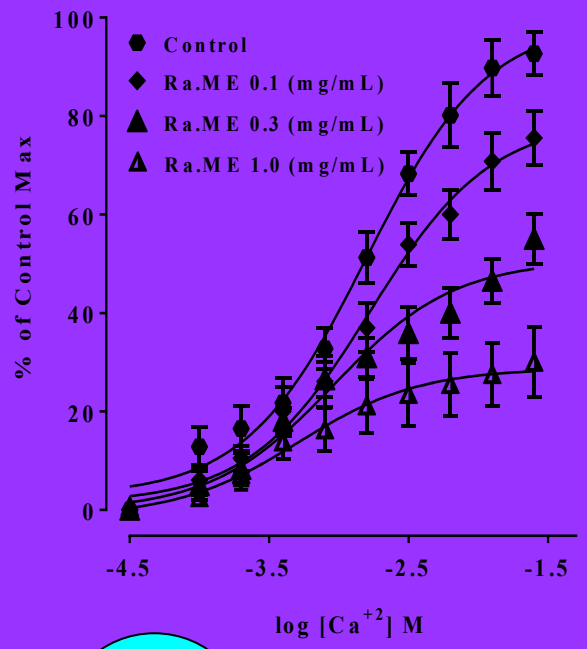

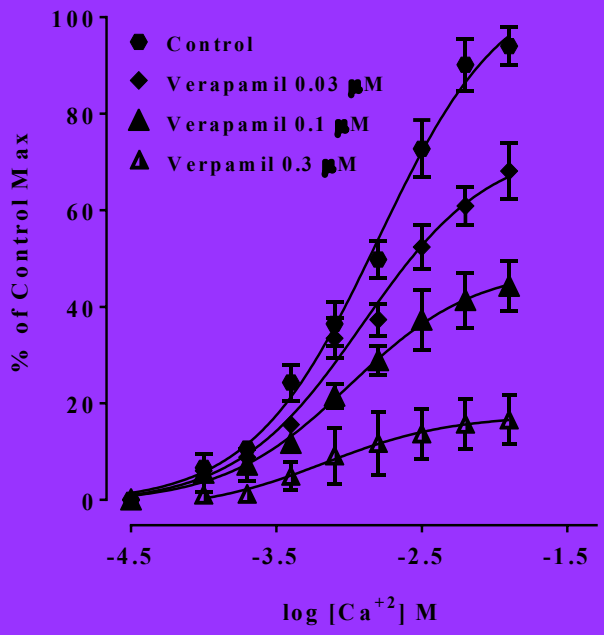

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Pharmacologically mechanistic basis for the traditional uses of *Rumex acetosa* in gut motility disorders and emesis

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Abstract

In vitro and *in vivo* studies were undertaken to evaluate the pharmacologically mechanistic background to validate the traditional uses of *Rumex acetosa* in the treatment of emesis and gastrointestinal motility disorders such as constipation and diarrhea. In rabbit jejunum preparation, methanolic extract of *R. acetosa* (0.01-1.0 mg/mL) caused a transient spasmogenic effect, followed by the spasmolytic effect (3-10 mg/mL). In presence of atropine, spasmogenic effect was blocked while spasmolytic effect was emerged, suggesting that spasmogenic effect was mediated through activation of muscarinic receptors. Extract inhibited the K^+ (80 mM)-induced contraction, suggesting Ca^{2+}-channel blockade, which was further confirmed when pretreatment of tissue with extract shifted the Ca^{2+} concentration-response curves to the right, similarly as verapamil. *R. acetosa* also exhibited the significant antiemetic activity ($p<0.05$) against different emetogenic stimuli, when compared with chlorpromazine. This study confirms the presence of gut modulator (spasmogenic and spasmolytic) and antiemetic activates, validating its traditional uses.

Introduction

Rumex acetosa Linn. (Polygonaceae) is a perennial herb; known by vernacular names of garden sorrel (English), chuukaa (Ayurvedic) and churkuy (Local) and is widely distributed in Asia and Western Himalayas from Kashmir to Kumaon (Khare, 2007).

