DIURETIC EVALUATION OF RHIZOMES OF *KYLLINGA NEMORALIS* (Hutch. & Dalz.)

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Summary

*Kyllinga nemoralis (Hutch.& Dalz.)*is an endemic species of the Ceylon and India, popularly known as "Nirbishi". This species has wide use in folk-medicine practice in these countries, especially as diuretic. So the aim of our study is to evaluate the diuretic activity of ethanol and petroleum ether extract of this species. Diuretic activity of ethanol and petroleum ether extract was observed at a dose of 100 mg/kg and 200 mg/kg body weight, respectively. Significant diuretic activity was evident till the fifth hour when rat were orally administrated with the extract at these concentrations. The diuretic activity of the extract was compared with that of the standard drug Fursemide at a dose of 20 mg/kg body weight. The maximum activity observed with both the extracts (200 mg/kg) doses at the fifth hour of study indicated its onset of diuretic action. The diuretic responses with its electrolyte excretion potency of the extract were remarkable in comparison with control animals. The extract, doses of 100 and 200mg/kg shows a dose dependent increase in volume of urine with moderate increase in Na+ and Ca^{2+} excretion, accompanied by the excretion of K+. In all cases the excretion of electrolytes and volume of urine comparable with the standard diuretic, Fursemide. The present results provide a quantitative basis explaining the traditional folk-medicine use of *Kyllinga nemoralis (Hutch.& Dalz.)*as a diuretic agent by the Ceylon Island and Indian population.

Keywords: Diuretic activity, *Kyllinga nemoralis* (Hutch.& Dalz.) rhizome, Ethanolic extract.

Introduction

Medicinal plants can be important sources of previously unknown chemical substances with potential therapeutic effects. The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry.
The World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for its basic health-care needs[1, 2]. *Kyllinga nemoralis (Hutch.& Dalz.)*(Family; Cyperaceae) [3], Nirbishi in Hindi, is a perennial herbs, grass-like in habit, the members this family grow chiefly in marshy and wet places. Underground sympodial rhizomes serve as organs of perennation. Stem, is an aerial shoot grass-like but in contrast to grasses, is solid and triangular. Leaves are simple, tristichous. Grass-like generally crowded near the base of the stem. This herb is found in India, Ceylon[3] and other part of the world and abundant in the plains. The paste of plant rhizome can be used to treat sprains and contusions[4]. Phytochemically, rhizomes of *Kyllinga nemoralis (Hutch.& Dalz.)*are found to contain glycosides, saponins, flavanoids, phenolic compounds, tri-terpinoides, carbohydrates and fixed oils and fat. Since the advocated diuretic potential of *Kyllinga nemoralis (Hutch.& Dalz.)* rhizomes were not tested rigorously by scientifically controlled experiments, this study was undertaken to investigate the diuretic potential of a ethanol and petroleum ether extract of *Kyllinga nemoralis (Hutch.& Dalz.)* rhizomes using albino rats.

Materials and methods

Plant material

The rhizomes of *Kyllinga nemoralis (Hutch.& Dalz.)* were collected from the Central Leather Research Institute (CLRI), Chennai, Tamilnadu. They were identified by Dr. P. Jeyaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The specimen has been preserved.

Extracts preparation

The rhizomes of *Kyllinga nemoralis (Hutch.& Dalz.)* were air-dried in an oven at 40 ◦C for 4 days and then the dry rhizome was cut and grinded to a powder by mechanical milling. The dried powdered plant material was subjected to continuous extraction in a Soxhlet extractor for 2 days using 100% ethanol and petroleum ether as solvents. The solvent was then recovered by vacuum distillation in a rotary vacuum evaporator (Buchler Corp.), representing a yield of 12.4% and 13.7% respectively of the dry material extracted. For pharmacological studies, the fractions were given orally to laboratory rats in a final volume of 100, 200 mg/kg bodyweight (bw).

Animals
Healthy adult crossbred male albino rats (weighing 150–180 gm) obtained from the King Institute, Chennai. These animals were used for the experiments.

Drugs
Frusemide (Sigma Chemical Co.) was used as a reference diuretic drug.

Acute Oral Toxicity
Five Wistar Albino rats of male sex having weight 150-180 gm were used for this study. Fixed dose levels of 50, 100, 200, 500, 1000 mg/kg were given initially to allow identification of a dose producing evident toxicity for the ethanolic rhizome extract of *Kyllinga nemoralis*. After giving the dose the toxic signs were observed within 48 hrs. food was withheld for a further 3-4 hrs after administration of the drug. The further 2000mg/kg was administered after the last dose and observed for the mortality. Same procedure is applied for the petroleum ether extract.

