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Original Articles

HYPOTENSIVE EFFECT OF ROYAL JELLY IN RAT

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ABSTRACT

The royal jelly elicited no significant change in heart rate ($P < 0.05$) Furthermore, the isolated right and left atria showed bradycardia and weaker force of contraction after royal jelly administration. Moreover, hexamethonium failed to block the hypotensive effect of royal jelly. The results indicated that royal jelly might be partly mediated the hypotensive effect via activation of muscarinic cholinergic receptors by its acetylcholine-like factor.

Keywords : royal jelly, acetylcholine, hypotensive effect, muscarinic cholinergic receptor

INTRODUCTION

Royal jelly is milky white highly viscous secretion produced from the hypopharyngeal glands and mandibular glands in the head of young worker honeybees. It is the essential food of all bee larvae for the first three days of life. After three days, only the future queen bees are continue to be fed on royal jelly, which is in some way responsible for their development into mature female insects. These queen bees can live ten to twenty times longer than other bees. Royal jelly contains many nutrients including carbohydrates, proteins, lipids, vitamins and minerals. The very important substance found only in royal jelly is 10-hydroxy-2-decinoic acid. (Townsend, 1940; Haydak, 1943; Johansson, 1955).

Royal jelly has been used popularly as food supplement for many years. Many reports showed some pharmacological properties of royal jelly such as antitumor (Townsend, 1959; Townsend, 1960), antibacterial (Kramer, 1977; O'Conner, 1985; Fujiwara, 1990), anti-inflammatory and wound healing (Fujii, 1990; Pongsakorn, 1992) and vasodilating effect (Henschle, 1956; Townsend, 1959; Bowem, 1977; Kivilaakso, 1978; Shinoda, 1987; Peungvicha, 1992). Nevertheless, the role of royal jelly on blood pressure has not been fully elucidated in normal rats. The objectives of this study were carried out to investigate the actions of royal jelly on blood pressure in both isolated and intact experimental animals.

MATERIALS AND METHODS

Animals

Male albino rats weighing 180-250 g were purchased from the National Laboratory Animal Centre, Mahidol University. The animals were maintained on laboratory pellets (Pokphabd Animal Feed Co., Thailand) and tap water *ad libitum*. They were fasted overnight with access to water before the experiment.

Royal jelly was obtained from Queen Living Products Co. Ltd. in the form of freeze-dried powder. In such form the weight was reduced to about one third of the fresh one.

Experimental design

The rats were anaesthetized with sodium pentobarbital at a dose of 40 mg/kg BW, intraperitoneally. Supplementary doses of the same drug were given whenever necessary to maintain the anaesthesia. The trachea was cannulated for spontaneous ventilation with room air and to facilitate respiration. Royal jelly and other agents were administered intravenously through a catheter inserted into a left femoral vein. Arterial blood pressure was monitored from right femoral artery with pressure transducer connected to MacLab. Electrocardiogram (ECG) was recorded by MacLab. The heart rate and blood pressure were examined for these experiments. The blood pressure response was measured at 20 seconds postinjection.

The effects of various doses of royal jelly on blood pressure and heart rate were determined. The animal was given slow bolus intravenous infusions of the doses 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 mg/kg BW of royal jelly. The infusion at each concentration lasted for 10 min before the next higher concentration was tested. The control group was given an equal volume of 0.9% sodium chloride solution.

The experiments studied on the effect of cholinergic agents. Firstly, the dose 0.8 mg/kg BW of royal jelly or 0.2 μg /kg BW of intravenous injection of acetylcholine was taken before and after 0.3 mg/kg BW of atropine injection in anaesthetized rats. Secondly, hexamethonium (3.5 mg/kg BW) infusion was performed before and after injection of royal jelly at 0.8 mg/kg BW in the anaesthetized rats. Finally, the effect of cholinesterase inhibitor response was taken before and after neostigmine injection at a dose of 0.01 mg/kg BW.

