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Antidiarrhoeal activity of leaf methanolic extract of *Rauwolfia serpentina*

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ABSTRACT

Objective: To evaluate the antidiarrhoeal property of methanol extract of the leaves of *Rauwolfia serpentina* (*R. serpentina*) in experimental diarrhoea induced by castor oil in mice. **Methods:** Doses of 100, 200 and 400 mg/kg *R. serpentina* leaf methanol extracts were administered to castor oil induced diarrhoea mice to determine its antidiarrhoeal activity. **Results:** All doses of the extract and the reference drug atropine sulphate (3 mg/kg, i.p.) produced a dose-dependent reduction in intestinal weight and fluid volume. The extracts also significantly reduced the intestinal transit in charcoal meal test when compared to diphenoxylate Hcl (5 mg/kg, p.o.). **Conclusions:** The results show that the extract of *R. serpentina* leaves has a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

1. Introduction

Large numbers of epidemiological and experimental researches indicate that acute-diarrhoeal diseases mainly occur in developing countries of the world, and are the foremost cause of death in children under five globally, causing about 1.5 million deaths yearly which are majorly associated with malnutrition[1]. The World Health Organization has formed a Diarrhoea Disease Control Program (CDD) in an attempt to wipe out the problem of diarrhoea in developing countries[1] which includes traditional remedies, health education evaluation as well as prevention approaches[2,3]. It may then be very important to identify and evaluate commonly available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects[4]. A wide range of medicinal plants with anti-diarrhoeal properties have been widely used by traditional healers; the pharmacological evaluation of some has shown the efficacy of some traditional medicines in treating diarrhoea[5]. However, most traditional remedies have not been precisely evaluated.

Rauwolfia serpentina (*R. serpentina*) (Apocynaceae), commonly known as Indian snake root, is a widely grown plant in Southeastern Nigeria and has been reported to possess medicinal uses such as hypotension, anticancer, central nervous system depressant, hypnotic, antidote against bite from poisonous reptiles, anti-dysentry, *etc*[6,7]. This study was therefore carried out with methanol extract of the leaves of *R. serpentina* to validate the folklore claims of its potent antidiarrhoeal activity using castor oil-induced diarrhoea model.

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2. Materials and methods

2.1. Plant material

Fresh leaves of *R. serpentina* were collected around Isieke Afaranta, Ibeku-Umuahia, Abia State, Nigeria in November, 2010. They were taxonomically identified by Dr. MC Dike of the Forestry Department of the Michael Okpara University of Agriculture, Umudike. Voucher specimen (VPP/CVM/MOUAU/33/2010) has been deposited at the Herbarium of the Department of Veterinary Physiology, Biochemistry and Pharmacology, Michael Okpara University of Agriculture, Umudike.

2.2. Preparation of the extract

The leaves were dried under shade and comminuted into coarse powder and 100 g of the dried plant was extracted with 80% MeOH and concentrated in vacuo (yield: 7.13% on dried weight). Preliminary qualitative phytochemical analysis of the methanol extract of *R. serpentina* (MERS) was performed.

2.3. Animals

Albino Swiss mice of either sex weighing (26–48 g) were used to evaluate the anti-diarrhoeal, anti-secretory and anti-intestinal transit activities. All animals were fed standard animal feed (Vital feed®, Nigeria) and clean drinking water *ad libitum* before the experiments. Each experimental group consisted of six animals housed in separate cages.

2.4. Acute toxicity test

The method of Lorke[8] was employed in this study. Twenty five mice of both sexes were randomly grouped into five with five mice in each group and were fed orally with graded doses (100, 500, 1 000, 1 500 and 2 000 mg/kg of MERS by gastric gavage. The animals were allowed free access to feed and water. They were observed over a 48 h period for acutely toxic signs and death.

2.5. Castor oil-induced diarrhoea

The methods described by Adeyemi and Akindele[9] were employed. Mice were divided into five groups of six animals each, diarrhoea was induced by administering 1 mL of castor oil orally to mice. Group 1 served as control (10 mL/kg, p.o. distilled water), group 2 received diphenoxylate Hcl (5 mg/kg, p.o.) served as standard and groups 3, 4, and 5 received MERS (100, 200 and 400 mg/kg, p.o.), respectively, 1 h before castor oil administration. The number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4 h. Mean of the wet stools passed by the treated groups were compared with those of the control groups.

2.6. Effect on castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method described by Yasmeen *et al*[10]. Animals were fasted overnight and divided into five groups of six animals each. Group 1 received distilled water (10 mL/kg, p.o), served as a negative control, group 2 received atropine sulphate (3 mg/kg, i.p., positive control) and groups 3, 4 and 5 received MERS of 100, 200 and 400 mg/kg orally, respectively 1 h before the oral administration of castor oil. Two hours later the animals were sacrificed by cervical dislocation, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by being milked into a graduated tube and their volumes were measured. The intestine was reweighed and the differences between full and empty intestines were calculated.

