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EVALUATION OF *MUSA* (PARADISIACA LINN. CULTIVAR) - "PUTTUBALE" STEM JUICE FOR ANTILITHIATIC ACTIVITY IN ALBINO RATS

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Abstract: The fresh juice of *Musa* stem (Puttubale) was tested for its antilithiatic activity. Zinc discs were implanted in the urinary bladder of albino rats to induce urolithiasis. The stones formed were mainly of magnesium ammonium phosphate with traces of calcium oxalate. *Musa* stem juice (3 mL/rat/day orally) was found to be effective in reducing the formation and also in dissolving the pre-formed stones.

Key words : *Musa* stem juice, foreign body implantation, antilithiatic activity, zinc disc

INTRODUCTION

In the indigenous system of medicine (Ayurveda), many plants have been claimed to be useful in urinary stones or calculus. Among these, stem juice of *Musa* species is most commonly used as lithontriptic (1-3). The Ayurvedic physicians of Dakshina Kannada district of Karnataka and Northern Kerala recommend a particular variety of *Musa paradisiaca*, locally known as *Puttubale* which is claimed to be very effective in dissolving urinary stones (4). Earlier studies on *Musa* stem juice have shown a lowering of liver glycolic acid oxidase activity and glycolic acid content of hyperoxaluric acid (5). The present study was planned to assess the efficacy of stem juice of *Musa* (*Puttubale*) in the dissolution of pre-formed stones and in preventing the formation of stones or calculi on zinc disc implants in the urinary bladder of rats.

METHODS

Adult albino rats of Wistar strain of either sex weighing between 150-200 g were used. The animals were fed with commercial rat feed (Lipton India) and were given water *ad libitum*.

Preparation of Musa stem juice: Innermost part of the stem of ripe *Puttubale* collected locally, crushed and fresh juice was obtained daily for administration.

Pre-operative investigations: Rats were fasted overnight and water was given *ad libitum*. Each rat was hydrated with water (5 mL) and 24 h urine was collected by keeping the rats in individual metabolic cages. The urine samples were analysed for calcium, magnesium, phosphate and oxalate. Subsequently food and water were given to the animals.

Insertion of foreign body: Foreign body insertion technique (6,7) was used here to induce stones in the urinary bladder. Zinc discs of 4 mm diameter weighing around 40-50 mg were used. Rats were anaesthetised with pentobarbitone sodium (30 mg/kg, i.p.). A suprapubic incision was made and the urinary bladder

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was exposed. A small cut was made, pH of the urine was determined using narrow range pH paper (BDH). Previously weighed sterile zinc disc was carefully inserted into the bladder. Bladder was closed with a single suture using absorbable 4-0 chromic catgut (Ethicon). The abdomen was closed in layers using silk thread. The rats were allowed to recover from the operation for one week.

Treatment schedules: The operated rats were divided into 4 groups of 20 animals (10 male and 10 female) each. Group 1 animals were kept as control and were given water (3 mL/rat/day orally) for 4 weeks. Group 2 animals were treated with *Musa* stem juice (3 mL/rat/day orally) for 4 weeks to see the prophylactic effect of the juice. Group 3 animals were left untreated for 4 weeks, later they were given *Musa* stem juice (3 mL/rat/day orally) for 4 more weeks to see the curative effect. Group 4 animals left untreated for 4 weeks and later they were given equivolume of water for another 4 weeks to see the spontaneous dissolution and/or washing of stone.

Rats were housed in individual cages. Food and water were provided *ad libitum*. Animals which died during the study were excluded from evaluation. 24 h urine samples were collected 5 and 9 weeks after implantation, to determine urinary electrolytes. Rats were sacrificed, pH of the urine was determined and the zinc discs from individual rats were removed at the end of 5 and 9 weeks after implantation. The discs were washed in distilled water and were allowed to dry. The dried discs were weighed and the difference between initial and final weight of zinc disc gives the weight of stone deposited. The stones were analysed qualitatively for calcium, oxalate, uric acid, cystine, magnesium, phosphate and carbonate using standard analytical procedures (8).

