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BIOCHEMICAL EFFECTS IN NORMAL AND STONE FORMING RATS TREATED WITH THE RIPE KERNEL JUICE OF PLANTAIN (MUSA PARADISIACA)

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ABSTRACT: *The effect of Musa paradisiaca stem kernel juice was investigated in experimental urolithiatic rats. Stone forming rats exhibited a significant elevation in the activities of two oxalate synthesizing enzymes - Glycollic acid oxidase and Lactate dehydrogenase. Deposition and excretion of stone forming constituents in kidney and urine were also increased in these rats. The enzyme activities and the level of crystalline components were lowered with the extract treatment. The extract also reduced the activities of urinary alkaline phosphatase, lactate dehydrogenase, r-glutamyl transferase, inorganic pyrophosphatase and β-glucuronidase in calculogenic rats. No appreciable changes were noticed with leucine amino peptidase activity in treated rats.*

INTRODUCTION

Urinary lithiasis is one of the most common diseases of mankind where in there is aggregation of crystalline components like oxalate, calcium, phosphate, uric acid etc., in the kidneys and urinary tract. This is likely to be associated with crystalluria (Hodgkinson, 1978).

The treatment of urolithiasis involves the dissolution of existing stones in the urinary tract and prevention of recurrence of further stone formation. Though many medical and sophisticated surgical therapies have been tried, they are associated with renal damage (Drach er al., 1986). In India, many traditional medicines have been used in the treatment of urinary stones from ancient times, and they need scientific evaluation to understand their mode of action.

The ripe kernel juice of *Musa paradisiacal* is popular among the rural people in South India and has been used to dissolve urinary stones (Nadkarni, 1976). It has no toxic effect, is readily available and easily consumable. The present work aims at understanding the administration of kernel juice to experimental calcium oxalate stone forming rats.

MATERIALS AND METHODS

Animals:

Male albino rats derived from Wistar strain weighing between 150 – 180g were used for the investigation. The animals were acclimatized to the laboratory conditions for a week and were fed with commercial pelleted diet (Gold mohur, Hindustan Lever Ltd, India). Water was given *ad libitum.*

Induction of calcium oxalate kidney stones:

In order to induce calcium oxalate lithiasis, the rat fee was mixed with 3% sodium glucollae and pelleted. These pellets were dried at room temperature for two days. This calculi producing diet (CPD) was fed to the rats for 30 days to induce calcium oxalate stones (Chow *et al.,* 1975).

The drug:

Fresh banana (*Musa paradisiacal Linn.,)* Kernel of the ripe plant (Valaithandu) was obtained locally. It was pulverized for the extract. The extract was administered through oral route by gastric intubation at a dosage of 1.5 ml/ rat/day, without any modification (Poonguzhali and Varalakshmi, 1992).

Experimental set up:

The animals were divided into six groups of six rats each.

Group I: Rats maintained on normal diet for 30 days (Control). The control rats received placebo (drinking water) by intragastric intubations to overcome the stress due to the mode of administration.

Group II: Rats fed with CPD for 30days (Stone formers).

Group III: Rats received normal diet and Musa stem extract during the last 15 days.

Group IV: Rats received normal diet and Kernel extract for 30 days.

Group V: Rats treated with CPD for 30 days and kernel juice during the last 15 days.

Group VI: Rats treated with CPD and kernel juice simultaneously for 30 days.

The initial and final body weight of the rats were recorded at the start of the experimental period and just before sacrifice.

The animals were acclimatized to metabolic cage for a week, to overcome the stress of the metabolic cage. Urine was collected in containers maintained at 0^0C in an ice bath, 24 hours before sacrifice. A portion of the urine sample was acidified with concentrated hydrochloric acid and used for the estimation of stone forming constituents – oxalate (Hodgkinsons and Williams, 1972), calcium (Hooper, 1956) and Phosphorus (Fiske and Subbarow, 1975). The other portions of the urine was centrifuged for 10 minutes and the sediment was discarded. The supernatant was then dialysed at 4^0C against distilled water for 3 hours. The aliquots of the dialysed urine was used for the assay of urinary enzymes.

Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated by the method of King (1965 a,b). Inorganic pyrophosphatase was determined by the method of Josse (1966). The method of Natelson (1971) was adopted for estimation leucine aminopeptidase (LAP). B-glucuronidase and gamma – glutamyl transferase were assayed by the methods of Kawai and Anno (1971) and Orlowski and Meister (1965). The enzyme activities were in terms of units/mg creatinine/hr.

At the end of the experimental period, the animals were sacrificed, their liver and kidneys were dissected and washed with ice cold saline and weighed. A 10% homogenate of these tissues were prepared, centrifuged at 12,000 g for 30 minutes and supernatant was used for the assay of the

two major oxalate synthesizing enzymes – glycolic acid oxidase (GAO) and lactate dehydrogenase (LDH) by the method of Lui and Roels (1970) and King (1965a) respectively. The protein content was estimated according to the method of Lowry et al, (1951).

Wet ashing of the kidney tissue was done according to the method of Ballentine and Barfoed (1957) and used for the estimation of renal stone forming constituents. Students 't' test was used to arrive at the statistical significance associated with various groups.

RESULTS AND DISCUSSION

Urinary super saturation with respect to the stone forming constituents is considered to be one of the prime factors in determining the pathogenesis of stone formation. In this context, mild hyperoxaluria has been reported to be more important factor than hypercalciuria (Antonacci et al., 1985).

Table – 1 shows the levels of oxalate, calcium and phosphorus in the urine and kidney of control and experimental rats.

The 24 hours urinary oxalate excretion was found to be significantly elevated ($p<0.001$) as compared to that of the controls. This increase may be due to that of the controls. This increase may be due to enhanced oxalate synthesis in the hepatic tissues by GAO from the orally administered sodium glycollate precursor. The extract treated calculogenic rats subsequently showed a marked reduction in oxalate level. The above results are comparable to that reported by Varalakshmi et al., (1990) and Baskar (1990) using the two indigenous durgs, Crataeva nurvala and Coleus aromaticus respectively, which also possess antilithic properties. Similarly, calcium and phosphorus excretions were also found to be elevated in the lithogenic rats. Hypercalciuria may be due to defective renal tubular reabsorption of dietary calcium or renal calcium leak (David et al., 1988). The increase in phosphorus excretion is in accordance with the observation of Varalakshmi et al., (1987) in sodium glycollate administered rats. The extract treatment significantly lowered the elevated levels of calcium ad phosphrous treated groups. This reduction in stone forming constituents due to the drug, is likely to prove beneficial in preventing calcium oxalate crystallization and subsequent stone formation.

Glycollate administration increased the levels of oxalate, phosphorus and calcium in the kidneys of stone forming rats. Calcium and oxalate concentration in renal tissues play key roles in the pathogeneisi of papillary calcification and eventual stone formation (Hautmann and Lehmann, 1980). Treatment with the extract decreased the kidney concentration of oxalate, phosphrous and calcium in the kidneys of stone forming rats. Calcium and oxalate concentration in renal tissues play key roles in the pathogeneisi of papillary calcification and eventual stone formation (Hautmann and Lehmann, 1980). Treatment with the extract decreased the kidney concentration of oxalate, phosphorus and calcium ($p<0.001$) respectively.

Oxalate synthesizing enzymes

Table 2 depicts the activities of oxalate synthesisng enzymes in liver and kidney of control and experimental animals.

GAO is a flavin linked peroxisomal enzyme, predominately localized in the liver (Master and Holmes, 1977), known to oxidize glycollate to glyoxylate, which in turn is

oxidized into oxalate. Another enzyme, LDH is a cytosolic enzyme present in most of the body tissue and fluids, catalising simultaneous oxidation and reduction of glyoxylate. A significant increase in the GAO and LDH activities were seen in the livers of the calculogenic rats which closely resembles the reports made in pyridoxine deficient rats (Varalakshmi and Richardson, 1983). The increade in GAO activity may be due to its greater affinity for glycollate than glyoxylate. Extracts administration reduced the activities of liver GAO and LDH in calculogenic rats assigning its efficiency in regulating oxalate synthesis. Similar reports were made in this laboratory with rats induced hyperoxaluria, for 7 days (Poonguzhali and Varalakshmi 1992).