Diuretic activity
Diuretic activity was determined by following the methods of Lipschitiz et al. (1943)[5]. Male rats were divided into six groups, of six animals each; each weighing 150 to 180 gm were fasted and deprived of water for 18hrs prior to the experiment. On the day of experiment all the animals are given normal saline orally (25 ml/kg bw). Immediately after dosing, the animals were placed in metabolic cages specially designed to separate urine and feces and kept at room temperature of 25 ± 0.5◦C. The urine was collected in measuring cylinder up to 5hrs after dosing. During this period no water or food are made available to the animals. The total volume of urine collected was measured for the control and treated groups. The parameters taken for each individual rat were, total urine volume, urine concentration of Na+, K+ and Ca^{2+} were measured by flame photometer.

Statistical analysis
The experimental results were expressed as the mean ± standard error of mean and the statistical significance was evaluated by using Student's t-test.

Results and Discussion

Diuretic activity
The urine volume analyzed for the ethanol and petroleum ether extract of *Kyllinga nemoralis (Hutch.& Dalz.)* in the test animals, as well as the electrolytes (Na+, K+ and Ca^{2+}) content of the urine samples tested, are included in Table 1.It shows the urinary volume (in ml) and the electrolyte (Na+, K+ and Ca^{2+}) content (meq/Lt) in the urine of animals treated with *Kyllinga nemoralis (Hutch.& Dalz.)* extracts, Frusemide and control groups, Table 1 also shows that the *Kyllinga nemoralis (Hutch.& Dalz.)* petroleum ether extract induced an important degree of urinary excretion beginning with the lowest dose administered; the two administered doses increase excretion levels of urine which is compared with the untreated controls.

This effect was repeated in rats receiving the ethanolic extract for the 100 and 200mg/kg doses respectively, the latter dosage producing a similar effect to that obtained with Frusemide. Electrolyte excretion induced by *Kyllinga nemoralis (Hutch.& Dalz.)* showed a dose-dependent increase for both the ethanolic and the petroleum ether extract when compared to the control group, in parallel with the urinary excretion.

Table 1: Diuretic activity of ethanolic and petroleum ether extract of rhizome of *Kyllinga nemoralis (Hutch.& Dalz.)*

	Dose	Urine volume (ml)	Na^+ meq/Lt	K^+ meq/Lt	Ca^{2+} meq/Lt
Control (Normal Saline)	25 ml/kg	2.52± .09	96.2±2.83	98.7 ± .94	86.2 ± 2.3
Ethanolic extract	100 mg/kg	2.72±0.14	120.2±v83	85.1 ± 0.28	86.7 ± 5.3
Ethanolic extract	200 mg/kg	3.25±0.25*	125.6±9.7*	87.7 ± 0.35*	90.25 ± 7.5*
Petroleum Ether extract	100 mg/kg	2.64±0.08	105.0±1.42	84.3±0.68	83.3 ± 1.41
Petroleum Ether extract	200 mg/kg	3.13±0.09*	114.3±9.3*	86.5 ± 4.1*	88.2 ± 6.8*
Furosemide	20 mg/kg	3.55±0.09*	138.8±0.19*	94.6 ± 1.49*	95.0 ±1.35*

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Fig. 1 Ion concentration of urine from different groups treated.

