DIURETIC ACTIVITY OF ROOT EXTRACT OF *RUBIA CORDIFOLIA* LINN.

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Summary

Rubia cordifolia Linn. (family: Rubiaceae) is known as Indian madder. The present investigation was carried out to evaluate the diuretic activity of the hydroalcoholic extract of roots of *Rubia cordifolia* in rats to support its folklore claim. Roots of *Rubia cordifolia* was coarsely powdered and extracted using 70% ethanol with soxhlet extractor for 22h. Four groups of rats were treated with vehicle (normal saline: 25 ml/kg), furosemide (20 mg/kg) and two doses of extract (286 mg/kg and 667 mg/kg body weight) orally. Urine excreted was collected up to 5h post-treatment and analyzed for urine volume, Na^+, K^+, Cl^- and creatinine content. The extract showed a significant ($p<0.01$) and dose dependent increase in urine volume and electrolyte excretion. Both doses of extract showed less influence on creatinine clearance than furosemide. The result indicates that hydroalcoholic extract of roots of *Rubia cordifolia* possess potent diuretic activity.

Key words: Diuretic activity, *Rubia cordifolia*, Root, Creatinine clearance.

Introduction

Rubia cordifolia Linn. (family: Rubiaceae) is a perennial, climber herb with very long cylindrical, flexuose roots with a thin red barks. It is distributed throughout the lower hills of Indian Himalayas and Western Ghats in India, Japan, Indonesia, Ceylon, Malay, Peninsula, Java and tropical Africa in moist temperate and tropical forests, up to an altitude of 3500 m [1]. In traditional system of medicine, roots are used as antidysenteric, astringent, antiseptic, antipyretic, analgesics, anti-inflammatory, anthelmintic, blood purifier, laxative, carminative and diuretic [1-3]. The root extract has been studied for its antioxidant, anti-inflammatory, anti-arthritic, analgesic, anti-cancer, hepatoprotective, hypoglycaemic, antihyperglycemic, antistress, nootropic, free radical scavenging, anti-platelet activating factor and immunomodulatory activities [4-17].

On the basis of the traditional use of the plant as a diuretic, the present study was carried out using hydroalcoholic extract of *Rubia cordifolia* Linn. root in an experimental model, to substantiate the folklore claim.

Method

Plant material: The roots of *Rubia cordifolia* were obtained from Sami labs Ltd. Bangalore, Karnataka, India as a gift sample for research purpose. The roots were dried under shade, powdered and stored in an airtight container.

Preparation of extract: The dried, powdered roots were extracted using 70% v/v ethanol in distilled water using a soxhlet extraction apparatus for 22 h. The extract was dried using vacuum oven. A brown-colored, semisolid mass was obtained with 24.8 % w/v yield. The extract was stored in desiccator for further study.

Phytochemical analysis of the extract: The hydroalcoholic extract of roots of *Rubia cordifolia* Linn. was subjected to qualitative analysis for the various phytoconstituents. Standard methods [18-19] were used for preliminary qualitative phytochemical analysis of extract. The analysis revealed the presence of carbohydrates, proteins and amino acids, fats and oils, cardiac glycosides, anthraquinone glycosides, saponins and steroids in hydroalcoholic extract of roots of *Rubia cordifolia* Linn.

Animals: Female Wistar albino rats weighing between 150-200 g each were used for this experiment. They were procured from Indian Institutes of Sciences, Bangalore, India. The animals were allowed for acclimatization for ten days under standard condition in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). They were housed in polypropylene cages and maintained at 27 ± 2°C, relative humidity 65 ± 10% under 12 h light/dark cycles. The animals were given standard diet supplied by Pranav Agro Industries Ltd, Sangli, India. The study protocol was approved from the Institutional Animal Ethics Committee (Ref. No.: IAEC/PP/03/2008-2009).