The effects of royal jelly on chronotropic and intropic responses were determined. Rats were killed by blowing on the head. The heart was quickly excised and placed in a petri-dish containing oxygenated Locke solution (of composition, in millimolar/liter: NaCl 155.8; $CaCl_2$ 2.15; KCl 5.6; $NaHCO_3$ 1.8 and glucose 5). The left and right atria were separated. The right atrium was transferred into 10 ml organ bath containing Locke solution continuously oxygenated bubble and maintained at 37° C. Each preparation was applied a tension of 1 g. The spontaneous heart rate was recorded with isometric force transducer connected to a MacLab. The atrium was allowed to equilibrate until the rate and amplitude of spontaneous contraction were stable. After 60 minutes of equilibrium period, the doses 40, 60, 80 and 100 μg/ml of royal jelly were administered to the bath fluid. The responses were recorded within 15 minutes of the each dose. After repeated washing and allowed the preparation to recover for at least 15 minutes, the 10^{-6} M of atropine was tested, and then the same doses of royal jelly were given.

The isolated left atrium was fixed on a platinum wire electrode. It was placed in 10 ml organ bath containing Locke solution at 37°C and continuously aerated with oxygenated bubble. The stimulus strength was 5 volts and the duration was 5 msec. The frequency of stimulation was kept constant at 250 pulses/min. The tissue was applied to a tension of 1 g and allowed to equilibrate until the force of contraction was stable before it was exposed to the drug in the same way as the isolated right atrial preparation. The force of contraction was recorded with isometric force transducer connected to a MacLab.

Analysis of data

Experimental data were expressed as mean ± SD. Statistical significance was tested according to Student's t-test. P value <0.05 was considered significant.

RESULTS

1. The effects of intravenous injection of royal jelly on systemic blood pressure and heart rate

A single dose of intravenous injection of 0.8 mg/kg BW of royal jelly reduced significant systolic blood pressure by 121.02 ± 12.24 to 73.45 ± 12.46 mmHg and diastolic blood pressure by 84.65 ± 10.62 to 40.68 ± 9.72 mmHg (P<0.05) (Fig. 1). The most reduction of hypotensive response was elicited within 10 minutes. A slow bolus intravenous infusion at the doses of 0.1 to 0.8 mg/kg BW of royal jelly caused significant and dose dependent reductions in both systolic and diastolic blood pressure (P<0.05). Most of the results showed more depression in diastolic than systolic blood pressure (Fig. 2). In contrast, an intravenous infusion of royal jelly elicited no significant changes in the heart rate (Fig. 3).

