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Antiurolithiatic Activity of *Parnabeeja* (*Bryophyllum Pinnata* Lamk.) on Ethylene Glycol (Eg) Induced *Mootrashmari* (Urolithiasis)- An Experimental Study

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Abstract:

Mootrashmari (Urolithiasis) remains a significant health problem in the adult population till date. Ethylene glycol induced hypercalciuria and hyperoxaluria model was used to assess the antiurolithiatic activity of *Parnabeeja* in male albino rats. The serum and histopathological results clearly revealed the antiurolithiatic activity of fresh juice of leaves of *Parnabeeja* particularly of calcium oxalate origin. The study suggested that *Parnabeeja (Bryophyllum pinnata)* protects the rats from *Mootrashmari* induced by ethylene glycol.

Key words: Antiurolithiatic activity, *Mootrashmari*, *Parnabeeja*

Introduction:-

Mootrashmari (Urolithiasis) is one of the oldest and most wide spread diseases known to man. The *Vedas* are considered as the oldest record of knowledge in our culture. Rigveda being the oldest and first among the *Vedas* have mentioned *Mootrashmari* [1]. References to *Mootrashmari* are made in the early Ayurvedic documents in India between 3000 and 2000 BC [2]. *Mootrashmari* have been found in the tombs of Egyptian mummies dating back to 4000 BC [3] and in the graves of north American Indians from 1500-1000 BC [4].

Fig. 1 Parnabeeja Plant

Calcium containing stones, especially calcium oxalate monohydrate (Whewellite), calcium oxalate dehydrate (Weddellite) and basic calcium phosphate (Apatite) are the most commonly occurring ones to and extent of 75-90% followed by Magnesium ammonium phosphate (Struvite) to an extent of 10-15%, Uric acid 3-10% and cystine 0.5-1%[5-6]. *Mootrashmari* is a common disorder estimated to occur in approximately 12% of the population with a recurrence rate of 70-81% in males, & 47-60% in females [7].

The incidence of kidney stones has been increased in western societies in the last five decades,

in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones [8].

Many remedies have been employed for the treatment and prevention of *Mootrashmari*. Many Ayurvedic *Aushadhi Dravyas* (medicinal plants) are proved to be useful, and they are reported to be effective with no side effects [9]. In Indian indigenous system of medicine *Parnabeeja* is reported to be useful in the treatment of urinary stones. Although the plant is claimed to be useful in the treatment of urinary stones, there is no record of systematic pharmacological studies on the plant. It has been reported that the kidneys are principle target organ for EG. Toxicity and administration of EG for four weeks resulted in insignificant urinary oxalate excretion and deposition of crystals in kidney. In the present study, an effort has been made to establish the scientific validity of *Parnabeeja.*

Materials and Methods:

Source of plant material and preparation of formulation:-

The leaves of *Parnabeeja* were collected from Kulish Smrutivan, Jaipur & Botanical garden, N.I.A, Jaipur, and authenticated in Department of Dravyaguna, N.IA, Jaipur & confirmed from Botany Department, RU, Jaipur. (Voucher specimen no. RUBL/68). Fresh leaves were cut to small pieces & fresh juice was prepared without adding water. Juice of leaves of *Parnabeeja* was prepared daily and the filtered fresh juice was used for experimental study.

Animal selection:-

Healthy adult male albino rats of Wister strain weighing between 150-200 gms were selected for experimental study. The animals were acclimatized to standard laboratory conditions (Temp. 25 ±2˚C) & maintained on 12 hour light: 12 hour dark cycle. They were provided with standard pelleted fed & drinking water ad libitum.

Ethylene glycol induced Urolithiasis model:-

Ethylene glycol induced hypercalciuria and hyperoxaluria model [10] was used to assess the antiurolithiatic activity in male albino rats.

Animals were divided into 3 groups and containing six animals in each group. Group I (control) served as control and was given regular rat food and drinking water ad libitum. Group II (EG) served as lithiatic group and given 0.75% ethylene glycolated water (solution) for induction of renal calculi till 28th day. Group III (EG+B.P) served as preventive group and given 0.75% ethylene glycolated water and fresh juice from leaves of *Parnabeeja* simultaneously till 28th day. Fresh juice of leaves of *Parnabeeja* given once daily by oral route.

