

Original Research Article

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## Nephroprotective Effect of Herbal Seed Extracts of *Vigna unguiculata* and *Hordeum vulgare* on Serum Biochemical Changes on Ethylene Glycol and Ammonium Chloride Induced Urolithiasis in Female Wistar Rats

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### ABSTRACT

The experimental work was undertaken to study the therapeutic efficacy of aqueous, alcoholic and biherbal extracts of *Vigna unguiculata* (V.U.) and *Hordeum vulgare* (H.V.) on ethyleneglycol and ammonium chloride induced urolithiasis in female wistar rats. Rats were divided into 4 groups. Each of 6 rats except lithiatic control group consist 8 rats. Group I and II served as lithiatic and vehicle control, respectively. In group I and III to XIV induction of urolithiasis was done 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 28<sup>th</sup> day, the rats of urolithiatic treatment Groups III to XIV were given aqueous and alcoholic seed extract of V.U. and H.V. @ 200 mg/kg and 300 mg/kg bwt orally as either single extracts or combination as biherbal extracts (1:1) in 0.5 % sodium bicarbonate using syringe and rat gavage needle. Blood samples were collected. Confirmation of urolithiasis was done by evaluating serum biochemical parameters. However, increase level of serum glutamic-pyruvic transaminase, Blood Urea Nitrogen, uric acid, creatinine, calcium and phosphorus, while decreased level of serum total protein and serum magnesium were observed in urolithiatic groups as compared to vehical control group on 28<sup>th</sup> day. Results of serum biochemistry reveals aqueous and alcoholic extract of V.U. and H.V. possess good therapeutic efficacy against urolithiasis. The effect of biherbal alcoholic extract of the seeds was much better in restoring the values on 63<sup>rd</sup> day after treatment.

#### Keywords

Biherbal extract, *Vigna unguiculata*, *Hordeum vulgare*, Ethylene glycol, Ammonium chloride, Sodium bicarbonate, Urolithiasis, Wistar rat

#### Article Info

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### Introduction

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are 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

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 treatment of urolithiasis (Nirumand *et al.*, 2018). The medical management of lithiasis, today, includes extracorporeal shock wave lithotripsy (ESWL) and surgical procedures which depends on the size and location of stones (Saha and Verma, 2015). Number of medicinal plants shows antiurolithiatic activity and play vital role in prevention of disease (Tiwari *et al.*, 2012).

Horsegram (*Vigna unguiculata*) supposed to have unique property of dissolving kidney stones, therefore, in many parts of the country it is given to prevent or cure urinary stones (Singla and Kumar, 1985). Medicinal use of *Hordeum vulgare* (Jav) as a diuretic. 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). Seed extract of *Hordeum vulgare* Linn. To experimentally CaOx-induced nephrolithiasic rats reduced the deposition of crystals into kidneys confirming it antilithiasic effect (Shah *et al.*, 2012).

## Materials and Methods

### Ethical approval

The project was approved by the Institutional Animals Ethics Committee (IAEC) of College of Veterinary Science and Animal Husbandry, Anand (Approval No: AAU/GVC/CPCSEA/IAEC/ 264/2017 dated 03/04/2017).

### Experimental design

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 in to 14 groups. Each of 6 rats except lithiatic control group consist 8 rats and were kept in separate cages. Group I and II served as lithiatic and vehicle control, respectively.

In group I and III to XIV induction of urolithiasis was done 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 28<sup>th</sup> day, the rats of urolithiatic treatment Groups III, VI, V, VI, VII, VIII, IX, X, XI, XII, XIII and XIV were given aqueous and alcoholic seed extract of V.U. and H.V. @ 200 mg/kg and 300 mg/kg bwt orally as either single extracts or combination as biherbal extracts (1:1) in 0.5% sodium bicarbonate using syringe and rat gavage needle.

### Preparation of plant extracts

The dried Seeds of H.V. were procured from Regional Research Centre (RRC) - Anubhav seeds, AAU, Anand and seeds of V.U. were purchased commercially from the local market of Vadodara, Gujarat. The air-dried seeds were powdered by mechanical grinder and stored in air tight containers. Exactly 100g of coarse powdered material of both the plants were successfully extracted with water and also with alcohol in soxhlet apparatus for 24 hours.