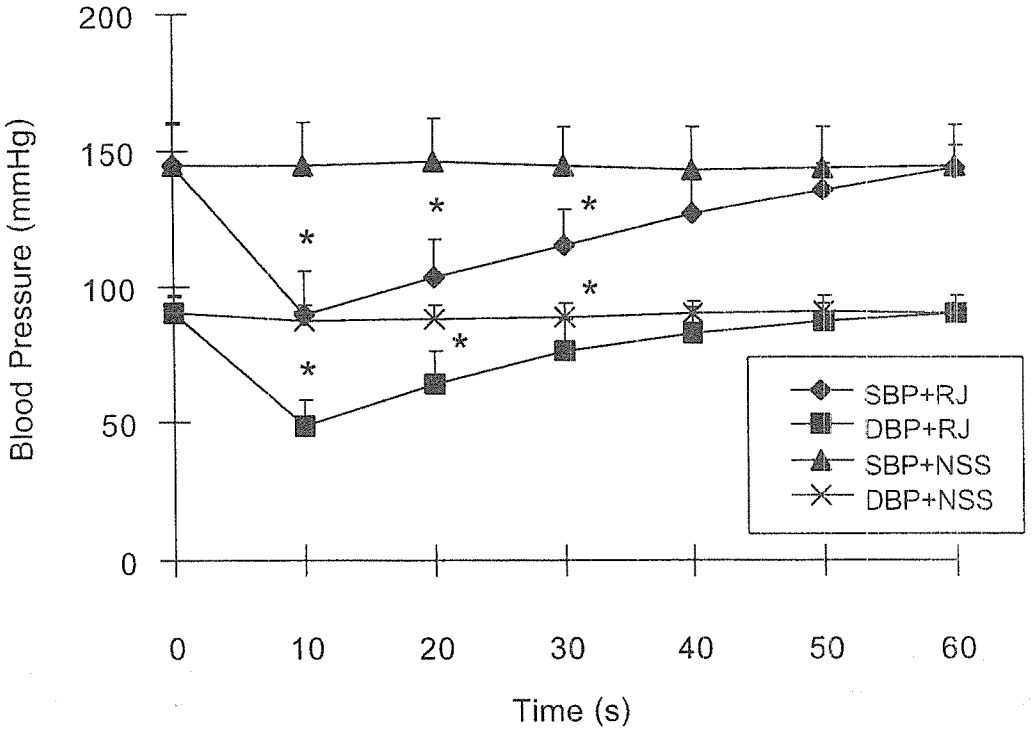

Figure 1 The effect of 0.8 mg/kg BW of royal jelly (RJ) and intravenous injection of normal saline solution (NSS) on systolic (SBP) and diastolic blood pressure (DBP) in anaesthetized rats. Values represent mean ± SD for 10 animals.
* P<0.05 and ** P<0.01

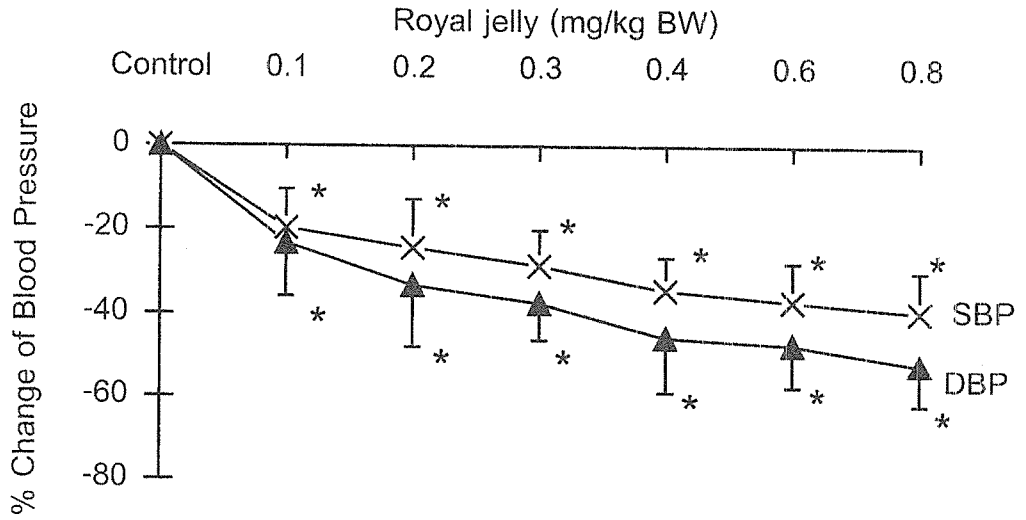

Figure 2 The effect of various doses of intravenous injection of royal jelly on percentage change in systolic (SBP) and diastolic (DBP) blood pressure in anaesthetized rats. Values represent mean ± SD for 10 animals. * P<0.05