Chemicals: Furosemide, a standard drug was supplied by Aventis Pharma Ltd, Ankleshwar. India. Sodium chloride AR (B. No. 0000025201) and potassium chloride AR (B. No. 0000030765) were purchased from Himedia Laboratories. Mumbai, India.

Acute toxicity study: Acute toxicity study of hydroalcoholic extract of root of *Rubia cordifolia* was determined by acute toxic class method of OECD Guidelines [20]. Two dose levels (286 and 667 mg/kg orally) were selected for evaluation of diuretic activity.

Evaluation of Diuretic activity: The methods of Biswas et al [21], Mamun et al [22] were employed for the assessment of diuretic activity. The animals were fasted with free access to water only for 18 h prior to the experiment and were divided into four groups of animals containing six each. First group served as vehicle control (0.5 % w/v Gum acacia in normal saline at dose of 25 ml/kg body weight). The second (furosemide: 20 mg/kg), third (Extract: 286 mg/kg) and fourth group (Extract: 667 mg/kg) received same amount of normal saline in which furosemide and extract was suspended using 0.5 % Gum acacia as suspending agent.
Immediately after the administration of the drug, the rats were placed in metabolic cages (Tecniplast, Italy). During this period of the experiment, animals were deprived of food and water. The urine excreted by the animals was collected and measured up to 5 hours and analysed for Na^+, K^+, Cl^- and creatinine content. Blood sample was collected by retro-orbital puncture and plasma was analyzed for creatinine content. Glomerular filtration rate (GFR) was evaluated by the clearance of creatinine. The concentration of sodium and potassium was analyzed by flame photometer (Systronics, Mumbai, India) and the amount of chloride was determined titrimetrically by silver nitrate solution using diphenylcarbazone as indicator [23]. Creatinine in urine and serum sample was estimated using kit by Reckon diagnostics, India in a semiautoanalyzer (Metrolab1600-DR).

Statistical Analysis: The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett comparison test. *p*-values were calculated against vehicle control groups and $p<0.05$ was considered significant.

Results

Table 1: Urinary volume and electrolyte excretion of control and treatment groups.

Groups	Urine Volume (ml/min/animal)	Na^+ Excreted in urine (meq/5h/animal)	K^+ Excreted in urine (meq/5h/animal)	Na^+/K^+ Ratio	Cl^- Excreted in urine (meq/5h/animal)	GFR = Creatinine clearance (ml/min)
Vehicle control	0.87±0.03	0.09 ± 0.004	0.07 ± 0.002	1.28	0.13 ± 0.004	0.18 ± 0.004
Furosemide (20 mg/kg)	6.60±0.24**	1.29 ± 0.047**	0.31 ± 0.002**	4.16	1.48 ± 0.054**	0.43 ± 0.009**
Extract (286 mg/kg)	1.97±0.04**	0.36 ± 0.009**	0.12 ± 0.002**	3.00	0.33 ± 0.013**	0.26 ± 0.007*
Extract (667 mg/kg)	3.33±0.12**	0.62 ± 0.020**	0.23 ± 0.011**	2.69	0.64 ± 0.022**	0.33 ± 0.031**

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Number of animals (N) = 6, Values are expressed as mean ± SEM.

