International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

EVALUATION OF DIURETIC AND ANTIUROLITHIATIC PROPERTIES OF ETHANOLIC EXTRACT OF *SIDA ACUTA* BURM F. IN WISTAR ALBINO RATS

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Received: 27 Jan 2016 Revised and Accepted: 15 Mar 2016

ABSTRACT

Objective: To evaluate the diuretic and antiurolithiatic properties of ethanolic extract of *Sida acuta* Burm. f. in albino rats.

Methods: Three different doses of *Sida acuta* (100 mg/kg, 200 mg/kg, 400 mg/kg) were tested for diuretic activity and compared with furosemide (25 mg/kg) and control group. 24 hr urine output and urinary concentrations of sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions were the parameters measured. Prophylactic and curative antiurolithiatic activity was tested for three doses (100 mg/kg, 200 mg/kg & 400 mg/kg) of *Sida acuta* and compared with control group and animals treated with standard herbal preparation Cystone (750 mg/kg) using zinc disc implantation model.

Results: *Sida acuta* (400 mg/kg) significantly increased the urine output ($p<0.001$), which was almost equivalent to furosemide. *Sida acuta* also caused a significant increase in the excretion of K^+ and Cl^-at higher doses (200 mg/kg, 400 mg/kg). Effects on Na^+excretion were not significant for all three doses of the test drug. *Sida acuta* (200 mg/kg, 400 mg/kg) showed statistically significant ($p<0.05$) reduction in depositions around zinc discs as compared to control when tested in prophylactically treated rats, but not in rats treated post-urolithiasis induction.

Conclusion: *Sida acuta* produced a dose-dependent increase in urine output, with no effect on sodium excretion. This probably indicates that the diuresis produced could be due to its aquarectic action. It also showed significant prophylactic antiurolithiatic activity, but further studies are necessary to evaluate fully its therapeutic potential.

Keywords: Zinc disc implantation, Calculi, Diuretic index, Cystone

INTRODUCTION

Urolithiasis has been one of the oldest medical conditions known to mankind. It refers to the formation of stone anywhere in the urinary tract and is the third most common condition of the urinary tract. Current global estimates show that urolithiasis affects around 12% of the population, with the recurrence rate being 70-80% in males and 47-60% in females [1]. Risk of developing urolithiasis is greater in the Western Hemisphere as compared to the east [2]. Calcium oxalate (CaOx) stones are the most predominant variety encountered [1]. Size and location of the stone dictates the therapeutic modality utilized for managing urolithiasis. Larger stones (more than 5 mm) or those which fail to pass through the urinary tract spontaneously require interventional procedures such as extracorporeal shockwave lithotripsy, percutaneous nephrolithotomy, etc. for their removal [3]. However, these procedures are expensive, and recurrences are quite common [4]. In spite of recent advancements, medical therapy of stone disease is limited and there is a dire need for the development of novel pharmacological agents in urolithiasis [5]. Moreover, because of its high recurrence rate, development of medical prophylactic regimens is also necessary [6].

Several plant extracts have been used to treat urolithiasis with promising effect. *Sida acuta* Burm. f. has been used in alternative systems of medicine to treat various diseases. Leaves of the plant are considered to possess demulcent, diuretic, anthelmintic and wound healing properties [7]. The roots are considered to possess stomachic, diaphoretic, antipyretic, astringent properties and also used the treatment of nervous and urinary diseases [8]. *In vitro* studies have shown the inhibitory capacity of methanolic and aqueous extracts of the root of *Sida acuta* on CaOx crystal growth [9]. This shows its potential as an antiurolithiatic agent. Many of the herbs with antiurolithiatic activity have also been observed to possess significant diuretic activity [10]. Diuretic activity, in turn, contributes to the antiurolithiatic action of these plants. Increased urine output increases the solubility of CaOx and other crystallizing salts and thus facilitates removal of small crystals and prevents their aggregation and growth [11]. However, a literature search revealed that no *in vivo* studies have been conducted with *Sida acuta* for evaluating these properties.

Hence, the following study was designed to evaluate the diuretic and antiurolithiatic properties of ethanolic extract of *Sida acuta* in Wistar albino rats.

MATERIALS AND METHODS

The study was started after obtaining clearance of the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal (IAEC/KMC/84/2013).

