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Research Paper

Acute and chronic diuretic effect of ethanolic extract of leaves of *Cocculus hirsutus* (L.) Diles in normal rats

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Abstract

Objectives The objective of this study was to evaluate the acute and chronic diuretic effect of the ethanolic extract of the leaves of *Cocculus hirsutus* (L.) Diles.

Methods The ethanolic extract was administered (100, 200 and 400 mg/kg, p.o.) in Wistar rats. In the acute study, rats received drugs orally and urine was collected after 1, 2, 3, 4, 5 and 6 h. The chronic study involved repeated administration of ethanolic extract for 28 days and urine was collected on day 1, 7, 14, 21 and 28. The parameters were total urine volume, concentration of Na^+, K^+ and Cl^- ions, creatinine in urine and serum. Urine output, electrolytes (Na^+, K^+ and Cl^- ions) and creatinine were determined on day 7, 14, 21 and 28.

Key findings The highest dose (400 mg/kg) of the ethanolic extract significantly ($P < 0.01$) enhanced urine output. Excretion of cations (Na^+ and K^+ ions) and anions (Cl^- ions) increased significantly with respect to the control (gum acacia 2% dissolved in saline, 10 ml/kg) group. The increase of cations in the urine after treatment with ethanolic extract was dose dependent. The ethanolic extract of the leaves of *C. hirsutus* (100, 200 and 400 mg/kg) and furosemide (10 mg/kg) did not significantly change the concentration of Na^+, K^+ and Cl^- ions in serum. The ethanolic extract of the leaves of *C. hirsutus* (100, 200 and 400 mg/kg) and furosemide (10 mg/kg) increased the excretion of creatinine in urine but with a corresponding decrease in serum.

Conclusions It was concluded that the ethanolic extract of the leaves of *C. hirsutus* (400 mg/kg) had significant diuretic effect in rats.

Keywords *Cocculus hirsutus*; diuretic; electrolytes; urine output

Introduction

Plant medicine has been used commonly for the traditional treatment of some renal diseases, and a number of plants have been reported to possess significant diuretic activity. Studies of herbal plants used as diuretics in traditional medicine have shown progressive growth in the last few decades.[1]

Cocculus hirsutus (L.) Diels (Family: Menispermaceae) is a widely growing plant found in the plains of India in dry localities. It is used medicinally by the Indian tribes for a wide range of ailments, including constipation and kidney problems.[2,3] Roots of *C. hirsutus* have been mentioned to possess anti-inflammatory and analgesic properties.[4] The juice from the leaves of *C. hirsutus* coagulates in water and forms mucilage, which is used externally as a cooling and soothing application in prurigo, eczema and impetigo, as a diuretic and in gout.[5] The aqueous extract of leaves of *C. hirsutus* possesses antihyperglycaemic activity in alloxan-induced diabetic mice.[6] The methanolic extract of the roots of *C. hirsutus* have been reported to possess hypoglycaemic and cardiotonic effects on diabetic rats and isolated perfused frog heart, respectively.[7] Sangameswaran and Jayakar[8] reported the antidiabetic and spermatogenic activity of *C. hirsutus*. The ethanolic extract of leaves of *C. hirsutus* have been reported to possess an antiurolithiatic effect.[9]

The aqueous extract of the aerial parts of *C. hirsutus* have been reported to possess diuretic and laxative activity in acute studies.[2] The objective of this study was to evaluate

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the acute and chronic diuretic effect of the ethanolic extract of leaves of *C. hirsutus* (CH-Eth) in normal rats.

Materials and Methods

Plant material and preparation of extract

The plant of *C. hirsutus* (locally called Vasanvel/Tana) was collected during April–June 2006 from the hilly area of Katraj Ghat, Pune. It was authenticated at Agharkar Research Institute, Pune, India and a voucher specimen was deposited at that Institute (specimen number WP-021). The leaves of *C. hirsutus* were shade-dried and powdered (mesh size-16) in a grinder. The leaf powder (500 g) was macerated in 99.9% ethanol (2.5 l) using a Soxhlet apparatus and filtered. The filtrate was dried on a tray dryer at 40°C (yield 4.29%, w/w). The semisolid ethanolic extract was suspended with 2% gum acacia in normal saline to prepare the drug solution (concentration 100 mg/ml) for the pharmacological study.

