INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

LITHOTRIPTIC EFFECT OF COMBINATION OF MATSYAKSHI (*ALTERNANTHERA SESSILIS* Linn. R.Br.,) AND TENDER COCONUT WATER IN ALBINO RATS

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Article Received on: 02/08/17 Approved for publication: 03/09/17

DOI: 10.7897/2230-8407.089158

ABSTRACT

Conversion of the traditional medicinal information into knowledge and its validation through scientific procedures is the need of the day. In the text Yogamrutha [Traditional Ayurvedic text in Kerala]; it is indicated that the kalka [paste] of Matsyakshi (*Alternanthera sessilis* Linn. R Br.) in narikelodakam (tender coconut water) has lithotriptic property. The present study evaluated the *In vivo* lithotriptic effect of Matsyakshi (*Alternanthera sessilis* Linn. R Br.) along with tender coconut water (*Cocos nucifera* Linn.) in ethylene glycol induced urolithiasis. For inducing calculi, 0.75 %(v/v) ethylene glycol was given along with drinking water for all groups except the normal control group (Group I) for 28 days. Then the treatment phase started and continued for next 28 days. The vehicle control group (Group II) received tender coconut water (0.86 ml/200g rat). The standard drug group (Group III) was treated with cystone (13.5mg/200g rat). Groups IV, V and VI received test drug (0.054g/100g, 0.108g/100g, and 0.216g/100g kalka respectively) along with tender coconut water. Parameters like serum calcium, serum uric acid serum creatinine, urine creatinine, urine uric acid and urine calcium were used to assess the activity. Animals treated with the test drug (Group IV, Group V and Group VI) showed highly significant reduction in all the six parameters. This suggests the ability of the test drug to reduce the biochemical changes induced by ethylene glycol and the lithotriptic effect of the drug.

Keywords: Lithotripsy, Matsyakshi, *Alternanthera sessilis* Linn. R.Br, Ethylene glycol.

INTRODUCTION

Ayurveda is one among the most ancient sciences in the world, in which most of the remedies have been taken from plants. In addition to classical ayurvedic literature, many regionally available traditional texts are there, which are the compilations of clinical experiences of physicians. These books have documented many medicinal formulations and their therapeutic indications. Yogamrutha is one among the important traditional books used by Ayurvedic physicians in Kerala. In this text, remedies using cost effective and regionally available herbal drugs are described. They are found to be useful, though the rationale behind their use is not well established through systematic pharmacological and clinical studies, except for some herbal drugs and plants. In Yogamrutha, it is indicated that the kalka (paste) of Matsyakshi [*Alternanthera sessilis* Linn. R.Br] in nalikerodakam (tender coconut water) has lithotriptic property[1].

The details regarding the synonyms and pharmacological properties of the plant matsyakshi are obtained from various Nighantu (Lexicons). As per the classical reference the plant can be used in the management of kapha-pitta-ratha vikaras. The plant is also observed to be an ingredient in the some of the formulations indicated for urinary system pathologies, in Chikitsamanjari, Yogamruta and some other traditional Ayurvedic texts in Kerala. According to Ayurvedic pharmacopeia of India, the botanical identity of Matsyakshi is *Alternanthera sessilis* Linn. R. Br[2]. It is a very common plant that is seen throughout India, mainly in moist places. It has proven anti inflammatory[3], anti hyperglycemic properties[4]; hematinic[5], analgesic activities[6] and also found effective in ocular therapy. But the lithotriptic property of the plant *Alternanthera sessilis* has not yet been proved.

The properties of Nalikera udaka [tender coconut water] is mentioned in almost all classical texts. Nalikera udaka has vasthi sodhana (diuretic) property[7]. It is sweet, and sterile and composed of unique chemicals such as sugars, vitamins, minerals, electrolytes, enzymes, amino acids, cytokine, and phyto-hormones. Tender coconut water has been shown to exhibit hepatoprotective, antioxidant and dyslipidemic actions[8]. It is also revealed to have anti-inflammatory[9], cardiopotective[10], antihypertensive[11] and renal protective activity[12].

Urolithiasis is a common disorder in global countries estimated to occur in approximately 12% of population, with a recurrence rate of 70-81% in males and 47-60% in females[13]. Renal stone disease is about 2-3 times more common in males than in females. Urinary calculi form as a result of physicochemical or genetic derangements leading to super saturation of the urine with stone forming salts or less commonly from recurrent urinary tract infection with urease producing bacteria. Urolithiasis is a challenging problem nowadays and many standard pharmaceutical drugs are available, they have a serious adverse effect that compromise long term use.

Urolithiasis can be correlated to the disease Muthrasmari mentioned in classical text books of Ayurveda. The description regarding the disease is seen even in Vedas. Ashmari has been

mentioned in all ayurvedic classical texts and has been mentioned one among the eight mahagadas (dreadful diseases)[14].

