RESEARCH ARTICLE

Effect of Seed Extracts of *Vigna unguiculta* and *Hordeum vulgare* on Kidney Homogenate and Rat Kidney Injury Molecule-1 in Ethylene Glycol and Ammonium Chloride Induced Urolithasis in Rats

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Abstract

The experiment was carried out to study the therapeutic efficacy of aqueous, alcoholic, and biherbal extracts of *Vigna unguiculata* (VU) and *Hordeum vulgare* (HV) in ethylene glycol and ammonium chloride-induced urolithiasis in female Wistar rats. Rats were divided into 14 groups, each of 6 rats, except the lithiatic control group, which consisted of 8 rats. Group I and II served as lithiatic and vehicle control, respectively. In group I and III to XIV urolithiasis were induced by administration of 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride along with drinking water for 28 days. Group II was given 0.5% sodium bicarbonate. After the 28th day, the rats of urolithiatic treatment Groups III to XIV were given aqueous and alcoholic seed extracts of VU and HV @ 200 mg/kg and 300 mg/kg b.wt. orally as either single extract or combination as biherbal extracts (1:1) in 0.5 % sodium bicarbonate for another 35 days using syringe and rat gavage needle. Blood samples were collected twice: on the 28th day of induction of urolithiasis and 63rd day experiment/herbal treatment. Significantly ($p < 0.01$) increased levels of calcium, oxalate, phosphate, and decreased levels of magnesium in the kidney homogenate were observed in the calculi induced groups as compared to the vehicle control group on 28th day. However, significantly increased rat Kidney Injury Molecule-1 (KIM-1) was observed in the calculi induced groups as compared to the vehicle control group in serum on the 28th day. Results of kidney homogenate and KIM-1 revealed that aqueous and alcoholic extracts of VU and HV possess good therapeutic efficacy against urolithiasis. The effect of biherbal alcoholic extract of the seeds at higher dose rate was much better in reducing or normalizing the values of most traits by 35 days of treatment, i.e., by 63rd day of the experiment and thus the continuation of treatment for some more days would be expected to restore the normal profile.

Keywords: Biherbal extract, Ethylene glycol, *Hordeum vulgare,* Urolithiasis, *Vigna unguiculata,* Wistar rat.

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Introduction

Urolithiasis refers to the presence of solid non-metallic minerals in the urinary tract. Among the several types of kidney stones, the most common is calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract (Purnima *et al.,* 2010). All over the globe, a large number of people are suffering from the urinary stone problem. The occurrence in some areas is so alarming that they are known as 'Stone Belts' (Chauhan *et al.,* 2009). The rate of occurrence is three times higher in men than women (Butterweck and Khan, 2009; Joy *et al.,* 2012). Various medicinal plants with diuretic activities exert inhibitory effects on crystallization, nucleation, and aggregation of crystals, making them useful for the treatment of urolithiasis (Nirumand *et al.,* 2018). A number of medicinal plants show antiurolthiatic activity and play a vital role in the prevention of disease (Tiwari *et al.,* 2012; Saha and Verma, 2015). As per Ayurveda, the seeds of *Hordeum vulgare* Linn. are reported to be useful in the treatment of a wide range of ailments, including urinary stones (Shah *et al.,* 2012[a]). The use of seed extract of *Hordeum vulgare* Linn to experimentally CaOx-induced nephrolithiasis rats reduced the deposition of crystals into kidneys, confirming it's antilithiaticeffect (Shah *et*

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al., 2012[b]). Hence this study was aimed to evaluate the effect of seed extracts of *Vigna unguiculta* and *Hordeum vulgare* on kidney homogenate and rat kidney injury molecule-1 in ethylene glycol and ammonium chloride-induced urolithasis in female rats.

Materials And Methods

Experimental Design

The project was approved by the Institutional Animals Ethics Committee (IAEC) of the College (Approval No: AAU/GVC/ CPCSEA/IAEC/ 264/2017 dated 03/04/2017). The work was carried out from November 2017 to April 2018 on 86 healthy mature (12-15 weeks) female Wistar rats. For induction of urolithiasis, ethylene glycol (EG) and ammonium chloride (AC) were used. Rats were divided into 14 groups, each of 6 rats, except lithiatic control group which consisted of 8 rats. Each group was then kept in separate cage. Group I and II served as lithiatic and vehicle control, respectively. In group I and III to XIV urolithiasis was induced by administration of 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride along with drinking water for 28 days. Group II was given 0.5% sodium bicarbonate. After 28th day, the rats of urolithiatic treatment Groups III to XIV were given aqueous and alcoholic seed extracts of VU and HV @ 200 mg/kg and 300 mg/kg b.wt. Orally as either single extract or combination as biherbal extracts in equal proportion (1:1) in 0.5% sodium bicarbonate solution as explained under therapeutic effect using syringe and rat gavage needle for next 35 days, *i.e.,* up to 63 days of the experiment.