Chemicals and apparatus

Creatinine kit (Accurex Biomedical Pvt. Ltd, Mumbai, India), furosemide (Lasix-Hoechst, Novartis Pharma, Pvt. Ltd, Mumbai, India), anaesthetic ether (TKM Pharma, Hyderabad, India), gum acacia (Research-Lab, Mumbai, India), ethanol (Changshu Yangyuan Chemicals, Shanghai, China) and centrifuge tubes (2.5 ml; Torson, Mumbai, India) were purchased from the respective vendors. Apparatus such as the metabolic cages (Technoplast, Italy), Biolyte 2000 (electrolyte analyser, Tiawan), auto-analyser (Secomam, Japan) and pH meter (model no- EQ 614, Lab India, Mumbai, India) were used in the study.

Animals and research protocol approval

Female Swiss albino mice (18–22 g) and male Wistar rats (150–250 g) were purchased from National Toxicology Centre, Pune, India. They were maintained at a temperature of 25 ± 1°C and relative humidity of 45–55% under a 12-h light : dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) and water was freely available. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

Acute oral toxicity study

The ethanolic extract was tested for its acute oral toxicity in healthy adult female mice as per guidelines (AOT No. 425) suggested by the Organization for Economic Co-operation and Development.[10] The ethanolic extract of the leaves of *C. hirsutus* at different doses (175, 550, 1750 and 2000 mg/kg, p.o.) were administered to different groups of mice ($n = 6$). The control mice received 2% gum acacia. Mortality and general behavioural, neurological and autonomic profiles of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following administration of the ethanolic extract of the leaves of *C. hirsutus*. The LD50 value (the dose required to kill 50% of the population) was determined using AOT software (Westat, EPA, US).

Diuretic activity of ethanolic extract of the leaves of *C. hirsutus*

The animals were randomly divided into five groups ($n = 6$): group 1, control (gum acacia 2% suspended in saline, 10 ml/kg); group 2, furosemide (10 mg/kg); group 3, ethanolic extract of the leaves of *C. hirsutus* (100 mg/kg); group 4, ethanolic extract (200 mg/kg); and group 5, ethanolic extract (400 mg/kg). The ethanolic extract and furosemide were given orally. The animals were placed individually in metabolic cages after administration of the ethanolic extract and furosemide. Rats were fasted overnight and allowed free access to water.

In the acute study, rats received ethanolic extract and furosemide orally and urine was collected after 1, 2, 3, 4, 5 and 6 h by placing them in metabolic cages.[11]

The chronic study involved repeated administration of the ethanolic extract and furosemide for 28 days (once a day) at a predetermined time. The animals were placed in metabolic cages on the 1st, 7th, 14th, 21st and 28th day and urine passed over the next 24 h was collected in graduated cylinders, filtered and urine volume was measured. Serum creatinine and electrolytes determination were obtained under anaesthesia. Blood samples were centrifuged at 7000 rev/min for 20 min at 4°C. Concentrations of Na^+, K^+ and Cl^- in the urine and serum were determined using the Biolyte 2000. Creatinine in urine and serum were determined by using an auto-analyser.[12,13]

Statistical analysis

Data was expressed as mean ± SEM and statistical analysis was carried out by one-way analysis of variance with post-hoc Dunnett's test performed using GraphPad InStat version 3.00 for Windows Vista BASIC, GraphPad Software, San Diego, California, US (www.graphpad.com). A P value was considered significant when < 0.05.

Results

Acute toxicity studies

Acute toxicity studies revealed that ethanolic extract of the leaves of *C. hirsutus* was safe up to 2000 mg/kg (p.o.) of body weight. The LD50 of the ethanolic extract was observed to be more than 2000 mg/kg. Based on the results obtained from this study, the doses for further pharmacological study were fixed to be 100, 200 and 400 mg/kg (p.o.).

Effect of the ethanolic extract of the leaves of *C. hirsutus* on urine output in rats

In the acute study, single oral administration of the ethanolic extract (100 mg/kg) significantly ($P < 0.01$) increased urine output at 2, 3 and 4 h as compared with the control group. The higher dose i.e. ethanolic extract 200 and 400 mg/kg, produced significant ($P < 0.01$) increase in urine output at 6 h compared with the control group. Furosemide (10 mg/kg) showed significant ($P < 0.01$) increase in urine output (13.15 ml) at 2 h compared with the control group. It was followed by a gradual decrease in urine output up to 6 h.

The onset and maximum effect revealed differences among the drugs. The onset of diuretic effect of furosemide was rapid and maximum diuretic effect was at 1 h and peak was reached in 2 h. The diuretic effect gradually decreased at the subsequent hours. On the other hand the ethanolic extract had a tardy onset and prolonged duration of action (Figure 1).

