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Original article

The Differences in the Relaxant Effects of Different Fractions of *Rosa damascena* on Guinea Pig Tracheal Smooth Muscle

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Abstract

Objective(s)
In the present study, the differences in the relaxant effects of aqueous, ethyl acetate and n-butanol fractions of *Rosa damascena* on tracheal smooth muscle of guinea pigs were examined.
Materials and Methods
The relaxant effects of three cumulative concentrations of each fraction (0.1, 0.2 and 0.4 g%) in comparison with saline and three cumulative concentrations of theophylline (0.1, 0.2, and 0.4 mM) were examined on precontracted tracheal chains of guinea pig by 60 mM KCl (group 1, n= 5) and 10 µM methacholine (group 2, n= 8).
Results
In group 1, all concentrations of theophylline, ethyl acetate fraction and two final concentrations of n-butanol fraction showed significant relaxant effects in comparison with saline ($p< 0.05$ to $P< 0.001$). In group 2, all concentrations of theophylline, ethyl acetate and aqueous fractions showed concentration dependent relaxant effects compared to that of saline ($p< 0.01$ to $P< 0.001$). In addition, the effect of ethyl acetate fraction in group 1 was significantly higher than those of theophylline ($p< 0.05$ to $p< 0.001$). However, the effects of other fractions were significantly lower than theophylline in both groups ($p< 0.01$ to $p< 0.001$). There were significant correlations between the relaxant effects and concentrations for theophylline and all fractions (except aqueous fractions in group 1) in both groups.
Conclusion
The results showed a potent relaxant effect for ethyl acetate fraction of *R. damascena* on tracheal smooth muscle comparable to that of theophylline but a relatively weak relaxant effect for aqueous and n-butanol fractions at concentrations used.

Keywords: Bronchodilatory agents, Fractions, Guinea pig, *Rosa damascene*, Trachea

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Introduction

The main constituents of *Rosa damascena* are; carboxylic acid (1), terpene, myrcene (2) and vitamin C (3).

Several therapeutic effects for this plant such as treatment of abdominal and chest pain, strengthening the heart (4), treatment of menstrual bleeding, digestive problems (5), and anti inflammation (1) as well as a gentle laxative (6) are reported.

Experimental studies showed different effects for this plant including anti HIV effect (7) as well as analgesic, hypnotic, antispasmodic and anti-inflammatory effects for essential oil of *R. damascena* (8, 9), antioxidant activity for phenolics-based fingerprinting of *R. damascena* (10) and antibacterial effects (11). The ability to counteract kindling acquisition (12) and increasing pentobarbital induced sleeping (13) were suggested for the ethanolic and aqueous extracts and essential oil from *R. damascena* respectively. The decreased mortality in adult Drosophila of the plant (14) and immunologic suppressive and psychological benefit of aromatherapy was also demonstrated (15). In addition, our studies showed antitussive (16) and relaxant effects of ethanolic extract and essential oil of this plant on tracheal smooth muscle (17).

With regard to antispasmodic and tracheal smooth muscle relaxant effects of the plant, the relaxant effect of three different fractions from *R. damascena* on guinea pig tracheal smooth muscle was examined in the present study.

Material and Methods

Plant and fractions

R. damascena was collected from the School of Pharmacy in spring 2005 and identified by a botanist. A voucher specimen was preserved in the Herbarium of the school of Pharmacy, Mashhad University of Medical Sciences (Herbarium No: 254-1804-01). Three different fractions of the plant were prepared according a previous study (19) as follows: Two hundred grams of the chopped, dried flowers of *R. damascena* was extracted for 3 days with 1500 ml 50% ethanol by digestion. The extract reduced to dryness with a vacuum rotary evaporator. A yield of 50 g (25%) was obtained. Forty five g of extract was dissolved in 50 ml water and the solution was extracted with ethyl acetate and n-butanol. The ethyl acetate fraction and n-butanol fraction were separated to obtain aqueous fraction. The ethyl acetate fraction yield was 9.7% (4.4 g), n-butanol fraction yield was 15.3% (6.9 g) and the aqueous fraction yield was 34.6% (15.6 g). The separation steps are shown in Figure 1.