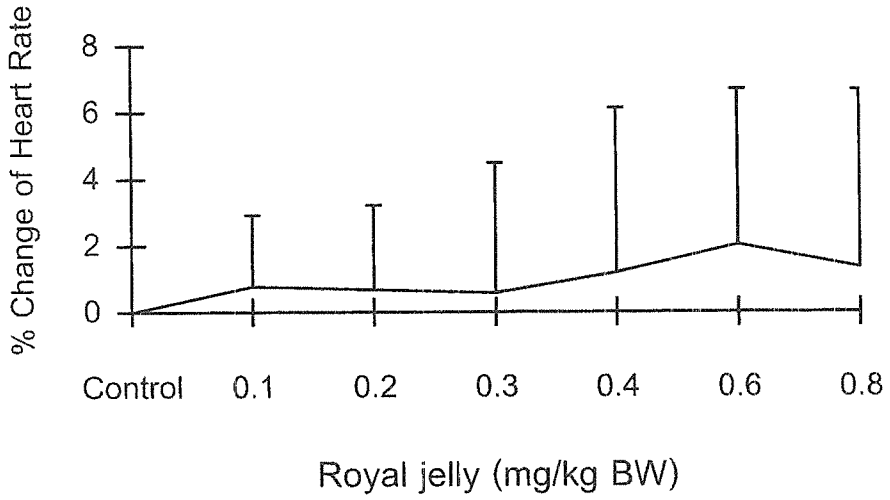

Figure 3 The effect of various doses of intravenous injection of royal jelly on percentage change in heart rate in anaesthetized rats. Values represent mean ± SD for 10 animals.

2. The effects of cholinergic blocking agent (atropine)

As shown in Fig. 4 and 5, atropine at a dose of 0.3 mg/kg BW administered 10 min before royal jelly (0.8 mg/kg BW) or acetylcholine (0.2 μg/kg BW) significantly decreased by both agents in anaesthetized rats ($P< 0.05$). It was noted that the hypotensive effects of royal jelly on the blood pressure were almost completely blocked but acetylcholine was completely blocked by atropine. Although, slight increase of heart rate from acetylcholine and royal jelly administration was not significant decreased by atropine blocking ($P>0.05$).

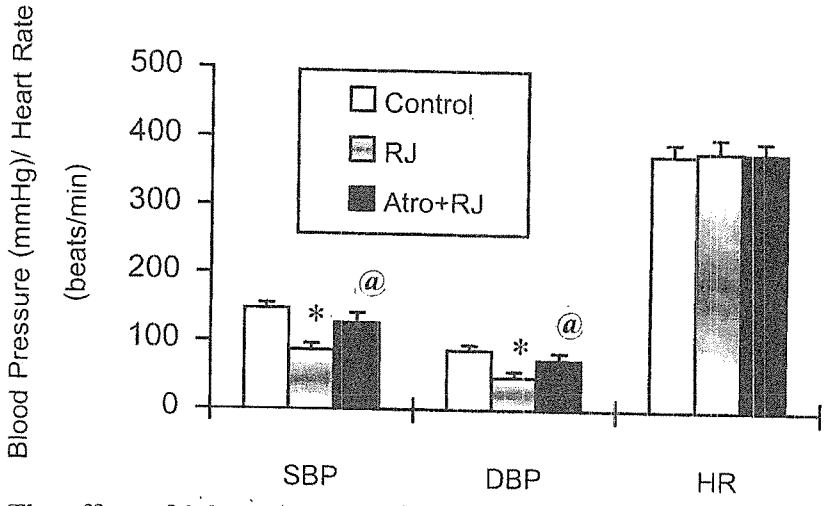

Figure 4 The effect of 0.8 mg/kg BW of intravenous injection of royal jelly (RJ) on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) before and after 0.3 mg/kg BW atropine (Atro) injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) and heart rate (beats / min) for 10 animals. *Significant compared royal jelly with control group. @ Significant compared atropine + royal jelly with royal jelly group. $P<0.05$

