

Effect of *Rosa damascena* Mill. flower extract on rat ileum

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Abstract

Rosa damascena flower is widely used for gastrointestinal (GI) disorders. However, its pharmacological action on isolated ileum has not been studied. In this research, the effect of extract of flower petals of *R. damascena* Mill. growing in Kashan, Iran, on ileum motility was investigated. Hydroalcoholic extract was prepared by percolation method. A section of rat ileum was suspended in an organ bath containing Tyrode's solution. The tissue was stimulated with electrical field stimulation (EFS), KCl and acetylcholine (ACh). The tissue was kept under 1g tension at 37°C and continuously gassed with O₂. Effect of the *R. damascena* extract was studied on ileum contractions induced by EFS, KCl and ACh and compared with that of atropine. *R. damascena* extract (10-100 µg/ml) induced a contraction in rat isolated ileum while at 1mg bath concentration it had relaxant effect on rat ileum. Hydroalcoholic extract of *R. damascena* (1-8 mg/ml) concentration dependently inhibited ileum contraction induced by KCl (IC₅₀=3.3 ± 0.9 mg/ml), ACh (IC₅₀=1.4 ± 0.1 mg/ml) and EFS (IC₅₀=1.5 ± 0.3 mg/ml). The vehicle had no significant effect on ileum contractions. From this experiment it was concluded that *R. damascena* extract at microgram concentrations had stimulatory effect on ileum smooth muscle. However, at milligram concentrations, it shows an inhibitory effect. This is most likely due to presence of different components in the extract. The stimulatory effect of the extract confirms its benefits for the treatment of constipation. Therefore, separation and identification of active components is recommended.

Keywords: *Rosa damascena*; Hydroalcoholic extract; Atropine; Ileum; Lidocaine

INTRODUCTION

Rosa damascena Mill. belongs to Rosaceae family which grows in many countries including Iran (1,2). *R. damascena* is a rose hybrid, derived from *Rosa gallica* and *Rosa moschata* and is more commonly known as the Damask rose, the Damascus rose, or sometimes as the Rose of Castile (3).

The uses of the dried Damascus rose in beauty products are numerous. The flower petals are sometimes used directly to flavour food and are considered safe for human consumption (4). Rose water containing traces of *R. damascena* essential oil are believed to alleviate bowel spasm. It has been shown that the essential oil of this herb is in fact a relaxant of isolated ileum (5). On the other hand, the dried flowers of *R. damascena* are being used

as folk medicine (6,7) and are believed to alleviate conditions like frigidity, chronic bronchitis, asthma, skin diseases, cancer, ulcers, wounds, wrinkles, infections, as well as constipation (8). The most popular use of dried flowers in Iran, however, is in yogurt as flavorant, and mild emollient. (4). Abdominal cramping and diarrhoea has been experienced as side effects when excessive flower petals are consumed as food dressing. As the essential oil is a relaxant of ileum contraction (5), the stimulating compound ought to be in the extract components. These effects could be due to a direct action on electrolytes secretion, enteric neurons and GI smooth muscle contraction. However, the pharmacological effect of *R. damascena* on GI tract is not well understood. Although, laxative effect of boiled extract of *R. damascena* is reported in rat (9),

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the pharmacological study of *R. damascena* on intestinal motility is not adequate. Therefore, the aim of this research was to investigate the effect of *R. damascena* hydroalcoholic extract on isolated ileum, in order to find any excitatory or inhibitory effect it may have on intestinal motility.

MATERIALS AND METHODS

Plant materials

Flowers of *R. damascena* were collected in May 2010 from Mashhad-ardehal (Kashan-Iran) and identified by Prof. Mohamad Reza Rahiminejad, Biology Department, Isfahan University. A voucher specimen was authenticated and then deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences (code number: 2261). The Petal parts of the flowers were dried in shade. The plant materials were powdered using electrical miller (Moulinex, France). The total hydroalcoholic extract was obtained by percolation (10) using 80% ethanol with solvent to plant powder ratio of 8:1. The percentage yield of the dried extract was calculated following evaporating the solvents.

Na⁺, K⁺, and Ca²⁺ ionic concentration of extract stock solution was determined using flame photometer (Corning 470, Japan) and atomic absorption spectrophotometer (Perkin-Elmer 2380, USA) respectively.

