Research Article

Effect of *Hordeum vulgare* Linn. Seeds on glycolic acid induced urolithiasis in rats

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ABSTRACT: **Objective:** To evaluate its anti-urolithic potential, the ethanolic extract of seeds of *Hordeum vulgare* was tested in an animal model of urolithiasis. **Materials and Methods:** Urolithiasis was induced by the addition of 3% glycolic acid to the normal diet of Wistar albino rats for a period of 42 days. Group I served as a normal control. Group II served as urolithiasis control. Group III, IV and V were treated with ethanolic extract of *Hordeum vulgare* (EHV) at 100, 250 and 500 mg/kg respectively. Group VI was treated with Cystone as a standard drug. The effects of EHV on various biochemical parameters were studied in urolithitic rats. **Results:** Glycolic acid induced hyperoxaluria in urolithiatic rats. And, there were significant elevated urine output, kidney weight loss and some renal injury markers in glycolic acid induced rats. In vivo antioxidant parameters including lipid peroxidation (MDA), superoxide dismutase (SOD) and catalase (CAT) were also determined. Oral administration of EHV 100, 250 and 500 inhibited CaOx crystal disposition in renal tubules and protected against associated changes in polyurea and kidney weight loss. EHV significantly maintained the urinary excretion of the calcium, phosphate, uric acid, urea, and oxalate and increased the excretion of citrate as compared to glycolic acid control animals. The increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly lowered by treatment with EHV. The extract also induced a significant decrease in MDA which increased in urolithiatic control rats. The extract also significantly increased SOD and CAT in urolithiatic rats which were markedly decreased in glycolic acid induced urolithiasis in rats. **Conclusion:** This study demonstrates the anti-urolithic activity of *Hordeum vulgare* seeds and rationalizes their medicinal use for the treatment of urolithiasis.

KEY WORDS: Glycolic acid; *Hordeum vulgare*; urolithiasis.

INTRODUCTION

Hyperoxaluria is one of the major risk factors of human idiopathic calcium oxalate (CaOx) urolithiasis. Oxalate is a natural byproduct of metabolism and harmlessly excreted through the urine in normal individuals. However, increased urinary excretion of oxalate (hyperoxaluria) can be highly toxic because of its propensity to crystallize at physiologic pH and form CaOx.[1] Urolithiasis is a complex process that results from a succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation, and retention within renal tubules.[2] Epidemiological data collected over several decades showed that the majority of stones (up to 80%) are composed mainly of calcium oxalate (CaOx).[3]

Urine is always supersaturated with common stone forming minerals. The crystallization inhibiting capacity of urine does not allow urolithiasis to happen in most individuals, whereas this natural inhibition is in deficit in stone formers.[4] The majority of urinary calculi are made up of calcium phosphate, calcium oxalate, uric acid (urates) or magnesium ammonium phosphate. Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches, i.e., percutaneous nephrolithotomy

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(PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics[5]. However, these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Hence, the search for herbo-mineral preparations is still ongoing.[6] A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they are reported to be effective with no side effects.[7]

Many Indian plants have been quoted to be useful as antilithiatic agents. They are effective with fewer side effects and are also inexpensive. Hence, the Indian plants are constantly being evaluated for possible antilithiatic effects in a systematic manner.[8] As per the indigenous system of Ayurveda medicine, the seeds of *Hordeum vulgare* Linn. have been reported to be useful in the treatment of a wide range of aliments, including urinary stones.[9] In the current study, an effort has been made to establish the scientific validity for the anti-urolithiatic activity of *Hordeum vulgare* seed extract using glycolic acid induced urolithiasis using male wistar albino rats.

