

**ANTI-DIARRHOEAL POTENTIAL OF ROOT EXTRACTS OF
RUBIA CORDIFOLIA, LINN. IN RATS**

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Summary

The present study was undertaken to evaluate the antidiarrhoeal property of alcoholic and aqueous extracts of *Rubia cordifolia* in experimentally induced diarrhoeal models like Castor oil induced diarrhoea, Charcoal meal test and Magnesium sulphate induced diarrhoea. Both the extracts of *Rubia cordifolia* (100 mg/kg, 200 mg/kg, and 400 mg/kg) were administered 30 min prior to induction of diarrhoea. The parameters like frequency and weight of the diarrhoeal dropping, percentage movement of charcoal in GIT were evaluated. Treatment with extracts has reduced the severity of diarrhoea in all the three experimental models by markedly reducing the fecal output, intestinal secretions and peristaltic movement of the gastrointestinal tract which confirms antidiarrhoeal potency.

Key words: *Rubia cordifolia*; Castor oil; Charcoal meal; Magnesium sulphate; diarrhoea.

Introduction

Herbal therapy provides rational means for the treatment of many diseases which are considered to be obstinate and incurable in other system of medicine. It lays a great deal of emphasis upon the maintenance of positive health of an individual and thus aims at both the prevention and cure of diseases.

Diarrhoea involves both an increase in the motility of the gastrointestinal tract, along with increased secretion of ions and a decrease in the absorption of fluid, and thus a loss of electrolytes, particularly Na⁺ and water [1]. Diarrhoea has long been recognized as one of the most important health problem in the developing countries. Worldwide, diarrhoea accounts for more than 5-8 million deaths in infants and small children less than 5 years each year. According to World Health Organization estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea [2].

It has been estimated that the incidence of diarrhoea in United States and other industrialized nations is, on average one episode per person annually [3]. It is of special concern in older because the majority of deaths in this population are associated with diarrhoea illness in the United States. In addition adults who care for infants in day care facilities, international travellers, homosexual men, immunosuppressed patients and those exposed to contaminated food and water are at a great risk. [4]

The major cause of diarrhoea among children in developing countries is malnutrition. To nullify the problem of diarrhoea which is a leading cause of mortality in developing countries, the World Health Organization has constituted a diarrhoeal disease control programme (CDD) which includes studies of traditional medicinal practices, together with the evaluation of health education and prevention approaches [5].

Considering the importance of plants as a source of medicine even today, in the present study a plant *Rubia cordifolia* (linn), often known as Common Madder or Indian Madder, family- Rubiaceae, which is in use for centuries in the treatment of many ailments was selected. However the plant is less explored for its varying activities, hence an effort has been made here to investigate the potential uses of this plant for treating diarrhea.

Materials and Methods

Animals:

Wistar albino rats of either sex (175-250g) were procured from Sri Venkateswara Enterprises, Bangalore, and were maintained under standard husbandry conditions (temperature of $25 \pm 1^{\circ}\text{C}$; RH 45 to 55% and 12: 12 light/dark cycle). The experiment was performed after prior approval of the study protocol by the institutional animal ethical committee of V.L. College of pharmacy, Raichur, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Materials:

The roots of *R. cordifolia* were purchased from Yucca Enterprises, Mumbai, Castor oil (Torrent Pharmaceuticals, Ahmedabad, India), Distilled water (Mysore Petro Chemicals, Raichur, India), Loperamide (Torrent Pharmaceuticals, Ahmedabad India).

Preparation of root extracts:

The shade dried roots were powdered to get a coarse granule. The powder was subjected for successive extraction in soxhlet apparatus. The powder was packed in a soxhlet apparatus and extracted with 95% alcohol at $60-80^{\circ}\text{C}$. The extract was dried on a water bath.

The marc from the above process was macerated with distilled water (containing 2% chloroform (10 ml), which acts as preservative) for 24 hours with occasional stirring for every 60 minutes. Then the resultant was filtered through muslin cloth. The filtrate was dried on a water bath maintained at 45°C to get a solid mass.

Preliminary phytochemical investigation:

Alcohol extracts of *R. cordifolia* (AERRC) and aqueous extracts of *R. cordifolia* (AQERRC) were subjected for the qualitative preliminary phytochemical identification by the standard methods described in practical Pharmacognosy [6,7].

Determination of acute toxicity (LD₅₀)[8]:

The acute toxicity of AERRC and AQERRC was determined in albino mice of either sex weighing between 18-22 g by following “up and down” (OECD guideline no.425) method of CPCSEA. 1/5th, 1/10th, 1/20th of the lethal dose of the individual extracts was taken as effective doses ED₅₀ and was used throughout the experimental studies [9].

Anti-diarrhoeal activity:

I. Castor oil induced diarrhoea [9]:

The method described by Awouters. F et al, was followed here with some modifications. In the present study albino rats of the either sex weighing 160-190 g were divided into 8 groups of 6 animals in each group. They were fasted overnight prior to the test with free access to water *ad libitum*.

