Symposium

Screening of two new herbal formulations in rodent model of urolithiasis

Abstract

Background: Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys. The recurrence of urolithiasis also represents a serious problem in patients. Not all standard pharmaceutical drugs used to prevent urolithiasis are effective in all patients, and many have adverse effects. The present study was undertaken to evaluate the antiurolithiatic potential of two new herbal formulations DRDC/AY/8080 (tablet) and DRDC/AY/8081 (syrup) against 28-day ethylene glycol (EG)-induced urolithiasis model in Wistar rats. **Materials and Methods:** Animals were divided into five groups ($n = 6$). The control group was given normal saline, and the toxicant group was given 0.75% EG with 1% w/v of ammonium chloride (AC) for 10 days followed by 0.75% w/v EG for next 18 days in drinking water. Treatment groups received respective oral co-treatment with DRDC/AY/8080 (265 mg/kg), DRDC/AY/8081 (2.65 ml/kg), and standard (2.65 ml/kg) for 28 days along with EG and AC as given in toxicant group. After 28th day urine, blood and kidney tissue were collected. Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} levels were estimated in urine, creatinine, and urea levels were estimated in serum whereas the extent of lipid peroxidation was measured in kidney tissue. Further, crystalluria and histopathological evaluation were carried out in urine and kidney tissue, respectively. **Results:** Toxicant group showed significant elevation ($P < 0.001$ vs. control) in serum creatinine, blood urea, tissue lipid peroxide, and urinary Mg^{2+} levels and significant reduction in ($P < 0.001$ vs. control) urinary Na^{+} and Ca^{2+} levels. Histopathology of the toxicant group showed damaged proximal tubules with deposits of refractile crystals and loss of tubular epithelium. Both tablet and syrup treated groups showed nephroprotective activity as evident from lower serum creatinine, blood urea, and lipid peroxide levels. Treatment with tablet and syrup formulations also showed significant ($P < 0.001$ vs. toxicant) elevation in urinary Na^{+}, Ca^{2+}, and reduction in Mg^{2+} levels. Histologically, both tablet/AY/8080) and syrup treatment showed protected against urolithiasis and nephrotoxicity. **Conclusion:** It can be concluded that the two herbal formulations DRDC/AY/8080 and DRDC/AY/8081 possess significant potential in the management of renal calculi.

Key words:
Calcium oxalate, ethylene glycol, urolithiasis

Introduction

Urolithiasis, also called calculi or uroliths, is a condition which involves the process of stone formation in the kidney, bladder, and/or urethra. It is one of the most common urological disorders which has affected humans since ancient times.[1] Urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation,

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and retention within the kidneys. Epidemiological data have shown that calcium oxalate (CaOx) is the predominant mineral in a majority of kidney stones.[2] The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Present treatment of urolithiasis, i.e., extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy are used for the surgical removal of stones. However, these techniques cause undesirable side effects such as tubular necrosis, hypertension, hemorrhage, and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation.[3-5] Not all standard pharmaceutical drugs used to prevent urolithiasis are effective in all patients, and many have adverse effects that compromise their long-term use.[6] DRDC/AY/8080 and DRDC/AY/8081 are two new herbal formulations developed by Dabur Research and Development Center. DRDC/AY/8080 contains the extract of Varun chaal (*Crataeva nurvala*), Punarnava extract (*Boerhavia diffusa*), Gokshur extract (*Tribulus terrestris*), Guduchi extract (*Tinospora cordifolia*), Daru haridra extract (*Berberis aristata*), Pashanbhed (*Bergenia ligulata*), Shwet parpati, Apamarg (*Achyranthes aspera*), Shuddha Shilajit, and Hajrul Yahood Bhasma, whereas DRDC/AY/8081 contains similar ingredients except Hajrul Yahood Bhasma. These formulations are expected to have potential anti-urolithiatic property. Therefore, the present study was designed to evaluate the effect of these two herbal formulations against ethylene glycol (EG)-induced urolithiasis in rats.

