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SHORT COMMUNICATION

Effect of Cystone, a Herbal Formulation, on Glycolic Acid-induced Urolithiasis in Rats

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The effect of cystone, a herbal formulation, was studied on experimentally induced urolithiasis in rats. Oxalate urolithiasis was produced by the addition of 3% glycolic acid to the diet for a period of 42 days. Glycolic acid treatment resulted in a significant increase in the levels of calcium and oxalate in the kidney as well as in the total kidney weight. Also, the urinary levels of calcium, oxalate and inorganic phosphorus were increased. Cystone treatment at 250, 500 and 750 mg/kg b.wt. p.o. for 42 days revealed a dose-related effect in the reduction of lithogenic substances, following glycolic acid induced urolithiasis. Simultaneous oral treatment with cystone at a dose of 500 and 750 mg/kg for 42 days, significantly reversed the glycolic acid-induced urolithiasis, presumably by preventing the urinary supersaturation of lithogenic substances, especially of oxalate and calcium. The reduction of urinary and kidney oxalate levels by cystone may be due to its inhibitory action on oxalate synthesizing liver enzyme glycolate oxidase. These observations indicate that cystone can play an important role in the prevention of disorders associated with kidney stone formation. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia (Hiatt and Friedman, 1982). The worldwide incidence of urolithiasis is quite high (Anderson *et al.*, 1967) and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis (Robertson and Peacock, 1980). Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis. In the present study, cystone, a polyherbal formulation, mainly comprising plant drugs which are widely used for antilithic activity in traditional medicine was evaluated for its effects on experimentally induced urolithiasis in rats.

MATERIALS AND METHODS

Forty male rats of Wistar strain weighing between 180–220 g were used in this study. The animals were acclimatized to standard laboratory conditions and maintained on 12 h light and dark cycle. The rats were fed with commercially available standard pelleted feed (Lipton India Ltd., Bombay) and water *ad libitum.*

The constituent plants of the formulation were procured from authentic sources and identified by Dr S. Farooq, botanist of The Himalaya Drug Co. A voucher specimen was deposited in the herbarium of the R & D Centre, Bangalore. The main constituents of this formulation and its proportion are shown in Table 1. All plant powders were individually weighed and mixed. The drug was administered as an aqueous oral suspension and the animals of the control group received water as vehicle.

The rats were divided into five groups of eight each. Rats of group I received the commercial diet and served as control, group II was fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 42 days (Chow *et al.*, 1975). Groups III, IV and V received 250, 500 and 750 mg/kg b.wt of cystone, respectively once a day orally in addition to the CPD for the same duration.

Collection and analysis of urine samples. On day 42, immediately after administration of the respective assigned doses, the rats were housed in metabolic cages for 24 h urine collection. A drop of concentrated hydrochloric acid was added to the collected urine and stored at 4°C. Levels of oxalate (Hodgkinson and Williams, 1972), calcium (Tsuyoshi Ohnishi, 1977) and inorganic phosphorus (Varley *et al.*, 1980) were determined spectrophotometrically. Sodium and potassium were estimated using a flame photometer.

Assay of renal tissue samples. At the end of the experiment, on day 43, the rats were killed by cervical dislocation and kidneys excised, washed with normal saline and weighed. The kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was

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Table 1. Main constituents of cystone

Plant name	Family	Voucher specimen	Part used	Proportion (mg/g)
Didymocarpus pedicellata	Gesneriaceae	Fa-235	Flower	290
Saxifraga ligulata	Saxifragaceae	Fa-236	Stem	220
Rubia cordifolia	Rubiaceae	Fa-237	Stem	72
Cyperus scariosus	Cyperaceae	Fa-238	Roots	72
Achyranthes aspera	Amaranthaceae	Fa-239	Whole plant	72
Onosma bracteatum	Boraginaceae	Fa-240	Whole plant	72
Vernonea cinerea	Compositae	Fa-252	Whole plant	72

Table 2. Effect of cystone on calculi-forming constituents in urine following 3% glycolic acid for 42 days

Parameter	Group I	Group II	Group III	Group IV	Group V
Oxalate (mg/24 h)	$12.30^{c} \pm 0.84$	25.12 ± 2.89	18.89 ± 3.37	$13.10^{b} \pm 2.95$	$12.92^{b} \pm 2.81$
Calcium (mg/24 h)	$4.26^{c} \pm 0.19$	7.18 ± 0.74	5.90 ± 0.92	$3.89^{b} \pm 0.36$	$3.87^{b} \pm 0.42$
Inorganic phosphorus (mg/24 h)	$0.923^{b} \pm 0.128$	1.596 ± 0.182	1.119 ± 0.120	$0.818^{b} \pm 0.136$	$0.800^{b} \pm 0.122$
Sodium (mEq/24 h)	$10.21^{c} \pm 0.94$	4.23 ± 0.79	$6.58^{a} \pm 0.82$	$10.13^{c,d} \pm 1.12$	$10.48^{c,d} \pm 1.30$
Potassium (mEq/24 h)	$11.76^{c} \pm 0.91$	6.49 ± 0.69	8.46 ± 1.54	10.81 ± 1.98	10.02 ± 2.14

Values are mean ± SE ($n = 8$).
[a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$ compared with Group II.