The various groups were treated as follows:

- Group I : Control (10 ml/kg distilled water p.o)
- Group II : Standard drug (Loperamide 3 mg/kg p.o)
- Group III : AERRC (100 mg/kg p.o)
- Group IV : AERRC (200 mg/kg p.o)
- Group V : AERRC (400 mg/kg p.o)
- Group VI : AQERRC (100 mg/kg p.o)
- Group VII : AQERRC (200 mg/kg p.o)
- Group VIII : AQERRC (400 mg/kg p.o)

30 minutes after treatment each rat received 1 ml of castor oil orally then housed separately in the metabolic cages with special provision to separate urine and faeces. During 4 h period number and weight of diarrhoeal droppings were noted. Percentage increase diarrhoea and percentage inhibition was calculated by making use of mean weight of the stools. Anti-diarrhoeal activity was determined in terms of percentage protection and was calculated by following formula:

$$\text{Percentage protection (\%)} = \frac{A-B}{A}$$

Where ‘A’ is the total weight of stools in control animals.

‘B’ is the total weight of stools in extracts treated animals.

II. Gastro-intestinal motility test [10]:

The method described by Pazhani G P. et al was used in this study. Albino rats of the either sex weighing between 160-200 g were divided into 8 groups of 6 animals each. They were fasted for 18 h prior to the test with free access to water *ad libitum*.

Treatment protocol was done as follows:

- Group I : Control (10 ml/kg distilled water p.o)
- Group II : Standard drug (Atropine sulphate 0.1 mg/kg i.p.)
- Group III : AERRC (100 mg/kg p.o)
- Group IV : AERRC (200 mg/kg p.o)
- Group V : AERRC (400 mg/kg p.o)
- Group VI : AQERRC (100 mg/kg p.o)
- Group VII : AQERRC (200 mg/kg p.o)
- Group VIII : AQERRC (400 mg/kg p.o)

30 minutes after the above treatment 1ml of charcoal meal (3% deactivated charcoal in 10% normal saline) was administered to all the animals orally. 30 minutes after this treatment, all rats were sacrificed and distance travelled by the charcoal meal in each animal's intestine from pylorus to caecum end was noted. Percentage travelled and percentage of inhibition was calculated by the following formula,

$$\% \text{ travelled} = (A/B) \times 100.$$

$$\% \text{ inhibition} = \{(B-A)/B\} \times 100.$$

Where 'A' is the distance travelled by the charcoal meal,
'B' is the total length of small intestine.

III. Magnesium sulphate induced diarrhoea [11] :

The method described by Mujumdar A M. et al was followed here with some modifications. In the present study albino mice of the either sex weighing between 20-25 gms were divided into 8 groups of 6 animals each. They were fasted overnight prior to the test with free access to water all the time.

Treatment protocol was done as follows:

- Group I : Control (10 ml/kg distilled water p.o)
- Group II : Standard drug (Magnesium sulphate 2 gm/kg p.o)
- Group III : AERRC (100 mg/kg p.o)

- Group IV : AERRC (200 mg/kg p.o)
Group V : AERRC (400 mg/kg p.o)
Group VI : AQERRC (100 mg/kg p.o)
Group VII : AQERRC (200 mg/kg p.o)
Group VIII : AQERRC (400 mg/kg p.o)

30 minutes after treatment each mouse received magnesium sulphate orally and then housed separately in the metabolic cages. During 4 h period number and weight of diarrhoeal dropping were noted. Percentage of diarrhoea and percentage inhibition was calculated by making use of mean weight of the stools. Anti-diarrhoeal activity was determined in terms of percentage protection, calculated by following formula given in the above method.

Statistical analysis:

Results are expressed as mean \pm SEM. The results were subjected to statistical analysis by using oneway ANOVA followed by Dunnett‘t’ test to calculate the significance differences among the groups and $P < 0.05$ was considered as significant.

Results

Phytochemical investigation:

Phytochemical screening of the plant extracts revealed that the presence of sterols, glycosides, carbohydrates, flavonoids, tannins, gums & mucilage, triterpenes.

Toxicity studies:

LD₅₀ studies reveal no mortality even at a dose of 2000 mg/kg for both extracts following OECD guidelines No.425 of CPCSEA.

Anti-diarrhoeal activity:

I. Castor oil induced diarrhoea in rats:

When compared with normal control (received castor oil with vehicle only), percentage inhibition of standard drug Loperamide was 94.98% and the percentage inhibition in

weight of the stool with AERRC (low, medium, high) and AQERRC (low medium, high) were 27.19%, 45.31%, 79.45% and 24.16%, 45.61%, 84.89% respectively. The potency of the anti-diarrhoeal activity was in the order Loperamide > AQERRC > AERRC. The results are shown in table no.1.

Table 1: Castor oil induced diarrhoea.