Preparation of Plant Extracts

The dried seeds of HV were procured from Main Forage Research Station (MFRS), AAU, Anand, and seeds of VU were purchased commercially from the local market of Vadodara, Gujarat. The air-dried seeds were powdered by the mechanical grinder and stored in airtight containers. Exactly 100 g of coarse powdered seed material of both the plants was extracted with water and also with alcohol in the soxhlet apparatus for 24 hours. The extract was evaporated under reduced pressure to give solid residue. The aqueous and alcoholic extracts (solid residues) were preserved in a refrigerator at 4°C for a subsequent experiment.

Therapeutic studies

After 28 days of induction of urolithiasis, the rats of group III and IV were given aqueous extract of VU @ 200 mg/kg, and 300 mg/kg, group V and VI with alcoholic extract of VU @ 200 mg /kg and 300 mg/kg, group VII and VIII with aqueous extract of HV @ 200 mg/kg and 300 mg/kg, and group IX and X were given alcoholic extract of HV @ 200 mg/kg and 300 mg/kg, respectively. The rats of group XI and XII were given aqueous biherbal extract of VU + HV (1:1) 200 mg/kg (*i.e.* 100+100 mg) and 300 mg/kg (*i.e.* 150+150 mg/kg), and those of group XIII and XIV were given alcoholic biherbal extract of VU + HV (1:1) 200 mg/kg and 300 mg/kg for another 35 days, respectively, in 0.5% sodium bicarbonate solution. Dose of extract was calculated according to body weight, it was weighed and dissolved in 0.5% sodium bicarbonate solution and was administered by oral route using sterile 1 mL syringe with oral rat gavage needle.

Kidney Homogenate Analysis

On 63rd day the abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were rinsed in an ice-cold normal saline solution after the extraneous tissue was removed. The left kidney was preserved in 10 % formalin. The right kidney was dried at 80° C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was separated. From the homogenate, calcium and phosphate were estimated as per Medeiros and Mustafa (1985) and Fiske and Subbarow (1925), respectively, while magnesium was estimated by Calmagite method using standard assay kits (Coral Clinical, Goa) with the help of *Visiscan* 167 Spectrophotometer. The homogenate oxalate was estimated by Chen *et al.* (2011).

Determination of Rat Kidney Molecule (KIM)-1

Blood samples were collected twice: after 28th day of induction of urolithiasis and then on 63rd day (after treatment) of the experimental period from all the rats by retro-orbital plexuses puncture under mild diethyl ether anesthesia with the help of a capillary tube. Serum was harvested by centrifugation at 3000 rpm for 15 minutes at 10°C (Eppendorf 5804 R, Germany) and stored -40°C for determination of Rat KIM-1 with the help of Rat KIM-1 ELISA kit (Elabscience Biotechnology Co., Ltd) as per the instructions of the manufacturer.

Statistical Analysis

Paired 't' test was used to compare kidney homogenate and Rat KIM-1 parameters before and after treatment, while a one-way analysis of variance (ANOVA) was used to compare the effects of V.U. and H.V. extracts with the vehicle control group, ethylene glycol model group, and groups were given plant extracts on different variables using software SPSS (Version 20). All the data have been presented as mean ± SE (Snedecor and Cochran, 1990).

Results And Discussion

The results of renal function evaluated by determination of rat KIM-1 and kidney homogenate in group I to XIV with induced urolithiasis and after 35 days of herbal therapy are presented in Tables 1 and 2.

KIM-1 is a type I trans-membrane structural glycoprotein located in the renal proximal tubule epithelial cells. These

Table 1: Changes in Rat KIM-1 concentration in different groups before and after treatment of induced urolithiatic rats (Mean ± SE)

