ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 6 (1): 67-75, 2013
ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.67.75

Cholinergic and Histaminergic Effects of the Aqueous Fraction of *Rosa damascena* Extract in Guinea Pig Ileum and Rabbit Jejunum

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ABSTRACT

Rosa damascena has been used as a traditional remedy for constipation and many other digestive disorders. In the current research, the effects of the aqueous fraction of *R. damascena* extract on the histaminergic and muscarinic receptors of the Guinea pig ileum and rabbit jejunum were investigated. First, the effects of different concentrations of the aqueous fraction on the contractions of ileum were examined. Then, the effects of the same concentrations of the aqueous fraction on ileal contractions, in the presence of chlorpheniramine and atropine, were recorded and measured. Furthermore, the effects of the aqueous fraction and acetylcholine on the contractions of jejunum were separately recorded, both before and after antagonizing the cholinergic receptors by atropine. To maintain a constant standard, the increase in contractile response of Guinea pig ileum to 0.1 μg mL^{-1} of histamine (equal to 10 cm increase in the amplitude of basal contractions) and increase in contractile response of rabbit jejunum to 1 μg mL^{-1} of acetylcholine (equal to 8 cm increase in the amplitude of basal contractions) were considered as 100% response. Aqueous fraction of *Rosa damascena* significantly increased the amplitude of basal contractions of rabbit jejunum and Guinea pig ileum. After antagonizing the histaminergic receptors, there was a considerable decrease in the contractile response of ileum to aqueous fraction. Also, contractile response of ileum and jejunum to aqueous fraction was decreased after antagonizing the cholinergic receptors. The reduction in contractile response to aqueous fraction following blockade of cholinergic receptors by atropine was more considerable in rabbit jejunum. These findings suggest that the stimulatory effect of the aqueous fraction of *R. damascena* on the intestinal contractions can be due to its excitatory effect on histaminergic and cholinergic receptors of ileum and jejunum.

Key words: *Rosa damascena*, ileum, jejunum, contraction, extract, histaminergic, cholinergic

INTRODUCTION

Rosa damascena is a plant from rosaceae family with aromatic flowers. This plant is cultivated in different parts of the world and used for preparing rose water, essential oils and other products (Yassa *et al*., 2009). In ancient medical books, *R. damascena* flowers have been described as a remedy for digestive disorders, joint pain, dysmenorrhea, constipation and urinary incontinence (Libester, 2002). A variety of *in vivo* and *in vitro* studies have been done on the constituents

isolated from flowers, petals and hips of plant. *Rosa damascena* flowers have been described as having antiseptic, antispasmodic, antiviral and antibacterial properties (Boskabady *et al*., 2011). Different preparations of *R. damascena* are used for different purposes in traditional medicine. For instance, petals of *R. damascena* are used to treat constipation while its essential oils are believed to alleviate bowel spasms (Sharafkandy, 1990; Mirheydar, 1993).

One important mechanism of action of *R. damascena* as a laxative may be its stimulant effect on intestinal motility which as a result, decreases the intestinal transit time. Various neurohormonal effects such as antiepileptic (Ramezani *et al*., 2008; Kheirabadi *et al*., 2008), hypnotic (Rakhshandah *et al*., 2007), antitussive (Shafei *et al*., 2003), antinociceptive (Rakhshandeh *et al*., 2008), bronchodilator (Boskabady *et al*., 2006) and hypoglycemic (Gholamhoseinian *et al*., 2009) properties have been described for *R. damascena*.

Additionally, some neurohormonal pathways, including enteric endocrine and nervous systems, have been described (Hansen, 2003; Barrett and Raybould, 2008). Modulation of the enteric nervous system is considered as one of the mechanisms of action for drugs that affect lower gastrointestinal function. However, these mechanisms are not well studied and understood for *R. damascena*. To date and based on available literatures no study has been done to investigate the effects of the aqueous fraction of *R. damascena* on intestinal cholinergic and histaminergic receptors. Therefore, in this research, the effects of the aqueous fraction on Guinea pig ileum and rabbit jejunum contractions were investigated, in order to find possible stimulatory or inhibitory effects they may have on these receptors.

