



DIURETIC ACTIVITY OF *ACHYRANTHES ASPERA* LEAVES EXTRACT

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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 *Achyranthes aspera* 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. *Achyranthes aspera* leaves (100mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as reference diuretic.

KEY WORDS: Diuretic activity, Furosemide, *Achyranthes aspera* leaves.

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 toxemia¹. 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⁺ reabsorption 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 justified⁴.

Achyranthes aspera Linn. belongs to the family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India and used by traditional healers for the treatment of fever, dysentery and diabetes^{5,6}. Leaf decoction for cardiovascular toxicity has been reported⁷, and the ethanol crude extract showed high larvicidal activity on the tick larvae against *Boophilis microplus*⁸. The root extract is well reputed for its pronounced insect molting hormonal activity⁹ and the ethanolic extract of the leaves and stem of the plant inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains¹⁰. Roots are used as astringents to wounds, in abdominal tumor and stomach pain¹¹. The benzene extract of the stem bark shows abortifacient activity in the rat¹². Leaf extracts were reported to possess thyroid stimulating, antiperoxidative and antifungal activity properties^{13,14}. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits¹⁵. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. The water soluble alkaloid achyranthine isolated from *Achyranthes aspera* possess anti-inflammatory activity¹⁶.

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 *Achyranthes aspera* leaves.

MATERIALS AND METHOD

Collection and preparation of Plant Extract

The leaves of *Achyranthes aspera* were collected in the month of October from the local field of Etawah, Uttar Pradesh state, India, and authenticated by Dr. Harish .K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at Institute's herbarium department for future reference (AN 104). Dried leaves were ground to coarse powder. Powder was first defatted with pet. 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 40-60°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^{17,18}.

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 intraperitoneally 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 mortality¹⁹.

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^{20, 21} 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 photometry²² and Cl⁻ concentration was estimated by titration²³ 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 ANOVA²⁴ at a probability level of P < 0.001.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethenolic fraction showed the presence of steroids, tannins and flavonoids. 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 *Achyranthes aspera* 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 *Achyranthes aspera* as a potential diuretic agent (Table 1).

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 pressure²⁵. 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 muscles²⁶. 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 recommended²⁷.

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⁺ excretion²⁸. 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 *Achyranthes aspera* 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 *Achyranthes aspera* have diuretic effect supporting the ethnopharmacological 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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Table 1. Diuretic activity of *Achyranthes aspera* leaves extracts.

| Extract | Dose | Electrolytic Labels | | |
|---------------|--------------|---------------------|------------------|-----------------|
| | | Na ⁺ | K ⁺ | Cl ⁻ |
| Aqueous | 250mg/kg b.w | 113.8 ± 2.042** | 66.60 ± 0.6429* | 127.3 ± 1.868** |
| Aqueous | 500mg/kg b.w | 127.8 ± 0.9849** | 73.60 ± 0.5196** | 155.6 ± 2.218** |
| Alcohol | 250mg/kg b.w | 120.5 ± 0.5196** | 71.20 ± 0.5033** | 147.5 ± 1.637** |
| Alcohol | 500mg/kg b.w | 136.2 ± 1.222** | 89.13 ± 0.2906** | 170.5 ± 1.947** |
| Furosemide | 20 mg/kg p.o | 145.2 ± 2.470** | 87.67 ± 1.782** | 174.3 ± 2.634** |
| Normal saline | 25 ml/kg p.o | 85.10 ± 2.892 | 59.03 ± 1.302 | 97.83 ± 1.126 |

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

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