JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Effect of Ethanolic Extract of HORDEUM VULGARE Seed on Calcium Oxalate Deposition by Surgericaly Induced Urolithiasis

Ravindrakumar Patel[1*], Jignesh G. Shah [2]

1. Department of Pharmacology, Smt. S. M.Shah College of Pharmacy, Amsaran, Mahemdabad, Gujarat, India.
2. Department of Pharmacology, Indukaka Ipcowala College of Pharmacy, New Vallabh vidyanagar, Anand, Gujarat, India.

Article history:

Received 16 June 2017
Revised 20 Aug 2017
Accepted 30 Aug 2017
Available online 01 Dec 2017

Citation:
Patel R. Shah J. G. Effect of Ethanolic Extract of HORDEUM VULGARE Seed on Calcium Oxalate Deposition by Surgericaly Induced Urolithiasis. **J Pharm Sci Bioscientific Res**. 2017. 7(5):335-340

For Correspondence:
Ravindrakumar K. Patel
Assistant Professor
Department of Pharmacology.
Smt, S. M. Shah College of Pharmacy,
Mobile no - +919979738804
Amsaran, Mahemdabad, Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

The present study was designed to investigate the ameliorating effect of ethanolic extract ofHordeum vulgare seeds in hyperoxaluria and renal cell injury. Materials and Methods: Wistar Albino rats weighing 250-300gm. All groups received regular rat food and drinking water ad libitum. Calcium oxalate crystals were placed in group III, IV, V and group VI by surgery while in group II surgery was performed but calcium oxalate crystal was not placed. Group IV and group V received Ethanolic extract of Hordeum vulgare 250 mg/kg and 500 mg/kg respectively. While Group VI was received standard drug cystone 750 mg/kg. All drugs were given orally from 1st to 14th day. Urinary volume, oxalate, calcium, inorganic phosphate, uric acid, citrate, magnesium, serum calcium, Phosphorus, BUN, uric acid, magnesium, was evaluated. Results: calcium oxalate feeding resulted in hyperoxaluria as well as increased renal excretion of calcium. Supplementation with EHVsignificantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. EHV significantly lowered the urinary excretion and kidney retention levels of oxalate, citrate, phosphate and calcium. Moreover, elevated serum levels of uric acid,BUN and calcium, phosphorus were significantly reduced by the extracts

KEY WORDS: Hordeum vulgare, Hyperoxaluria; Urolithiasis; caicium oxalate.

INTRODUCTION:

Urinary calculi are the third prevalent disorder of the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Lithiasis is a male predominant disorder, with a recurrence rate of 70–80% in males and 47–60% in females. Currently, no allopathic medications are available for urolithiasis, Surgery, lithotripsy, and local calculus disruption using a high-power laser are used to treat calculi. However, these procedures are expensive and recurrence is quite common[1]. As per Ayurveda, the seeds of *Hordeum vulgare Linn.* are reported to be useful in the treatment of a wide range of aliments including urinary stones[2]. However, no scientific data are available to establish the antiurolithiasis property of the seed extract of *H. vulgare Linn.* In the present study, an effort has been made to establish the scientific validity of the antiurolithiasis activity of *Hordeum vulgare* seed extract using calcium oxalate-induced urolithiasis using male Wistar albino rats.

MATERIALS AND METHODS

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/03-2010/04 dated 29.03.2010) 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.

Chemical

Calcium oxalate crystal was prepared by supersaturation reaction. In 300 ml of distilled water, 100 ml calcium chloride solution (0.4mol/l) was added followed by drop by drop addition of 100 ml potassium oxalate (0.4mol/l) solution up to 2 hr with constant stirring at 75 ^{o}C. Then it was maintained at 75 ^{o}C additionally for 5 hr, after which crystals obtained were washed and maintained at 37 ^{o}C for 2 weeks to allow aggregation and crystal growth. After 2 weeks when the material had pasty consistency, it was transferred to a metallic model to a template containing cylinders of 3 mm diameter to obtain small disks of calcium oxalate and was put again to a stove at 37°C for 24hrs. Care was taken that material turn rigid but not brittle. The crystals which were rigid but not brittle were used for implantation

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 ºC for subsequent experiments. The yield of the extract was 4.82 % w/w.

