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EVALUATION OF DIURETIC POTENCY OF ROOT EXTRACTS OF *HEMIDESMUS INDICUS* IN RATS

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ABSTRACT

Background and objectives: The plant *Hemidesmus indicus* syn. Sariba, Family- Asclepiadaceae, is a perennial, slender, laticiferous, prostrate or semi-erect, twining, wiry shrub. Root part of this plant is commonly used as tonic, alternative, blood purifier, diaphoretic, diuretic and skin diseases. Crude hydro ethanolic extract of root of *Hemidesmus indicus* (CHEERHI) was evaluated for its diuretic activity in a dose of 250 and 500 mg/kg body weight by oral route. Methods: Lipschitz model Results and interpretation: CHEERHI showed significant increase in urine volume along with increased excretion of sodium, potassium, creatinine and chloride ions as compared to control group. CHEERHI showed delayed diuretic effect (after 4 hours of administration) as compared to furosemide. Conclusion: It is diuretic and thus blood purifier.

KEY WORDS: *Hemidesmus indicus*, Diuretic activity, Metabolic cage, Furosemide.

INTRODUCTION

The crude hydro ethanolic root extract of *Hemidesmus indicus* (CHEERHI) was evaluated for *in vivo* acute diuretic activity in Rats by Lipschitz model. So, Ethnobotanical and Ayurvedic claims of *Hemidesmus indicus* for their diuretic activity is to be proved by modern techniques is the main objective of present work. Most of the therapeutically useful diuretics act mainly by modifying various functions of the nephron. It is well known that all available allopathic drugs gives quick action but on the other hand they leave the body to suffer from large numbers of adverse reactions which are also required to treat separately or need

discontinuation of treatment. There is an extreme need to investigate drugs of plant origin having less adverse effects and more acceptable by society.

MATERIALS AND METHODS

1.1 Collection and identification of plant material: The roots of *Hemidesmus indicus* were collected in the month of August-September, 2008 from the Gir forest region of Junagadh district in Gujarat state and identified and authenticated by Joint Director, Botanical survey of India (BSI), Jodhpur. The voucher specimen number SU/DPS/Herbs/10 of the same was deposited in the Department of Pharmaceutical sciences, Saurashtra University, Rajkot, for the further reference. The root was collected fresh and then dried in hot air oven at about 55^0C.

1.2 Materials, Instruments and chemicals: Soxhlet apparatus, solvent recovery apparatus, desiccator, distillation apparatus, TLC chamber, sprayer, digital electronic weighing instrument, chromatographic paper, TLC plates, Camag HPTLC system consists of TLC scanner III, Application device Linomat IV, twin trough plate development chamber and wins cats software was used, china dish, beakers, conical flasks were used.

Petroleum ether, ethyl acetate, benzene and chloroform, methanol, ethanol, Furosemide, DMSO, Metabolic cage, autoclave.

All the chemicals and reagents used were obtained from SD fine-chem Limited, Mumbai; Sisco Research Laboratory Ltd., Mumbai; LOBA chemical Pvt. Ltd., Mumbai. Furosemide was procured from Hem deep organics, Ankleshwar.

1.3 Preparation of extract: The CHEERHI obtained by above mentioned procedure was used for the study of diuretic activity. Required amount of dried extract was dissolved in deionised water and DMSO and administered to rats orally.

1.4. Reference drug: Furosemide, a high-ceiling loop diuretic, was used as the reference drug (positive control). It was dissolved in deionised water prior to administration.

1.5. Experimental animals: Albino rats of either sex of Wistar strain, weighing 150-200 gm were used for study. The animals were housed in a group of 3 rats per cage under well controlled conditions of temperature (25 ± 1^oC), humidity (55±5%) and 12 hr/12 hr light-dark cycle. Animals had access to standard pellet diet and water given ad libitum (Sanaa Lahlou et

al., 2006) The protocol of the experiment was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidelines of the Committee for the purpose of Control and Supervision of Experiments of Animals (CPCSEA). Animals was provided by Department of Pharmaceutical Sciences, Saurashtra University, Rajkot and its registration number is 1155/ac/07/CPCSEA.

1.6. Biochemical methods: Urine was collected and urinary levels of sodium and potassium were quantitated by ions selective electrode method. Concentration of creatinine in urine was determined by Jaffe alkaline picrate method and chloride concentration by colorimetric estimation.(Derasari et al., Bio chemistry practical book).