The increasing incidence of urolithiasis has created an incentive for the research. The present study plans to systematically evaluate and verify the use of *Alternanthera sessilis Linn. R. Br* along with tender coconut water in kidney and bladder pathologies; as the literature survey showed that no previous research was done to support the above use. The convertion of these traditional medicinalinformation into knowledge and its validation through scientific procedures is the need of the day.

MATERIALS AND METHODS

Experimental animals

36 Healthy adult albino rats of either sex with body weight between 150gm – 250gm were procured from the Animal house of Govt. Ayurveda College, Thiruvananthapuram. The animals were housed in polypropylene wired gauge cages (42×26.5×15cm) with a clean bedding of husk. They were acclimatized for a period of 14 days prior to performing the experiment. They were maintained under standard laboratory conditions (temperature24-28º C, relative humidity 60-70%, and 12 hour dark and light cycles). The animals were provided standard laboratory pelleted rat feed and water *ad libitum*. The experimental protocols were approved by the Institutional Scientific and Ethical Committee (Order no: IEC.133, dated 28/04/2015) and a written permission from Institutional Animal Ethical Committee, Govt. Ayurveda College, Thiruvananthapuram (Order no: IAEC No: 28; dated: 24/4/2015) was obtained. The study was taken and carried out as per the standard guidelines for the maintenance and the use of experimental animals.

Research design

Ethylene glycol induced urolithiasis model, in Wistar Albino rats using modified Sunitha et al method[15].

Drugs and chemicals

Ethylene glycol (EG), Di ethyl ether. All the chemicals used were of analytical grade. Cystone tablets (The Himalaya Drug Company, Bangalore, India; Batch No. 19300426F; Mfg. Date: Sep.-2013; Exp. Date: Sep-2018)

Study drugs

Plant material

The plant drug *Alternanthera sessilis* Linn. R.Br. was procured from the Botanical garden, Government Ayurveda College, Thiruvananthapuram.

The test drug, Matsyakshi [*Alternanthera sessilis* Linn. R.Br.] was given in the form of Kalka [fine paste of macerated fresh plant material] along with tender coconut water. Kalka was prepared based on the procedure mentioned in API[16]. Dose of kalka for 200g rat comparable to adult human therapeutic dose calculated using Paget & Barnes conversion factor (1964)[17]. Thus the animal dose of kalka was calculated as 0.216mg/200g rat as the adult therapeutic dose of kalka is 12g[18].

The tender coconut water was collected daily. The tender coconut was procured from a local vendor, and it was then opened and the liquid endosperm was collected and kept in a clean vessel. In human, the Kalka (macerated paste) is administered with four times anupana (adjuvant) [18]. So for 12g kalka, 48 ml anupana is needed. Therefore, the effective animal doses of anupana as per Paget and Barns conversion factor for 200g rat was 0.864ml.

The stock solution for test drug administration was prepared by mixing 5g matsyakshi kalka with 20 ml tender coconut water.

For the vehicle control group (Group II), 0.864ml/200g rat was given as per Paget and Barns Conversion factor, as the human dose for anupana (adjuvant) is 48ml (for 12 g kalka)

Standard drug: The human dose of cystone was taken as 750mg/kg [19]. From this, the rat dose was calculated by using the Paget and Barnes conversion factor. Based on this the dose was 13.5 mg/200g body weight. The stock solution was prepared by dissolving 67.5 mg drug in 1 ml water (67.5mg/1kg/1ml).

Ethylene glycol induced urolithiasis in albino rats

The acclimatized albino rats were weighed and grouped into six (Group I, II, III, IV, V and VI) with 6 animals in each group. Selection was done randomly so as to assure equal distribution of sex, body weight etc. in each group. Then, the animals were marked for proper identification and kept each group in separate cages. Each cage was labeled separately for group identification.

Procedure

Group I was the normal control group and was maintained on regular rat feed and drinking water *ad.libitum* throughout the study period. All remaining groups (Group II-Group VI) received calculi inducing treatment for 28 days, comprised 0.75 % v/v ethylene glycol in drinking water. On 28th day, the ethylene glycol administration was stopped. From the next day onwards, the treatment phase started. The albino rats of each group were again weighed; and the respective doses were calculated. The animals were administered with the test drug, standard drug and the adjuvant for a period of 28 days. The group II; which is the vehicle control group received tender coconut water (0.86 ml/200g rat) for the subsequent 28 days. The Group III; which was the standard drug group; was treated with 13.5 mg/200g of cystone once daily for 28 days. Group IV; which was the test drug group and the animals in this group received the test drug in half dose. Group V was the test drug group and was administered with the test drug in effective therapeutic dose. Group VI was also the test drug group; which received the test drug in double dose. Microscopic examination of urine samples was done again for checking the stage of crystals. Blood samples and urine samples were collected and the parameters were analyzed.

Experimental groups and their treatment schedule is illustrated in Table 2.

