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Evaluation of antipsychotic and anti-diarrhoeal activities of ethanolic extract of roots of *Rubia cordifolia* Linn

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SUMMARY

The objective of the present study was to assess the antipsychotic and antidiarrhoeal activities of ethanolic extract of roots of *Rubia cordifolia* in mice and rats. The antipsychotic activity of ethanolic extract of roots of *Rubia cordifolia* (ERC) was evaluated by observing its effect on amphetamine-induced stereotyped behavior in mice. Effect of ERC was also studied on motor coordination and locomotion in mice. The antidiarrhoeal activity was evaluated using castor oil-induced diarrhoea and excretion of sodium and potassium ions in the intestinal secretion in rats and gastrointestinal transit in mice. The ERC inhibited amphetamine-induced stereotyped behaviour, diminished locomotion and impaired motor coordination. ERC inhibited castor oil-induced diarrhoea, decreased both sodium and potassium excretion in the intestine and decreased gastrointestinal transit. Thus the present study confirms the anti-diarrhoeal activity of *Rubia cordifolia*. Further studies are necessary to evaluate the potential of *Rubia cordifolia* as an antipsychotic.

Key words: *Rubia cordifolia* roots extract; Antipsychotic; Antidiarrhoeal

INTRODUCTION

Rubia cordifolia Linn (Rubiaceae) known as Manjista or Indian Madder is a climber growing in northwest Himalayas, Nilgiris, and other hilly districts of India. It is commonly used in the Indian system of Medicine for treatment of obstructions in the urinary tract, and as astringent, and diuretic. It is also useful in external inflammation, ulcers, diarrhoea, and certain skin diseases (Nadkarni, 1982). Roots of *Rubia cordifolia* contain anthraquinones (Wang *et al*., 1992), lucidin having mutagentic activity (Poginsky *et al*., 1983), and napthahydroquinones dimer (Itokawa *et al*., 1989; Itokawa and Ibraheim, 1993) having cytotoxic activity. Previous studies have reported anti-inflammatory, anticonvulsant, hypoglycemic, antistress, anxiolytic, and nootropic activities (Kasture *et al*., 2001, 2002; Kasture and Kasture, 2004). The ethyl acetate fraction of ethanolic extract of roots of *Rubia cordifolia* (ERC) has anti-oxidant and lipoxygenase-inhibiting activity (Tripathi and Sharma, 1995). The methanolic extract of *Rubia cordifolia* has anticancer (Advankar and Chitnis, 1982) and antiviral activity against Herpes viruses (Jin *et al*., 1989). The alcoholic extract also has immunomodulatory activity (Joharapurkar *et al*., 2003).

Rubia cordifolia contains anthraquinones. Though the anthraquinones are commonly known to cause diarrhoea (as in case of Senna leaves and Cascara bark), *Rubia cordifolia* is used in folklore medicine as an antidycentric. During our previous studies (Kasture *et al*., 2002; Kasture and Kasture, 2004) we noticed a central nervous system depressant effect of ERC. It is known that antipsychotics cause

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constipation by inhibiting cholinergic and dopaminergic transmission and also decrease gastric secretion and motility (Baldesarrini and Tarazi, 1996). Therefore in the present study we evaluated antipsychotic and antidiarrhoeal activity of ERC. ERC was given intraperitoneally for assessment of antipsychotic activity and orally for antidiarrhoeal activity.

MATERIALS AND METHODS

Drugs

d-Amphetamine (Sigma, USA), haloperidol (Searle, India), chlorpromazine (Intas, India), Analar grade polyethylene glycol-400 (PEG 400) and castor oil (Ipca, India) were used in this study. All drug solutions were prepared in distilled water immediately before experiment.

Animals

Albino Swiss mice (22 - 25 g) and Wistar rats (150 - 180 g) of either sex were housed in groups of five under standard laboratory conditions of temperature (25 ± 2 °C), humidity (55 ± 5%), and 12/12 h light/ dark cycle. The animals had free access to standard pellets chow (Lipton, India ltd.) and distilled water. Food, but not water was deprived overnight and during the experiment. The Institutional Animal Ethical Committee approved the protocol of this study.

