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STUDY OF DIURETIC ACTIVITY OF ETHANOLIC EXTRACT OF SEEDS OF HORDEUM VULGARE IN ALBINO RATS

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ABSTRACT

Background: Diuretics, which encourage the excretion of water and electrolytes, are extremely beneficial for treating a variety of illnesses, including those that cause edema, such as congestive heart failure, kidney problems, pregnancy-related toxemia, premenstrual tension, and hypertension. **Objectives:** To investigate, the diuretic efficacy of an ethanolic seed extract of Hordeum vulgare (EEHV) using the Lipschitz method on albino rats,. **Methods:** Five groups of albino rats were used to evaluate the diuretic activity of EEHV by using metabolic cages. Group I served as the normal control and received vehicle (CMC 2%).Group II received frusemide 10 mg/kg.The groups III, IV, and V were given low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of EEHV in vehicle, respectively.All the rats were immediately hydrated with saline (15 ml/kg, po) and housed in metabolic cages. At the end of the fifth hour, the total amount of urine collected was measured. Total urine volume and sodium, potassium, and chloride ion concentration in the urine, were measured and estimated. **Results:** The EEHV at various dose levels (100,200, and 400 mg/kg) significantly increased urine volume and increased sodium, potassium, and chloride ion elimination in urine as compared to the vehicle-treated control group. **Conclusion:** The amount of urine produced and the concentrations of sodium, potassium, and chloride increased significantly after a single dosage of standard frusemide (10 mg/kg) and EEHV (100, 200, and 400 mg/Kg). The higher diuretic efficacy caused by EEHV 400 mg/Kg was equivalent to that of conventional furosemide (10 mg/kg).

Keywords: Diuretic Activity, *H.* Vulgare , Seeds, Ethanolic Extract, Hydrated Rats

INTRODUCTION

In a variety of clinical settings, sodium is utilised to maintain the volume and make up of body fluids whereas diuretics are used to promote excretion of urine flow. Premenstrual syndrome, nephritic syndrome, cirrhosis, renal failure, toxaemia of pregnancy, premenstrual dysphoric disorder, and hypertension are among the many life-threatening disorders that can be treated by drug-facilitated urine excretion[1,2]. Diuretics that are available in the market, such as thiazides and loop diuretics, have a variety of negative side effects, including electrolyte imbalance and metabolic changes[3]. A large number of medicinal plants mentioned in the ayurvedic medical system are known to have diuretic properties, including Abelmoschus esculentus, Achyranthus aspera,

Steganotaemia araliacea, Boerhavia diffusa, Anisochilus carnosus, Bixa orellana, Costus speciosus, Benincasa hispida, Morinda citrifolia (Noni).

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

METHODOLOGY

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

Preparation of extract: The seeds were properly washed with clean tap water, shade-dried, and powdered by using mechanical grinder. Ethanolic extract of H. vulgare seeds was prepared by using soxhlatation[4,5] in the department of pharmacology at maharajahs institute of medical sciences,Vizianagaram. 200 g of root powder was placed in the soxhlet device, and ethanol (95% alcohol) was used to extract the substance. The extraction procedure was run for 18 to 20 hours until a colourless solvent appeared in the side tube. The solvents from the extracted extract were evaporated on a water bath that was kept at a temperature of 500°C to dry it, and the yield of the alcoholic extract as a percentage of the total amount of powder used for the extraction was recorded. The extract's phytochemical composition was evaluated utilising accepted practises.

Experimental Design:

Experimental Animals: The study was conducted by using albino rats from the Central Animal House, MIMS, Vizianagaram, Andhra Pradesh, weighing between 140-200 g of either sex. These mice were used to assess the diuretic action of EEHV.The experimental protocol was approved by the institutional animal ethical committee. Before conducting the studies, the animals were acclimated for a total of 15 days under routine husbandry settings. All rats were kept in six-rat metallic cages, with the temperature kept at 22+2C.

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

Acute toxicity study[6,7]: Determination of $LD_{50,}$ Albino mice of either sex, weighing 16–20 g, were used to test the acute toxicity of the EEHV. They were kept in typical husbandry conditions. Before the experiment, the animals were fasted for three hours. A single dose of the extract was given and the animals' deaths were tracked for up to 48 hours. (short term toxicity). The subsequent dose of the extract was determined in accordance with OECD guidelines No. 420 based on the short-term toxicity profile. The LD50 maximum dose was 2000 mg/kg. To conduct this investigation, doses like 1/20th, 1/10th, and 1/5th of the LD50 were chosen and regarded as low, medium, and high doses, or 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively.