MATERIAL AND METHOD

Animal selection
For acute toxicity studies, albino mice of either sex weighing between 25-30 gm and for the study of antiurolithisis activity adult male albino rats of Wistar strain (150-200 gm) were procured form Zydus Research Centre, Ahmedabad. The animals were acclimatized to standard laboratory conditions (temp: 23 ± 2 °C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow (VRK Nutritional Solutions, Pune, India) with free access to drinking water *ad libitum* for the period of 28 days. Institutional Animal Ethics Committee (IAEC) approval (Protocol no.: IICP/PH/02-2010/05 dated 15.03.2010) was obtained before the experiment and care of animal was taken as per guidelines of CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

Chemicals
Ethylene glycol was obtained from SD Fine Chemical Limited, Mumbai, India. Cystone (Himalaya Health Care, India) was procured from the local market. All other chemicals and reagents used were of analytical grade.

Plant material
The dried seeds of *Hordeum vulgare* Linn. were received from commercial supplier, Anand, Gujarat, India. The seeds were identified by Dr. G. C. Jadeja, Department of Agricultural Botany, B. A. College of Agriculture, Anand Agricutlture University, India. A Voucher specimen (voucher no. IICP/11-JGS/03-HV) was deposited in the herbarium of the Department of Pharmacognosy, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India.

Preparation of plant extract
The air-dried powdered seeds (500 g) were extracted with ethanol in soxhlet apparatus for 24 h. The extract was evaporated to dryness under reduced pressure to give solid residues. The residue was stored below 4 °C for subsequent experiments. The yield of the extract was 4.82 % w/w.

Acute toxicity studies
The acute toxicity was performed as per WHO guideline and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (no.420) using albino mice of either sex prior to the evaluation of anti-urolithiatic activity. The EHV was tested using graded doses (500, 1000, 2000 and 5000 mg/kg) in mice. Furthermore, the general behaviour of mice was recorded continuously for 12 h, and daily for a further 2 weeks for any eventual mortality.[10] The EHV did not show mortality, or any remarkable symptoms of toxicity and/or any significant changes in general behaviour in mice.

Experimental Design:
Urolithiasis induced by glycolic acid model was used to study the anti-urolithiatic activity in Wistar albino rats. Animals were divided into six groups containing six animals in each. Animals of group I received the commercial diet and served as control, group II-IV was fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 42 days. Group III, IV and V administered orally 100, 250 and 500 mg/kg b.w. of extract respectively. Group VI received standard anti-urolithiatic drug, cystone (750 mg/kg b.w.; p.o.).[11] All the extracts and standard drug were given once a day orally in addition to the CPD for the duration of 42 days.

Collection and analysis of urine
On the completion of treatment, all animals were kept in individual metabolic cages. Animals had free access to drinking water during the urine collection period. At the completion of treatment of 42nd day, the urine sample of each animal was collected. Volume of urine and its pH were measured immediately after the collection of urine. The collected urine were admixed with a drop of concentrated hydrochloric acid and stored at 4°C. Urine was analyzed as previously described for calcium,[12] phosphate,[13] oxalate,[14] urea,[15] uric acid[16] and citrate.[17]

Collection and analysis of serum
Blood was collected by retro-orbital puncture under mild anesthetic conditions. Serum was separated by centrifugation at 15000 rpm for 20 min and analyzed for calcium,[12] phosphate,[13] urea,[15] uric acid[16] and creatinine.[18,19]

Kidney homogenate analysis

The animals were sacrified by cerival dislocation. The abdomen was cut open to remove both kidneys from each animal. The weight of dry and wet kidney was measured. Isolated kidneys were cleaned of extraneous tissue and rinsed in ice-cold physiological saline. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was separated. The concentration of calcium,[12] phosphate[20] oxalate,[21] and uric acid[16] in kidney homogenate were determined.

Enzyme assay

A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of protein,[22] superoxide dismutase (SOD),[23] catalase (CAT)[24] and malondialdehyde (MDA).[25]

Statistical analysis

Results were expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett's multiple comparison tests (Graphpad Prism software for Windows, Version 2.03.1998). $p < 0.05$ was considered to be statistically significant.