Urinary enzymes

Urinary excretion of marker enzymes have found a very high diagnostic value in understanding the biochemical mechanisms underlying stone formation.

Table 3 summarises the levels of urinary marker enzymes in the control and experimental rats. Alkaline phosphatase activity is regularly present in human urine and is an intergral part of the brush border membrane. Its activity is important for the process of calcification (Majeska and Wuthier, 1975). An increase in alkaline phosphatase activity was observed in stone forming rats which closely parallels the work done by Khan et al., (1989) in hyperoxaluric conditions. This increase in enzyme activity could be correlated with decrease in kidney enzyme in caculogenic rats (Jayanthi et al., 1990). Extract treatment was found to reduce the enzyme activity in CPD fed rats.

Lactate dehydrogenase is present in rat urine in readily measurable amounts. Stone forming rats showed an increase in the urinary levels of the enzyme activity, which can be considered as a biochemical marker of nephron cell damage. This might be due to the necrosis of proximal tubules (Hofmeister et al., 1986). Supplementation of the kernel juice moderately decreased the enzyme activity.

Calcium phosphate lithiasis would be favoured by an alkaline urine because it would contain less pyrophosphate and newly released phosphate as a result of its pyrophosphatase activity. A reduction in pyrophosphatase activity was observed by us similar to that reported by Schneider and Wasmund (1971) in nephrolithiatic rats. This reduction may be due to the fact that dialyzable components in urine like oxalate and sulphare ions are found to inhibit the enzyme activity (Wakid et al., 1970). The activity was further reduced in treated groups with extract treatment.

LAP is a brush border enzyme and its activity is present mainly in the proximal tubules (calcamuggi et al., 1988). Kidney is the major contributor of urinary LAP. The reduction in LAP activity in cacogenic rats closely parallels the lowered fibrinolytic activity observed in other studies, (Subha, 1992; Charlton and Osmond, 1986). Kernel juice administered produced a slight increase in LAP activity in treated groups.

Urinary β-glucuronidase, in kidneys and epithelial cells of the urinary tract, is elevated during acute inflammation and toxic lesions (Bank and Bailine, 1965). Similar increase in the enzyme activity was observed here in stone forming rats. The results are comparable to that made in calculogenic rats with L(+) – tartrate administration (Padmaja and Varalakshmi, 1989). The extract treatment resulted in

moderate decrease in β-glucuronidase activity in CPD treated groups.

Gamma glutamyl transferase (_-GT) is deeply localized in the brush border membrane, which catalyses the transfer of glutamyl residue from glutathione and other glutamly peptides to various acceptors. Elevated urinary excertion of _-GT was observed in stone forming rats as in patients with idiopathic calcium lithiasis (Baggio et al., 1983). Patients subjected to extracorporeal shock wave lithotripsy also showed a closely resembling picture after 24 hours (Karlin *et al.,* 1990). The extract was found restore the activity to that of normal in CPD treated rats.

Thus, the reduction in urinary oxalate levels along with reduced GAO and LDH activities brought about by Musa stem kernel extract in rat lithogenesis is note worthy and are likely to contribute to the antilithic action of the herbal plant. Further work is in progress to evaluate its actions in humans.

TABLE – 1

EFFECT OF *MUSA PARADISIACA* KERNEL EXTRACT ON STONE FORMING CONSTITUENTS IN URINE AND KIDNEY OF CONTROL AND CALCULOGENIC RATS

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Urine (mg/24 hours)						
Oxalate	1.49 ± 0.22	2.74 ± 0.22[a]***	1.32 ± 0.25	1.02 ± 0.12[c]**	1.73 ± 0.04[f]***	1.27 ± 0.25[a]***
Calcium	0.49 ± 0.03	0.66 ± 0.06[a]***	0.39 ± 0.05	0.35 ± 0.04[c]*	0.45 ± 0.04[f]***	0.38 ± 0.03[g]***
Phosphorus	3.71 ± 0.60	5.74 ± 0.81[a]***	2.62 ± 0.30[b]***	2.23 ± 0.17[c]***	3.62 ± 0.50[f]***	3.16 ± 0.13[g]***
Kidney (mg/g wet tissue)						
Oxalate	0.75 ± 0.07	1.45 ± 0.18[a]***	0.68 ± 0.11	0.57 ± 0.06[c]***	1.28 ± 0.13[a]***	0.82 ± 0.08[g]***
Calcium	2.93 ± 0.66	3.76 ± 0.39[a]***	2.01 ± 0.10	1.81 ± 0.08	2.51 ± 0.05[b]***	1.55 ± 0.09[g]***
Phosphorus	0.22 ± 0.10	0.40 ± 0.01[a]*	0.20 ± 0.06[b]*	0.19 ± 0.01[c]*	0.26 ± 0.02[f]***	0.22 ± 0.01[c]***[g]***