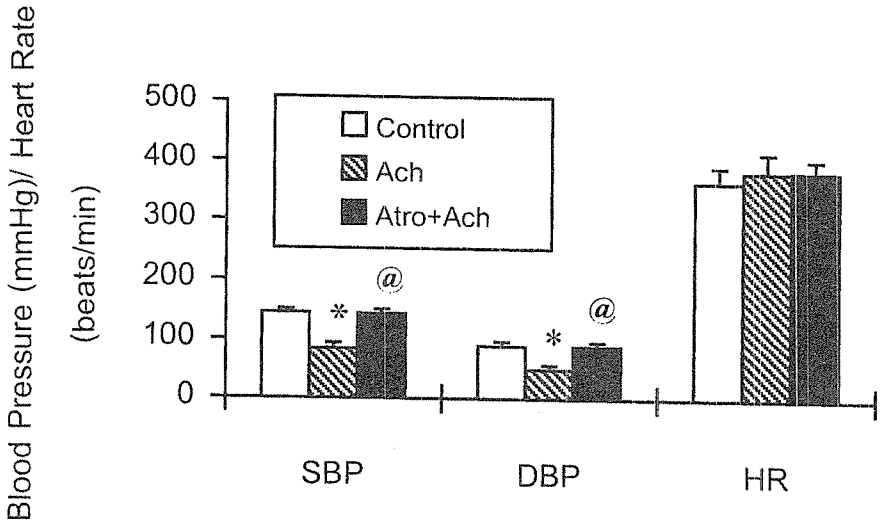

Figure 5 The effect of 0.2 μg/kg BW of acetylcholine (Ach) intravenous injection on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) before and after 0.3 mg/kg BW of atropine (Atro) injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) and heart rate (beats / min) for 10 animals. *Significant compared acetylcholine with control group. @ Significant compared atropine + acetylcholine with royal jelly group. $P<0.05$

3. The effects of ganglionic blocking drug (hexamethonium)

At the dose 3.5 mg/kg BW of hexamethonium injected 10 minutes prior to royal jelly failed to block royal jelly action, although a slight enhancement on the hypotensive effect was no significantly shown after royal jelly at a dose of 0.8 mg/kg BW ($P>0.05$) (Fig. 6).

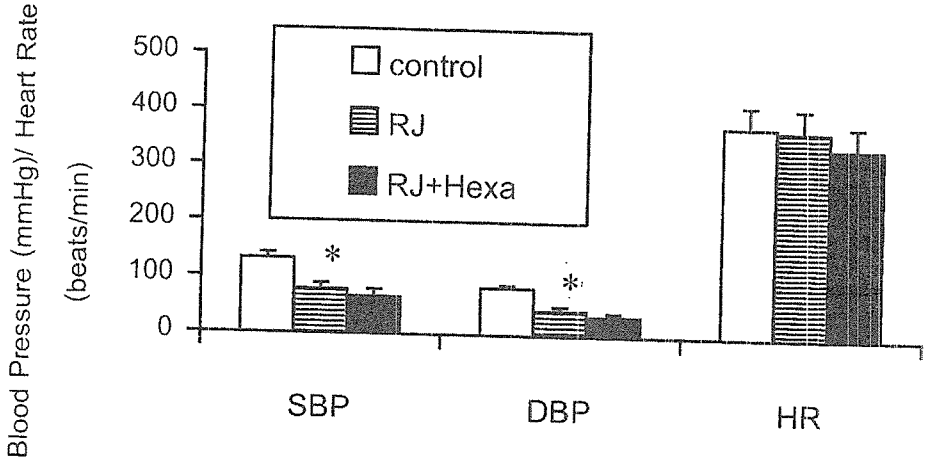

Figure 6 The effect of 0.8 mg/kg BW of royal jelly (RJ) intravenous injection on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) before and after 3.5 mg/kg BW of hexamethonium (Hexa) in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) and heart rate (beats / min) for 10 animals. * Significant compared royal jelly with control group. $P<0.05$

4. The effects of cholinesterase inhibitor (neostigmine)

Administration of 0.01 mg/kg BW of neostigmine 2 minutes before royal jelly (0.2 mg/kg BW) or acetylcholine (0.2 μg/kg BW) infusion significantly enhanced more hypotensive effect of both agents in anaesthetized rats ($P<0.05$) (Fig. 7 and 8). On the other hand, royal jelly or acetylcholine injection after neostigmine administration elicited no significant change of heart rate.