Chemicals, drugs, and instruments

Ethylene glycol (Merck, India), Furosemide (Sanofi-Aventis Pharma, India), Cystone (Himalaya Herbal Healthcare, Bangalore, India) and biochemical kits (Agappe Diagnostics Limited, Ernakulam, India) were purchased from authorized retailers. Star 21 Plus Semi Auto-analyser (Rapid Diagnostics Group, India) was used for the biochemical tests.

Plant material

The plant material was procured from Udupi, Karnataka, India and authenticated by a botanist from Mahatma Gandhi Memorial College, Udupi. The roots were shade dried and powdered using a mechanical grinder and sieved. 500 ml of 90% ethanol (solvent) was taken in a round bottom flask, which was attached to a Soxhlet extractor and condenser. 250 g of powdered plant material was loaded in a thimble, and placed inside the Soxhlet extractor. The extraction was done at 40 °C for 24 h. Then, the extract was subjected to water bath evaporation to remove ethanol. The concentrate obtained was weighed and stored in desiccators at room temperature for further use [12].

Three different doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were selected for the study as per previously reported data in literature [13].

Animals

Inbred male albino rats of Wistar strain weighing 150-200g were used for the study. The animals were housed under standard environmental conditions and were fed with standard pellet feed and water *ad libitum*.

Diuretic study

The rats were randomly divided into five groups of six rats each. They fasted overnight before the day of the experiment. All the rats received 25 ml/kg body weight of normal saline before drug administration to impose uniform salt and water load. They then received single dose of vehicle (control group), furosemide 20 mg/kg (standard group) and three different doses (100 mg/kg, 200 mg/kg, 400 mg/kg) of ethanolic root extract of *Sida acuta* (test groups), prepared in the same volume of vehicle (0.5% carboxymethyl cellulose). Immediately after drug administration, each rat was kept in a separate metabolic cage under standard housing conditions, and urine was collected over the next 24 h. During this period, rats had free access to water but not to food. The volume of urine collected over 24 h was measured and biochemically analysed for sodium, potassium and chloride levels using respective kits [14].

Calculation of diuretic index, saliuretic index and Na^+/K^+ratio

The following formulae were used for the calculation of different urinary parameters:

$$\text{Diuretic index} = \frac{\text{Mean urine volume of the test group}}{\text{Mean urine volume of the control group}}$$

$$\text{Saluretic index} = \frac{\text{Concentration of electrolyte in urine of the test group}}{\text{Concentration of electrolyte in urine of the control group}}$$

$$\frac{Na^+}{K^+}\text{ratio} = \frac{\text{Concentration of } Na^+ \text{in urine of a group}}{\text{Concentration of } K^+ \text{in urine of the same group}}$$

Antiurolithiatic study

Zinc disc implantation model was used for assessment of antiurolithiatic activity. Zinc discs were inserted in the urinary bladder of rats as per the method described by Vermeulen *et al.* [15]. Before anaesthesia, the rats were orally administered 5 ml of water to dilate their urinary bladder for easier identification. They were then anaesthetized with a combination of intraperitoneal ketamine (80 mg/kg) and xylazine (8 mg/kg). The abdominal skin was shaved and cleaned with surgical spirit. After making a suprapubic incision and opening the abdomen, the urinary bladder was carefully exposed. A small nick was made at the apex of the bladder, and the sterile zinc disc (10±2 mg) was carefully inserted into the bladder. The bladder was then closed with 1 or 2 stitches using chromic catgut (4-0), and then the abdomen was closed in layers. The skin was sutured with sterile silk thread. The rats were allowed to recover for 1 w during which food and water were given *ad libitum*.

Procedure of study

Three doses (100 mg/kg, 200 mg/kg & 400 mg/kg) of ethanolic extract of roots of *Sida acuta* were tested for antiurolithiatic activity and compared with the control group and animals treated with standard herbal preparation Cystone [16].

The prophylactic activity of *Sida acuta* against urolithiasis was tested in five groups of rats, each containing six rats. After recovery following zinc disc insertion, the control group was administered 1% solution of ethylene glycol in drinking water *ad libitum* for 4 w. Prophylactic treatment group (Standard) received Cystone 750 mg/kg orally daily along with 1% solution of ethylene glycol in drinking water *ad libitum* for 4 w. Prophylactic treatment groups (Test) received three different doses of ethanolic extract of *Sida acuta* (100 mg/kg, 200 mg/kg, 400 mg/kg) orally daily along with 1% solution of ethylene glycol in drinking water *ad libitum* for 4 w.