In vitro contractility assessment

Male Wistar rats (Bred in School of Pharmacy animal house, Iran), weighing 180–250 g were killed and their ileums were cut out and placed in oxygenated Tyrode's solution at room temperature. All animals were handled in accordance with the internationally accepted principles for laboratory animal use and care, as recommended by university authority (11).

The ileum was then cut to 2-3 cm long strips and mounted in 50 ml organ bath (Harvard, England) filled with Tyrode's solution at 37°C and continuously bubbled with O₂. Smooth muscle contraction of the ileum was measured using a Harvard isotonic transducer under 1g weight and recorded on a Harvard Universal Oscillograph (England) pen recorder device.

Following resting time of at least 15 min, initially effect of extract (1, 10, 100 and 1000 µg/ml) was examined on established stable ileum basal tension. In addition, the effect of equivalent concentration of Na⁺, K⁺, and Ca²⁺ ions of the extract was determined and examined on rat ileum to be compared with hydroalcoholic extract solution.

In separate experiments, the ileum was contracted by applied electrical field stimulation (EFS) using trains of 6 V and 50 Hz rectangular pulses from a stimulator (made in school of Pharmacy workshop) for 1s duration. The EFS was delivered at 15 min intervals to the ileum via a couple of platinum electrodes wires placed on either side of the ileum inside the organ bath. In another group of experiments, contraction was induced in rat ileum by addition of KCl (80 mM) into the bath. In the third group of experiments, contraction was induced by acetylcholine (ACh, 2µM). ACh was in contact with the tissue for 20 s before it was washed off with fresh Tyrode's solution.

After equilibration period of 15 min, effect of the extract was determined on above induced contractions using two fold increments in concentration until a full concentration response effect was achieved. Extract effects were evaluated after at least 10 min contact with the tissue. Experiments were performed alongside time-matched vehicle treated controls with the tissue from same animal.

Similarly, effect of atropine was examined on contraction induced by KCl, ACh and EFS using four fold concentration increments and compared with the effect of the extract. Furthermore, effects of the local anesthetic lidocaine (5, 50 & 500 µM) added in the organ bath were evaluated on EFS elicited activity and the contraction induced by ACh.

Drugs and solutions

Tyrode's solution composed of (mM): NaCl, 136.9; KCl, 2.68; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.42 and glucose, 5.55, was made up in distilled water. The extract was made up as 250 mg/ml stock solution in dimethyl sulphoxide (DMSO), dilution was made in 50% DMSO. Atropine sulfate (1 mM), lidocaine hydrochloride (10

mM) and KCl (2 M) stock solutions were made up in distilled water. Acetylcholine hydrochloride (Sigma) was made up as 100 mM stock solution and acidified by 1% acetic acid. Further serial dilutions were made in distilled water. Unless stated, all chemicals and drugs were from Merck.

Measurements and statistical analysis

The contractile response to extract was measured relative to tissue baseline and expressed as the percentage of the initial response in each experiment. All the values are expressed as mean \pm standard error of the mean (SEM). Whenever appropriate, the IC₅₀ value (drug concentration causing 50% of maximum response), was calculated.

Statistical significance was assessed using a one-way analysis of variance (ANOVA) for repeated measures and when appropriate, were compared with the control groups using unpaired *Student's t-test*. Differences were considered statistically significant for $P < 0.05$. Sigma Plot computer program was used for statistical analysis and calculation of IC₅₀.

RESULTS

Plant materials analysis

The yield of the extract was 16.9% (w/w) with brownish-red colour. The K⁺, Na⁺ and Ca²⁺ ionic contents of the extract stock solutions were 2.35 g/l, 2.3 g/l and 54 mg/l, respectively.

In vitro contractility assessment

Application of electrical field stimulation (EFS) caused a rapid contraction within few seconds followed by relaxation and a further contraction which lasted few minutes (Fig. 1). The EFS responses were the same as the EFS biphasic response reported by Ekblad & Sundler (12). Lidocaine at 5 and 50 μ M bath concentration reduced both EFS responses without affecting the ileum response to ACh. Nevertheless, with 500 μ M lidocaine concentration in the bath, ACh response was reduced by $31 \pm 13.6\%$ while, the initial and the secondary EFS responses were reduced further by $84 \pm 6.7\%$ and $98 \pm 1.2\%$ respectively (Fig. 2). Atropine (200 nM to 50 μ M), at concentration which blocked ACh responses in rat ileum, had no effect on KCl induced contraction but only partially attenuated the EFS responses at its highest used concentration (Fig. 3).