Table 3. Effect of cystone on kidney weight and calculi-forming constituents in kidney following 3% glycolic acid for 42 days

Parameter	Group I	Group II	Group III	Group IV	Group V
Wet weight (g/100 g b.wt)	$0.340^{c} \pm 0.0054$	0.423 ± 0.0084	$0.400^{b} \pm 0.019$	$0.358^{c} \pm 0.012$	$0.350^{c,e} \pm 0.009$
Dry weight (g/100 g b.wt)	$0.087^{c,d} \pm 0.0018$	0.119 ± 0.0015	$0.112^{b} \pm 0.0020$	$0.096^{c,g} \pm 0.0022$	$0.094^{c,g} \pm 0.0016$
Oxalate (mg/100 mg tissue)	$0.449^{c,d} \pm 0.018$	1.103 ± 0.056	$0.895^{a} \pm 0.061$	$0.563^{c,g} \pm 0.031$	$0.548^{c,f} \pm 0.039$
Calcium (mg/100 mg tissue)	$0.168^{c,d} \pm 0.0061$	0.359 ± 0.012	$0.309^{a} \pm 0.016$	$0.216^{c,g} \pm 0.013$	$0.211^{c,f} \pm 0.020$

Values are mean ± SEM ($n = 8$).
[a] $p < 0.05$, [b] $p < 0.02$ and [c] $p < 0.001$ compared with Group II; [d] $p < 0.01$ Group I vs Group IV and V; [e] $p < 0.05$, [f] $p < 0.01$ and [g] $p < 0.001$ Group III vs Group IV and V.

boiled in 10 mL of 1 N hydrochloric acid for 30 min. The kidneys were then homogenized (Chow *et al.*, 1974). The homogenate was centrifuged at 2000 rpm for 10 min, and the supernatant separated. The estimation of oxalate and calcium was carried out by the method of Hodgkinson and Williams (1972) and Tsuyoshi Ohnishi (1977), respectively.

Statistical analysis. The data of urinary and renal parameters were expressed as mean ± SEM. The results were analysed statistically using ANOVA followed by Dunnett's *t*-test. The minimum level of significance was fixed at $p < 0.05$.

RESULTS AND DISCUSSION

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. In this context, the changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). In the present study, feeding 3% glycolic acid resulted in hyperoxaluria, which is known to be due to the ready conversion of glycolic acid to oxalate by the oxalate synthesizing liver enzyme glycolate oxidase (Richardson and Tolbert, 1961). Hyperoxaluria is usually the initiating factor of oxalate urolithiasis. Glycolic acid, the precursor of oxalic acid, is known to increase significantly the incidence of oxalate lithiasis (Runyan and Gershoff, 1965). Our results are in agreement with these studies, as shown by the significant increase in kidney weight. The increase in urinary calcium and oxalate levels were also found to be highly significant. Cystone treatment at a dose of 250, 500 and 750 mg/kg b.wt. revealed a dose related response. Cystone treatment at dose levels of 500 and 750 mg/kg b.wt showed a better protective effect. However, there was no significant difference observed between 500 and 750 mg/kg b.wt of cystone treatment. These findings revealed that 500 mg/kg b.wt of cystone is the minimum dose required for eliciting an optimal activity. Cystone treatment significantly lowered the oxalate values ($p < 0.01$) probably by its inhibitory action on glycolate oxidase. Urinary sodium excretion was significantly elevated in the drug treated animals. Urinary potassium excretion was also elevated, though not significantly (Table 2). The reduction in the urinary oxalate level will be beneficial in preventing the urinary supersaturation with respect to oxalate. Calcium and phosphorus play a vital role in renal calculogenesis. Calcium and inorganic phosphorus levels were also elevated in the rats receiving a calculi-producing diet. The increase in calcium excretion may be due to defective tubular reabsorption in the kidneys (Varalakshmi *et al.*, 1990). Cystone treatment markedly reduced the levels of calcium and phosphorus ($p < 0.01$) in urine (Table 2).

There was a significant increase in the kidney weight of animals receiving 3% glycolic acid which was almost normalized in the cystone treated animals (Table 3). Glycolic acid feeding for 42 days resulted in renal tissue

deposition of calcium and oxalate. The increased deposition of calcium and oxalate in the renal tissue is known to lead to papillary calcification and eventual calculi formation (Heutmann and Lehmann, 1980). A similar elevation in renal stone forming constituents in rats fed with CPD has been reported earlier (Baskar, *et al*., 1996.). Cystone administration significantly reduced both calcium and oxalate levels in kidneys, which is known to prove beneficial in preventing calculi formation due to supersaturation of these lithogenic substances (Table 3).

The reduction in the stone forming constituents in urine and renal tissue brought about by cystone treatment in calculosis is noteworthy. These effects could contribute to the antilithic and lithotriptic property of this formulation.

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