Table 1:

Statistical analysis of serum sodium, serum chloride, serum potassium and serum calcium among Control, EG and EG + BP groups

Parameters	Control (Mean±SEM)	0.75% EG (Mean±SEM)	0.75% EG + BP (Mean±SEM)	Statistics One way ANOVA
Serum sodium	134.16±0.6009	141±1.390	135±0.4773	F=15.099 P< 0.0003 Significant
Serum chloride	97.26±0.768	101.5±0.4382	98.33±0.6009	F=12.103 p <0.0007 Significant
Serum potassium	5.7±0.1826	6.016±0.1167	5.983±0.1014	F=1.587 P<0.2370 Not significant
Serum calcium	10.4±0.278	9.016±0.1222	9.93±0.1145	F=14.099 P<0.0004 Significant

Values are Mean±SEM, n=6 in each group, EG = Ethylene Glycol, BP = *Bryophyllum pinnata*, p value less than 0.05 was considered as significant. Test Analysis of variance (One way ANOVA) followed by Turkey-Kramer multiple comparisons test

Table 2: Statistical analysis of serum creatinine, serum uric acid, blood urea, BUN among Control, EG and EG + BP group.

Parameters	Control (Mean±SEM)	0.75% EG (Mean±SEM)	0.75 EG + BP (Mean±SEM)	Statistics One way ANOVA
Serum creatinine	0.7383±0.017	1.216±0.06009	0.753±0.02171	F=50.517 P<0.0001 Significant
Serum uric acid	6.816±0.1167	6.8±0.2082	6.883±0.1973	F=0.060 P<0.9412 Not Significant
Blood urea	27.33±0.8819	41.16±3.710	27.67±0.8819	F=12.2 P<0.0007 Significant
Blood urea nitrogen	12.25±0.2349	18.75±1.675	12.33±o.4014	F=13.806 P<0.0004 Significant

Values are Mean±SEM, n=6 in each group, EG = Ethylene Glycol, BP = *Bryophyllum pinnata*, p value less than 0.05 was considered as significant. Test Analysis of variance (One way ANOVA) followed by Turkey-Kramer multiple comparisons test.

Statistical Analysis:-

Results were expressed as mean ± SEM differences. The data were analysed using Analysis of variance (one-way ANOVA) followed Turkey-Kramer multiple comparison test and differences between the data were considered significant at $p<0.05$ [11].

The concentration of serum sodium, serum chloride, serum potassium and serum calcium are showm in Table 1. The level of Sodium was significantly elevated in rats treated with EG (Gr.II) compared with control value (Gr.I), there were significant decrease in the level of Sodium in rats treated with EG plus BP (Gr.III). Statistically increase in the level of chloride was noted in rats treated with EG (Gr.II) when compared with control value (Gr.I), while there was significant decrease in the values of chloride in rats treated with EG plus BP (Gr.III). The values of potassium were remarkably unchanged in Group II & III when compared with control values (Gr.I). The leval of calcium was significantly decrease in rats treated with EG (Gr.II) when compared with control value (Gr.I), while there was significant increase in the values of calcium in rats treated with EG plus BP (Gr.III).

The levels of BUN, blood urea, serum creatinine, serum uric acid are shown in Table 2. The levels of serum creatinine, BUN, blood urea were significantly increased in rats treated with EG (Gr. II) when compared with control group(Gr.I), while there were significant decrease in values of these parameters in rats treated with EG plus BP (Gr.III). There were no significant differences in the values of Serum uric acid in rats treated with EG (Gr.II) and EG plus BP (Gr.III) when compared with control values (Gr.I).

In the present study the chronic administration of 0.75% v/v ethylene glycolated water to male albino rats resulted in hypercalciuria & hyperoxaluria, as the decrease of serum calcium indicates an increase of urinary calcium & calcium oxalate stone formation.

Histological study

On histopathological observation EG induced lithiatic group (Gr.II) showed presence of polymorphic irregular calcium oxalate crystals in lumina of tubules accompanied by edema and cast formation which causes dilation of proximal tubules along with interstitial inflammation. This might be attributed to oxalate formation and also causes extensive intertubular hemorrhages and congestion of blood vessels. Atmani et. al.[12] have also shown that crystal deposits are intensively birefringent, polycrystalline & arranges in rosette characteristic of

calcium oxalate crystals. The presence of such deposits is an evidence of adhesion and retention of pentids with in renal tubules. These histological observations support the presence and growth of renal calculi in renal medulla regain as observed in human urolithiasis (fig. 1,2,3).

