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Antidiarrheal activity of methanolic leaf extract of *Rumex vesicarius*

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Abstract

This study evaluates the antidiarrheal activity of *Rumex vesicarius* (leaf) by using *in vitro* and *in vivo* assays. Antidiarrheal effect of *R. vesicarius* was evaluated using castor oil-induced diarrhea model in rat. Weight and volume of the intestinal content were assessed using the enteropooling method. Atropine (3 mg/kg, i.p) was used as positive control. *R. Vesicarius* at the doses of 200 and 400 mg/kg p.o. significantly retarded castor oil-induced enteropooling and intestinal transit. The gastrointestinal transit rate was studied and *R. vesicarius* at the doses of 200 and 400 mg/kg significantly inhibited ($p<0.001$) weight and volume of intestinal content. *R. vesicarius* caused concentration-dependent (0.01–1 mg/mL) relaxation of spontaneous contractions in isolated rabbit jejunum tissue preparation and inhibited K^+-80 induced contractions (0.01-5 mg/mL), similar to verapamil, suggestive of calcium channel blockade. Results obtained herein indicate that *R. vesicarius* may contain effective compounds which can be used as an antidiarrheal agent.

Introduction

Diarrhea affects 70% of the world population (Ouyang and Chen, 2004), especially in the third world countries where sanitary conditions are alarming. Acute diarrhea being the most common, is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions can contributing factors (Thapar and Sanderson, 2004). Rotavirus is the major causative agent of infectious diarrhea, particularly in young children now-a-days, however, other viral (*Enterovirus, norovirus* and adenovirus), bacterial (*Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens (Allen et al., 2004).

Rational use of herbal medicines is accepted universally; here we attempt to investigate the folklore claim of *Rumex vesicarius* L. leaf extracts for antidiarrheal activity. *R. vesicarius* has been in use cooling agent, astringent, anti-venom agent and appetizer for the treatment of allaying pain of toothache, nausea, and insect bite, seeds were used for dysentery (Dymoke, 1972).

In the Ayurvedic system of medication, it was used as stomachic (Ahirrao et al., 2011), antitumor, analgesic, flatulence, spleen disease, high cough, asthma, laxative, bronchitis, dyspepsia, heart troubles, alcoholism and biliousness (Kirtikar and Basu, 1987). In the Unani system of medication, it was used as a tonic, in leucoderma, scabies and as diuretic (Kirtikar and Basu, 1987). In other folk medicines, it was used to eradicate piles, constipation and hiccup (Hariparasad, 2011). Reptile insect, urinary affection, hepatoprotective, dysmenorrhea, blood purifier, depurative, sedative, alkalinity, chronic catarrh, renal disorders, dyspepsia, bloody dysentery and coronary (Madhavashetty et al., 2008), vomiting (Khan et al., 2013), leucoderma, antiviral, lymphatic glyndular system disease, antidiabetic, rectal prolapsus, aphrodisiac anticholesterol, impetigo and carbubuncles (Pullaiah and Ali, 1999), antioxidant (Rao, 2003), anthelmintics (Rao et al., 2012), cancer and inflammation (Aggarwal et al., 2006), spasmogenic and

spasmolytic (Khan et al., 2014a), diuretic (Rao et al., 2011), antifungal (Amira et al., 2011), and antipyretic (Khan et al., 2014b). This study reports the antidiarrheal activity of methanolic leaf extracts of *R. vesicarius*.

Materials and Methods

Plant material

R. vesicarius was collected in December, 2012 from the sandy fields of Mondka Shahjamal District, Muzaffar Garh, Pakistan. The plant material was authenticated by expert taxonomist, Prof. A. H. Dasti at the Department of Botany, Bahauddin Zakariya University, Multan, Pakistan (voucher F.P.ST-215). The plant material was made free from foreign adulterants and vegetative debris by hand picking and leaves were detached from the plant, washed and shade dried. Within 8 days, the leaves became crispy. The special electrical herbal grinder was used to form the coarse powder. Uniform dark green powder was obtained with a characteristic smell.

