

Research Article



Evaluation of Diuretic and Anti Urolithiatic Activity of the Whole Plant *Abutilon indicum* Ethanolic Extract in Albino Rats

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ABSTRACT

The ethanolic and chloroform extract was tested for diuretic and antiurolithiatic activity. The parameters taken for diuretic activity were total urine volume and concentration of ions like Na⁺, K⁺ and Cl⁻. Furosemide was used as reference diuretic. Urolithiatic activity was induced in rats by using 0.75% of ethylene glycol in drinking water for 28 days. Then in the assessment of urolithiatic activity electrolytic analysis, urine analysis and kidney homogenate analysis were taken into consideration. The effect of diuretic and anti urolithiatic activity for chloroform and ethanolic extract was studied at a dose level of 400mg/kg. The ethanolic extract had a significant diuretic and anti urolithiatic activity which proved the folk lore claim and can be used as a potent drug for urolithiasis.

Keywords: Abutilon indicum, ethylene glycol, chloroform extract, ethanolic extract, diuretic, anti urolithiatic activity.

INTRODUCTION

Kidney stones occur more frequently in men than women and are common between 20 to 40 years. People who took excess of protein, calcium and oxalate in their diet are more prone to stone formation. People who do not take enough oral fluids may also be prone to stone formation.

70% of people with a rare hereditary disease called renal tubular acidosis, cases of hyper uricosuria, excess intake of vitamin D and blockage of the urinary tract develops kidney stones¹.

Most of the plants have a significant role in the reduction of kidney stones and proves to be effective without any side effects.

One such plant in the traditional system of Indian medicine is *Abutilon indicum* belonging to the family Malvaceae commonly called Tutti in Tamil. The drug is extensively used for treatment of piles, leucoderma, leprosy, bronchitis and stones in bladder².

The chemical constituents reveal the presence of alkaloids, glycosides, steroids, tannins, terpenoids, flavonoids and quinines³⁻⁵.

The present study is to ensure the possible therapeutic potential of the whole plant of *Abutilon indicum* on the basis of scientific background.

Plant Collection and Authentication

The plant material of whole part used for the investigation was collected from Chennai in the month of November 2006. The plant was identified and authenticated by Prof. P. Jayaraman. Ph.D., Plant Anatomy Research Centre (PARC) Medicinal Plant Research Unit No. 4-II Street, Sakthi Nagar, West Tambaram, Chennai, 600 045.

Animals

Rats were produced by Vels University, Chennai, India. They were housed in standard micro loan boxes with standard laboratory diet and water ad libitum.

The experimental protocol was approved by the institutional animal ethics committee (IAEC) Vels University, Chennai, India.

MATERIALS AND METHODS

Animals

Healthy adult male albino rats of Wister strain weighing 150-120 gms were selected for the study.

The animals were acclimatized to standard condition temperature 25 ± 2 °C and fed with standard animal feed (Hindustan lever limited) and water ad libitum.

The protocol was approved by animal ethics committee constituted for the purpose as per CPCSEA guideline.

Drug Treatment and Experimental Design

Plant Material

The whole plant of *A.indicum* was collected in month of September from Chennai and authenticated by Prof. Dr. Jeyaraman, Director, plant anatomy research center, Chennai and a voucher specimen is kept in department of Pharmacognosy, Vel's College of Pharmacy for future reference.

Preparation of Extract⁶

The shade dried coarsely powdered entire plant of *A.indicum* was extracted by chloroform, ethanol(95%) and water by soxhlet apparatus at a room temperature of 60-70 °C. the extracts were dried using vacuum flash evaporator to yield a solid mass.



A suspension of EEA1 and CEA1 in 10% aqueous. Tween 80% was prepared for oral administration by gastric intubation method.

Experimental Design

Diuretic Activity⁷

The method adopted was lipschitz test. Four groups of sin rats weighing 150-250 gm were fasted and deprived of water for 24 hrs prior to the experiment on the day of experiment animals were given normal saline orally (10ml/kg body weight). Group one received 10ml of saline served as vehicle control. Group II was treated with standard (furosemide) 10mg/kg per oral route. Group III and group IV were treated with CEA1 and EEA1 at a dry level of 400mg/kg per oral route. Then the chemicals were placed in metabolic cage specially designed to separate the urine and faeces. At the end of 6th and 24th hr urine collected was measured and by using flame photometer (Elico pvt. Ltd model CL 22A) Sodium and potassium was estimated. Chloride was estimated by Schales and Schales method (Robert H.Carmen M.D. 1993).