In the chronic study, repeated oral administration of the ethanolic extract increased the urinary output in a dose-dependent manner. The calculated difference in urine output observed after 24 h of treatment on day 28 and day 0 with 100, 200 or 400 mg/kg of the ethanolic extract was 15.60, 18.75 and 23.80 ml, respectively. The corresponding urine output value difference in the case of furosemide was 22.45 ml and the control group was 14.5 ml. The highest dose (400 mg/kg) of the ethanolic extract showed similar diuresis as compared with furosemide (10 mg/kg, p.o.) (Figure 1).

Effect of the ethanolic extract of the leaves of *C. hirsutus* on the concentrations of sodium in rat urine and serum

Administration of the ethanolic extract (100, 200 and 400 mg/kg, p.o.) resulted in increased excretion of sodium (Na^+) in urine compared with control group at all time intervals. The highest dose (400 mg/kg, p.o.) caused maximum Na^+ concentration excretion on day 1. Furosemide on the other hand caused an increase in Na^+ excretion on day 1 followed by gradual decrease during the subsequent days. The result indicated natriuretic activity of the ethanolic extract (Figure 2).

Chronic administration of the ethanolic extract (100, 200 and 400 mg/kg, p.o.) did not change Na^+ concentration in serum compared with control-treated group (Table 1).

Effect of the ethanolic extract of the leaves of *C. hirsutus* on the concentrations of potassium in rat urine and serum

Single administration of furosemide (10 mg/kg, p.o.) increased excretion of potassium (K^+) at 4 h compared with 4 h by ethanolic extract. The ethanolic extract (400 mg/kg) appeared to be more effective than furosemide in the excretion of K^+.

Chronic administration of the ethanolic extract (100, 200 and 400 mg/kg, p.o.) resulted in significant increase ($P < 0.01$) in excretion of K^+ in a dose-dependent manner in urine compared with the control group. Furosemide (10 mg/kg) showed significant change in K^+ excretion in urine on