Crude extract

The powdered plant material (1 kg) was subjected to maceration in 70% methanol in an amber colored glass bottle at room temperature (25°C) for 8 days with occasional shaking (Aziz et al., 2013a). The soaked material was passed through muslin cloth to remove the vegetative material and the fluid obtained was filtered through Whatman No. 1 Filter paper. The filtrate was evaporated on a rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) at 37°C under reduced pressure. The approximate yield was 11% and the extract obtained was stored at -4°C in airtight jars in lab refrigerator.

Preliminary phytochemical screening

Major phytochemical classes were screened by the method described by Aziz et al. (2013b).

Chemicals and drugs

All the chemicals, solvents, and drugs used were of analytical grade. Atropine was purchased from Ethical Laboratories (Pvt.) Ltd. Pakistan. Verapamil was purchased from Aventis Pharmaceuticals (Pvt.) Ltd. Pakistan. All other chemicals used were of the available analytical grade.

Animals and housing condition

Five adults locally breed rabbits (1.0-1.5 kg) of either sex, purchased from the animal market Hussain Agahi Multan, Pakistan, with age limit between 6 to 7 months were used for the experiments. Fifteen rats (200–240 g) were purchased from the animal house Islamia University, Bahawalpur, Pakistan. Animals were provided with fresh green fodder and tap water *ad libitum* and maintained in an air-conditioned room (23-25°C) at the Faculty of Pharmacy, Bahauddin Zakariya University, Multan. All rabbits were kept in fasting condition for at least 12 hours before the commencement of experiments, but had free access to water. The experiments were approved by the Ethical Committee of the Bahauddin Zakariya University, Multan, (EC/12/2012 dated 07 December 2012).

Castor oil-induced diarrhea

Rats were divided into 4 groups of 3 animals each, diarrhea was induced by administering 1 mL of castor oil orally to rats. The Group I treated as control (2 ml/kg, i.p. saline); Group II received atropine (3 mg/kg, i.p.) served as standard and Group III and IV received *R. vesicarius* (200 and 400 mg/kg, i.p.) 1 hour before castor oil administration. The number of both wet and dry diarrheal droppings was counted every hour for a period of 4 hours mean of the stools passed by the treated groups were compared with that of the positive control groups consisted of animals given an intraperitoneal injection of saline and atropine (Aziz et al., 2014).

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert et al. (1976). Overnight fasted rats were divided 4 groups of 3 animals each. The Group I received normal saline (2 mL/kg, i.p.) served as a control; Group II received atropine (3 mg/kg, i.p.) and Groups III and IV received *R. vesicarius* 200 and 400 mg/kg orally respectively. 1 hour before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Small intestinal transit

Rats fasted for 18 hours were divided into 5 groups of 3 animals each. Group I received 2 mL normal saline orally; Group II received 2 mL of castor oil orally with saline 2 mL/kg intraperitoneally; Group III received atropine (3 mg/kg, i.p.); Group IV and V received *R. vesicarius* 200 and 400 mg/kg intraperitoneally respectively, 1 hour before administration of castor oil. One mL of the marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 hour after castor oil treatment. The rats were sacrificed after 1 hour and the distance traveled by the charcoal meal from the pylorus was measured and expressed as the percentage of the total length of the intestine from the pylorus to cecum (Izzo et al., 1999).

Isolated rabbit jejunum preparations

The rabbit was starved overnight and was sacrificed subsequently to a blow on the head. The abdomen was opened and jejunum was dissected out and cut into

segments of about 2 cm in length following removal of adhering mesenteries. The segments were mounted between two stainless steel hooks in a 10 mL tissue bath, containing the normal Tyrode solution (pH 7.4), maintained at 37°C and aerated with carbogen (5% CO_2 + 95% O_2). A preload of 1 g was applied and the tissue was allowed to equilibrate for a period of 30 min during which the tissue was washed with fresh fluid at an interval of every 10 min prior to exposure to any test material. The spontaneous contractions were recorded isotonically through a Power Lab Data Acquisition System (AD Instruments, Sydney, Australia) (Khan et al., 2014a).

