

Full Length Research Paper

Pharmacological evaluation of *Rumex vesicarius* Linn leaf extract and fractions in rabbit gastrointestinal ailment

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Received 13 February, 2014; Accepted 25 March, 2014

The aqueous-methanolic leaf extract and fractions of the *Rumex vesicarius* L. (Rv.Cr) was evaluated for the possible presence of spasmogenic and spasmolytic constituents to rationalize its traditional uses in gastrointestinal disorders. In rabbit jejunum, *R. vesicarius* caused a dose-dependent (0.03 to 0.3 mg/ml) weak stimulatory effect on spontaneous contractions, followed by relaxation at the next higher dose (1 mg/ml). In the presence of atropine (0.03 µM), the spasmogenic effect was abolished and the relaxant effect was obtained at lower doses (0.1 to 1.0 mg/ml) shifting the dose response curves to the left. The spasmolytic effect on the spontaneous and K⁺-induced contractions in atropinized preparations was mediated at doses 0.03 to 1.0 mg/ml and 0.3 to 5 mg/ml, respectively which explained the involvement of calcium channel blocking (CCB) effect. The CCB effect was confirmed when pretreatment of the tissue with *R. vesicarius* produced a dose-dependent shift in the Ca⁺⁺ dose-response curves to the right in a similar manner as verapamil. Activity-directed fractionations revealed that the spasmolytic effect was concentrated in methanolic fraction, while spasmogenic activity in the aqueous fraction which was remarkably stronger than aqueous-methanolic extract. This data shows that the crude leaf extract of *R. vesicarius* L. contains spasmogenic and spasmolytic constituents mediating their effect through cholinergic and CCB actions, respectively, which explains its traditional use in the gastrointestinal disorders such as abdominal colics and cramps, constipation and diarrhea, gastroesophageal reflux disease and irritable bowel syndrome/inflammatory bowel disease.

Key words: *Rumex vesicarius* Linn, spasmogenic, Spasmolytic, Cholinergic, CCB.

INTRODUCTION

Constipation and diarrhea, colic and cramps are affecting 70% of the population worldwide (Quyang and Chin, 2004). Developing countries such as Pakistan, India and Bangladesh are commonly facing gastrointestinal disorders due to low standard sanitary conditions. Acute

diarrhea is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions can contribute a lot (Thapar and Sanderson, 2004). Rotavirus is the major causative agent for infectious diarrhea, especially in young ones. However, other

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microbial agents viral (adenovirus, enterovirus and norovirus), bacterial (*Shigella* species, *Escherichia coli*, *Salmonella* species, *Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) also disturbs the normal tone of gastrointestinal tract (Allen et al., 2004). *Rumex vesicarius* Linn., locally known as "Khat palak". *R. vesicarius* L. has been used traditionally for ally pain of toothache, as astringent for nausea, cooling agent, anti-venom, and insect bite, and as appetizer, seeds were used for dysentery (Dymoke, 1972). In Ayurvedic system of medication, it was used as stomachic (Ahirrao et al., 2012), analgesic, anti tumor, laxative, flatulence, spleen disease, high cough, asthma, bronchitis, dyspepsia, heart troubles, alcoholism, and biliousness (Kirtikar and Basu, 1987). In Unani system of medication, it was used as analgesic, tonic, leucoderma, for scabies, and diuretic (Kirtikar and Basu, 1987). In other folk medicines, it was used to eradicate, hiccup, piles, and constipation (Hariparasad, 2011). Reptile insect, hepatoprotective, depurative, dysmenorrhoea, blood purifier, sedative, alkalinity, dyspepsia, urinary affection, chronic catarrh, renal disorders, bloody dysentery, and coronary (Madhavashetty et al., 2008), vomiting (Khan et al., 2013), antiviral, antidiabetic, leucoderma, lymphatic glandular system disease, rectal prolapsus, aphrodisiac anti-cholesterol, impetigo, and carbuncles (Nardkarnis, 2008; Pullaiah and Ali, 1999), antioxidant (Rao, 2003), stomach ache (Rao and Patil, 2012), diuretic (Rao et al., 2011), cancer and inflammation (Aggarwal et al., 2006), anti-fungal (Amira et al., 2011), anthelmintics (Rao et al., 2012), and antipyretic (Khan et al., 2013).

This study reports the spasmodic and antispasmodic activity of aqueous methanolic leaf extract of *R. vesicarius* Linn and its fractions.

MATERIALS AND METHODS

Plant

Indigenous medicinal plant *R. vesicarius* L. known by a local name of "Khat palak" belongs to family Polygonaceae. The plant was collected from the sandy fields of Mondka Shahlamal District, Muzaffar Garh, Pakistan. The plant material was authenticated by expert taxonomist, Professor Dr. 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, leaves became crispy. Special electrical herbal grinder was used to form coarse powder. Uniform dark green powder was obtained with characteristic smell.

