ORIGINAL ARTICLE

Diuretic activity of extracts of *Centratherum anthelminticum*

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In the present study petroleum ether, chloroform and alcohol extracts of *Centratherum anthelminticum* (L.) Kuntze (family: Asteraceae) seeds (200 mg/kg b.w., p.o.) were tested for diuretic activity. The animals were grouped into five of six animals each. All the animals received priming dose of 0.9% sodium chloride solution (25 ml/kg b.w.). The first group served as control and the second group received the standard drug spiranolactone (20 mg/kg body weight) in 0.9% sodium chloride solution. The other three groups received petroleum ether, chloroform, and alcohol extracts of *C. anthelminticum* seeds in a dose of 200 mg/kg body weight suspended in 0.9% sodium chloride solution (p.o.). Urine volume was recorded for all the groups for 5 h. The highest diuretic activity was presented by alcohol extract followed by that of chloroform, petroleum ether extract has no significant diuretic activity. Alcohol and chloroform extracts significantly decreased K^+ excretion. We observed a potent diuretic and electrolyte excretion activity of chloroform and alcohol extracts of *C. anthelminticum* seeds. These findings suggest the possible traditional use of this plant in hypertension as diuretics are used in the management of hypertension.

Key words: *Centratherum anthelminticum*, diuretic activity, electrolyte excretion, hypertension, spiranolactone

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 toxaemia.[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^+ 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 justified.[4]

The plant *Centratherum anthelminticum* (L.) Kuntze is highly reputed in Hindu medicine as remedy for leucoderma and other skin diseases. The seeds have a hot sharp taste, acrid, astringent to the bowels, anthelmintic and cure ulcers. The seeds are used as purgative, for asthma, kidney troubles and hiccough, applied in inflammatory swelling, remove blood from liver, good for sores and itching of the eyes. In Punjab, it is considered as antipyretic. The seeds are also credited with tonic, stomachic, and diuretic properties.[5,6]

However, no systematic pharmacological studies have been carried out in order to confirm its diuretic activity. Hence, in the present study diuretic activity of three different extracts of *C. anthelminticum* seeds were investigated to justify the rationale behind using this plant as diuretic in hypertension.

MATERIALS AND METHODS

Animals

Albino Wistar rats weighing 150-200 g and albino mice weighing 20-30 g procured from registered breeder, CPCSEA approved, were used for diuretic and acute toxicity studies. Animals were kept at room temperature (26 ± 2°C) for one week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed but 18 h prior to the experiment, the rats were deprived of food but water *ad libitum*.

Plant Material

The seeds of *C. anthelminticum* (L.) Kuntze were collected from Hubli, Karnataka, and were authenticated by Dr. B. D. Huddar, Head, Department of Botany, S. K. Arts and H.S. Kothambri Science Institute, Hubli and voucher specimen has been deposited at the herbarium

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Received: 28-05-08; **Accepted:** 12-09-2008

for further reference.

Processing of Plant Material

The seeds of *C. anthelminticum* were shade dried at room temperature and were subjected to size reduction to get coarse powder of desired particle size. The powdered material was subjected to successive extraction in a Soxhlet apparatus using petroleum ether (60-80°C), chloroform and alcohol. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste. The yield was 13.60% w/w, 3.71% w/w and 14.21% w/w for petroleum ether, chloroform, and alcohol extract, respectively.

Drugs and Chemicals

All the drugs, chemicals, and reagents were procured from S.D. Fine Chemicals, (Mumbai, India). All the chemicals were of analytical grade.

Phytochemical Investigation

Qualitative chemical tests were conducted for above extracts of *C. anthelminticum* seeds to identify the various phytoconstituents.[7,8] The results of preliminary phytochemical investigation are shown in Table 1.

Acute Toxicity Studies

Approval of the Institutional Animals Ethics Committee was obtained prior to experimentation on animals. Acute toxicity studies were carried out as per OECD guidelines by employing the Up and Down method prior to evaluating each of the extracts for diuretic activity.

Diuretic Activity

The modified method of Rao was employed for the assessment of diuretic activity.[9] The animals were grouped into five of six animals each and they were fasted and deprived of food and water for 18 h prior to experiment. All the animals received priming dose of 0.9% sodium chloride solution (25 ml/kg b.w.). The first group received vehicle (saline) served as control and the second group served as the standard group, received the standard drug spiranolactone (20 mg/kg b.w.). The other three groups received petroleum ether, chloroform, and alcohol extracts of *C. anthelminticum* seeds in a dose of 200 mg/kg b.w. suspended in saline.

After oral administration, each animal was placed in an individual metabolic cage specially designed to separate faeces and urine at room temperature. The observed parameters were total urine volume for 5 h, urine pH, the electrolytes Na^+, K^+, and Cl^- excreted in urine.

The concentration of the electrolytes in urine is expressed in terms of mmol/L and the urine volume is expressed in ml/100g/5 h. Na+ and K^+ concentrations were measured by Flame photometer and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate as an indicator.[10,11] The ratio of the concentration of Na^+/K^+ and diuretic index at the end of 5 h, were calculated to assess the diuretic potential of the extracts of *C. anthelminticum*.

