HYPOTENSIVE EFFECT OF ROYAL JELLY IN DIABETIC RATS

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ABSTRACT

Royal jelly is a secretion produced from worker honeybees. Intravenous injection of royal jelly 0.1-0.8 mg/kg body weight caused significantly dose dependent hypotensive effects in normal and streptozotocin (STZ) diabetic rats. Additionally, the isolated right and left atria were reduced heart rate and force of contraction by royal jelly administration, respectively. Moreover, the endothelium-dependent relaxation which was produced by royal jelly in aortic strips were significantly attenuated in diabetic rats, compared with control vessels of nomal Wistar rats. Furthermore, The effects including: hypotensive, vasodilatoyr, negative inotropic, and negative chronotropic response were significantly inhibited by atropine. The mechanisms of this result may be mainly mediated via mascarinic cholinergic receptors by action of acetylcholine that consisted in royal jelly. Endothelium-mediated vasodilation was impaired in STZ-diabetic rats. Diminished vasodilation was elicited by royal jelly in diabetic rats was due to a defect in endothelial dysfunction.

Keyword:- royal jelly, diabetic rat, hypotensive effect, endothelium-mediated vasodilation, endothelial dysfunction

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 (Karmer, 1977; O'Conner, 1985; Fujiwara, 1990), anti-inflammatory and wound healing (Fujii, 1990; Pongsakorn, 1992) and vasodilating effect (Henschler, 1956; Townsend, 1959; Bowem, 1977; Kivilaakso, 1978; Shinoda, 1987; Peungvicha, 1992). Nevertheless, the role of royal jelly on blood pressure has not been elucidated in diabetic rats. The objectives of this study were carried out to investigate the actions of royal jelly on hypotensive effect in experimental diabetic animals.

METERIAL AND METHOD

Animals male albino rats weighing 150-180 gm were purchased from the 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. They were randomly divided into two groups as normal and diabetic rats. After a week adaptation period, diabetes was induced by a single tail-vein injection of 65 mg/kg body weight of streptozotocin (STZ) (Sigma Chemical Co.) fresh preparation. Age-match control rats received only an equivalent volume of normal saline. In the next morning 24 hours after injection, the blood sugar analysis of normal and diabetic animals were done by using the Precision Plus Electrodes of MediSense, Inc. The blood glucose less than 100 mg% of normal rats and at least 300 mg% of diabetic rats were considered to use as a criteria in choosing for the experiments. The blood glucose were detected every week in two months as a period of experiment. On

the day of experiment, after an overnight fasted rats were weighed and determined blood glucose before the start of 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.

For blood pressure studies, the rats were anaesthetized with 30 mg/kg BW of sodium pentobabital intraperitoneal injection. 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. Drugs 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 a MacLab. Electrocardiogram (ECG) was recorded by a MacLab. The parameters were examined for these experiments. The hypotensive response was measured at 0.5 min postinjection. Animals were not reused poststudy.

The effect of various doses of royal jelly on blood pressure and heart rate were determined. The normal and diabetic animals were taken slow bolus intravenous infusions of the doses 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg BW of royal jelly and the control group was given an equal volume of 0.9% sodium chloride solution.

The effect of acetylcholine on blood pressure and heart rate were examined by intravenous infusion of the doses 0.1, 0.2, 0.4, 0.6 and 0.8 μg/kg BW of the both groups of animal and the control group was given and equal volume of 0.9% sodium chloride solution.

The experiments studied on the effect of royal jelly on mascarinic receptors. The dose 0.5 mg/kg BW of royal jelly or 0.8 μg /kg BW of acetylcholine intravenous injection was taken before and after 0.3 mg/kg BW of atropine injection in anaesthetized rats.

The effect of royal jelly on chronotropic and intropic response were determined. Rats were killed by blowing on the head. The heart was quickly excised and placed in a

petri-dish contraining oxygenated Locke solution (of composition, in millimolar/liter : NaCl 155.8; $CaCl_2$ 2.15; KCI 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 gm. The rate and contractility were recorded with isometric force transducer connected to a Mac Lab. The atrium was allowed to equilibrate until the rate and amplitude of spontaneous contraction were stable and then the experiments began. The doses 0.05, 0.1 and 0.15 mg of royal jelly were administered to the bath fluid. The responses were recorded within 5 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 gm and allowed to equilibrate until the force of contraction was stable before it was exposed to the drug. The doses 0.1, 0.2 and 0.3 mg of royal jelly were administered to the bath fluid. The responses were recorded within 5 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 effect of royal jelly on vascular activity was determined. Rats were killed by blowing on the head of animal. The thoracic aorta was quickly excised and placed in a petri-dish contraining oxygenated Kreb solution (of composition, in millimolar/liter : NaCl 118; $CaCl_2$ 2.52; KCI 4.7; $NaHCO_3$ 24.88 $MgSO_4$ 1.64; KH_2PO_4 1.18 and glucose 5.55). The intract endothelium of spiral thoracic aorta were separated and transferred into 10 ml organ bath containing Kreb solution continuously oxygenated bubble and maintained at