Acute Toxicity Study^{8,9}

The acute oral toxicity study was carried as per the guidelines set by organization for economic cooperation and development (OECD) received from committee for the purpose of control and supervision of experiments on animals (CPCSEA). The chloroform and ethanolic extract were administered at the dose level of 4000mg/kg. One tenth of the median lethal dose (LD50) was taken as an effective dose (Anumpame and Handa, 1990).

Ethylene Glycol Induced Urolithiasis Model¹⁰⁻¹²

Ethylene glycol induced hyperoxaluria model (Atmani) was used to assess the antiurolithiatic activity in albino rats. Animals were divided into five group containing sin animals each. Group I served as vehicle control and received regular rat food and drinking water and libitum. Ethylene glycol (0.75%) in drinking water was fed to group II – V for induction of renal calculi till 28th day. Group II served as lithiatic control (0.75%). Group III received standard drug cystone (750mg/kg body weight) from 15th day till 28th day. Group IV received CEA1 400mg/kg body weight and group V received EEA1 400mg/kg body weight from 1st day till 28th day. All the extracts and standard drug were given daily by oral route.

Assessment of Anti Urolithiatic Activity

Analysis of Urine¹³

All animals were kept in individual metabolic cages and urine samples of 24 hr were collected on 28th day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Then the urine collected was analyzed for calcium, oxalate, phosphate and protein content (Robert 1993).

Serum Analysis¹⁴

After the experimental period, blood was collected from the retro orbital region under anesthetic condition and animals were sacrificed by cervical decapitation. Serum was separated but centrifugation at 10,000×gm for 10 min and analyzed for creatinine, uric acid, urea, nitrogen and albumin content (Robert, 1993).

Kidney Homogenate Analysis¹⁵

The abdomen was cut open to remove both kidneys from each animal. Isolated kidney were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 minutes and homogenized. The homogenate was centrifuged at 2000×g for 10 minutes and the supernatant was separated (Chow). The calcium (Medeiros and Mustafa 1985), phosphate (Fiske and Subbarao 1925) and oxalate (Hodgkinson and Williams, 1972) content in kidney homogenate were determined.

Statistical Analysis

All data were expressed as mean ±SEM. Statistical significant test was done by one way ANOVA followed by TUKEY KRAMER multiple companion test (Spiegel and Meddis, 1980). Differences between the data were considered significant at p<0.05.

DISCUSSION

In the present study of assessment of diuretic activity Lipschitz method was employed. Results predicted that the ethanolic extract of *Abutilon indicum* possess the potent diuretic activity. The diuretic activity was comparable to that of standard drug furosemide. The ethanolic extract of *Abutilon indicum* significantly increase the total volume of urine output and excretion of Na⁺, K⁺ and Cl⁻ (Table 1).

The diuretic activity of the extract was indicated by increase in water excretion and excretion of sodium, potassium and chloride. The preliminary phytochemical analysis of the plant showed the presence of alkaloids, steroids, tannins, phenolic compound, terpenoids and flavonoids.

It can be suggested that the above phytoconstituents may be responsible for diuretic activity. For urolithiasis from the acute toxicity study the LD50 dose was found to be 4000mg/kg body weight of the extracts. So the therapeutic dose was taken as 400mg/kg body weight.

The chronic administration of 0.75 %v/v ethylene glycol to the Wistar rats resulted in hyperoxaluria. The intake of ethylene glycol is rapidly absorbed and metabolized in the liver via alcohol or aldehyde dehydrogenase to glycolic acid. The glycolic acid is oxidized to glyoxylic acid which is further oxidized to oxalic acid or oxalate by glycolate oxidase or by lactate dehydrogenase thereby resulting in hyperoxaluria. So there is further increase in calcium, uric acid, phosphate excretion in calculi induced animals.



However treatment with the ethanolic extract of whole plant of *Abutilon indicum* significantly lowered the elevated levels of calcium and phosphate in urine and in kidney which was compared with cystone treated group (Table 3). Since the phosphate and uric acid level increase in urine, serum enhances crystallization which combine with urine to form stone due to super saturation in ethylene glycol induced group where as the group receiving the ethanolic extract of *Abutilon indicum* showed significant results in formation of hyperoxaluric acid and stone formation.

Further the standard and ethanolic extract group showed good diuretic activity with non-formation of calculi which prevented pain and reduction of body weight which was reverted in group receiving ethylene glycol (i.e.) reduction in consumption of food due to intense pain and further decrease in body weight was observed.

The serum, uric acid and BUN were remarkably increased in calculi induced animals. Due to induced nephro toxicity waste nitrogenous substance like BUN accumulate thereby resulting in decreased excretion of urea nitrogen in urine and creatinine. But the standard and test treatment group significantly prevent the damage to the kidney which was observed by the decline in urea and creatinine level. The administration of the ethanolic extract of *Abutilon indicum* prevents the impairment of renal function.

