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An Experimental Study of Pasanabheda (*Bryophyllum pinnatum*) in the Management of Ashmari (Urinary Bladder Stone)[1]

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ABSTRACT

Ashmari (Urolithiasis) is a frequently encountered urological disorder in its different forms. Since many years it has been a challenging problem facing by the urologist to combat the disease and find its solution an experimental study has been carried out. In the present study the alcoholic extract of the whole plant and the fresh juice of the leaves of the plant Pasanabheda (*Bryophyllum pinnatum*) were studied for its antiurolithiatic activity against experimentally produced urinary bladder stones in albino rats in the laboratory. The entire study was conducted in three groups of 08 rats. Bladder stone was produced by implanting the glass bead in the urinary bladder of all rats as a foreign body. Group-A rats were treated with plain water, group-B rats were treated with alcoholic extract of the drug whereas, group-C rats were treated with fresh juice of the leaves of the plant in calculated doses, for a period of 30 days. In this research, different parameters of blood and urine, related to the pathogenesis of the stones, were tested. The trial drug has clearly shown the effectiveness in increasing the urine output, lowering the pH of urine, decreasing the excretion of urinary calcium and phosphorus and preventing the growth of urinary bladder stone and over all its formation in experimental model of albino rats.

Key words: Ashmari, Urolithiasis, Pasanabheda, *Bryophyllum pinnatum,* Antiurolithiatic activity, Urinary bladder stones.

INTRODUCTION

Ashmari[2] (Urolithiasis) is a painful clinical entity attributed by multiple causative factors and reflecting the features of pain in abdomen of various degrees with or without the complaint of dysurea. A urinary calculus has been considered to be the pathology of disturbed metabolism as per the modern medical science. Despite of the advancement made in the field of investigation and making diagnosis in modern medicine, the basic mechanism of stone formation is obscure.

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Urinary stone formation is triggered often due to disturbed metabolic condition of hypercalcaemia with increased calcium excretion in urine and ultimately responsible for the urolithiasis. It is thought that hypercalcaemia may contribute for the formation of renal stone but need not necessarily be the primary factor. Apart from that, many other constituents of urinary stone like phosphate, magnesium, uric acid, cystine etc. play important role in the formation of various types of urinary stones and many other physico-chemical factors are also responsible for urolithiasis. As it happens to be a complex phenomenon, it has drawn the attention of researchers in the experimental field to produce a suitable experimental urinary stones model for analyzing the factors involved in it.

To combat such problem, a suitable drug for preventing as well as curing the problems of urolithiasis is yet to be explored. Ideally, the drug should have the antilithogenic properties

without disturbing the physiology as the drug must have the diuretic property and should not interfere with normal water and electrolyte balance as well as the pH of the body, should be free from toxic effects on the various systems even if it is administered for a long time and the drug should not be contraindicated also in renal disease as well as should not be precipitated renal disease as well.

To answer the above factors, the present study was planned to study the role of *Pasanabheda/ Parnabeeja* (*Bryophyllum pinnatum*)[3] in management of *Ashmari*[4]. The objective was to find out an effective and safe drug for treatment of urinary calculus. A critical evaluation of its effect on normal physiology of urinary system and on experimentally induced bladder stone and also study the effect of drug on the serum as well as urinary level of electrolytes in albino rats. In the earlier research work the herbs namely *Saxifraga ligulata, Berginea ligulata, Coleus aromaticus* and *Aerva lanata* are considered as the botanical source for Pasanabheda and were reported to possess litholitic activity. *Bryophyllum pinnatum,* another variety of Pasanabheda, was selected for this study, based on the experience and therapeutic usefulness of Orissa and Bengali Vaidyas.

STUDY PLANNING

The entire experimental study was carried out in two parts.

EXPERIMENT (PART-I)

AIMS AND OBJECTIVES

1) To determine the pre-experimental standard values of serum as well as urinary electrolytes with pH.