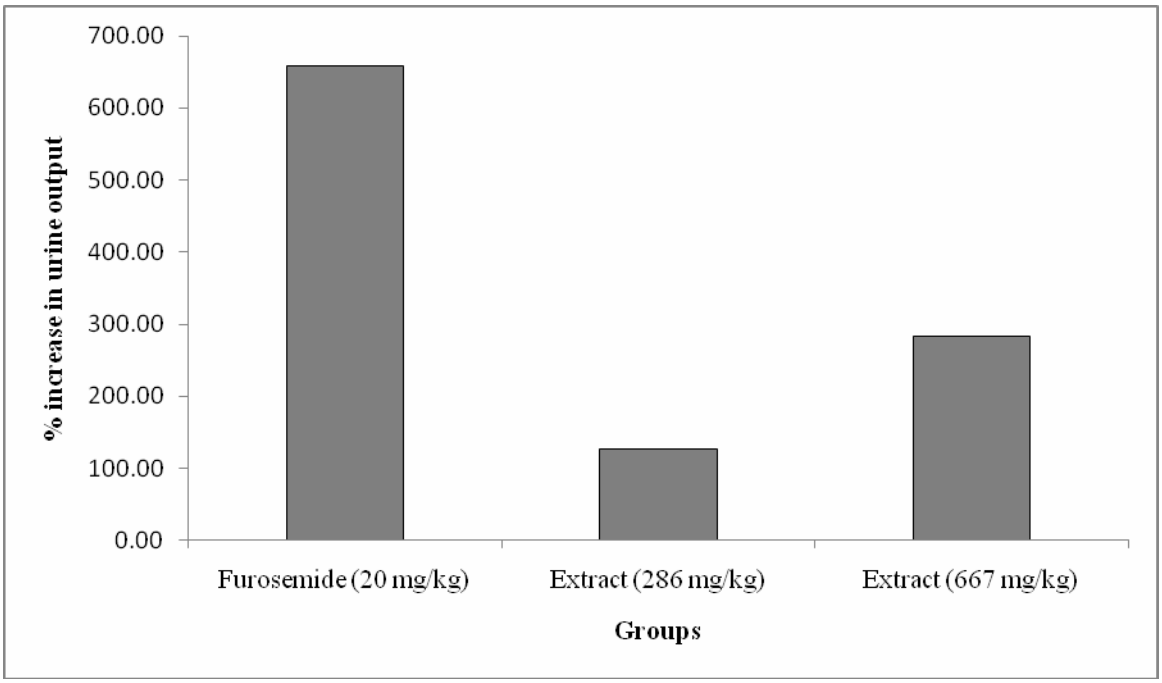
Urinary output and electrolyte excretion of control and treatment groups are presented in Table no. 1. The results showed that the reference diuretic furosemide and all doses of extract tested in the study caused a very significant ($p < 0.01$) increase in volume and electrolyte excretion as compared to vehicle control. There was 126.44 % (from 0.87±0.03 to 1.97±0.04) and 282.76 % (from 0.87±0.03 to 3.33±0.12) increase in urine output by the 286 and 667 mg/kg extract treatment respectively (Bar chart. 1).

The lower dose of extract (286 mg/kg) increased the Na^+, K^+ and Cl^- excretion by 300 % (from 0.09 ± 0.004 to 0.36 ± 0.009 meq/5h/animal), 71.43 % (from 0.07 ± 0.002 to 0.12 ± 0.002 meq/5h/animal) and 153.85 % (from 0.13 ± 0.004 to 0.33 ± 0.013 meq/5h/animal) respectively than that produced by vehicle control (Bar chart. 2).