RESULTS

There were no any behavioral changes, signs of toxicity as well as any mortality were observed after the administration of a dose up to 5000 mg/kg. So, From the acute toxicity study, the LD_{50} cut-off dose was found to be 5000 mg/kg body weight for the extract. Hence, the therapeutic dose was taken as 100 mg/kg, 250 mg/kg, and 500 mg/kg body weight for the ethanolic extract.

In the present study, urolithiasis was induced by the supplementation of normal commercial diet with glycolic acid for the 42 days. Table 1 indicates the various physical parameters that were measured including the volume of urine collected at the end of the treatment, the pH of urine and the weight of dry and wet kidney. The volume of urine was reduced in calculi induced animals (Group II) as compared to normal control group. The treatment with EHV 500 (Group V) and standard drug Cystone (Grop VI) increase the volume of urine in calculi induced animals when compared with control (Group II). The pH of urine of calculi controlled animals (Group II) were significantly ($p<0.05$) increased as compared to normal control rats which was slightly acidic. The treatment with EHV 500 was significantly ($p<0.05$) decreased the pH of urine near to neutral in calculi induced animals. There was a significant ($p<0.05$) increase in the kidney weight (both dry and wet weight) of animals receiving 3% glycolic acid which was significantly ($p<0.05$) reduced by the treatment with Cystone and the EHV 250 & 500. The kidney weight was significantly ($p<0.05$) higher in kidneys with crystal deposits.

Due to the hyperurolxaluria there was an increased urinary excretion of calcium, phosphate, uric acid, urea and oxalate in calculi control animals (Table 2, Group II). However, supplementation with EHV 500 (Table 2, Group V) significantly ($p<0.05$) reserved these changes in urinary excretion of calcium, phosphate, uric acid, urea and oxalate

Table 1: Effect of ethanolic extract of seeds of *H. vulgare* Linn. on various physical parameters of control and experimental animals

Parameters	Grp I Normal Control	Grp II Calculi induced	Grp III EHV 100	Grp IV EHV 250	Grp V EHV 500	Grp VI Cystone Treated
Volume; ml/24 hr	18.17 ± 0.87	17.83 ± 1.40	19.17 ± 1.07	20.17 ± 0.87	22.17 ± 0.87	20.67 ± 1.08
pH	6.38 ± 0.08	8.22 ± 0.07*	8.01 ± 0.25	7.79 ± 0.01	7.01 ± 0.12**	7.33 ± 0.11**
Wet weight (g/100 g b.wt)	0.64 ± 0.03	1.12 ± 0.01*	0.91 ± 0.24	0.87 ± 0.04	0.79 ± 0.04**	0.84 ± 0.03**
Dry weight (g/100 g b.wt)	0.24 ± 0.01	0.41 ± 0.03*	0.35 ± 0.02	0.20 ± 0.01	0.18 ± 0.07**	0.30 ± 0.01**

EHV = Ethanolic extract of *Hordeum vulgare*; n = 5 in each group; Values are expressed as mean ± SEM; Data are analyzed by One-way ANOVA followed by Dunnett's test; *p < 0.05 significantly different as compared to normal control group, **p< 0.05 significantly different as compared to calculi induced group.

Table 2: Effect of ethanolic extract of seeds of *H. vulgare* Linn. on various parameters in urine of control and experimental animals

