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An experimental evaluation on Ashmari bhedana property of giri kadali (*ensete superbum* (roxb.) cheesm.) and narikela pushpa (*cocos nucifera l.)* – A comparative study.

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Abstract: In Ayurveda classics many drugs have been told to have Ashmari bhedana property. At the same time, many extra pharmacopoeial drugs are also used in Folk practice. This study aim to analysis the effect of , Cliff banana seeds- *Ensetesuperbum*(Roxb.)Cheesm. and Narikelapushpa- *Cocos nucifera* L. as they are widely used by the Folk practitioners of Kerala and Karnataka respectively. It was an experimental study, Albino rats was the source. The 2 trial groups of Curative showed Ashmari bhedana Property in comparison to the control group. Out of these folklore drugs, Narikelapushpa- *Cocos nucifera L.*Choorna showed better Ashmari bhedana property than that of Cliff banana- *Ensete superbum*(Roxb.) Cheesm.

Keyword: Ashmari; Ashmari bhedana; Urolithiasis; Cliff banana [*Ensete superbum*(Roxb.)Cheesm.]; Narikela pushpa [*Cocos nucifera*L.]; pain; Bladder stones; Basti.

1. INTRODUCTION:

The fast development in Science and Technology in this 21st century has motivated man to live a sophisticated life. So tinned and fast foods have replaced the traditional healthy foods, leading to many ailments and stress related disorders. Mutrashmari (Urinary calculus) is no more an unfamiliar disease. Mutrashmari is one among them which has been delineated as a 'Mahagadha' in Ayurveda[1,2,3] and recent researches discover that 'Global Warming' would increase its incidence by 30% in the near future[4]. Mutrashmari is a disorder in which Asmari is formed in the urinary tract. It has been estimated that 5% of global population suffer from urinary calculi. At some period of their life it has also been noted that men are twice as likely as women to develop calculi[5].The phenomenon of lithofication termed as Asmari Sanghtana, has been clearly explained in all the Ayurvedic texts since ancient times. In these texts, four types of Asmari have been described[2,6].Based on the concept of Anukta Dravyas, evaluating and scientific updating of new drugs used in ethno medicine would help to increase the bulk of Ayurvedic Pharmacopoeia and also serves in documenting and proving the folklore claims which is a part of Ethno-medical study. Hence, a comparative experimental study would be done to scientifically validate and evaluate the AshmariBhedana property of the seed of wild plantain [*Ensetesuperbum*(Roxb.) Cheesm. (=*Musa superb* Roxb.)][7, 8] and flowers of *Cocos nucifera* L., which is highly used in folk medicine in the treatment of Mutrashmari. These drugs are easily available, cost effective, and could be easily administered.

2. MATERIALS AND METHODS :

Source of data

This being an experimental study the source will be grouped as follows.

Experimental Source:

Table no.1 **Method of collection of data**

1.	**Sample**	18 male albino rats weighing 150-200gms will be selected in Random
2.	**Inclusive Criteria**	Healthy, male active rats each weighing in between 150-200gms.
3.	**Exclusive criteria**	Diseased, Female rats, Rats under trial for other experiments and Rats below 150gms and more than 200gms.