Preparation of extract

About 100 g of root powder of *Rubia cordifolia* was defatted with petroleum ether (60 - 80 °C) and extracted with ethanol (90% v/v) by cold maceration. The extract was vacuum distilled and the residue obtained was dried in air, at room temperature. The percentage yield of the ethanol was 11.50% w/w.

Pharmacological evaluation

ERC was used for pharmacological screening. The ERC (10 - 100 mg/kg) was dissolved in minimum volume of polyethylene glycol-400 (PEG 400) and used for pharmacological evaluation.

Behavioral study

Motor coordination

The effect of ERC on motor coordination was assessed in mice using Rotarod apparatus (INCO-Medicraft, India). Mice were divided into seven groups, five in each group. The control group received vehicle (PEG 400, 10 ml/kg, i.p.) and haloperidol (1 mg/kg, i.p.) whereas other groups received ERC (10, 25, 50, 100 mg/kg, i.p.) with haloperidol. One group received only ERC (50 mg/kg). Animals were previously trained to remain on rotating rod (20 rev/min) for 5 min. In order to test the motor coordination, these rats were placed individually on rotating rod and fall off time was noted as described by Dunham and Miya (1957).

Spontaneous motor activity

The effect on spontaneous motor activity was studied using Actophotometer (INCO, India) as described by Kulkarni (1989). Mice were treated with ERC (50 mg/kg i.p.) or ERC (100 mg/kg) along with haloperidol (1 mg/kg i.p.) 30 min before the test. The mice placed in the activity cage for 5 min for acclimatization and the basal activity was recorded in 5 min-observation period. The difference in activity (that is, before and after drug treatment) was noted for each dose of ERC. The effect of ERC on locomotion was compared with vehicle treated mice.

Amphetamine-induced stereotypical behavior

The mice were divided into six groups each containing five. D-amphetamine (1 mg/kg, i.p) was given 30 min after either the vehicle (PEG 400, 10 ml/kg, i.p.) or the ERC (10 -100 mg/kg i.p.) treatment. The mice were observed for latency to biting, grooming, rearing and repetitive head movements (RHM) as described earlier by Kandi *et al.* (1998).

Anti-diarrhoeal activity

Castor oil-induced diarrhoea

Twenty four hour food deprived rats were randomly allocated to five groups of six animals each. Group I received vehicle (Distilled Water (D.W.) 10 ml/kg, p.o.), group II received castor oil alone, group III received ERC alone (100 mg/kg p.o.) while groups IV and V received ERC (50 and 100 mg/kg p.o.) respectively. Loperamide (5 mg/kg p.o.) was used as a reference standard. After 60 min of these administrations, each animal was given 2 ml of castor oil using orogastric tube and the rats were placed individually in a cage and observed for 4 h for percentage incidence of diarrhoea and weight of faecal matter (Awouters *et al.*, 1978).

Gastrointestinal transit

Mice were divided into groups of six. To each animal, 1ml of activated charcoal meal (12.5% charcoal plus 12.5% gum acacia in water) was given orally 60 min after an oral dose of vehicle or ERC. Group I was administered with vehicle (5 ml/kg p.o.) and mice in group II and III received ERC (50 and 100 mg/kg p.o. respectively). After 30 min of administration of charcoal meal animals were killed by cervical dislocation and the intestine was removed without stretching and placed lengthwise on moist filter paper. The distance traveled by charcoal from pyloric sphincter to caecum was measured. The percentage of the total length traveled was evaluated for each mouse (Lutterodt, 1989).