The extract was evaporated under reduced pressure to give solid residue. The aqueous and alcoholic extracts were preserved in refrigerator at 4° C for subsequent experiment.

## Studies of therapeutic effect

After 28 days of induction of urolithiasis group III and IV was given aqueous extract of V.U. 200 mg/kg and 300 mg/kg, group V and VI was given alcoholic extract of V.U. 200 mg/kg and 300 mg/kg, group VII and VIII was given aqueous extract of H.V. 200 mg/kg and 300 mg/kg, group IX and X was given alcoholic extract of H.V. 200 mg/kg and 300 mg/kg, group XI and XII was given aqueous biherbal extract V.U. + H.V. (1:1) 200 mg/kg and 300 mg/kg, group XIII and XIV was given alcoholic biherbal extract of V.U. + H.V.(1:1)200 mg/kg and 300 mg/kg for another 35 days. Dose was administered by oral route using sterile 1ml syringe with oral rat gavage needle. Dose was calculated according to body weight.

## Blood collection and analysis of serum biochemical

Blood samples were collected thrice: first 0 day, after 28<sup>th</sup> days of induction of urolithiasis and then on 63<sup>rd</sup> day (after treatment) of experimental period. Blood samples were collected from all the rats by retro-orbital plexuses puncture under mild diethyl ether anaesthesia with the help of capillary tube.

Serum was harvested by centrifugation at 3000 rpm for 15 minutes at 10° C (Eppendorf 5804 R, Germany) and stored - 40° C used for biochemical analysis using standard procedure and assay kits (Coral Clinical, Goa, India) with the help of *Visiscan 167* Spectrophotometer.

## Parameter studied

The biochemical parameters viz. serum glutamic-pyruvic transaminase (SGPT), Blood Urea Nitrogen (BUN), uric acid, creatinine, calcium and phosphorus, total protein (TP), Magnesium were studied during the study.

## Statistical analysis

Paired T-test was used to compare serology parameters before and after treatment, while One-way-analysis of variance (ANOVA) was used to compare the effects of V.U. and H.V. extracts with vehical control group, ethyleneglycol model group and group given plant extract on different variables using software SPSS (Version 20). All the data have been presented as mean  $\pm$  SE (Snedecor and Cochran, 1990).

## Results and Discussion

Renal function was evaluated by measuring serum total protein, SGPT (serum glutamic-pyruvic transaminase), BUN (Blood Urea Nitrogen), uric acid, creatinine, calcium, phosphorus and magnesium in group I to XIV (Table 1 and 2) (Figure 1, 2, 3, 4, 5, 6, 7 and 8).

In present study, decreased the level of serum Total protein and increased level of serum SGPT, BUN and Uric acid were observed in the urolithiasis induced group as compared to the 0 day (when experiment was started) and vehicle control group by day 28 of treatment (Table 1) (Figure 1, 2, 3 and 4). However the single extract or the co-treatment with aqueous and alcoholic extracts of V.U. and H.V.for 28 day significantly restored these changes, i.e., by 63<sup>rd</sup> day of experiment in most of the groups. Biherbal alcoholic extract compared to mono-herbal extract of the said seeds was much better in restoring the values of serum Total protein, SGPT, BUN and Uric acid and serum SGPT and Total protein values were came down nearer to vehicle control group by 63<sup>rd</sup> day.

Patel (2018) stated that significant decreased in total protein level following administration of EG + AC while significant increased following administration of extracts of

*Bryophyllum calycinum* and *Solanum xanthocarpum*. Vasanthi, *et al.*, (2017) stated that Ethylene glycol induction induces renal cellular damage. Renal injury was also evidenced by the increased activities of SGPT. This might be due to the leakage of these enzymes into the general circulation from the collateral circulation. Administration of cystone or plant extract at all doses prevented the leakage of these marker enzymes and maintained its level in comparison to lithiatic

rats. Lakshmi *et al.*, (2014) stated that in ethylene glycol and ammonium chloride induced rats, significant raise in uric acid and BUN were observed in serum, because of decreased glomerular filtration rate due to obstruction in the urine flow in urinary system with the deposition of calcium oxalate in renal tubules. *Trianthema portulacasrum* Linn. and *Gymnema sylvestre* showed decrease in uric acid and BUN level.