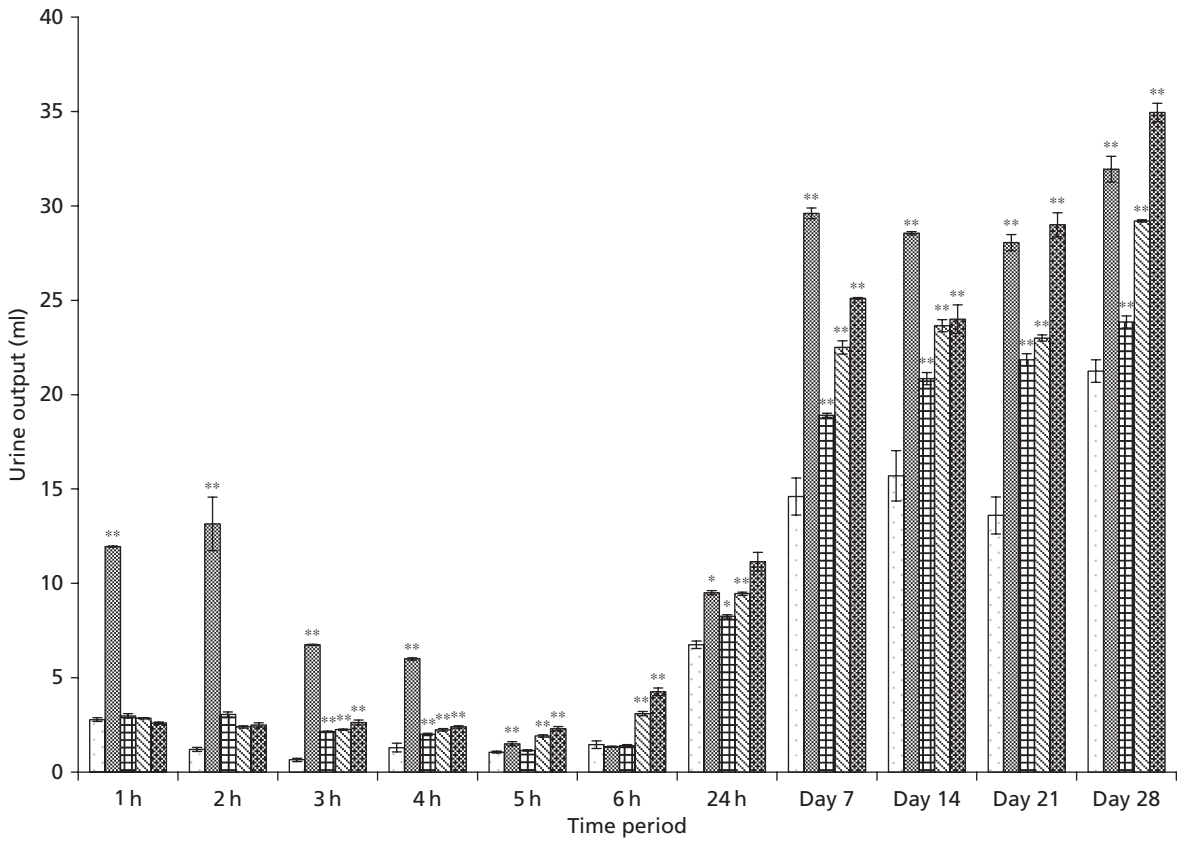

Figure 1 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on urine output in rats. CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*. Values are mean ± SEM, $n = 6$ in each group. Statistical analysis by one-way analysis of variance followed by post-hoc Dunnett's test using Graphpad Instat software. *$P < 0.05$, **$P < 0.01$ compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). Where, (□) control; (▨) Furosemide (10 mg/kg); (▦) CH-Eth (100 mg/kg); (▧) CH-Eth (200 mg/kg); (▩) CH-Eth (400 mg/kg).

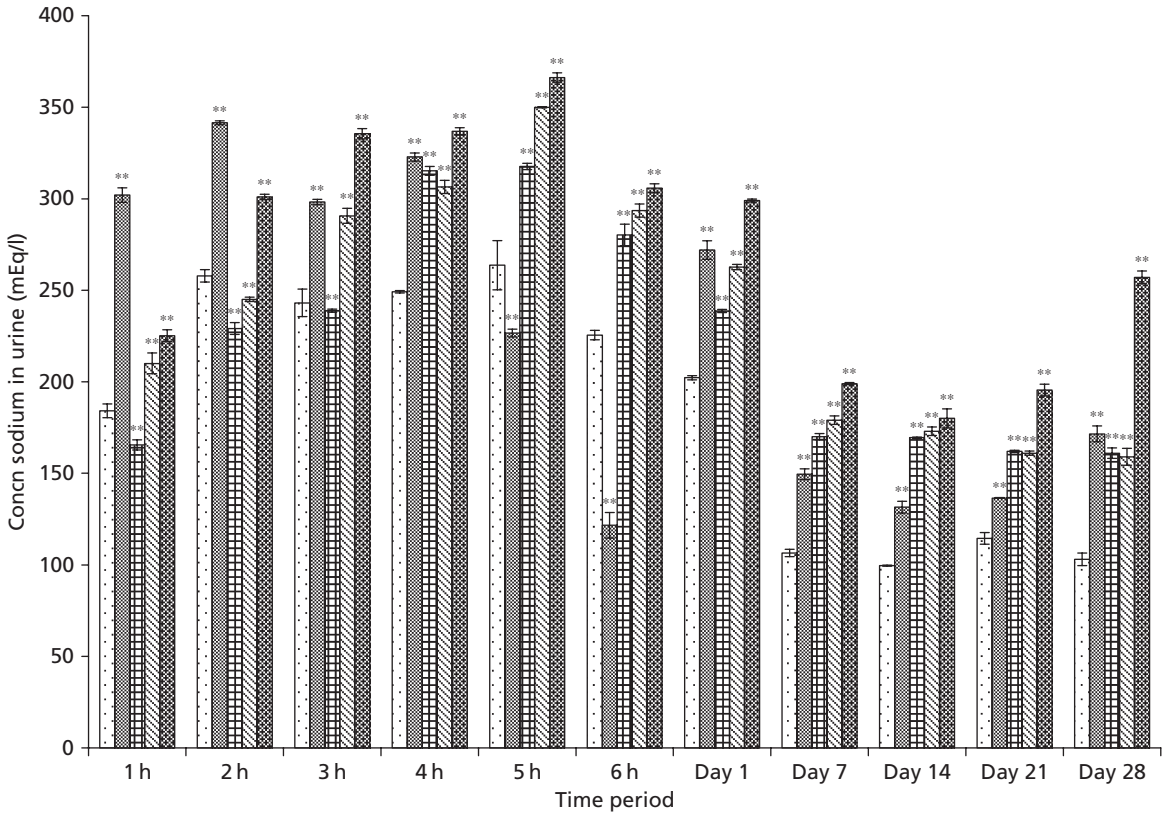

Figure 2 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on the concentrations of sodium in rat urine. CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*. Values are mean ± SEM, $n = 6$ in each group. Statistical analysis was by one-way analysis of variance followed by post-hoc Dunnett's test using Graphpad Instat software. $^{**}P < 0.01$ compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). Where, (□) control; (▨) Furosemide (10 mg/kg); (▦) CH-Eth (100 mg/kg); (▧) CH-Eth (200 mg/kg); (▩) CH-Eth (400 mg/kg).