2) To standardize the apparatus, materials and methods for experimental study on urinary stone.

MATERIALS AND METHODS

TECHNIQUES AND METHODOLOGY IN GENERAL:

(a) Method for Collection of Urine

The urine for 24 hours was collected by using the metabolic cage, modified by Singh L.M. (Dept. of Shalya- Shalakya, I.M.S., BHU, Varanasi, India).

(b) Method for pH recording of Urine

The pH of the 24 hours collected urine was recorded by using pH paper prior to addition of the preservative in the sample.

(c) Method for Preservation of Urine

The collected urine was kept into a glass bottle with intact cork. The decomposition of urine and precipitation of electrolytes were avoided by adding 1 N Hcl of 100 ml / litter of urine. The chance of evaporation was avoided by adding a few drops of liquid paraffin to the urine sample.

(d) Method for Collecting Blood Sample

To estimate the serum electrolytes (Calcium & Phosphorus), uric acid and creatinine, at least 4 ml blood was required. Because the rats were so small, it was difficult to take out 4 ml blood by usual procedure without sacrificing the rats. So, the rats were anaesthetized by injecting Nembutal (Sodium pentothal) intra - peritonial and opened the thoracic cavity to take the blood from the heart by direct syringing. After collection, the blood samples were allowed to coagulate and serum was separated by centrifuging the blood and separated serum was preserved for further required investigations.

(e) Animals

Total 12 male albino rats of Charles foster strain were procured from the animal house. The weight of those animals was in the range of 270 gm to 300 gm. The rats were housed in four separate metabolic cages at rate of three rats in each cage.

EXPERIMENTAL PROTOCOL

The selected 12 albino rats were weighed and allotted to four different groups. The rats were housed in 04 separate metabolic cages by distributing 03 rats in each cage.

The rats were provided with usual laboratory diet and water through measuring polythene bottle having metallic nozzle attached and suspended in to the cages. No extra liquid or diet was provided. Sufficient time was given to the rats to get them adjusted with laboratory environment. Then the experiment was carried further.

ANALYSIS OF URINE

a) The urine was collected for 24 hours by using the modified metabolic cage and daily urine output chart was recorded.

b) The pH of urine was recorded prior to adding the preservative and paraffin oil.

c) The sample was send to the laboratory for estimation of urinary calcium and phosphorus, urinary creatinine and uric acid.

d) The entire steps were repeated three times at the intervals of ten days.

ANALYSIS OF SERUM

a) The blood samples were collected by direct syringing from the heart after opening the chest wall under anaesthesia and sacrificed there on.

b) 4 ml blood was taken out from only one rat of each group to estimate serum calcium, phosphorus, creatinine and uric acid.

c) The procedure was repeated three times at the interval of ten days.

WATER CONSUMPTION ANALYSIS

1. The quantity of the consumed water was also recorded for 24 hours by each rat.

2. The apparatus were cleansed daily to avoid contamination of stool and waste food materials so that chances of error in urine collection could be minimized.

DURATION OF EXPERIMENTAL STUDY

The whole experiment was continued for 30 days.

OBSERVATIONS AND RESULT

Table - 1, Urine output, Urinary pH and Water Intake in Pre-Experimental Rats:

Sr. No.	Subject	On 10th Day	On 20th Day	On 30th Day	Mean
1.	Mean Urinary Output (ml/24 hour)	07.75	07.75	08.75	08.08
2.	Mean Urinary pH	09	10	09	09.30
3.	Mean Water Intake (ml/24 hour)	17.00	20.00	20.00	19.00

Table 2: Estimation of Serum calcium, phosphorus, uric acid and creatinine in Pre Experimental Rats

Sr. No.	Subject	On 10th Day	On 20th Day	On 30th Day	Mean
1.	Value of serum calcium (mg %)	10.75	9.50	10.30	10.18
2.	Value of serum phosphorus (mg %)	10.10	9.75	9.70	9.85
3.	Value of serum uric acid (mg %)	8.70	8.07	7.90	8.22
4.	Value of serum creatinine (mg %)	19.10	18.80	19.45	1912