Phytochemical investigations of aerial parts revealed the presence of ascorbic acid, oxalic acid and flavones such as rutin, vitexin, kaempferol, oxymethylanthraquinone, flavones C-glycosides and flavones O-glycolsides (Kato and Morita, 1990), whereas hyperin, chrysophanein, proanthocyanidin and phloroglucinol derivates with anthraquinones, such as chrysophanol, physcion and emodin anthrones were isolated from the roots (Bicker et al., 2009; Lim et al., 2011).

R. acetosa has been reported to have diaphoretic, insecticidal, diuretic, antimicrobial, anticancer, antiseptic and antipyretic activities (Lee et al., 2005; Gescher et al., 2011; Wegiera et al., 2011). Decoction of the plant has been used as a folkloric medicine to treat constipation, bronchitis, scurvy, arthritis, gastric ulcer, cramp, diarrhea, sore throat and water retention while seeds are used as astringent in hemorrhages (Kirtikar and Basu, 1984; Duke et al., 2002). Plant has been evaluated pharmacologically for the presence of anticancer (Leonard et al., 2006), anti-ulcerogenic and anti-inflammatory activities (Bae et al., 2012).

Although *R. acetosa* has traditionally been used to treat emesis and gastrointestinal ailments, while scientific investigation to validate such traditional uses are so far lacking. The purpose of present study was to investigate its therapeutic potential and possible mechanism of action which may explains the folkloric uses of the plant in emesis and gastrointestinal motility disorders such as constipation and diarrhea.

Materials and Methods

Plant collection and extraction

Whole plant of *R. acetosa* was collected from northern areas of Pakistan and was identified by the Curator, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. Electrically grinded coarse powdered material (#40) was subjected to triple maceration (Hussain et al., 2013), with 70% methanol-aqueous mixtures to produce the methanolic extract of *R. acetosa*; named Ra.ME.

Animals

Animals including, chicks (80-95 g, 10-12 days old) Swiss albino mice (35-45 g, 3-4 weeks old,) and rabbits (1.0-1.5 kg, 7-10 months old) of either sex were locally purchased and placed under controlled environmental condition (25-28°C). Chicks were housed in plastic cages with sawdust as bedding under room temperature with 12 hours/12 hours dark-light cycle, whereas animal were provided with standard diet and *ad libitum* with tap water. Before the commencement of experiments, food was withdrawn from all animals but had free access to water.

Standard drugs and chemicals

Research grade and highest purity chemicals, solvents, and drugs were used in the experimental study. Carbachol, acetylcholine chloride, potassium chloride, magnesium chloride, ethylenediamine tetraacetic acid, and verapamil were purchased from Sigma Chemicals Co. (USA), whereas dimethysulfoxide, glucose, magnesium sulfate, calcium chloride, sodium bicarbonate, potassium dihydrogen phosphate, tween-80, sodium dihydrogen phosphate, and methanol were obtained from Merck, Germany. Ammonium hydroxide, sodium chloride, and sodium hydroxide were purchased from BDH Laboratory Supplies, England. Antiemetic drug (chlorpromazine) and emetogenic stimuli (copper sulfate and cisplatin) were obtained from Highnoon pharmaceutical (Pvt.) Ltd. Pakistan and Sharlab S. L, Spain, respectively.

Phytochemical screening

Methanolic extract was subjected to phytochemical screening for the detection of alkaloids, carbohydrates, tannins, saponins, anthraquinones, steroids and flavonoids as possible important constituents of the plant, according to standard method (Evans, 2006).

Acute toxicity test

For the acute toxicity testing, mice were fasted 24 hours prior to test but had free access to water. Twenty mice were divided into four groups (each 5). Group 1 (negative control) was given normal saline (0.9% NaCl; 10 mL/kg) orally whereas group 2, 3, and 4 (test groups) were orally administered with 2, 4, and 6 g/kg of methanolic extract of plant, respectively. All 4 groups were kept under regular observation for possible mortality and toxic effects as diarrhea, anorexia, gastrointestinal spasms and behavioral changes for 24 hours.