R. damascena extract at low concentrations (10-100 μ g/ml) induced contraction in rat ileum, suspended in organ bath (Fig. 4). Maximum contraction was observed with 100 μ g/ml extract in the bath. At higher concentration (1 mg/ml) the contraction amplitude was attenuated. The corresponding concentration of ionic content of the extract only caused a minor contraction on the ileum in comparison with the time matched control tissues (Fig. 4).

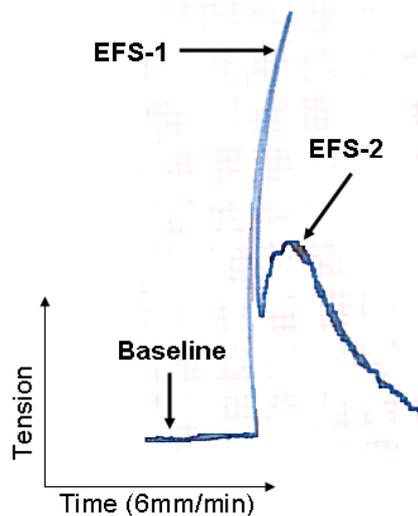


Fig. 1. Contractile record of electrical field stimulation (EFS) in rat isolated ileum.

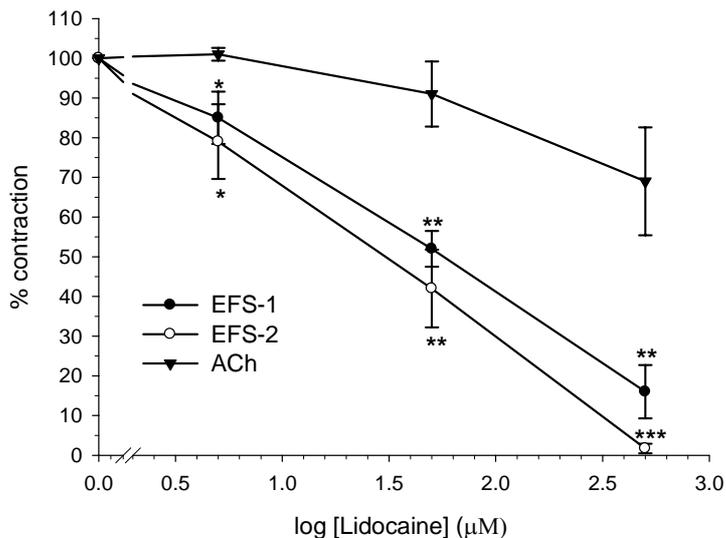


Fig. 2. Effect of lidocaine on first (EFS-1) and secondary (EFS-2) contractile responses to electrical field stimulation (EFS, 6 V, 50 Hz, 1 s duration) and on tension development to acetylcholine (ACh, 2 µM) in the isolated ileum of rats. Ordinate scale: ileum contractile response expressed as % of initial control response. Abscissa scale: log₁₀ concentration of lidocaine. Lines drawn through the points, using ten fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). Stars show statistical differences in comparison with ACh response, *P<0.05, **P<0.01, ***P<0.001 (*Student's t-test*).

