



PHYTOCHEMICAL INVESTIGATION AND DIURETIC ACTIVITY OF ECILEPTA PROSTRATA LEAVES

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Abstract: Kidney, as excretory organ of our body serves important function of excretion of waste products, regulation of fluid volume and electrolyte content etc. Damage to kidney can lead to severe life threatening complications. Diuretics are drugs capable of increasing levels of urine. Aqueous and alcoholic extracts of the leaf of *Ecilepta prostrata* leaves were tested for diuretic activity in rats. The parameters studied on individual rat were body weight before and after test period, total urine volume urine concentration of Na⁺, K⁺ and Cl⁻. In the present study alcoholic and aqueous extracts of was investigated. *Ecilepta prostrata* leaves (100mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as reference diuretic, The plant extracts did not appear to have renal toxicity or any other adverse effects during the study period.

Keywords: Diuretic activity, Furosemide, *Ecilepta prostrata* leaves, Aqueous and alcoholic extracts

INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia [1]. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na⁺ re absorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH [2, 3]. Although most of the diuretics proved to be very effective in promoting sodium excretion, all cause potassium loss and prompted the search for potassium sparing diuretic. Hence search for a new Diuretic agent that retains therapeutic efficacy and yet devoid of potassium loss is justified [4]

Ecilepta prostrata Hassk [Asteraceae] is a small genus of herbs commonly known as Bringaraja [Sanskrit], Maka [Marathi] and Bhangra [Hindi]. The plant is distributed throughout India in wet or moist wastelands, ascending upto 2000m on the hills. It is an erect or prostrate, much branched herb with white flowers. The plant has a bitter, hot, sharp, dry taste and is used in Ayurveda [a primary health care system of India], for the treatment of vitiated conditions of kapha and vata. Traditionally, it is extensively used against jaundice, in treatment for night blindness, headache and diseases pertaining to hair and its growth. It is also considered as a rejuvenator [5].

Eclipta prostrata is a source of coumestan-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis [6]. *Eclipta prostrata* is widely used in India as a cholagogue and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder [7].

Coumestan-type compounds, wedelolactone and dimethyl wedelolactone, have been isolated as the main active principles of *Eclipta prostrata*, both constituents exhibiting anti-hepatotoxic activity [8-9]. *In vivo* tests indicate that wedelolactone neutralizes the lethal and myotoxic activities of rattlesnake venom [10]. Wedelolactone (WL) and dimethyl wedelolactone (DWL) showed potent activity when were tested in the trypsin inhibition bioassay (*in vitro*) [11]. The roots have emetic and purgative properties and it have been applied externally as an antiseptic to ulcers and wounds in cattle. The shoot extract shows antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. From the whole plant of *Eclipta prostrata*, a new triterpene saponin, namely eclalbatin, together with alpha-amyrin, ursolic acid and oleanolic acid have been isolated [12]. In Ayurveda a large number of indigenous drugs have been mentioned possessing analgesic properties. The total ethanol extract of *Eclipta prostrate* have been shown to possess analgesic properties [13].

No systematic studies have been reported for its diuretic activity. Hence an effort has been made to



establish the diuretic activity of aqueous and alcoholic extracts of *Ecilepta prostrata* leaves.

MATERIALS AND METHOD

Collection and preparation of Plant Extract:

The leaves of were *Ecilepta prostrata* collected in the month of December from the local field of Etawah, Uttar Pradesh state, India, and authenticated by Dr. SP Agrawal Narain College, Sheikhabad India. A voucher specimen was submitted at Institute's herbarium department for future reference (SMGI 241 / 10). Dried leaves were ground to coarse powder. Powder was first defatted with petroleum ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract.

Extraction and phytochemical screening of plant:

The powdered plant materials (500g) were extracted with petroleum ether at 40-600 C, by continuous hot percolation using soxhlet apparatus. The extraction was carried out by using solvent of increasing polarity starting from petroleum ether and methanol respectively. The extraction was carried out for 72 hours. The petroleum ether extract was filtered and concentrated to dry mass by using vacuum distillation. A dark greenish brown residue was obtained. The marc left, after petroleum ether extraction was taken and then subsequently extracted with methanol for 72 hours. The methanolic extract was then filtered and concentrated to dry mass. A dark greenish residue was obtained. Phytochemical screening was performed using standard procedures [14, 15].

Experimental animals:

In bred colony strains of Wistar rats of either sex weighing 150-250 g procured from the animal house were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 28±1° C and standard 12 hour: 12 hour day night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd) and water *ad libitum*. Prior to the experiment the animals were acclimatized to the laboratory conditions. All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC) and followed the guidelines of IAEC.

Drug: Furosemide tablet was collected from local market of Etawah U.P. was used as known Diuretic agent. The standard solution was prepared by dissolving the tablet in the solvent. The dose of was Furosemide maintained 100 mg/kg body weight.

Acute Toxicity Study: Acute toxicity study was carried out by using graded doses of drug were administered intra peritoneally in graded doses (200 to 1000 mg/kg body weight). They were observed

continuously for the first 2 h for toxic symptoms and up to 24 h for mortality [16].