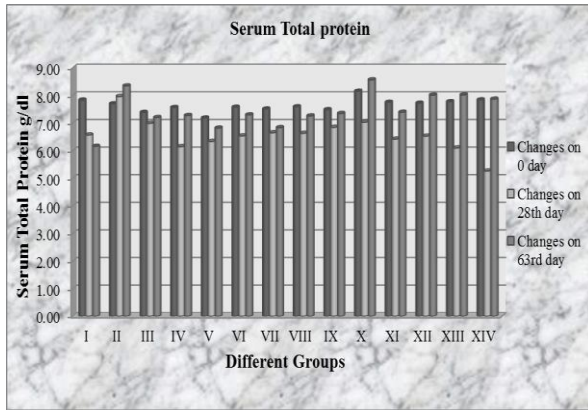


Figure 1: Changes in Serum Total protein (g/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day

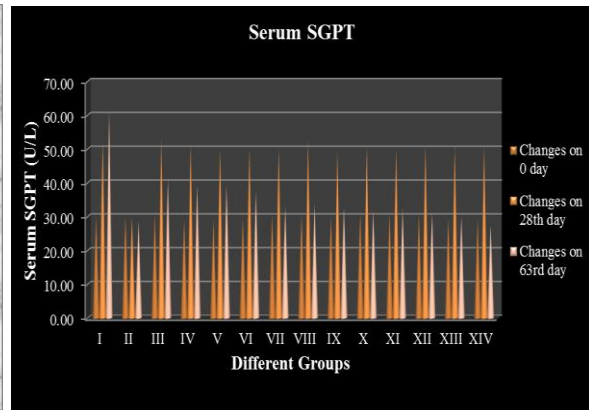


Figure2: Changes in Serum SGPT (U/L) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day

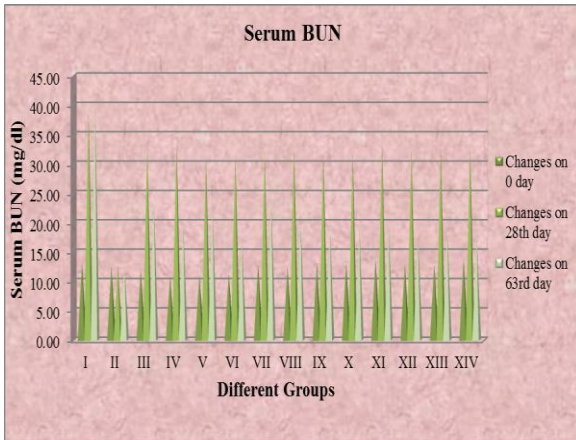


Figure3: Changes in Serum BUN (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day

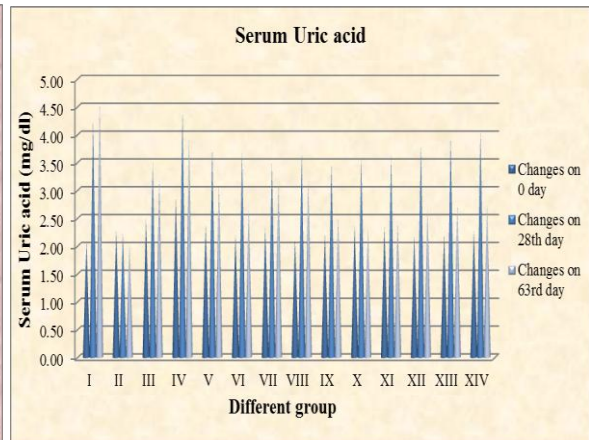
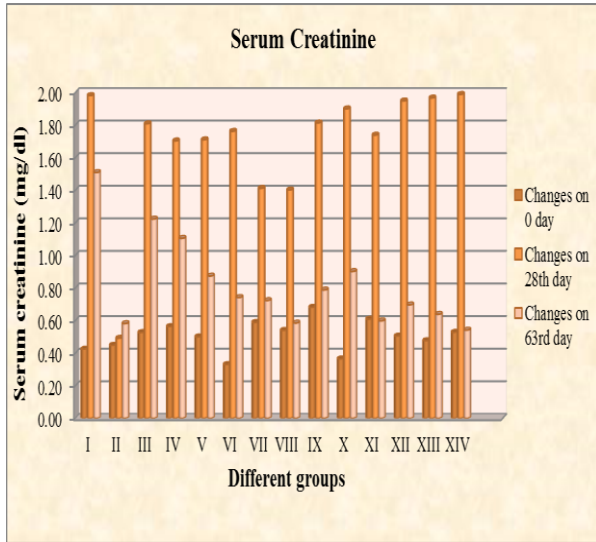
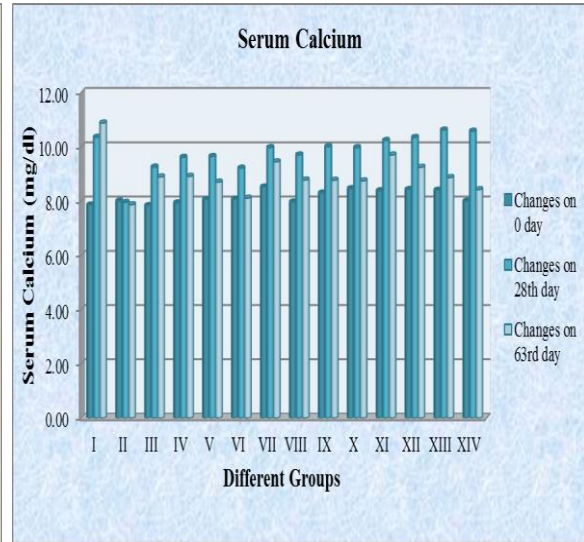