2.7. Effect on small intestinal transit

Mice were fasted for 18 h and divided into five groups of six animals each. Group 1 received 2 mL of castor oil orally with distilled water 10 mL/kg orally. Group 2 received diphenoxylate Hcl (5 mg/kg, p.o.) groups 3, 4 and 5 received 100, 200 and 400 mg/kg orally of the MERS, respectively, 1 h before administration of castor oil. One mL of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The animals were sacrificed after 1 h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum[11,12].

2.8. Statistical analysis

The experimental results were represented as mean ± standard error of the mean (SEM). ANOVA and *post hoc* LSD were used for the evaluation of data and $P<0.05$ was considered significant.

3. Results

Preliminary qualitative phytochemical analysis of MERS showed the presence of alkaloids, saponins, flavonoids, phenols, tannins, riboflavin, niacin and terpenes in trace amounts. No acutely toxic signs and death were observed in acute toxicity test.

Oral administration of 100, 200 and 400 mg/kg doses of MERS to mice reduced in a dose dependent manner the frequency as well as the wetness of faecal droppings when compared with the untreated control (Table 1).

Castor oil caused an accumulation of water and electrolytes in intestinal loop. The reference drug atropine sulphate (3 mg/kg, i.p.) and the 200 and 400 mg/kg doses of the extract produced a reduction in intestinal weight and fluid volume. However, the reference drug produced a better result. This could be attributed to the crude nature of the extract as further fractionation may produce a better effect. The results showed that 100 mg/kg, p.o. dose of extract produced 30.04% inhibition of volume of intestinal content, while, 200 and 400 mg/kg, p.o. doses produced 32.73% and 39.97% inhibition of volume of intestinal content, respectively. The weight of intestinal content was also reduced at all the doses employed (Table 2).

The movement of the orally administered charcoal meal through the small intestine at the highest dose of 400 mg/kg was significantly decreased from 70.35% to 60.03% when compared with the controls (Table 3).

Table 1
Effect of the extract of *R. serpentina* on castor oil-induced diarrhoea (mean±SEM).

Groups	Treatments (mg/kg)	Faeces (*n*/mice)	Wet faeces (*n*/mice)	% Antidiarrhoeal activity
1	Distilled water (10 mL/kg)	6.02±0.03	17.03±0.02	73.91
2	Diphenoxylate (5)	21.60±0.65	5.12±0.11	19.23
3	MERS (100)	8.19±0.06	5.46±0.13**	40.02
4	MERS (200)	15.61±0.01	7.85±0.02	33.47
5	MERS (400)	12.06±0.13*	6.12±0.08	27.36

*: $P<0.05$, **: $P<0.01$ when compared with control.

Table 2
Effect of *R. serpentina* extract on castor oil-induced enteropooling in mice (mean±SEM).

Groups	Treatments (mg/kg)	Volume of intestinal content (mL)	Weight of intestinal content (g)	% Inhibition
1	Distilled water (10 mL/kg)	0.39±0.03	0.43±0.03	0.00
2	Atropine (3)	0.23±0.02	0.21±0.04	45.35
3	MERS (100)	0.40±0.01	0.53±0.03	30.04
4	MERS (200)	0.29±0.01	0.35±0.04	32.73
5	MERS (400)	0.23±0.03	0.22±0.10	39.97

Table 3
Effect of *R. serpentina* extract on charcoal transit time in mice (mean±SEM).

Groups	Treatments (mg/kg)	Length of stomach (cm)	Distance covered (cm)	% Average travel
1	Distilled water (10 mL/kg)	38.82±3.02	27.44±1.15	70.35
2	Diphenoxylate (5)	36.97±1.21	13.28±0.33	54.65
3	MERS (100)	33.69±0.13**	23.56±0.27	69.96
4	MERS (200)	39.27±0.21**	26.41±1.14*	67.27
5	MERS (400)	41.02±0.09*	24.62±1.01	60.03

*: $P<0.05$, **: $P<0.01$ when compared with control.

4. Discussion

The prevention of intraluminal fluid secretion, caused by castor oil was observed with the reference drug and the extract at 200 and 400 mg/kg doses which may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut. Suppression of intestinal fluid accumulation by extracts may also suggest inhibition of gastrointestinal function[13,14]. Previous studies have incriminated tannins, flavonoids, reducing sugars/glycosides among others as potent antidiarrhoeal and antidysentery agents[15]. Flavonoids have also been shown to have inhibitory actions on intestinal motility[16]. These aforementioned chemical constituents are present in MERS, thus, may be associated with the antidiarrhoeal findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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