Castor oil-induced fluid accumulation and estimation of Na^+ and K^+ secretion

The rats fasted for 24 h but access to water were randomized and allocated to five groups, six rats in each. Group I received vehicle (D.W. 10 ml/kg, p.o.) and Group II received castor oil only (2 ml p.o.), group III and IV received oral administration of 50 and 100 mg/kg of ERC respectively 1 h prior to castor oil administration. Group V received loperamide (5 mg/kg, p.o.) as a standard antidiarrhoeal agent 30min prior to castor oil. After 30 min, the rats were killed by cervical dislocation and the small intestine was ligated both at pyloric sphincter and at the ileocaecal junction. The entire small intestine was dissected out, its contents were collected into a graduated measuring cylinder and the volume of the contents was recorded. The fluid samples were analyzed for Na^+ and K^+ concentrations as described earlier (Di Carlo *et al.*, 1994) using flame photometer (Model CL 22D of Elico, India).

Statistical analysis

All observations were presented as mean ± standard error of the mean (S.E.M.). The data were analyzed by Student's *t* test or one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered as significant.

RESULTS

Effect on motor coordination

The vehicle treated mice remained on the rotating rod for 295.25 ± 2.02 s. The animals remained on rotating rod for 147.0 ± 10.5, 109.0 ± 10.0 and 47.5 ±

Table 1. Effect of *Rubia cordifolia* on motor coordination in mice

Group No.	Treatment (mg/kg i.p.)	Latency to fall from rotating rod (mean ± S.E.M.)
1	Vehicle	295.25 ± 2.02
2	Haloperidol (1)	169.7 ± 2.53*
3	ERC (10) + Halo	147.0 ± 10.05*
4	ERC (50) + Halo	109.0 ± 10.0*
5	ERC (100) + Halo	47.5 ± 12.97*
6	ERC (50)	200.7 ± 26.95*

N = 5, $F_{5, 24} = 38.1$, $P < 0.001$, *$P < 0.01$ compared to vehicle treated group (Dunnett's test).

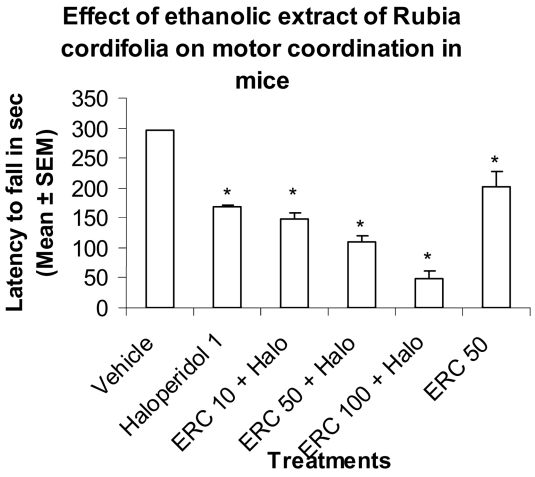

Fig. 1. Effect of ERC on motor coordination in mice. N=5, $F_{5, 24}$=38.1, $P < 0.001$, *$P < 0.01$, compared to the vehicle treated group (Dunnett's test).

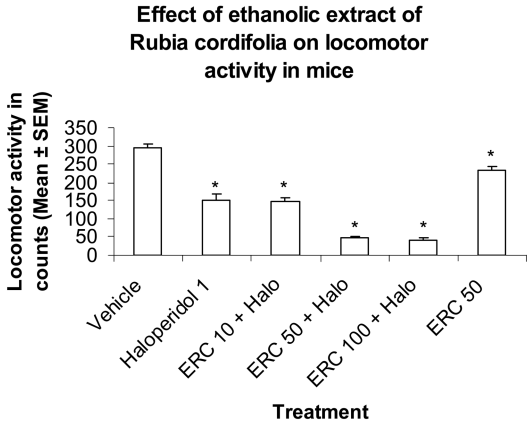

Fig. 2. Effect of ERC and haloperidol on locomotor activity in mice. N=5, $F_{5,24}$ = 78.48, $P < 0.0001$, *$P < 0.01$, compared to the vehicle treated group (Dunnett's test).

12.97 s respectively when they received ERC in doses of 10, 50, 100 mg/kg along with haloperidol. The animals treated with ERC alone in a dose of 50 mg/kg stayed for 200.7 ± 26.95 s while haloperidol treated mice stayed on the rotating rod for 169.7 ± 2.53 s. (Table 1, Fig. 1).