Values are expressed as Mean ± SD of six animals

Group I - Control – Normal diet
Group II - Stone formers – CPD
Group III - Control + extract for 15 days
Group IV - Control + extract for 30 days
Group V - CPD + extract for last 15 days
Group VI - CPD + extract for 30 days

Comparisons made between groups are:

a – Group I and II; b – Group I and III; c – Group I and IV; d – Group I and V; e-Group I and VI; f – Group II and V; g – Group II and VI

Statistical significance *p<0.05, **p<0.01, ***p<0.001

TABLE – 2

EFFECT OF *MUSA PARADISIACA* KERNEL EXTRACT ON OXALATE SYNTHESIZING ENZYMES IN LIVERS AND KIDNEYS OF CONTROL AND CALCULOGENIC RATS

Enzymes	Group I	Group II	Group III	Group IV	Group V	Group VI
Liver						
GAO	1.47 ± 0.16	2.53 ± 0.36[a]***	1.29 ± 0.01[b]*	1.15 ± 0.03[c]**	2.26 ± 0.09[d]**[h]***	2.05 ± 0.16[e]**[g]*
LDH	1.10 ± 0.09	1.83 ± 0.12[a]***	1.06 ± 0.12	1.02 ± 0.10	1.79 ± 0.10[d]***[h]***	1.63 ± 0.16[e]***[g]*
Kidney						
LDH	1.51 ± 0.29	1.67 ± 0.33	1.36 ± 0.34	1.07 ± 0.05c*	1.61 ± 0.05	1.24 ± 0.13g*

Values represent the Mean ± SD of six animals

Enzymes units are as follows : One unit of GAO is defined as the enzyme required for the production of one manomole of glyoxylate per minute at 37^0C.

One unit of LDH is defined as the enzyme required for the production of one micromole of pyruvate per minute at 37^0C.

Comparisons made between groups as same as in Table.1.

Statistical significance : *p < 0.05; ** p < 0.01; *** p<0.001.

TABLE 3

EFFECT OF *MUSA PARADISIACA* KERNEL EXTRACT ON UPINARY ENZYMES OF CONTROL AND CALCULOGENIC RATS

Enzymes	Group I	Group II	Group III	Group IV	Group V	Group VI
ALP	0.78 ± 0.03	1.02 ± 0.09[a]***	0.71 ± 0.08	0.61 ± 0.14[c]*	0.90 ± 0.11[d]*	0.68 ± 0.15[g]**
LDH	1.61 ± 0.40	3.16 ± 0.40[a]***	1.46 ± 0.05	1.34 ± 0.60	3.01 ± 0.70[d]**	2.80 ± 0.16[e]***[g]*
Inorganic pyrophosphatase	1.48 ± 0.08	1.05 ± 0.15[a]***	1.20 ± 0.09[b]**	1.16 ± 0.13[c]**	0.87 ± 0.06[d]***[f]*	0.47 ± 0.08[e]***[g]*
LAP	1.24 ± 0.20	0.97 ± 0.15[a]*	1.30 ± 0.11	1.39 ± 0.18	1.08 ± 0.09	1.19 ± 0.25
β-glucuronidase	0.05 ± 0.003	0.09 ± 0.016[a]**	0.043 ± 0.002[b]***	0.039 ± 0.005[c]**	0.087 ± 0.010[d]***	0.083 ± 0.005[e]***[g]
Glutamyl transferase	5.04 ± 0.32	9.84 ± 0.53[a]***	4.36 ± 0.28[b]**	4.21 ± 0.18[e]**	6.93 ± 0.68[d]***[f]***	5.20 ± 0.15[g]***

Values represent the Mean ± SD of six animals

Enzymes units are as follows : ALP – micromoles of phenol; LDH – micromoles of pyruvate; Inorganic pyrophosphatase – micromoles

LAP – micromoles of β – naphthylsnine; β – glucuronidase – micromoles of p – nitrophenol; glutamyl transferase – micrnitroaniline per mg creatinine per hour at 37^0C.