Statistical analysis

The data expressed are the mean ± standard error of the mean (SEM) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). The statistical parameter applied in the castor oil-induced diarrhea test is chi-square test. $p<0.05$ was noted as significantly different. Concentration-response curves were analyzed by nonlinear regression using Graph Pad program version 6 for Windows (Graph Pad, USA).

Results

Phytochemical analysis of *R. vesicarius* methanolic extract shown the presence of alkaloids, glycosides, saponins, tannins, anthraquinones, coumarins and phenols.

Castor oil-induced diarrhea, 30 min after administration of castor oil, the diarrhea was clinically apparent in all the animals of the control group, for the next 4 hours. This was markedly reduced by the intraperitoneal injection of atropine, 3 mg/kg (74.3%). A similar marked reduction in the number of defecations after 4 hours was achieved with *R. vesicarius* the doses of 200 or 400 mg/kg i.p. *R. vesicarius* 200 and 400 mg/kg significantly inhibited the defecation (39.2% and 66.3%). The dose of extract delayed the onset of diarrhea and only 30% of animals showed diarrhea at first hour ($p<0.001$) (Table I). While, in castor oil induced interpooling both the doses 200 and 400 mg/kg significantly inhibited the weight of intestinal content, 26.3 and 49.1 respectively (Table II). Furthermore, on castor oil-induced small intestinal transit both the doses of *R. vesicarius* shown 29.9 and

Table I

Caster oil-induced diarrhea in rats

Group	Treatment	Number of defecation (within 4 hours)	%Inhibition of defecation
I	Castor oil (1 mL p.o) + saline (2 mL/kg i.p)	27.2 ± 2.1	---
II	Castor oil (1 mL p.o) + atropine (3 mg/kg i.p)	6.9 ± 0.2[b]	74.3
III	Castor oil (1 mL p.o) + *R. vesicarius* (200 mg/kg i.p)	16.5 ± 0.9[a]	39.2
IV	Castor oil (1 mL p.o) + *R. vesicarius* (400 mg/kg i.p)	9.1 ± 0.7[b]	66.3

Effect of *R. vesicarius* on castor oil-induced diarrhea in rats: *R. vesicarius* was administered i.p 1 hour before castor oil administration. Values are expressed as mean ± SEM from the experiments; [a]$p<0.01$, [b]$p<0.001$ when compared with castor oil + saline-treated group

Table II

Effect of *R. vesicarius* on castor oil-induced enteropooling in rats

Group	Treatment	Weight of intestinal content (g)	%Inhibition of weight intestinal content
I	Castor oil (1 mL p.o) + saline (2 mL/kg i.p)	2.4 ± 0.1	---
II	Castor oil (1 mL p.o) + atropine (3 mg/kg i.p)	1.8 ± 0.1[b]	24.4
III	Castor oil (1 mL p.o) + *R. vesicarius* (200 mg/kg i.p)	1.7 ± 0.1[a]	26.1
IV	Castor oil (1 mL p.o) + *R. vesicarius* (400 mg/kg i.p)	1.2 ± 0.1[b]	49.3

Effect of *R. vesicarius* on castor oil-induced enteropooling in rats: *R. vesicarius* was administered i.p 1 hour before castor oil administration. Values are expressed as mean ± SEM from the experiments; [a]$p<0.01$, [b]$p<0.001$ when compared with castor oil + saline-treated group

Table III

Effect of *R. vesicarius* on castor oil-induced small intestinal transit in rats

Group	Treatment	Total length of intestine (cm)	Distance travelled by marker (cm)	%Intestinal transit inhibition
I	Saline (2 mL/kg i.p)	82.8 ± 1.6	45.5 ± 1.7	44.9
II	Castor oil (1 mL p.o) + saline (2 mL/kg i.p)	80.2 ± 2.9	72.8 ± 1.2	9.2
III	Castor oil (1 mL p.o) + atropine (3 mg/kg i.p)	83.5 ± 2.8	35.8 ± 1.3[b]	57.0
IV	Castor oil (1 mL p.o) + *R. vesicarius* (200 mg/kg i.p)	80.3 ± 1.8	56.2 ± 1.7[a]	29.9
V	Castor oil (1 mL p.o) + *R. Vesicarius* (400 mg/kg i.p)	82.0 ± 1.2	45.1 ± 1.3[b]	45.0