Materials and Methods

Male Wistar rats age (10–12 weeks) weighing 180–220 g were used. Animals were obtained from and kept at the Central Animal House of Hamdard University in colony cages at an ambient temperature of 25 ± 2°C and relative humidity of 45–55% with 12 h light/dark cycles after initial acclimatization for about 1 week. They had free access to standard rodent pellet diet and water *ad libitum*. The experimental study was approved by the Institutional Animal Ethics Committee of the University. Rats were divided into different groups and treated as mentioned in Table 1.

On 28th day, animals were kept in urine collection cage, and morning 3 h urine was collected for crystalluria study.

Further, 24 h urine was collected before and after treatment. Twenty-four hours urine samples were divided into two parts A and B. Part A was with 5 ml HCL to pH-2, whereas Part B was used as such.

After collection of urine, blood was withdrawn on the 29th day from retrobulbar plexus and animals were sacrificed under high dose of pentobarbitone sodium anesthetic and kidneys were excised, washed with ice-cold saline and tissue homogenate were prepared for biochemical estimation. Kidney tissue from each group was also fixed in formalin for 48 h. Thereafter, slides were prepared with hematoxylin and eosin staining. Tissue slides were evaluated by a pathologist who was blind to the treatment groups.

Table 1: Treatment schedule proposed for antiurolithiatic activity of formulation DRDC/AY/8080, DRDC/AY/8081, and Neeri

Group	Treatment
Control	Normal saline for 28 days
Toxicant	(0.75% EG + 1% w/v of CA) for 10 days followed by 0.75% w/v EG for next 18 days
DRDC/AY/8080 (tablet)	(0.75% EG + 1% w/v of CA) for 10 days followed by 0.75% w/v EG for next 18 days + tablet at a dose of 265 mg/kg/day given for 28 days
DRDC/AY/8081 (syrup)	(0.75% EG + 1% w/v of CA) for 10 days followed by 0.75% w/v EG for next 18 days + syrup at a dose of 2.65 ml/kg/day given for 28 days
Neeri (standard)	(0.75% EG + 1% w/v of CA) for 10 days followed by 0.75% w/v EG for next 18 days Neeri syrup at a dose of 2.65 ml/kg/day given for 28 days

EG – Ethylene glycol; AC – Ammonium chloride

Followed by a collection of urine, blood and kidney tissue estimation of urine Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, serum creatinine, blood urea, and tissue thiobarbituric acid-reactive substances (lipid peroxidation) were carried out and hematology was evaluated. Qualitative evaluation of urine for crystalluria and histopathology of kidney tissue was also conducted.

Results

EG is a metabolic precursor of oxalate. EG is oxidized to glycolic acid which is, in turn, oxidized to oxalic acid. Administration of EG to rats results in hyperoxaluria, CaOx crystalluria, and occasional deposition of CaOx crystals in the kidney. At present, it seems clear that renal epithelial cell injuries play a decisive role in renal calculi development,[7,8] and in fact the lithogenic effect caused by EG must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by EC. Thus, although EG rat model is considered an interesting model to evaluate renal papillary stone development, at least for those stones which genesis is linked to oxidative cell damage. Ammonium chloride (AC) ingestion, which induces metabolic acidosis, has been used in conjunction with EG ingestion to promote the deposition of CaOx crystals in rat kidneys. At different doses AC in combination with EG, all rats form kidney CaOx depositions within 4–7 days.[9,10] Therefore, we investigated the effect of the test sample on EG-induced urolithiasis model in rats which is a well-established model and used by several investigators.[11-13] In the present study, chronic administration of 0.75% EG aqueous solution to male Wistar rats resulted in hyperoxaluria. This stone formation

is the result of supersaturation caused by salts like CaOx. However, all the treatment groups were able to reverse this effect and following results were observed.

