Anti-diarrhoeal activity of crude aqueous extract of *Rubia tinctorum* L. roots in rodents

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Abstract

The Anti-diarrhoeal effect of aqueous extract of *Rubia tinctorum* L. (Rubiaceae) roots in rodents was examined. At doses 300, 600 and 800 mg/kg aqueous extract protected rats, in a dose-dependent fashion, against castor oil-induced diarrhoeal dropping by 37, 59 and 64% respectively. Furthermore, it has significantly inhibited by 41% the gastrointestinal transit of charcoal in mice at 800 mg/kg dose of extract. These data suggest that *Rubia tinctorum* showed antidiarrhoeal activity by inhibiting intestinal motility which was concordant with its use in traditional medicine.

Key words: *Rubia tinctorum* L., antidiarrhoeal effect, transit intestinal

Introduction

Diarrhoea is one of the leading causes of mortality in developing countries and the major cause of this disease is malnutrition. The World Health Organisation (WHO) has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices (Cynthia *et al.*, 2008; WHO, 1999). In Morocco *Rubia tinctorum* L. roots have been used as antidiarrheic, it has been also used in blood diseases, and reputed as aphrodisiac (Bellakhdar, 1997). The plant is reported to possess antimicrobial (Kalyoncu *et al.*, 2006), antifungal activities (Manojlovic *et al.*, 2005) and endowed with diuretic and stone inhibitory activities (Wijnsma *et al.*, 1986).

The aim of the present study was to assess the antidiarrhoeal effects of the crude aqueous extract of *Rubia tinctorum* L. roots on rodent.

Materials and Methods

Plant material

Rubia tinctorum L. roots were collected locally from north eastern area of Morocco region. The botanical identification was done by Professor B. Haloui at the department of Biology, Faculty of Science University Mohammed I Oujda, Morocco. A voucher specimen (N° 50759) was previously deposited in Scientific Institute of Rabat.

Preparation of the aqueous extract

50 g of roots of this plant was boiled in 1L of distillate water for 30 min, as traditionally used. The extract was filtered and evaporated to dryness using vacuum rotary evaporator (yield 12.5 %).

Animals

Wistar rats (200–250 g) and albino Swiss mice (20–25 g) either sex were used for this study. The animals were housed at the animal house of the Department of Biology, Mohammed the First University, Oujda, Morocco. A commercial diet and tap water were provided ad libitum. Animals were fasted for 24 hours before the study with free access to water. All procedures concerning animals were carried out in an ethically proper way by following guidelines as set by the World Health Organization.

Castor oil-induced diarrhoea

Overnight, rats were fasted. The animals were randomized into six groups, control, positive control and test groups containing six rats in each group. Control group received distilled water 1 ml/kg orally. The positive control group received loperamide hydrochloride at the dose of 5 and 10 mg/kg orally by gavage. Test groups received by gavage the crude aqueous extract at the doses of 300, 600 and 800 mg/kg. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed at every defecation. Diarrhoea was induced by oral administration of 1 ml castor oil to each rat, 1 hour after the above treatments. During an observation period of 4 hours, diarrhea onset time was determined by the time that diarrhea began after administration of castor oil. Fecal output was assessed by collecting fecal material for 4 hours after the administration of castor oil, and this was dried at 70°C for overnight before weighing. The percentage fecal output (FOP) was calculated as follows (Pillai, 1992; Akah *et al.*, 1999):

$$\% \text{ FOP} = \frac{ft}{fc} \times 100$$

where ft is the mean fecal weight of each treatment group, and fc is the mean fecal weight of the control group.

The percentage of inhibition was calculated as follows:

$$\% \text{ of inhibition} = 100\% - \% \text{FOP}$$

Small intestinal transit study

The effects on intestinal propulsion in Swiss albino mice were tested using the charcoal method (Abdullahi *et al.*, 2001). Animals were fasted for 24 hour but allowed free access to water. They were randomized into five groups of six animals each. Group 1 (control) received distillate water 0.8 ml by gavage, groups 2, 3 and 4 were treated with *Rubia tinctorum* L. extract 300, 600 and 800 mg/kg *p.o.* respectively; group 5 was given orally with loperamide hydrochloride 5 mg/kg as a standard. After 15 min, each mouse was administered 0.5 ml charcoal meal which contained 3% activated charcoal suspended in 0.5% aqueous cellulose orally by gavage. All the mice were killed 30 min later by cervical dislocation and bled, and the small intestine was rapidly dissected out and placed on a clean surface. The intestine was carefully inspected and the distance traversed by the charcoal meal plug from the pylorus to caecum was measured. The length of the whole small intestine was also measured. The distance traveled by the charcoal plug from pylorus to caecum was expressed as a percentage of the total length of the small intestine (Rao *et al.*, 1997).

$$\text{Intestinal propulsion \%} = \frac{\text{Distance moved by the suspended charcoal head}}{\text{Whole length of small intestine}} \times 100$$

The percentage of inhibition compared with the control group was determined by using the following equation (Aye-Than *et al.*, 1989):

$$\text{Inhibition \%} = \frac{\text{Intestinal propulsion \% (Extract)} - \text{Intestinal propulsion \% (Control)}}{\text{Intestinal propulsion \% (Control)}}$$

Toxicity study

The extract was studied for acute oral toxicity (7 days) using Swiss albino mice (3 males and 3 females in each group), the test was performed using increasing doses (0.0, 5.0 and 10.0 g/kg), given orally, the mice were allowed food and water *ad libitum* and kept under regular observation.