The serum and histopathological results clearly revealed the antilithiatic activity of fresh juice of leaves of *Parnabeeja,* particularly of calcium oxalate origin.

Discussion and Conclusion:

In general population, or consumer exposure occurs primarily from the use of EG in automotive antifreeze. These have been a number of acute human poisoning from accidental or intentional ingestion of antifreeze, with the kidney being the most sensitive target organ. Regimens for the treatment of acute EG poisoning are designed to prevent metabolism to the toxic acidic metabolites to treat acidosis and to prevent kidney damage [13.14]. EG has in itself a low toxicity. But is *in vivo* broken down to four organic acids: Glycoaldehyde, glycolic acid, glyoxylic acid and oxalic acid. The metabolites are cell toxins that cause CNS depression and cardiopulmonary and renal failure. Glycolic acid causes severe acidosis and oxalate is precipitate as calcium oxalate in the kidneys and other tissues [15]. Urolithiasis and Hepatotoxicity induction by EG was established in many researchers [16-23]. It has been reported that the kidneys are principle target organ for EG. Toxicity and administration of EG for four weeks resulted in insignificant urinary oxalate excretion and deposition of crystals in kidney[24]. Hence in our study EG was chosen to induce urolithiasis. In the present study the chronic administration of 0.75% v/v ethylene glycolated water to male albino rats resulted in hypercalciuria & hyperoxaluria, as the decrease of serum calcium indicates an increase of urinary calcium and calcium oxalate stone formation. The decrease of serum calcium concentration indicates an increase of urinary calcium and calcium oxalate stone formation. This suggestion is an agreement with several studies like Rajagopal et. al.(1977) [25] who reported that the level of serum calcium was decreased and urinary calcium increased in rats treated with EG. Moreover, Soundararajan et. al. (2006) [26] showed that calcium oxalate excretion was significantly increased in urine of EG induced urolithic rats. Additionally, they stated that EG disturbs oxalate metabolism by way of increased the substrate availability that increase the activity of oxalate synthesizing enzymes in rats. Sodium and chloride excretion from the body is a function of arterial blood pressure [27]. Sodium depletion stimulates rennin release and subsequence production of Angiotensin II, a potent vasoconstrictor. Increased blood sodium levels inhibit rennin release from the juxtaglomerular cells and consequent withdrawal of Angiotensin II [28]. When modulation of the rennin-angiotensin system is pharmacologically prevented, Changes in salt take markedly affect long term levels of arterial blood pressure [29]. There is therefore a need to strike a balance in levels of extreme of hypotension or hypertension. Kang et. al. (2002) [30] reported that the hypernatremia is rare but does occur when there is loss of body fluid containing less sodium than blood along with water intake restriction or if there is excessive sodium intake with limited liquid intake. Vogal et. Al. (2009) [31] reported that the hypernatremia almost always indicate water depletion. The present increase of serum sodium level is suspected to be due to the inability of the kidneys to excreate adequate sodium from the tubular fluid. Also, the levels of serum creatinine, blood urea & blood urea nitrogen were significantly increased as a strong indication of renal & hepatic impairment.

References:

[1]N. S. Sontakke, Rigvedasamhita (Sayanbhashya): part 1-5, Vedic sanshodhana mandala, Puna.1933-1957.

[2]Butt A. J. Etiologic factors in renal lithiasis, Charles. C. Thomson publishers, Springfield, Illinosis, USA, 1954;3.

[3]Riches E. The history of lithotomy & lithotrity. Ann. R. Coll. Surg. Engl, 1968;43:185.

[4] Beck C. W, Mulvane W. P. Apathetic urinary calculi from early American Indians. JAMA, 1996;195:168-169.

[5] Dietrich B.L, Blaschke R, Schmidt W. "Results of 5035 stone analysis. A contribution to epidemiology of urinary stone disease". Scand. J.Urol.nephrol, 1990;24:205-210.

[6] Williams H.E, Wandzilak T.R. Oxalate synthesis, transport & the hyperoxaluric syndromes. J. Urol. 1989;141:742.

[7] Smith C.L, Guay D.R.P. Nephroliyhiasis, in pharmacotherapy & pathophysiologic approach, 2nd edition editated by Dipitro, Talbert, Hayes, Yee, Matzke, Posey (Elsevier, Newyork) 1992:720.