Results were expressed as mean ± SEM. Significance between 'Control' and 'Drug-treated' animals were determined by Student's t-test.

RESULTS

The average weight of stones obtained from different groups of animals is shown in Table I. The amount of stone deposition in females was less when compared to that in males ($P<0.01$ in group 1 and $P<0.001$ in group 4). Qualitative analysis of stones showed presence of ammonium, magnesium, phosphate and traces of calcium and oxalate. Treatment with *Musa* stem juice caused a significant reduction in stone deposition in group 2 in comparison to group 1 in both males and females ($P<0.02$ in males and $P<0.01$ in females). This shows *Musa* stem juice has a significant effect in reducing the formation of stones. Similarly with *Musa* stem juice treatment, there was significant reduction in stone deposition in group 3, when compared to group 4 in both the sexes ($P<0.001$ in males and females). This indicates that *Musa* stem juice has a significant effect in dissolving the pre-formed stones. pH of urine was found to be 6-7 before and after completion of the study.

The electrolyte concentration in urine before

TABLE I : Weight of stone deposited on zinc discs in different groups of rats.

Group	*No. of animals*		*Weight of stones mg ± SEM*	
	Male	*Female*	*Male*	*Female*
1	8	7	92.00 ± 14.65	38.14 ± 11.44[b]
2	8	8	46.38 ± 12.44[a]	23.37 ± 3.94[b]
3	9	8	122.55 ± 8.65[c]	42.12 ± 7.33[c]
4	8	7	226.62 ± 22.19	78.85 ± 8.81[c]

[a] $P < 0.02$ between male rats of group 1 and 2.

[b] $P < 0.01$ between male and female rats of group 1 and between female rats of group 1 and 2.

[c] $P < 0.001$ between group 3 and 4 in both sexes and between male and female rats of group 4.

implantation, 5 weeks after implantation and after completion of the study are shown in Tables II and III. After 5 weeks of zinc disc implantation, there was a marked increase in magnesium and oxalate levels in both sexes of group 1, 3 and 4 alongwith a decrease in phosphate level in group 1, when compared to normal

TABLE II : Influence of urolithiasis and *Musa* stem juice treatment on urinary electrolytes in male rats.

Group	Time of urine analysis	Calcium	Magnesium	Phosphate	Oxalate
		← (mg/dl) →			
1	A	1.9±0.4	2.5±0.2	98.3±6.8	1.6±0.08
	C	2.9±0.2[b]	12.2±0.9[d]	73.9±9.7[a]	2.1±0.1[d]
2	A	2.3±1.1	2.3±0.2	87.4±6.0	1.0±0.02
	C	5.0±0.9[b]	13.9±0.8	116.0±12.3[c]	5.3±0.6[d]
3	A	1.6±0.2	1.3±0.2	92.4±8.2	1.1±0.1
	B	3.1±0.5[c]	16.5±1.9[d]	86.7±9.2	3.1±0.2[d]
	C	5.2±1.9	20.9±2.9	71.3±10	18.0±2.4b
4	A	2.8±0.6	2.3±1.0	91.6±5.2	1.1±0.1
	B	3.4±0.9	13.9±1.9[d]	90.0±9.8	4.5±0.8[d]
	C	4.1±1.9	15.6±1.7	86.0±12	11.71±1.4

A = Before implantation; B = 5 weeks after implantation ; C = After treatment

[a]P < 0.05 - in group 1 before and 5 weeks after implantation.

[b]P < 0.02 - in group 1 before and 5 weeks after implantation, between group 1 & 2 and 3 & 4 after treatment.

[c]P < 0.01 - in group 3 before and 5 weeks after implantation, between group 1&2 after treatment.

[d]P < 0.001 - in group 1, 3 and 4 before and 5 weeks after implantation, between group 1& 2 after treatment.

TABLE III : Influence of urolithiasis and *Musa* stem juice treatment on urinary electrolytes in female rats.