Collection and analysis of urine

The urine samples were collected after the lithogenic phase (after the administration of EG for 28 days) and after the treatment period. All animals were kept in individual metabolic cages and urine samples of 24 hour were collected. Animals had free access to drinking water during the urine collection period. After urine collection, the urine samples were analyzed for total urinary excretion of calcium, uric acid and creatinine

Table 1: Different therapeutic doses of Matsyakshi kalka

	Half	Therapeutic	Double
Dose (g/100g body weight)	0.054	0.108	0.216

Table 2: Experimental groups and their treatment schedule

Name of group	Treatment of animals of each group
Group I	Animals on normal diet and water
Group II	Adjuvant control of test drug- animals on 0.75 % ethylene glycol in drinking water for 28 days and then the adjuvant – tender coconut water from day 28- 56.
Group III	Standard drug treated control group- animals on 0.75 % ethylene glycol in drinking water for 28 days and then the standard drug cystone from day 28- 56
Group IV	Test drug in half of therapeutic dose- animals on 0.75 % ethylene glycol in drinking water for 28 days and then the test drug in half of therapeutic dose from day 28- 56
Group V	Test drug in half of therapeutic dose- animals on 0.75 % ethylene glycol in drinking water for 28 days and then the test drug in effective dose of therapeutic dose from day 28- 56
Group IV	Test drug in half of therapeutic dose- animals on 0.75 % ethylene glycol in drinking water for 28 days and then the test drug in double of therapeutic dose from day 28- 56

Table 3: Estimation of Serum Calcium

	Serum Calcium (mg/dl)				Paired t test	
	Before treatment		After treatment			
	Mean	SD	Mean	SD	t	P
Group I	5.53	0.60	5.43	0.77	0.218	0.836 ns
Group II	10.33	0.23	8.12	1.87	3.320	0.021*
Group III	10.55	0.75	6.48	0.77	9.339	0.000**
Group 1V	10.07	1.15	7.98	1.06	7.118	0.001**
Group V	10.82	0.33	6.42	0.48	16.472	0.000**
Group VI	11.00	1.01	6.43	0.78	11.564	0.000**

*: Significant at 5% level (P<0.05), **: Significant at 1 % level (P<0.01)

Table 4: Percentage change in Serum Calcium

	Change in S. Calcium between the treatment		Percentage change in S. calcium			ANOVA	
	Mean	SD	Mean	SD	se	F	p
Group I	0.10	1.12	0.3	21.7	8.9	8.187	0.001**
Group II	2.51	1.85	24.2	18.1	7.4		
Group III	4.07	1.07	40.6	8.1	3.3		
Group IV	2.69	0.93	24.3	7.9	3.2		
Group V	4.40	0.65	36.2	5.2	2.1		
Group VI	4.00	0.85	38.3	6.0	2.4		

*: Significant at 5% level (P<0.05), **: Significant at 1 % level (P<0.01)

Table 5: Estimation of Serum creatinine

	Serum Creatinine (mg/dl)				Paired t test	
	Before treatment		After treatment			
	mean	sd	mean	Sd	t	P
Group I	0.25	0.08	0.24	0.04	0.169	0.872 ns
Group II	3.61	0.54	2.02	0.28	10.180	0.000**
Group III	3.64	0.77	0.71	0.15	8.685	0.000**
Group IV	3.67	0.65	1.9	0.49	6.731	0.001**
Group V	3.55	0.82	0.81	0.27	8.092	0.000**
Group VI	3.42	1.44	0.63	0.09	4.455	0.007**

Table 6: Percentage change in Serum Creatinine

	Change in S. creatinine between the treatment		Percentage change in S creatinine			ANOVA	
	Mean	SD	Mean	SD	se	F	p
Group I	0.01	0.12	0.7	86.5	35.3	5.870	0.001**
Group II	2.58	0.62	44.4	9.8	4.0		
Group III	2.93	0.83	80.4	7.4	3.0		
Group IV	2.55	0.93	48.6	17.1	7.0		
Group V	2.74	0.83	77.3	10.8	4.4		
Group VI	2.65	1.46	81.2	28.9	11.8		

Table 7: Estimation of Serum Uric acid

	Serum Uric acid (mg/dl)				Paired t test	
	Before treatment		After treatment			
	mean	Sd	mean	Sd	t	P
Group I	1.28	0.19	1.36	0.55	0.737	0.494ns
Group II	3.73	0.67	2.95	0.58	2.311	0.007**
Group III	3.83	1.17	1.95	0.61	4.504	0.006**
Group IV	3.35	0.39	2.57	0.69	4.960	0.004**
Group V	3.37	0.69	1.98	0.34	4.383	0.007**
Group VI	3.35	0.74	1.53	0.18	3.927	0.001**

