

Original Research Article

Evaluation of Diuretic activity of aqueous seed extract of *Hordeum Vulgare* in albino rats :An Experimental Study

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ABSTRACT

Background: Diuretics are the drugs that promote the excretion of water and electrolytes are highly useful in various diseases including those exhibiting edema such as congestive cardiac failure, kidney disorders, toxemia of pregnancy, premenstrual tension and hypertension.

Aims: To evaluate the diuretic activity of aqueous seed extract of *Hordeum Vulgare* (AQSEHV) by Lipschitz method in albino rats. **Methods:** Albino rats were divided into 5 groups. These 5 groups were used to evaluate the diuretic activity of AQSEHV by using metabolic cages. The I group serves as normal control received vehicle (CMC 2% in normal saline), the II group Furosemide (10 mg/Kg, p.o) in vehicle; other groups III, IV and V were treated with low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of AQSEHV in vehicle. Immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg.p.o) and placed in the metabolic cages (3 per cage), specially designed to separate urine and faeces, kept at 21°C±0.5°C. The total volume of urine collected was measured at the end of 5th h. During this period, no food and water was made available to animals. Various parameters like total urine volume and concentration of Sodium, Potassium, Chloride ions in the urine were measured and estimated respectively. **Results:** When compared to vehicle treated control group the AQSEHV at different dose levels (100,200 and

400 mg/kg) has significantly increased the urine volume and also enhanced the elimination of Sodium, Potassium and Chloride ions in urine. **Conclusion :** Single dose administration of AQSEHV as 100, 200 and 400 mg/Kg and standard Frusemide (10 mg/kg b.wt) has significantly ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$) increased the urine output along with an increase in concentration of Sodium, Potassium, and Chloride. AQSEHV 400 mg/Kg produced a greater diuretic activity which is comparable to the effect of standard Furosemide (10 mg/kg).

Keywords : *H.vulgare* , seeds, aqueous extract, hydrated rats, diuretic activity

INTRODUCTION

Diuretics are the drugs that are used to increase excretion of urine flow and sodium are used to maintain the volume and composition of body fluids in a variety of clinical situations. Drug facilitated excretion of urine is mainly useful in many life-threatening diseases such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, toxemia of pregnancy, premenstrual tension and hypertension^{1,2}. The presently available diuretics such as thiazides and loop diuretics exhibit various adverse effects such as electrolyte imbalance and metabolic alterations³ etc. A vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as *Abelmoschus esculentus*, *Achyranthus aspera*, *Steganotaemia araliacea*, *Boerhavia diffusa*, *Anisochilus carnosus*, *Bixa orellana*, *Costus speciosus*, *Benincasa hispida*, *Morinda citrifolia* (Noni), *Xanthium strumarium*, *Kigella pinnata*, *Bacopa monnieri*, *Barbara vulgaris* and *Cissampelos pareira* and some of the diuretics are derived from these medicinal plants⁴.

The seeds of *H.vulgare* traditionally used as a diuretic but scientifically many research studies are not available as diuretic agent. Hence the present study was undertaken to evaluate diuretic activity of aqueous extract of seeds of *H.Vulgare* in hydrated (Modified Lipschitz test) albino rats.

Collection of Seeds: The seeds of *H.vulgare* were obtained from the general stores in market, Vizianagaram, Andhra Pradesh and were identified and authenticated by the botanist of maharajahs degree college, Vizianagaram, Andhra Pradesh.

Preparation of extract: Seeds were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation of aqueous extract of seeds of *H.vulgare* was done by using maceration. About 200 g of seed powder was subjected to cold maceration with chloroform water in a conical flask for about 7 days at room temperature .the flask was securely plugged with absorbent cotton and shaken periodically. Then the material was filtered through a muslin cloth and mark was pressed .The filtered was refiltered through whatman filter paper to get the clear filtrate .The filtrate was concentrated to dry residue in a desicator over anhydrous sodium sulphate. The resulting extract was weighed and filled into the sample containers. Phytochemical evaluation for the extract was performed using standard procedures⁵.

Experimental Design:

Experimental Animals:

Albino rats weighing between 140-200 g of either sex was used in the study and were obtained from the Central Animal House, MIMS, Vizianagaram, Andhra Pradesh. The

experimental protocol was approved by the Institutional Animal Ethical Committee and these animals were used to evaluate the diuretic activity of AQSEHV. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments. All rats were housed in metallic cages 6 in each and temperature maintained at $22\pm 2^{\circ}\text{C}$.

Drugs used: Furosemide 20 mg/ml (Sanofi Aventis, Andheri East, Mumbai.)

Acute toxicity study^{6,7}:

Determination of LD_{50} : The acute toxicity of AQSEHV was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 h study period (short term toxicity). Based on the short term toxicity profile, the next dose of the extract was determined as per OECD guidelines No.420. The maximum dose tested (2000 mg/kg) for LD_{50} . From the LD_{50} , doses like $1/20^{\text{th}}$, $1/10^{\text{th}}$ and $1/5^{\text{th}}$ were selected and considered as low, medium and high dose i.e: 100 mg/kg, 200 mg/kg, 400 mg/kg respectively to carry out this study.