Crude extract

The powdered plant material (1 kg) was subjected to maceration in 70% methanol in amber coloured glass bottle at room temperature for 8 days with occasional shaking (Harborne, 1973). The soaked material was passed through muslin cloth to remove the vegetative

material and the fluid obtained was filtered through Whatman-1 Filter paper. The filtrate was evaporated on a rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) at 37°C under reduced pressure. Approximate yield was 11% and the extract obtained was stored at -4°C in air tight jars in lab refrigerator.

Preliminary phytochemical screening

Phytochemical screening was done for the presences of vital phytochemical classes by the method described by Tona et al. (1998).

Chemicals and drugs

All the chemicals, solvents, and drugs used were of analytical grade. Acetylcholine chloride and atropine sulfate were purchased from Ethical Laboratories Pvt. (Ltd) Pakistan. Dimethylsulfoxide, ethylenediamine tetraacetic acid, glucose, magnesium chloride, magnesium sulfate, potassium chloride, potassium dihydrogenophosphate, sodium chloride, sodium bicarbonate, and sodium dihydrogenophosphate were purchased from Sigma Chemical Company, St. Louis, MO, USA. Calcium chloride was purchased from Merck (Merck, Darmstadt, Germany).

Animals and housing condition

Ten adult albino rabbits (1.0 to 1.5 kg) of either sex, purchased from the local market with age limit between 6 and 7 months were used for the experiments. Animals were provided with fresh green fodder and tap water *ad libitum* and maintained in air conditioned room (23 to 25°C) at the Faculty of Pharmacy, Bahauddin Zakariya University, Multan. All rabbits were kept in fasting condition for at least 24 h 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, with reference number EC/12/2012 dated 07 December 2012.

Plant extract solution

The plant extract (0.3 g) was dissolved in 1 ml of methanol to produce stock solution. From this stock solution further dilutions were made. Solutions were freshly prepared on the day of experiment.

Antispasmodic activity

We used the procedure described by Farre et al. (1991) to screen spasmolytic activity. Contractions in the intestine portions were produced by high KCl (80 mM) to depolarize the intestine portions (Farre et al., 1991). The extracts were then applied in similar fashion to relax the tissues and percentage relaxation response on KCl induced contractions was recorded as shown in Figure 1. The following formula was used for calculations:

$$\text{Inhibition/Stimulation (\%)} = 100 - \frac{\text{Average height of normal contraction (mm)}}{\text{Average height of contractions after extract (mm)}} \times 100$$

Spasmodic activity

The extract was screened for possible cholinomimetic activity as per procedure mentioned. Tyrode's solution was prepared having