Table 8: Percentage change in Serum Uric acid

	Change in Serum uric acid between the treatment		Percentage change in Serum uric acid			ANOVA	
	mean	sd	Mean	sd	se	F	p
Group I	0.17	0.55	2.25	41.7	17.0	6.529	0.001**
Group II	0.78	0.82	20.9	20.7	8.5		
Group III	2.40	1.30	48.8	18.8	7.7		
Group IV	0.78	0.39	23.2	13.6	5.6		
Group V	1.38	0.77	41.2	16.5	6.7		
Group VI	1.39	0.87	54.3	20.3	8.3		

Table 9: Estimation of Urine Creatinine

	Urine creatinine (mg/dl)				Paired t test	
	Before treatment		After treatment			
	Mean	Sd	Mean	sd	t	P
Group I	22.24	3.21	21.67	1.78	0.225	0.843ns
Group II	82.53	7.65	72.57	3.11	1.608	0.024*
Group III	82.07	2.12	45.37	2.40	7.804	0.01**
Group IV	78.13	7.45	58.63	3.56	4.594	0.044*
Group V	63.97	16.6	37.83	9.49	2.877	0.003**
Group VI	71.87	1.99	38.17	2.53	13.352	0.006**

Table 10: Percentage change in Urine creatinine

	Change in urine creatinine between the treatment		Percentage change in urine creatinine			ANOVA	
	mean	sd	Mean	sd	se	F	p
Group I	0.58	4.43	0.9	18.3	10.6	6.414	0.004**
Group II	9.97	10.74	12	11.4	6.6		
Group III	19.50	4.33	44.7	5.0	2.9		
Group IV	26.13	9.85	24	11.1	6.4		
Group V	36.70	22.10	40.8	18.4	10.6		
Group VI	33.70	4.37	46.8	4.8	2.8		

Table 11: Estimation of Urine Calcium

	Urine Calcium (mg/dl)				Paired t test	
	Before treatment		After treatment			
	Mean	sd	mean	sd	t	P
Group I	1.87	0.74	1.60	0.36	0.977	0.431ns
Group II	7.83	1.23	5.41	0.25	1.326	0.030*
Group III	8.03	0.42	2.87	0.35	16.005	0.004**
Group IV	8.10	1.11	5.02	0.75	3.186	0.020*
Group V	6.10	1.20	3.53	1.64	3.017	0.049*
Group VI	7.23	0.40	4.52	1.71	0.250	0.032*

Table 12: Percentage change of Urine calcium

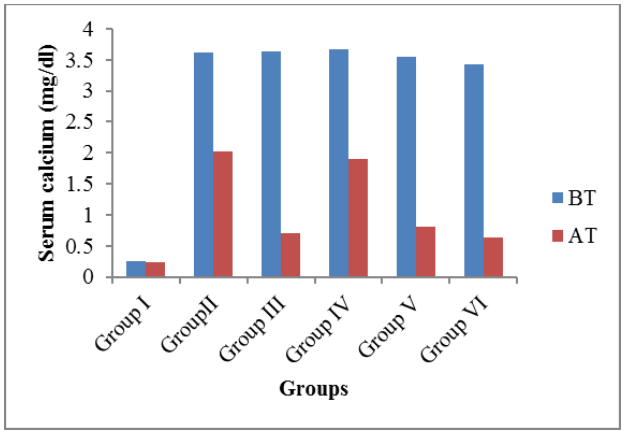
	Change in urine calcium between the treatment		Percentage change in urine calcium			ANOVA	
	mean	sd	Mean	sd	se	F	p
Group I	0.27	0.47	0.07	18.0	10.4	3.270	0.043*
Group II	0.77	1.00	30.9	10.8	6.2		
Group III	4.17	0.45	64.2	4.3	2.5		
Group IV	1.97	1.07	38.0	11.7	6.7		
Group V	1.13	0.65	42.1	14.9	8.6		
Group VI	0.30	2.08	45.3	29.4	17.0		

Table 13: Estimation of Urine uric acid

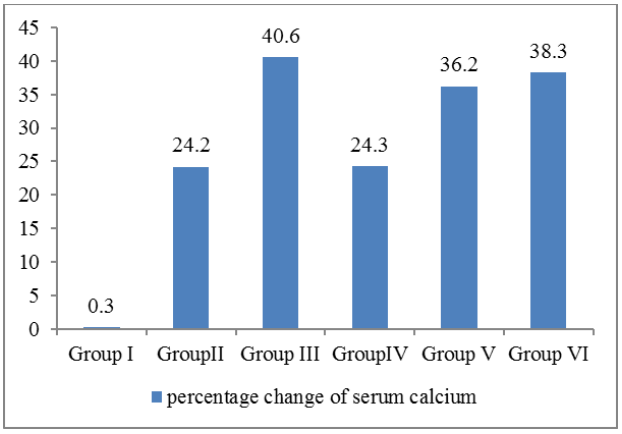
	Urine uric acid (mg/dl)				Paired t test	
	Before treatment		After treatment			
	mean	Sd	mean	Sd	t	P
Group I	2.77	0.67	3.33	0.71	17.0	0.096^{ns}
Group II	21.77	2.11	13.67	1.42	7.324	0.018*
Group III	17.50	0.53	7.7	2.19	6.947	0.002**
Group IV	18.87	1.75	9.93	3.51	2.992	0.003**
Group V	18.80	2.63	9.30	2.19	7.615	0.007**
Group VI	18.93	1.50	7.80	3.05	10.599	0.009**