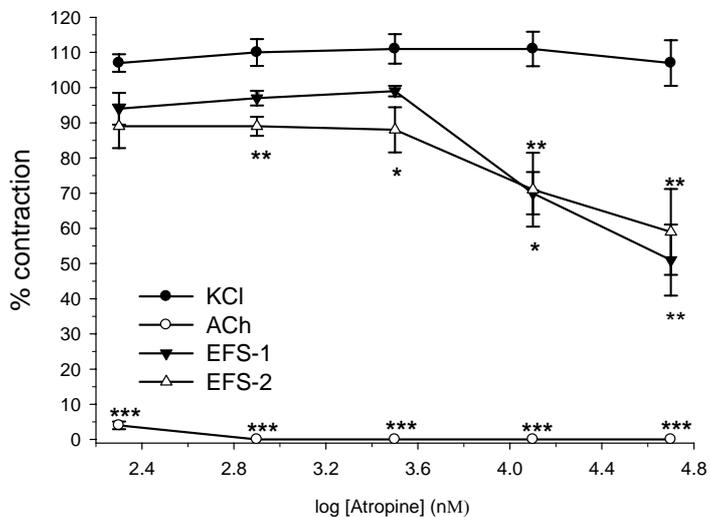


Fig. 3. Effect of atropine on tension development to potassium chloride (KCl, 80 mM), acetylcholine (ACh, 2 µM) and first and secondary contractile responses to electrical field stimulation (EFS, 6 V, 50 Hz, 1 s duration) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as % of initial control response. Abscissa scale: log₁₀ concentration of atropine. Lines drawn through the points, using four fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). Stars show statistical differences in comparison with KCl response, *P<0.05, **P<0.01, ***P<0.001 (*Student's t-test*).

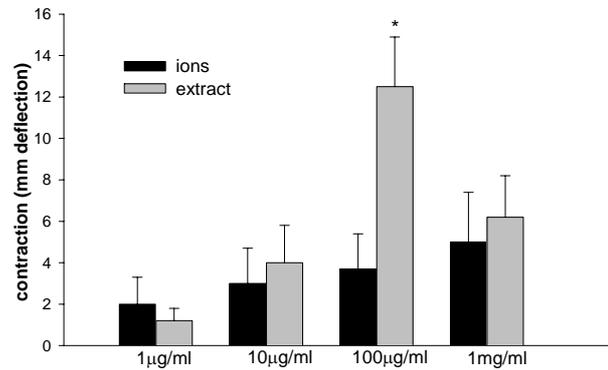


Fig. 4. Tension development in isolated rat ileum following addition of low concentrations of hydroalcoholic extract of *R. damascena* or its corresponding K^+ , Na^+ and Ca^{2+} ionic contents (control). Ileum contractile response was measured as maximum amplitude of response from tissue baseline. Columns are mean and the vertical bars show the SEM (n=6). *P<0.05, in comparison with corresponding ionic contents (*Student's t-test*).

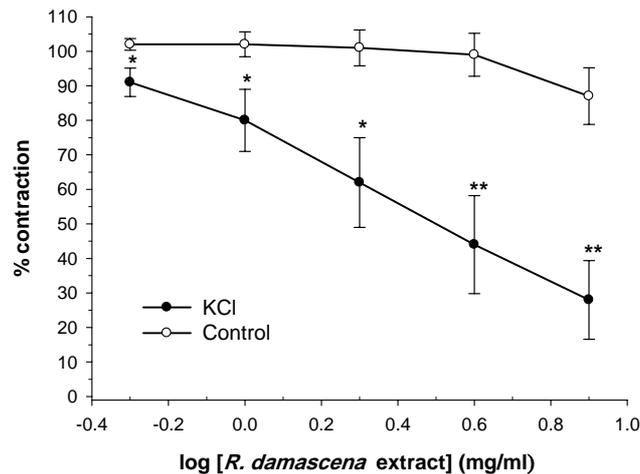


Fig. 5. Effects of *R. damascena* hydroalcoholic extract on tension development to potassium chloride (KCl, 80 mM), in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as % of initial control response. Abscissa scale: \log_{10} concentration of the extract. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). There was no statistically difference in vehicle treated time-matched control tissues contractions over the course of study (ANOVA). Stars show statistically significant differences in KCl responses in comparison with its corresponding vehicle treated time matched control tissues *P<0.05, **P<0.01 (*Student's t-test*).

The extract of *R. damascena* (0.5-4 mg/ml) concentration dependently inhibited contraction induced by 80 mM KCl with $IC_{50}=3.3 \pm 0.9$ mg/ml (n=6) (Fig. 5). Relaxation of the ileum began with 0.5 mg/ml extract in the bath, reduced to $28 \pm 11.4\%$ with 8 mg/ml bath concentration. Hydroalcoholic extract of *R. damascena* in a concentration-dependent manner also inhibited rat ileum contraction induced by ACh ($IC_{50}=1.4 \pm 0.1$ mg/ml), complete inhibition

was achieved with 4 mg/ml extract concentration in the bath (Fig. 6). The extract vehicle had no significant effect on ileum contraction induced by either ACh or KCl (ANOVA).