Effect of *R. vesicarius* on castor oil-induced small intestinal transit in rats: *R. vesicarius* was administered i.p 1 hour before castor oil administration. Values are expressed as mean ± SEM from the experiments; [a]p<0.01, [b]p<0.001 when compared with castor oil + saline-treated group

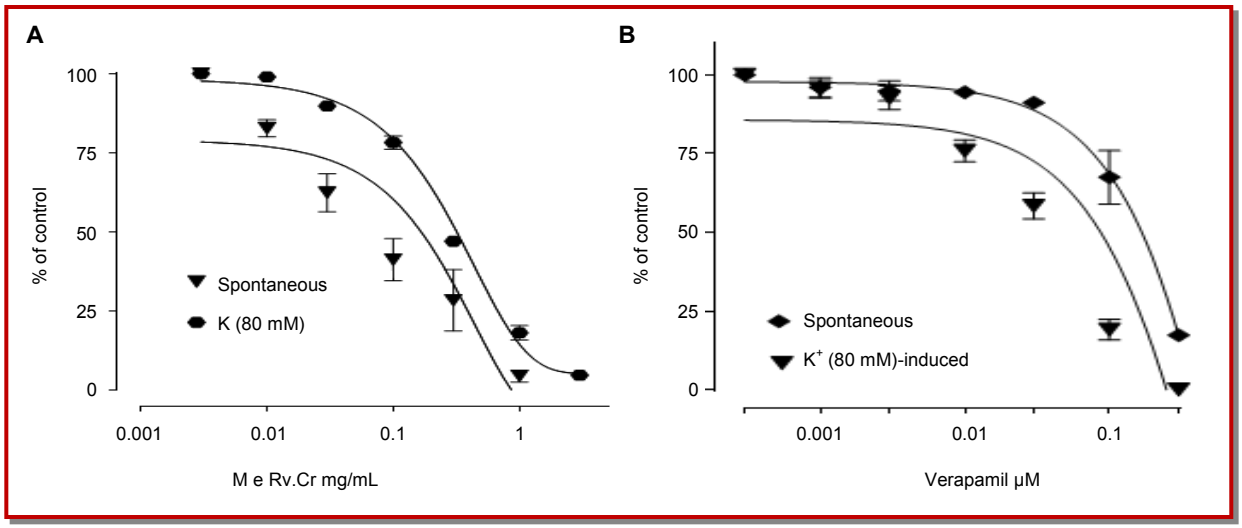

Figure 1: Concentration response curves showing the inhibitory effect of *R. vesicarius* (A) and verapamil (B) on spontaneous and K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. The values shown are mean ± SEM of 5 observations

45% inhibition (Table III). *R. vesicarius* caused concentration-dependent (0.01–1 mg/mL) relaxation of spontaneous contractions in isolated rabbit jejunum tissue preparation and inhibited K^+-80 induced contractions (0.01-5 mg/mL), similar to verapamil, suggestive of calcium channel blockade (Figure 1). The calcium channel blockade effect was confirmed when pretreatment of the jejunum preparation with *R. vesicarius* produced a concentration-dependent (0.03-1 mg/mL) rightward shift in Ca^{++} concentration response curves, as caused by verapamil (Figure 2).

Discussion

R. vesicarius exhibited a dose-dependent protective effect against diarrhea. It is also noted that *R. vesicarius* significantly inhibited castor oil-induced intestinal fluid accumulation and the volume of intestinal content, dose dependently more than atropine. *R. vesicarius* significantly reduced the castor oil induced intestinal transit. In this study, atropine produced a significant reduction in the number of stools and increased intestinal transit time possible due to its anti-cholinergic effect (Croci et al., 1997). However, it did not inhibit castor oil induced enteropooling and gain in weight of intestinal content, suggesting thereby that mediators other than acetylcholine are involved in castor oil induced enteropooling. An increase in intestinal transit time with atropine could also result due to the reduction in gastric emptying (Pierce et al., 1997). Castor oil is also reported to induce diarrhea by increasing the volume of intestinal content by prevention of the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Iwao and Terada, 1962), thereby prevents the reabsorption of NaCl and water