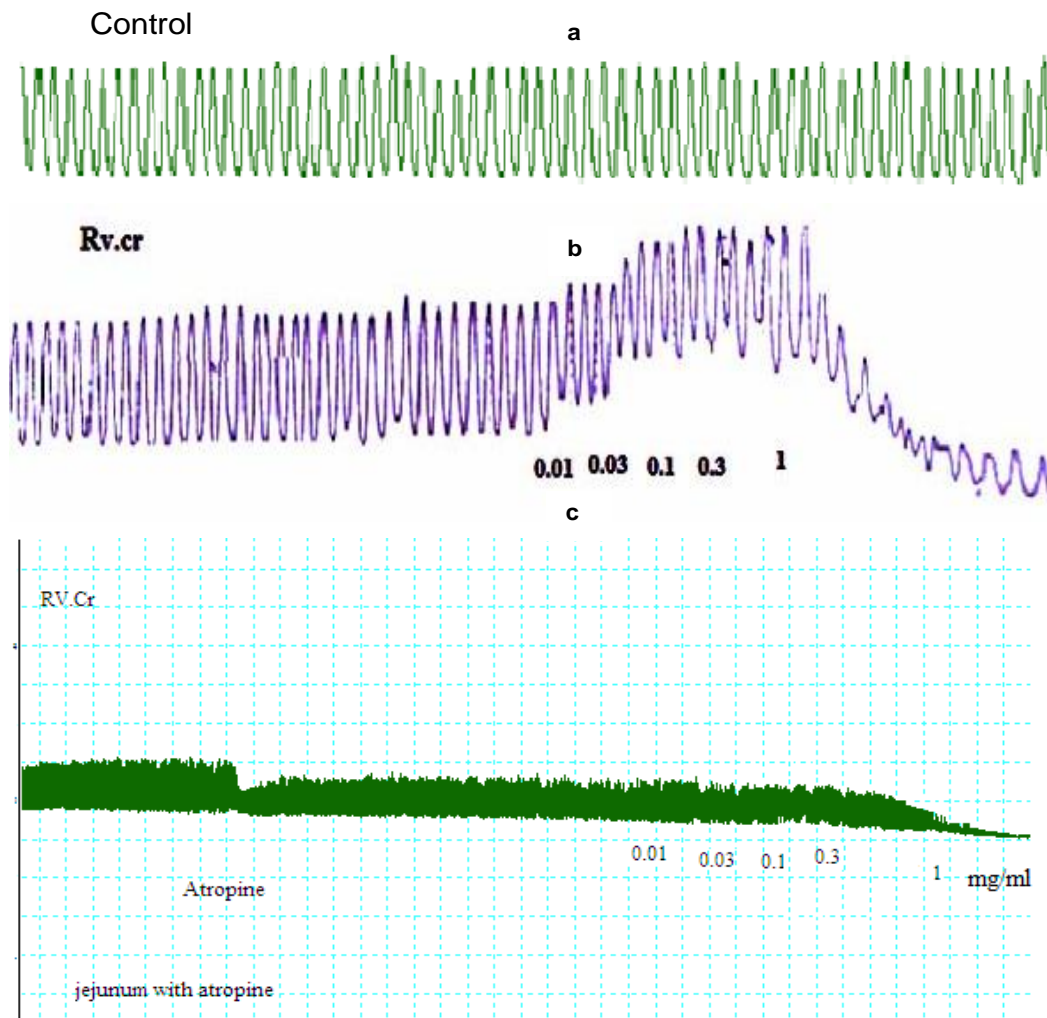


Figure 1. Tracing showing control (a) and the effect of crude methanolic extract of *Rumex vesicarius* leaf (Rv.Cr) on isolated rabbit jejunum preparations (b) and with atropine (c).

the following concentration (mM): KCl 2.68, NaCl 136.9, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, CaCl₂ 1.8 and glucose 5.55. The animals were then slaughtered and their abdomens were opened. Rabbit's jejunum portion(s), of about 1.5 to 2 cm length, was isolated and mounted in the tissue bath containing 10 ml of Tyrode's solution maintained at 37°C and supplied with carbogen gas (5% carbon dioxide and oxygen mixture). These portion(s) were kept in Tyrode's solution previously aerated with the carbogen gas (Qayum, 2004). Earlier, the tissues were stabilized for normal activity for a period of about 25 to 40 min. For possible pharmacological screening on the tissues through series of experiments, methanolic leaf extract of *R. vesicarius* was tried at doses of 0.01 to 05 mg/ml. All the doses were applied in cumulative manner and the results were recorded (Farre et al., 1991). The spasmogenic and spasmolytic activity were recorded.

Assay method

Isolated rabbit jejunum preparations

The rabbit was starved over night and was sacrificed subsequent to a blow on the head. The abdomen was opened and jejunum was

dissected out and cut to 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 normal Tyrodes solution (pH 7.4), maintained at 37°C and aerated with carbogen (5% CO₂ + 95% O₂). 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 isotonicly through a Power Lab Data Acquisition System (AD Instruments, Sydney, Australia) (Gilani et al., 2005).

Determination of Ca⁺⁺ channel blocking activity

The Ca⁺⁺ channel blocking activity was determined by application of the methanol extract on K⁺-(80 mM) induced spastic contractions in isolated rabbit jejunum preparations (Fare et al., 1991). Isolated rabbit jejunum preparations exposed to K⁺ (80 mM) showed a sustained contraction. Extract was added in cumulative manner to demonstrate relaxation behaviour in jejunum preparation (Van-Rossum, 1963). Repeatedly, speculated that such relaxation was mediated through blockade of calcium channels (Bolton, 1979).

Table 1. Phytochemical analysis of *Rumex vesicarius* (leaf) crude extracts (Rv.Cr).

S/N	Test	Observation	Result
1	Alkaloid	No precipitation	Negative
2	Saponins	1 cm froth	Positive
3	Tannins	Light purple	Positive
4	Anthraquinones	Pink	Positive
5	Coumarins	Yellow fluorescence	Positive
6	Phenols	Light purple	Positive
7	Flavanoid	Light yellow colour	Positive

CCB effect of methanol extract was further confirmed by the previously reported method (Gilani et al., 2005). The isolated preparations were set to stabilized in normal Tyrode's solution, which was subsequently substituted with K⁺-normal, but Ca⁺⁺-free Tyrode's solution. EDTA (0.1 mM) was added to remove Ca⁺⁺ from the tissues. Solution was further replaced with Ca⁺⁺-free and K⁺-rich Tyrode's solution. Incubated for 30 min, Ca⁺⁺ was added to tissue bath to get control Ca⁺⁺ dose-response curves (CDRCs) in a cumulative manner. The gradual increase in contractile activity of the tissue depicted that the strength of contractions was dependent on the availability of extracellular Ca⁺⁺ for K⁺-induced influx of Ca⁺⁺. After successive CDRCs, Ca⁺⁺ were found to be super imposable, and tissue was washed, and 60 min incubated for methanol extract. Then concentration response curves of Ca⁺⁺ were reconstructed and compared to the CDRCs. The concentration response curves for Ca⁺⁺ were developed in the presence of various concentrations of the methanol extract to assess a possible Ca⁺⁺ channel blocking effect (Bolton, 1979).

Statistical analysis

The results for spasmolytic and spasmogenic activities are expressed as the mean \pm standard error of mean (SEM). EC₅₀ values with 95% confidence interval were calculated using the computer software GraphPad Prism program version 6.0 for Windows (GraphPad, and San Diego, USA). Dose-response curves were analyzed by nonlinear regression sigmoidal response curve (variable slope).