Relaxant effect of the extract of *R. damascena* at concentration ranges which inhibited the KCl responses were also examined on biphasic contractions induced by EFS. The extract of *R. damascena* (0.5 mg/ml to 4 mg/ml) concentration dependently

inhibited both the initial ($IC_{50}=1.5 \pm 0.3$ mg/ml) and the secondary ($IC_{50}=1.8 \pm 0.3$ mg/ml) contractile responses to EFS. At its highest used concentration the extract almost removed the EFS response (see Fig. 7).

Inhibitory effect of the extract was more or less the same on the both initial and the

secondary contraction induced by EFS. There was no statistically difference in vehicle treated time-matched control tissues contractions over the course of study (ANOVA). The inhibitory effect of the extract on KCl, ACh and EFS responses was reversible following washing the tissue with fresh Tyrode's solution.

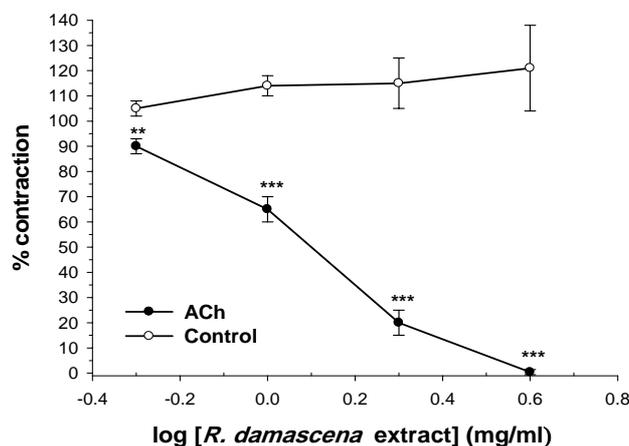


Fig. 6. Effects of *R. damascena* hydroalcoholic extract on tension development to acetylcholine (ACh, 2 μ M) in the isolated ileum of rats. Ordinate scale: ileum contractile response expressed as % of initial control response. Abscissa scale: \log_{10} concentration of the extract. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). There was no statistically difference in vehicle treated time-matched control tissues contractions over the course of study (ANOVA). Stars show statistically significant differences in ACh responses in comparison with its corresponding vehicle treated time matched control tissues **P<0.01, ***P<0.001 (Student's t-test).

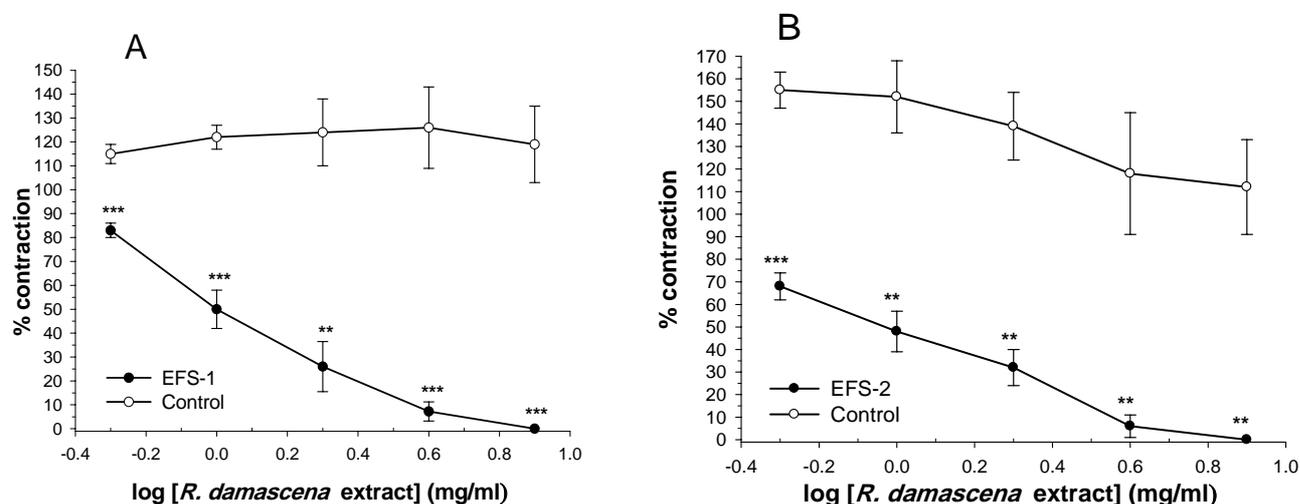


Fig. 7. Effect of *R. damascena* hydroalcoholic extract on tension development to first (A) and second (B) contractile responses to electrical field stimulation (EFS, 6 V, 50 Hz, 1 s duration) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as % of initial control response. Abscissa scale: \log_{10} concentration of the extract. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). There was no statistically difference in vehicle treated time-matched control tissues contractions over the course of study (ANOVA). Stars show statistically significant differences in EFS responses in comparison with its corresponding vehicle treated time matched control tissues **P<0.01, ***P<0.001 (Student's t-test).