Locomotor activity

The vehicle treated mice crossed the beam of light in the Actophotometer 294.0 ± 11.37 times during the observation period of 5 min. The ERC showed significant dose dependent reduction in locomotor activity in doses of 10, 25, 50, 100 mg/kg given along with haloperidol 150.0 ± 17.88, 146 ± 11.21, 47.5 ± 4.57, and 40.0 ± 6.64 respectively. The ERC *per se* in a dose of 50 mg/kg reduced locomotion by 61.0 ± 11.33 (Table 2, Fig. 2).

Amphetamine antagonism

Following the administration of amphetamine (1 mg/kg i.p.) all mice exhibited a stereotypical behavior (SB). After treatment with amphetamine, latencies to various SB were: biting - 3.825 ± 0.04 s; grooming - 131.9 ± 12.08 s; rearing - 11.0 ± 1.19 s, and RHM - 68.6 ± 5.18 s. Administration of ERC (25, 50, and 100 mg/kg) delayed the latency to bite, groom, rear and RHM significantly. There was no significant effect on the stereotyped behavior when ERC was administered in a dose of 10 mg/kg. Haloperidol, used as a positive control inhibited amphetamine induced SB (Table 3, Fig. 3).

Antidiarrhoeal activity

Castor oil-induced diarrhoea

Castor oil (2 ml p.o.) produced diarrhoea in 83.3% of rats and the mean weight of feces was 1,120.25 ± 133.5 mg. The percent incidence of diarrhoea in

Table 2. Effect of ERC on locomotor activity in mice

Group No.	Treatment (mg/kg i.p.)	Number of counts of actophotometer (mean ± S.E.M.)
1	Vehicle	294.0 ± 11.37
2	Haloperidol (1)	150.0 ± 17.88*
3	ERC (10) + Halo	146.0 ± 11.21*
4	ERC (50) + Halo	47.5 ± 4.7*
5	ERC (100) + Halo	40.0 ± 6.64*
6	ERC (50)	233.0 ± 11.33*

N = 5, $F_{5, 24}$ = 78.48, $P < 0.0001$, *$P < 0.01$ compared to vehicle treated group (Dunnett's test).

Table 3. Effect of *Rubia cordifolia* on amphetamine-induced stereotyped behavior in mice

Group No.	Treatment (mg/kg)	Latency in second (mean ± S.E.M.) to			
		Biting	Grooming	Rearing	Repetitive Head movements
1	Vehicle	3.8 ± 0.04	131.9 ± 12.08	11.0 ± 1.97	68.6 ± 5.18
2	ERC (10)	4.62 ± 0.2	168.2 ± 21.11	13.33 ± 1.72	85.4 ± 4.41
3	ERC (25)	17.2 ± 0.14*	205.0 ± 22.2*	87.0 ± 11.4*	112.0 ± 11.23
4	ERC (50)	18.8 ± 0.96*	221.0 ± 19.1*	198.0 ± 17.2*	129.0 ± 21.23*
5	ERC (100)	19.7 ± 1.61*	239.0 ± 13.1*	241.0 ± 21.3*	136.0 ± 29.3*
6	Halo (1)	23.2 ± 4.28*	277.0 ± 31.3*	287.0 ± 23.2*	154.0 ± 16.6*
$F_{5,24}$ =		18.67	6.12	59.53	3.55
P		0.0001	0.0001	0.0001	0.015

N = 5, *P < 0.001 one way ANOVA followed by Dunnett's test. Halo: Haloperidol.