Experimental Model:

Lipschitz Test[8,9]: albino mice of either sex that were kept under regular husbandry circumstances were used, the acute toxicity of EEHV was done using OECD guidelines. The animals were starved for three hours prior to the experiment, and a single dose of the extract was given. The animals' deaths were then tracked for up to 48 hours during the study period (Short term toxicity). According to OECD guidelines No. 420, the subsequent dose of the extract was calculated based on the short-term toxicity profile. The highest dose tested for LD50 (2000 mg/kg). To conduct this investigation, doses like 1/20th, 1/10th, and 1/5th of the LD50 i.e., 100 mg/kg, 200 mg/kg, and 400 mg/kg—were chosen and regarded as low, medium, and high doses, respectively. Five groups of albino rats were used to evaluate the diuretic activity of EEHV by using metabolic cages. Group I served as the normal control and received vehicle (CMC 2%).Group II received frusemide 10 mg/kg.The groups III, IV, and V were given low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of EEHV in vehicle, respectively.All the rats were immediately hydrated with saline (15 ml/kg, po) and housed in metabolic cages. At the end of the fifth hour, the total amount of urine collected was measured. Total urine volume and sodium, potassium, and chloride ion concentration in the urine, were measured and estimated.

Estimation of urinary electrolytes: According to the user handbook for the biochemical kits, the Ion Selective Electrode technique was used to measure the electrolytes in urine (sodium, potassium, and chloride). (Roche, Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana.)

Statistical Analysis: The mean $\pm$ SEM (n=6) was used to express the experimental results. One-way ANOVA was used for statistical analysis, followed by the Dunnett's t test.

RESULTS

To determine the phytoconstituents in the EESV, qualitative phytochemical analyses was performed. The results showed that phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate were present.All of the rats in the acute toxicity investigation survived even 14 days later. As a result, it can be concluded that the extract is safe up to the highest dose level examined (2000 mg/kg). No significant alterations were noticed during this study period.

Table No.1 displays the findings from the study of the EEHV's diuretic activity.When compared to control, EEHV has demonstrated a considerable diuretic activity by increasing urine output and increased excretion of sodium, potassium, and chloride levels. It was discovered that the effect of EEHV was dose dependent, meaning that among the three doses examined, the higher dose generated a greater effect. The diuretic effect following treatment with EEHV was found to be considerable in terms of urine output, sodium, potassium, and chloride concentrations after comparison with the common diuretic medicine furosemide. Urine electrolyte concentrations for the three ions Na+, K+, and Cl^- measured and showed that EEHV was successful in raising urinary electrolyte concentrations for all three ions.

DISCUSSION

Historically, medicinal plants have been utilised to treat a variety of illnesses. They are also used as adjuvants for the treatment of mild to moderate hypertension. Diuretics were used to treat peripheral oedema and pulmonary congestion. Diuretic medications are extremely helpful in lowering fluid overload and relieving orthopnea and paroxysmal nocturnal dyspnoea in congestive heart failure and acute left ventricular failure[10,11]. These medications reduce plasma volume, which in turn reduces venous return to the heart. This reduces the pressure on the heart, the need for oxygen, the volume of plasma, and the oxygen demand. As a result, diuretics are essential for hypertension patients[12,13]. In cases of aspirin and morphine poisoning, they are used to induce forced diuresis (forced alkaline diuresis and forced acidic diuresis). Diuretics are additionally helpful in preventing recurring calculi.[14]

The results of the current study showed that EEHV considerably increased urine output as well as the excretion of urinary electrolytes[15,16] in a dose-dependent manner. Ethanolic extract from the seeds of H. vulgare was previously found to have antiurolithic and antioxidant activities by Jignesh G. Shah et al[3]. In this study diuretic efficacy of ethanolic extract of H. vulgare seeds was evaluated. The seeds of H. vulgare contain phenolic acids, flavonoids, lignans, tocols, phytosterols.[17,18] and folate, according to phytochemical[3] investigations. In earlier research, phenolic acids, flavonoids, lignans, tocopherols, and phytosterols were reported as phytochemical compounds with diuretic properties.[18,19] Most of these plant phytochemicals described above also present in the EEHV. Therefore, it can be stated that the above phytoconstituents are responsible for the observed diuretic activity.

CONCLUSION

From the results it can be concluded that EEHV at doses of 100, 200, and 400 mg/Kg as well as conventional furosemide (10 mg/Kg) increased urinary output and also increased the levels of sodium, potassium, and chloride ions in urine. The diuretic action of EEHV 400 mg/kg was higher and comparable to that of conventional furosemide (10 mg/kg).The current study validates and supports the traditional usage of H. vulgare seed for diuretic action.

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Conflict of interest: None declared

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TABLES

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

SN	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)	11.34±0.01	109.01 ± 1.14	45.09 ± 1.40	78.92 ± 1.31
2	Standard (Frusemide 10 mg/kg b.wt)	20.21±0.02***	183.04±2.04***	84.68±1.40***	123.05±1.46***
3	EEHV Low (100 mg/kg b.wt)	12.56±0.03***	112.02±2.44***	51.74±1.34***	89.21 ±2.04***
4	EEHV Medium (200 mg/kg b. wt)	14.54±0.02***	139.74±1.44***	69.12±1.20***	100.14±1.70***
5	EEHV High (400 mg/kg b.wt)	17.74±0.02***	178.21±1.70***	79.21±1.34***	116.64±1.58***

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)