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Effect of *Lagenaria siceraria* fruit powder on sodium oxalate induced urolithiasis in Wistar rats

Rahul V. Takawale, Vishal R. Mali, Chinmay U. Kapase, Subhash L. Bodhankar

Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune, Maharashtra, India

ABSTRACT

Background: In spite of advances in the present practice of medicine, the formation and growth of calculi continues to trouble mankind, as there is no satisfactory drug to treat kidney stones. In India, many indigenous drugs are in use for the treatment of urinary calculus disease. **Objective**: The present study was intended to determine anti-urolithiatic effect of *Lagenaria siceraria* fruit powder (*LSFP*) against sodium oxalate (NaOx) induced urolithiasis in rats. **Materials and Methods:** Animals were grouped as Vehicle Group (received vehicle gum acacia 2% w/v 1 mL/kg/p.o.), NaOx Group(Sodium oxalate 70 mg/kg,i.p.), LSFP Group (500 mg/kg, p.o. LSFP suspended in gum acacia 2% + Sodium oxalate 70 mg/kg), Cystone Group (500 mg/kg, p.o. Cystone suspended in gum acacia 2% + Sodium oxalate 70 mg/kg). **Result**: The increased severity of microscopic calcium oxalate (CaOx) crystals deposition along with increased concentration in the kidney was seen after 7 days of NaOx (70 mg/kg, i.p.) pre-treatment. *LSFP* (500 mg/kg, p.o.) and standard marketed formulation Cystone (500 mg/kg, p.o.) caused a significant reversal of NaOx-induced changes in ion excretion and urinary CaOx concentration in 7 days treatment. **Conclusion**: From the results, it was concluded that *LSFP* showed beneficial effect against urolithiasis by decreasing CaOx excretion and preventing crystal deposition in the kidney tubules.

Key words: Cystone, *Lagenaria siceraria*, sodium oxalate, urolithiasis

INTRODUCTION

Urolithiasis is a worldwide problem, sparing no geographical, cultural, or racial groups. Occurrence of primary bladder stones (cystolithiasis) has substantially reduced over the past two decades, but they are still reported in parts of the developing world predominantly in children and in patients with neurogenic bladders and benign prostatic hypertrophy.[1] Approximately 80% of these calculi are composed of calcium oxalate (CaOx) and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system.[2] Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are costly and with these procedures recurrence is quite common.[3] The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year, and 50% at 10 years.[4] Various therapies including thiazide diuretics and alkali-citrate are being used in an attempt to prevent recurrence but scientific evidence for their efficacy is less convincing.[5] Traditional practitioners are prescribing the plant preparations; however, the rationale behind their use is not well established through systemic pharmacologic and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects.[3]

Lagenaria siceraria (*LS*) (Cucurbitaceae) is known as bottle gourd, calabash (Eng), Lauki (Hindi), and Dudhi (Marathi). The fruit is eaten as vegetable and is rich in various chemical constituents, such as ascorbic acid, beta-carotene, vitamin B complex, pectin, dietary soluble fibers, and also good source of minerals and amino acid. It is widely distributed from north to south India. Medicinally it is used as diuretic, cardiotonic, in urinary infection, hepatoprotective, antihyperlipidemic, anti-inflammatory, antidote to certain

Address for correspondence:
Dr. Subhash L. Bodhankar, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune - 411 038, Maharashtra, India.
E-mail: sbodh@yahoo.com

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poisons, purgative, ulcer and fever. It is also for treatment of pectoral cough, asthma and other bronchial disorders. The *Lagenaria siceraria* fruit powder (LSFP) has been reported in the literature to be useful as cardioprotective[6] and diuretic.[7] The antiurolithiasis activity of LSFP has not been mentioned in the literature. The objective of this study was to evaluate the antiurolithic activity of LSFP in sodium oxalate (NaOx)-induced urolithiasis in rats.

MATERIALS AND METHODS

Authentication and preparation of samples

The fruit was identified and authenticated at Agharkar Research Institute, Pune, India (Auth.07-47). *Lagenaria siceraria* fruits were processed at the manufacturing facilities of Ayush Wheatgrass Remedies Pvt. Ltd. (Pune, India). Whole fruits were cut in small flakes and dried on tray dryer at the temperature of 40°C. The dried flakes were processed in pulverizer to obtain the powder, which was labeled as LSFP.