Group	Treatment mg/kg (p.o)	Stools wt mean± SEM (g)	% Inhibition
Control	vehicle 10 ml/kg	3.316±0.12	-
Toxicant	Castol oil 1 ml	3.55± 0.20	-
Standard	Loperamide 3.0	0.166±0.16**	94.98%
AERRC	100	2.416±0.10**	27.19%
AERRC	200	1.816±0.11**	45.31%
AERRC	400	0.683±0.16**	79.45%
AQERRC	100	2.516±0.21**	24.16%
AQERRC	200	1.8±0.11**	45.61%
AQERRC	400	0.5±0.13**	84.89%
n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant			

II. Effect on gastro intestinal motility:

When compared with motility of normal control animals percentage reduction of gastro intestinal motility with the standard, atropine sulphate was 66.21%, and AERRC (low, medium, high) and AQERRC (low, medium, high) had shown 21.94%, 43.70%, 54.07% and 19.51%, 39.75%, 53.58% respectively. According to the percentage inhibition of charcoal movement in intestine the order of the potency of anti-diarrhoeal activity was atropine sulphate > AERRC > AQERRC. The results are shown in table no.2.

Table 2: Gastro-Intestinal motility test.

Group	Treatment mg/kg (p.o)	total length mean±SEM (cms)	Distance traveled mean±SEM (cms)	% movement of charcoal mean±SEM(cms)	% Inhibition
Control	vehicle 10 ml/kg	91.83±0.74	81.16±0.65	88.41±1.05	-
Toxicant	-	-	-	-	-
Standard	Atropine 0.1 i.m	92.33±0.91 ^{ns}	31.16±0.54 ^{**}	34.17±0.77 ^{**}	66.21
AERRC	100	92.33±0.918 ^{ns}	72±1.26 ^{**}	78.04±1.91 ^{**}	21.94
AERRC	200	91.83±0.749 ^{ns}	51.83±0.47 ^{**}	56.44±0.67 ^{**}	43.70
AERRC	400	91.83±0.749 ^{ns}	42.16±0.70 ^{**}	45.91±0.70 ^{**}	54.07
AQERRC	100	92.83±1.01 ^{ns}	74.66±0.88 ^{**}	80.47±1.32 ^{**}	19.51
AQERRC	200	91.33±0.42 ^{ns}	55±1.22 ^{**}	60.23±1.40 ^{**}	39.75
AQERRC	400	92.66±0.80 ^{ns}	43±0.96 ^{**}	46.40±1.01 ^{**}	53.58
n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant					

III. Magnesium sulphate induced diarrhoea in mice:

When compared with normal control (received magnesium sulphate with vehicle only) the percentage inhibition in weight of the stool with standard drug Loperamide was 85.83% and with AERRC (low, medium, high) and AQERRC (low medium, high) it was 24.46%, 58.79%, 69.95% and 33.04%, 63.51%, 75.96% respectively. The potency of the anti-diarrhoeal activity was in the order Loperamide > aqueous > alcoholic. The results are shown in table no.3.

Table 3: Magnesium Sulphate induced diarrhoea.

Group	Treatment mg/kg (p.o)	Stools wt mean± SEM (g)	% Inhibition
Control	vehicle 10 ml/kg p.o	2.33±0.13	-
Toxicant	Magnesium sulphate	3.92±0.16	-
Standard	Loperamide 3	0.33±0.21**	85.83
AERRC	100	1.76±0.06*	24.46
AERRC	200	0.96±0.15**	58.79
AERRC	400	0.7±0.11**	69.95
AQERRC	100	1.56±0.09**	33.04
AQERRC	200	0.85±0.18**	63.51
AQERRC	400	0.56±0.14**	75.96
n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant			

Discussion

Castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and induces peristaltic changes in the mucosal fluid and electrolyte transport that result in a hypersecretory response and diarrhoea [12, 13]. Ricinoleic acid liberation results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [14, 15].

The osmotic properties of MgSO₄ prevent the reabsorption of water and ions, leading to an increase in the volume of the intestinal content. This compound also promotes the production of cholecystokinin from duodenal mucosa, which further increases the secretions and has a positive motor effect on the small intestine [16].

Charcoal is used to evaluate efficacy of a test compound to prevent gastrointestinal propulsive motility. These tests are based on the intestinal transport of a

charcoal meal along the small intestine. The effect of drugs on change in propulsion of charcoal meal is good indicator of clinical activity.

Loperamide, works by decreasing the activity of the myenteric plexus, which decrease the motility of the circular and longitudinal smooth muscles of the intestinal wall. This increases the amount of time that the substances stay in the intestine, allowing more water absorption out of fecal matter. It also decreases colonic mass movements and suppresses the gastrocolic reflex.

Tannins [17], reducing sugars, sterols, flavanoids [18] and triterpenes [19] are reported for their anti-diarrhoeal activity. The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electric secretion, which are known to be altered in this intestinal condition. *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by prostaglandin-E₂. In addition, flavonoids possess antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism. Both the AERRC and AQERRC contained tannins, flavonoids, sterols and triterpenes; these may have contributed for the anti-diarrhoeal activity exhibited.

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