Statistical analysis

Student's *t*-test was used for statistical analysis and $P < 0.05$ was considered to be significant.

Results and Discussion

The crude aqueous extract of *Rubia tinctorum* was orally administered in mice up to the 10 g/kg for 7 days, during this time nothings was indicated and no mortality was observed. Ino *et al.* (1995) have found that the maximum tolerated dose of *Rubia* extract was between 3,500 and 5,000 mg/kg body weight of (C57BL/6 × C3H) F₁ mice after an acute toxicity test performed during 14 days. This difference with our result is due probably to period of treatment which was longer by 7 days or to the fact that content of the two extracts was different.

In the *in vivo* study, the extract (300, 600 and 800 mg/kg) protected in a dose-dependent fashion the rats against castor oil-induced diarrhoeal dropping by 37.16, 59.81% and 64.62%

Table 1. Effect of crude aqueous extract of *Rubia tinctorum* L. roots on fecal output (FOP) in castor oil-induced diarrhoea of Wistar rats

Treatment	Means of dry feces weight (g)	% FOP	% of inhibition	Onset time (min)
Water (1 ml/Kg)	5.43 ± 0.79	—	—	59.67 ± 0.33
<i>Rubia tinctorum</i> L. 300 mg/kg	3.41 ± 0.36	62.83	37.16*	115.4 ± 17.57*
<i>Rubia tinctorum</i> L. 600 mg/kg	2.18 ± 0.25	40.18	59.81*	134.4 ± 23.85**
<i>Rubia tinctorum</i> L. 800 mg/kg	1.92 ± 0.18	35.38	64.62**	185.7 ± 11.46**
Loperamide (5 mg/Kg)	0.54 ± 0.08	36.40	63.58***	187.15**
Loperamide (10 mg/Kg)	0.10 ± 0.10	1.89	98.1***	234.9 ± 2.57***

Values are ± S.E.M (n=6). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, *vs.* control; Student's *t*-test. % of inhibition = 100% minus %FOP.

Table 2. Effect of crude aqueous extract of *Rubia tinctorum* L. roots on gastrointestinal transit in mice

Treatment	Distance traveled by charcoal (as % of total length of small intestine)	% inhibition
Control	72.14 ± 2.83	—
<i>Rubia tinctorum</i> L. (300 mg/kg)	58.05 ± 3.97	-19.52
<i>Rubia tinctorum</i> L. (600 mg/kg)	54.74 ± 4.52*	-24.12
<i>Rubia tinctorum</i> L. (800 mg/kg)	42.23 ± 2.36**	-41.46
Loperamide (5 mg/kg)	49.70 ± 1.25***	-31.10

Values are means ± S.E.M (n=6). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, *vs.* control; Student's *t*-test.

respectively and twice increased the onset time, whereas, 98.1% of the protection was observed in the case of treatment by the standard antidiarrhoeal agent loperamide (10 mg/kg) (Table 1).

Furthermore, the extract significantly has inhibited the gastrointestinal transit of charcoal in mice at 800 mg/kg dose of extract by 41.46% as compared to control, whereas when the loperamide (5 mg/kg) was used, an inhibition of 31.1% was observed (Table 2).

The active component of castor oil is the ricinoleic acid. The latter has induced diarrhoea by a hypersecretory response and stimulating peristaltic activity (Ammon *et al.*, 1974; Zavala *et al.*, 1998). The pharmacological effect of the standard antidiarrhoeal agent is due to its antimotility and antisecretory properties (Couper, 1987). From this investigation, it is likely that the extract mediated its effect through similar mechanisms or antispasmodic effect which reduced intestinal contractions and hence allowing a greater time for absorption of water.

Major constituents of *Rubia Tinctorum* are more than 30 anthraquinones, such as alizarin, lucidin, lucidin primeveroside, ruberythric acid (Goverdina *et al.*, 2002). It is also worth to note that the plant Senna (*Cassia acutifolia* pods) has been used as laxative for centuries (Ben, 1972) thanks to its anthraquinones content. Further investigations are necessary to determine which anthraquinones or other products are responsible for this biological activity.

The results of this study seem to provide a support for the traditional medicine use of *Rubia tinctorum* as antidiarrhoeal agent. This support is accented by an Antimicrobial activity study which revealed that the aqueous extract of *Rubia tinctorum* L. roots exhibited an antibacterial activity against a number of pathogenic bacterial strains that cause diarrhoea (Kalyoncu, *et al.*, 2006).

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