MATERIALS AND METHODS

Plant and extracts: *R. damascena* flowers were collected from the market and identified by botanists in the Herbarium of the School of Pharmacy of Mashhad University of Medical Sciences (Herbarium No: 254-1804-01). Flowers were then air-dried and a 200 g sample of it, including petals and hips, was milled and mixed with 1500 mL of 50% ethanol for 72 h. The ethanol used for obtaining the extract was then removed using a rotary evaporator. The final ethanolic extract weighed 50 g (Rakhshandeh *et al*., 2004). Five grams of the ethanolic extract was taken for another study and the remaining 45 g was used to obtain the ethyl acetate, n-butanol and the aqueous fractions. For this purpose, 45 g of ethanolic extract was mixed with 50 mL of distilled water and 50 mL of ethyl acetate as solvent. A separatory funnel was used to separate the ethyl acetate phase. The solvent then was removed under reduced pressure and the remaining part was the ethyl acetate fraction which weighed 4.4 g. The same technique was carried out on the remaining part of the extract using n-butanol as solvent. The extracted n-butanol fraction weighed 10 g. The aqueous phase which was separated from the n-butanol phase was then gradually heated up, using a water bath, to evaporate the water, thus producing the aqueous fraction which weighed 30.6 g. In the present study only the aqueous fraction was used (Voon *et al*., 2012).

Tissue preparation: White rabbits weighing 2000-2500 g and Guinea pigs weighing 800-1000 g which were housed under standardized environmental conditions (20-22°C, 12 h light/dark cycle and 50% relative humidity) and free access to food and water, were killed by a blow on the neck. Then 3 cm of jejunum from rabbit or ileum from Guinea pig was removed and immediately transferred to a 60 mL organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK). The lower end of the tissue segment was fixed to the bottom of the organ bath chamber and the other end connected to a transducer. Organ bath contained Tyrode solution

of the following composition: NaCl; 8 g L^{-1}, KCl; 0.2 g L^{-1}, $CaCl_2$; 0.2 g L^{-1}, $MgCl_2$; 0.1 g L^{-1} NaH_2PO_4 0.05 g L^{-1}, $NaHCO_3$; 1.00 g L^{-1} and glucose; 1.00 g L^{-1}. The Tyrode solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for about 1 h while it was washed with tyrode solution every 15 min.

Protocols: Contractile effects of the aqueous fraction of *R. damascena* extract on Guinea pig ileum and rabbit jejunum were separately recorded and measured after fixation. Additionally, the effects of the aqueous fraction on Guinea pig ileum and rabbit jejunum after antagonizing the muscarinic receptors by atropine were studied. Similarly, the effects of acetylcholine on Guinea pig ileum and rabbit jejunum, both before and after antagonizing their muscarinic receptors by atropine, were recorded and measured. Furthermore, stimulatory effects of histamine and the aqueous fraction separately on Guinea pig ileum were examined. Their effects were recorded both before and after antagonizing the histaminergic receptors of ileum by chlorpheniramine. The concentrations of extract and drugs and also the design of the experiments are as follows:

Rabbit jejunum:

- The aqueous fraction of *R. damascena* extract (0.16, 0.33, 0.50, 0.66 and 0.83 mg mL^{-1}) alone and also in the presence of atropine (0.08 μg mL^{-1}) as acetylcholine antagonist
- Acetylcholine (0.16, 0.33, 0.50 and 0.66 μg mL^{-1}) alone and also in the presence of atropine (0.001 μg mL^{-1}).

Guinea pig ileum:

- The aqueous fraction (0.25, 0.66 and 1.00 mg mL^{-1}) alone and also in the presence of chlorpheniramine (1.6 μg mL^{-1}) as histamine antagonist
- Histamine (0.005, 0.05 and 0.1 μg mL^{-1}) alone and in the presence of chlorpheniramine (0.03 μg mL^{-1})
- The aqueous fraction (0.66, 0.83 and 1.3 mg mL^{-1}) alone and in the presence of atropine (0.001 μg mL^{-1})
- Acetylcholine (0.01, 0.03, 0.06 and 0.1 μg mL^{-1}) alone and also in the presence of atropine (0.008 μg mL^{-1})

All responses were recorded on a kymograph implementing the following procedure: The kymograph was turned on to record basal contractions of the tissue for 30 sec. Then, specific concentration of the aqueous fraction or appropriate drug (acetylcholine or histamine) was added to the 60 mL organ bath and the effect on contractions was recorded for 30 sec. Finally, the kymograph was turned off and the tissue was washed with tyrode solution.