4.	**Grouping**	**Group**	**No of Rats**	**Drug**	**Form**	**Dose**	**Purpose**
		Control	6	Distilled water	-	1ml.	To serve as prophylactic control
		Trial 1	6	Seeds of*Ensetesuperbum* (Roxb.) Cheesm.	Churna with distilled water	0.216gm/2oogm body wt.(B.D)	To bring about Uro-Lithotriptic effect.
		Trial II	6	Flowers of *Cocos nucifera* L.	Churna with distilled water	0.216gm/2oogm body wt.(B.D)	To bring about Uro-Lithotriptic effect.
5.	**Procedure**	The experiment will be carried out by following technique developed by Vermulen(1950).First, operative procedures are to be carried out. After Anaesthetizing the rats with Ketamine, Vertical incision measuring less than 1cm is to be made over the Supra-pubic area in the middle, above the roof of penis. Bladder is to be identified and a small incision(30mm)should be made and sterilized zinc bead is to be inserted and bladder is to be closed with 2 stitches and layer by layer, abdominal layers are to be closed. During the treatment, rats are supplied with normal diet and water. From 8th day the drug is to be administered assuming that in 7 days Mutrashmari will be formed. The drug is to be administered for 21 days and bladder is to be re-operated and formed stone will be weighed and analyzed.						
6.	**Dosage**	Control-Distilled water Trial-1 - seeds of *Ensete superbum* (Roxb.) Cheesm. -0.216gm/200gm body wt.(B.D) Trial-II -flowers of *Cocos nucifera* L.-0.216gm/ 200gm body wt.(B.D)					
7.	**Duration of the Treatment**	21 days					
8.	**Observations**	After 21 days of drug administration rats are to be sacrificed and formed stones are to be weighed and the difference in weight of zinc bead before insertion and after treatment are to be noted. The difference gives the weight of stone formed.					
9.	**Statistical Test**	Appropriate student's 't' test.					

3. EXPERIMENTAL STUDY :

The present study is aimed to compare the efficacy of Narikela pushpa and Giri kadali beeja in selected Albino rats in Ashmari induced by the following method -Foreign body Insertion technique, developed by Vermeulen et al., 1950; Vermeulen, 1962).

Source of Animals:

Fifty Wister Albino male rats were procured in the Animal house attached to A.L.N.Rao memorial Ayurvedic medical college.

Rat maintenance:

All the animals were bred and maintained at the animal house of A.L.N. Rao memorial Ayurvedic medical college, Koppa, under identical conditions of place, light, temperature, food and other conditions.

All the cages used for the experiment were cleaned before commencement of the experiment and was changed every day to maintain antiseptic measures for the post operative rats till the end of the experiment. All the cages were washed

with detergent followed by disinfectant phenol solution and painted with spirit to maintain hygiene and for aseptic measures. Paddy husk bedding was provided only after the post operative Period and healing of the surgical wound.

Feeding schedule:

The quantity of food suggested for rats weighing 150-200gms. Was about 15-20gms/day and water was provided as required. Readymade standard laboratory rat feeds prepared by Lipton India was procured and used.

Examination of the animals prior to the experiment:

All the Wister Albino rats were subjected to general check up for sex and weight.

- Animals of 3 months of age as specified by breeders were selected.
- Sex is determined by looking at the external genitalia.
- Weight of each animal was checked by using spring balance.
- Heart rate was counted as number of beats/minute by feeling the heart rate by thumb.
- Respiratory rate was counted as number of inspiration and expirations/minute observing the movement of the abdomen.
- Each rat in the experiment was identified by colouring the forehead by using permanent markers.
- The cages were labeled with the name of the group and dosage.

Table No: 2 showing groups of experiment

Group	Drug	Purpose
I-Control	Distilled water. From the 8th day.	To serve as Prophylactic control
II-Treated group I	Girikadali beeja choorna. From the 8th day.	To bring about Urolithotriptic effect
III-Treated group II	Narikela pushpa choorna. From the 8th day.	To bring about Urolithotriptic effect
Group	Drug	Purpose
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Dose fixation:

The generalized dose for the animals has been calculated based on the conversion formula **rat dose/kg.bd.wt. = 0.018 × human dose × 5.** All the above preparations were administered according to this formula.

Mode of administration of the drug:

Table No: 3 showing Treatment Protocol:

GROUP	TREATMENT DOSE	DURATION
I.	Distilled water (1ml/200gms/day orally)	for 4 weeks
II.	Giri kadali beeja choorna Dose 0 .216gm/200gm body wt(B.D)	for 3 weeks
II.	Narikela pushpa choorna Dose 0.216gm/200gm body wt(B.D)	for 3 weeks

Urolithotriptic study:

Urolithotriptic property can be analyzed in albino rats by the following method (Foreign body Insertion technique, developed by Vermeulen et al., 1950; Vermeulen, 1962).

Study can be divided in to the following stages.