		Rat KIM-1 concentration (pg/ml)		
Group No	*Group Name*	*on day 28th*	*on day 63rd*	*Percent Change*
I	Lithiatic Control	1141.42 ± 6.98	1189.24^{j}± 2.38**	+4.21
II	Vehicle Control	88.20 ± 2.41	91.63^{a}± 2.02	+3.89
III	AQ. EX. VU 200 mg/kg	1207.40 ± 6.83	1020.31^{i}±5.61**	-15.49
IV	AQ. EX. VU300 mg/kg	1173.43 ± 5.32	950.10^{h}± 7.76**	-19.03
V	AL. EX. VU 200 mg/kg	1136.78 ± 8.97	939.03^{h}± 8.45**	-17.40
VI	AL. EX. VU 300 mg/kg	1141.10 ± 7.36	846.82^{e}± 5.29**	-25.78
VII	AQ. EX. HV 200 mg/kg	1112.52 ± 3.71	912.04^{g}± 4.53**	-18.02
VIII	AQ. EX. HV 300 mg/kg	1063.30 ± 5.45	883.56^{f}± 8.78**	-16.90
IX	AL. EX. HV 200 mg/kg	1085.37 ± 6.62	796.19^{d}± 4.66**	-26.64
X	AL. EX. HV 300 mg/kg	1110.76 ± 6.04	793.68^{d}± 7.94**	-28.55
XI	BIH.AQ.EX. (VU+HV) 200 mg/kg	1103.37 ± 4.80	798.61^{d}± 3.53**	-27.62
XII	BIH.AQ.EX. (VU+HV) 300 mg/kg	1029.34 ± 5.41	705.13^{b}± 6.01**	31.50
XIII	BIH.AL.EX. (VU+HV) 200 mg/kg	1140.70 ± 6.87	764.97^{c}± 4.85**	-32.94
XIV	BIH.AL.EX. (VU+HV) 300 mg/kg	1112.24 ± 2.93	711.95^{b}± 4.09**	-35.99

*(p < 0.05), **(p < 0.01), VU = *Vigna Unguiculata,* HV = *Hordeum Vulgare*
Means with different superscript differ significantly (p < 0.05).

Table 2: Comparison of different herbal treatments on calcium, phosphate, oxalate, and magnesium levels in kidney homogenate on 63rd day in induced urolithiatic rats.

Group no	*Group Name*	*Calcium (mg/dL)*	*Phosphate (mg/dL)*	*Oxalate (mg/dL)*	*Magnesium (mg/dL)*
I	Lithiatic Control	7.18^{f} ± 0.13	4.77^{d} ± 0.14	5.01^{e} ± 0.30	1.76^{e} ± 0.06
II	Vehicle Control	4.04^{a} ± 0.13	2.88^{a} ± 0.15	2.56^{a} ± 0.32	3.01^{a} ± 0.04
III	AQ. EX. VU 200 mg/kg	6.58^{e} ± 0.16	4.35^{cd} ± 0.17	4.36^{de} ± 0.19	2.13^{de} ± 0.06
IV	AQ. EX. VU 300 mg/kg	6.04^{cde} ± 0.10	4.04^{bc} ± 0.07	4.01^{cd} ± 0.33	2.23^{cd} ± 0.05
V	AL. EX. VU 200 mg/kg	6.41^{de} ± 0.16	4.08^{bc} ± 0.11	4.07^{cd} ± 0.11	2.34^{cd} ± 0.06
VI	AL. EX. VU 300 mg/kg	5.94^{cde} ± 0.10	3.86^{bc} ± 0.12	3.85^{cd} ± 0.33	2.63^{cd} ± 0.08
VII	AQ. EX. HV 200 mg/kg	6.55^{e} ± 0.05	4.31^{bcd} ±0.29	3.89^{cd} ± 0.13	2.18^{cd} ± 0.06
VIII	AQ. EX. HV 300 mg/kg	6.02^{cde} ± 0.06	3.94^{bc} ± 0.18	3.73^{bcd} ± 0.23	2.40^{bcd} ± 0.04
IX	AL. EX. HV 200 mg/kg	6.18^{cde} ± 0.14	4.00^{bc} ± 0.33	3.75^{bcd} ± 0.14	2.32^{bcd} ± 0.07
X	AL. EX. HV 300 mg/kg	5.87^{cd} ± 0.11	3.73^{bc} ± 0.20	3.54^{bc} ± 0.17	2.37^{bc} ± 0.06
XI	BIH.AQ.EX. (VU+HV) 200 mg/kg	6.12^{cde} ± 0.13	4.26^{bcd} ±0.29	3.71^{bcd} ± 0.22	2.32^{bcd} ± 0.01
XII	BIH.AQ.EX. (VU+HV) 300 mg/kg	5.62^{c} ± 0.24	3.81^{bc} ± 0.13	3.44^{bc} ± 0.28	2.51^{bc} ± 0.01
XIII	BIH.AL.EX. (VU+HV) 200 mg/kg	4.95^{b} ± 0.44	3.95^{bc} ± 0.20	3.01^{ab} ± 0.13	2.57^{ab} ± 0.01
XIV	BIH.AL.EX. (VU+HV) 300 mg/kg	4.84^{b} ± 0.39	3.70^{b} ± 0.15	2.57^{a} ± 0.20	2.90^{a} ± 0.06

Means with different superscript differ significantly (p < 0.05).