Effect on urinary ion level

Effect of different treatment on urinary ion levels is shown in Table 2. Results of urinary calcium level showed reduced level in toxicant group ($P < 0.001$ vs. control) compared to control group. However, treatment with tablet formulation (DRDC/AY/8080) showed significant elevation ($P < 0.001$ vs. toxicant) in urinary calcium level compared to the toxicant group. Similarly, treatment with syrup (DRDC/AY/8081) also showed significant ($P < 0.01$ vs. toxicant) elevation in calcium level compared to the toxicant group. Neeri standard group significantly elevated ($P < 0.05$ vs. toxicant) calcium level compared to toxicant. Comparison of the tablet (DRDC/AY/8080), syrup (DRDC/AY/8081), and Neeri showed an insignificant difference. Results showed that toxicant treatment resulted in reduced sodium excretion therefore significantly lower urinary sodium level ($P < 0.001$ vs. control) compared to control group were observed. Treatment with tablet formulation (DRDC/AY/8080) significantly elevated ($P < 0.001$ vs. toxicant) sodium excretion compared to the toxicant group. Similar results were obtained with syrup (DRDC/AY/8081) which showed significantly elevated ($P < 0.001$ vs. toxicant) urinary sodium level compared to toxicant group. Comparison of treatment groups, i.e., tablet (DRDC/AY/8080), syrup (DRDC/AY/8081), and Neeri showed that natriuresis showed by Neeri was significantly lower ($P < 0.001$ vs. tablet) compared to the tablet as well as the syrup ($P < 0.0001$ vs. syrup) treated groups. Toxicant group showed significant elevation ($P < 0.001$ vs. control) in urinary magnesium level. However, treatment with tablet formulation (DRDC/AY/8080) further elevated magnesium level to significantly higher ($P < 0.001$ vs. toxicant) level compared to the toxicant group. Similarly, treatment with syrup (DRDC/AY/8081) also showed significant elevation ($P < 0.01$ vs. toxicant) compared to toxicant group. The standard group, treated with Neeri, showed opposite results and reduced urinary magnesium level significantly ($P < 0.001$) compared to the toxicant group. Treatment with Neeri syrup showed a significant reduction ($P < 0.001$ vs. toxicant) in elevated urinary magnesium level compared to toxicant group. Comparative evaluation of treatment groups showed that urinary magnesium level was significantly lower ($P < 0.001$ vs. tablet) compared to tablet (DRDC/AY/8080) group as well as the syrup group (DRDC/AY/8081).

Effect on markers of nephrotoxicity

Effect of treatment of test and standard drugs is shown in Table 3. Treatment with EG and AC (toxicant group) showed significant elevation ($P < 0.001$ vs. control) compared to control group. Treatment with tablet formulation (DRDC/AY/8080) showed a significant reduction ($P < 0.001$ vs. toxicant) in serum creatinine level compared to the toxicant group. Creatinine level in syrup formulation (DRDC/AY/8081) were significantly lower ($P < 0.001$ vs. toxicant) compared to toxicant group. Neeri syrup (standard) treated group also showed a significant reduction ($P < 0.001$ vs. toxicant) in serum creatinine level compared to toxicant group. There was an insignificant difference between Neeri and control group.

Table 2: Results of urinary ion levels and evaluation of oxalate crystals in urine samples of different treatment groups

	Calcium level	Sodium level	Magnesium level	Oxalate crystals
Control	32.00±2.23	203.6±5.91	6.15±0.21	Nil
Toxicant	17.20±1.60[a]	140.74±4.55[a]	14.65±0.63[a]	+ + +
Tablet (DRDC/AY/8080)	26.40±2.50[c,d]	172.33±6.34[a,d]	18.09±1.22[a,d]	Nil[d]
Syrup (DRDC/AY/8081)	26.01±1.60[c,e]	173.56±1.89[a,d]	16.55±0.36[a,e]	Nil[d]
Neeri syrup	23.36±1.18[b,f]	125.51±4.92[a,d]	7.77±0.41[c,d]	Nil[d]

[a]$P<0.001$ versus control; [b]$P<0.01$ versus control; [c]$P<0.05$ versus control; [d]$P<0.001$ versus toxicant; [e]$P<0.01$ versus toxicant; [f]$P<0.05$ versus toxicant

Table 3: Results on serum creatinine, blood urea, and tissue measured as nanogram levels of different treatment groups