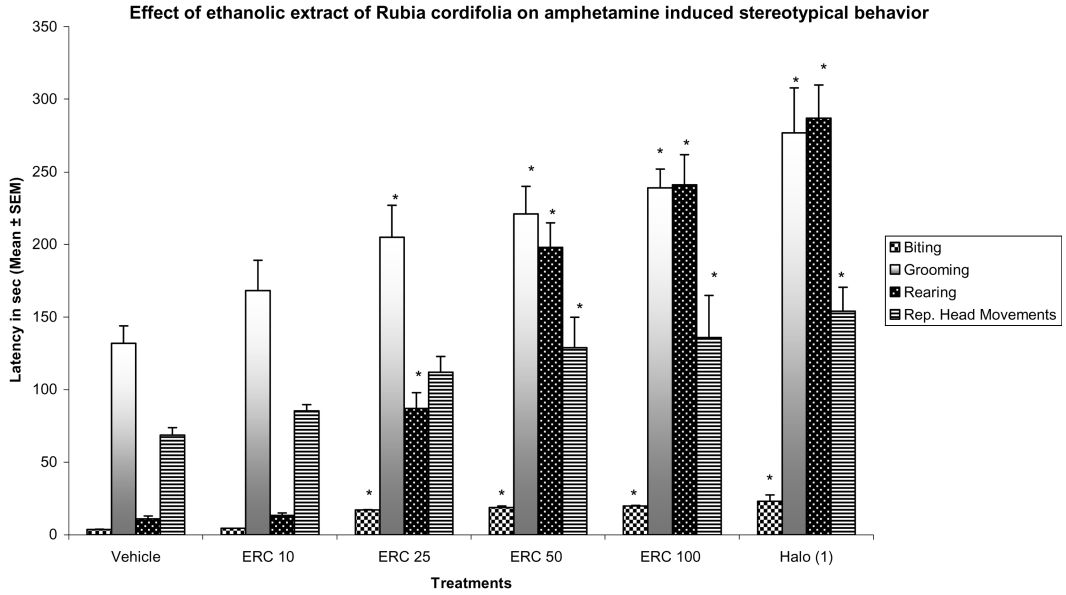

Fig. 3. Effect of ERC on amphetamine induced SB in mice. *P < 0.01, compared to the vehicle treated group (Dunnett's test).

rats treated with ERC (50 and 100 mg/kg p. o.) and castor oil was 48.11% and 22.83% respectively. ERC also decreased the fecal weight from 1,120.25 ± 133.25 to 864.5 ± 55.3mg and 581.25 ± 146.71 mg respectively. Loperamide, a positive control, reduced the incidence of diarrhoea to 16.6% and the weight of stools was reduced to 401.5 ± 51.2 mg (35.85% decrease as compared to castor oil treated rats) (Table 4, Fig. 4).

Gastrointestinal transit

In vehicle treated rats the extent of gastrointestinal transit after 30 min of administration was 72.9 ± 11.2 cm. The effect of the ERC at the dose of 50 and 100 mg/kg caused a reduction in the gastrointestinal transit to 34.24 ± 3.02, 32.48 ± 4.17% respectively (Fig. 5).

Castor oil-induced fluid accumulation and estimation of Na^+ and K^+ secretion

The volume of intestinal fluid accumulated in the vehicle treated rats was 1.25 ± 0.13 ml whereas in castor oil treated group it was 3.47 ± 0.25 ml. Treatments with ERC 50 and 100 mg/kg significantly

Table 4. Effect of ERC on castor oil induced diarrhea

Group No.	Treatment (mg/kg)	% Incidence of diarrhoea	Weight of stool in mg (mean ± S.E.M.)
I	Vehicle	-	651.0 ± 135
II	Castor oil (2 ml)	83.3	1120.25 ± 133.52[@]
III	ERC (100)	-	417.66 ± 62.25[*]
IV	ERC (50) + Castor oil (2 ml)	48.11[#]	864.5 ± 55.33
V	ERC (100) + Castor oil (2 ml)	22.83[#]	581.25 ± 146.71[*]
VI	Loperamide (5) + Castor oil	16.6[#]	401.5 ± 51.2[*]
	$F_{5, 30} = 6.87$, $P = 0.0001$		

- : Absence of Diarrhoea. N = 6, [@]$P < 0.001$ as compared to group I, *$P < 0.001$ as compared to group II, One Way ANOVA followed by Dunnett's test , [#]$P < 0.05$, Fisher exact test.