Diuretic Activity:

Male rats (Wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et al [17, 18] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline (25ml/Kg,p.o.); the second group received furosemide (100mg/Kg,i.p.) in saline; the third, fourth groups received the Alcohol and Aqueous extract at the doses of 100 mg/Kg, respectively, in normal saline. Immediately after administration the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feces, kept at room temperature of 25± 0.5°C throughout the experiment. The urine was collected in measuring cylinders up to 3 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na⁺, K⁺, and Cl⁻ in the urine. Na⁺, K⁺ concentrations were measured by Flame photometry [19] and Cl⁻ concentration was estimated by titration [20] with silver nitrate solution(N/50)using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± SD, the test of significance (p<0.01 and p<0.05) was statically.

Statistical Analysis:

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA [21] at a probability level of P < 0.001.

RESULTS AND DISCUSSIONS

Table No.1: Phytochemical Screening Of *Ecilepta Prostrata* Leaves

Test for Phytoconstituent	Powder	Aqueous	Methanol
Alkaloids	+ve	-ve	+ve
Glycoside	+ve	-ve	-ve
Saponins	+ve	+ve	+ve
Tannins & Phenolic comp.	-ve	-ve	-ve
Steroids & Terpenoids	+ve	+ve	+ve
Carbohydrates	-ve	-ve	-ve
Protein & Amino acid.	-ve	-ve	-ve

The preliminary phytochemical screening of the ethenolic fraction showed the presence of steroids, tannins and flavonoids etc. are given in Table no-1.

In acute toxicity study, it was found to be safe and no mortality was observed to a dose as high as 800 mg/kg. Present study shows that the aqueous and alcoholic extract of *Ecilepta prostrata* leaves possess good diuretic activity. Urine volume, cation and anion excretion were increased, Na⁺/K⁺ ratio of 2.04 and 2.18 were obtained for aqueous and alcoholic extract respectively. The normal value for Na⁺/K⁺ ratio is reported to be 2.05-2.83. The concentration of aldosterone is found to be dependent on Na⁺/K⁺ ratio. If the Na⁺/K⁺ ratio falls below the normal in plasma the aldosterone secretion will be decreased and if the ratio rises above the normal value the aldosterone secretion will be increased. Significant increase in Na⁺, K⁺ and Cl⁻ excretion was observed in aqueous and alcoholic extract treated animals but it was less than the furosemide control. Further studies are required to assess the medicinal value of leaves of *Ecilepta prostrata* as a potential diuretic agent (Table-2).

Table.2: Diuretic Activity of *Ecilepta Practsostrata* Leaves.

Extract Drug	Dose Mg/kg	Urine volume	Diuretic Potency	Electrolytic Excretion		
				Na ⁺	K ⁺	Cl ⁻
Methnol Extract	250mg/kg b.w	0.91±0.0 30	0.42	222.05± 0.903	6.07 ± 0.075	115.19±0 .742
Aqueous Extract	250mg/kg b.w	0.02 ± 0.79	0.82	247.90±2.2 46	5.73± 0.193	137.32± 0.495
Frusamide	11mg/kg g b.w	3.74 ± 0.26	1.3	252.34±1.3 85	4.817±0.11 0	141.24± 8.173
Normal saline	0.87	0.32±0.0 2	0.11	212.64±0.8 7	4.92±0.05 4	110.82± 0.84

Each Value represents the mean ± SEM of six rats
P < 0.05*, P < 0.01**, P < 0.001***,

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure [22]. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol and aqueous extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles [23]. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [24].

In present study aqueous and alcohol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavanoids, saponins, Results of present investigation showed that ethanol is most

effective in increasing urinary electrolyte concentration of all the ions i.e. Sodium, Potassium and Chloride followed by alcohol and aqueous extracts while other extracts did not show significant increase in urinary electrolyte concentration.

A complex set of interrelationships exists among the cardiovascular system, the kidneys, the central nervous system (Na⁺, appetite, thirst regulation) and the tissue capillary beds (distribution of extracellular fluid volume), so that perturbation at one of these sites can affect all the remaining sites. A primary law of the kidneys is that Na⁺ excretion is a steep function of mean arterial blood pressure (MABP) such that small increase in MABP cause marked increase in Na⁺ excretion [25]. One of the earliest strategies for the management of hypertension was to alter Na⁺ balance by restriction of salt in the diet. Diuretic agents having antihypertensive effects were used alone and had greater efficacy than all other antihypertensive drugs. In this study pharmacological evaluation of diuretic action of aqueous and alcoholic extracts of *Ecilepta prostrata* was evaluated using furosemide under controlled laboratory condition. As diuretic therapy may lead to number of life threatening electrolytic disorder and toxicities, so safety profile studies are carried out following a sub chronic administration of extracts.

CONCLUSION

The extracts of *Ecilepta prostrata* have diuretic effect supporting the ethno pharmacological use as diuretics. This effect may be explored in the use of the plant in the management of inhibit bacterial growth.

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