RESULTS

Preliminary phytochemical screening detected the presence of tannins, phenols, saponins, anthraquinones and coumarins as constituents of the crude aqueous-methanolic extract of *R. vesicarius* (Rv.Cr), while it tested negative for the presence of alkaloid.

When tested on isolated rabbit jejunum, Rv.Cr showed spasmogenic as well as spasmolytic effect. The spasmogenic effect was observed at lower doses (0.03 to 0.3 mg/ml), while at the next higher dose (1 mg/ml) relaxation of spontaneous and high K⁺ (80 mM) induced contractions (0.03 to 5 mg/ml) with respective EC₅₀ values of 1.886 mg/ml (1.576 to 2.256), 95% CI (n=4) and 1.73 mg/ml (0.9740 to 3.68, n=04) and 2.088 (1.576 to 2.767), 95% CI (n=4). Verapamil relaxed the spontaneous and high K⁺ (80 mM) induced contractions with

respective EC₅₀ vales of 0.13 uM (0.104 to 0.114, n=4) and 0.013 uM (0.0096 to 0.0179, n=4).

DISCUSSION

Phytochemical analysis of crude leaf extract of *R. vesicarius* (Rv.Cr) showed the presence of saponins, tannins, anthraquinones, coumarins, phenols, and flavonoid, while the alkaloid was absent as methanolic soluble constituents (Table 1).

As per Figure 1, there is moderate spasmogenic activity in the absence of atropine. That means muscarinic receptors were not blocked and the extract produced stimulation on the receptors, whereas, in pretreated atropine tissues (Figure 1; with atropine), the spasmogenic activity (25 \pm 88% of control, n=6, p \leq 0.05) was abolished. Its mechanism was attesting through muscarinic receptors because atropine is an antimuscarinic drug (Gilman et al., 1990). Thus, the left shift in EC₅₀ values in the activity (EC₅₀ without atropine=1.886 mg/ml (1.576 to 2.256), 95% CI, n=4) and EC₅₀ with atropine=1.73 mg/ml (0.9740 to 3.68, n=4) confirm the cholinomimetic activity (Figure 2). The most interesting phenomenon was observed with and without atropine complete relaxation of the tissue preparation observed at similar dose (1 mg/ml); although, the earlier doses in the absence of atropine spasmodic activity was observed, while all phenomenon replaced by spasmolytic activity in the presence of atropine. For further evaluation, tissues were depolarized with high potassium level (80 mM bath concentration) that produced a sustained contraction (Farre et al., 1991). Rv.Cr was then tried in cumulative manner to observe the spasmolytic effect on the tissues. As it has been regarded in previous multiple studies that contractions induced by potassium are mediated through calcium channels by influx of calcium from extra cellular fluid and a substance which will inhibit the contraction produced by KCl is considered to have calcium channel blocking activity (Bolton, 1979; Janbaz et al., 2012; Gilani et al., 2005; Brown and Taylor, 1996). According to Figure 3, the extract produced a spasmolytic effect on the KCl depolarized tissues, which attestifies further its calcium channel blocking activity depicted in

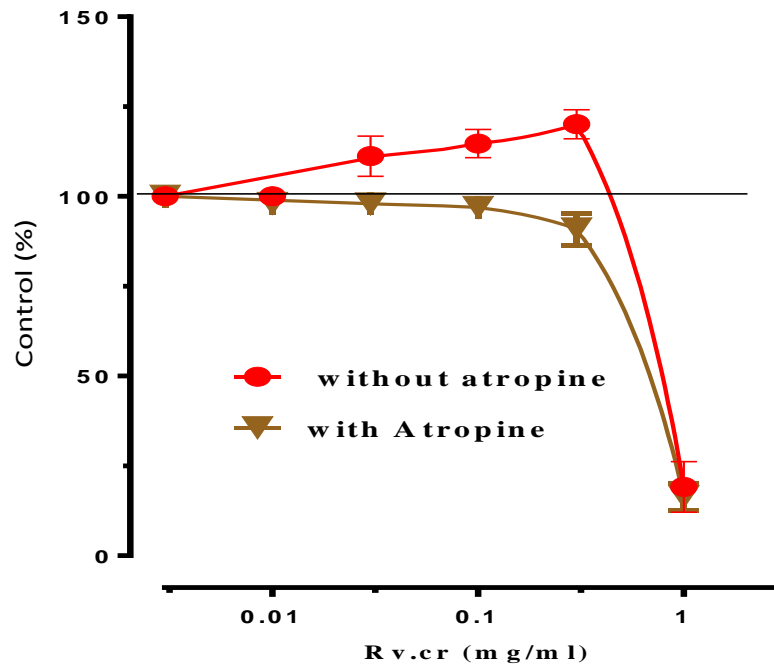


Figure 2. Effect of methanolic extract of Rv.Cr on atropinized and non-atropinized rabbit jejunum preparations. Values are mean±SEM, n=5.