Acute toxicity studies

The acute toxicity was performed as per Organisation of Economic Cooperation and Development (OECD) guideline (no.420) using albino mice of either sex prior to the evaluation of anti-urolithiasis activity. The Ethanolic extract of Hordeum vulgare Seeds (EHV) was tested using graded doses (500, 1000, 2000 and 5000 mg/kg) in mice. Furthermore, the general behavior like changes in awareness, mood, motor activity, posture, motor-coordination, muscle tone and reflexes were mice was recorded continuously for 12 h, and daily for a further 2 weeks for any eventual mortality[3]. The EHV did not show mortality, or any remarkable symptoms of toxicity and or any significant changes in general behavior in mice.

Method:

Healthy male Wistar Albino rats (30 rats) weighing 250-300gm were divided into five groups containing six animals in each. Duration of treatment of standard and test drugs was 14 days. All groups received regular rat food and drinking water ad libitum. Calcium oxalate crystals were placed in group III, IV, V and group VI by surgery while in group II surgery was performed but calcium oxalate crystal was not placed. Treatment was started after 24 hr of surgery. Group IV and group V received Ethanolic extract of *Hordeum vulgare* 250 mg/kg and 500 mg/kg respectively. While Group VI was received standard drug cystone 750 mg/kg. All drugs were given orally from 1st to 14th day. Antibiotics, painkiller medicines and any other medicines were strictly avoided during the study period.

Surgical procedure

Rats were anesthetized using mixture of ketamine (40-87 mg/kg, i.p. or i.m.) and xylazine (5-13 mg/kg, i.p. or i.m.). The bladder was exposed through a suprapubic incision and a CaOx crystal (seed) of <3 mm in diameter was introduced into the bladder. After suturing the bladder, muscle and skin, the animals were kept under 24 hr observations in individual cage. After 24 hours of the surgery pelvic X-rays were taken to evaluate the presence of crystal. After completion of treatment X-rays of animals were taken before treatment but before being sacrificed.

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 14thday, the urine sample of

A: Sham Operated (Before) **B: Sham Operated (After)** **C: Calculi Controlled (before)**

D: Calculi Controlled (After) **E: EHV 250 mg/kg treated (Before)** **F: EHV 250 mg/kg treated (After)**

G: EHV 500 mg/kg treated (Before) H: EHV 500 mg/kg treated (After) I: Cystone treated (Before) J: Cystone treated (After)

Table 1 Effect of Ethanolic extract of Hordeum vulgare Linn. Seeds on various physical parameters in surgically induced renal stone.

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 4^0C Urine was analyzed as previously described for calcium[4], inorganic phosphate[5], oxalate[6], uric acid[8] and citrate[9], magnesium.

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[4], inorganic phosphate[5], urea[7],magnesium, (**Blood Urea Nitrogen)**BUN.

Statistical analysis

Results were expressed as mean ± SEM (Standard Error Mean). 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. **(Figure-1,2,3,4.)**

RESULTS

The X-ray examination of urinary bladder at the beginning and at the end of experimental period was carried out. The x-ray examination of urinary bladder at the end of treatment, there was clearly observed increased in growth of crystals in calculi control animals. Animals treated with the extract and the standard drug showed inhibition of the crystal growth in bladder. This crystal

growth was significantly prevented by the treatment of EHV 500 and standard Cystone (Fig. A-J).

After two weeks of insertion of stone in the bladder, there was significant decreased in body weight of calculi control animals. The weight loss was not observed in sham (calcium oxalate treated animals) operated animals and was significantly prevented by the treatment with standard drug cystone and the EHV 250. Average daily water intake was significantly reduced in the calculi control animals as compare to normal control and sham(calcium oxalate treated animals) operated animals, whereas improvement in water intake was observed with the treatment of standard and test drugs. There was also significant decreased in urine output in calculi control animals but treatment with test as well as standard drugs significantly caused diuresis. Slight shift of urine pH from acidic to alkaline was observed with calculi control animals as compared to normal control animals. The treatment with standard cystone and EHV significantly prevented this change of pH.

Analysis of urine

At the end of study period, the urine of surgically induced urolithiasis control animals showed significantly higher elimination of calcium, oxalate, phosphate, uric acid as compare to normal control animals. There were significantly decreased elimination of citrate and magnesium of calculi control animals as compared to normal control animals. Sham(calcium oxalate treated animals) operated animals showed the similar results to normal control animals. The treatment with standard drug cystone 750 and EHV 500 significantly decreased the elimination of calcium (Fig. 1-A), oxalate (Fig. 1-B), phosphate (Fig. 1-C) and uric acid (Fig. 2-A) when compared with calculi control animals. Whereas, the treatment with standard drug cystone, produced significantly increased the levels of citrate (Fig. 2-B) and magnesium (Fig.2-C). The treatment with EHV 100, 250 and 500 were failed to produce significant effect on such urolithiasis inhibitory parameters.