1.7. Treatment protocols in study of diuretic activity: The experimental animals were divided into four groups, six animals in each group and drugs were given in a manner shown in table 1 (Sanaa Lahlou et al., 2006.)

Table 1 Treatment protocol

No.	Group	Group specification
1	Control	Vehicle (0.9% NaCI + deionised water-oral)
2	Standard	Furosemide (10 mg/kg-oral)
3	Test 1	CHEERHI (250 mg/kg-oral)
4	Test 2	CHEERHI (500 mg/kg-oral)

1.8. Assessment of diuretic activity: Experimental design: The diuretic activity was evaluated as follows: each animal was placed in an indiviidual metabolic cage (figure 1) 24 hr. prior to commencement of the experiment for adaptation. The animals were divided into four groups of six rats each for the acute (single dose) study. Rats were fasted overnight with free access to water and subjected to the stated treatment as described below. The rats were observed occasionally for apparent toxicity (Sanaa Lahlou et al., 2006)

Figure 1 indicating metabolic cage and collected urine in control, standard and 250mg/kg dose of extract

Acute diuretic activity

Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5 ml/100 gm body weight (BW), to impose a uniform water and salt load (Benjumea et al., 2005) The first group received orally distilled water 10 ml/Kg BW, and served as the control group. The second and third groups were administered orally 250 mg/kg and 500 mg/kg BW of *Hemidesmus indicus* extract. The fourth group was treated with an oral dose of 10 mg/kg BW of furosemide. Urine was collected and measured at 1, 2, 4, 6, and 24 hr. after the dose. Sodium, potassium, chloride and creatinine concentrations were determined in the 24 hr urine sample (Sanaa Lahlou et al., 2006).

Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) (Graph Pad instat software) or by Student's t-test. Significant differences were indicated by P values lower than 0.05.

RESULTS

Phytochemical screening of the extract revealed the presence of coumarin (Harbone, 1976 and Stahl, 1969), β-sitosterol, α and β-amyrins, lupeol, tetracyclic triterpene alcohols, flavanoids, terpenoids, saponins. a glycoside and a ketone. Ash of powder showed positive test for iron. (Prajapati et al).

***In vivo* diuretic activity**

Study of diuretic activity was carried by LIPSCHITZ model (Vogel). Crude hydroethanolic extract of root of HI of the dose of 250 mg/kg and 500 mg/kg (Nadana Saravanan et al., 2008) was taken in study.

Results of animal experiments are shown in tables

Table 2: Effect of CHEERHI on urinary volume during first 4 hours.

Group name	Mean ± SEM (ml)
Control	5.933 ± 0.8106
Standard	6.817 ± 2.603
Test 500 mg/kg	3.450 ± 1.033
Test 250 mg/kg	3.633 ± 1.143

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05, **P< 0.01, ***P<0.001 compared to controls using student's t-test.

Table 3: Effect of CHEERHI on urinary volume after 4 hours. (4 – 24 hrs)

Group name	Mean ± SEM (ml)
Control	1.650 ± 0.5584
Standard	7.517 ± 1.508
Test 500 mg/kg	9.067 ± 1.201**
Test 250 mg/kg	8.117 ± 1.504**

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05, **P< 0.01, ***P<0.001 compared to controls using student's t-test.

Table 4: Effect of CHEERHI on urinary sodium concentration (mmol/lit)

Group Name	Mean ± SEM (mmol/lit)
Control	108.217 ± 5.36
Standard	152.067 ± 14.115
500 mg/kg	130.933 ± 10.675
250 mg/kg	114.155 ± 15.551

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05, **P< 0.01, ***P<0.001 compared to controls using student's t-test.

Table 5: Effect of CHEERHI on urinary potassium concentration (mmol/lit)

Group Name	Mean ± SEM (mmol/lit)
Control	77.167 ± 7.715
Standard	136.817 ± 12.201
500 mg/kg	119.667 ± 12.392*
250 mg/kg	123.767 ± 15.08*

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05, **P< 0.01, ***P<0.001 compared to controls using student's t-test.

Table 6: Effect of CHEERHI on urinary creatinine excretion (mmol/lit)

Group Name	Mean ± SEM (mmol/lit)
Control	9.675 ± 3.065
Standard	39.85 ± 5.035
500 mg/kg	30.567 ± 5.64*
250 mg/kg	21.853 ± 5.039*

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05, **P< 0.01, ***P<0.001 compared to controls using student's t-test.