Figure4: Changes in Serum Uric acid (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day

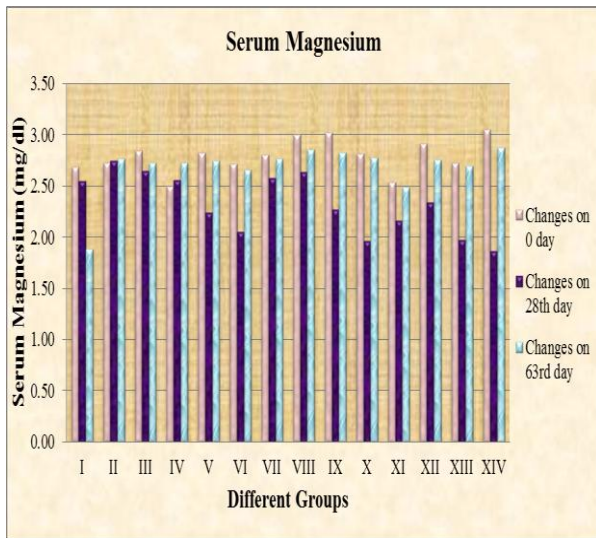




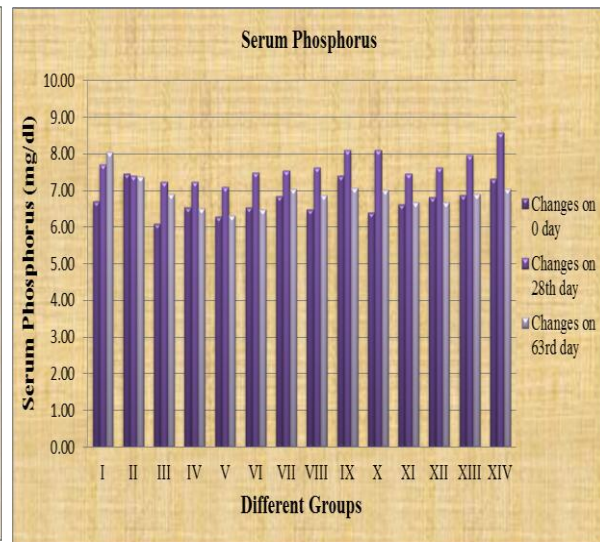
**Figure5:** Changes in Serum Creatinine (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day



**Figure6:** Changes in Serum Calcium (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day



**Figure7:** Changes in Serum Magnesium (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day



**Figure 8:** Changes in Serum Phosphorus (mg/dl) concentration in different group on 0, 28<sup>th</sup> and 63<sup>rd</sup> day

**Table.1** Changes in serum Total protein, serum SGPT (serum glutamic-pyruvic transaminase), serum BUN (blood urea nitrogen) and serum Uric acid concentration on day 0, 28<sup>th</sup> and 63<sup>rd</sup>