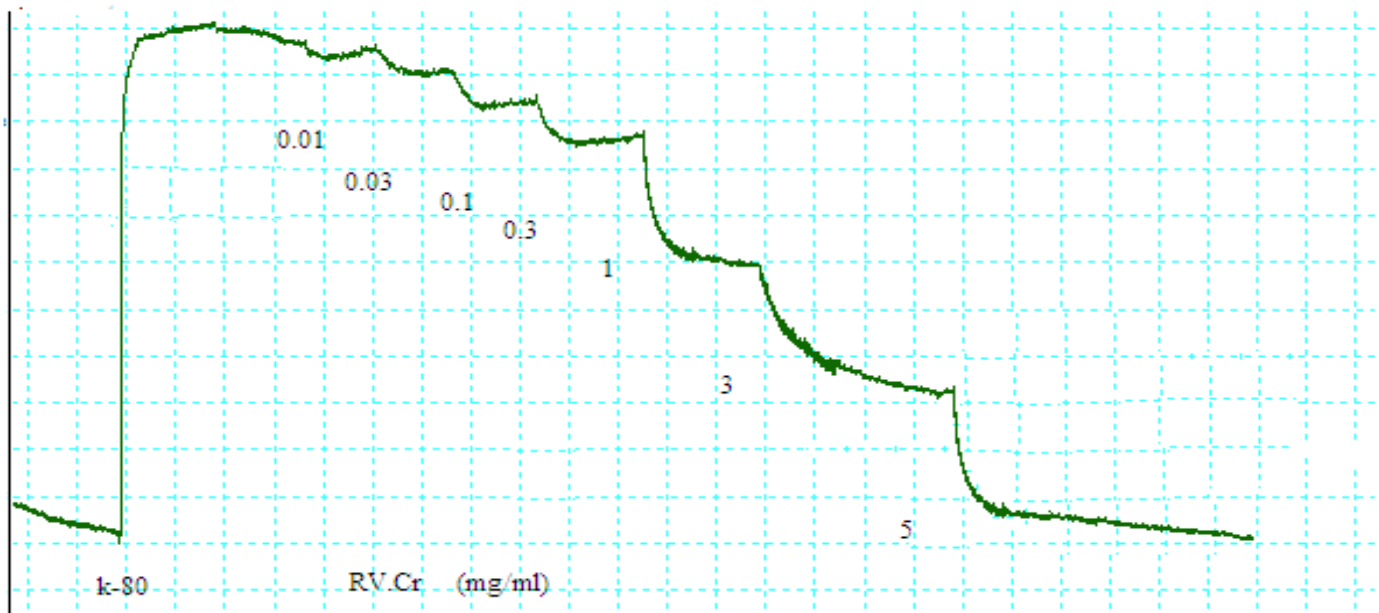


Figure 3. Trace showing the effect of crude methanolic extract of *Rumex vesicarius* leaf (Rv.Cr) on K-80 induced contractions on isolated rabbit jejunum preparations.

Figure 4. Furthermore, methanolic fraction of Rv.Cr was found capable of complete relaxation at 1 mg/ml, most likely by calcium channel blockade (Figure 6). It was

confirmed when it demonstrated the same pattern of activity as verapamil a standard calcium channel blocker (Figures 8 and 9). Furthermore, it shifted the calcium