Table 3: Estimation of urinary calcium, phosphorus, uric acid and creatinine in Pre-Experimental Rats

Sr. No.	Subject	On 10th Day	On 20th Day	On 30th Day	Mean
1.	Value of urinary calcium (mg %)	10.75	9.50	10.30	10.18
2.	Value of urinary phosphorus (mg %)	10.10	9.75	9.70	9.85
3.	Value of urinary creatinine (mg %)	19.10	18.80	19.45	1912

The pre-experimental observations in rats show the following results:-

1) The average urine output was 08.08 ml /24 hours in male albino rats of average weight of 280 gm.

2) The mean water intake was 19.30 ml /24 hours by each rat.

3) The mean urinary pH was 09.30 which show the alkaline nature of urine.

4) The mean serum calcium was 10.16 mg % and urinary excretion of calcium was 01.10 mg /24 hours.

5) The mean serum creatinine was 19.12 mg % and mean urinary excretion of creatinine was 20 mg/24 hours.

6) The mean serum uric acid was 08.22 mg%.

The values mentioned in table No. 1, 2, and 3 were considered as standard values in the study of Experiment Part-II. In view of small sample size the mean values of frequent observations were taken at the intervals of 10 days to minimize the possible errors.

EXPERIMENT (PART-II)

AIMS AND OBJECTIVE

1- Induction of urinary bladder stone in albino rats.

2- To assess the effect of trial drug on the inducted bladder stones.

3- To assess the effect of trail drug on certain serum and urinary parameters like 24 hours urine output, urine pH and serum and urinary Calcium, Phosphorus value along with uric acid and creatinine values.

MATERIAL AND METHOD

(a) The Test Formulation

Two forms of Pasanabheda (Bryophyllum pinnatum) i.e. alcoholic extract of whole plant and fresh juice of plant's leaves (Patra Swarasa) were used in two groups of albino rats.

(b) Dose fixation

The dose of the drug was calculated by converting human dose of drug on the basis of body surface area ratio by referring to the table of Pagets and Barnes (1969). Human dose x conversion factor (0.018) for rat = 'x'/200 g. Alcoholic extract and Fresh juice of Pasanabheda leaves: Human dose 20 g./ day, hence, rat dose = 20,000 x 0.018 = 360 mg / 200g.

(c) Route of drug administration

The test drug was administered orally with the help of gastric catheter connected with measured glass syringe nozzle to all the groups.

(d) Animals grouping

Three groups were made:

1. **Group A**: Water control
2. **Group B**: Alcoholic extract treated group (1800 mg / kg)
3. **Group C**: Fresh juice of leaves treated group (1800 mg / kg)

(e) Method of urinary stone formation in bladder: Various methods have been evolved for the production of calculi in experimental animals by the previous research workers (Implantation of zinc pellets etc). For the present study, the method of implanting foreign body i.e. glass bead has been chosen to produce the stone in the bladder of the rats because the procedure was found easy, the formation of stone was almost definite, the growth of stone could be assessed at short intervals by non-invasive methods, the procedure could be adopted in small laboratory setup with animals such as albino rats and repetition of the experiment consumes less time.

EXPERIMENTAL PROTOCOL

Total 24 male albino rats of Charles Foster strain, weight about 270 to 300 gm each, were taken and allocated in to three groups of eight rats in each group. The rats were provided usual and similar laboratory diet with plenty of water as per the Experiment Part-I and allowed them to be adjusted in laboratory atmosphere for one week.

After one week, urinary bladder calculi were produced by putting a small glass bead, approximately 1.5 mm of diameter and 6-9 mg in weight as a foreign body in to the urinary bladder of each rat considering the foreign body as one of the most reliable method for induction of experimental urolithiasis[1],[2],[3].