Table 1 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on the concentrations of electrolytes in rat serum

Treatment (mg/kg)	Na^+ in rat serum (mEq/l) after				K^+ in rat serum (mEq/l) after				Cl^- in rat serum (mEq/l) after			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Control (10 ml/kg)	140.28 ± 0.47	139.68 ± 1.13	142.08 ± 1.12	142.48 ± 0.63	6.34 ± 0.11	6.74 ± 0.16	6.03 ± 0.08	5.62 ± 0.12	103.17 ± 1.70	104.50 ± 1.43	102.50 ± 1.72	105.67 ± 1.38
Furosemide (10)	142.48 ± 0.90	141.95 ± 0.68	141.38 ± 0.68	144.15 ± 1.14	5.81 ± 0.04	6.03 ± 0.04	6.13 ± 0.05	5.46 ± 0.14	106.17 ± 1.70	104.33 ± 1.28	105.50 ± 1.00	103.67 ± 1.23
CH-Eth (100)	142.02 ± 0.51	141.07 ± 0.41	143.00 ± 1.00	141.55 ± 0.53	6.11 ± 0.05	6.06 ± 0.04	6.12 ± 0.04	6.14 ± 0.29	102.50 ± 0.96	104.83 ± 1.24	104.00 ± 1.23	106.33 ± 0.96
CH-Eth (200)	141.25 ± 0.90	140.73 ± 0.39	140.90 ± 0.87	143.02 ± 0.50	5.86 ± 0.08	6.87 ± 0.07	5.91 ± 0.09	6.18 ± 0.06	101.67 ± 0.49	103.00 ± 1.10	104.83 ± 1.30	103.83 ± 0.83
CH-Eth (400)	138.70 ± 0.58	141.50 ± 0.54	140.05 ± 1.40	143.28 ± 0.45	5.96 ± 0.16	6.20 ± 0.14	6.48 ± 0.33	6.58 ± 0.33	104.67 ± 1.20	102.17 ± 1.08	105.83 ± 1.08	102.83 ± 1.14

Values are mean ± SEM, $n = 6$ in each group. Statistical analysis was by one-way analysis of variance followed by *post hoc* Dunnett's test using Graphpad Instat software. All values were nonsignificant as compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*.

day 7, 14, 21 and 28. Furosemide (10 mg/kg) significantly increased ($P < 0.01$) K^+ excretion in urine with a gradual decrease after day 1. In this context, the ethanolic extract caused less K^+ excretion on day 1 but a gradual increase thereafter indicating a prolonged kaluretic effect. The kaluretic effect of both the drugs reached a peak at 4 h (Figure 3).

Chronic administration of the ethanolic extract (100, 200 and 400 mg/kg, p.o.) did not change K^+ concentration in serum compared with the control-treated group (Table 1).

