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Original article

Effect of Aqueous-Ethanolic Extract from *Rosa damascena* on Guinea Pig Isolated Heart

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Abstract

Objective(s)
In the present study, the effects of aqueous-ethanolic extract from *Rosa damascena* on heart rate and contractility were examined.
Materials and Methods
Isolated guinea-pig hearts were perfused through aorta in a Langendorff mode. Heart rate (HR) and contractility were determined in the presence of four concentrations of the extract (0.1, 0.2, 0.4 and 1.0 mg %) and isoprenaline (1, 10, 100 nM and 1 μM) in comparison with baseline values in the presence and absence of propranolol (n= 10 for each group).
Results
Both isoprenaline and the extract caused increase in heart rate and contractility ($P< 0.05$ to $P< 0.001$). The percent increased in HR due to the final concentration of isoprenaline in the absence of propranolol was significantly greater than that of the extract ($P< 0.01$). Propranolol caused significant reduction in both HR and contractility ($P< 0.05$ for both) but this effect was significantly reversed by isoprenaline and the extract ($P< 0.05$ to $P< 0.001$). The percent increased in heart contractility due to the final concentration of the extract in the absence and presence of propranolol was significantly greater than that of isoprenaline ($P< 0.05$ for both cases). There was significant correlation between both HR and heart contractility with concentration of isoprenaline and the extract ($P< 0.05$ to $P< 0.001$).
Conclusion
In conclusion this study showed a relatively potent inotropic and chornotropic effect for *Rosa damascena* on isolated guinea-pig heart.

Keywords: Aqueous-ethanolic extract, β-adrenoceptor, Guinea-pig, Isolated heart, *Rosa damascena*

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Introduction

Rosa damascena is an erect shrub 1 to 2 meter in height with large, showy and colorful flowers. This plant contains vitamin C (1), carboxylic acid (2) terpene and myrcene (3).

Different therapeutic effects were described for *R. damascena* including: treatment of abdominal and chest pain, strengthening the heart (as described for this plant in ancient medical books) (4), treatment of menstrual bleeding, digestive problems (5), anti inflammation (2), cough (1) and laxative (6). Several pharmacological effects such as anti HIV (7), analgesic, hypnotic, antispasmodic and anti-inflammatory effects (1, 8), analgesic and antitussive effects (9, 10) and the relaxant effect on guinea pig trachea (11) were reported for this plant.

A new flavonoid glycoside, derived from this plant significantly suppresses angiotensin I-converting enzyme (ACE) activity, which indicates that *R. damascena* and its flavonoids may be effective to improve the cardiovascular system (12). Therefore, in the present study, the effect of aqueous-aqueous-ethanolic extract from *R. damascena* on heart rate and contractility of guinea pigs hearts was examined.

Materials and Methods

Plant and extracts

R. damascena was collected from Kashan (middle part of Iran) in spring 2005. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences (Herbarium No: 254-1804-01). The Aqueous-ethanolic extract was prepared as follows: 50 grams the chopped, dried flowers of the plant was extracted with 150 ml distilled water and 150 ml ethanol by soxhelat apparatus. The solvent of the extract was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final extract was 10 g %.

Preparation of the isolated hearts

Dunkin Hartley guinea pigs of either sex, with a body weight of 400-500 g, were provided by Razi Istitute, Mashhad, Iran. Guinea pigs were killed by a blow on the neck, the hearts were rapidly isolated, and were washed in ice-cold saline. The coronary circulation was perfused through aorta on a modified Langendorff apparatus at a constant perfusion pressure of 70 mm Hg (13, 15). The hearts were perfused with Krebs Henselite (K-H) buffer solution (37 °C, pH 7.4, saturated with 95% O_2 and 5% CO_2). The K-H buffer solution contained the following (in mmol/L): NaCl 118, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0. All the hearts were first perfused with K-H solution for 20-30 min for stabilization in a Langendorff apparatus and then the effects of extracts from *R. damascena* and isoprenaline were studied.

Protocol of experiments

The effect of different solutions on heart rate and heart contractility compared to baseline values was examined in following groups (n= 10 for each condition):

1. The effects of four concentrations of aqueous-ethanolic extract from *R. damascena* (0.1, 0.2, 0.4 and 1.0 mg%) in the absence of propranolol.
2. The effects of four different concentrations of isoprenaline (1, 10 nM, 0.1 and 1 μM) in the absence of propranlol
3. The effects of four concentrations of the extract in the presence of 1 μM propranlol
4. The effects of four concentrations of the isoprenaline in the presence of 1 μM propranlol

Each concentration of the solutions was given as one-minute intracoronary infusion and the heart rate and contractility were recorded in the last 10 sec. During the experiments each heart served as its own control before injection of each solution. In the absence of pharmacological intervention, both heart rate and contractility were reproducible.