Statistical Analysis

All values are expressed as mean ± SEM. The values obtained for the above parameters in case of the extracts were compared with standard drug and control group by using One-Way ANOVA followed by Dunnett's test. The values of $P<0.05$, $P< 0.01$, and $P< 0.001$ were considered to indicate a significant difference between groups.

RESULTS

In the present study, petroleum ether, chloroform, and alcohol extracts of the *C. anthelminticum* (L) seeds were subjected to preliminary phytochemical chemical tests as shown in Table1. These tests revealed the presence of glycoside and sterols in petroleum ether extract, while chloroform extract was found to contain only sterols and alkaloids. Alcohol extract showed the presence of flavonoids, sterols, phenolic compounds, and alkaloids.

From acute toxicity studies, it was observed that animals were found to be safe up to a maximum dose of 2000 mg/kg b.w. But, there were few changes in the behavioral response like alertness, touch response, and restlessness. Therefore, 1/10th of the maximum tolerated dose 200 mg/kg b.w. was chosen for further the studies.

Table 1: Phytochemical constituents present in different extracts of *Centratherum anthelminticum* seeds

Chemical constituents	Petroleum ether extract	Chloroform extract	Alcohol extract
Flavonoids	—	—	+
Glycosides	+	—	—
Sterols	+	+	+
Saponins	—	—	—
Phenolic compounds	—	—	+
Alkaloids	—	+	+

+Present, —Absent

Table 2: Effect of oral administration of *Centratherum anthelminticum* extracts on urinary volume, electrolytic excretion and pH

Groups	Urine volume (ml/100g/5 h)	Urine pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^-	Na^+/K^+	T/C (Diuretic index)
Control	4.68 ± 0.62	6.58 ± 0.11	104.80 ± 1.30	92.17 ± 0.80	58.38 ± 5.30	1.14	—
Standard	8.20 ± 0.44***	7.10 ± 0.14	126.80 ± 1.30***	61.92 ± 2.10***	78.27 ± 1.72*	2.05	1.75
Pet ether extract	5.73 ± 0.38^{ns}	6.67 ± 0.67	113.00 ± 3.68^{ns}	89.34 ± 2.95^{ns}	55.36 ± 3.26	1.26	1.22
Chloroform extract	6.86 ± 0.30*	6.73 ± 0.50	118.30 ± 3.10**	78.90 ± 1.33 ***	60.88 ± 5.39	1.50	1.47
Alcohol extract	7.62 ± 0.58***	7.00 ± 0.06	122.80 ± 2.64 ***	72.54 ± 1.32***	62.47 ± 5.67	1.70	1.63

Values are given as mean ± SEM; n = 6, * $P < 0.05$,** $P < 0.01$, *** $P < 0.001$ considered for significance, (ANOVA followed by Dunnett's test). 'T' stands for urine collected for extracts, 'C' stands for urine collected for control

Significant diuretic activity was observed with chloroform and alcohol extracts of *C. anthelminticum* seeds comparable to the standard drug spiranolactone (20 mg/kg b.w.). Of the two extracts, alcohol extract was found to have more significant diuretic activity ($P< 0.001$). But, petroleum ether extract has no significant diuretic activity at the dose tested [Table 2]. There was no significant change in the urine pH in all the groups tested.

An increase in the excretion Na^+ was observed in all the extract treated groups, but significant only in chloroform and alcohol extracts ($P< 0.01, 0.001$).

There was a decrease in the excretion of K^+ in all the three extracts but again significant only in chloroform and alcohol extracts ($P< 0.001$).

Significant change was not observed with the excretion of Cl^- except in the standard group treated with spiranolactone, where there was a significant increase in Cl^- excretion ($P< 0.05$).

The present result shows significant diuretic potency and their effect on electrolyte excretion of chloroform and alcohol extracts of *C. anthelminticum* (L.) Kuntze seeds comparable to the standard drug spiranolactone.

DISCUSSION

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.[12]

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.

The results of the present study revealed that the chloroform and alcohol extracts of *C. anthelminticum* seeds posses a potent diuretic activity. The diuretic potency was comparable to that of the standard drug spiranolactone. Here, the chloroform and alcohol extracts of *C. anthelminticum* seeds increases the Na^+ excretion but decreases K^+ excretion, which may be acting like potassium sparing diuretic. The preliminary phytochemical analysis revealed that flavonoids, phenolic compounds, sterols, and alkaloids are present in alcohol extract while the chloroform extract was found to contain only alkaloids and sterols. These natural products might be acting individually or synergistically to produce diuresis. It is also possible that the alcohol extract might manifest cumulative effect of several active principles in the extract.[13] Since hypertension can be treated with diuretics, this study will provide basis for the traditional use of this plant in hypertension.

In conclusion, our results demonstrate both chloroform and alcohol extracts of *C. anthelminticum* seeds administered at the dose of 200 mg/kg b.w. (p.o.) has significant effects on urinary excretion of electrolytes and support the claims of diuretic efficacy of the title plant. The present study also provides basis for the traditional use of *C. anthelminticum* in hypertension.

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Source of Support: Nil, **Conflict of Interest:** None declared.

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