Group No	Group Name	Total protein (g/dl)			SGPT (U/L)			BUN			Uric acid		
		0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day
I	Lithiatic Control	29.99 ± 0.59	52.40 ± 0.80**	61.41 <sup>g</sup> ± 0.51**	29.99 ± 0.59	52.40 ± 0.80**	61.41 <sup>g</sup> ± 0.51**	13.26 ± 1.20	38.99 ± 0.48**	40.53 <sup>f</sup> ± 1.20	2.07 ± 0.07	4.28 ± 0.25**	4.61 <sup>d</sup> ± 0.21
II	Vehicle Control	30.17 ± 0.36	29.68 ± 0.62	28.61 <sup>ab</sup> ± 0.64	30.17 ± 0.36	29.68 ± 0.62	28.61 <sup>ab</sup> ± 0.64	12.67 ± 1.06	12.75 ± 0.98	12.69 <sup>a</sup> ± 1.10	2.28 ± 0.24	2.23 ± 0.25	2.03 <sup>a</sup> ± 0.12
III	AQ. EX. V.U. 200mg/kg	30.67 ± 0.23	52.81 ± 1.51**	41.06 <sup>f</sup> ± 0.28*	30.67 ± 0.23	52.81 ± 1.51**	41.06 <sup>f</sup> ± 0.28*	12.07 ± 1.17	32.73 ± 0.69**	23.28 <sup>de</sup> ± 0.97**	2.50 ± 0.47	3.50 ± 0.45	3.25 <sup>bc</sup> ± 0.36
IV	AQ. EX. V.U.300mg/kg	29.06 ± 0.25	51.33 ± 0.49**	39.27 <sup>ef</sup> ± 0.87*	29.06 ± 0.25	51.33 ± 0.49**	39.27 <sup>ef</sup> ± 0.87*	10.93 ± 0.31	34.00 ± 0.85**	19.76 <sup>bcde</sup> ± 1.14**	2.88 ± 0.45	4.42 ± 0.50*	3.94 <sup>cd</sup> ± 0.64*
V	AL. EX. V.U. 200mg/kg	29.07 ± 0.34	50.28 ± 0.39**	39.43 <sup>ef</sup> ± 2.27*	29.07 ± 0.34	50.28 ± 0.39**	39.43 <sup>ef</sup> ± 2.27*	11.08 ± 0.76	31.32 ± 0.42**	22.21 <sup>cde</sup> ± 1.56**	2.39 ± 0.17	3.76 ± 0.33**	3.14 <sup>bc</sup> ± 0.41
VI	AL. EX. V.U. 300mg/kg	29.52 ± 0.56	50.48 ± 1.06**	37.55 <sup>e</sup> ± 0.61**	29.52 ± 0.56	50.48 ± 1.06**	37.55 <sup>e</sup> ± 0.61**	11.42 ± 0.74	31.54 ± 0.38**	16.86 <sup>b</sup> ± 1.62**	2.21 ± 0.18	3.69 ± 0.31**	2.68 <sup>ab</sup> ± 0.38**
VII	AQ. EX. H.V. 200mg/kg	30.64 ± 0.24	50.08 ± 0.57**	32.90 <sup>d</sup> ± 0.77**	30.64 ± 0.24	50.08 ± 0.57**	32.90 <sup>d</sup> ± 0.77**	13.03 ± 0.64	31.71 ± 0.22**	23.80 <sup>e</sup> ± 1.31**	2.33 ± 0.21	3.50 ± 0.33**	3.20 <sup>bc</sup> ± 0.39*
VIII	AQ. EX. H.V. 300mg/kg	30.58 ± 0.45	52.17 ± 0.80**	33.67 <sup>d</sup> ± 0.25**	30.58 ± 0.45	52.17 ± 0.80**	33.67 <sup>d</sup> ± 0.25**	12.46 ± 0.96	33.12 ± 0.54**	20.45 <sup>bcde</sup> ± 2.34**	2.12 ± 0.27	3.67 ± 0.28**	3.21 <sup>bc</sup> ± 0.27*
IX	AL. EX. H.V. 200mg/kg	29.99 ± 0.78	49.23 ± 1.15**	32.81 <sup>d</sup> ± 1.02**	29.99 ± 0.78	49.23 ± 1.15**	32.81 <sup>d</sup> ± 1.02**	13.17 ± 0.47	31.81 ± 0.45**	18.89 <sup>bcd</sup> ± 0.81**	2.23 ± 0.23	3.51 ± 0.33**	2.52 <sup>ab</sup> ± 0.15*
X	AL. EX. H.V. 300mg/kg	31.15 ± 0.25	51.01 ± 0.75**	31.91 <sup>cd</sup> ± 0.61**	31.15 ± 0.25	51.01 ± 0.75**	31.91 <sup>cd</sup> ± 0.61**	13.33 ± 0.22	32.07 ± 0.23**	19.06 <sup>bcd</sup> ± 2.14**	2.37 ± 0.22	3.59 ± 0.36*	2.32 <sup>ab</sup> ± 0.20*
XI	BIH.AQ.EX. (V.U.+H.V.) 200mg/kg	30.93 ± 0.31	50.17 ± 0.69**	32.40 <sup>cd</sup> ± 0.71**	30.93 ± 0.31	50.17 ± 0.69**	32.40 <sup>cd</sup> ± 0.71**	13.98 ± 0.21	33.52 ± 0.16**	19.81 <sup>bcde</sup> ± 1.49**	2.35 ± 0.26	3.62 ± 0.41*	2.46 <sup>ab</sup> ± 0.34**
XII	BIH.AQ.EX. (V.U.+H.V.) 300mg/kg	30.92 ± 0.84	51.00 ± 0.82**	31.06 <sup>bcd</sup> ± 0.39**	30.92 ± 0.84	51.00 ± 0.82**	31.06 <sup>bcd</sup> ± 0.39**	12.99 ± 0.25	32.26 ± 0.23**	18.13 <sup>bc</sup> ± 1.07**	2.18 ± 0.33	3.82 ± 0.25**	2.64 <sup>ab</sup> ± 0.33**
XIII	BIH.AL.EX.(V.U.+H.V.) 200mg/kg	29.51 ± 0.64	50.57 ± 1.08**	30.08 <sup>bc</sup> ± 0.68**	29.51 ± 0.64	50.57 ± 1.08**	30.08 <sup>bc</sup> ± 0.68**	12.93 ± 0.20	32.95 ± 0.21**	18.35 <sup>bc</sup> ± 0.81**	2.21 ± 0.20	3.97 ± 0.09**	2.75 <sup>ab</sup> ± 0.21**
XIV	BIH.AL.EX. (V.U.+H.V.) 300mg/kg	29.05 ± 0.62	50.62 ± 0.83**	27.48 <sup>a</sup> ± 0.77**	29.05 ± 0.62	50.62 ± 0.83**	27.48 <sup>a</sup> ± 0.77**	13.87 ± 0.25	33.10 ± 0.39**	17.62 <sup>bc</sup> ± 1.07**	2.28 ± 0.40	4.06 ± 0.20**	2.75 <sup>ab</sup> ± 0.15**