The individual rat was anaesthetized by giving the intra - peritonial injection Nembutal (0.5 mg/kg) and the suprapubic cystotomy were performed. The urinary bladder was closed after implanting a glass bead in it by using the stay suture to minimize the trauma and finally the abdominal wall was

Table 4: Effect of Drug on the Weight of the Stone in Control Groups-A

Sr. No.	Wt. of glass beads (mg) - a	Wt. of formed stones (mg) – b	Net wt. of stones (mg) a – b
1-	08.00	45.00	37.00
2-	09.00	365.00	356.00
3-	08.50	2129.00	2120.00
4-	08.00	08.00	00.00
5-	07.00	16.00	09.00
6-	06.00	13.00	07.00
TOTAL	**46.50**	**2576.00**	**2529.00**
MEAN	**07.75**	**429.33**	**421.50**

Table 5: Effect of Drug on the Weight of the Stone in Alcoholic Extract Treated Groups-B

Sr. No.	Wt. of glass beads (mg) - a	Wt. of formed stones (mg) – b	Net wt. of stones (mg) a – b
1-	07.00	07.00	00.00
2-	08.00	58.00	50.00
3-	06.00	855.00	849.00
4-	09.00	09.00	00.00
5-	07.50	603.00	595.50
6-	08.50	20.50	20.00
TOTAL	**46.00**	**1552.50**	**1514.50**
MEAN	**07.67**	**258.75**	**252.40**

Table 6: Effect of Drug on the Weight of the Stone in Fresh Juice of Leaves Treated Groups-B

Sr. No.	Wt. of glass beads (mg.) - a	Wt. of formed stones (mg.) – b	Net wt. of stones (mg.) a – b
1-	06.00	06.00	00.00
2-	06.50	06.50	00.00
3-	09.00	11.50	02.50
4-	08.00	08.00	00.00
5-	09.00	15.00	06.50
6-	08.50	104.00	95.50
TOTAL	**47.00**	**151.50**	**104.50**
MEAN	**07.83**	**25.25**	**17.40**

Table 7: Percentage of Stone Formation in Different Groups

Sr. No.	Group	Number of rats with implanted bead	Number of rats found dead	Number of rats with formed stone	Number of rats without stone formation	% of rats with formed stones
1-	Control group -A	08	02	05	01	83.33
2-	Alcoholic extract treated group-B	08	02	04	02	66.66
3-	Fresh juice treated group-C	08	02	03	03	50.00

Table 8: Effect of the Drug on the Mean Weight of the Formed Stones in Different Groups

Sr. No.	Group	Mean Wt. of glass beads (mg.) – a	Mean Wt. of formed stones (mg.) - b	Mean Net wt. of stones (mg.) a – b
1-	Control group - A	07.75	429.33	37.00
2-	Alcoholic extract treated group-B	07.76	258.75	252.40
3-	Fresh juice treated group-C	07.83	25.25	17.40

Table 9: Effect of Drug on Urine Output, Water Intake and Urine pH in Different Groups

Sr. No.	Parameters	Pre-Experimental group	Control Group-A	Alc. Ext. treated Group-B	Fresh Juice Treated Group-C
		Mean Value	Mean Value	Mean Value	Mean Value
1-	Urine output (ml. / 24 hours)	08.08	07.90	10.50	13.50
2-	Water Intake (ml. / 24 hours)	19.00	19.50	21.50	24.00
3-	Urine pH	09.30	09.50	08.00	06.00

Table 10: Effect of Drug on Serum Calcium, Phosphorus, Creatinine and Uric Acid in Different Groups

Sr. No.	Parameters	Pre-Experimental group	Control Group-A	Alc. Ext. treated Group-B	Fresh Juice Treated Group-C
		Mean Value	Mean Value	Mean Value	Mean Value
1-	Serum Calcium (mg. %)	10.18	10.10	08.30	09.30
2-	Serum Phosphorus (mg. %)	09.85	09.85	08.56	08.15
3-	Serum Creatinine (mg. %)	19.12	12.50	11.66	12.50
4-	Serum Uric acid (mg. %)	08.22	10.44	11.18	10.79