In vitro assessment of spasmogenic and spasmolytic activity

For the assessment of the possible presence of spasmogenic and spasmolytic activity, methanolic extract was tested on isolated rabbit jejunum preparations, as described previously (Arshad et al., 2012; Hussain et al., 2014). A preload of 1 g was applied to mesenteries free isolated rabbit jejunum segments (2-3 cm), suspended in isolated tissue baths containing 10 mL of Tyrode's solution (KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8, and glucose 5.55 mM), aerated with carbogen (37°C) and tissues response were recorded via displacement transducers (model FT-03) coupled with an oscillograph and powerlab data acquisition system (AD Instruments, Australia) linked to a computer installed with labchart software (version 6) (Janbaz et al., 2012; Janbaz et al., 2014). Each tissue was allowed to equilibrate for at least 30 min, with double change of Tyrode's solution. Methanolic extract was applied to tissue bath containing isolated rabbit jejunum preparations in a cumulative fashion, without prior addition of any agonist (Gilani et al., 1994). Contractile effect of the methanolic extract was assessed as the percent of the maximum effect produced by the control drug, acetylcholine (1.0 µM).

Confirmation of Ca^{2+} channel blocking activity

To assess whether the spasmolytic activity of the methanolic extract was through calcium channel blockade (CCB), the tissue preparations were depolarized by exposing to high concentration of KCl, i.e. K^+ (80 mM), resulting in appearance of sustained contraction (Farre et al., 1991). Methanolic extract was applied to K^+ (80 mM)-induced contraction in a cumulative manner, to achieve concentration-dependent inhibitory response. The K^+(80 mM)-induced smooth muscle contraction has been reported to be mediated through influx of Ca^{2+} from extracellular fluid, and the substances capable to inhibit such contractions are speculated to be acting through the blockade of Ca^{2+} channels (Bolton, 1979).

For confirmation of calcium channel blocking activity of the methanolic extract, the isolated rabbit jejunum preparations were allowed to stabilize in normal Tyrode's solution, and then allowed to subsequently contract on exposure to K^+ (80 mM)- which were subsequently replaced with Ca^{2+}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min to ensure the removal of calcium from the tissues (Janbaz et al., 2013). After 30 min, tissues were further exposed to EDTA containing Tyrode's solution which was K^+-rich and Ca^{+2}-free, having the following chemical composition (g/L): KCl (3.72), NaCl (5.72), $MgCl_2$ (0.1), $NaHCO_3$ (1.0), NaH_2PO_4 (0.05), glucose (1.0), and EDTA (0.37).

Subsequent to an incubation period of 40 min, cumulative Ca^{2+} concentrations were applied to the tissue bath to obtain control calcium concentration-response curves (CRCs). When control CRCs of Ca^{2+} were found superimposed, the tissue was pretreated with methanolic extract for 50 min. The Ca^{2+} channel blocking activity was further confirmed when methanolic extract caused shifting of the CRCs in calcium-free medium towards right in a concentration-dependent manner (Gilani et al., 2005).

In vivo assessment of antiemetic potential

Chick emetic model was slightly modified to assess the antiemetic potential of the methanolic extract (Florczyk et al., 1982; Akita et al., 1998; Eda et al., 2005; Hussain et al., 2015b). Chicks were divided in 4 groups of 5 chicks each. Each chick was set aside in a large beaker for the 15 min to acclimatize. Chicks of group 1 (control group) were given an oral dose of normal saline (0.9%), while the chicks of group 2 and 3 (experimental groups) were given an oral dose of methanolic extract at the dose range of 50 and 100 mg/kg body weight in a volume of 10 mL/kg in 0.9% saline containing 5% DMSO and 1% tween 80, whereas chicks of group 4 (standard group) were given an oral dose of chlorpromazine (antiemetic drugs) at the dose range of 150 mg/kg body weight. After 15 min, copper sulfate was administered orally at 50 mg/kg body weight to chicks of all groups. Same process was repeated by using cispaltin (10 mg/kg; IV) and fresh juice of *Brasica* (10 mg/kg; oral), instead of copper sulfate while other protocols remain same as before, then number of retches (an emetic action without emitting gastric material) was counted for the next 15 min. The percentage retching inhibition was calculated as:

Retching inhibition (%) = [(A-B)/A] x 100

Where, A and B represents the frequency of retching in control and experimental groups respectively

Statistics and data analysis

All data was expressed as mean ± SEM of triplicate. EC_{50} values were given with 95% confidence intervals and the logarithmic dose response curves of different treatments were then plotted using computer software "Graph pad Prism" version 6, (Graph Pad Software, San Diego, CA, USA). Concentration-response curves were analyzed by nonlinear regression sigmoidal response curve (variable slope). Unpaired Student *t*-test was used for assessment of the observations. $p<0.05$ and $p<0.005$ were believed to be statistically significant and most significant values respectively.

Results

Phytochemical screening of R. acetosa

Methanolic extract of R. acetosa (Ra.ME) was found to contain alkaloids, saponin, tannin, carbohydrates, soluble starch, anthraquinones and flavonoids, while coumarin was absent.

Acute toxicity of R. acetosa

Acute toxicity of methanolic extract of *R. acetosa* was tested at different doses (2, 4 and 6 g/kg); there was no change in mice behavior and mortality up to the dose as high as 6 g/kg, indicating that the methanolic extract is safe up to the maximal tested dose and is higher than the normal therapeutic dose.

Spasmogenic and spasmolytic effect of R. acetosa on rabbit jejunum preparations

Methanolic extract of *R. acetosa* at the dose range of 0.01 -1.0 mg/mL showed the spasmogenic effect on spontaneously contracting isolated rabbit jejunum preparations, which did not sustained and was subsequently followed by spasmolytic effect at the next higher dose (3-10 mg/mL) with EC_{50} value of 2.87 mg/mL (1.52-3.25, 95% CI: n=3) (Figure 1). The observed contractile responses to Ra.ME was expressed as percentage of the maximal response to acetylcholine (0.3 µM), i.e., 12.57 ± 1.7, 20.26 ± 0.76, 25.30 ± 1.7, 31.3 ± 1.2, and 46.23 ± 5.2 (mean ± SEM, n=3), at the concentration range of 0.01, 0.03, 0.1, 0.3, and 1.0 mg/mL, respectively (Figure 2). In the presence of atropine (0.1 µM), spasmogenic effect was abolished while the spasmolytic effect was observed with EC_{50} value of 1.57 mg/mL (95% CI: 1.12-1.95, n = 3) as shown in Figure 1.

When tested against K^+ (80 mM)-induced contractions, Ra.ME caused the dose dependent relaxation with EC_{50}

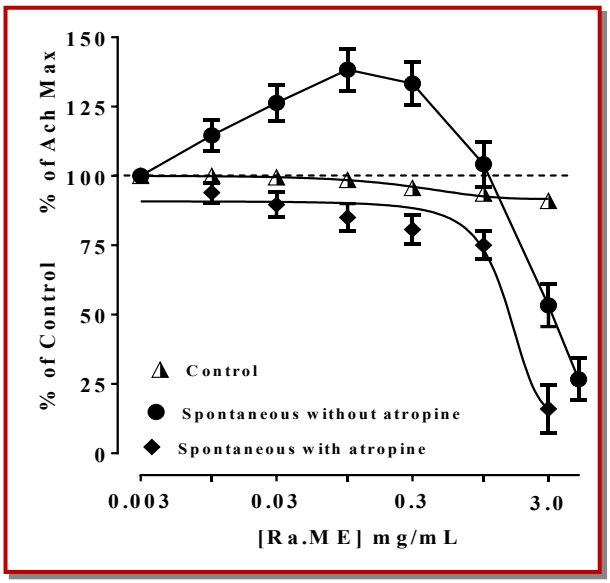

Figure 1: Concentration–response curves, showing the effect of Ra.ME in the absence and presence of atropine (0.1 µM) on spontaneously contracting isolated rabbit jejunum. The spasmogenic responses are expressed as percent of acetylcholine maximum (Ach Max) (Data are mean ± S.E.M, n = 3)