AQ. EX/AL. EX. = aqueous/alcoholic extract, V.U./H.V. = *Vigna unguiculata* and *Hordeum vulgares* ; BIH = Biherbal

\*p<0.05, \*\*p<0.01 between days, (Means with different superscript differ significantly)

**Table.2** Changes in serum creatinine, serum calcium, serum magnesium and serum phosphorus concentration on day 0, 28<sup>th</sup> and 63<sup>rd</sup>

Group No	Group Name	Creatinine			Calcium			Magnesium			Phosphorus		
		0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day	0 day	28 <sup>th</sup> day	63 <sup>rd</sup> day
I	Lithiatic Control	0.43 ± 0.08	1.98 ± 0.06**	1.51 <sup>d</sup> ± 0.21*	7.86 ± 0.21	10.36 ± 0.18**	10.88 <sup>f</sup> ± 0.22	2.68 ± 0.10	2.55 ± 0.12	1.88 <sup>a</sup> ± 0.10**	6.70 ± 0.30	7.71 ± 0.41**	8.05 <sup>b</sup> ± 0.37**
II	Vehicle Control	0.45 ± 0.08	0.49 ± 0.08	0.58 <sup>a</sup> ± 0.06	8.01 ± 0.15	7.96 ± 0.05	7.87 <sup>a</sup> ± 0.13	2.73 ± 0.23	2.75 ± 0.09	2.76 <sup>bc</sup> ± 0.07	7.45 ± 0.31	7.42 ± 0.24	7.39 <sup>ab</sup> ± 0.25
III	AQ. EX. V.U. 200mg/kg	0.53 ± 0.07	1.81 ± 0.19**	1.22 <sup>cd</sup> ± 0.10**	7.84 ± 0.25	9.26 ± 0.55*	8.90 <sup>bcd</sup> ± 0.51	2.85 ± 0.10	2.65 ± 0.05	2.73 <sup>bc</sup> ± 0.04	6.10 ± 0.32	7.25 ± 0.45*	6.90 <sup>a</sup> ± 0.40*
IV	AQ. EX. V.U.300mg/kg	0.56 ± 0.10	1.70 ± 0.10**	1.11 <sup>bcd</sup> ± 0.03**	7.94 ± 0.16	9.62 ± 0.46*	8.92 <sup>bcd</sup> ± 0.47**	2.50 ± 0.20	2.56 ± 0.09	2.73 <sup>bc</sup> ± 0.13	6.55 ± 0.49	7.24 ± 0.46**	6.51 <sup>a</sup> ± 0.24
V	AL. EX. V.U. 200mg/kg	0.50 ± 0.08	1.71 ± 0.11**	0.87 <sup>abc</sup> ± 0.10**	8.06 ± 0.12	9.65 ± 0.23**	8.71 <sup>abcd</sup> ± 0.15**	2.82 ± 0.04	2.24 ± 0.10**	2.75 <sup>bc</sup> ± 0.04**	6.29 ± 0.22	7.10 ± 0.29**	6.32 <sup>a</sup> ± 0.15*
VI	AL. EX. V.U. 300mg/kg	0.33 ± 0.04	1.76 ± 0.07**	0.74 <sup>ab</sup> ± 0.10**	8.08 ± 0.24	9.22 ± 0.15**	8.12 <sup>ab</sup> ± 0.28*	2.72 ± 0.17	2.05 ± 0.12*	2.66 <sup>bc</sup> ± 0.10**	6.53 ± 0.30	7.50 ± 0.35**	6.47 <sup>a</sup> ± 0.34**