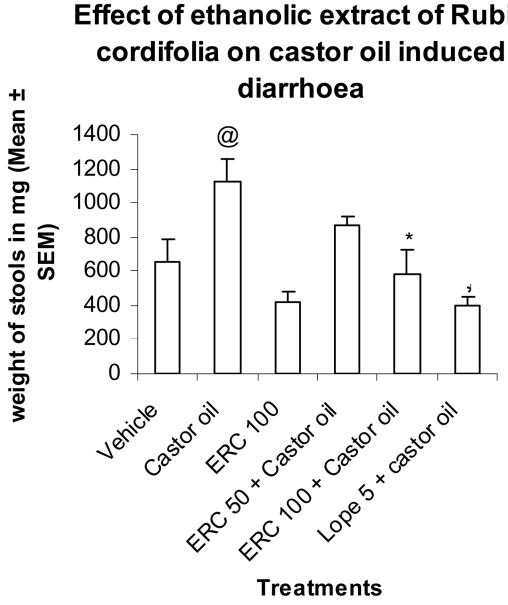

Fig. 4. Effect of ERC on castor oil induced diarrhea. @: compared to vehicle treated group. *P<0.01, compared to the castor oil treated group (Dunnett's test).

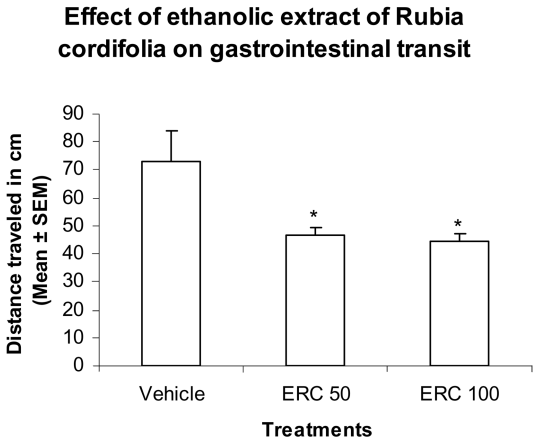

Fig. 5. Effect of ERC on gastrointestinal transit. N = 6, $F_{2,15}$=5.29, $P < 0.0182$, *$P < 0.05$ compared to the castor oil treated group (Dunnett's test).

reduced the intestinal fluid volume to 2.27 ± 0.17 ml and 1.87 ± 0.13 ml respectively ($P < 0.0001$). The K^+ concentration in the intestinal fluid of ERC treated

Table 5. Effect of ERC on Castor oil-induced fluid accumulation and Estimation of Na^+ and K^+ secretion

Sr. No.	Treatment (mg/kg)	Intestinal fluid in ml (mean ± S.E.M.)	Na^+ (mmol/l) (mean ± S.E.M.)	K^+ (mmol/l) (mean ± S.E.M.)
I	Vehicle	1.25 ± 0.13	15.25 ± 1.70	6.4 ± 0.78
II	Castor oil	3.47 ± 0.25[#]	39.25 ± 3.32[#]	10.836 ± 1.14[#]
III	CPZ (2) + CO	1.92 ± 0.13[*]	17.55 ± 1.78[*]	6.95 ± 0.5[*]
IV	ERC (50) + CO	1.87 ± 0.13[*]	18.84 ± 2.15[*]	6.42 ± 0.56[*]
V	ERC (100) + CO	2.27 ± 0.17[*]	22.78 ± 2.19[*]	7.37 ± 0.29[*]
VI	Lope (5) + CO	2.01 ± 0.2[*]	19.5 ± 1.5[*]	6.8 ± 0.4
$F_{5, 24}$		15.9	15.84	6.33
P		0.0001	0.0001	0.001

N = 5, CPZ: Chlorpromazine, Lope: Loperamide, CO: Castor oil. [#]$P < 0.05$ as compared to the vehicle treated group. [*]$P < 0.001$, One Way ANOVA followed by Dunnett's test, as compared to the castor oil treated group.