The experiments with extract, acetylcholine and histamine were also repeated, after antagonizing the cholinergic and histaminergic receptors of Guinea pig ileum and rabbit jejunum. For this purpose, the kymograph was turned on to record the basal contractions for 30 sec. Then the specific concentration of atropine or chlorpheniramine was added to the organ bath and the tissue was incubated for 4 min in the organ bath which contained tyrode solution and either

atropine or chlorpheniramine. The incubation of tissue for 4 min was done to allow the antagonist drug to take effect and to block the cholinergic or histaminergic receptors, respectively. Then the prepared concentration of extract, acetylcholine or histamine as agonist drugs, was added to the organ bath and kymograph was turned on again to record the contractions in response to the extract, acetylcholine or histamine. The experiments with extract and each of the agonist and antagonist drugs were repeated at least 3 times. At the end of each experiment, after recording the tissue contractions, the kymograph was turned off and tissue was washed with Tyrode solution.

To maintain a constant standard, contractile response of Guinea pig ileum to histamine (0.1 μg mL^{-1}) which was equal to 10 cm increase in the amplitude of basal contractions was considered as 100% and the contractile responses of ileum to the extract and to other drugs were compared to it. Similarly, contractile response of rabbit jejunum to 1 μg mL^{-1} of acetylcholine which was equal to 8 cm increase in the amplitude of the basal contractions was considered as 100% and the contractile responses of jejunum to the extract and to other drugs were compared to it. In each experiment, highest amplitude of the recorded basal contractions was considered as the baseline to measure any alteration in contractions, in response to the aqueous fraction or to drugs.

Statistical analysis: All the data were expressed as Mean±SEM. Student's t-test was used to compare the two groups. Significance was accepted at $p<0.05$.

RESULTS

Effect of the aqueous fraction of *R. damascena* on histaminergic receptors of Guinea pig ileum: As illustrated in Fig. 1, increase in the contractile response of ileum to 0.25, 0.66 and 1.00 mg mL^{-1} of the extract was 30±1, 50±2 and 70±3%, respectively. However, contractile response of ileum to the same concentrations of the extract in the presence of chlorpheniramine (1.6 μg mL^{-1}) was significantly reduced to 3±1, 35±5 and 52±4%, respectively. The p-value, in the increasing order of extract concentration, was <0.001, <0.008 and <0.003.

Effect of histamine on Guinea pig ileum contractions: Increase in contractile response of ileum to 0.005, 0.05 and 0.1 μg mL^{-1} of histamine was 15±3, 60±5 and 99.3±1.1%, respectively. However, as depicted in Fig. 2, adding chlorpheniramine (0.03 μg mL^{-1}) resulted in significant reduction of the contractile response to 0±0.0, 3±0.5 and 15±3.0%, respectively ($p<0.001$).

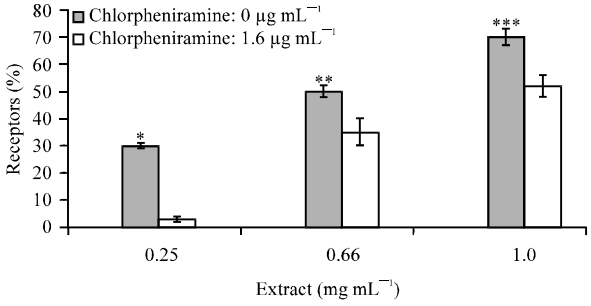

Fig. 1: Effects of the aqueous fraction on the histaminergic receptors of Guinea pig ileum in the presence and absence of chlorpheniramine, Values are Mean±SEM, *$p<0.001$, **$p<0.008$ and ***$p<0.003$

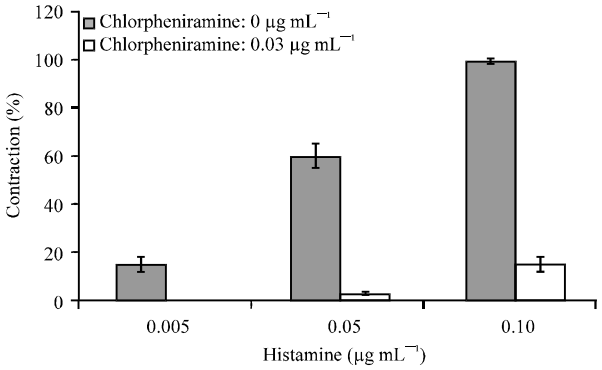

Fig. 2: Effects of different concentrations of histamine on Guinea pig ileum contraction the presence and absence of chlorpheniramine, $p<0.001$