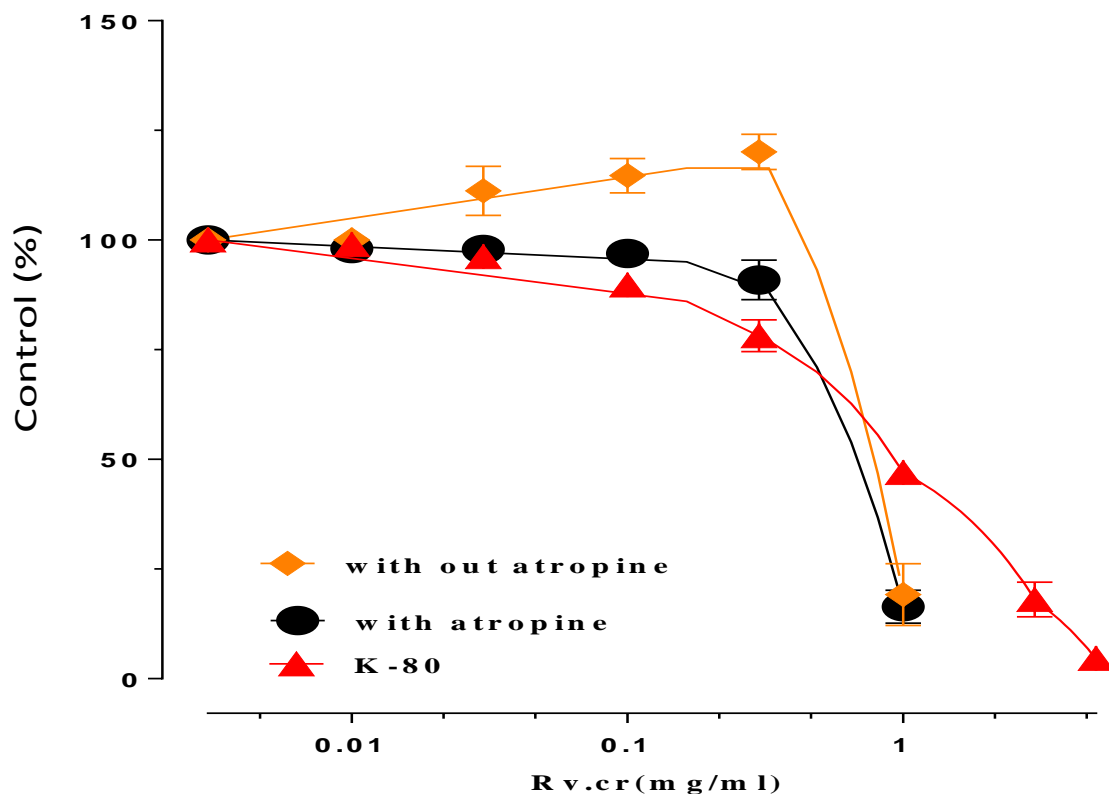


Figure 4. Effect of different concentrations of methanolic extract of Rv.Cr on atropinized and non-atropinized rabbit jejunum preparation as well as effect on K-80 induced contractions. (Value \pm SEM, n=4).

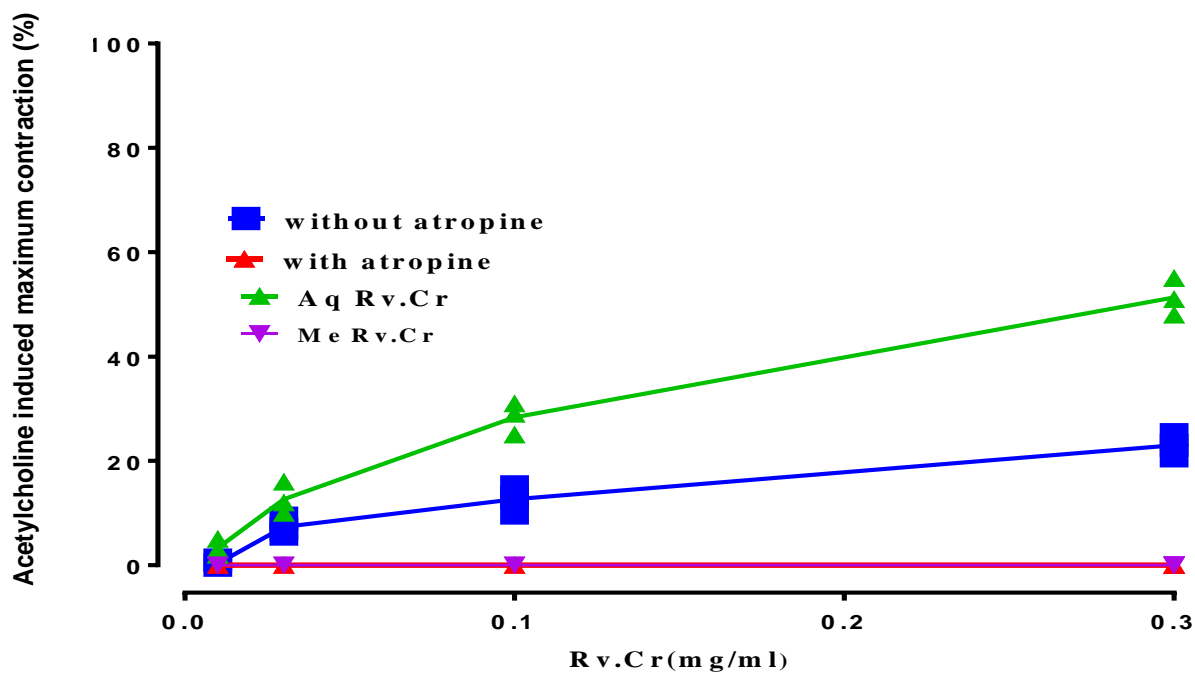


Figure 5. Concentration dependent spasmogenic effect of *Rumex vesicarius* leaf extract and fractions. The responses are given as percent of acetylcholine (10 μ M) induced maximum contraction. The values are shown as mean \pm SEM, n=6.

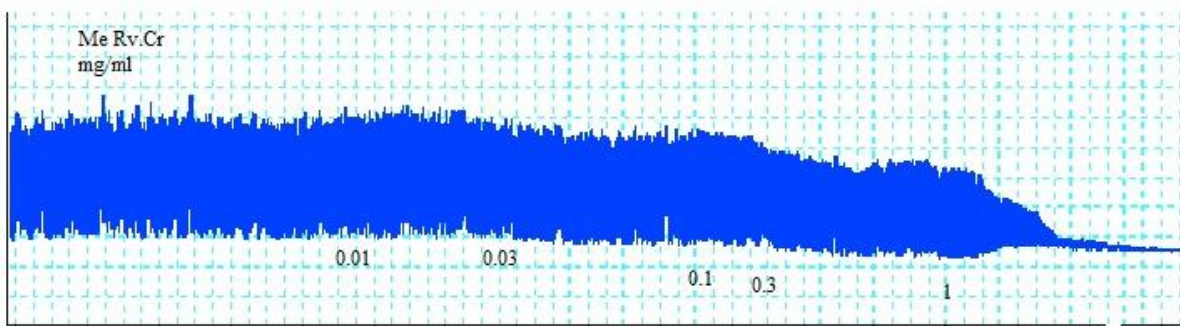


Figure 6. Trace showing the effect of crude methanolic fraction of *Rumex vesicarius* leaf (Me Rv.Cr) on isolated rabbit jejunum preparations.