Chemicals

NaOx (Batch No: 1404271109) was obtained from Fine Chemical Industry, Mumbai. Creatinine and uric acid estimation kit were purchased from Accurex Biomedical Pvt. Ltd., Mumbai. The measurement of oxalate, magnesium, sodium, potassium, chloride, and calcium was carried out by the following methods. All chemicals used were of analytical grade. Cystone (Himalaya Herbal Healthcare, Bangalore, India) was used as standard drug. The detailed composition and antiurolithic action of Cystone formulation is well documented. Cystone contains extracts of Shilapushpa (*Didymocarpus pedicellata*) 130 mg, Pashanbheda (*Saxifraga ligulata*) 98 mg, mangishta (*Rubia cordifolia*) 32 mg, Nagarmusta (*Cyperus scariosus*) 32 mg, Apamarga (*Achyranthes aspera*) 32 mg, Gojiha (*Onosma bracteatum*) 32 mg, Sahadevi (*Vernonia cinerea*) 32 mg, Hajrul yahood bhasma (*Limesilicate calyx*) 32 mg, and Shilajeet (mineral pitch) 26 mg.[8]

Animals

Male Wistar rats (250–300 g) were purchased from National Institute of Biosciences, Pune, India. Animals were housed under standard conditions of temperature 24°C±2°C and relative humidity of 30%–70% with 12:12 h light:dark cycles. The animals were fed with standard pellet diet (Chakan Oil Mills, Pune) and provided water ad libitium. All the experiments were carried out between 9:00 and 16:00 hours. The protocol was approved by Institutional Animal Ethics Committee (IAEC).

Preparation of drug suspension

Suspensions of LSFP and Cystone in gum acacia (2%) solution were prepared. The dose administered of both these suspensions was 500 mg/kg. The route of administration was oral by using oral gavage.

Experimental design and protocol

A total number of 24 animals were randomly divided into following 4 groups comprising 6 animals per group.

Groups	Treatment
Vehicle Group	Vehicle gum acacia 2% w/v 1 mL/kg/p.o.
NaOx Group	Sodium oxalate 70 mg/kg, i.p.
LSFP Group	500 mg/kg, p.o. LSFP suspended in gum acacia 2% + Sodium oxalate 70 mg/kg
Cystone Group	500 mg/kg, p.o. Cystone suspended in gum acacia 2% + Sodium oxalate 70 mg/kg

The treatment period was 7 days. Rats from each group were placed individually in metabolic cages (Techniplast, Milan, Italy) for 24 h for urine collection. Food and water was available during experimentation in the cages. Urine samples after collection were preserved by addition of 2 drops thymol as a preservative.

Methods

Urine parameters

Urine samples were analyzed for Creatinine (Jaffe method) Uric acid (Uricase method), Magnesium,[9] and oxalate[10] concentration.

Serum parameter

On day 7 blood was withdrawn by retro-orbital puncture from each rat. The serum was separated by centrifugation at 10,000 rpm at 4°C using cryocentrifuge machine (serial No. 5810, Eppendorff, USA) for the estimation of serum parameter (Na^+, K^+, creatinine, uric acid).

Histopathology

On day 7 the animals were sacrificed by cervical dislocation. Both kidneys were dissected and one kidney from each rat was placed in 10% formalin solution. The organ specimen was subjected to dehydration with xylene (1 h each) and alcohol of strength 70%, 90%, and 100%, respectively, each for 2 h. The infiltration and impregnation was carried out by treatment with paraffin wax twice, each time for 1 h and paraffin wax was used to prepare paraffin L molds. Specimens were cut into sections of 3–5 μm thickness and stained with hematoxylin and eosin (H and E). The sections were mounted by diestrene phthalate xylene. The Tubulointerstitial Damage Index was estimated. The other kidney was processed to determine CaOx deposition.

Statistical analysis

The results are expressed as mean±SEM. Comparison

between the groups was made by analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

Urine parameter

The urinary output of NaOx Group rats was (13.8 ± 3.361 mL/24 h/rat) on day 7, whereas in LSFP Group and Cystone group it was (22.5 ± 4.209 mL/24/rat) and (17 ± 3.296 mL/24 h/rat), respectively.