The kidney stone inducers like calcium, phosphate and uric acid concentration significantly increased in serum of calculi induced rats as compare to normal control rats. Urolithiasis inhibitors like Magnesium concentration decreased significantly in calculi induced rats. The sham operated group of animals showed the parallel effect as compared to normal control rats. On completion of drug therapy, the concentration of kidney stone inducers were significantly reversed by the standard drug Cystone 750 and EHV 500 as compared to calculi induced rats (Fig.3-B & C). The calcium level did not significantly reversed by the EHV at any dose (Fig. 3-A). The concentration of magnesium was not significantly increased by the EHV (Fig. 4-A).

The renal functioning parameters like urea and blood urea nitrogen level were significantly elevated in calculi induced rats. The treatment with standard drug cystone produced significantly reduction in elevated levels of urea and blood urea nitrogen as compared to calculi control animals. Here, the EHV did not produced any significant effects on urea and blood urea nitrogen levels in calculi induced rats as compared to calculi control rats (Fig. 4-B & C).

DISCUSSION

According to Biom et al.[10], in the experimental model of urolithiasis (surgical model), insertion of visceral foreign body i.e. calcium oxalate crystal/seeds in urinary bladder leads to crystal growth after fourteen days in the calculi control animals i.e. urolithiasis with no significant metabolic and systemic alterations. Many factors are involved in the pathogenesis of urolithiasis. The visceral CaOx crystals/seeds acts as a supporting surface, allowing organic and inorganic material to precipitate over the central nidus, thereby mimicking a spontaneous calculus growth. Although the presence of a supersaturated milieu is necessary for precipitating CaOx (present in most calculi) acting as a promoter of crystal formation, this is not enough to form a stone, as urine is normally a supersaturated solution and only some individuals are prone to this disease. In fact, increase of diuresis could reduce supersaturation of the urine with precipitating substances which is normally associated with formation of urinary calculi[11,12]. The growth of the crystals was confirmed by x-ray analysis. With the help of x-rays analysis confirmed the presence of CaOx in main calculi, this suggest that the satellite probably grew on small fragments released from main stone The treatment with test drug *Hordeum vulgare* seeds and standard drug interfered with crystals deposition and substantially modified stone shape. *Hordeum vulgare* seeds also caused significant diuresis and preventing the growth of stone.

Urinary stones were accompanied by a proportional hypertrophy of the urinary bladder smooth musculature.

Such effect indicates increased contraction of the musculature probably to overcome obstruction of the bladder outlet by the formed calculi. Partial obstruction of the urinary bladder outlet leads to a compensatory growth of the detrusor smooth muscle cells, and occurs as a response to the increased intravesical pressure required to empty the bladder [13,14]. In surgery model similar preventive changes were observed in levels of promoters and inhibitors to that of various treatment groups in ethylene glycol and glycolic acid induced urolithiasis model. Kidney function tests also showed significant prevention in damage with the treatment of test drug and standard drugs. This finding raises the possibility for an alternative use of *Hordeum vulgare* seeds, to induce changes in calculi that might aid in elimination and/or dissolution of calculi.Stone inducing treatment caused hypertrophy and extensive CaOx crystal deposition in kidneys of untreated rats accompanied by oxidative damage as reflected from increased levels of markers of oxidative injury: Malondialdehyde (MDA), and diminished level of total protein content and reduced activities of antioxidant enzymes like Superoxide dismutase (SOD) and catalase (CAT) in kidneys. Membrane damage due to lipid peroxidation or the depletion of cellular antioxidant has been suggested to be a predisposing factor for CaOx crystal deposition[15]. lipid peroxidation has been observed to correlate with hyperoxaluria and renal tubular damage, indicating that hyperoxaluria can induce tubular cell injury and that injury may be caused by the production of free radicals in patients with CaOx stones[16]. The significant increase in the lipid peroxidation contents with CaOx stones and hyperoxaluria in the calculi induced rats agree with the findings of previous report. In addition, another study has reported that free radical damaged cells produce a favorable environment for crystal development and that phytic acid prevents CaOx crystallization by its antioxidant properties[17]. Therefore, the antioxidant action of herbal extracts could be of importance in explaining their antilithiatic action, particularly if the formations of these calculi are induced by lesions that have been caused by cytotoxic substances with oxidative capacities [18].