The effects of the extract and isoprenaline in the presence and absence of propranolol were examined in two different series of animal heart. The effects of aqueous-ethanolic extract and isoprenaline in each heart were performed randomly with a 30 min resting period of the heart between examining of the effect of each two solution while the heart is perfused with ordinary Krebs. In all experiments, the HR and contractility were recorded on a kymograph (ET8 G-Boulitt, Paris) and were

measured after fixation. The HR was measured using the number of recorded contraction curve during last 10 sec of each concentration of the extract, isoprenaline for 10 sec before injection (as the baseline value). For measuring heart contractility, kymograph was calybrated before each experiment as the height of the recording curve/g and the height of the recording curve due to each testing solution was then converted to gram-contraction. The concentrations of isoprenaline was chosen according to a previuos study (16) and those of the extract according to our previous studies (11, 17, 18) and pilot experiments. The local Animal Research Committee of Mashhad University of Medical Sciences approved the experimental procedures used in the present study.

Statistical analysis

The data of the HR and contractility were expressed as mean±SEM. The data obtained in the presence of different concentrations and baselines were compared using ANOVA with Tukey-Kramer post-hoc test in the presence and absence of propranolol. The data of heart rate and contractility obtained in the presence of each concentrations of the extract were compared with those of isoprenalin using paired "t" test.

The percent increase in HR and contractility due to final concentrations of the extract and isoprenaline were also compared using paired "t" test. The correlation between the effect of aqueous extract and isoprenaline and their concentrations were also evaluated using least square regression. Significance level was set at $P< 0.05$.

Results

Effect of aqueous-ethanolic extract on heart rate and heart contractility

All concentrations of isoprenaline and the extract caused significant increase in HR and heart contractility ($P< 0.05$ to $P< 0.001$). Propranolol caused significant reduction in both HR and contractility ($P< 0.05$ for both cases). However, isoprenaline and the extract concentration significantly reversed the effect of propranolol on HR ($P< 0.05$ to $P< 0.001$), (Table 1 and 2).

Differences between the effect of isoprenaline and aqueous-ethanolic extract

The percent increased in HR due to the final concentration of isoprenaline (1 μM) in the absence of propranolol was significantly greater than that of the extract ($P< 0.01$). However, the percent increased in heart contractility due to the final concentration of the extract (1.0 mg %) in the absence and presence of propranolol was significantly greater than that of isoprenaline ($P< 0.05$ for both cases), (Table 3).

Table 1. The effect of four different concentrations of isoprenaline and extract from *Rosa damascene* on heart rate of isolated guinea pig's hearts in the presence and absence of propranolol.

Experimental design		Pre-antagonist	St Dif *vs* B	Post-antagonist		St Dif *vs* B
Baseline		210.00±17.90	-	Propranolol	170.50±12.83 *	-
	1 nM	269.50±19.14	$P< 0.05$		253.00±16.80	$P< 0.002$
Isoprenaline	10 nM	308.00±20.42	$P< 0.002$	Propranolol	319.00±13.72	$P< 0.0001$
	0.1 μM	357.50±26.25	$P< 0.001$	+	368.50±18.43	$P< 0.0001$
	1 μM	412.50±26.25	$P< 0.001$	Isoprenaline	423.50± 21.77	$P< 0.0001$
Baseline		260.91±21.28	-	Propranolol	209.55±17.50 *	-
	0.1 mg %	310.00±24.90	NS		295.00±25.98	$P< 0.05$
Extract	0.2 mg %	330.00±23.45	$P< 0.05$	Propranolol	355.00±26.08	$P< 0.001$
	0.4 mg%	360.00±18.71	$P< 0.002$	+	400.00±16.73	$P< 0.001$
	1.0 mg%	395.00±17.89	$P< 0.001$	Extract	465.00±17.18	$P< 0.001$

Data were expressed as mean±SEM. St Dif: Statistical differences, B: Baseline. Statistical differences between the data in the presence and absence of propranolol; * $P< 0.05$.

Table 2. The effect of different concentrations of isopernaline and aqueous-ethanolic extract of *Rosa damascene* on contractility of guinea pig's hearts in the presence and absence of propranolol.

Experimental design		Pre-antagonist	St Dif *vs* B	Post-antagonist		St Dif *vs* B
Baseline		0.45±0.10	-	Propranolol	0.29±0.04 *	-
	1 nM	0.55±0.10	NS		0.43±0.05	*P*< 0.05
Isoprenaline	10 nM	0.62±0.09	NS	Propranolol	0.53±0.05	*P*< 0.002
	0.1 µM	0.72±0.09	*P*< 0.05	+	0.64±0.05	*P*< 0.001
	1 µM	0.78±0.10	*P*< 0.05	Isoprenaline	0.81±0.05	*P*< 0.001
Baseline		0.26±0.05	-	Propranolol	0.16±0.04 *	-
	0.1 mg %	0.38±0.09	NS		0.33±0.05	*P*< 0.05
Extract	0.2 mg %	0.45±0.09	*P*< 0.05	Propranolol	0.46±0.06	*P*< 0.001
	0.4 mg%	0.52±0.08	*P*< 0.01	+	0.61±0.06	*P*< 0.001
	1.0 mg%	0.69±0.09	*P*< 0.002	Extract	0.73±0.07	*P*< 0.001

For abbreviations see Table 1. Statistical differences between the data in the presence and absence of propranolol; **P*< 0.05.