Comparisons made between tables are same as in Table – 1 Statistical significance : *p , 0.05; ** $p < 0.01$; *** $p < 0.001$.

REFERENCES

1. Hodgkinson, A. Evidence of increased oxalate absorption in patients with calcium containing renal stones. Clin. Sci. Mol. Med., 54, 291 – 294 (1978).

2. Drach, G.W., Dretler, S., Fari, W., Finlayson, B., Gillenwater, J., Griffith, D., Lingeman, J. and Newman D. Report of the United States cooperative study of extracorporeal shockwave lithotripsy, J. Urol., 135, 1127 – 1130 (1986).

3. Nadkarni, K.M., The medicinal and nutritional properties of Musa species. Indian Materia Medica, Vo. I: 822 – 827 (1976).

4. Chow, F.H.C., Dysart, M.I., Harmar, D.N. and Udall, R.H. Control of oxalate urolithasis by DL-alanine. Invest. Unol., 13, 113 – 116 (1975).

5. Poonghuzali P. and Varalakshmi, P. Effect of banana stem juice on biochemical change in liver of normal and hyperoxaluric rats. Ind. J. Exp. Biol., 30, 440 – 442 (1992).

6. Hodgkinsons, A. and Williams, A. An improved colorimetric procedure for urine oxalate. Clin. Chem. Acta, 36, 127 – 132 (1972).

7. Hooper J., Microdetermination of urine calcium, J. Med. Lab. Tech., 13, 40 – 473 (1956).

8. Fiske, C.H . and Subbarow, Y., The calorimetric determination of phosphrous. J. Biol. Chem, 66, 375 – 400 (1975).

9. King, J.C. Lactate dehydrogenase in: Practical clinical enzymology, D. Van. Nostrand Co., Ltd., London, 83 – 93 (1965a).

10. King J.C. The phosphatase in: Practical Clinical Enzymology D. Van. Nostrand Co. Ltd., London, 191 – 208 (1956b).

11. Josse, J. Constitutive inorganic pyrophosphatase activity of E. Coli. J. Biol. Chem., 241, 1938 – 1947 (1966).

12. Natelson, S. Estimation of leucine aminopeptidase in : Techniques of Clinical Chemistry (Springfield, T.Ed), 3, 462 – 464 (1971).

13. Kawai, Y. and Anno, K. Mucopolysaccharide – degrading enzymes from the liver of the squid, Ommastrepties slonipacificus. Biochem. Biophys. Acta, 242, 428 – 438 (1971).

14. Orlowski, M. and Meister, A. Isolation of Gamma – glutamyl transpeptidase from dog kidney. J. Biol. Chem., 240, 338 – 347 (1965).

15. Lui, N.S.T. and Roels, O.A. An improved method for determining glyoxylic acid. Anal. Biochem., 38, 202 – 209 (1970).

16. Lowry, O.H., Rose brought, N.J., Farr, A.L. and Randall, R.J., Protein measurements with the Folin's phenol reagent. J. Biol. Chem.., 193, 265 – 275 (1951).

17. Ballentine, R. and Barford, D.D. Determination of metals in: Methods in Enzymology. Colowick, P and Kaplan, N.O., (Academic Press, New York), 3, 1002 – 1035 (1957).

18. Antonacci, A. Colussi, G., De Ferrai, M.E., Rombola G., Pontoneiro, G., Surion, M., Malberti, F and Minetti, L. Calcium oxalate urine supersaturation in calcium stone formere: hypercalciuria versus hyperoxaluria in: Urolithiasis Clinical & Basic research, Schwille, P.O., Smith, L.H., Robertson, W.G., and Finlayson, B. Plenum press, New York, London, 283 – 286 (1985).

19. Varalakshmi, P., Sharmila Y. and Latha, E. Effect of Crataeva nurvala in experimental urolithiasis J. Ethanopharmacol., 28, 313 – 321 (1990).