	Creatinine (mg/dL)	Blood urea (mg/dL)	MDA (nmol/mg)
Control	0.12±0.03	19.3±10.50	3.56±0.25
Toxicant	0.71±0.05[a]	134.7±14.86[a]	8.81±0.23[a]
Tablet (DRDC/AY/8080)	0.25±0.02[b,d]	70.61±3.34[a,d]	5.62±0.22[a,d]
Syrup (DRDC/AY/8081)	0.28±0.01[a,d]	81.97±2.88[a,d]	5.08±0.30[a,d]
Neeri syrup	0.17±0.01[d]	27.11±10.2[d]	5.32±0.21[a,d]

[a]$P<0.001$ versus control; [b]$P<0.01$ versus control; [c]$P<0.05$ versus control; [d]$P<0.001$ versus toxicant. MDA – Malondialdehyde

The toxicant group showed significantly high blood urea level ($P < 0.001$ vs. control) compared to control group. Treatment with tablet formulation (DRDC/AY/8080) showed a significant reduction ($P < 0.001$ vs. toxicant) of elevated urea level compared to toxicant group. Urea level in syrup formulations (DRDC/AY/8081) was also significantly reduced ($P < 0.001$ vs. toxicant) compared to toxicant group. Neeri syrup (standard) treated group also showed a significant reduction ($P < 0.001$ vs. toxicant) in blood urea level compared to toxicant group. There was an insignificant difference between Neeri and control group.

Lipid peroxidation was measured as a nanogram of malondialdehyde (MDA) per mg of protein level in the kidney tissue. Toxicant group showed significantly higher tissue MDA level ($P < 0.001$ vs. control) compared to control. Tablet formulation (DRDC/AY/8080) treated

group had significantly lower (P < 0.001 vs. toxicant) level of tissue MDA compared to toxicant group. Similar results were obtained with syrup (DRDC/AY/8081) which showed significantly lower (P < 0.001 vs. toxicant) MDA level compared to toxicant group. Neeri treated group (standard) showed significant lowering (P < 0.001 vs. toxicant) of lipid peroxidation as evident from lower MDA level. Comparison of treatment groups, i.e., tablet (DRDC/AY/8080), syrup (DRDC/AY/8081), and Neeri showed an insignificant difference.

Crystalluria study (for oxalate crystals) as shown in Table 3 showed nil crystals were observed in groups treated with DRDC/AY/8080 (tablet) and DRDC/AY/8081 (syrup) supported the preventive effect of these formulations on the induction of urolithiasis.

The present study showed that oral treatment with both DRDC/AY/8080 and 8081 prevented the CaOx crystal aggregation. On administration of both tablet and syrup, the reduction in calcium deposition in the kidneys and their urinary excretion implies the efficacy of both formulations preventing the formation of CaOx stones. Further, formulation 8080 and 8081 also caused a significant reduction in the elevated urinary creatinine thus, reflects the improvement in renal impairment. Lipid peroxidation is another critical cause of injury that occurs during urolithiasis. Increased MDA level is consistent with the occurrence of damage mediated by free radicals.[14] The biochemical alterations were further supported by histopathological observations of the kidney [Figure 1]. Hematological examination of different groups showed normal red blood cell, white blood cell, and hemoglobin levels and no hemolysis was observed in any group.