Curative property was tested in five groups of rats with six rats in each group. After recovery following zinc disc insertion, rats in the control group were administered 1% solution of ethylene glycol in drinking water *ad libitum* for 4 w followed by water for the next 4 w. Curative treatment (Standard) group received 1% solution of ethylene glycol in drinking water for 4 w *ad libitum* followed by Cystone 750 mg/kg orally daily for the next 4 w. Test group animals received 1% solution of ethylene glycol in drinking water *ad libitum* for 4 w followed by ethanolic extract of *Sida acuta*, in three doses (100 mg/kg, 200 mg/kg, 400 mg/kg) orally daily for the next 4 w.

At the end of the study period (4 w for prophylactic activity and 8 w for the curative property), rats were sacrificed and zinc disc implant/vesical calculi collected, weighed and statistically evaluated. The difference between initial and final weights indicated the amount of stone formed.

Statistical analysis

Data was analysed using analysis of variance (ANOVA) in SPSS version 20, followed by post hoc Tukey's test. Skewed data was analysed using Mann-Whitney test, preceded by Kruskal-Wallis test for significance. p-value less than 0.05 was considered significant.

RESULTS

Assessment of diuretic activity

Table 1 below shows the effect of different drugs on the urine output. The reference diuretic, furosemide, significantly increased 24 hr urine output when compared to control group ($p<0.001$), the diuretic index being 1.64. The test drug (*Sida acuta*), at a dose 400 mg/kg, showed a significant increase in urine volume as compared to control ($p<0.001$) and was comparable with furosemide ($p = 0.991$). *Sida acuta* at 100 mg/kg and 200 mg/kg showed an increase in urine output which was not statistically significant ($p = 0.414$, 0.051 respectively) when compared to control group. The diuretic index of *Sida acuta* 400 mg/kg (1.59) was less than that of furosemide (1.64).

Table 1: Effect of *Sida acuta* root extract on urine output

Group	Urine output (mL/24hr) [mean±SEM]	Diuretic index
Control	9.28±0.34	-
Furosemide 20 mg/kg	**15.22±0.74***	1.64
Sida acuta 100 mg/kg	11.22±0.95	1.20
Sida acuta 200 mg/kg	12.50±1.05	1.34
Sida acuta 400 mg/kg	**14.72±0.60***	1.59

* = $p<0.05$ as compared to control (One-way ANOVA followed by post-hoc Tukey's test); n (number of rats) = 6 in each group; Diuretic index = Volume of test group/Volume of control group; ANOVA = Analysis of variance; SEM = Standard error of mean

Table 2 shows the effect of furosemide and *Sida acuta* at varying doses on urinary excretion of Na^+, K^+ and Cl^-. Furosemide showed a significant increase in sodium ($p = 0.002$), potassium ($p<0.001$) and chloride ($p<0.001$) excretion as compared to control. *Sida acuta,* at 200 mg/kg and 400 mg/kg, showed significant increase in the urinary excretion of potassium ($p<0.001$ for both) and chloride ($p = 0.043$ and $p = 0.006$ for *Sida acuta* 200 mg/kg and 400 mg/kg respectively) as compared to the control group. However, *Sida acuta* 200 mg/kg was less efficacious than furosemide in its potassium excretory capacity ($p = 0.023$). Sodium excretion, on the other

hand, was not significantly higher in *Sida acuta* 200 mg/kg and 400 mg/kg groups as compared to the control group (p = 0.196 and p = 0.086 for *Sida acuta* 200 mg/kg and 400 mg/kg respectively). *Sida acuta* at 100 mg/kg showed a significant increase in the excretion of only potassium (p<0.001) when compared with control. The effect on sodium (p = 0.807) and chloride (p = 0.071) excretion was not statistically significant. *Sida acuta* 100 mg/kg was also significantly less efficacious when compared to furosemide in its capacity to excrete sodium (p = 0.022), potassium (p = 0.017) and chloride (p = 0.036). There was no statistically significant difference between furosemide and *Sida acuta* 400 mg/kg groups in their capacity to excrete Na^+, K^+ and Cl^-. Furosemide showed higher saluretic effect as compared to the test drug in different doses.