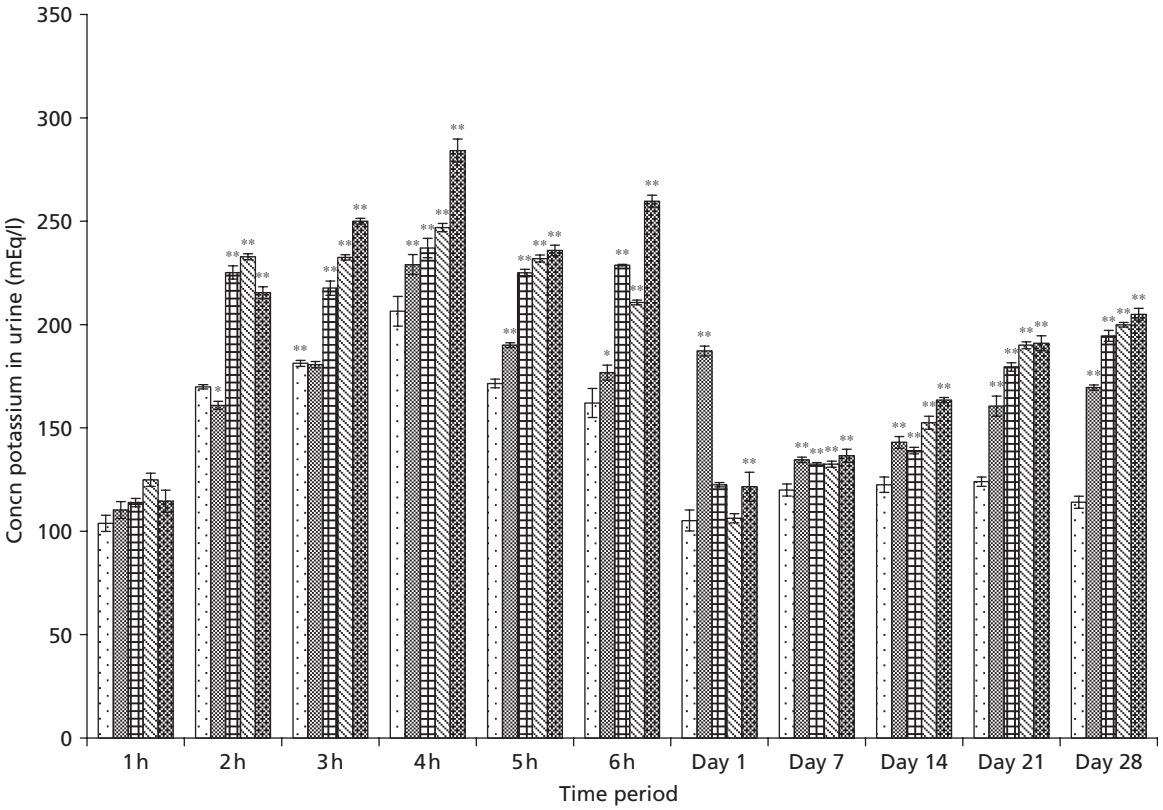

Figure 3 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on the concentrations of potassium in rat urine. CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*. Values are mean ± SEM, $n = 6$ in each group. Statistical analysis was by one-way analysis of variance followed by post-hoc Dunnett's test using Graphpad Instat software. *$P < 0.05$, **$P < 0.01$ compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). Where, (□) control; (▨) Furosemide (10 mg/kg); (▦) CH-Eth (100 mg/kg); (▧) CH-Eth (200 mg/kg); (▩) CH-Eth (400 mg/kg).

Effect of the ethanolic extract of the leaves of *C. hirsutus* on the concentrations of chloride in rat urine and serum

In the acute study, furosemide (10 mg/kg, p.o.) increased excretion of Cl^- at 5 h compared with 4 h with ethanolic extract. The ethanolic extract appeared to be equipotent with furosemide in the excretion of Cl^-.

Chronic administration of furosemide produced maximum chloruretic activity on day 1 but a decrease in chloruretic activity during subsequent days. The ethanolic extract appeared to be a more effective chloruretic agent than furosemide (Figure 4).

Chronic administration of ethanolic extract (100, 200 and 400 mg/kg, p.o.) did not change Cl^- concentration in serum compared with the control-treated group (Table 1).

Effect of the ethanolic extract of the leaves of *C. hirsutus* on the concentrations of creatinine in rat urine and serum

Chronic administration of the ethanolic extract (100, 200 and 400 mg/kg, p.o.) and furosemide (10 mg/kg, p.o.) resulted in a significant increase ($P < 0.01$) in excretion of creatinine in urine, but significantly decreased ($P < 0.01$) serum creatinine concentration compared with the control-treated group (Table 2).

Discussion

Diuretics are drugs that increase the rate of urine flow. Clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and of an accompanying anion, usually Cl^-. Diuretics not only alter the excretion of Na^+, but also modify renal handling of cations (e.g. K^+, H^+, Ca^{2+} and Mg^{2+}), anions (e.g. Cl^-, HCO_3^-, and $H_2PO_4^-$) and uric acid.[14]

This study indicated that the ethanolic extract of the leaves of *C. hirsutus* (100, 200 or 400 mg/kg) caused a significant ($P < 0.01$) and dose-dependent increase in urine and electrolyte (Na^+, K^+, Cl^-) excretion in rats at 3 h. It was noted that ethanolic extract treatment increased both urine and electrolyte excretion qualitatively similar to furosemide, which is a potential saluretic and diuretic drug.[15,16]

Furosemide is a rapidly acting diuretic. The onset of furosemide was rapid and maximum diuretic effect was achieved at 1 h and the peak effect was reached at 2 h. The diuretic effect gradually decreased at the subsequent timings. Ethanolic extract (200 and 400 mg/kg) on the other hand showed peak effect at 6 h. The cumulative volume during 6 h was less compared with furosemide. The results of chronic administration indicated that a latent period of a week was required to exhibit a stable diuretic action. These result suggested mild diuretic action of the ethanolic extract. The site of action appeared to be different to furosemide.