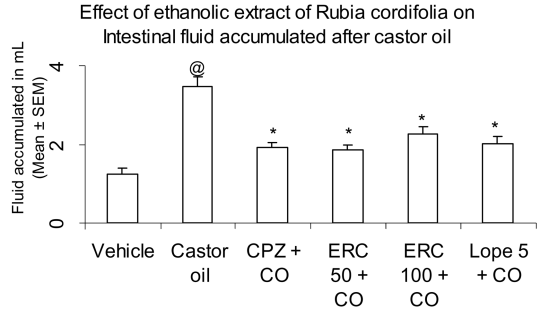

Fig. 6. Effect of ERC on intestinal fluid accumulated after castor oil. N=5, $F_{5,24} = 15.9$, $P < 0.0001$, @: Compared to vehicle treated group. *P<0.05 compared to the castor oil treated group (Dunnett's test).

group was 7.37 ± 0.29 and 6.42 ± 0.56 mmol/l in dose of 50 and 100 mg/kg which was significantly different from the value 10.83 ± 1.14 mmol/l obtained from castor oil administration (P < 0.0001). Na^+ and K^+ concentration in the intestinal fluid revealed that ERC had inhibitory effect on Na^+ and K^+ concentration. The administration of ERC (50 and 100 mg/kg p.o.) significantly reduced Na^+ concentration in castor oil-induced intestinal fluid to 22.78 ± 0.17, 18.84 ± 2.15 mmol/l (P < 0.001). The reference standard loperamide reduced the volume of intestinal fluid, Na^+ and K^+ contents of intestinal fluid (Table 3, Fig. 6).

DISCUSSION

The results of the present study indicate that the ethanolic extract of roots of *Rubia cordifolia* decrease motor coordination, diminish locomotion, and inhibited amphetamine-induced SB. The extract also exhibited antidiarrhoeal activity as shown by the inhibition of castor oil-induced diarrhoea, reduced gastrointestinal transit, and decreased Na^+ concentration in castor oil treated animals.

On rotating rod the ERC impaired the motor performance. It is known that weak postsynaptic dopamine DA antagonist reduces the spontaneous locomotor activity and the general depressants of nervous system decrease locomotion (Munkavad *et al.*, 1968). The typical antipsychotic agents impair motor coordination by blockage of dopamine D_1 and D_2 receptors (Wang *et al.*, 1992). The ability to block amphetamine-induced agitation is generally considered to reflect the sedative property of compound, while blockade of SB is much more specific in indicating the antipsychotic activity of the drug (Wang *et al.*, 1992). In present study ERC antagonized the action of amphetamine by increasing latency to bite, groom, rear, and repetitive head movement.

Castor oil induced diarrhoea is characterized by semisolid droppings in all animals treated with vehicle. Ricinoleic acid of the castor oil has an irritant action on the gastrointestinal tract (Awouters *et al.*, 1978). ERC significantly inhibited castor oil-induced diarrhoea as shown by reduction in number as well as the weight of fecal matter. (P < 0.001) Treatment with the ERC produced a significant reduction in the intestinal fluid accumulation (P < 0.0001). ERC also reduced the gastrointestinal transit. ERC had an inhibitory effect on Na^+ and K^+ concentrations in the intestinal fluid after administration of castor oil. Castor oil increases the peristaltic activity and produces changes in the intestinal mucosal membrane permeability to electrolyte and water (Di Carlo *et al.*, 1994). Membrane bound enzyme Na^+ and K^+-ATPase has been related to Na^+ and K^+ transport in the intestine. It is known that synthetic antipsychotics cause constipation by reducing cholinergic and dopaminergic transmission (Baldesarrini and Tarazi, 1996), however antidycentric drugs do not show antipsychotic activity. This study has shown that *Rubia cordifolia* has both antipsychotic and antidiarrhoeal activities. Further studies are necessary to isolate the active ingredient responsible for these activities and to find out the adverse effects which are related to antipsychotics and antidiarrhoeal agents.

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