Table 2: Effect of *Sida acuta* root extract on urinary excretion of sodium, potassium & chloride ions

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Saluretic index			Na^+/K^+
				Na^+	K^+	Cl^-	
Control	89.36±5.38	52.82±0.83	82.19±4.88				
Furosemide 20 mg/kg	126.16±6.62*#	93.05±3.15*#¶	107.45±1.68*#	1.41	1.76	1.31	0.80
Sida acuta 100 mg/kg	98.54±5.85	82.37±3.10*	94.15±2.02	1.10	1.56	1.15	0.70
Sida acuta 200 mg/kg	108.17±6.26	82.77±1.10*	95.16±1.83*	1.21	1.57	1.16	0.77
Sida acuta 400 mg/kg	111.79±5.42	86.00±1.75*	98.8±3.54*	1.25	1.63	1.20	0.77

* = p<0.05 as compared to control, # = p<0.05 as compared to *Sida acuta* 100 mg/kg, ¶ = p<0.05 as compared to *Sida acuta* 200 mg/kg (One-way ANOVA followed by post-hoc Tukey's test); n (number of rats) = 6 in each group; Saluretic index = Concentration of test group/Concentration of control group; ANOVA = Analysis of variance; Na^+= Sodium, K^+= Potassium, Cl^-= Chloride; Na^+, K^+, Cl^-values expressed as mean±Standard error of mean

Assessment of antiurolithiatic activity

Table 3 shows the effect of different treatment groups on prophylactic antiurolithiatic activity. Standard drug Cystone (p = 0.013), *Sida acuta* at 200 mg/kg (p = 0.016) and 400 mg/kg (p = 0.013) showed significant decrease in crystal deposition around zinc disc as compared to control but with *Sida acuta* at 100 mg/kg (p = 0.109) the difference was not statistically significant. There was no statistically significant difference between the median weights of deposit around zinc discs in the Cystone and *Sida acuta* 200 mg/kg (p = 0.337), 400 mg/kg (p = 0.575) groups.

Results of testing for curative antiurolithiatic property among different treatment groups are given in table 4. There was no statistically significant difference between control, Cystone and *Sida acuta* at three different doses (100 mg/kg, 200 mg/kg, 400 mg/kg) for the curative effect on urinary stones (p = 0.587).

Table 3: Prophylactic activity of *Sida acuta* root extract on crystal deposition around zinc disc

Group	Deposit weight in mg [Median (Q_1, Q_3)]	p value
Control	133 (91.5, 240.25)	-
Cystone 750 mg/kg	47 (21.25, 88.50)	**0.013***
Sida acuta 100 mg/kg	86.5 (30, 127.25)	0.109
Sida acuta 200 mg/kg	59 (48, 80.25)	**0.016***
Sida acuta 400 mg/kg	58.5 (27.5, 89.25)	**0.013***

* = p<0.05 as compared to control (Mann-Whitney test); n (number of rats) = 6 in each group

Table 4: Curative activity of *Sida acuta* root extract on crystal deposition around zinc disc

Group	Deposit weight in mg [Median (Q_1, Q_3)]
Control	168 (38.75, 398.5)
Cystone 750 mg/kg	81.50 (12, 137.75)
Sida acuta 100 mg/kg	97 (90.50, 119.75)
Sida acuta 200 mg/kg	99 (30.50, 131.75)
Sida acuta 400 mg/kg	57.5 (16.75, 141.75)

p = 0.587 (Kruskal-Wallis test); n (number of rats) = 6 in each group

DISCUSSION

This study evaluated the diuretic and antiurolithiatic activity of orally administered ethanolic root extract of *Sida acuta* Burm. f. in normal albino Wistar rats. *Sida acuta* was selected for the study since it has been used for the same purpose in traditional medicine and in *in vitro* studies. Furosemide, a widely used diuretic, was used as the reference diuretic and Cystone was used as the standard antiurolithiatic drug. All the drugs were administered orally, since oral route is the most common route of drug administration, especially with traditional herbal medicine.

Diuretics are drugs that increase the rate of urine flow. Clinically useful diuretics also increase the excretion rate of Na^+ion, which is a major determinant of extracellular fluid volume. Furosemide acts by inhibiting Na^+-K^+-$2Cl^-$ symporter and thereby, interfering with their absorption [17].

Even though *Sida acuta* is said to possess diuretic activity [7, 18], no study has been published till date to validate the diuretic activity of ethanolic extract of its root, to the best of our knowledge.