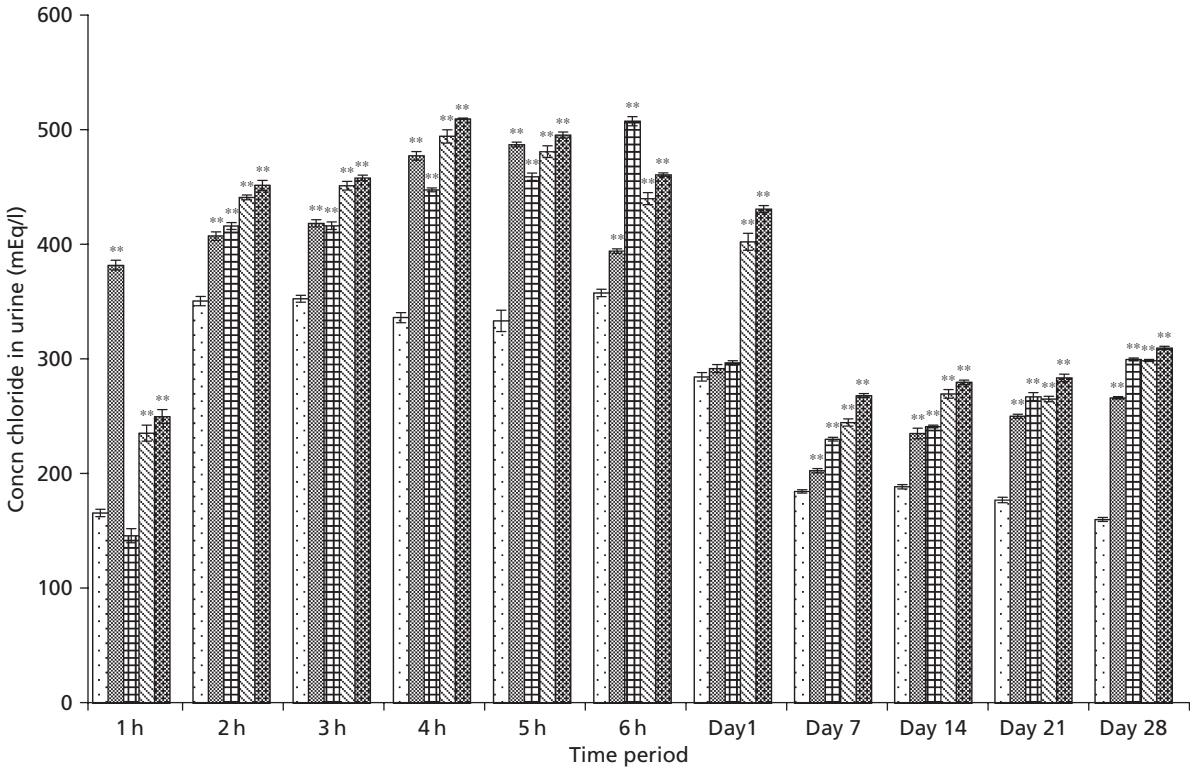

Figure 4 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on the concentrations of chloride in rat urine. CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*. Values are mean ± SEM, $n = 6$ in each group. Statistical analysis was by one-way analysis of variance followed by post-hoc Dunnett's test using Graphpad Instat software. $^{**}P < 0.01$ compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). Where, (□) control; (▨) Furosemide (10 mg/kg); (▤) CH-Eth (100 mg/kg); (▧) CH-Eth (200 mg/kg); (▩) CH-Eth (400 mg/kg).

