



Evaluation of Gokhru and Pashanbhed for management of experimental urolithiasis in rabbits

AMARPAL¹, P KINJAVDEKAR², H P AITHAL³ and A M PAWDE⁴

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Various chemicals used for management of urolithiasis have one or the other side effects; hence medicinal plants were tried by many workers for dissolution or prevention of urolithiasis. The gokhru (*Tribulus terrestris*) fruit is used in bladder disorders as a diuretic and to treat urinary calculi, hepatitis, rheumatism and skin complaints (Anand *et al.* 1994). Major constituents of the fruit are glycosides like protodioscin, terrestrosins A-E etc. The pharmacological activity of the plant is believed due to inhibition of enzyme glycolic acid oxidase, responsible for conversion of glycolate to glyoxylate, which is finally converted into oxalates (Elizabeth 2002). Sangeeta *et al.* (1994) reported that administration (5 g / kg p.o.) of an aqueous extract of *T. terrestris* fruits to glycolate treated rats reduced urinary oxalate excretion, increased glyoxylate excretion, normalized kidney lactate dehydrogenase activity and decreased the activities of glycolate oxidase and glycolate dehydrogenase in the liver.

Similarly pashanbhed (*Bergenia ligulata*), a medicinal plant, has an anti-urolithiatic and antilithic (Seth *et al.* 1974) activity and is widely used in the treatment of dysuria, renal failure, cystitis and crystalluria. In our earlier studies, the mixture of gokhru and pashanbhed was effective in the dissolution of calculi in goats. Therefore, the present study was designed to evaluate the efficacy of these drugs against experimentally produced struvite and oxalate calculi in rabbits.

The experimental study was conducted in 48 male Angora rabbits aged between 6–8 months; weighing 1–1.5 kg and randomly divided into 2 equal groups as OX (oxalate) and ST (struvite) groups. The animals of OX group were further divided into 3 equal subgroups, viz. OXN, OXG and OXP and kept in pairs in rabbit cages after giving identification. The feed and water was provided in the feeding trough in individual cages. The animals of OX group were given

ammonium chloride powder mixed in their feed for 1 week (1% of feed) and ethylene glycol in drinking water (1% of water) for 1 month to induce oxalate calculi. Before feeding of the calculogenic diet, the animals were operated surgically and a plastic bead of 5–6 mm diameter was placed in the bladder of each animal to provide nidus for the deposition of crystals. The surgical procedure was conducted under general anaesthesia using xylazine @ 5 mg/kg and ketamine @ 50 mg/kg, intramuscularly. During surgery, urine was collected from the bladder for microscopic examination.

The animals of subgroup OXN were not given any preventive drug. Whereas, subgroup OXG received 1% gokhru fruit powder mixed in feed. Similarly, the animals of group OXP were provided with 1% pashanbhed rhizomes powder, mixed in feed. All the animals of OX group were observed for 1 month and then sacrificed by over dose of intra-cardiac thiopental. Plastic bead from the bladder was collected after dissection, while urine was collected in sterile syringes and biopsy samples from bladder, kidney and liver were collected in 10% formalin for histopathological examination.

The animals of ST group were also sub-grouped as the animals of group OX and named as STN, STG and STP. These animals were given magnesium phosphate as 1% concentration of their diet. The animals of subgroup STN did not receive any drug for the prevention of calculogenesis but the animals of subgroup STG and STP were given 1% gokhru powder and 1% pashanbhed powder, respectively, in their diet feed as in group OX.

Microscopic examination: Microscopic examination of urine was done for determining crystals, microscopic casts, leukocytes and pus cells. One drop of urine sediment, obtained by centrifugation of 5 ml urine, was mixed with methylene blue stain (diluted in distilled water) in 1:5 proportion on a slide. Another drop of urine sediment was kept on other slide without staining. Both the slides were examined by conventional light microscopy, first at low power (×100) and then at high power (×450) magnification

Present address: ^{1,3,4}Senior Scientist (¹e mails: amarpal@ivri.up.nic.in; ³aithal@ivri.up.nic.in; ⁴apawde@ivri.up.nic.in), ²Principal Scientist (e mail: pk@ivri.up.nic.in), Division of Surgery.

for recording the presence and type of cells, epithelial casts and crystals.