Table 3. Increased heart rate and contractility due to the final concentration of the extract and isoprenaline (precent propertion to baseline values) and the statistical differences between the extract and isoprenaline.

Parameter	Isoprenaline	Extract	St Dif	Isoprenaline+An	Extract + An	St Dif
Rate	213.20±12.20	169.10±12.41	*P*< 0.05	279.80±24.94	255.80±24.70	NS
Contractility	247.20±34.81	369.90±42.69	*P*< 0.05	360.70±55.19	572.50±74.58	*P*< 0.05

An: antagonist, for additional abbreviations see Table 1.

Table 4. Correlation between the effects of aqueous-ethanolic extract from *Rosa damascene* and isoprenaline with concentrations on heart rate and contractility of isolated guinea pig heart in four groups of experiments.

Parameter	Extract		Extract+A		Isoprenaline		Isoprenaline+A	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Rate	0.378	*P*< 0.05	0.610	*P*< 0.001	0525	*P*< 0.001	0.609	*P*< 0.001
Contractility	0.338	*P*< 0.05	0.548	*P*< 0.001	0.594	*P*< 0.001	0.574	*P*< 0.001

A: agonist, for additional abbreviations see Table 1.

Relationship between concentration and the effect of aqueous-ethanolic extract and isoprenaline

There were significant correlations between both HR and heart contractility with concentration of isoprenaline and the extract (*P*< 0.05 to *P*< 0.001), (Table 4).

Discussion

The results of the present study showed a concentration dependent increase in both HR and heart contractility due to aqueous-ethanolic extract from *R. damascena*. However, the effect of the extract on heart contractility (inotropic effect) was more pronounced.

To explore the mechanism(s) of the effect of extract of this plant (stimulatory effect on adrenergic receptors), its effect on pre-treated heart with propranolol was examined. The extract in a dose dependent manner and significantly reversed the effect of propranolol on both HR and contractility which was more pronounced on heart contractility. The effect of the extract on contractility of pre-treated heart with propranolol was even greater but its effect on HR was less than that of isoprenaline.

The significant correlation between concentrations of different solutions and their effect on HR and heart contractility supports concentration dependent effects of hydro-ethanolic extract similar to isoprenaline. Therefore the results of the present study indicated that hydro-ethanolic extract of *R. damascena* has a significant choronotropic and a more potent inotropic effect. With regard

to the effect of the extract on pre-treated heart with propranolol, the possible mechanism of action of the extract from *R. damascena* on heart is its β-adrenoceptor stimulatory effect (16). Although, the reduction of HR and contractility due to propanolol in the baseline condition could be due to its membrane stabilization, but similar abolishment of the effect of propranolol by isoprenaline and the extract suggests a β-adrenoceptor stimulatory effect for the plant.

Therefore, with regard to the results of the present study, the most possible mechanism of action of hydro-ethanolic extract on heart is suggested to be the stimulatory effet on β-adrenoceptor because the extract increased both HR and contractility. In addition, if the extract has a stimulatory effet on β-adrenoceptor it can reverse the effect of both methacholine and diltiazem with functional antagonism manner. Although, phosphodiestrase III inhibitors, could have similar effect, but it is unlikely to reverse the effect of propranolol exactly similar to isoprenaline which was seen for the extract in the present study.

Other possible mechanisms of the effect of plant on heart rate and contractility such as opening effect on calcium channels, increased cAMP levels like phosphodiestrase III inhibition (19) or forskolin-like action (20) should be investigated in more detailed studies. In addition the effects of different fractions of the extract also need to be studied on heart activities and the mechanism(s) of their actions should be explored in further studies.

The results of the present study are novel and may represent a pharmacological action that is attractive under various conditions of cardiac impairment. In hypocalcemia conditions (21, 22), which results in low systolic intracellular free calcium concentration and in heart failure patients, *R. damascena* would increase the contractile response, thereby improving cardiac pumping function. Further studies will be needed to isolate and structurally identify the active ingredient(s) of *R. damascena* plant.

Conclusion

This study showed novel and potent inotropic and chornotropic effects for *R. damascena* on isolated guinea pig heart. The results of the present study suggest a possible stimulatory effect of aqueous-ethanolic extract from *R. damascena* on β-adrenoceptor of isolated guinea pig heart.

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