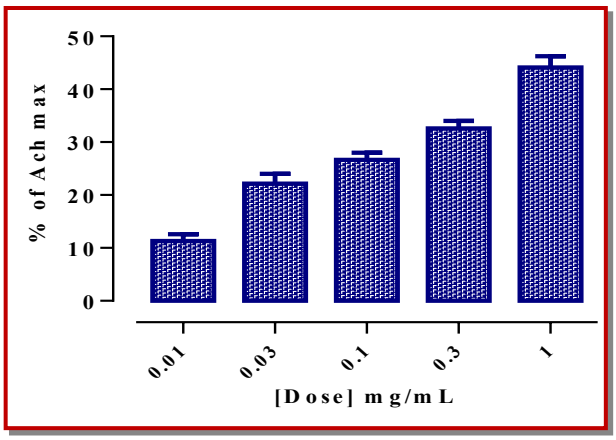

Figure 2: Bar diagram showing the effect of Ra.ME in comparison to the acetylcholine maximum response in isolated rabbit jejunum preparation (values are expressed as mean ± SEM, n=3)

value of 1.3 mg/mL (95% CI: 0.92-1.75, n=3), while verapamil, (positive control) inhibited the K^+(80 mM)-induced contractions, with EC_{50} value of 0.15 mg/mL (95% CI: 0.01-0.65, n=3) (Figure 3). Ra.ME shifted the Ca^{+2}-concentration response curves at the dose range of (0.1-1.0 mg/mL, n=3) to the right, like that caused by verapamil at 0.03-0.3 µM (n=3), as shown in Figure 4.

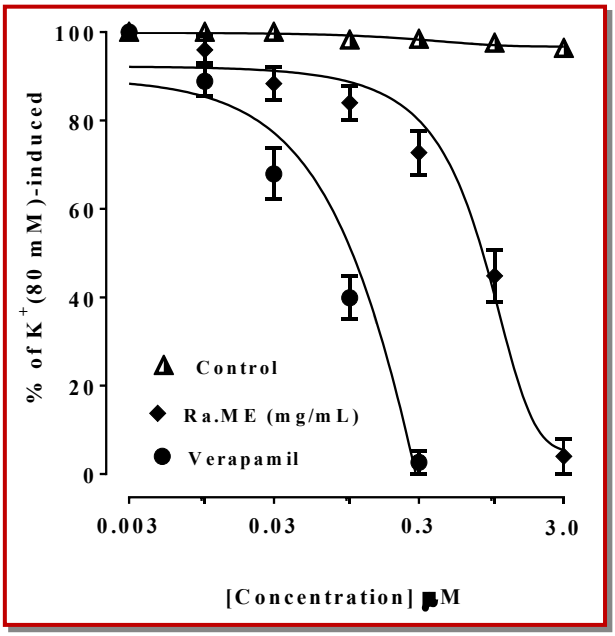

Figure 3: Concentration-dependent inhibitory effect of Ra.ME and verapamil against K^+(80 mM)-induced contractions in isolated rabbit jejunum preparations (values are expressed as mean ± SEM, n=3)

Antiemetic potential of R. acetosa

In control groups, numbers of retches were 72 ± 0.7, 74 ± 0.9 and 75 ± 0.6 to whom normal saline (10 mL/kg) was administered, followed by copper sulphate, *Brasica compestris* and cisplatin respectively. While in the experimental groups (50 mg/kg), number of retches were reduced with %inhibition of emesis of 50.1, 46.0 and 25.5, whereas at the dose of 100 mg/kg number of retches were significantly reduced with %inhibition of emesis of 75.5, 78.0 and 34.1 followed by copper sulfate, *Brasica compestris* and cisplatin respectively, which were comparable to standard drug chlorpromazine (Figure 5).