Administration of NaOx in rats resulted in increased oxalate (1.93 ± 0.09 mg/dL), uric acid (2.31 ± 0.04 mg/dL), and creatinine (3.76 ± 0.10 mg/dL) excretion in NaOx group. In LSFP treated group reduction in oxalate (0.66 ± 0.161 mg/dL), uric acid (0.97 ± 0.14 mg/dL), and creatinine (2.58 ± 0.07 mg/dL) values was observed when compared with the NaOx group. Magnesium excretion was decreased 0.01 ± 0.0 mg/dL significantly by NaOx administration. However, treatment with *LSFP* prevented urinary loss of magnesium and restored it to normal value [Table 1].

Serum parameters

Elevated serum sodium and potassium levels were observed in NaOx Group. Elevated serum uric acid (2.26 ± 0.04 mg/dL) indicate impaired renal functions in NaOx Group. Creatinine level was also elevated (3.322 ± 0.247 mg/dL) in NaOx Group, which was not observed in LSFP Group (1.927 ± 0.3896 mg/dL). In rats treated with *LSFP* or Cystone the serum sodium, potassium, uric acid, and creatinine values were significantly reduced compared with the NaOx group [Table 2].

Histopathology

Histopathology of Vehicle Group did not reveal any crystals deposition or histologic damage [Figure 1a].

In NaOx group, maximum deposition of the crystals and also maximum histologic damage were seen. The changes were interstitial fibrosis with dense infiltration by mononuclear cells and few eosinophils. Renal tubules showed tubular cell swelling loss of nuclei and shedding of cells in the lumen. Tubular atrophy was focal and patchy. The crystals were large and in groups. The crystals were seen in the interstitium, tubular lamina lining, tubular epithelium, and in blood vessels [Figure 1b].

Cystone-treated group showed minimum crystal deposition and also showed minimal histological damage. Interstitial fibrosis with infiltration by mononuclear cells and few eosinophils were seen. Tubules showed tubular cell swelling

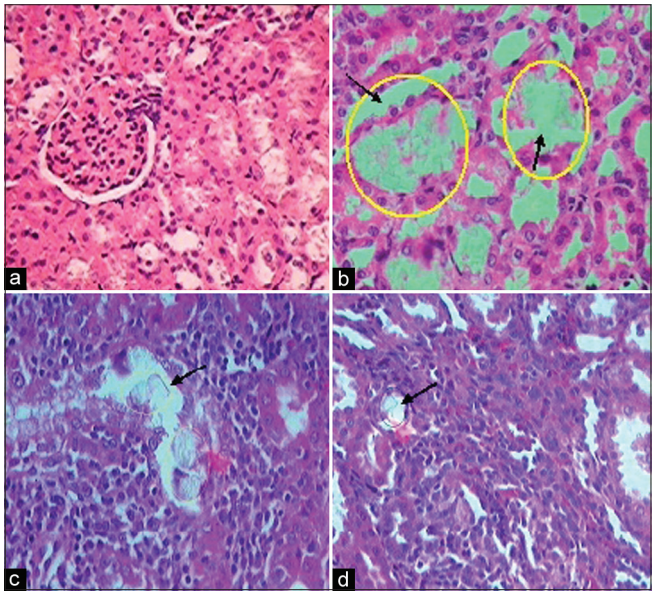

Figure 1: Light microscopicarchitecture and calcium oxalate deposits in the kidney section. (a) VehicleGroup: does not showed histological damage, (b) NaOx Group: maximum depositionof the crystals (c) Cystone: showed minimum deposition crystals (d) *Lagenaria siceraria* (500 mg/kg, p.o.) medium deposition of the crystals (H and E stain, 200×)

Table 1: Effect on urine parameters

Parameter	Vehicle	NaOx	*LS*+NaOx	Cystone+NaOx
Urine output	12.66 ± 2.171	13.8 ± 3.16	22.5 ± 4.20**	17 ± 3.29
Urine pH	6.98 ± 0.41	7.23 ± 0.35	7.37 ± 0.34	6.68 ± 0.43
Oxalate (mg/dL)	1.73 ± 0.07	1.93 ± 0.09	0.66 ± 0.16**	0.48 ± 0.12**
Uric acid (mg/dL)	0.15 ± 0.07	2.31 ± 0.04	1.97 ± 0.14*	1.89 ± 1.59**
Magnesium	0.03 ± 0.0	0.01 ± 0.0	0.04 ± 0.0	0.04 + 0.0**
Creatinine (mg/dL)	2.54 ± 0.06	3.76 ± 0.10	2.58 ± 0.07	1.54 ± 0.12**

Values are expressed as mean S.E.M., (*n*=6), data analyzed by one-way ANOVA followed by Dunnett's test. **P<0.01, as compared with vehicle group