VII	AQ. EX. H.V. 200mg/kg	0.59 ± 0.08	1.41 ± 0.19**	0.72 <sup>ab</sup> ± 0.12*	8.53 ± 0.38	9.98 ± 0.28*	9.45 <sup>dc</sup> ± 0.30*	2.80 ± 0.17	2.58 ± 0.07	2.76 <sup>bc</sup> ± 0.06	6.86 ± 0.30	7.56 ± 0.18**	7.05 <sup>ab</sup> ± 0.38
VIII	AQ. EX. H.V. 300mg/kg	0.54 ± 0.05	1.40 ± 0.20*	0.59 <sup>a</sup> ± 0.15*	7.97 ± 0.25	9.71 ± 0.30*	8.78 <sup>bcd</sup> ± 0.29*	3.00 ± 0.12	2.63 ± 0.08*	2.86 <sup>c</sup> ± 0.03*	6.48 ± 0.62	7.63 ± 0.44*	6.86 <sup>a</sup> ± 0.37*
IX	AL. EX. H.V. 200mg/kg	0.68 ± 0.05	1.81 ± 0.17**	0.79 <sup>abc</sup> ± 0.19**	8.30 ± 0.27	10.01 ± 0.28**	8.78 <sup>bcd</sup> ± 0.15*	3.03 ± 0.06	2.27 ± 0.10**	2.83 <sup>c</sup> ± 0.05**	7.41 ± 0.34	8.10 ± 0.31*	7.06 <sup>ab</sup> ± 0.31*
X	AL. EX. H.V. 300mg/kg	0.37 ± 0.05	1.90 ± 0.18**	0.90 <sup>abc</sup> ± 0.20**	8.46 ± 0.20	9.98 ± 0.17**	8.75 <sup>bcd</sup> ± 0.33**	2.82 ± 0.13	1.96 ± 0.08**	2.77 <sup>bc</sup> ± 0.09**	6.38 ± 0.19	7.93 ± 0.52	6.86 <sup>a</sup> ± 0.45**
XI	BIH.AQ.EX. (V.U.+H.V.) 200mg/kg	0.61 ± 0.08	1.74 ± 0.25**	0.60 <sup>a</sup> ± 0.14**	8.39 ± 0.28	10.24 ± 0.22**	9.81 <sup>e</sup> ± 0.18**	2.54 ± 0.12	2.16 ± 0.28	2.49 <sup>b</sup> ± 0.15	6.62 ± 0.24	7.47 ± 0.29*	6.68 <sup>a</sup> ± 0.32*
XII	BIH.AQ.EX. (V.U.+H.V.) 300mg/kg	0.51 ± 0.08	1.95 ± 0.18**	0.70 <sup>ab</sup> ± 0.18**	8.43 ± 0.22	10.35 ± 0.14**	9.25 <sup>cde</sup> ± 0.23*	2.92 ± 0.15	2.34 ± 0.14	2.75 <sup>bc</sup> ± 0.09*	6.81 ± 0.41	7.63 ± 0.48	6.67 <sup>a</sup> ± 0.33**
XIII	BIH.AL.EX.(V.U.+H.V.) 200mg/kg	0.48 ± 0.04	1.97 ± 0.24**	0.64 <sup>ab</sup> ± 0.10**	8.41 ± 0.25	10.63 ± 0.19**	8.87 <sup>bcd</sup> ± 0.17**	2.73 ± 0.08	1.97 ± 0.13**	2.69 <sup>bc</sup> ± 0.11**	6.88 ± 0.21	7.98 ± 0.46*	6.90 <sup>a</sup> ± 0.36**
XIV	BIH.AL.EX. (V.U.+H.V.) 300mg/kg	0.53 ± 0.09	1.99 ± 0.24**	0.54 <sup>a</sup> ± 0.15**	8.01 ± 0.37	10.58 ± 0.08**	8.43 <sup>abc</sup> ± 0.17**	3.06 ± 0.13	1.86 ± 0.08**	2.87 <sup>c</sup> ± 0.04*	7.32 ± 0.19	8.58 ± 0.45*	7.04 <sup>ab</sup> ± 0.28**