The histopathological studies showed changes like damaged epithelial cells at the inner layer dilation of tubules and presence of crystals in the tubules. However kidney section of group treated ethanolic extract of *Abutilon indicum* shows improvement in dilation of tubules, tubule interstitial inflammatory in filtration and reduced crystal deposition.

Table 1: Diuretic activity of whole plant of *Abutilon indicum*

Dose mg/kg	Treatment	Volume of urine mg/lit	Sodium meq/l	Potassium meq/l	Chloride meq/l
10ml 10% tween 80 p.o	Control	4.5 ± 0.0308	72.5 ± 0.619	27.0 ± 0.365	46.00 ± 0.365
10ml p.o	Standard (Furosemide)	7.18 ± 1.005	98.16 ± 0.477	94.166 ± 0.400	103.33 ± 0.421
400mg p.o	CEAI	6.1 ± 0.042	85.833 ± 0.477	76.114 ± 0.601	86.44 ± 0.333
400mg p.o	EAI	6.3 ± 0.114**	103.66 ± 0.401**	88.166 ± 0.494**	92.33 ± 0.601**

Data were analyzed by one way ANOVA followed by Dunnett's 't' test. Multiple comparison test n=6 value are expressed as mean ± SEM significance level ** p<0.01.

Table 2: Effect of *Abutilon indicum* for diuretic activity

S. No	No. of Animals	Treatment	Dose	Urine Volume (ml/rat)				
				2hr	4hr	6hr	8hr	24hr
1	6	Control	10ml/kg oral route	1.1 ± 0.363	2.1 ± 0.476	2.6 ± 0.0334	3.1 ± 0.0223	4.5 ± 0.0308
2	6	Standard (furosemide)	10ml/kg oral route	3.3 ± 0.054	4.3 ± 0.0996	4.9 ± 0.048	5.9 ± 2.449	2.5 ± 1.005
3	6	Test-I (chloroform extract)	400mg/kg oral route	2.1 ± 0.054	3.2 ± 0.087	3.9 ± 0.605	4.6 ± 0.149	1.8 ± 0.042
4	6	Test-II (ethanolic extract)	400mg/kg oral route	2.6 ± 0.060**	3.6 ± 0.057**	4.3 ± 0.051**	5.6 ± 0.036**	2.2 ± 0.114**

Statistical significant test for comparison was done by ANOVA, followed by Dunnett's 't' test (n=6). **p<0.01

Table 3: Effect of *Abutilon indicum* extracts on serum, kidney and urinary parameters in experimental animals

S. No.	Parameter	Group-I Normal	Group-II Control ethylene glycol induced	Group-III Standard Cystone treated	Group-IV Test-I Chloroform extract	Group-V Test-II Ethanol extract
1	Urine (mg/dl) calcium phosphate	3.01 ± 0.119	8.31 ± 0.152	3.43 ± 0.130	4.17 ± 0.079	3.96 ± 0.076**
		3.63 ± 0.049	7.71 ± 0.060	4.61 ± 0.130	5.15 ± 0.042	4.95 ± 0.067**
2	Kidney (mg/gm) Calcium phosphate	1.65 ± 0.042	7.21 ± 0.151	3.48 ± 0.083	5.11 ± 0.043	3.81 ± 0.119**
		1.58 ± 0.047	6.31 ± 0.047	2.51 ± 0.047	3.11 ± 0.151	2.66 ± 0.135**
3	Serum (mg/dl) Nitrogen (BUN)	24.5 ± 0.428	90.5 ± 0.428	38.5 ± 0.428	44.38 ± 0.872	38.99 ± 2.629**
	Creatinine	0.72 ± 0.017	2.75 ± 0.042	0.90 ± 0.028	1.86 ± 0.016	1.05 ± 0.048**
	Uric acid	1.30 ± 0.010	3.7 ± 0.048	1.725 ± 0.026	2.86 ± 0.049	1.82 ± 0.048**

Statistical significant test for comparison was done by ANOVA, followed by Dunnett's 't' test (n=6) **p<0.01



CONCLUSION

The evaluated extract showed significant diuretic activity after an administration of 400mg/kg body weight. They showed best diuretic and superior urine excretion of Na⁺ and K⁺ ion excretion compared with standard and negative group. The observed diuretic activity seems to be effective in preventing the deposition of calculi which showed the plant extract prevents formation of urinary stone thus confirming the folklore claim as anti urolithiatic activity. Further a detailed investigation for the isolation of constituents responsible for diuretic and anti urolithiatic study has to be confirmed.

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