ORIGINAL ARTICLE

PREVENTION AND DISSOLUTION EFFECTS OF *SANG SARMAHI* ON CALCIUM OXALATE CRYSTALS INDUCED BY GLYOXALATE TREATMENT IN RATS

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Background: Development of cost-effective therapy for safe removal of kidney stones is very much desired. The purpose of this study was to evaluate litholytic and anti-urolithic effects of *Sang sarmahi* on calcium oxalate crystals induced by glyoxalate treatment in rats. **Methods:** Thirty male Wistar rats were randomly divided into five equal groups; A, B1, B2, C1 and C2. Group A rats were untreated and served as negative controls. The rats in groups B1 and B2 after induction of calcium oxalate crystals were respectively given placebo and *Sang sarmahi* treatment and served as positive control and curative groups. The rats in groups C1 and C2 were simultaneously administered glyoxylate+placebo, and glyoxylate+*Sang sarmahi* treatments and respectively served as positive controls and preventive group. Serum samples of rats in groups A, C1 and C2 were measured for the levels of superoxide dismutase, glutathione peroxidase, catalase, calcium, magnesium, oxalate and creatinine. Similarly, left kidney tissue homogenates of rats in groups A, B1 and B2 were analyzed for the levels of reduced glutathione, malondialdehyde, superoxide dismutase, glutathione peroxidase and catalase. Histological slides of right kidney sections of all 30 rats were prepared for microscopic examination. **Results:** Histological examination of renal tissue sections showed that *Sang sarmahi* failed to prevent crystallization in kidneys of groups B and C rats. No significant differences were seen in serum and tissue homogenate parameters between positive controls and *Sang sarmahi* treated rats of both prevention and curative groups. **Conclusion:** *Sang sarmahi* failed to exhibit any significant litholytic and anti-urolithic activity.

Keywords: Litholytic, Antiurolithic, Calcium oxalate crystals, *Sang Sarmahi*

Pak J Physiol 2018;14(3):21–4

INTRODUCTION

Development of stones in kidney, ureter, urinary bladder and/or urethra is collectively called as urolithiasis. According to classification of kidney stones by chemical composition, calcium oxalate (CaOx) is the most common type of kidney stones worldwide, followed by calcium phosphate, struvite, urate, cystine and other rare forms.[1,2]

Pure calcium oxalate stones are formed in sterile acidic urine and their solubility is largely independent of *p*H.[2–3]

The main crystalline forms of calcium oxalate stones include calcium oxalate- monohydrate (COM) and calcium oxalate-dihydrate (COD). From the two hydrated forms of CaOx, COM is more stable and prevalent than the COD.[4]

Formation of CaOx stones in kidneys is a serious health problem in both developed and developing countries. The worldwide prevalence of kidney stones in men by age 70 years varies from approximately 1 to 5% in Asia, 4% in England, 13% in North America and 20% in Saudi Arabia.[5–7]

No treatment option for getting safe rid of calcium oxalate kidney stones is available in medical literature as yet except for surgical interventions. This can have many adverse effects as surgical procedures can cause physical trauma and if kidney stone is recurrent, then repeated surgery is problematic. Therefore, a search focused on an alternative way of safe removal of calcium oxalate kidney stones is highly wanted to be carried out.

Sang sarmahi is believed to be having ability to break and dissolve kidney stones.[8] No scientific study has been carried out on the dissolution and prevention effects of *Sang sarmahi*. The present study was designed to evaluate the litholytic and antiurolithic effects of *Sang sarmahi* on calcium oxalate crystallization induced in rat kidneys by glyoxalate treatment.

MATERIAL AND METHODS

This experimental animal study was carried out at the animal house of Sindh Agriculture University, Tando Jam from Oct 2014 to Apr 2015. Rat urolithiasis model was employed in this study, to investigate the antiurolithic activity and kidney stone dissolution power of *Sang sarmahi*. Rat urolithiasis model was chosen because of many resemblances between experimental nephrolithiasis induced in rats and nephrolithiasis caused in human beings.[9,10]

Thirty male Wistar rats of eight weeks age (weighing 250–350 g each) were kept in polypropylene rat cages type1 (SRP01) under a controlled 12 h light/ dark cycle at 23–24 °C, and 50–60% humidity. All animals had *ad libitum* access to standard chow and tap

water. After 3 days in cages, the rats were randomly divided into 5 groups namely A, B1, B2, C1, and C2. Group A untreated rats served as negative controls. Group B1 and B2 rats were given intraperitoneal injection of 60 mg/Kg of glyoxylate five times a week to induce calcium oxalate crystal deposition in the kidneys. After one week, B1 rats were given double distilled deionized water as placebo only and served as positive controls for curative group, while group B2 rats were given 30 mg/Kg[7] *Sang sarmahi* and served as curative group.