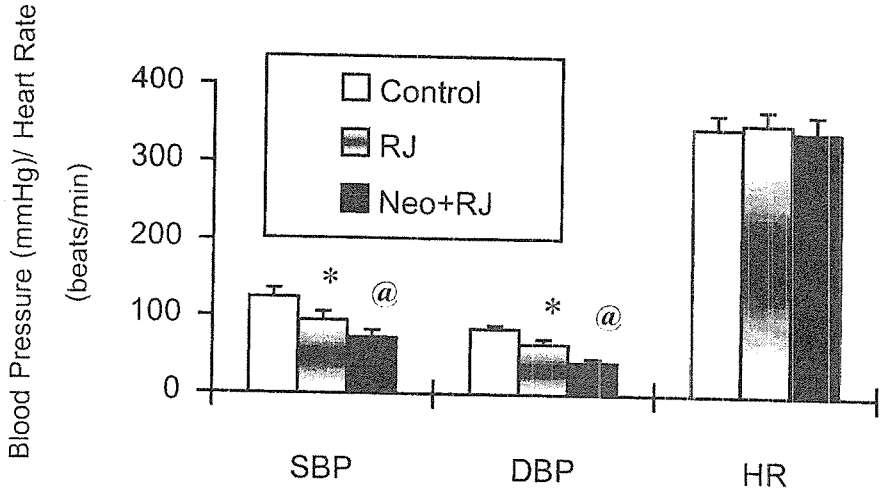

Figure 7 The effect of 0.2 mg/kg BW of royal jelly (RJ) intravenous injection on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) before and after 0.01 mg/kg BW of neostigmine (Neo) injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) and heart rate (beats / min) for 10 animals. * Significant compared royal jelly with control group. @ Significant compared neostigmine + royal jelly with royal jelly group. $P<0.05$

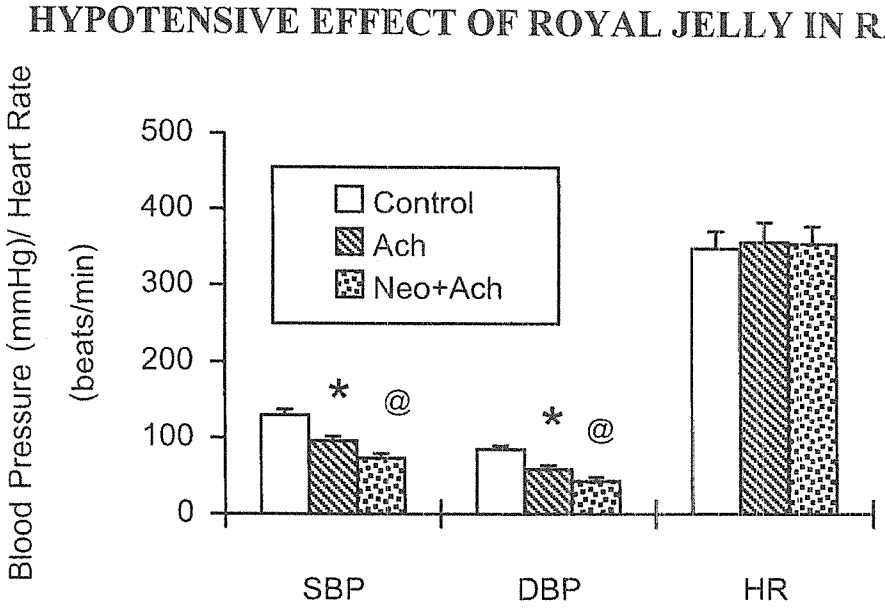

Figure 8 The effect of 0.2 μg/kg BW of acetylcholine intravenous injection on systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) before and after neostigmine (Neo) 0.01 mg/kg BW. injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) and heart rate (beats / min) for 10 animals.
* Significant compared acetylcholine with control group. @ Significant compared neostigmine + acetylcholine with the acetylcholine group. $P<0.05$

5. The effect of royal jelly and cholinergic blocking agent (atropine) on chronotropic and inotropic action.

As shown in Fig.9 and 10, royal jelly at doses of 40, 60, 80 and 100 μg/ml caused significant reduction of the chronotropic and inotropic responses ($P<0.05$). It should be noted the effetcts of royal jelly were almost completely blocked by atropine.