Parameters (mg/day)	Grp I Normal Control	Grp II Calculi induced	Grp III EHV 100	Grp IV EHV 250	Grp V EHV 500	Grp VI Cystone Treated
Calcium	3.69 ± 0.33	5.50 ± 0.54*	4.45 ± 0.30	4.11 ± 0.31	3.57 ± 0.41**	4.08 ± 0.22**
Phosphate	3.11 ± 0.23	7.22 ± 0.17*	7.18 ± 0.45	6.32 ± 0.44	5.11 ± 0.53**	4.23 ± 0.41**
Uric acid	2.06 ± 0.29	4.96 ± 0.37*	4.67 ± 0.38	4.13 ± 0.34	3.28 ± 0.37**	2.91 ± 0.35**
Urea	9.47 ± 0.37	15.15 ± 1.04*	15.17 ± 0.75	14.24 ± 0.49	12.28 ± 0.78**	9.72 ± 0.48**
Oxalate	3.48 ± 0.51	11.43 ± 0.81*	10.65 ± 1.08	8.13 ± 0.79**	5.58 ± 0.61**	4.65 ± 0.39**
Citrate	16.54 ± 1.67	10.36 ± 0.87*	13.28 ± 1.17	16.21 ± 0.92**	21.46 ± 1.36**	19.44 ± 1.87**

EHV = Ethanolic extract of *Hordeum vulgare*; n = 5 in each group; Values are expressed as mean ± SEM; Data are analyzed by One-way ANOVA followed by Dunnett's test; *p < 0.05 significantly different as compared to normal control group, **p< 0.05 significantly different as compared to calculi induced group.

excretion dose-dependently in EHV treated animals (Table 2, Group III-V). Urinary citrate flow was decreased by glycolic acid inducing treatment. However, supplementation with EHV 250 and 500 (Table 2, Group IV, V) significantly ($p<0.05$) maintained the elevated level of citrate and restores it near to normal value. The observed results were comparable with cystone treated animals (Table 2, Group VI).

Renal stone induction caused impairment of renal functions of the control (Group II) rats as evident from the markers of glomerular and tubular damage as reflected by the elevated levels of serum creatinin, uric acid and urea in calculi induced animals (Table 3, Group II), which were significantly ($p<0.05$) reduced in the animals treated with EHV 500 (Table 3, Group V). The serum calcium and inorganic phosphate were remarkably increased in calculi-induced animals (Table 3, Group II). However, treatment with EHV 500 mg/kg significantly ($p < 0.05$) lowered the elevated serum level of calcium and inorganic phosphate in calculi induced animals.

Table 4 shows the parameters of the kidney homogenate. The calcium, phosphate, uric acid and oxalate level were significantly ($p<0.05$) increased in kidney homogenate of calculi induced animal group (Group II). The EHV (500 mg/kg) and cystone treatment ($p<0.05$) maintained the levels of all parameters mentioned above. The EHV (100 and 250 mg/kg) failed to exhibit significant reduction in calcium, uric acid and oxalate level in kidney homogenate except phosphate level.

For *in vivo* antioxidant activity, ethylene glycol treatment significantly ($p<0.05$) increased MDA and decreased SOD and CAT levels in calculi- induced animals (Table 5, Group II). The treatment with EHV 500 produced significant ($p < 0.05$) reduction in MDA and antioxidant enzymes like SOD and CAT as compared to control group.

DISCUSSION

In urolithiatic study, male rats were selected as a model system to induce renal stones because the urinary system of male rats resembles that of humans.[24] Here 3% glycolic acid used to provoke hyperoxaluria, which is known to be due to the ready conversion of glycolic acid to oxalate by

Table 3: Effect of ethanolic extract of seeds of *H. vulgare* Linn. On various parameters in serum of control and experimental animals

Parameters (mg/day)	Grp I Normal Control	Grp II Calculi induced	Grp III EHV 100	Grp IV EHV 250	Grp V EHV 500	Grp VI Cyston Treated
Calcium	7.17 ± 0.79	12.61 ± 0.98*	12.89 ± 0.64	11.66 ± 0.99	8.77 ± 0.70**	7.52 ± 0.47**
Phosphate	3.21 ± 0.25	10.52 ± 0.40*	10.22 ± 0.60	9.02 ± 0.35	6.38 ± 0.65**	4.35 ± 0.24**
Uric acid	3.53 ± 0.72	7.74 ± 0.60*	7.83 ± 0.65	6.60 ± 0.43	5.43 ± 0.48**	4.38 ± 0.30**
Urea	28.80 ± 1.83	44.94 ± 2.88*	45.46 ± 4.29	41.09 ± 2.33	33.59 ± 1.72**	24.27 ± 2.04**
Creatinin	0.83 ± 0.13	1.73 ± 0.12*	1.82 ± 1.02	1.43 ± 0.12	1.32 ± 1.49**	1.02 ± 0.06**