Experimental Model:

Lipschitz Test^{8,9} :

Male Albino rats were divided into 5 groups of 6 rats in each. The I group serves as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt), the II group received Furosemide (10 mg/Kg, p.o) in vehicle; other groups III, IV, V were treated with low, medium, and high doses of AQSEHV in vehicle and immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at $21^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$. The total volume of urine collected for 5h was measured at the end. During this period no food and water was made available to animals. Various parameters like total urine volume and concentration of Sodium, Potassium and Chloride in the urine were measured and estimated respectively.

Estimation of urinary electrolytes:

Urine electrolytes (sodium, potassium and chloride) were determined by Ion Selective Electrode method as described by the user instruction manual of the biochemical kits (Roche, Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana.)

Statistical Analysis:

Experimental results were expressed as mean \pm SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnett's 't' test.

Results:

The AQSEHV was subjected to qualitative phytochemical tests to identify the phytoconstituents and it revealed the presence of phenolic acids, flavonoids, lignans, tocopherols, phytosterols and folate.

In acute toxicity study all the animals were survived even after 14 days. This indicates that the extract was found to be safe up to the maximum dose level tested (2000 mg/kg). No major behavioural changes were observed during this period of study.

The results obtained with evaluation of diuretic activity of AQSEHV was shown in Table No.1. AQSEHV has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride levels when compared to control. The effect of AQSEHV was found to be dose dependent, i.e, among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide, the diuretic effect observed after treatment with AQSEHV was found to be significant in terms of urinary output, sodium, potassium, chloride concentrations. Determination of urinary electrolyte concentration revealed that AQSEHV was effective in increasing urinary electrolyte concentrations for all the three ions tested (Na^+ , K^+ , Cl^-).

Discussion :

Traditionally medicinal plants are used in the treatment of various diseases. Diuretics are used as adjuvants in the treatment of mild to moderate hypertension. pulmonary congestion and peripheral oedema were relieved by diuretics. In Congestive cardiac failure and acute left ventricular failure diuretic drugs are highly useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnoea¹⁰ These drugs decrease plasma volume and subsequently venous return to the heart. This decreases the cardiac work load, oxygen demand and plasma volume and also decreases blood pressure. Thus diuretics play a vital role in hypertensive patients¹¹. They are used to induce forced diuresis (forced alkaline diuresis and forced acidic diuresis) in cases of aspirin and morphine poisoning. Diuretics are also useful in prevention of recurrent calculi. The present study revealed that AQSEHV significantly increased the urinary out, as well as the elimination of urinary electrolytes in a dose dependant manner. Earlier Jignesh G Shah et al, 2012 reported antiurolithic and antioxidant activity with aqueous extract of seeds of *H.vulgare*^{3,12}. In the present work aqueous extract of seeds of *H.vulgare* was studied for its diuretic activity. The phytochemical³ studies reveals that the seeds of *H.vulgare* contains phenolic acids, flavonoids, lignans, tocopherols, phytosterols^{13,14,15} and folate. Earlier studies reported phytochemical substances phenolic acids, flavonoids, lignans, tocopherols, phytosterols. AQSEHV was identified with most of these plant phytochemical substances mentioned above. Hence it can be reported that the observed diuretic activity is due to these above phytoconstituents.

Conclusion :

From the results it can be concluded that single dose administration of AQSEHV as 100, 200 and 400 mg/Kg and standard Frusemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium, Potassium and Chloride ions in urine. AQSEHV 400 mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg). The present study supports and justify the rationale behind the traditional use of seeds of *H.vulgare* for diuretic activity.

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Table No: 1 Effect of AQSEHV on urine volume and electrolyte concentration in hydrated rat model(Lipschiz test) in albino rats

S.No	Groups	Total Urine Vol (ml/kg b.wt/5 h)	Na ⁺ mmol/L	K ⁺ mmol/L	Cl ⁻ mmol/L
1	Control (10 ml/Kg b. wt)	12.43±0.01	110.02 ± 1.16	49.09 ± 1.42	79.94 ± 1.32
2	Standard (Frusemide 10 mg/kg b.wt)	21.22±0.01***	185.05±2.05***	85.71±1.50***	125.06±1.56***
3	AQSEHV Low (100 mg/kg b.wt)	13.47±0.02***	115.00±2.46***	58.84±1.35***	91.23 ±2.05***
4	AQSEHV Medium (200 mg/kg b.wt)	15.55±0.03***	147.77±2.44***	70.12±1.21***	101.24±1.71***
5	AQSEHV High (400 mg/kg b.wt)	18.85±0.01***	180.22±1.80***	81.22±1.36***	114.66±1.68***

n=6, Values expressed as mean ± SEM., Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One Way ANOVA followed by Dunnett`s 't' test)