AQ. EX/AL. EX. = aqueous/alcoholic extract, V.U./H.V. = *Vigna unguiculata* and *Hordeum vulgares* ; BIH = Biherbal  
 \*p<0.05, \*\*p<0.01 between days, (Means with different superscript differ significantly)

In the urolithiasis induced groups, increased serum Creatinine, Calcium, Phosphorus and decreased level of serum Magnesium levels were observed as compared to compared to the 0 day (when experiment was started) and vehicle control group by day 28 of treatment (Table 2) (Figure 5, 6, 7 and 8).

However the single extract or the co-treatment with aqueous and alcoholic extracts of V.U. and H.V. for 28 day significantly restored these parameters, i.e., by 63<sup>rd</sup> day of experiment in most of the groups.

Biherbal alcoholic extract compared to mono-herbal extract of the said seeds was much better in restoring the values of serum Magnesium, Creatinine, Calcium, Phosphorus and serum Magnesium, Creatinine, Phosphorus values were came down nearer to vehicle control group by 63<sup>rd</sup> day.

Mashiyava *et al.*, (2015) was observed significant increase in mean values of serum Magnesium following administration of EG, while significant restoration following administration of extracts of *Bryophyllum calycinium* and *Tribulus terretris*.

Khan *et al.*, (2017) concluded that there was significant increase in mean values of serum Phosphorus following administration of EG, while significant following administration of extracts *Gum arabic*. Rathva (2016) concluded significant increase the Creatinine and Calcium levels following administration of EG, while significantly decreased following administration of extracts of *Solanum xanthocarpum* and *Acaraynthus aspera*. Increased levels were indicative the necrosis of renal epithelia and damage at the collecting tubules and nephron.

From the results of this experiment, it is concluded that alcoholic extract of V.U. and H.V. at the dose rat of 300mg/kg compared to

aqueous extracts and single herbal aqueous and alcoholic extract of the said seeds was much better in restoring the changes in biochemical parameters EG + AC induced urolithiasis in Wistar rats.

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### Conflict of Interest

Authors declare no conflict of interest for this research work.

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