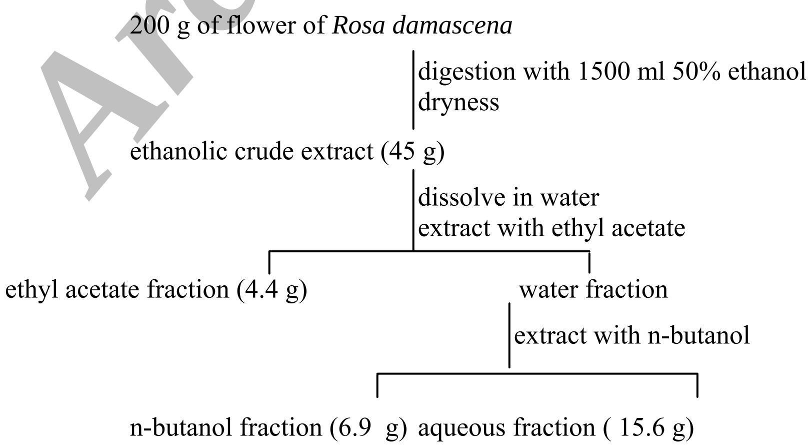

Figure 1. Separation of flowers of *Rosa damascena*

Tissue preparation

Male guinea pigs (400-700 g) were sacrificed by a blow on the neck and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain (18, 19). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent UK) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37 ^oC and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 hr while it was washed with Krebs solution every 15 min.

Protocols

The relaxant effects of three cumulative concentrations of ethyl acetate, n-butanol and aqueous fractions (0.1, 0.2 and 0.4 g%) and theophylline anhydrous (Sigma Chemical Ltd UK) (0.1, 0.2 and 0.4 mM), and saline (0.4 ml) as negative control were examined. Different concentrations of fractions were produced by adding 0.1 ml of 10 g% to a 10 ml organ bath respectively two times and 0.2 ml was added one more time. For theophylline, 0.1 ml of 10 mM concentrated solution was added to organ bath 2 times and 0.2 ml another time. The consecutive volumes were added to organ bath at five min intervals. Ethyl acetate and n-butanol fractions were dissolved in saline by adding few drop of tween 80 and the same amount of tween was added to the other solutions.

In each experiment the effect of three cumulative concentrations from each fraction, theophylline or saline on contracted tracheal smooth muscle was measured after exposing tissue to each concentration of the solution for 5 min. A decrease in tone was considered to be a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction. An increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change (13).

The relaxant effect of different solutions was tested with two different experimental designs as follows:

1. On tracheal chains contracted by 60 mM KCl (group 1 experiments, n= 5).
2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 2 experiments, n= 8).

The relaxant effects in two groups of experiments were examined in two different series of tracheal chains. All of the experiments were performed randomly with a 1 hr resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation.

Statistical analysis

Data of relaxant effect were expressed as mean±SEM. The relaxant effects of different concentrations of each fraction were compared with the results of negative and positive control using ANOVA. The relaxant effects obtained in two groups of experiments were compared using unpaired t test. Correlation between the relaxant effects and concentrations of three fractions and theophylline were examined using least square regression. Significance was accepted at $P< 0.05$.

Results

Relaxant (bronchodilatory) effect

All concentrations of theophylline, ethyl acetate fraction and two final concentrations of n-butanol fraction showed significant relaxant effects compared to that of saline ($P< 0.05$ to $P< 0.001$) in groups 1 experiments. However, aqueous fraction showed significant contraction effect on tracheal chains ($P< 0.001$ for all concentrations). The effect of ethyl acetate fraction was significantly higher than those of theophylline ($P< 0.05$ to $P< 0.001$) but, the effects of other fractions were significantly lower than theophylline ($P< 0.001$ for all cases) in this group (Table 1).

All concentrations of theophylline, ethyl acetate and aqueous fractions showed concentration dependent relaxant effects compared to that of saline ($P< 0.01$ to $P< 0.001$) in group 2. However, n-buthanol fraction did not show any significant relaxant effect on tracheal chains in this group. The effect of ethyl acetate fraction in group 2 was non-significantly higher than those of theophylline but the effects of other fractions were significantly lower than theophylline in this group ($P< 0.01$ to $P< 0.001$) except for the first concentration of aqueous fraction (Table 2).

Comparison of the relaxant effect between different fractions

The effects of all concentrations of aqueous fraction were significantly lower than other two fractions ($P< 0.001$ for all cases) in group 1. In addition the effects of all three concentrations of n-butanol fraction were significantly lower than that of ethyl acetate fraction ($P< 0.05$ to $P< 0.001$).

In group 2, the effects of final concentration (0.4 g%) of aqueous fraction and all concentrations of n-butanol fraction were significantly lower than the effect of ethyl acetate fraction ($P< 0.01$ to $P< 0.001$). In addition the effects of all three concentrations of aqueous fraction were significantly higher than that of n-butanol fraction ($P< 0.01$ for all concentrations).

Comparison of the relaxant effect between two groups of experiments

There was no significant difference in the relaxant effect of different concentrations of theophylline and ethyl acetate fraction between group 1 and 2 (Figure 1a and b).