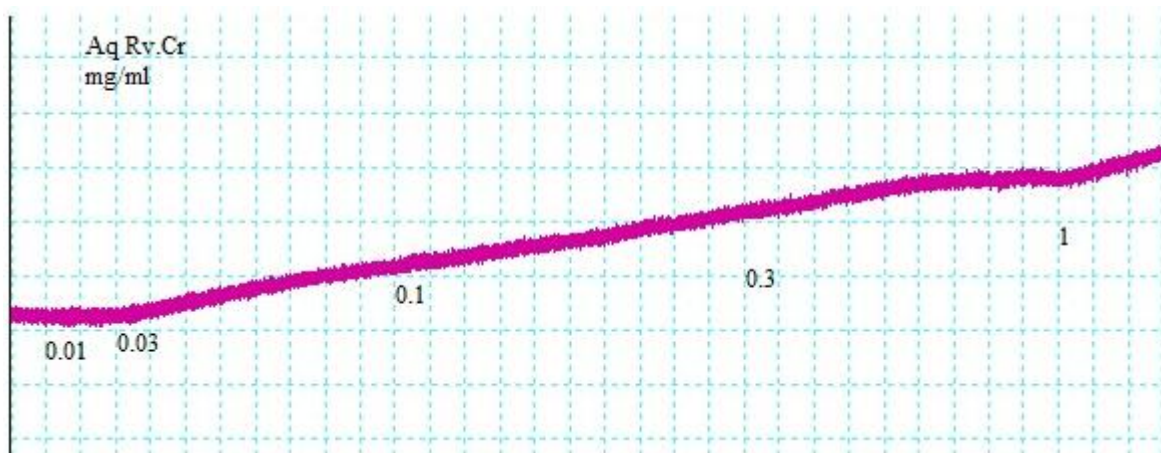


Figure 7. Tracing showing the effect of crude aqueous fraction of *Rumex vesicarius* leaf (Aq Rv.Cr) on isolated rabbit jejunum preparations.

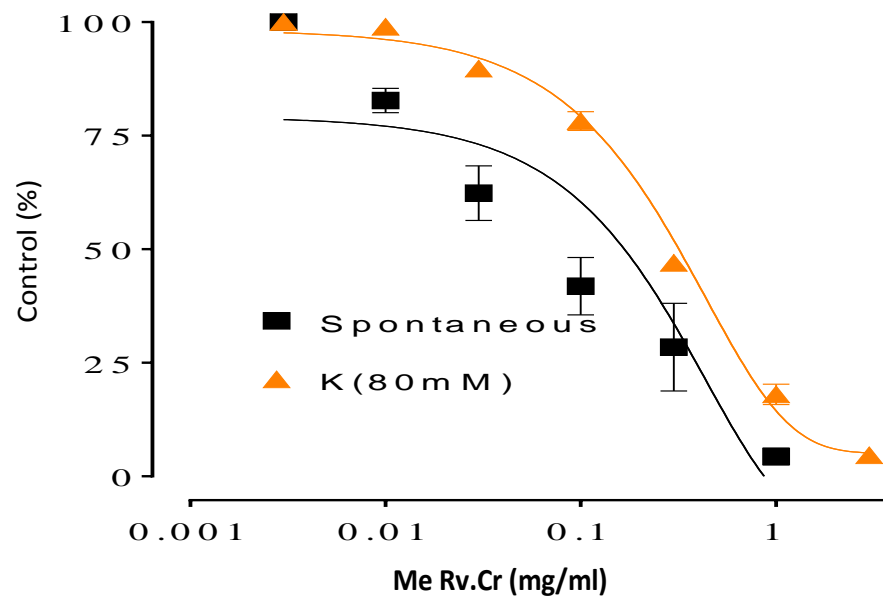


Figure 8. Concentration response curves showing the inhibitory effect of Me Rv.Cr on spontaneous and K⁺ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values shown are mean±SEM of 5 observations.