Table 2: Effect on serum parameters

Parameter	Vehicle	NaOx	*LS*+NaOx	Cystone+NaOx
Sodium (eEq/L)	3601.19 ± 164.23	5536.11 ± 276.90	4120 ± 10.88**	4202.22 ± 11.24**
Potassium (eEq/L)	25.69 ± 1.38	54.54 ± 0.92	30.22 ± 1.08**	28.486 ± 0.83**
Uric acid (mg/dL)	2.16 ± 0.02	4.26 ± 0.04	2.76 ± 0.04*	2.99 ± 0.73**
Creatinine (mg/dL)	1.318 ± 0.062	3.322 ± 0.24	1.927 ± 0.38**	1.817 ± 0.058***

Values are expressed as mean S.E.M., (*n*=6), data analyzed by one-way ANOVA followed by Dunnett's test. **P<0.01, as compared with vehicle group

loss of shedding of cells in the lumen. Tubular atrophy was focal and patchy. The crystals were large and seen in the interstitium, tubular lamina lining, and the tubular epithelium and in blood vessels [Figure 1c].

The *LSPF* group showed medium deposition of the crystals and also showed less histologic damage. The numbers of crystals in LSFP group were more compared with that of the Cystone-treated group. Histologic damage was more than that for Cystone-treated group [Figure 1d].

DISCUSSION

Previous reports showed that NaOx administration results in hyperoxaluria in untreated group.[11,12] Since it is accepted that hyperoxaluria is a major risk factor in the pathogenesis of renal stones,[13] the observation that oxalate levels were significantly decreased by *LSFP* at 500 mg/kg dose justify the rationale for use.

In urolithiasis, the glomerular filtration rate decreases due to the obstruction of the outflow of the urine by stones in kidney. The waste products, particularly creatinine and uric acid, accumulate in the blood.[14] In NaOx alone group urine and serum creatinine were increased, which indicated that there was a marked renal damage; however, in *LSFP*-treated group urine creatinine was significantly decreased ($P<0.01$) and also serum creatinine was lowered, which indicated that there was arrest of breakdown of protein due to renal damage in *LSFP* group. Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate plays an important role in stone formation and has about 15-fold greater effect than urinary calcium.[15] In earlier studies cystone was used as a standard drug for comparison of antiurolithiatic activity of plant *Crataeva magna* Lour. Bark, *Mimusops elengi*, and *Bergenia ciliata* leaves.[16-18] Clinical study on cystone described the efficacy and safety in the management of urolithiasis in human.[19] In this study, cystone was used as a standard drug. There was increase in urinary oxalate after NaOx administration. Decreased level of oxalate in Cystone and *LSFP* group was seen. This effect may be due to the inhibition of formation of oxalate by the *LSFP* treatment. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with CaOx solubility and it binds and reduces the inhibitory activity of glycosaminoglycans.[14] The urine and serum uric acid levels of NaOx group increased, which indicated that there was a renal damage; however, in *LSFP*-treated group urine and serum uric acid were significantly lower, which confirm arrest of renal damage. Normal urine contains inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats.[14] Magnesium complexes with oxalate and reduces the supersaturation of CaOx by reducing the saturation of CaOx and as a consequence reduces the growth and nucleation rate of CaOx crystals.[20,21] In NaOx group the level of Mg^{2+} was significantly decreased. In *LSFP* group decrease in Mg^{2+} levels were not observed, which may probably be because of the protective effect of *LSFP* on kidney. The presence of C-glycosylflavonoids, such as orientin and isoroientin, have been reported in *LSFP*.[22] Chen *et al*. reported isolation of four new D: C-friedooleanane triterpenes in the stem of *LSFP*.[23] Budzianowski *et al*. reported antioxidant property of orientin and vitexin.[24] Phytochemical constituents, such as triterpenes[17,25-27] and C-glycosylflavonoids[28] containing plants showed antiurolithiatic effect. The mechanism underlying this effect is still unknown, but it is apparently related to its diuretic properties and lowering of urinary concentrations of stone-forming constituents, which may be attributed to the presence of triterpenoids and flavonoids.[25] It is thus apparent that the flavonoid and triterpenes present in *LSFP* might have been responsible for the reduction of CaOx crystal aggregation and stone formation in kidney as observed in the present study. The results support the use of *LSFP* as an effective alternative in treating NaOx-induced urolithiasis.

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