1. Pre-operative preparation
2. Insertion of foreign body
3. Experimental protocol

1. Pre- operative preparation:

Rats were fasted overnight and water was given ad libitum. Each rat was hydrated with water (5ml) and all the rats kept in individual cages.

2. Insertion of foreign body:

Rats were anaesthetized with Ketamine 120mg/kg intra peritoneal. When the rats were sufficiently anaesthetized they were brought to the dissection block. The hair on lower abdomen was removed with hair removing cream. The area

was washed with soap water. The animal was fixed in supine position to the dissection block by tying its limbs with thread to the block.

A vertical incision measuring less than one centimeter was put over the supra pubic area in the middle, a few millimeters above the roof of the penis. First the skin was exposed and was held in position by mosquito forceps on either side. Later the muscular layer was cut and was clamped in position by artery forceps. Then the lower abdominal contents were visible. The vesicle vessels are the finding points. On either side of the bladder was held carefully by means of fine blunt forceps causing minimum of tissue injury. Then a fine curved needle mounted on the needle holder was passed through the bladder wall. A small incision just sufficient to allow the bead was introduced in the bladder very carefully by means of fine forceps. Before that the bead was weighed and recorded. With one or two stitches the incision of the bladder was closed. The bladder was put in position. The skin was closed and stitched using threads and the skin was painted with tincture iodine. The animals were put back to the concerned cages. The same procedure was adopted for all the rats used in this experiment. Each group has got six rats.

3. Experimental protocol:

The Experimental protocol was followed as shown in the Table No.15 in Page.82.

Drug schedule:

For the rats normal diet and water given for 7days. From the 8th day the drug is to be administered, assuming that with in 7 days Mootrashmari will be formed. On 7th day the rats were again anaesthetized and urinary bladder opened to collect the formed stone. After weighing the stone it was replaced into the bladder. Visceral and abdominal layers were sutured accordingly. From 8th day onwards the drug was administered at appropriate dose.

Criteria for assessment of the results:

The difference of the weight of the zinc disc, before insertion and after the treatment schedule is weighed. This difference gives the weight of stone formed.

These values will be subjected for statistical analysis to evaluate the lithotriptic activity.

4. DISCUSSION:

Ashmari is one the Ashtamahagadas. It is considered as Maharoga as it is affecting the basti (one of the important Trimarmas) and also the seat for the Vata (prime among the Tridoshas). Both Vagbhata and Sushruta had mentioned this Ashmari as Mahagada .

Even though there are a lot of treatment regimens are available for urinary stones. Still the cheap and safe technique is lacking. In case of urinary stones even after advanced surgeries like ESWL and Percutaneous surgeries recurrence is quite common.

The drugs were selected due to two main reasons.

1. It is very commonly used in folk practice for lithotriptic activity.
2. Among such tribal drugs, Ensete and coconut flower are easily available and cheaper.

We need to systematically prove any tribal claim through research experimentations and then document it with details and probable mode of action so as to accept that new piece of information to the scientific world, and also to utilize it for the welfare of the society. Here this study also aims at scientifically evaluating the action of two tribally used drugs and accept them to the main realm.

5. RESULTS:

The study was conducted in Curative phase; the following are the results of the Experimental study

Table No: 4 Showing the weight of stones formed in *Ensete superbum* (Roxb.) Cheesm. Group before and after treatment

SI no	Weight of stones after 1 week(B.T) in mgs	Weight of stones after 4 weeks (A.T) in mgs
Rat 1	120	30
Rat 2	110	50
Rat 3	130	70
Rat 4	125	45
Rat 5	115	40
Rat 6	110	35
Mean	118.3	45

Table No: 5 Showing the effectiveness of *Ensete superbum* in reducing the stone weight in curative group from 7th day to 28th day.

Test	Group 1	Group 2	Mean G.p 1	Mean G.p 2	T value	degrees of freedom	P value	Remark
Paired T	Trial I BT	Trial I AT	118.3	45	15.4	5	>.001	Highly significant

There is a significant difference between AT and BT of group 1 which shows that the weight of stone has reduced to a greater extent from 7th day to 28th day. This indicates the effectiveness of *Ensete superbum* in curing the bladder stones.