Rats in group C1 simultaneously received intraperitoneal injection of 60 mg/Kg of glyoxylate+ placebo and served as positive controls for preventive group. Rats in group C2 simultaneously received intraperitoneal injection of 60 mg/Kg of glyoxylate+30 mg/Kg of *Sang sarmahi* and served as prevention group.

Calcium oxalate crystal deposition in the kidney of rats was induced by intraperitoneal injection of glyoxylate (from Sigma-Aldrich). Glyoxylate was selected for this purpose because this compound has been shown to be a faster and more reliable method for induction of calcium oxalate crystals than the previous experimental rat models.[11]

On day 8 of experiment, rats in groups A, C1 and C2 were anaesthetized (by administering a combination of Ketamine 80 mg/Kg and Diazepam 10 mg/Kg intraperitoneally)[12] and blood samples were collected from the retro-orbital region. These samples after clotting at room temperature (for 15–30 minutes) were centrifuged at 1,000 G for 15 minutes in a refrigerated centrifuge (Labnet Z326 Hi-Speed Centrifuge). The resulting supernatant (serum), stored at -80 °C was analyzed within a month for the activity of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) by kit methods[13,14] using a SpectraMax Plus 384® micro-plate reader. Serum levels for calcium, magnesium, oxalate and creatinine were measured by commercial kit methods[14,15] employing a Microlab 300® Semi-automatic biochemistry analyzer.

At the end of 14-day treatment, all rats in groups A, B1 and B2 were sacrificed by cervical dislocation and their abdomen was opened to excise both kidneys. The left kidney of these rats was rinsed with phosphate buffer saline to remove any RBCs. Thereafter, a 10% homogenate was prepared by homogenizing the tissue in 5–10 ml of ice cold buffer, *p*H 7.4, i.e., 50 mM phosphate buffer saline (PBS) containing 1 mM of EDTA per gram tissue. The contents were centrifuged at 10,000 G for 15 min at 4 ºC, and the supernatant thus obtained was analyzed for reduced glutathione (GSH), malondialdehyde, superoxide dismutase, glutathione peroxidase and catalase by standard kit methods.[16,17]

The right kidneys of rats recovered similarly were fixed in bouin liquid for 24–48 hours, dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin and were cut at 3–4 µm intervals on a rotary microtome floated in hot water bath at 42 °C and mounted on thoroughly cleaned gelatinized glass slides appropriately numbered. The slides were placed on hot plate at 37 °C for 24 hours for fixation of renal tissue slices and thereafter stained with hematoxylin and eosin (H&E) for histological examination. Representative slides were photographed using optical microscopy under polarized light.[18–21]

SPSS-22 was used for statistical analysis. Results were reported as Mean±SD. One-way analysis of variance (ANOVA) and post hock multiple comparison tests were applied to find out the significance of differences in means, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Table-1 presents mean serum levels for SOD, GPX, CAT, Ca^{2+}, Mg^{2+}, oxalate and creatinine in the 3 groups of rats. Comparison of serum parameters between untreated rats (Group A) and rats treated with glyoxalate+placebo (Group C1) showed highly significant differences ($p<0.0001$) in the mean serum levels for SOD, GPX, CAT, Ca^{2+}, Mg^{2+} , oxalate and creatinine. Serum SOD, GPX, CAT, Ca^{2+} and Mg^{2+} levels were significantly higher and of oxalate and creatinine lower in untreated rats as compared to rats given glyoxalate+placebo treatment. A similar comparison of serum parameters between untreated rats and rats treated with glyoxalate+SS showed highly significant differences ($p<0.0001$) in the mean serum levels for SOD, GPX, CAT, Mg^{2+}, oxalate and creatinine. Serum SOD, GPX, CAT, Ca^{2+} and Mg^{2+} levels were significantly higher and of oxalate and creatinine lower in untreated rats as compared to rats given glyoxalate+*Sang sarmahi* treatment. Comparison of serum parameters between Group C1 with C2 showed that there were no significant differences ($p>0.05$) in mean serum levels for SOD, GPX, CAT, Ca^{2+}, Mg^{2+}, oxalate and creatinine, although *Sang sarmahi* treatment caused an increase in serum levels of SOD, GPX, CAT, Ca^{2+} and Mg^{2+}, and a decrease in oxalate and creatinine levels.