EHV = Ethanolic extract of *Hordeum vulgare*; n = 5 in each group; Values are expressed as mean ± SEM; Data are analyzed by One-way ANOVA followed by Dunnett's test; *p < 0.05 significantly different as compared to normal control group, **p< 0.05 significantly different as compared to calculi induced group.

Table 4: Effect of ethanolic extract of seeds of *H. vulgare* Linn. on various parameters in kidney homogenate of control and experimental animals

Parameters (mg/day)	Grp I Normal Control	Grp II Calculi induced	Grp III EHV 100	Grp IV EHV 250	Grp V EHV 500	Grp VI Cystone Treated
Calcium	3.04 ± 0.38	6.12 ± 0.55*	5.88 ± 0.50	5.05 ± 0.48	4.43 ± 0.35**	3.67 ± 0.27**
Phosphate	3.38 ± 0.37	6.42 ± 0.60*	6.62 ± 0.40	5.39 ± 0.37	4.47 ± 0.42**	3.94 ± 0.37**
Oxalate	1.08 ± 0.13	3.11 ± 0.20*	3.01 ± 0.20	2.58 ± 0.17	1.96 ± 0.30**	1.46 ± 0.26**
Uric acid	1.85 ± 0.23	4.25 ± 0.36*	4.40 ± 0.16	3.95 ± 0.16	2.55 ± 0.25**	1.90 ± 0.27**

EHV = Ethanolic extract of *Hordeum vulgare*; n = 5 in each group; Values are expressed as mean ± SEM; Data are analyzed by One-way ANOVA followed by Dunnett's test; *p < 0.05 significantly different as compared to normal control group, **p< 0.05 significantly different as compared to calculi induced group.

Table 5: Effect of ethanolic extract of seeds of *H. vulgare* Linn. on antioxidant enzymes of control and experimental animals

Parameters (mg/day)	Grp I Normal Control	Grp II Calculi induced	Grp III EHV 100	Grp IV EHV 250	Grp V EHV 500	Grp VI Cystone Treated
Melondialdehyde	01.37 ± 0.25	03.11 ± 0.61*	03.07 ± 0.28	02.33 ± 0.22	01.87 ± 0.14**	01.50 ± 0.24**
Superoxide dismutase	00.65 ± 0.07	00.13 ± 0.05*	00.35 ± 0.08	00.45 ± 0.11**	00.66 ± 0.05**	00.57 ± 0.04**
Catalase	03.29 ± 0.69	00.99 ± 0.12*	01.19 ± 0.38	01.77 ± 0.08**	02.59 ± 0.19**	02.96 ± 0.22**

EHV = Ethanolic extract of *Hordeum vulgare*; n = 5 in each group; Values are expressed as mean ± SEM; Data are analyzed by One-way ANOVA followed by Dunnett's test; *p < 0.05 significantly different as compared to normal control group, **p< 0.05 significantly different as compared to calculi induced group.

the oxalate synthesizing enzyme, glycolate oxidase in liver. Glycolic acid (the precursor of oxalic acid) is known to increase significantly the incidence of oxalate lithiasis. Our results are in concord with these studies, as shown by the significant increase in kidney weight.[13]

As postulate in previous studies, following a stone-inducing regimen, volumes of 24 h urine and water intake were observed higher in the untreated group compared with those of the control animals and the urine pH was slightly reduced.[25] In the present investigation, the volume of urine and the pH were measured at the end of treatment. The volume of urine was not significantly reduced in the Cystone and the EHV treated animals. This indicates that the EHV had no diuretic activity. While, the pH were significantly affected by the drug treatment. There was a good correlation between wet tissue calcium and oxalate concentrations by compared to kidneys without CaOx crystals; in wet tissue such salt concentrations were observed significantly higher.