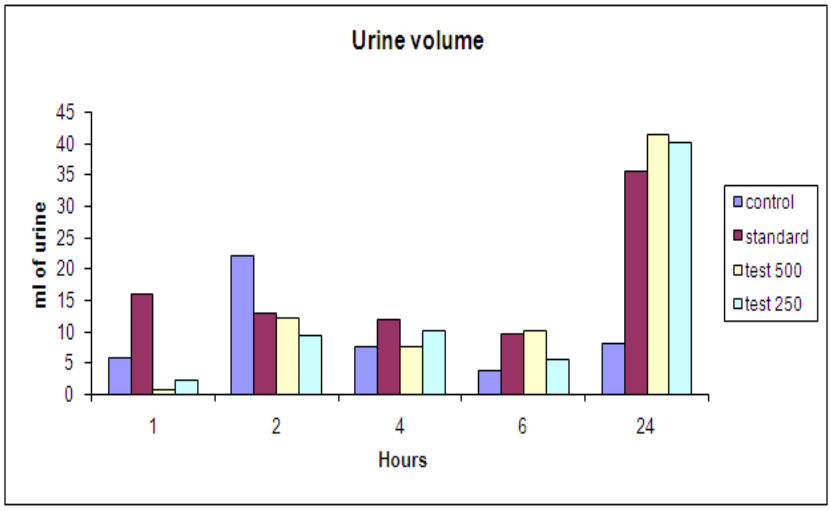
Table 7: Effect of CHEERHI on urinary chloride excretion (mmol/lit)

Group Name	Mean ± SEM (mmol/lit)
Control	171.72 ± 8.291
Standard	147.67 ± 6.211
500 mg/kg	156.83 ± 8.440
250 mg/kg	139.33 ± 6.469*

Values are expressed as ± S.E.M. of 6 rats in each group. *P<0.05,**P< 0.01, ***P<0.001 compared to controls using student's t-test.

The P value is 0.0348, considered significant.

Urinary volume excretion during 24 hrs of dose administration is also depicted in graphical manner which is shown below



Graph 1: Urine volume excretion

Effect on urine volume: Treatment with a single dose of the CHEERHI increased diuresis. It was found that during first 4 hours after treatment with HI extract, there was no rise in urine volume (extract 500 mg/kg 3.450 ± 1.033 and extract 250 mg/kg 3.633 ± 1.143 versus control 5.933 ± 0.8106; P=0.1846 and considered not significant).

Urine output was found increased significantly after 4 hours i.e. between 4 to 24 hours (Extract 500 mg/kg 9.067 ± 1.201 and extract 250 mg/kg 8.117 ± 1.504 versus control 1.650 ± 0.5584; P=0.0007, which is considered extremely significant.

Thus, Hydro ethanolic root extract of *H. indicus* displayed diuretic activity, with an excretion of sodium and potassium ions in laboratory animals. Potassium loss is one drawback with this extract because it leads to adverse effect of hypokaelemia and cardiovascular arrest.

CONCLUSION

In the present study, the diuretic effect of orally administered hydro ethanolic extract of HI root was evaluated in normal rats after one dose. The pharmacological response was compared with that produced by furosemide, a widely used diuretic in clinical practice. The plant material selected for the study is used as flavouring agent and in the preparation of a sherbet, which is reported to have cooling properties and also has demulcent and diuretic properties. Oral route were chosen because that is the way people use this medicine. The effect on urinary volume, electrolyte balance and urinary creatinine concentration was determined. The mechanism of action by which diuresis was induced by this extract was also assessed by comparing the effect with that of furosemide, a high ceiling loop diuretic.(Jackson, 1996).

Diuretics has two components: increase in urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine (Jackson, 1996). The reference drug, furosemide, increases urine output and urinary excretion of sodium by inhibiting Na+/K+/2CI- symporter in the thick ascending limb of loop of henle.(Jackson, 1996).

In the saline primed rats (LIPSCHITZ MODEL), a dose of 250 mg/kg and 500 mg/kg of CHEERHI extract took 4 hour after the dose to increase urine output significantly. In comparision, a single extract of furosemide induced a brisk and significant diuresis. The difference in the time of onset of the diuretic action of these substances may be related to the gastrointestinal absorption characteristics of the active principle(s). Stimulation of diuresis by single dose of extract continued for atleast 24 hr.

Extract also showed increase in sodium, potassium and creatinine concentration in urine. Because, extract showed increase in creatinine excretion, it is best used as blood purifier. For authentification of Establishment of its use as diuretic, it require to conduct sub acute and chronic experiment in rats for in vivo screening of diuretics.

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