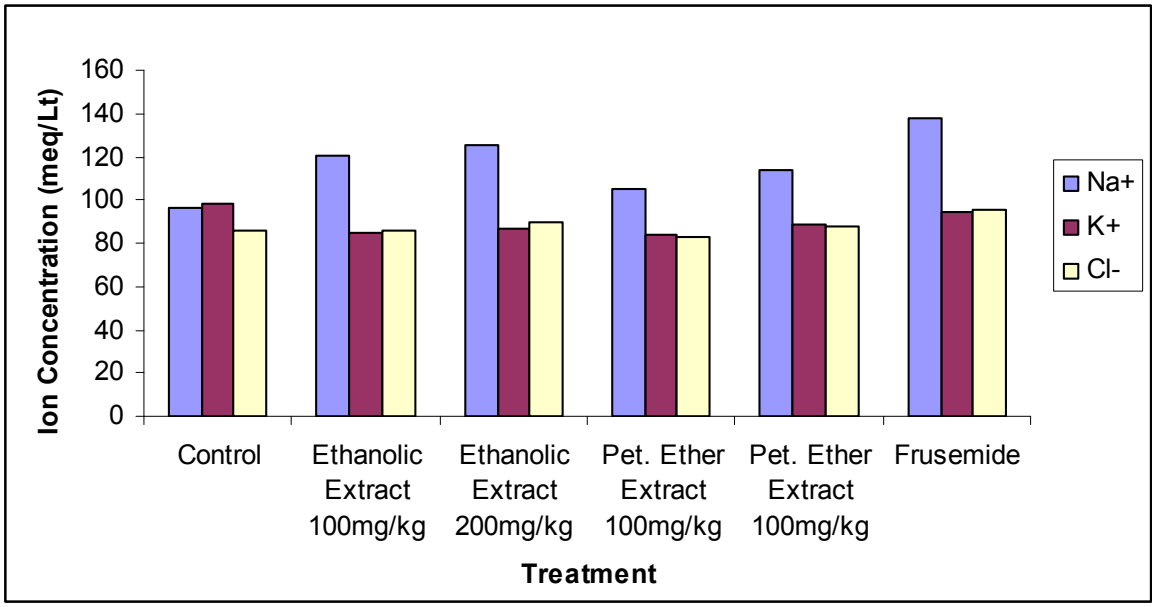
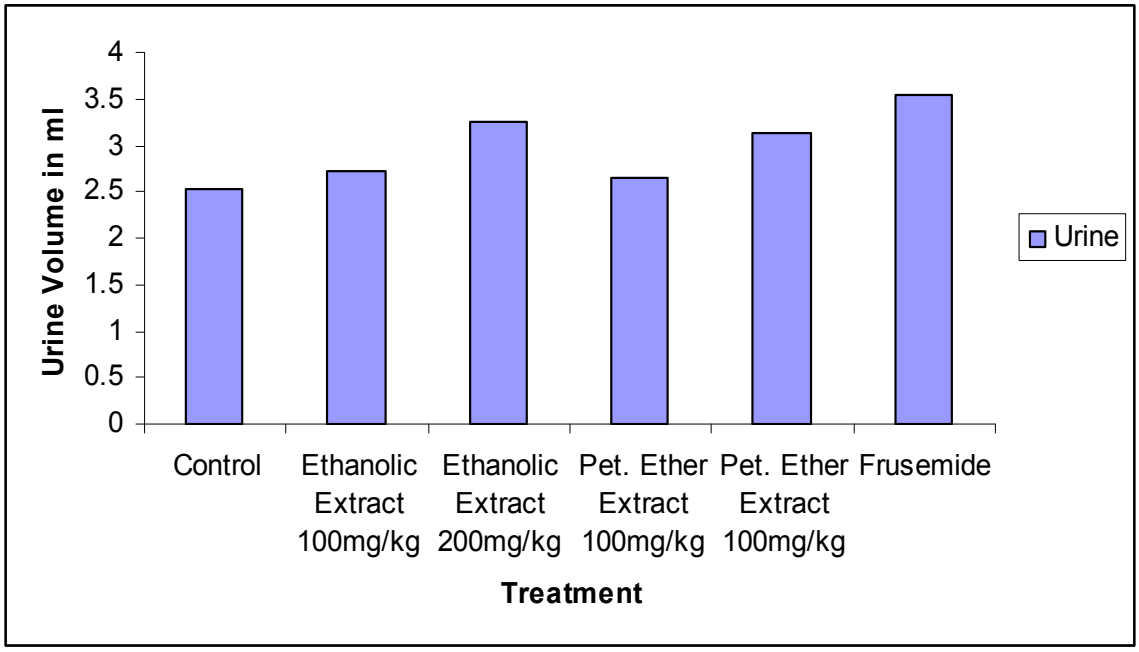


Fig. 2 Volume of urine from different treated groups.



Acute Oral Toxicity

LD_{50} of the ethanol and petroleum ether extract of *Kyllinga nemoralis (Hutch.& Dalz.)* was done as per OECD guidelines (Revised draft 423). The ethanolic and petroleum ether extracts of *Kyllinga nemoralis (Hutch.& Dalz.)* falls under class 4 (LD_{50} > 2000 mg/kg). The animals did not show any signs of toxicity and behavioral changes.

Conclusion

The method of Lipschitiz et al. (1943) was employed for the assessment of diuretic activity. Ethanolic extract 200 mg/kg shows significant diuretic activity near to standard drug. Ethanolic extract shows better result when compared with petroleum ether extract.

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