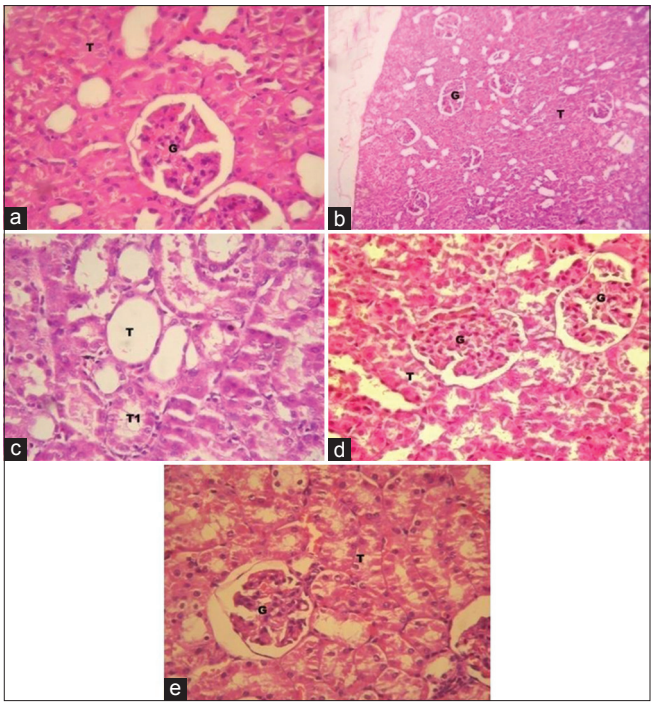

Figure 1: High power photomicrograph of section of kidney from (a) normal control group showing a normal glomerulus and tubules; (b) toxic control group showing damaged proximal tubules (T) with deposits of refractile crystals (C) and loss of tubular epithelium; (c) Neeri group showing a few dilated tubules (T) along with a number of undamaged tubules (T1). (d) Formulation DRDC/AY/8080 showing a normal glomerulus and tubules. No evidence of tubular damage was seen in this sample (e) tablets showing a normal glomerulus and tubules. No evidence of tubular damage was seen in this sample; G = Glomerulus, T = Tubule (H and E, ×400)

Discussion

EG is a metabolic precursor of oxalate. EG is oxidized to glycolic acid which is, in turn, oxidized to oxalic acid. Administration of EG to rats results in hyperoxaluria, CaOx crystalluria, and occasional deposition of CaOx crystals in the kidney. At present, it seems clear that renal epithelial cell injuries play a decisive role in renal calculi development,[7,8] and in fact the lithogenic effect caused by EG must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by EC. Thus, although EG rat model is considered an interesting model to evaluate renal papillary stone development, at least for those stones which genesis is linked to oxidative cell damage. AC ingestion, which induces metabolic acidosis, has been used in conjunction with EG ingestion to promote the deposition of CaOx crystals in rat kidneys. At different doses AC in combination with EG, all rats form kidney CaOx depositions within 4–7 days.[9,10] Therefore, we investigated the effect of the test sample on EG-induced urolithiasis model in rats which is a well-established model and used by several investigators.[11-13]

Results of urinary calcium level showed reduced level in toxicant group (P < 0.001 vs. control) compared to control group. However, treatment with tablet formulation (DRDC/AY/8080) showed significant elevation (P < 0.001 vs. toxicant) in urinary calcium level compared to toxicant group. Comparison of the tablet (DRDC/AY/8080), syrup (DRDC/AY/8081), and Neeri showed an insignificant difference. Results showed that toxicant treatment resulted in reduced sodium excretion therefore significantly lower urinary sodium level (P < 0.001 vs. control) compared to control group were observed.

Treatment with EG and AC (toxicant group) showed significant elevation (P < 0.001 vs. control) compared to control group. Treatment with tablet formulation (DRDC/AY/8080) showed a significant reduction (P < 0.001 vs. toxicant) in serum creatinine level compared to toxicant group.

Toxicant group showed significantly high blood urea level ($P < 0.001$ vs. control) compared to control group. Lipid peroxidation was measured as a nanogram of MDA per mg of protein level in the kidney tissue. Crystalluria study (for oxalate crystals) supported the preventive effect of these formulations on the induction of urolithiasis.

Lipid peroxidation is another critical cause of injury that occurs during urolithiasis. Increased MDA level is consistent with the occurrence of damage mediated by free radicals.[14] Formulations DRDC/AY/8080 and DRDC/AY/8081 showed good antiurolithiatic activity rats, and hence it carries potential to be developed as a preventive intervention in the treatment of urolithiasis.

Conclusion

The results of both the formulations tested against EG model of urolithiasis attributed good antiurolithiatic potential and hence these formulations can be used in prevention and management of urolithiasis.

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Conflicts of interest
There are no conflicts of interest.

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