CONCLUSION

Deposition of calcium oxalate crystals in urinary bladder by surgical method in rats showed increase salt deposition, crystal aggregation within fourteen days. At the end of experiment, it was confirmed by X-ray analysis. Ethanolic extract of *hordeum vulgare* seeds as well as standard drug cystone treatment significantly prevented alteration in these parameters.

REFERENCES

1. P rasad K, Sujatha D, Bharathi K. Herbal drugs in urolithiasis – a review. Pharmacognosy Res 2007;1:175-9.
2. Khare CP. Indian medicinal plants - An illustrated dictionary. Heidelberg: Springer Publishers; 2007. p. 314-5.
3. Ghosh MN, Fundamentals of Experimental Pharmacology. In M. N. Ghosh, editor. 4th edition. Hilten & company, Calcutta, 2008; p. 156-157.
4. Caraway WT. Uric acid In. Standard Methods in Clinical Chemistry. 4th ed. D. Selegson editors. New York: Academic Press; 1963. p. 4239.
5. Fossati P, Tarenghi G. & Musitelli C, Enzymatic Colorimetric Method for the Determination of Inorganic Phosphorus in Serum and Urine, J. Clin.Chem. Clin. Biochem 1988 ; 26 :399.
6. Young D. Effect of Preanalytical Variables on clinical Laboratory Tests. 2nd ed. editor. Washington: AACC press; 1997. p. 493-97.
7. Godkar PD, Godkar DB, Text book of medical laboratory technology. 2nd ed., editor. Bhalani publishing home, India; 2003. p. 409-18.
8. Hodgkinson A, Williams A. An improved colorimetric procedure for urine and kidney homogenate. Clinica Chimica Acta 1972; 36:127.
9. Kaur T, Bijarnia RK, Singla SK, Tandon C. In vivo efficacy of Trachyspermum ammi anticalcifying protein in urolithiatic rat model. J of Ethnopharmacology 2009; 126:459-62.
10. Biom MA, Freitas AM,Schor N:the effect of phyllnthus niruri on urinary inhibitors of calcium oxalate crystallization and other factor associated with renal stone formation. BJU International2002; 89:829-34.
11. Fleisch H:Inhibitors and promoters of stone formation. Kidney International 1978; 13:369-71.
12. Smith, C.L., Guay, D.R.P., 1992. Nephrolithiasis. In: Di Piro, J.T., Talbert, R.L., Hayes, P.E., Yee, G.C., Matzke, G.R., Posey, L.M. (Eds.), Pharmacotherapy and Pathophysiology Approach. 2nd Ed. Elsevier, New York, pp. 720-736.
13. Gabella, G., Uvelius, B., 1990. Urinary bladder of rat: fine structure of normal and hypertrophic musculature. Cell Tissue Research 262, 67-79.

14. Levin, R.M., Haugaard, N., Levin, S.S., Buttyan, R., Chen, M.W., Monson, F.C., Wein, A.J., 1995. Bladder function in experimental outlet obstruction: pharmacologic responses to alterations in innervation, energetics, calcium mobilization, and genetics. Advances in Experimental Medicine and Biology 385, 7-19.
15. Selvam, R., Bijikurien, T., 1991. Methionine feeding prevents kidney stone deposition by restoration of free radical mediated changes in experimental rat urolithiasis. Journal of Nutritional Biochemistry 2, 644-651.
16. Huang, H.S., Ma, M.C., Chen, C.F., Chen, J., 2003. Lipid peroxidation and its correlation with urinary levels of oxalate citric acid and osteopontin in patients with renal calcium oxalate stones. Journal of Urology 62, 1123-1128.
17. Grases, F., Garcia'Ferragut, L., Costa'Bauza, A., 1998. Development of calcium oxalate crystals on urolithium: effect of free radicals. Nephron 78, 296-301.
18. Grases, F., Prieto, R.M., Gomila, I., Sanchis, P., Costa'Bauzá, A., 2009. Phytotherapy and renal stones: the role of antioxidants, A pilot study in Wistar rats. Urological Research 37, 35-40.

.