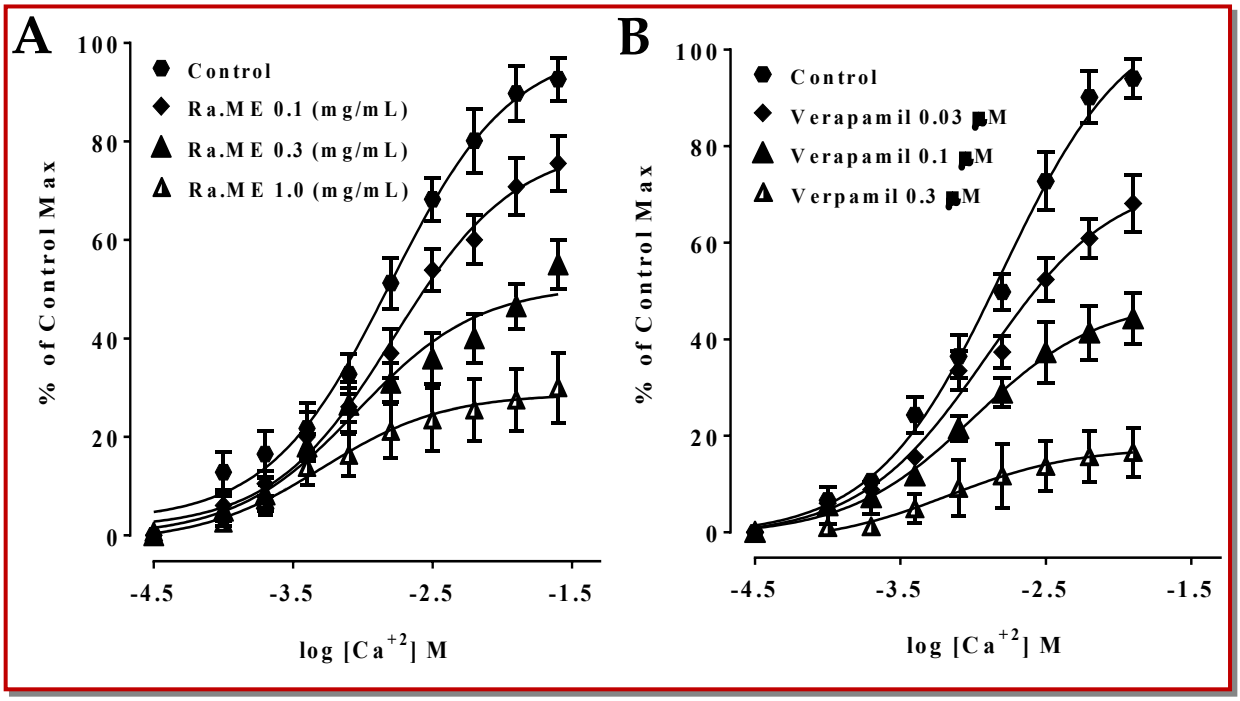

Figure 4: Concentration-response curves of Ca^{+2} in the absence and presence of different concentrations of (A) Ra.ME and (B) verapamil in isolated rabbit jejunum preparations (values are expressed as mean ± SEM, n=3)