Group B1 and B2 against Group A had significantly decreased kidney tissue mean levels for SOD, GPX, CAT, and GHS, and increased for MDA ($p<0.0001$). It can also be noted that with exception to renal tissue SOD activity ($p<0.05$), no significant differences ($p>0.05$) in the mean levels for GPX, CAT, GSH, and MDA were detected between Groups B1 and B2. The histological examination of the kidney sections showed calcium crystals, atrophic tubules and tubular epithelial degeneration in both preventive and

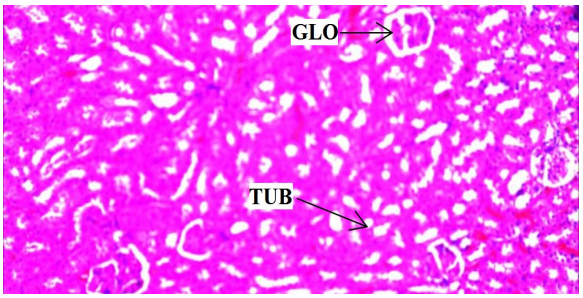
dissolution groups of SS treated rats (Fig-1 c, e). *Sang sarmahi* had neither stone dissolving nor preventing effects at the concentration used in this study.

Table-1 Serum chemistry of 18 rats distributed equally into A, C1 and C2 Groups

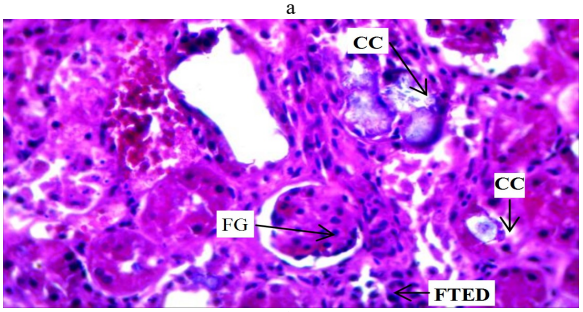
Serum variable	Sub-group	Mean±SD	*p*	
SOD (U/ml)	Group A	185.5±5.9	A vs C1	0.0001
	Group C1	68.6±13.5	A vs C2	0.0001
	Group C2	81.8±13.0	C1 vs C2	0.151
GPX (ηM/min/mL)	Group A	236.5±36.2	A vs C1	0.0001
	Group C1	74.5±11.5	A vs C2	0.0001
	Group C2	87.8±17.3	C1 vs C2	0.614
CAT (ηM/min/mL)	Group A	861.0±87.0	A vs C1	0.0001
	Group C1	357.3±11.9	A vs C2	0.0001
	Group C2	392.3±12.6	C1 vs C2	0.611
Calcium (mg/dl)	Group A	10.0±0.6	A vs C1	0.0001
	Group C1	7.1±1.17	A vs C2	0.021
	Group C2	8.2±1.0	C1 vs C2	0.175
Magnesium (mg/dl)	Group A	2.3±0.36	A vs C1	0.0001
	Group C1	1.4±0.12	A vs C2	0.0001
	Group C2	1.5±0.07	C1 vs C2	0.856
Oxalate (µM/L)	Group A	7.7±1.14	A vs C1	0.0001
	Group C1	41.3±1.37	A vs C2	0.0001
	Group C2	39.3±1.6	C1 vs C2	0.414
Creatinine (mg/dl)	Group A	0.6±0 14	A vs C1	0.0001
	Group C1	2.3±0.19	A vs C2	0.0001
	Group C2	2.0±0.20	C1 vs C2	0.101

Table 2 Renal tissue chemistry of 18 rats distributed equally into A, B1 and B2 groups

Renal variable	Sub-group	Mean±SD	*p*	
SOD (U/mg)	Group A	195.0±5.7	A vs B1	0.0001
	Group B1	87.8±3.9	A vs B2	0.0001
	Group B2	95.6±6.4	B1 vs B2	0. 050
GPX (ηM/min/mg)	Group A	290.1±8.3	A vs B1	0.0001
	Group B1	107.1±21.0	A vs B2	0.0001
	Group B2	112.8±18.4	B1 vs B2	0.830
CAT (ηM/min/mg)	Group A	904.6±39.0	A vs B1	0.0001
	Group B1	300.8±37.5	A vs B2	0.0001
	Group B2	311.8±45.1	B1 vs B2	0.889
GSH (µM/mg)	Group A	5.0±0.7	A vs B1	0.0001
	Group B1	3.6±0.1	A vs B2	0.0001
	Group B2	3.5±0.15	B1 vs B2	0.774
Malondialdehyde (µmol/gWTW)	Group A	2.3±0.5	A vs B1	0.0001
	Group B1	7.8±1.3	A vs B2	0.018
	Group B2	5.5±2.7	B1 vs B2	0. 100



a



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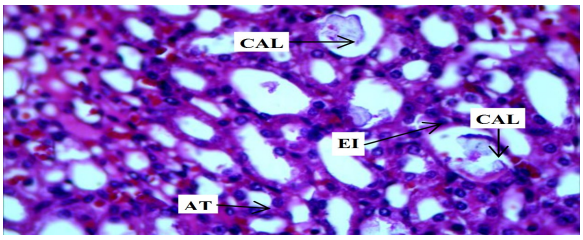