Table No: 6 Showing the weight of stones formed in *Cocos nucifera* L. group before and after treatment.

SI no	Weight of stones after 1 week(B.T) in mgs	Weight of stones after4 weeks (A.T) in mgs
Rat 1	120	30
Rat 2	125	20
Rat 3	135	50
Rat 4	130	45
Rat 5	120	30
Rat 6	115	25
Mean	124.16	33.3

Table No:7 Showing the effectiveness of *Cocos nucifera* in reducing the stone weight in curative group from 7th day to 28th day.

Test	Group 1	Group 2	Mean G.p 1	Mean G.p 2	T value	degrees of freedom	P value	Remark
Paired T	Trial II B T	Trial II A T	124	33.3	30.2	5	>.001	Highly significant

There is a significant difference between AT and BT of group 1 which shows that the weight of stone has reduced to a greater extent from 7th day to 28th day. This indicates the effectiveness of *Cocos nucifera* L. in curing the bladder stones

Table No: 8 showing the mean of weight of stone on 7th day in control group, trial drug 1 and trial drug 2

Stone weight on 7th Day				
Group	Mean	N	Std. Deviation	Std. Error of Mean
Control	112.5	6	13.00385	5.3088
Trial Drug 1(Ensete superbum)	118.33	6	8.16497	3.33333
Trial Drug 2(Cocos nucifera)	124.17	6	7.3598	3.00463

Table No: 9 showing the one way Anova analysis the means of stone weight on 7th day in control, trial 1 and trial 2

ANOVA					
Stone weight on 7th Day					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	408.333	2	204.167	2.113	0.155
Within Groups	1449.667	15	96.644		
Total	1858	17			

One way Anova was done followed by post hoc scheffe test to evaluate the level of difference in their mean weight of the stone formed on the 7th day in between the group at 95% confidence interval or p value less than 0.05 .

Table No: 10 showing the mean of weight of stone on 28th day in control group, trial drug 1 and trial drug 2

Stone weight at 28 days				
Group	Mean	N	Std. Deviation	Std. Error of Mean
Control	136.67	6	19.91649	8.13087
Trial Drug 1(Ensete superbum)	45	6	14.14214	5.7735
Trial Drug 2(Cocos nucifera)	33.3333	6	11.69045	4.77261

Table No: 11 showing the one way Anova analysis the means of stone weight on 28th day in control, trial 1 and trial 2

ANOVA					
Stone weight on 28th Day					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	38433.333	2	19216.667	78.614	Less than 0.05
Within Groups	3666.667	15	244.444		

One way Anova was done followed by post hoc scheffe test to evaluate the level of difference in the mean of the reduced weight of the stone formed after 28th day of our curative study in between the group at 95% confidence interval or p value less than 0.05

Table No:12 Showing the percentage of efficacy treatment in different groups

SI no	Name of the curative group	Average weight of stone 1week in mg(B.T)	Average weight of stone 3 week in mg (A.T)	BT-AT	Percentage of efficacy
1	Control	112.5	136.6	-24.1	-21.4%
2	*Ensete superbum*	118.3	45	73.3	61.85%
3	*Cocos nucifera*	125.16	33.3	91.86	73.39%

The difference in efficacy between trial 1 and trial 2 is not statistically significant both are almost equally effective. When percentages of efficacy or mean values were compared, *Cocos nucifera* appears to be slightly superior in action.

6. CONCLUSION:

Animal Experimental study was conducted to know the Ashmari Bhedana action of the two trial drugs by zinc bead insertion technique introduced by Vermeulen et al 1950. Trial 1 and ll have shown significant action in reducing stone size individually when compared with control group. Both are significant in reducing bladder stone individually by 21 days of treatment when stone size was compared between 7th and 28th day of each group. The difference in efficacy between trial 1 and trial 2 is not statistically significant – both are almost equally effective. When percentage of efficacy or mean values were compared, *Cocos nucifera* appears to be slightly superior in action

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