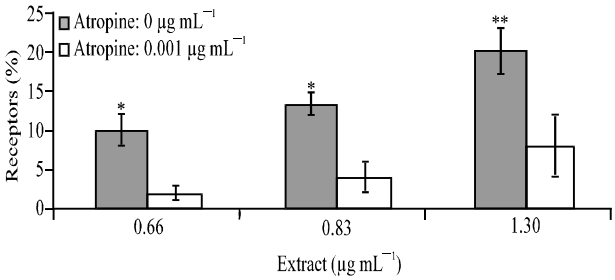

Fig. 3: Effect of the aqueous fraction of *R. damascena* on cholinergic receptors of Guinea pig ileum in the presence and absence of atropine,*$p<0.003$, **$p<0.014$

Effect of the aqueous fraction of *R. damascena* on cholinergic receptors of Guinea pig ileum: Increase in contractions of ileum in response to 0.66, 0.83 and 1.3 mg mL^{-1} of aqueous fraction was 10±2, 13.3±1.5 and 20±3%, respectively. In the presence of atropine (0.001 μg mL^{-1}), however, as shown in Fig. 3, the increase in contractions, in response to same concentrations of extract, was significantly reduced to 2±1, 4±2 and 8±4%. The p-value in the increasing order of the extract concentration was <0.003,<0.003 and <0.014, respectively.

Effect of acetylcholine on Guinea pig ileum contractions: Acetylcholine in concentrations of 0.01, 0.03, 0.06 and 0.1 μg mL^{-1} resulted in 15±3, 25±5, 45±3 and 60±8% increase in ileum contractions, respectively. As shown in Fig. 4, However, in the presence of atropine (0.008 μg mL^{-1}), the increase in contraction amplitude in response to the same concentrations of acetylcholine was predictably reduced to 3±1, 5±1, 18±2 and 25±5%, respectively. The p-value in the increasing order of acetylcholine concentration was <0.003, <0.002, <0.001 and <0.003.

Effect of acetylcholine on contractile response of rabbit jejunum: As it is illustrated in Fig. 5, acetylcholine in concentrations of 0.16, 0.33, 0.50 and 0.66 μg mL^{-1} resulted in 25±5, 35±3, 37±2 and 60±4% increase in contractions of rabbit jejunum, respectively. This increase was however reduced to 17±4, 21±3, 25±3 and 45±2% in response to the same concentrations of acetylcholine in the presence of atropine (0.001 μg mL^{-1}). This reduction was only statistically significant for acetylcholine concentrations of 0.33, 0.50 and 0.66 μg mL^{-1}. The p-value in the increasing order of acetylcholine concentration was 0.096, 0.005, 0.004 and 0.004.

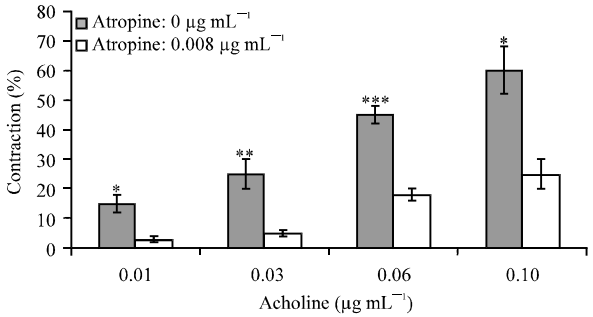

Fig. 4: Effects of different concentrations of acetylcholine on the contractions of Guinea pig ileum in the presence and absence of atropin, *p<0.003, p = 0.005 and ***p<0.001

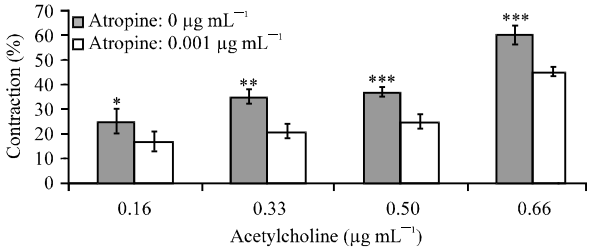

Fig. 5: Effects of acetylcholine on contractile response of rabbit jejunum in the presence and absence of atropine, *p = 0.096, **p = 0.005 and ***p = 0.004