cells undergo regeneration after various forms of injury and shed KIM-1 antigen into the serum. Thus KIM-1 is an early and specific biomarker for tubular kidney injury. In the present study, an increased level of Kidney Injury Molecule was observed in urolithiasis induced groups as compared to the vehicle control group on 28th day (Table

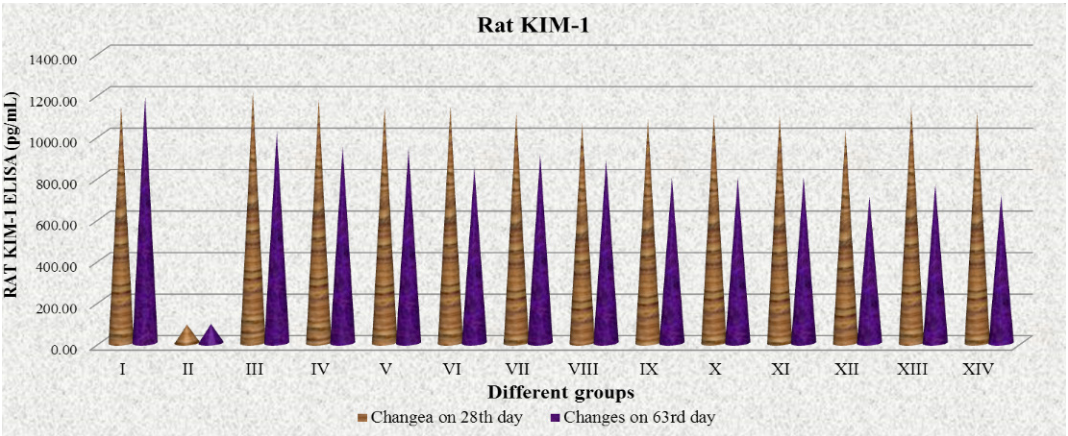

Fig. 1: Changes in rat KIM-1 concentration in different groups before and after herbal treatment

1, Fig. 1). However, the single extract or the co-treatment with aqueous and alcoholic extracts of VU and HV for 35 days decreased KIM1 concentration by 15 to 36%, being more significant with a higher dose, and the decrease was statistically highly significant by 63rd day of the experiment in all the lithiatic groups. Biherbal alcoholic extract compared to mono-herbal extract of the said seeds was much better in reducing the values of KIM-1 by 63rd day in comparison to 28th day of the experiment. Alcoholic extract was more effective in all groups as compared to aqueous extract. Patel (2018) stated that rat KIM-1 is a specific biomarker for tubular kidney injury. Kidney injury molecules were increased in lithiatic groups in comparison to the normal group. At the same time, KIMs were decreased due to treatment given in these groups with aqueous, alcoholic, and biherbal extracts of *Brophyllum calycinum* and *Solanum xanthocarpum*, which have therapeutic potential against urolithiasis. The present results were in agreement with the findings of Patel (2018).

In calculi induced groups, a significant increase in calcium, phosphate, oxalate, and decreased magnesium levels in kidney homogenate were observed on 63rd day in comparison to the vehicle control group (Table 2). The differences were the greatest between lithiatic control and vehicle control groups. Further, either the single extract or the co-treatment with biherbal aqueous and alcoholic extracts of VU and HV also significantly decreased these parameters by 63rd day of the experiment in all the groups compared to vehicle control and even lithiatic control group. Biherbal alcoholic extract compared to mono-herbal extract of the said seeds was much better in decreasing the values of kidney calcium, phosphate, oxalate, and magnesium. The oxalate and magnesium contents of kidney homogenates were reduced and restored to near normal/vehicle control group by the 63rd day of biherbal alcoholic groups (Table 2). The trend of observations shows that the continuation of treatment for some more days would be expected to restore the normal profile of macro-minerals and oxalate in the kidneys of induced lithiatic rats.

Goyal *et al.* (2018) reported calcium and phosphate to be stone promoting factors. In the present study, ethylene glycol and ammonium chloride significantly elevated the calcium level in the kidney. Hypercalciuria favored the nucleation and precipitation of CaOx in urine that leads to crystal growth. Patel (2018) found that treatment with *Bryophyllum calycinum* and *Solanum xanthocarpum* restored oxalate in urine and kidney homogenate. The reduction in oxalate excretion was observed on extract treatment. This decreased excretion of oxalate may be due to the inhibition of some steps of oxalate synthesis from ethylene glycol by the plant extract. Das and Malipeddi (2016) observed decreased magnesium levels in the renal tissues of the stone induced groups. Magnesium is considered as a potent inhibitor of CaOx crystals because it decreases supersaturation and metabolic acidosis.

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