[8] Prien E.L. Jr. Composition & structure of urinary stone. Am. J Med, 1968;45:654-672.

[9] Nadkarni K.M. In Indian materica medica, volume 1, 3rd edition (Popular Book Depot, Bombay) 199976:371.

[10] Atmani F., Silmani Y., Mimouni M., Hacht B.; Prophylaxis of calcium oxalate stone bt Herniaria hirsute on experimentally induced nephrolithiasis in rats. BJU Int., 2003, 92: 137-40.

[11] Kulkarni S.K. Handbbok of experimental pharmacology. 2nd edition: Mumbai: Vallabh Prakashan. 1993:172-189.

[12] Atmani F, Silmani Y, Mimouni M, Aziz M, Haccht B, Ziyyat A, Effect of aqueous extract from from Herniaria hirsute L on experimentally nephrolithic rats. J Ethno Pharmacol, 2004;87-95.

[13] Barceloux, D. G., E. P. Krenzelok, K. Oison & Watson, 1999; American academy of clinical toxicology practical guidelines on the treatment of ethylene glycol poisoning. J. Toxicol. Clin. Toxicol., 37:537-560.

[14] Brent, J., K. Mc Martin, S. Pillips, K.K Burrkhat & J.W. Donovan et. Al., 1999. Fomepizole for the treatment of ethylene glycol poisoning. N. Engl. J. Med.,340:832-838.

[15] Leth, P. M. & M. Gregerson, 2005. Ethylene glycol poisoning. Forenscic Sci. Int., 155:179-184.

[16] Christina, A. J., L. M. Packia, M. Nagarajan & S. Kurian, 2002. Modulatory effect of Cyclea peltata Lam. On stone formation induced by ethylene glycol treatment in rats. Methods find. Exp. Clin. Pharmacol., 24:77-79.

[17] Huang H. S., Ma M.C., Chen J., Chen C. F.; Changes in the oxidant antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J. Urol., 2002; 167:2584-93.

[18] Karadi R. V., N. B. Gadge, K. R. Alagawadi & R. V. Savadi, 2006. Effect of Moringa oleifera Lam. Root-wood on ethylene glycol induced urolithiasis in rats. J. Ethnopharmacol., 105:306-311.

[19] Celik, I. & H. Suzek, 2007. Effects of subacute treatment of ethylene glycol on serum marker enzymes & erythrocyte & tissue antioxidant defence systems & lipid peroxidation in rats. Chem. Biol. Interact., 167:145-152.

[20] Hadjzaden, M. A., A. Khoei, Z. Hadjzadech & M. Parizady, 2007. Ethanolic extract of Nigella sativa L. seeds on ethylene glycol induced kidney calculi in rats. Urol. J., 4:86-90.

[21] Hadjzaden, M. A., N. Mohammadian, Z. Rahmani & F.B. Rassouli, 2008. Effect of thymoquinone on ethylene glycol induced renal calculi in rfats. Urol. J., 5:149-155.

[22] Verma, N.K., S.S. Patel, T.S.M. Saleem , A.J.M. Cristina 2009. Modulatory effect of noni-herbal formulation against ethylene glycol induced renal calculi in albino rats. J. Pharma. Sci. Res., 1:83-89.

[23] Divaka, K., A. T. Pawar, S. B. Chandrasekhar, S. B. Dighe & G. Divakar, 2010. Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats. Food Chem. Toxicol.

[24] D. Anantha K.C, M.S., A.M.R., 2010Antiurolithiatic activity of Macrotyloma uniflorum seed extract ethylene glycol induced urolithiasis in rats.

[25] Rajagopal , G. K. Venkatesen, P. Ranganathan & S. Ramakrishnan, 1977. Calcium & phosphorous metabolism in ethylene glycol toxicity in rats. Toxicol., Applied Pharmacol., 39:543-547.

[26] Soudararajan P., R. Mahesh, T. Ramesh & V. H. Begum, 2006. Effect of Aerva lanata on calcium oxalate urolithiasis in rats. Indian J. Exp. Biol. , 44:981-986.

[27] Guyton, A,C. & J.E. Hall,. Textbook of medical physiology. 11th ed. 2006, Elsevier & Saunders, Philadelphia.

[28] Jackson, B.A & T.A. Kotchen, 1984. Salt sensitive hypertension contribution of chloride. Science, 223:1430-32.