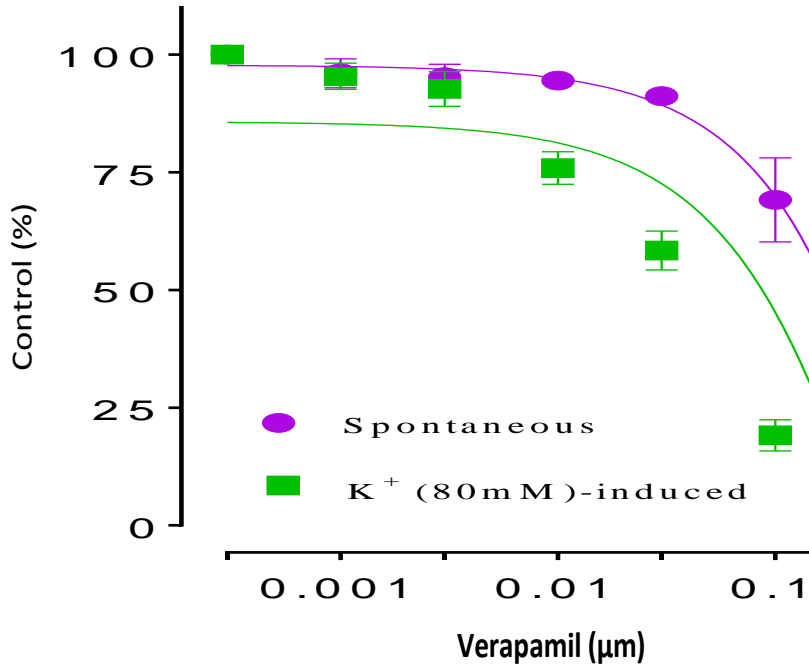


Figure 9. Concentration response curves showing the inhibitory effect of verapamil on spontaneous and K⁺ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values shown are mean±SEM of 5 observations.

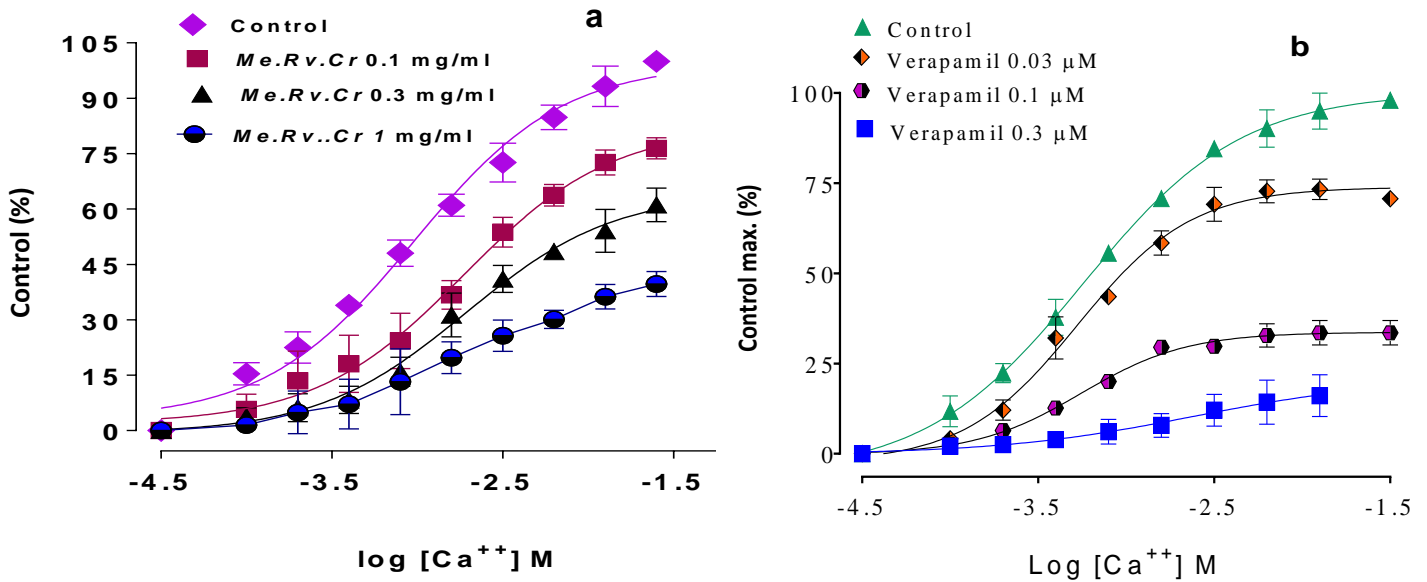


Figure 10. Effect of (a) In.Cr and (b) verapamil (standard) on concentration-response curve of Ca²⁺ on isolated rabbit jejunum preparations (values are expressed as mean ± SEM, n=5).

channel curves to the right same as verapamil (Figure 10a and b). In contrast, its aqueous fraction showed more aggregated spasmogenic activity (51%) than aqueous-methanolic extract (25%) by cholinergic stimulations (Figure 5, 7). Aqueous methanolic extract and methanol and aqueous fractions all are acidic in nature (Table 2)

which provides pharmacological reason for its use in alkalinity.

Rv.Cr was found safe in mice up to dose 2000 mg/kg indicating safe drug relatively to others in use. This data indicates that spasmogenic activity mediated gut stimulation through muscarinic receptors, while

Table 2. Nature and percentage spasmogenic and spasmolytic activity of methanolic extract of extract *Rumex vesicarius* L. and its fractions.

Extract	Spasmogenic activity (%)	Spasmolytic activity (%)	Nature
Rv.Cr	25	99	Acidic
Aq Rv.Cr	51	8	Acidic
Me Rv.Cr	0	99	Acidic

spasmolytic activity was through CCB.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Khalid Hussain Janbaz (Dean) for his guidance throughout the experiment

Conflict of Interests

The author(s) have not declared any conflict of interests.

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