However, the effects of all concentrations of aqueous fraction in group 1 were statistically lower than those of group 2 experiments ($P< 0.001$ for all concentrations), (Figure c). In addition the effect of final concentration (0.4 g%) of n-butanol fraction in group 2 was significantly higher than group 1 ($P< 0.01$), (Figure d).

Correlation between concentrations of solutions and their relaxant effect

There were significant positive correlations between the relaxant effects and concentrations for theophylline and all fractions (except for aqueous fractions in group 1) in both groups. (for all cases $P< 0.001$, Table 3).

Table 1. Relaxant effect of three different fractions (aqueous, ethyl acetate and n-butanol) from *Rosa damascena* in group 1 experiments (contracted tracheal chains with 60 mM KCl, n=5) and their comparisons with negative control (saline) and positive control (theophylline).

Different concentration	Saline	Aqueous fraction	Ethyl acetate fraction	N-buthanol fraction	Theophylline
0.1	-	-3.50±1.17	33.80±2.13	3.48±1.20	12.44±1.53
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.001$	NS	$P< 0.001$
St. Dif. *vs* Theophylline		$P < 0.001$	$P< 0.001$	$P< 0.001$	
0.2	-	-6.30±0.50	48.20±3.50	6.20±0.46	34.82±3.98
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.001$	$P< 0.05$	$P< 0.001$
St. Dif. *vs* Theophylline		$P< 0.001$	$P< 0.001$	$P< 0.001$	
0.4	1.74±0.94	-6.60±0.98	68.42±4.48	24.00±3.77	61.15±1.53
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.001$	$P< 0.001$	$P< 0.001$
St. Dif. *vs* Theophylline		$P< 0.001$	$P< 0.05$	$P< 0.001$	

Values are presented as mean±SEM. St. Dif.: Statistical difference. The unit of concentration for fractions was g%, and for theophylline, mM.

Table 2. Relaxant effect of three different fractions (aqueous, ethyl acetate and n-butanol) from *Rosa damascena* in group 2 experiments (contracted tracheal chains by µM methacholie, n= 8) and their comparisons with negative control (saline) and positive control (theophylline).

Different concentration	Saline	Aqueous fraction	Ethyl acetate fraction	N-buthanol fraction	Theophylline
0.1	-	18.25±2.40	21.50±5.37	1.56±0.87	21.73±4.23
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.01$	NS	$P< 0.001$
St. Dif. *vs* Theophylline		NS	NS	$P< 0.001$	
0.2	-	26.75. ±3.32	44.81±11.55	3.50±0.87	38.40±4.56
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.001$	NS	$P< 0.001$
St. Dif. *vs* Theophylline		$P< 0.01$	NS	$P< 0.001$	
0.4	1.74±0.94	34.88±4.37	77.89±9.14	5.00±1.17	65.45±6.31
St. Dif. *vs* Saline		$P< 0.001$	$P< 0.001$	NS	$P< 0.001$
St. Dif. *vs* Theophylline		$P< 0.001$	NS	$P< 0.001$	

For abbreviations see Table I.

Table 3. Correlation (r) between the relaxant effect s of three different fractions (aqueous, ethyl acetate and n-butanol) from *Rosa damascena* and theophylline with their concentrations in two groups of experiments

Different substances	Aqueous fraction		Ethyl acetate fraction		N-buthanol fraction		Theophylline	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Group 1	-0.512	NS	0.961	$P< 0.001$	0.873	$P< 0.001$	0.977	$P< 0.001$
Group 2	0.583	$P< 0.005$	0.692	$P< 0.001$	0.460	$P< 0.01$	0.843	$P< 0.001$