Glycolic acid administration increased the urinary calcium level. It has been stated that hypercalciuria favors precipitation of calcium oxalate from urine[26] Thus, the high oxalate and calcium ion concentration in urine tends to form calcium oxalate crystals. According to previous reports, not only calcium and oxalate excretion but excretion of inorganic phosphate is also important in the formation of urinary stone.[27] In the present study, oxalate, calcium and phosphate excretion observed in glycolic acid–treated rats is likely to have formed calcium phosphate crystals. Conversely, EHV declines the levels of oxalate, calcium as well as phosphate execretion which is known to prove valuable in preventing calculi formation due to supersaturation of these urolithiagenic substances.

It has been reported that uric acid interferes with calcium oxalate solubility. It also reduces the inhibitory activity of glycosaminoglycans.[28] The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation.[28,29] In comparison with normal controls there was significant increased in uric acid level in serum as well as in urine in glycolic acid control group. While in case of groups received treatment of standard and EHV, there was significant maintain the level of uric acid observed.

In urine, citrate complexes calcium, reducing the free calcium- ion activity. This effect is pH-dependent, with maximum complex formation at or above pH 6.5. The citrate is as a specific inhibitor of crystal growth and aggregation. Citrate is one of the major inhibitors of calcium phosphate- crystal growth. In the calcium oxalate system,

its effect is thought to be small and pH-dependent.[30] In the present study, EHV showed protective effect mediated by their influence on the urinary excretion of some citrate, in a model of experimental lithiasis in rats.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Thus the waste products particularly nitrogenous substances such as urea, uric acid and creatinin get accumulated in blood.[30] In comparison with the normal control there was significant increased in urea, uric acid and creatinine levels in serum as well as in urine in glycolic acid control group. While in case of groups received treatment of standard and EHV there was significant decreased in the level of urea, uric acid and creatinine.

Elevated oxalate concentrations in urine has been reported to induce lipid peroxidation and cause renal damage by reacting with polyunsaturated fatty acids in cell membrane.[26] The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR and the anti-lipid peroxidative property. Stone inducing treatment caused hypertrophy and extensive calcium oxalate crystal deposition in kidneys of untreated rats accompanied by oxidative damage as reflected from increased levels of markers of oxidative injury: MDA and protein carbonyl content, and decreased activities of antioxidant enzymes and GSH levels in kidneys as well as deteriorated renal functions.[31] The systemic circulation may damage some part of the kidney, probably the mesengial or glomerular epithelial cells, and cause the kidney to produce more reactive oxygen species than systemic blood. Therefore, the excessive oxidative stress would have been completely compensated for by elevated antioxidant enzymes in the kidney during this period.[32] The decreased activities of catalase in the nephrolithiasis in this model may have led to more H_2O_2 accumulation in the kidney, resulting in more hydroxyl radical formation; because catalase is the only enzyme that regulates the potent hydroxyl radical.[33] The EHV 500 may prevent the lipid peroxidation induced renal damage caused by calcium oxalate crystals deposition in the kidney. Hence, EHV prevented calcium oxalate crystal attachment as well as stone formation. And in addition, Administration of EHV 500 results in significant increase in the catalase and increase levels of SOD and CAT levels in kidney as compared to the control animals, which suggests its efficacy in preventing free radical-induced damage.

CONCLUSION

Overall, the presented data in this paper indicates that administration of seed extract of *Hordeum vulgare* Linn. to experimentally CaOx-induced nephrolithiasic rats reduced the deposition of crystals into kidneys confirming its

antilithiasic effect. This effect is possibly mediated through an antioxidant, nephroprotective property and lowering the concentration of urinary stone forming constituents.

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