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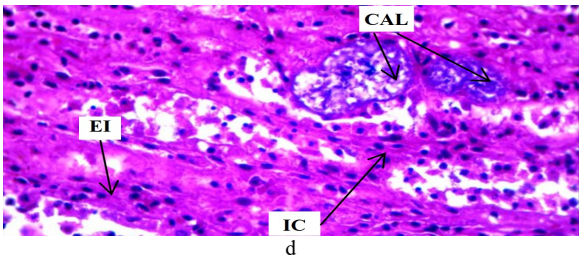

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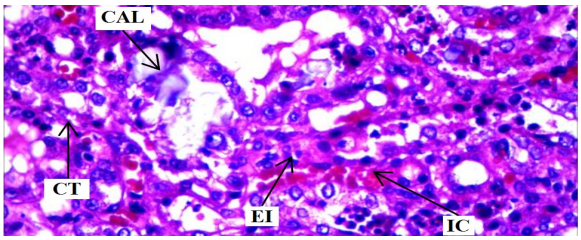

e

Figure-1: Histology of renal tissue

a: Group A showing essentially normal histological structure with normal glomeruli (GLO) and tubules (TUB) (H&E stain ×100); **b:** Group B1 showing calcium crystals (CC), focal glomerulosclerosis (FG) and focal tubular epithelial degeneration (FTED) (H&E stain ×400); **c:** Group B2 (Sang sarmahi treated group) showing edematous interstices (EI) and atrophy of renal tubules (AT), tissue details are faintly visible. Calcification (CAL) is visible (H&E stain ×400); **d:** Group C1 (Glyoxylate+placebo treated group) showing severe edematous interstices (EI), large vacoulations, hydropic changes, and shrinkage of renal tubules, Calcification (CAL) and inflammatory cells (IC) (H&E stain x 400); **e:** Group C2 (Glyoxylate+Sang sarmahi treated group) showing edematous interstices (EI), vacoulation, and collapsed tubules (CT), Glomeruli are collapsed, tissue details are not clearly visible. Calcification (CAL) and inflammatory cells (IC) are visible. (H&E, ×400)

DISCUSSION

In present study preventive and curative effects of *Sang sarmahi* have been evaluated in glyoxylate induced rat urolithiasis model. We observed that positive controls of both dissolution and prevention groups of rats, i.e., Groups B1 and C1 respectively as against the negative controls (Group A) had significantly decreased SOD, GPX, CAT levels both in renal tissue and in serum samples, GSH in renal tissue, and calcium and magnesium in serum samples, while increased levels of MDA in tissue samples and of oxalate and creatinine in serum samples. This confirms the observation that glyoxylate increases oxalate excretion resulting in super-saturation of urine and hence precipitation of calcium oxalate crystals in acidic sterile urine.[22–24] The presence of calcium oxalate crystals in renal tissue triggers oxidative stress by producing intracellular oxygen derived free radicals that have the potential to damage biological membranes.[25–27] Damage to kidney

due to oxidative stress is evident from the significantly raised MDA levels in tissue samples of glyoxalate treated rats compared to untreated rats, and also from the renal tissue section of glyoxylate treated rats which shows glomerulosclerosis, tubular epithelial degeneration and calcification (Fig-1 b and c).

Sang sarmahi experimented first time in present study is found to be less effective in the dissolution and prevention of kidney stones at the dose used.[8] As compared to placebo control groups of rats, *Sang sarmahi* treated rats were found to have significantly raised SOD activity in tissue samples. Also increased levels of SOD, GPX, CAT, Ca^{2+} and Mg^{2+} and decreased of oxalate and creatinine were noticed in serum samples. Tissue MDA levels were found to be lower in *Sang sarmahi* treated rats as against the placebo controls which suggest some antioxidant effect of *Sang sarmahi*. This could also possibly be due to inhibition of the enzyme glycolate oxidase that converts glyoxalate into oxalate.[15] The serum creatinine levels although decreased by *Sang sarmahi* treatment were however, quite high, indicating obstruction to the urinary outflow due to calcium oxalate crystals in the renal tubules. The histological examination of the kidney sections showed calcium oxalate crystals, atrophic tubules and tubular epithelial degeneration in both preventive and dissolution groups of SS treated rats (Fig-1 c, e).

CONCLUSION

Sang sarmahi had neither stone dissolving nor stone preventing effects at the concentration used in present study.

ACKNOWLEDGEMENT

We thank Higher Education Commission of Pakistan for providing research grant for this study under NRPU project #4701.

CONFLICTS OF INTEREST

We hereby declare that we do not have any conflict of interest related to publication of this article.

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Received: 27 Apr 2018 Reviewed: 25 May 2018 Accepted: 31 May 2018