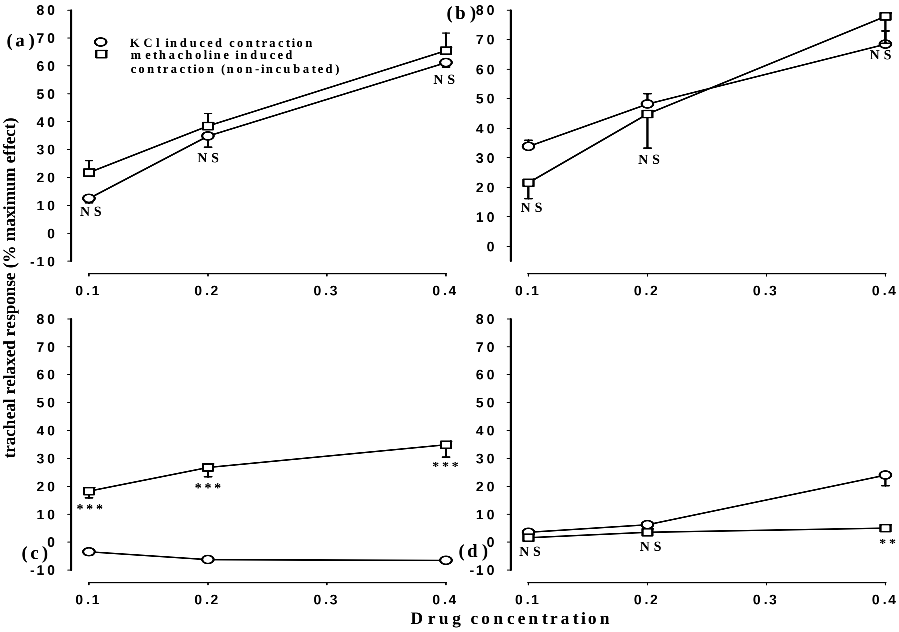

Figure 2. Concentration response curves of the relaxant effect of theophylline (a), ethyl acetate (b), aqueous (c), and n-butanol fractions (d), in two groups of experiments.
group 1; KCl induced contraction of tracheal chains (o, n= 5) and group 2; methacholine induced contraction of tracheal chains (□, n= 8). Statistical differences in the relaxant effect of different solutions between group 1 with those of group 2; NS: non-significant difference, **; $P< 0.01$, ***; $P< 0.001$.

Discussion

The differences in the relaxant (bronchodilatory) effects of three different fractions (aqueous, ethyl acetate and n-butanol) from *R. damascena* were studied in this study. All concentrations of theophylline, ethyl acetate fraction and two final concentrations of n-butanol fraction showed significant relaxant effects in both groups and aqueous fractions from *R. damascena* showed relatively potent relaxant effect in group 2.

Several different mechanisms including stimulation of β-adrenergic receptors (19, 20), inhibition of histamine H_1 receptors (21) or an anticholinergic property of this plant (22) could contribute in the relaxant effect of different fractions from *R. damascena* on tracheal smooth muscle. The absence of relaxant effect of aqueous extract in group 1 and relatively potent relaxant effect in group 2 suggests an inhibitory effect of this fraction on muscarinic receptors. In addition the non significant difference in relaxant effect of acetyl acetate fraction between two groups may suggest an antagonist activity of this fraction on muscarinic receptors. However, to confirm the antagonistic effects of these fractions, the concentration-response curves to a muscarinc agonist should be performed in the presence of saline and fractions.

The obvious relaxant effect of ethyl acetate fraction and relative effect of N-butanol fraction from *R. damascena* in group 1 may indicate an inhibitory effect of this plant on calcium channels of guinea pig tracheal chain. In fact, bronchodilatory effect of calcium channel blockers (23, 24) has been shown and KCl affects calcium channels (25, 26). The result of the present study may also suggest an opening effect for aqueous fraction of this plant on potassium channels since the bronchodilatory effect of potassium channel opening has been demonstrated previously (23). In fact, if the aqueous fraction from *R. damascena* had a potassium channel opening effect, they would not have a relaxant effect on tracheal chains contracted by KCl, while they could show a relaxant effect when the tracheal chain was contracted by metacholine.

The greater relaxant effect of ethyl acetate fraction compared to the other two fractions suggests that lipid soluble (non-polar) constituents of this plant are mainly responsible for its relaxant effect on tracheal smooth muscle. The results of this study confirmed those of the previous one (17) indicating the relaxant effect of this plant on smooth muscles and showed that mainly acetyl acetate fraction is responsible for its relaxant effect. However, more studies are required to reveal the different therapeutic effect, exact effective substance(s), and mechanism(s) of action of *R. damascena.*

In our previous study the relaxant effect of ethanolic extract and essential oil of the plant was studied (17). However, in the present study, the effect of three different fractions of the plant were studied which were different in composition. The results of the present study showed that the relaxant effect of ethyl acetate fraction of the plant was similar or even higher than the effect of theophylline in both groups of experiments. The results of our previous study (17) also showed similar relaxant effect for the essential oil of the plant and theophylline. These results indicate that non-polar constituents of *R. damascena* specially those present in the essential oil of the plant are responsible for its relaxant effect on tracheal smooth muscle.

Conclusion

The results of this study indicated a relatively potent relaxant (bronchodilatory) effect for mainly ethyl acetate fraction from *R. damascena* on tracheal chains of guinea pig which was greater than that of theophylline. The results also suggest a muscarinc antagonistic effect for aqueous and acetyl acetate fractions. The inhibitory effect of ethyl acetate and n-butanol fractions on calcium channels and an opening effect of aqueous fraction on potassium channels are also suggested.

Acknowledgment

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