In the present study, although all the test groups showed an increase in urine output as compared to the control, the increase was significant only with high dose *Sida acuta* (400 mg/kg). The diuretic activity is considered to be good when the diuretic index values are higher than 1.50, moderate when the values are between 1.00 to 1.50, mild when the values are from 0.72 to 1.00 and no diuretic activity exists with values below 0.72 [19]. Both furosemide group and *Sida acuta* 400 mg/kg group showed a diuretic index value>1.50, indicating good diuretic activity.

Furosemide produced a significant increase in the excretion of all the three major urinary electrolytes, Na^+, K^+ and Cl^- with respect to control. This shows its saluretic capacity. All the three doses of *Sida*

acuta produced a dose-dependent increase in the excretion of Na^+, K^+ and Cl^-. However, significance was noted with respect to K^+ excretion in all the groups, and Cl^-excretion in medium (200 mg/kg) and high dose (400 mg/kg) of the test drug. This probably indicates a different mode of action for *Sida acuta* compared to furosemide. The mineral content of herbs can also be a confounding factor contributing to the diuretic effect of plant extracts. Plant extracts with diuretic activity were observed to have a high ratio of potassium to sodium content [20]. Hook *et al.*, in a study conducted with dandelion, concluded that its high potassium content was responsible for the diuretic activity [21]. Herbal diuretics without significant action on the excretion of urinary electrolytes are considered aquarectics [22]. Increased urine output by aquarectics may prove to be beneficial in kidney stones, and for the treatment of mild urinary infections [22]. Na^+/K^+ ratio greater than 10.0 indicate potassium sparing effect [23]. Since all the treatment groups had lesser values, potassium-sparing action can be ruled out.

Zinc disc implantation induced urinary bladder calculi model is one of the commonly used models for preclinical evaluation of the antiurolithiatic activity. This model induces urinary calculi without severe renal damage and mimics the etiology of stone formation in humans. The main component of crystals formed is magnesium ammonium phosphate [16]. Ethylene glycol administration following zinc disc implantation promotes the development of CaOx crystals [24]. Vimala *et al.*, demonstrated the *in vitro* inhibitory effect on CaOx crystal growth by aqueous and methanolic extracts of roots of *Sida acuta* [9].

There was a high degree of variation in the size of stones formed in all the treatment groups. Test for prophylactic antiurolithiatic activity showed a significant decrease in crystal deposition with a medium and high dose of *Sida acuta,* which was comparable with standard drug Cystone. This confirms the potential of *Sida acuta* as a prophylactic antiurolithiatic agent. The diuretic action of the extract could have led to the inhibition of stone formation.

Due to very high intra-group variation in stone sizes among rats tested for curative antiurolithiatic activity, there was no statistically significant difference between the groups. However, median weight of stones in the different groups displayed a trend towards dose-dependent curative effect on the formed stones. High variation in stone size could be due to physiological variation among individual rats or due to the long duration of the study. Singh *et al.*, demonstrated that there is sufficiently large depositions around zinc discs even within 10 d of implantation [25]. They also showed that the rats suffer from severely compromised renal functions due to increasing crystal size for studies beyond 20 d [25], which may further promote the development of large stones. Further studies, either of a shorter duration or using other models, need to be conducted to evaluate the complete antiurolithiatic potential of *Sida acuta.*

Oxalate, one of the commonest ion implicated in urolithiasis, is known to produce renal tubular epithelial cell death by oxidative stress, and may promote the formation of stones [26, 27]. Londonkar *et al.*, identified the presence of various alkaloids, steroids, carbohydrates, glycosides, amino acids, saponins, flavonoids, anthocyanins, fatty acids and phenolic compounds in the ethanolic extract of *Sida acuta* Burm. f [28]. Many of these compounds are known to possess antioxidant activity, and may have a protective effect in urolithiasis [29, 30]. Hence, further research is needed to identify the exact compound in *Sida acuta* responsible for its diuretic and antiurolithiatic activity.

CONCLUSION

Even though *Sida acuta* showed significant diuretic activity, with the high dose (400 mg/kg) being almost equivalent to furosemide in increasing urine output, it didn't produce a significant increase in the excretion of major urinary electrolyte, sodium. Hence, it can be considered to be an aquarectic rather than a diuretic. *Sida acuta* also showed significant activity as a prophylactic antiurolithiatic agent, with the higher dose being almost equi-efficacious as Cystone. However, the results were inconclusive when tested for curative anturolithiatic activity. Further studies are required to evaluate completely the antiurolithiatic potential of *Sida acuta* Burm. f. and the active constituents responsible for the same.

CONFLICT OF INTERESTS

Declared none

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