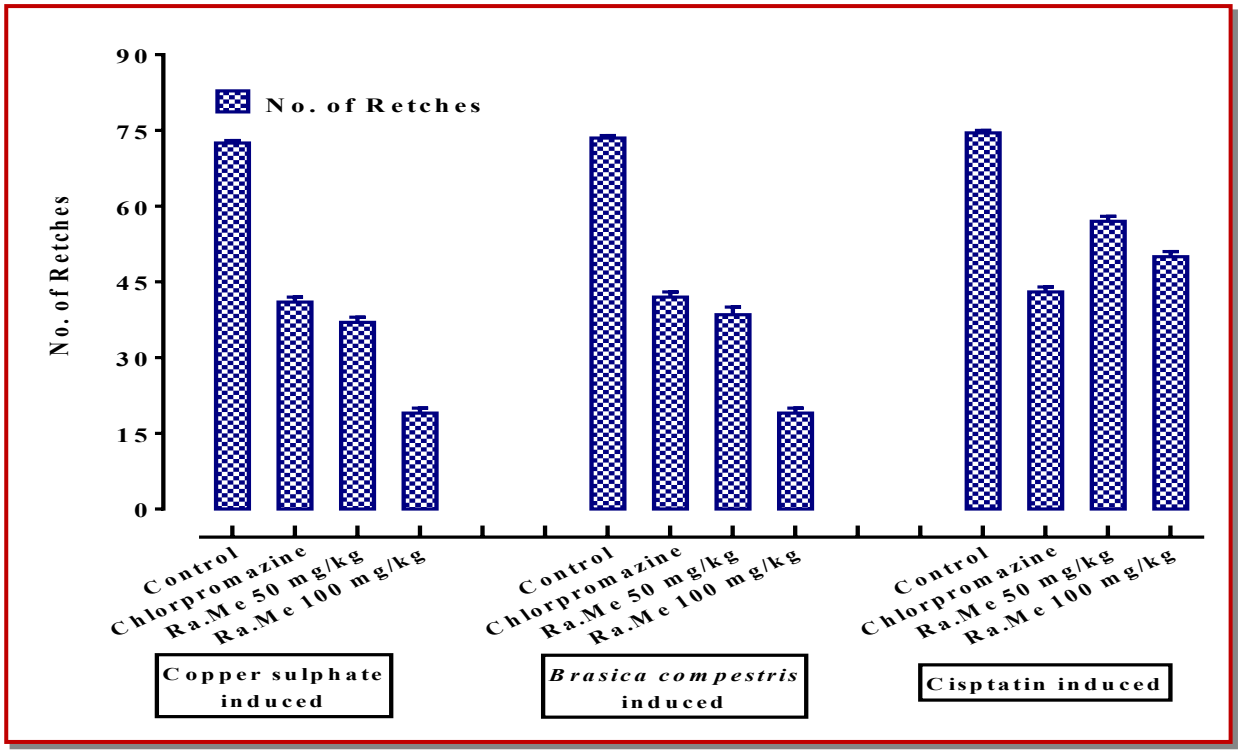

Figure 5: Antiemetic effect of Group 1: Control (normal saline); Group 2: standard drug (chlorpromazine); Group 3; *R. acetosa* (50 and 100 mg/kg) against copper sulfate, *Brasica compestris* and cisplatin induced emesis in young chicks

Discussion

Methanolic extract of *R. acetosa* was tested for the presence of different phytoconstituents and it was found to contain alkaloids, saponin, tannin, carbohydrates, soluble starch, anthraquinones and flavonoids, while coumarins was absent. Oral dose of extract of *R. acetosa* as high as 6 g/kg, did not produced the lethality among the treated groups of mice.