Table 14: Percentage change of Urine Uric acid

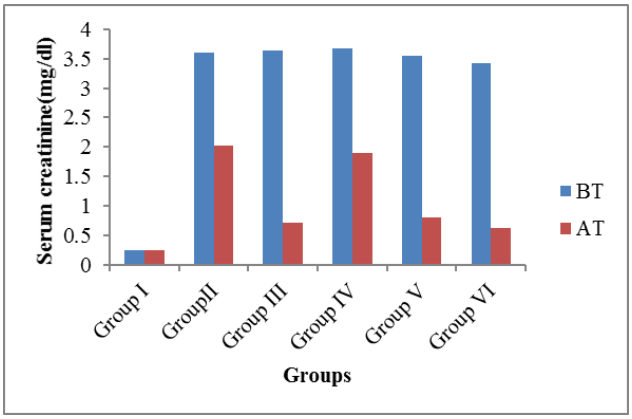
	Change in urine uric acid between the treatment		Percentage change in urine uric acid			ANOVA	
	Mean	SD	Mean	SD	se	F	p
Group I	0.57	0.06	21.0	3.4	1.9	18.66	0.00**
Group II	8.93	2.11	42.1	8.4	4.9		
Group III	9.80	2.44	51.2	13.3	7.7		
Group IV	8.10	4.69	43.7	18.8	10.8		
Group V	11.13	2.53	55.9	10.0	5.8		
Group VI	9.50	1.55	58.8	12.4	7.2		



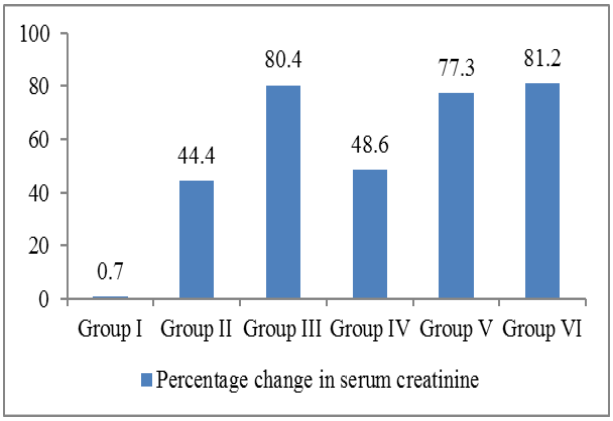
Graph 1: The bar diagram showing the before treatment and after treatment values of Serum Calcium



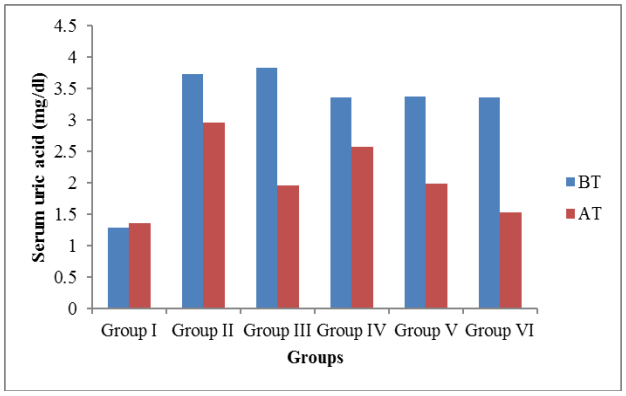
Graph 2: Bar diagram showing the Percentage change of Serum Calcium



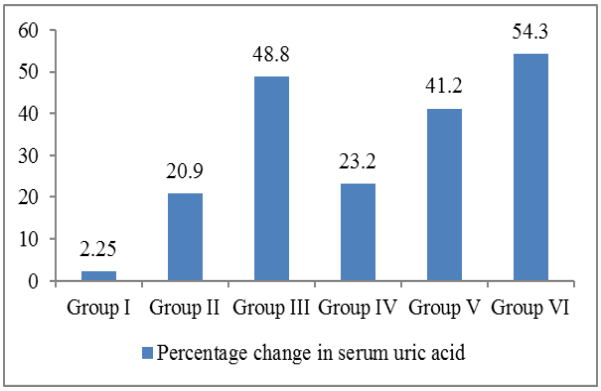
Graph 3: The comparative bar diagram showing the before treatment and after treatment values of Serum Creatinine