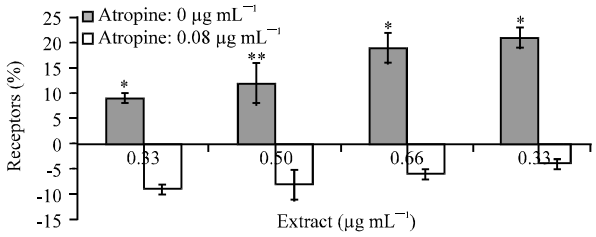

Fig. 6: Effect of different concentration of aqueous fraction of *R. damascena* extract on cholinergic receptors of rabbit jejunum in the presence and absence of atropine, *p<0.001, **p<0.002

Effect of the aqueous fraction of *R. damascena* on cholinergic receptors of rabbit jejunum: The aqueous fraction of extract in concentrations of 0.33, 0.50, 0.66 and 0.83 mg mL^{-1} resulted in 9±1, 12±4, 19±3 and 21±2% increase in jejunum contractions, respectively. As demonstrated in Fig. 6, the contractile responses of jejunum to the same concentrations of extract in the presence of atropine (0.08 µg mL^{-1}) were -9±1, -8±3, -6±1 and -4±1%, respectively. The negative numbers indicate that not only there was no increase in the amplitude of contractions, but also a decrease in the amplitude of basal contractions was observed. The p-value in the increasing order of extract concentration was <0.001, <0.002, <0.001 and <0.001.

DISCUSSION

Normal function of digestive system depends, in part, on normal intestinal movements. These coordinated movements are regulated by complex mechanisms involving different receptors within the gut wall and substances with inhibitory or excitatory effects on them. These receptors

can maintain gut reflex activity and peristalsis even when disconnected from the CNS, (Blackshaw *et al*., 2007). Study of these receptors and various substances which affect their function is one of the leading topics in gastroenterology research. Although the results of these studies have been successfully used by pharmaceutical companies to develop different promotility or antimotility drugs, there are always concerns regarding the side effects, cost and availability of these medications among prescribing physicians and patients. Therefore, substitution of these drugs with effective herbal formulations with better safety profile is of great interest for both physicians and patients.

In the present study, the effects of the aqueous fraction of *R. damascena* on histaminergic and cholinergic receptors, as two main receptors involved in Guinea pig ileum and rabbit jejunum peristalsis, were investigated. This experimental design demonstrated that the aqueous fraction of *R. damascena* extract increases the amplitude of contractions in both ileum and jejunum. On the other hand, there was a significant decrease in the contractile response of ileum and jejunum to the extract, after antagonizing cholinergic receptors by atropine. These findings imply that the aqueous fraction of *R. damascena* extract has a stimulatory effect on these receptors.

Additionally, some studies suggest that substances like vasoactive intestinal peptide (Gordon *et al*., 1990), neurotensin (Nguyen-Le *et al*., 1997) and prostaglandins (Fukunaga *et al*., 1993) exert their stimulatory effect on contractions of isolated ileum through acetylcholine release and muscarinic stimulation. It has also been shown that stimulation of α-adrenoreceptors of Guinea pig ileum has an inhibitory effect on ileal contractions through reduction of acetylcholine release (Burks, 1994; Fuder and Muscholl, 1995; De Ponti *et al*., 1996; Stebbing *et al*., 2001).

Considering the various pathways involved in stimulation of muscarinic receptors in Guinea pig ileum, further research is needed to verify whether the aqueous fraction of *R. damascena* extract stimulates muscarinic receptors directly, through modulation of other excitatory mediators or by exerting α-blocker properties.

This study also demonstrated that the stimulatory effects of the aqueous fraction on ileal contractions were significantly reduced after antagonizing the histaminergic receptors by chlorpheniramine. This finding implies that aqueous fraction has an excitatory effect on histaminergic receptors.

The *R. damascena* extract contains several components and the responsible compound for its stimulatory effect on intestinal movements cannot be determined by the results of the current study. More research on the aqueous fraction and other fractions of *R. damascena* extract such as n-butanol and ethyl acetate is needed to find the most effective component and to use it for developing new medications.

CONCLUSION

This experimental study shows that the aqueous fraction of *Rosa damascena* significantly increases the basal contractions of Guinea pig ileum and rabbit jejunum. These results indicate that the aqueous fraction of *Rosa damascena* could be used as a promising herbal medication especially for treatment of the functional gastrointestinal movement disorders.

ACKNOWLEDGMENT

This study was financially supported by Vice Chancellery of Research of Mashhad University of Medical Sciences. We are grateful to Dr. Habibollah Esmaeeli, Dr. Kang Wang and Ms. Helya Rahvar for their kind help in preparing this article.

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