Table 11: Effect of Drug on Urinary Excretion of Calcium, Phosphorus and Creatinine in Different Groups

Sr. No.	Parameters	Pre-Experimental group	Control Group-A	Alc. Ext. treated Group-B	Fresh Juice Treated Group-C
		Mean Value	Mean Value	Mean Value	Mean Value
1-	Serum Calcium (mg. %)	10.18	10.10	08.30	09.30
2-	Serum Phosphorus (mg. %)	09.85	09.85	08.56	08.15
3-	Serum Creatinine (mg. %)	19.12	12.50	11.66	12.50
4-	Serum Uric acid (mg. %)	08.22	10.44	11.18	10.79

approximated after replacing the bladder inside the abdominal cavity.

From third post operative day, rats of control group-A were treated with distilled water as a single dose per day where as the rats of treated group – B were provided alcoholic extract of trial drug as single dose per day and the rats of treated group – C, provided fresh juice of the leaves of the trial drug in a single dose per day by adopting the method described in Experiment Part-I. All dosage of water and drugs were as per the calculated as per the dose mentioned above and according to individual body weight of every rat.

The experiment was carried out for total 30 days after implantation of glass bead. The growth of stones was observed along with the analysis of urine and serum parameters at the end of the experiment.

During this period, two rats of each group were found to be dead due to post operative complications. On 30th day, the rats were sacrificed and the stone/s was collected by performing suprapubic cystolithotomy and weighed after drying it. Before that the recording of urine output for 24 hours, pH of urine, water intake for 24 hours were recorded along with the collection of urine and blood sample for required investigations.

OBSERVATIONS

A successful effort was made to produce experimental bladder stone in every rat by implantation of an insoluble foreign body (glass bead) in urinary bladder.[1]

It was observed that the stone was not formed in all rats which suggested that inspite of putting a foreign body in urinary bladder of each rat. Out of 18 rats of different groups, stones were formed in only 12 rats (Table No. 7) and showed uneven in size, shape, surface and even in number which suggested that the stone formation is not a simple phenomenon. Foreign body in the urinary tract is one of the important factors which acts as a nidus and often leads to formation of stones, but it is not always found correct and proved the fact again that the only one factor is not sufficient for development of stone. So, the present findings differ from the finding of other workers i.e. P. Sankaran Nayer (1966), P. Kumar (1971).

The formation of stones was found highest in control group (83.33%) and lowest in fresh juice treated group (50.00%) followed by 66.66% in alcoholic treated group (Table No.7) which showed that the fresh juice of the leaves of drug having more beneficial effect in comparison to alcoholic extract of the drug.

The mean net weight of stones in control group was highest (421.50 mg) whereas it was lowest in fresh juice treated group (17.40 mg) followed by alcoholic treated group 252.40 mg as shown in the Table No.8 which showed the anti - urolithiatic effect of the drug, particularly fresh juice of leaves of the drug .

On gross examination, stones of control group (A) were larger, smooth, shiny and hard, whereas stones of the fresh juice treated group (B) were smaller with uneven surface and brittle in consistency. In the alcoholic extract treated group (C), the stones were relatively bigger with smooth surface than fresh juice treated group which were smaller and rough than the control group.

The drug altered the pH of the urine towards acidic which was more marked in fresh juice treated group and also increase in the 24 hours urine output as well along with the increase of water intake proportionately were noted (Table No. 9). The increased output of urine and acidic pH were suggested towards the prevention of the stone formation in albino rats.

The drug showed lithotriptic effect on vesical calculus in rats probably due to its multi-dimensional action. It showed the diuretic effect which is helpful to prevent the deposition of crystals in urinary tract and further growth of the stone by flushing out the crystals from the urinary tract.[2]

The drug decreased the pH of urine towards acidic. As most of stones are formed in alkaline medium,[3] the drug by changing the pH of urine prevented the formation as well as growth of the stones.