Plastic bead examination: Plastic bead was visually examined for the deposition of minerals.

Histopathological examination: The tissue samples were processed by standard procedures for H & E staining. The stained samples were examined for the morphological changes and presence of crystals in bladder and kidney, and pathological changes in the liver.

Feeding of ethylene glycol and ammonium chloride to the animals of OX group and magnesium phosphate to the animals of group ST did not show any effect in the normal behaviour or feeding pattern of these animals. Although, the appetite remained normal but the water intake increased significantly in the animals of group OX.

The urine colour in all the animals was yellow to dark yellow. However, the pH in OX and ST group, ranged from 6.5–7.0 and 6.5–8.0, respectively. The beads were smooth, without deposition of any crystals in all the animals of subgroups OXG, OXP, STG and STP, however 2 beads collected from the animals of subgroup OXN and 1 from subgroup STN were whitish in appearance and showed the deposition of minerals on their surfaces. The samples collected from the urinary bladder, kidney and liver appeared smooth and normal in shape, size and texture.

Microscopic examination of urine samples collected preoperatively did not show the presence of any crystals or casts. Out of total 24 animals of OX group, the oxalate crystals were present in only two animals of subgroup OXN. Similarly, none of the animal of ST group except 1 from subgroup STN showed the presence of any struvite calculi in microscopic examination of urine. Epithelial casts and WBC were present abundantly in all the animals showing the presence of crystals in their urine. However, in rest of the animals, the urinary casts and WBC were scarcely seen. Histopathological examination of bladder showed smooth mucosa of normal thickness with subjacent normal lamina propria and normal muscle layer. Similarly, the kidney samples also showed the normal renal architecture without deposition of any crystal or calculi. Histological examination of liver revealed normal hepatic cellular arrangement without any microscopic changes indicative of absence of toxicity or necrosis of hepatic tissue.

Of many types of stone formed in animals, the most common are struvite and calcium oxalates (Clark 1974). The formation of calculi encompasses several physico-chemical events beginning with crystal nucleation, growth aggregation and ending in the retention within urinary tract. Various chemical medicaments have been shown to have some preventive effects but all of them are associated with one or the other toxic effect. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants (Atmani 2003). The model of struvite and oxalate calculi formation in rabbits is not reported earlier.

However 0.75% ammonium chloride and 1% ethylene glycol (Atmani *et al.* 2003) and ethylene glycol and vit D₃ (Yasui *et al.* 1999) induced oxalate uroliths in rats. The struvite calculi formation was also reported following implantation of bacteria *Ureaplasma urealyticum* and zinc dics into the bladder of Wister mice (Arai *et al.* 1996). High phosphorus diet was used to produce experimental urolithiasis in goats (Sato and Omori 1977). Feeding of phosphorus mill diet was also considered as one of the important causes of urolithiasis in small ruminants (Amarpal *et al.* 2004).

In the present study feeding of ammonium chloride and ethylene glycol could produce urinary crystals only in 25% of rabbits, however, the same treatment resulted in development of urinary crystals in all the rats in the study of Atmani *et al.* (2003). High phosphorous diet given to the animal of ST group led to formation of calculi only in 12.5% of the rabbits. The difference in the species might be responsible for lower success in the induction of calculi in rabbits. There seems to be no earlier study reporting the experimental inductions of urolithiasis in rabbits. When the Gokhru or Pashanbhed were fed to the rabbits maintained on calculogenic diet, there was no crystal formation in the urine of rabbits of any subgroup. It was suggestive of protective role of both gokhru and pashanbhed on the development of oxalate as well as struvite calculi in rabbits. In our earlier study (Singh 2006), we found a positive effect of the mixture of gokhru and pashanbhed on the dissolution of preformed calculi in clinical cases in goats.

On the basis of our results it was concluded that both gokhru and pashanbhed can potentially inhibit the formation of oxalate and struvite calculi, however, further studies are needed to elucidate the mechanism and efficacy of these drugs in a more suitable model before recommending for field use.