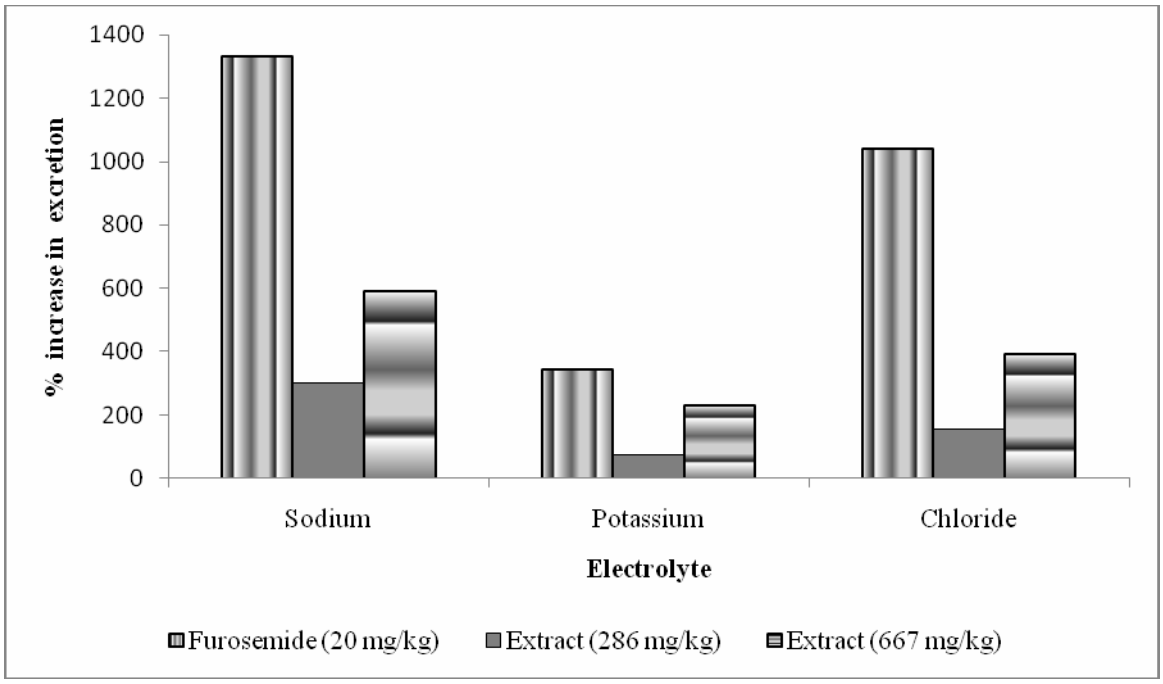
The higher dose of extract (667 mg/kg) increased the Na^+, K^+ and Cl^- excretion by 588.88 % (from 0.09 ± 0.004 to 0.62 ± 0.020 meq/5h/animal), 228.57 % (from 0.07 ± 0.002 to 0.23 ± 0.011 meq/5h/animal) and 392.31 % (from 0.13 ± 0.004 to 0.64 ± 0.022 meq/5h/animal) respectively than that produced by vehicle control (Bar chart. 2).

Both doses of plant extract (667 and 286 mg/kg) and furosemide caused a very significant ($p < 0.01$) increase in creatinine clearance (GFR). At 667 and 286 mg/kg doses, the GFR increased to 83.33 and 44.44 percent of the control value respectively. Furosemide treatment produced a larger increase (138.88 %) in the GFR (Bar chart. 3).

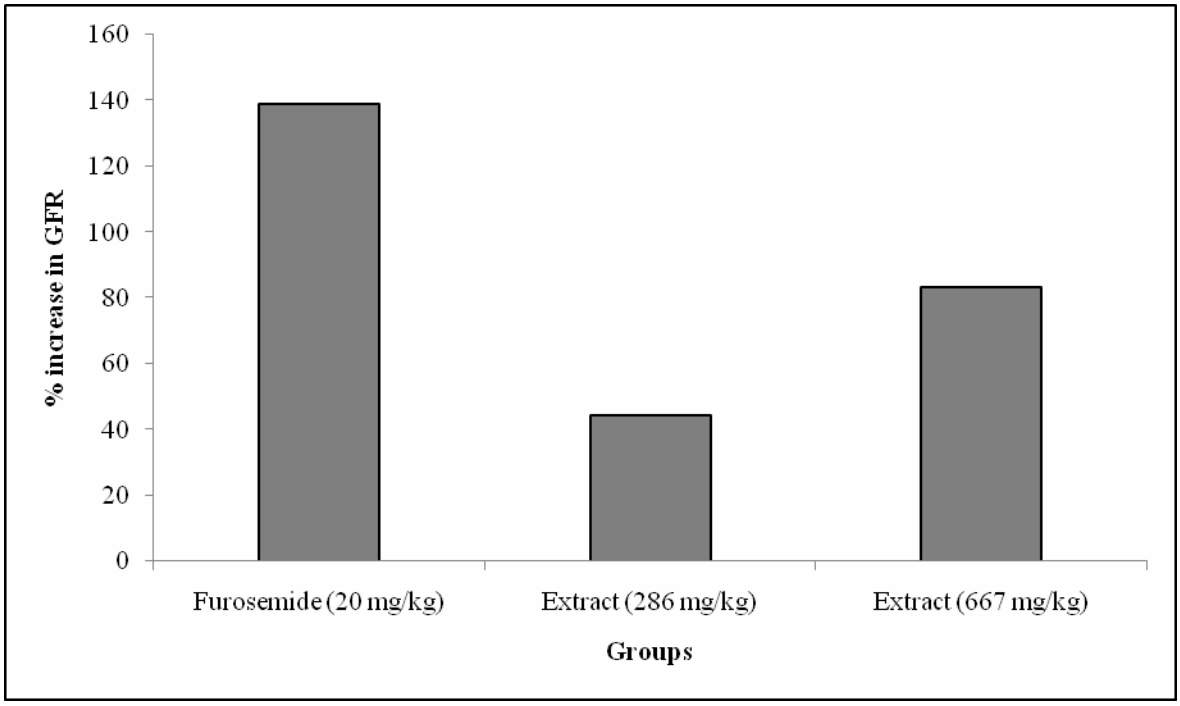
There was an increase in the ratio of concentration of excreted sodium and potassium ions after plant extract treatment. The sodium/potassium excretion ratios were 2.69, 3 and 4.16 for 286 and 667 mg/kg of the plant extract and furosemide respectively.