Gross appearance of the stones showed multiple eroded areas which indicated the lithotriptic action of the drug. This might have happened due to the drug which excreted through urine and might have acted upon the stones and eroded it partially by dissolution effect.

These findings can be well explained from Ayurvedic prespective. Though, *Kapha* (*Shleshma*) plays the key role in the formation of *Ashmari* (Stone), other *Doshas* like *Vata* and *Pitta* are also essential for further deposition of stones[4]. Due to properties like *Guru*, *Snigdha* and *Pichchhila*,[5] the *Kapha Dosha* not only acts as a nidus but also binds other *Doshas* and gets consolidated to form *Ashmari*. Pasanabheda is attributed with Sheetaveerya, Tikta, kashaya

Rasas which have pro- Vatic and anti- Kapha properties. The diuretic property of Pasanabheda with other attributers may be concluded that *Ashmaribhedana Karma* might be having *Prabhava* action.

Calcium is the commonest ingredient of the urinary stones and can be present in the form of calcium oxalate, calcium phosphate, calcium carbonate etc. These compounds of the calcium are important for stone formation because it's less solubility and easy precipitation. Irrespective of other factors, hypercalciurea alone is an important factor in the etiopathogenesis of urinary stones. In the present study, the mean value of serum calcium was within normal limit and a little difference was noticed among all the groups (Table No.10) whereas, the mean value of urinary excretion of calcium was significantly higher in alcoholic extract treated group in comparison to control and fresh juice treated groups (Table No.11).

Since, hypercalciurea is one of the known predisposing factors for formation of urinary stones but it is not always essential factor for formation of urinary stones[6]. The animals of alcoholic extract treated group showed hypercalciurea (Table No.10) whereas in control group, the urinary excretion of calcium was within normal limit (Table No.11) but in control group the formation of stones were higher than the alcoholic treated group. These findings suggested that the drug in the form of alcoholic extract enhances the urinary excretion of calcium.

Due to the litholytic properties of the drug the formation of stone in fresh juice treated group and in alcoholic extract treated group was less in comparison to control group. Not only the number of stones formed were less but also the size of stones were smaller and relatively brittle in consistency in drug treated groups.

The alcoholic extract of the drug not only enhanced the urinary excretion of calcium but also prevented the deposition of calcium salts on glass bead. Hence, the drug both, in the form of fresh juice and alcoholic extract reduced the chance of formation of stone in urinary bladder and inhibited the growth of the stones. This was more marked in fresh juice of the drug.

The 24 hours excretion of urinary phosphorus was also estimated in all groups of rats at different intervals and findings suggested that both alcoholic extract and fresh juice of the drug reduced the urinary excretion of phosphorus in comparison to control group (Table No.11).

Excess of urinary phosphorus helped in growth of urinary stones[7]. The mean value of serum phosphorus was showed no significant difference in animals of all groups (Table No.10). So, this finding suggested that even normal serum phosphorus may cause urinary stone[8]. As the drug reduced the urinary excretion of phosphorus it might be helpful in preventing the urinary stone formation.

CONCLUSION

The result of this study has revealed that the trial drug, Pasanabheda (*Bryophyllum pinnatum*), has diuretic and lithotriptic properties in fresh juice of the leaves of the drug. The fresh juice of the drug has also shown the reducing effective of urinary excretion of the calcium, phosphorus and creatinine in experimental rat. The drug has the property to lowering the level of the serum calcium, phosphorus and creatinine but alcoholic extract of the drug has shown better effect. The pH of urine was found reduced in trial drug along with rendering not only the inhibitory effect on the formation of urinary stone but also prevent the further development of stones in urinary bladder. Finally, it can be concluded that the trial drug has the lithotryptic properties by increasing the urine output, lowering the pH of urine, decreasing the excretion of urinary calcium and phosphorus.

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