DISCUSSION

Dried flower petals of *R. damascena* traditionally are used to alleviate constipation (6,7). Drugs that affect lower GI function may act on smooth muscles and/or work by modulating the activity of the enteric nervous system (ENS). The ENS is embedded in the lining of the GI system. The neurotransmitters acetylcholine (ACh), serotonin (5-HT) and a number of peptides including opioid peptide are the important regulators of motility and water absorption (13). It has been reported that boiled extract of *R. damascena* induced diarrhoea in mice and increased intestinal transient time (9). This effect of *R. damascena* could be due to increase in gut motility and/or secretion by acting on ENS. In this research we have shown that depending on concentration used, hydroalcoholic extract of *R. damascena* could have stimulatory and/or inhibitory effects on rat ileum smooth muscle. The excitatory effect of *R. damascena* extract at low concentration could confirm its emollient action, since we have shown that *R. damascena* extract can increase ileum motility. This excitatory effect of the extract was not related to the ionic content of the extract. The mechanism of excitatory effect of the extract is not clear but a direct effect on the smooth muscles of ileum is more likely since it only caused an increase in basal tension rather than increasing the EFS responses. At higher concentrations, however, *R. damascena* extract exhibited an inhibitory effect on rat ileum contractions, indicating presence of inhibitory substances in the extract. Therefore, the stimulatory effect could concurrently be covered by the presence of inhibitory components in the extract. Nevertheless, at milligram concentrations the inhibitory effect of the extract was the dominant response.

Stimulation of the myenteric plexus (nerve net) situated between the longitudinal and the circular muscle layers of the GI tract are mainly responsible for muscle contraction seen with transmural stimulation (EFS). Inhibition of EFS responses by the local anesthetic 'lidocaine' at selective concentration without affecting the ACh induced contraction support

the suggestion that EFS responses were mainly due to activation of these neurons within the ENS.

The main known pharmacological agents that decrease GI motility are opioids and muscarinic receptor antagonists (14). Although atropine completely abolished the contractile effect of ACh on the ileum, it only incompletely inhibited the effect of transmural stimulation (EFS). The remaining contraction is most likely due to release of other excitatory neurotransmitters from the enteric plexus. This indicates that excitatory transmitters other than ACh are also important in normal function of myenteric plexus. Removal of ACh response by atropine, indicates that ACh action is mainly mediated through muscarinic receptors, activation of which result in release of Ca^{2+} ions from intracellular stores mediated through M_3 receptors (15). Inhibition of KCl response by the extract indicates that the inhibitory effect is post-synaptic but it is not like muscarinic receptor antagonists, meanwhile atropine as an antimuscarinic drug only removed the responses due to ACh. Atropine only causes partial inhibition of EFS responses, because the remaining contraction is due to release of neurotransmitters other than ACh nonadrenergic-noncholinergic (NANC). The *R. damascena* extract however, removed the entire responses to EFS indicating that it inhibits contraction due to release of all neurotransmitters. Another assumption could be that there are different constituents in the extract that are affecting the NANC neurotransmitters. So far there is not confirmation of the constituents of *R. damascena* extract but the extract contain substances like tannins, gallic acid, malic acid, resins and flavanoids including kaempferol and quercetin (16).

CONCLUSION

In conclusion, we have demonstrated both excitatory and inhibitory effects of *R. damascena* extract on isolated rat ileum smooth muscles. The stimulatory effect of the low concentration of *R. damascena* is in consistent with its use as laxative. But it is

likely that the extract may affect the secretion of electrolytes, which needs to be further investigated. The inhibitory effect of the extract at higher concentrations, confirms that its laxative effect is dose limited. Further research for isolation and identification of compounds responsible for excitatory and inhibitory action on ileum smooth muscles are recommended.

ACKNOWLEDGMENT

We would like to acknowledge Isfahan University of Medical Sciences, Isfahan, Iran, because of financially support of this research.

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