[29] Hall, J.E., M.W. Brands & J.R. Heneger,1999. Angiotensin II & long term arterial pressure regulation J. Am. Soc. Nephrol., 10:s256-265.

[30] Kang S., W. Kim, M.S. Oh, 2002. Pathogenesis & treatment of hypernatremia. Nephron, 92:14-17.

[31] Vogal, B. L., M. Burnier, 2009. Hypernatremia. Ther. Umsch., 66:753-757.

[32] Bapalal Vaidya; Some controversial drugs in Indian Medicine, Chaukhamba Sanskrit Series, Varanasi, 1982.

[33] Vaidya, Bapalal: Nighantu Adarsha, Chaukhamba Bharti Academy, Varanasi, vol I, Reprint. 1998.

[34] Bhavamishra: Bhavaprakash Nighantu, Commentry by K. C. Chunekar, Edited by Dr. G. S. Pandey, Chaukhamba Orientalia, Varanasi, 8th Ed. 1988.

[35] Sharma Prof. P.V: Dravyaguna Vigyana part 1 and 5, Chaukhamba Vishwabharati, Varanasi, reprint, 2006.

[36] Watt, George: A dictionary of the economic products of India, Cosmo publication Delhi, 1972. vol VI, part IV.

[37] Anonymous : The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health & Family

Welfare ,Department of Health New Delhi, part-1, vol I, 1989, p.47-48.
[38] Bhandari, Chandra Raj: Vanaushadhi Chandrodaya, Chaukhamba Sanskrit Series, Varanasi, vol X, 10th Ed.1993.
[39] Dr. Ram Sushil Singh: Vanaushadhi-Nidarshika (Ayurvedic Pharmacopea), 2nd edition, Jivan Shiksha Mudranalaya, Varanasi.
[40] Mishra, Banwari Lal: Dravyaguna Hastamalak, Premlata Nathani Publication, Jaipur, 2nd Ed. 1986.
[41] Athervavedasamhita (Sayanbhashya): part 1-4, Sanskrit Vishwabandhu, Hashiyarpur. 1960-1962.

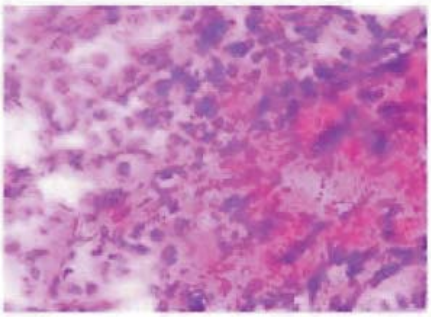

Fig-1

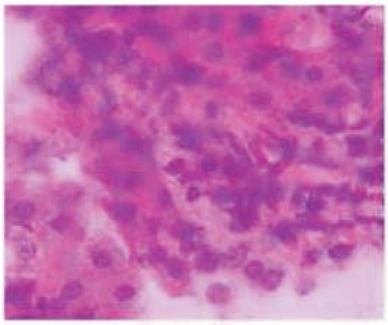

Fig-2

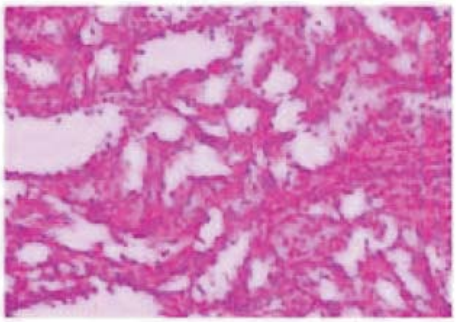

Fig- 3

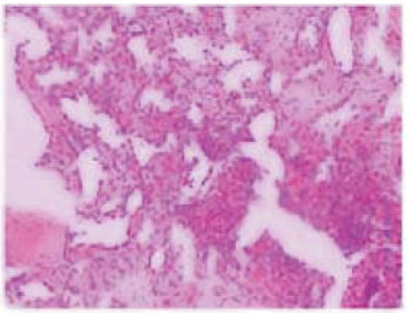

Fig- 4

Photo micrographs of kidney tissue sections from Fig-1 normal rat, Fig- 2 a rat receiving ethyleneglycol (0.75% in drinking water) Fig- 3 a rat receiving *Macrotyloma uniflorum* aqueous extract (250mg/kg body weight) Fig- 4 a rat receiving *Macrotyloma uniflorum* alcoholic extract (250mg/kg body weight)