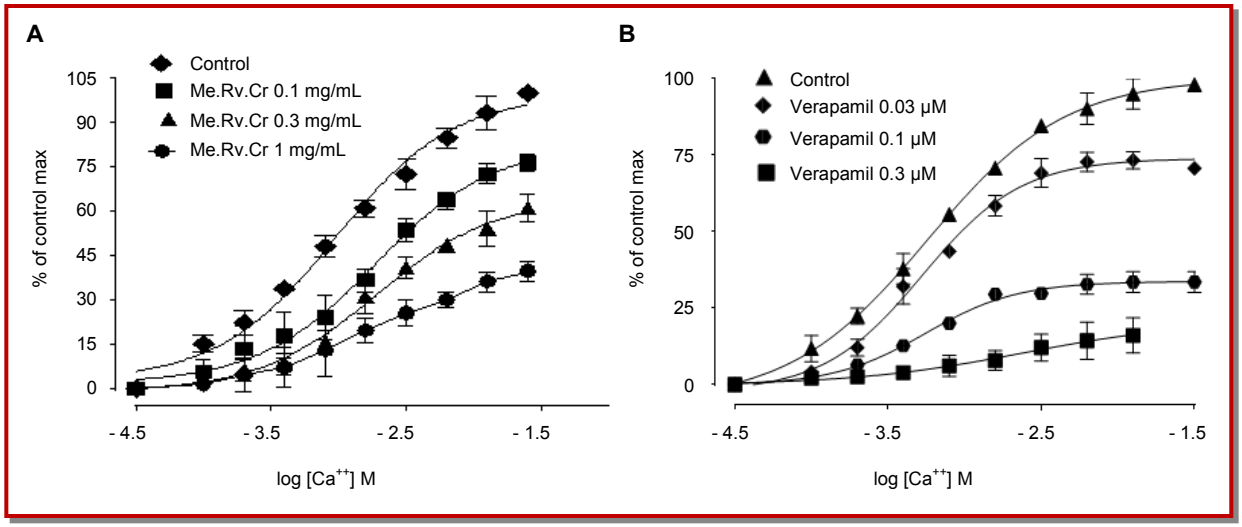

Figure 2: Effect of the *R. vesicarius* on concentration-response curve of Ca^{2+} on isolated rabbit jejunum preparations (A), and effect of the verapamil (standard) on concentration–response curve of Ca^{2+} on isolated rabbit jejunum preparations (B); Values are expressed as mean ± S.E.M.; n=5

(Mascolo et al., 1994). Probably R. vesicarius increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

The secretary diarrhea is associated with an activation of Cl- channels, causing Cl- efflux from the cell, the efflux of Cl- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea (Galvez, 1993). *R. vesicarius* may inhibit the secretion of water into the lumen by altering this mechanism. Antidiarrheal property of medicinal plants is found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Ammon et al., 1985). The phytochemistry of R. vesicarius revealed the presence of alkaloids, triterpinoids, tannins, flavonoids, phenols, gums, carbohydrates and mucilage (Hariparasad, 2011). These phytoconstituents may mediate the antidiarrheal property of the *R. vesicarius*. Although the antidiarrheal properties of the reported active terpenoids are well established. Sesquiterpenes, diterpenes, flavonoids, terpenes, and terpenoid derivatives are known for inhibiting release of autocoids and prostaglandins, thereby inhibit the motility and secretion induced by castor oil (Vimala et al., 1997; Longanga et al., 2000). *R. Vesicarius* is reported effective in many antimicrobial studies against the bacteria and fungus causing infectious diarrhea (Mostafa and ElBakry, 2011; Sahli and Abdulkhair, 2011).

Conclusion

The presence of antidiarrheal activity *in vivo* and *in vitro* assays in *R. vesicarius* mediated possibly through a calcium antagonistic mechanism, which might explain the traditional use of the plant in abdominal cramps, diarrhea and dysentery.

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