Control Ethylene Glycol (0.75%)	6.04±0.43	7.73±0.52	4.67±0.03	1.63±0.01	3.48±0.29	54.04±3.69
Group III Reference Control Cystone (750mg/kg)	1.55±0.01***	4.03±0.32***	0.63±0.05***	0.95±0.07***	1.48±0.01**	40.52±3.72**
Group IV <i>Luffa acutangula</i> (200mg/kg)	4.66±0.02*	5.03±0.25**	2.74±0.01*	1.08±0.01*	2.63±0.02	48.64±2.27*
Group V <i>Luffa acutangula</i> (400mg/kg)	2.42±0.03***	4.35±0.42***	1.03±0.01***	0.98±0.05***	1.84±0.01**	41.32±3.95**

Values are in mean ± SEM (n=6),

*P<0.05 , **P<0.01, ***P<0.001 Vs Urolithiatic Control

CONCLUSION

The ethanolic root extract of *Luffa acutangula* studied for its antiurolithiatic activity against ethylene glycol induced urolithiasis in rats. From the results it was concluded that, *Luffa acutangula* root extract exhibited antiurolithiatic activity. Further study may be requiring in order proving its mechanism of action.

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