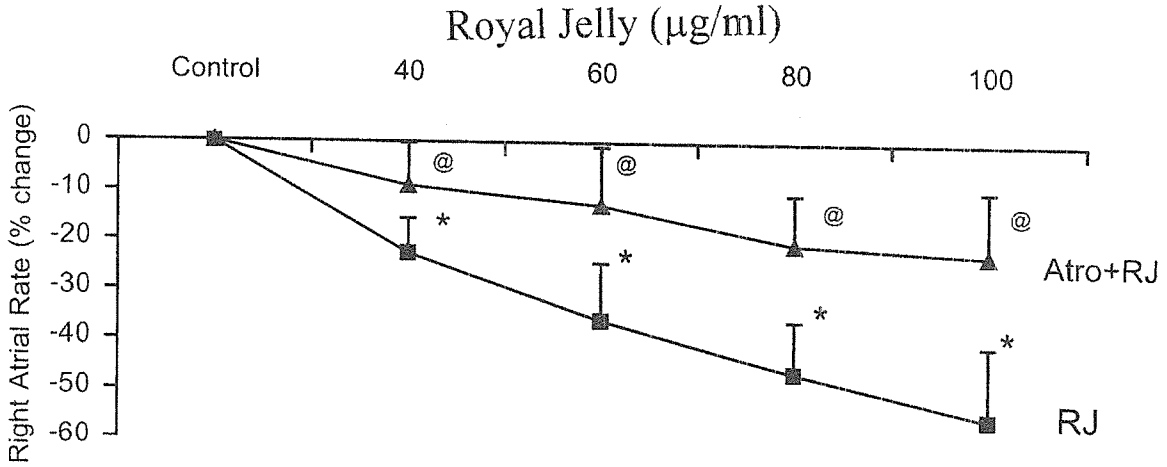

Figure 9 The accumulative dose response curve of royal jelly (RJ) before and after 10^{-6} M of atropine(atro) administration on isolated right atrial rate. Each point on the curve represents the mean value ± SD of the percentage change from the control values for 10 animals.
* Significant compared royal jelly with control group. @ Significant compared atropine + royal jelly with royal jelly group. $P<0.05$