Bar chart 1. Comparison of percent increase of urine output in treatment groups.
Data are expressed as mean ± SEM., n = 6 rats per group.



Bar chart 2. Comparison of percent increase of urinary electrolyte excretion in treatment groups.
Data are expressed as mean ± SEM., n = 6 rats per group.



Bar chart 3. Comparison of percent increase in GFR in control and treatment groups.
Data are expressed as mean ± SEM., n = 6 rats per group.

Discussion

The graded doses of the root extract of *Rubia cordifolia Linn* in normal saline showed a very significant increase in diuresis, natriuresis, kaliuresis, chloride excretion and GFR at all examined doses.

The diuretic action of root extract of *Rubia cordifolia Linn* was compared with the effect of furosemide, the reference drug. The hydroalcoholic extract of roots of *Rubia cordifolia* Linn. produced an effect on diuresis and urinary excretion of Na^+, K^+ and Cl^- similar to that of furosemide, but at a lower potency which may be due to the crude nature of the extract. Because of the similar diuretic and saluretic activity of the extract, it is likely that the active component (s) of the roots of *Rubia cordifolia* Linn. has a furosemide-like action.

There was an increase in the ratio of concentration of excreted sodium and potassium ions after plant extract treatment. This indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect.

The observed diuretic effect may be due to the effect of extract on the glomerular filtration rate and the direct inhibitory effect on the reabsorption mechanism of the salt.

The *Rubia cordifolia Linn* root extract and furosemide increased GFR significantly. The increase in GFR by the extract is possibly due to (a) a detergent like interaction with structural components of glomerular membranes (b) a decrease in renal perfusion pressure, attributable to decrease in the resistance of the afferent arteriole, and/or an increase in the resistance of the efferent arteriole and/or (c) the direct effect on the arteriole wall affecting glomerular blood flow [24].

The direct inhibitory effect of the extract on the reabsorptive mechanism of the salt can be attributed to the presence of saponins. It has been shown that saponins, in general, have emulsifying-detergent properties and affect cell membrane permeability by inducing alteration in the lipidic structural organization. In addition, detergents affect the activity of Na^+-K^+-ATPase and alter Na^+ transport, water permeability, hormone receptor, ionic channel activities [25-26].

This study validated the diuretic use of root of *Rubia cordifolia* Linn in traditional system of medicine. The precise site(s), the molecular and cellular mechanism(s) and the active component(s) responsible for the diuretic activity remain to be elucidated.

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