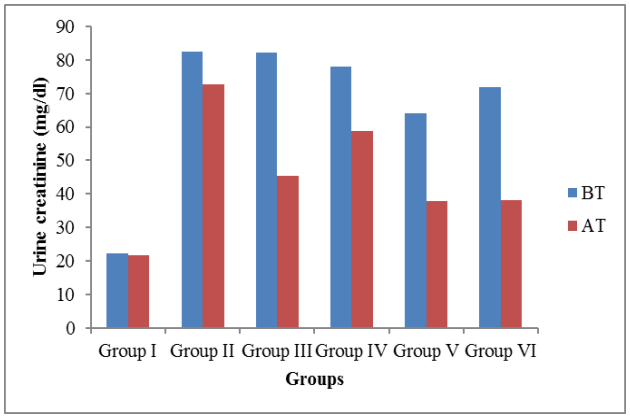
Graph 4: Bar diagram showing the Percentage change in Serum Creatinine



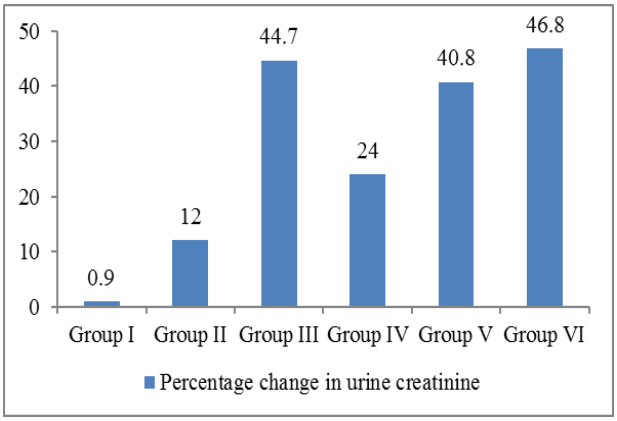
Graph 5: The comparative bar diagram showing the before treatment and after treatment values of Serum Uric acid



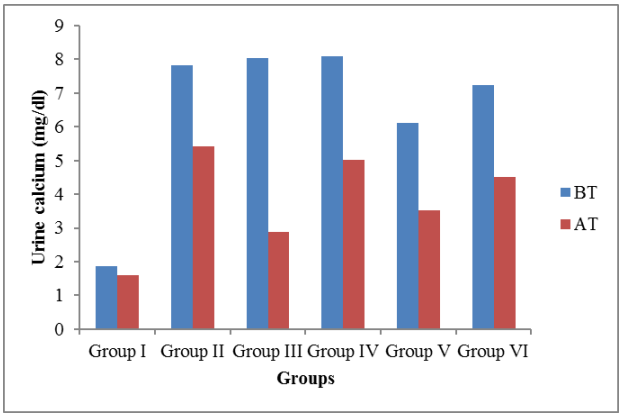
Graph 6: Bar diagram showing the Percentage change in Serum Uric acid



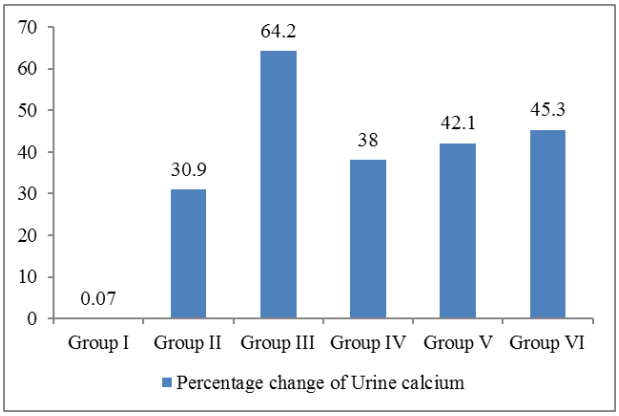
Graph 7: The bar diagram showing the before treatment and after treatment values of Urine Creatinine



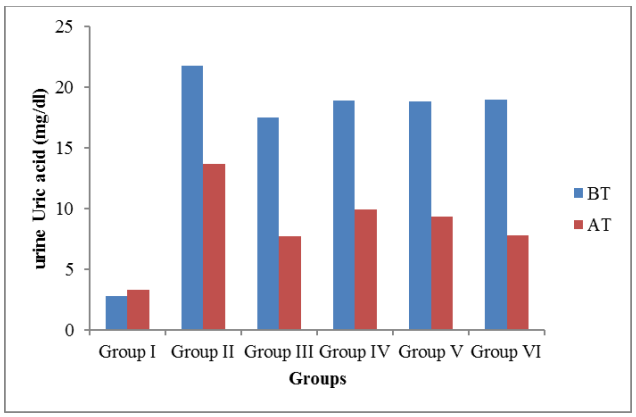
Graph 8: Bar diagram showing the Percentage change in Urine Creatinine