20. Baskar, R. M.Phill thesis entitled "Role of Coleus aromaticus in experimental urolithiasis". University of Madras, 1990.

21. David, A.B., Ben Johnson, R., Carol e. Nalbantain and Murray J. Flavus. Increased calcium absorption and retention without elevated serum, 1, 25 $(OH)_2$ D_3 in genetically hyperoxaluric rats. Kidney, 33, 129 – 164 (1988).

22. Varalakshmi, P., Poonguzhali, P. and Selvam, G.S. Effects of the ripe kernel juice of plantain (Musa paradisiacal) in experimental hyperoxaluric rats in: Proceedings of IV National Conference – Urolithiasis Society of India, Chandigarh, pp 26 (1987).

23. Hautmann, R. and Lehmann, A. Intrarenal distribution of oxalic acid, calcium, sodium and potassium in man. Eur. J. Clin. Invest., 10, 173 – 176 (1980).

24. Masters, C.J. and Holmes, R.S. Peroxisomes: New aspects of Cell physicology and Biochemistry Physiol. Rev., 57, 816 – 882 (1977).

25. Varalakshmi, P., and Richardson, K.E. The effect of vitamin B_6 deficiency and hepatectomy on the synthesis of oxalate from glycollate in the rat. Biochem. Biophys. Acta, 757, 1 – 7 (1983).

26. Majeska, R.J. and Wuthier, R.E. Studies on matrix vesicles isolated from chick epiphyseal cartilage: Association of pyrophosphatase and ATPase activities with alkaline phosphatase. Biochim. Biophys. Acta, 391, 51 – 60 (1975).

27. Khan, S.R., Shevock, P.N. and Hackett, R.D. Urinary enzymes and calcium oxalate urolithasis. J. Urol., 142 (3), 846 – 849 (1989).

28. Jayanthi, S., Subha, K. and Varalakshmi P. Enzymes of the kidney brush border membrane in experimental urolithiasis and effect of L(+) tartrate. Med. Sci. Res., 18, 345 – 348 (1990).

29. Hofmeister, R., Bhargava, A.S and Gunzel, P. Value of enzyme determinations in urine for the diagnosis of nephrotoxicity in rats Clin. Chem. Acta, 160, 163 – 167 (1986).

30. Schneider, H.J., and Wasmund, H.G. Mechanism of action of phosphate in kidney stone disorders. J. Urol. Nephrol., 64, 343 – 349 (1971).

31. Wakid, N.W., Kutayli, F. and Buri, H. Inorganic pyrophosphatase in human urine. Clin. Chem. Acta, 30, 527 – 529 (1970).

32. Calcamuggi, G., Cestonaro, G., Gatte, G., Antossi, G., Cotto, A. and Emmanelli, G. Urinary enzymes release following aminoglycoside administration in single low dose. Enzyme, 39, 119 – 122 (1988).

33. Subha, K., Ph.D. Thesis entitled "Effect of an exogenous glycosaminoglycan (Sodium pentosan polysulphate) in experimental urolithiasis in rats" (1992).

34. Charlton, C.A.C and Osmond, C. Deficient urinary fibrinolysis in renal stone disease. Br. Med., J., 292, 1239, 1240 (1986).

35. Bank, N. and Bailine, S.H. Urinary β-glucuronidase activity in patients with urinary tract infections. New Eng. J. Med., 272, 70 – 75 (1965).

36. Padmaja, T.C., and Varalakshmi, P. Urinary enzymes in experimental calcium oxalate lithiasis and effect of L(+) – tartrate. Med. Sci. Res., 17(14), 589 - 590 (1989).

37. Baggio, G., Gambaro, G., Ossi, E., Favaro, S. and Borsatti, A. Increased urinary excretion of renal enzymes in idiopathic calcium oxalate nephrolithiasis J. Urol., 129, 1161 – 1162 (1983).

38. Karlin, G.S., Schulsinger, D., Urivetsky, M. and Smith, A.D. Absence of persisting parenchymal damage after extracorporeal shockwave lithotripsy as judged by excretion of renal tubular enzymes. J. Urol., 144, 13 – 14 (1990).