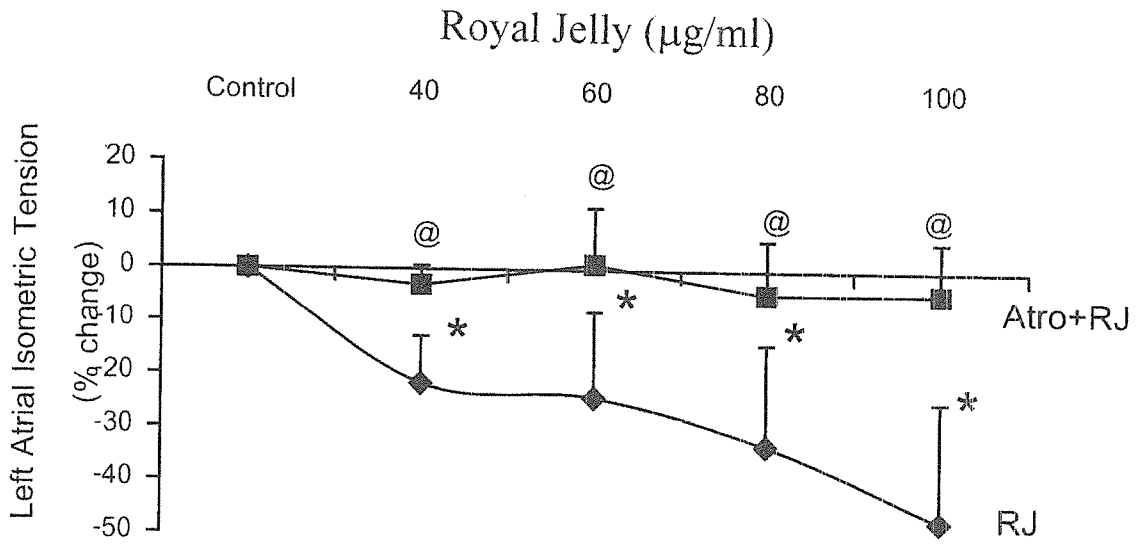

Figure 10 The accumulative dose response curve of royal jelly (RJ) before and after 10^{-6} M of atropine (atro) administration on isolated left atrial isometric tension. Each point on the curve represents the mean value ± SD of the percentage change from the control values for 10 animals.
*Significant compared royal jelly with control group.
@ Significant compared atropine + royal jelly with royal jelly group. $P<0.05$

DISCUSSION

The results of the present study demonstrated that intravenous infusion of royal jelly produced a dose dependent reduction of systemic blood pressure in anesthetized rats. The blood pressure rapidly fell followed the administration of royal jelly and sustained for 1.0 minute. Diastolic blood pressure showed more reduction than systolic blood pressure, with no significant changes of heart rate in intact preparation. On the other hand, the *in vitro* studies showed a reduced heart rate and force of atrial contraction in response to royal jelly administration. The hypotensive effect of royal jelly could be explained, in part, by the reduction of force of contraction and total peripheral resistance from dilatation of blood vessels by the action of royal jelly as previous studies in isolated vessel preparations (Shinoda, 1987; Peungvicha, 1992).

Cholinergic agonists and some other substances which act on muscarinic cholinergic receptors have been reported to cause vasodilatation and decrease in cardiac rate and force of contraction (Weiner, 1980). However, this action is blocked by atropine, which is a competitive antagonist of acetylcholine at muscarinic cholinergic receptors (Shutt, 1979). In the present study, administration of atropine produced a significant inhibition of hypotensive effect of royal jelly and acetylcholine in the anesthetized rats. Additionally, the hypotensive effect of royal jelly elicited more reduction after cholinesterase inhibitors (neostigmine) administration. The results suggested that the hypotensive effect of royal jelly may be partly mediated via muscarinic cholinergic receptors. In order to determine the hypotensive effect of royal jelly, which may act through the hypothalamic or brain stem centers, ganglionic blocking drug was used to prevent the impulses from these centers. Hexamethonium, which blocks the transmission of impulses from the preganglionic axon by occupying receptor sites at the postganglionic sites (Colhoun, 1960, Rang, 1982) was applied. It

was found that hexamethonium could not significantly reduce the hypotensive effect of royal jelly in anesthetized rats. On the other hand, it slightly enhanced no significant reduction of the systemic blood pressure. This suggested that the royal jelly might not act upon the central nervous system to produce hypotension. The result of hypotension in the animals in this study emphatically established by the effect of acetylcholine in royal jelly (Dayan, 1960).

Interestingly, the cholinergic blocking agent was completely blocked the dose-dependent effect of negative inotropic response but it could partially block negative chronotropic response by royal jelly administration in isolated preparation. These might be caused by the different mechanisms of royal jelly on right and left atria. The first mechanism was the direct inotropic effect of royal jelly on cholinergic receptors which could be blocked by cholinergic antagonists. The other mechanism might be an indirect chronotropic action of royal jelly on some mediators in the neurons innervating the heart.

In conclusion, the present investigation demonstrated that royal jelly caused a dose dependent and reversible reduction in blood pressure in anesthetized rats. This effect might be mediated through muscarinic cholinergic rather than nicotinic cholinergic receptors. The hypotensive effect should be caused by the action of acetylcholine in royal jelly. Further investigations should be required to elucidate the details and other mechanisms on cardiovascular system, especially, royal jelly might directly stimulate the vascular endothelial cells to secrete nitric oxide (NO), which in turn possessed acetylcholine activity to relax smooth muscle cells of the blood vessel.

ACKNOWLEDGEMENT

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