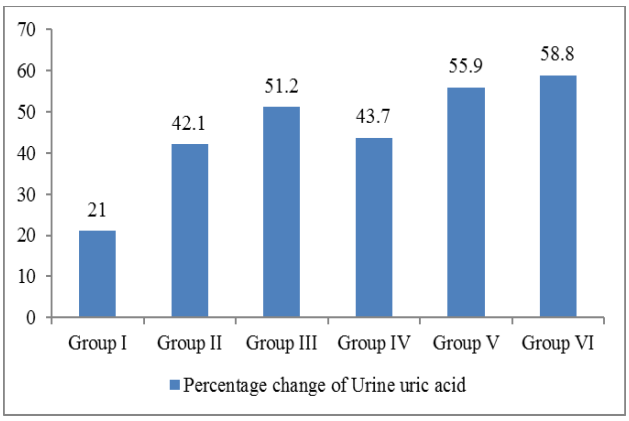
Graph 9: The bar diagram showing the before treatment and after treatment values of urine calcium



Graph 10: Bar diagram showing the Percentage change in Urine Calcium



Graph 11: The bar diagram showing the before treatment and after treatment values of Urine Uric acid



Graph 12: Bar diagram showing the Percentage change in Urine Uric acid

Microscopic examination of urine

The urine samples were analyzed microscopically to check the stage of crystals formed by ethylene glycol administration.

Serum analysis

The serum samples were collected after the lithogenic phase (after the receiving EG 28 days) and after the treatment period. After urine collection on the next day, blood was collected by retro-orbital method under mild anesthetic condition using diethyl ether. The blood samples were analyzed for serum values of calcium, uric acid and creatinine.

Statistical analysis

All data were expressed as mean ± SD (Standard Deviation). The results were analyzed by one-way analysis of variance (ANOVA), followed by comparison between test and control groups using Student's t-test. Differences between groups were considered significant at $p < 0.05$.

RESULTS

In the present study chronic administration of 0.75% (v/v) of ethylene glycol in drinking water resulted in increased concentration of calcium, creatinine and uric acid levels in serum and urine of calculi induced rats when compared to normal animals. However, the treatment with test drug significantly lowered the elevated levels of parameters.

Estimation of Serum Calcium (Table 3; Graph 1): For the group II, there is a significant reduction of serum calcium values ($p<0.05$). There is highly significant reduction in serum calcium values for groups III- VI ($p<0.01$). The percentage change of each group (Table 4; Graph 2) was calculated to compare the efficacy among the groups in reducing the serum calcium level. On analyzing the percentage change values of serum calcium among the six groups, group III shows maximum efficacy. Among the test drug groups (Group IV, Group V and Group VI), the group which receives the double dose (Group VI) shows better efficacy.

Estimation of Serum Creatinine (Table 5; Graph 3): There is highly significant reduction in serum creatinine values for groups II- VI ($p<0.01$). The percentage change values among groups were depicted in (Table 6; Graph 4).

Estimation of serum uric acid (Table 7; Graph 5): There is highly significant reduction in serum calcium values for groups II- VI ($p<0.01$). The percentage change values of Serum uric acid among groups were represented in (Table 8; Graph 6).

Estimation of Urine Creatinine (Table 9; Graph 7): Groups II and IV reduced the urine excretion values of creatinine significantly ($p<0.05$). There is highly significant reduction in urine creatinine values for groups III, V and VI ($p<0.01$). The percentage change values among groups in reducing elevated levels of Urine creatinine were tabulated. (Table 10; Graph 8).

Estimation of Urine Calcium (Table 11; Graph 9): The group II and the test drug groups (group IV group V and group VI), showed significant reduction of urine calcium values ($p<0.05$). There is highly significant reduction in urine calcium values for groups III ($p<0.01$). The percentage change values of urine

calcium among the six groups were depicted in (Table 12; Graph 10).

Estimation of Urine Uric acid (Table 13; Graph 11): For the group II, there is a significant reduction of urine uric acid values ($p<0.05$). There is highly significant reduction in urine uric acid values for Group III and all the test groups (Group IV, Group V and Group VI) ($p<0.01$). The percentage change values of urine excretion values of uric acid among the six groups were tabulated (Table 14; Graph 12)

DISCUSSION

The present study evaluated the *In vivo* lithotriptic effect of Matsyakshi (*Alternanthera sessilis* Linn.R Br.) along with tender coconut water in ethylene glycol induced urolithiasis. Experimental study design was selected since no pre clinical studies for assessing the lithotriptic effect of combination of the drugs has undergone till date. Renal calcium oxalate crystal deposition induced by ethylene glycol is the most appropriate animal model that is frequently used to mimic the stone formation in humans. Ethylene glycol (0.75%) was given along with drinking water for 28 days. Previously reported studies showed that 28 days administration of ethylene glycol (0.75% v/v) in the drinking water of laboratory animals significantly cause renal stone formation by increasing the urinary concentration of oxalate[20,21].

Parameters taken for the study were serum calcium, serum uric acid, serum creatinine, urine calcium, urine uric acid and urine creatinine. The lithotriptic effect was assessed by statistically analyzing the before treatment and after treatment (BT and AT respectively) values of these parameters by paired t test. The efficacy among the groups was analyzed by percentage change values of each group.