Keeping in view of traditional uses of *R. acetosa* in gastrointestinal motility disorders, pharmacological study of methanolic extract was carried out on spontaneously contracting isolated rabbit jejunum preparations, to evaluate its possible effect, initially produced the spasmogenic effect (contractile effect), which is usually mediated through cholinergic mechanism as like by acetylcholine (Gilani et al., 2005; Hussain et al., 2014), so for the conformation of this mechanism, spontaneous contracting jejunum preparations were pretreated with muscarinic receptor antagonist (0.1 µM of atropine) (Delmendo et al., 1989), abolished the stimulatory effect of the methanolic extract, which indicates that the *R. acetosa* causes the gut stimulation via cholinergic pathway (Hussain et al., 2015a). Acetylcholine is a neurotransmitter released by the parasympathetic nervous system; regulate the peristaltic movement of the gut by acting on M_3 muscarinic receptor while atropine (antagonist) blocks muscarinic receptors (Brown and Taylor, 1996). The observed spasmogenic effect of *R. acetosa* validates its traditional use as anti-constipative agent in the hypomotility disorder of the gut. The spasmogenic effect was followed by the spasmolytic effect at next higher doses of the methanolic extract, indicating the co-existence of spasmogenic, and spasmolytic constituent(s), which is probably meant by the nature not to allow the spasmogenic effect going beyond the constipation, particularly at higher doses (Ghayur and Gilani , 2005).

It has been previously observed that spasmolytic effect of medicinal plants is usually mediated through Ca^{2+} channels blockade (Janbaz et al., 2012; Hussain et al., 2014). To elucidate whether the spasmolytic effect of *R. acetosa* is also mediated through the Ca^{2+} channels blockade, methanolic extract was tested on K^+(80 mM)-induced contractions which is known to cause smooth muscles contractions through opening of the voltage dependent L-type Ca^{2+} channels, thus allowing the influx of extracellular Ca^{2+} causing a contractile effect (Bolton, 1979; Karaki et al., 1997), and substances inhibiting the K^+(80 mM)-induced contraction are known as Ca^{2+} channels blockers, i.e. inhibitor of Ca^{2+} influx (Godfraind et al., 1986; Okumura et al., 1993). Methanolic extract, like verapamil (standard Ca^{+2} channel blocker), relaxed the K^+(80 mM)-induced contractions, indicating the Ca^{2+} channels blocking action. The Ca^{2+} channels blocking effect of *R. acetosa* was further confirmed when it shifted the Ca^{2+}-concentration response curves to the right, like that caused by verapamil, used as positive control.

The observed anticholinergic and Ca^{2+} antagonist effects of the *R. acetosa* might be due to presence of alkaloids and flavonoids, evident in preliminary phytochemical investigation, as these constituents have been reported to possess the antimuscarinic (Broadley, 1996) and Ca^{2+} channel blocking (Revuelta et al., 1997) activities, respectively, while other mechanisms and constituents cannot be ruled out.

Based upon the traditional use of *R. acetosa* for the ailment of emesis, *in vivo* pharmacological study was carried out by adopting chick emetic model to evaluate its possible antiemetic effect. As emesis is caused by the activation of the vomiting centre located in medulla oblongata, directly by activation of motor pathway and/or indirectly following input from four principal areas, i.e., chemoreceptor trigger zone (CRTZ), GIT, cortex, thalamus, cerebral and vestibular region. The CRTZ is in the proximity to medulla and it is not protected by the blood brain barrier (Becker, 2010). On the basis of results it can be inferred that antiemetic response of *R. acetosa* (100 mg/kg) was more significant for the copper sulphate and *Brasica compestris* induced emesis which was comparable to chlorpromazine. So, it can be hypothesized that the antiemetic effect of *R. acetosa* can be likely mediated through inhibition of chemoreceptor trigger zone while exact mechanism is not clearly known.

Conclusion

This study shows the presence of cholinomimetic and calcium antagonistic constituents in the methanolic extract of *R. acetosa.* The cholinomimetic activity is likely to act as aperients (mild laxative) and provides the sound mechanistic back ground for the possible use of the plant in the traditional medicine for the constipation while the calcium antagonistic activity pharmacological rationale for its use in diarrhea, though additional mechanisms cannot be ruled out.

Financial Support

Self-funded

Ethical Issue

All experiments were performed by following ruling of Institute of Laboratory Animal Resources, Commission on Life Sciences (National Research Council, 1996); with reference number EC/06/2012.

Conflict of Interest

Authors declare no conflict of interest

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