SUMMARY

The present study was conducted to evaluate the efficacy of gokhru (*Tribulus terrestris*) and pashanbhed (*Bergenia ligulata*) against experimentally produced struvite and oxalate calculi in rabbits. The rabbits (48) were randomly divided in 2 equal groups and were named as OX (oxalate) and ST (struvite). Animals of OX group were given ammonium chloride powder mixed in feed (1% of feed) for 1 week and ethylene glycol in drinking water (1% of water) for 1 month to induce oxalate calculi. These animals were further divided into 3 equal subgroups, viz. OXN, OXG and OXP. The animals of group ST were given magnesium phosphate in 1% concentration of their diet. These animals were also divided in 3 equal subgroups, viz. STN, STG and STP. Subgroups OXN and STN were not given any preventive drug whereas, subgroup OXG and STG were provided with 1% Gokhru fruit powder in their feed. Similarly, the animals of groups OXP and STP were provided with the 1% Pashanbhed rhizomes powder. The appetite of the animals remained normal but the water intake increased

significantly in the animals of OX group. The urinary pH was between 6.5 and 7.0 in the animals of OX group, and from 6.5 to 8 in the animals of group ST. After 1 month urine collected from 2 animals of subgroup OXN showed the presence of oxalate crystals and 1 animal of STN subgroup showed the presence struvite calculi. None of the animals of treatment subgroups showed the presence of calculi in their urine. Hence, it was concluded that both gokhru and pashanbhed can potentially inhibit the formation of oxalate and struvite calculi. However, further studies are warranted to elucidate the mechanism and efficacy of these drugs in a more suitable model before recommending for field use.

REFERENCES

- Amarpal, Kinjavdekar P, Aithal H P, Pawde A M, Singh T and Pratap K. 2004. Incidence of urolithiasis: A retrospective study of five years. *Indian Journal of Animal Sciences* **74**: 175–77.
- Anand R, Patnaik G K, Kulshreshtha D K and Dhawan B N, 1994. Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. *Indian Journal of Experimental Biology* **32**: 548–52.
- Arai Y, Takeuchi H, Okada Y, Tomoyoshi T. 1996. Experimental bladder stone production by *Ureaplasma urealyticum*. *Hinyokika Kiyo* **42**: 729–34. (Abstract).
- Atmani F, Slimani Y, Mimouni M, Hacht B. 2003. Prophylaxis of calcium oxalate stones by *Herniaria hirsuta* on experimentally induced nephrolithiasis in rats. *British Journal of Urology International* **92**:137–40.
- Atmani F. 2003. Medical management of urolithiasis, what opportunity for phytotherapy? *Frontiers in Bioscience* **1**: 507–14.
- Clark W T. 1974. The distribution of canine urinary calculi and their recurrence following treatment. *Journal of Small Animal Practice* **15**: 437–44.
- Elizabeth M W. 2002. Major herbs of Ayurveda. *Dabur Research Foundation and Dabur Ayurved Limited- 2002*.
- Sangeeta D, Sindhu H, Thind S K and Nath R. 1994. Effect of *tribulus terrestris* on oxalate metabolism in rats. *Journal of Ethnopharmacology* **44**: 61–66.
- Sato H and Omori S. 1977. Incidence of urinary calculi in goats fed a high phosphorus diet. *Japanese Journal of Veterinary Science* **39**: 531–35.
- Seth S D and Prabhakar M C. 1974. Preliminary pharmacological investigations of *Tribulus terrestris*, Linn (gokhru). 1. *Indian Journal of Medical Science* **28**: 377–80.
- Singh M. 2006. 'Percutaneous tube cystotomy and medical dissolution for treatment of obstructive urolithiasis in goats.' M.V.Sc. Thesis submitted to Indian Veterinary Research Institute, Izatnagar.
- Yasui T, Fujita K, Sato M, Sugimoto M, Iguchi M, Nomura S and Kohri K. 1999. The effect of takusha, a kampo medicine, on renal stone formation and osteopontin expression in rat urolithiasis model. *Urological Research* **27**: 194–99.