In the lithogenic phase, ethylene glycol induced urolithiasis resulted in significant elevation of urine and serum levels of calcium, creatinine and uric acid($p<0.01$) compared to normal control group; indicating disrupted renal homeostasis. Also, plenty of calcium oxalate crystals were observed in urine microscopic examination. EG administration caused precipitation of the oxalate in the urine as calcium oxalate (CaOx) due to its poor solubility. Previous studies reported that the EG is metabolized to hippuric acid, oxalic acid etc which can cause metabolic acidosis and impair the proximal tubule bicarbonate reabsorption. This acidosis may result in renal calcium leak associated with the gut absorption of calcium and bone calcium release. All these can lead to hypercalciuria and hypercalcemia[22]. Also, it is reported that increased urinary calcium favors the nucleation, precipitation and subsequent aggregation of calcium oxalate and calcium phosphate crystals in urine. High oxalate levels in nephrons damage epithelial cells, and also result in heterogeneous crystal nucleation and aggregation.[23]. These calcium oxalate crystals decrease the Glomerular Filtration Rate due to the obstruction to the urine out flow. This leads to the accumulation of nitrogenous substances such as uric acid and creatinine in blood and urine[24].

Animals treated with the test drug in all the three doses (Group IV, Group V and Group VI) showed highly significant reduction in all the six parameters as compared to the lithogenic phase. Among the test drug groups, Group VI showed better efficacy in reducing all the six parameters. Group VI also showed better efficacy than the standard drug cystone in reducing the serum and urine values of creatinine and uric acid. All the test drug groups showed better efficacy in reducing the urine and serum parameters as compared to the vehicle control group. This suggests the ability of the drug to reduce the biochemical changes induced by ethylene glycol and the lithotriptic effect of the drug.

Urinary calculi formation is a complex process resulting from a succession of several physicochemical events including super saturation, nucleation, growth, aggregation, and retention of organic salts within renal tubules. The herbal drugs that have several phytoconstituents and exert their beneficial effects on urolithiasis by multiple mechanisms[25].

Studies indicate that mucoproteins exhibit significant affinity for calcium oxalate surface, thus promote the growth of crystals and cement them; which result in the formations of large crystals of calcium oxalate. Thus, the lithotriptic activity is mainly by disintegrating the binding mucin (mucoprotein) of calculi to prevent crystal aggregation to form a large stone. Saponins disintegrate the mucoproteins, thereby preventing calcium oxalate formation and retention. Saponin is a major chemical constituent of *Alternenthera sessilis* Linn. R.Br, which was observed in preliminary phytochemical analysis.

Previous studies indicate that the flavonoids in plants help in the relaxation of smooth muscle of the urinary tract and this facilitate the expulsion of stones from the kidney. The test drug is found to be a rich source of flavonoids.

Lupeol is found to be efficient in reducing the risk of stone formation by way of preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents. Lupeol has the ability to restore the antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase. The compound Lupeol is a phytochemical found in the roots of *Alternanthera sessilis* Linn. R.

After the treatment with the test drug, the uric acid and creatinine values of serum and urine were restored. This suggests that the drug has the potential to improve the renal function. These results show that the test drug dissolves the preformed calculus and prevent the formation of new calculi in the urinary system.

Diuretic action is also needed to increase the amount of fluid going through the kidneys and flush out the deposits. Increase in volume decreases the saturation of salts and prevent the precipitation of crystals. Previous researches also prove the diuretic activity of *Alternanthera sessilis.*

Animal and cellular studies have revealed that calcium oxalate crystals cause injury to the kidney cells. Epithelial injury is considered to be a risk factor for the crystallization and crystals deposition in the kidney, as it promotes crystal nucleation, aggregation, retention and stone development. The injury of the urinary tract also leads to the clinical manifestations like haematuria. Previous studies confirmed the wound healing property of *Alternanthera sessilis*, which may help to repair the epithelial injury.

Antioxidants have been shown to provide protection against oxidative injury by oxalate and crystal deposition. Earlier studies showed antioxidant potential of both *Alternanthera sessilis* and tender coconut water. This can be counted as an added benefit.

CONCLUSION

The present study was conducted to evaluate the lithotriptic effect of matsyakshi [Alternanthera sessilis Linn. R.Br and tender coconut water in ethylene glycol induced urolithiasis model. The efficacy of the drug in urolithiasis is a traditional knowledge. The lithotriptic activity of the drug may be probably due to multiple mechanisms like diuretic activity, crystallization inhibition activity, improving renal function and anti oxidant activity of the drugs. Phytoconstituents like flavonoids, saponins and lupeol present in the drugs may be responsible for the lithotriptic activity.

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Cite this article as:

Dhanya V T *et al*. Lithotriptic effect of combination of Matsyakshi (*Alternanthera sessilis* Linn. R.Br.,) and tender coconut water in albino rats. Int. Res. J. Pharm. 2017;8(9):56-64 http://dx.doi.org/10.7897/2230-8407.089158

Source of support: Nil, Conflict of interest: None Declared