Table 2 Effect of the ethanolic extract of the leaves of *Cocculus hirsutus* on the concentrations of creatinine in rat urine and serum

Treatment (mg/kg)	Creatinine concentration in rat urine (mg%) after				Creatinine concentration in rat serum (mg%) after			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Control (10 ml/kg)	2.14 ± 0.01	2.79 ± 0.03	3.17 ± 0.01	3.71 ± 0.02	0.43 ± 0.02	0.69 ± 0.02	0.72 ± 0.02	0.71 ± 0.03
Furosemide (10)	3.84 ± 0.03	12.48 ± 0.01**	14.14 ± 0.03**	15.65 ± 0.03**	0.22 ± 0.01**	0.65 ± 0.02**	0.54 ± 0.02**	0.56 ± 0.01**
CH-Eth (100)	2.36 ± 0.02	6.87 ± 0.02*	6.78 ± 0.03**	6.97 ± 0.02**	0.57 ± 0.02**	0.45 ± 0.02**	0.57 ± 0.01**	0.47 ± 0.01**
CH-Eth (200)	2.94 ± 0.02	12.32 ± 0.01**	12.93 ± 0.02**	12.89 ± 0.02**	0.56 ± 0.02**	0.46 ± 0.01**	0.57 ± 0.01**	0.46 ± 0.02**
CH-Eth (400)	3.45 ± 0.03	14.44 ± 0.01**	14.33 ± 0.03**	15.10 ± 0.01**	0.66 ± 0.01**	0.58 ± 0.02**	0.49 ± 0.03**	0.4 ± 0.02**

Values are mean ± SEM, $n = 6$ in each group. Statistical analysis by one-way analysis of variance followed by *post hoc* Dunnett's test using Graphpad Instat software. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group (2% gum acacia suspended in normal saline; 10 ml/kg, p.o.). CH-Eth, the ethanolic extract of the leaves of *C. hirsutus*.

Furosemide acts on the thick ascending loop of Henle.[17] The slow onsets of the ethanolic extracts indicated a site further away from the loop of Henle.

Dilutional hyponatraemia occurs in patients with cardiac heart failure in whom vigorous diuresis is induced with ceiling agents. The kidney tends to retain water, although it is unable to retain salt due to the diuretic effect; extracellular fluid gets diluted, hyponatraemia occurs and oedema persists despite natriuresis. Hearing loss occurs with high ceiling diuretics. Brisk diuresis induced in patients with cirrhosis may precipitate mental disturbances and hepatic coma. It may be due to hypokalaemia, alkalosis and increased blood ammonia levels.[16]

In ethanolic extract-treated-animals serum Na^+, K^+ and Cl^- levels were similar to that of the control group. Creatinine concentration decreased in serum and increased in urine on day 28. Loop diuretics cause acidification of urine.[18,19] Acidosis did not develop after treatment with the ethanolic extract of the leaves of *C. hirsutus*.

Earlier study by Ganapaty *et al.*[2] reported the diuretic effect of the aqueous extract of the aerial parts of *C. hirsutus*. The authors only carried out an acute study (one day) and reported the change in urine volume and excretion of Na^+, K^+ and Cl^- in urine. Our study thus appeared to be more elaborate as it included a chronic study and a greater number of parameters.

Previous phytochemical analysis of *C. hirsutus* reported essential oil, ginnol, sterols, alkaloids, flavonoids phenolic compounds and glycosides.[9,20] The ethanolic extract of the whole plant showed the presence of isoquinoline alkaloid, D-trilobine and DL-coclaurine, jamitine-*N*-oxide, cohirsine, hirsudiol, shaheenine, hirsutine, cohirsinine, cohirsutine, corsitinine, jamtinine and haiderine.[21–30] It was also possible that the ethanolic extract might have manifested cumulative effect of several substances in the extract due to secondary active metabolites.[31] The diuretic activity of the ethanolic extract of the leaves of *C. hirsutus* may have been attributed to its alkaloids, flavonoids or phenolic compounds. It appeared that the ethanolic extract caused diuresis and enhanced excretion of electrolytes though a mechanism different to that for furosemide.

Conclusions

We concluded that treatment with the ethanolic extract of the leaves of *C. hirsutus* produced a marked diuresis in rats after acute and chronic treatment. In our study, no lethality was observed at least for the dose and duration used. However, advanced toxicological studies need to be performed in mice and rats. It remains necessary to study eventual adverse effects of this plant such as alteration of some neural, metabolic and hormonal parameters, before its recommendation for clinical use. The precise sites and the molecular and cellular mechanisms of action of the ethanolic extract of the leaves of *C. hirsutus* remain to be elucidated in further studies. The marked and prolonged activity necessitates a more comprehensive chemical and pharmacological investigation to elucidate the exact mechanism and to isolate and identify the active principles.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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