Group	Time of urine analysis	Calcium	Magnesium	Phosphate	Oxalate
		← (mg/dl) →			
1	A	2.1±0.8	3.8±0.4	110.0±8.9	1.7±0.06
	C	2.9±0.9	11.6±1.6[d]	78.3±7.2[c]	2.2±0.09[d]
2	A	2.2±0.7	2.6±0.6	91.6±5.4	1.9±0.07
	C	4.9±1.1	15.2±0.9[a]	148.0±30[b]	4.9±0.4[d]
3	A	2.2±0.8	2.1±0.3	99.2±10.5	0.9±0.01
	B	3.9±0.9	14.1±2.3[d]	74.9±14.7	2.3±0.4[d]
	C	4.8±0.8	18.5±1.4	89.8±9.6	15.9±3.2[c]
4	A	2.2±0.4	2.9±0.2	93.0±7.4	1.9±0.2
	B	2.9±0.6	15.3±2.1[d]	86.8±11	4.0±0.7[c]
	C	3.8±0.8	17.9±0.9	97.0±9.9	9.4±1.2

A = Before implantation; B = 5 weeks after implantation; C = After treatment

[a]P < 0.05 - between group 1 & 2 after treatment.

[b]P < 0.02 - between group 1&2 after treatment.

[c]P < 0.01 - in group 1 and 4 before and 5 weeks after implantation, between group 3 & 4 after treatment.

[d]P < 0.001 - in group 1, 3 and 4 before and 5 weeks after implantation, between group 1& 2 after treatment.

values. Calcium level was increased in male animals of group 1 and 3 in comparison to normal values, 5 weeks after implantation.

Musa stem juice treatment increased the urinary levels of calcium, phosphate and oxalate in group 2 animals when compared to group 1 in both the sexes, whereas magnesium levels were unaffected. In both male and female animals of group 3, given *Musa* stem juice treatment, there was an increase in the level of oxalate when compared to group 4. Calcium, magnesium and phosphate levels were not changed significantly in group 3 animals when compared to group 4 after treatment with *Musa* stem juice.

DISCUSSION

In both male and female animals, in which zinc discs were implanted, considerable amount of stone deposit was observed and the stones formed were of magnesium ammonium phosphate with traces of calcium and oxalate. This was similar to the observation of Vermeulen et al (9). The amount of stone deposition in females was less in both group 1 and 4 when compared to that in males. This observation was similar to that made by Joyamma Varkey et al (10). This difference in stone formation between male and female may be due to the fact that estrogens antagonize and androgens favour urinary calculus formation (11). Factors like stress also play an important role in stone formation in males when compared to females (12).

Urinary electrolyte estimation showed an increase in levels of magnesium and oxalate in both the sexes and calcium in males, 5 weeks after implantation. This is in line with the observation of Vermeulen and Goetz (13), who observed an increase in magnesium and a decrease in calcium levels along with the formation of magnesium ammonium phosphate type of stones. Similarly with calcium oxalate type of stones they observed an increase in calcium and a decrease in magnesium levels. The increase in levels of magnesium and calcium in our study may be due to formation of mixed type of stones, mainly magnesium ammonium phosphate and traces of calcium oxalate.

On treatment with *Musa* stem juice, there was an increase in the levels of calcium, phosphate and oxalate in group 2 when compared to group 1 animals. In group 3 animals there was an increase in the level of oxalate with *Musa* stem juice treatment when compared to group 4. This shows the ability of *Musa* stem juice in dissolving calcium, phosphate and oxalate ions from the stones. The fact that the concentration of the calculogenic ions have not reversed to normal as expected indicates incomplete dissolution of the calculi with one month treatment. pH of urine does not seem to exert any significant effect on stone dissolution since the urine pH was found to be 6-7 in all the groups.

The results obtained in this study prove the efficacy of *Musa* (*Puttubale*) stem juice in reducing the incidence of urolithiasis to a considerable extent, thus justifying the claim made in the indigenous system of medicine.

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