37°C. Special care was taken to avoid damage to the endothelial layer. In without endothelial experiment, the spiral thoracic aorta was gently rubbed the intimal surface with a cotton wool and the loose endothelium was confirmed by 10^{-4} M of acetylcholine (Furchgott, 1980) . A resting tension of 1 gm was maintained throughout the experiment. The contractility was recorded with isometric force transducer connected to a MacLab. The vessel was allowed to equilibrate until stable about 60 minutes, and then the experiments began. The doses 0.2, 0.4, 0.6, 0.8 and 1.0 mg of royal jelly were administered to the bath in a cumulative manner after induced the vessel contraction by 10^{-4} M of phenylephrine. 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. Relaxant effects of these agents were expressed as percent of the maximal contraction obtained with phenylephrine.

Analysis of data

Experimental data were expressed as mean ± SD. Statistical significance was tested according to Student's t-test for paired variety.

RESULT

Eight weeks after streptozotocin treatment, The blood glucose level at the time before sacrifice was 378.77 ± 60.21 mg % (21.00±3.3 mg/dl) in diabetic rats and 83.72 ± 10.27 mg % (4.64 ± 0.57) in normal rats. The hypotensive effect of royal jelly in diabetic rats was shown in the following circumstances.

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

A slow bolus intravenous infusion of acetylcholine caused significantly ($P<0.01$) and dose dependent reductions in systolic and diastolic blood pressure in both groups of animal (Fig 1 and 2). As shown in Fig 1, at the doses of 0.6 and 0.8 microgram/kg BW

systolic blood pressure was similarly depressed but the systolic blood pressure of normal rats was significantly ($P<0.05$) depressed more than diabetic rats (Fig 1). At the dose higher 0.3 microgram/kg BW most of results showed depression in diastolic blood pressure of normal animals more than diabetic animals (Fig 2). In contrast, an intravenous infusion of acetylcholine elicited no significant changes in the heart rate in the both groups of animal (Fig 3). Similarly, both systolic and diastolic blood pressure were significantly ($P<0.01$) elucidated dose response of hypotensive effect by a bolus intravenous infusion of royal jelly in the both groups of animal (Fig 4 and 5). The doses of 0.1 to 1.0 mg/kg of royal jelly depressed systolic blood pressure of normal rats more than diabetic rats (Fig 4). As same as, the depressed diastolic blood pressure of normal rats was significantly ($P<0.05$ and 0.01) was more than diabetic rats (Fig 5). Additionally, an intravenous infusion of royal jelly elicited no significant changes in the heart rate too (Fig 6).

2. The effects of cholinergic blocking agent (atropine) on the hypotensive effect of royal jelly

As shown in Fig 7 and 8, atropine at a dose of 0.3 mg/kg BW that the dose was almost completely blocked hypotensive activity of acetylcholine (0.8 microgram/kg BW) administered 15 min before royal jelly (0.5 mg/kg BW). The atropine significantly ($P<0.01$) reduced hypotensive effect of both groups of anaesthetized animal

3. The effect of royal jelly on vascular activity

As shown in Fig 9, royal jelly induced a concentration-dependent relaxation of the precontracted aorta from control animals and also diabetic animals. This response was, however, significantly ($P<0.05$) attenuated in the diabetic rat aorta. When aortas were denuded mechanically, the acetylcholine-induced relaxation was abolished in both the control and diabetic aorta preparations (data not shown). They should be noted the vascular relaxation activity of royal jelly significant ($P<0.01$) blocked by atropine in both groups of animal (Fig 10). Similarly, royal jelly caused significant ($P<0.05$) more vascular

relaxation of intact endothelium than absent endothelial preparation in both groups of rats (Fig 11).

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

As shown in Fig 12 and 13 royal jelly caused significant ($P<0.05$) elicited no significant change of the chronotropic and inotropic responses in both groups of animal. They should be noted the chronotropic and inotropic effect of royal jelly was significantly ($P<0.05$ and 0.01) blocked by atropine in their groups.

DISCUSSION

This experiment used streptozotocin to induecd diabetes. This experiment model has been used widely to examine physiological alteration during insulin dependent diabetes mellitus (IDDM). Although control and diabetic rats had similar body weights initially, diabetic animals. As a result, bodyweight at the time of study was approximately one third lower in diabetic rats than the control rats. We assume that differences in vascular responses in two groups of animals in this study were due to the presence of diabetes, but we cannot exclude a nonspecific effect of illness.

The results of the present study demonstrated that intravenous infusion of acetylcholine and royal jelly produced a dose dependent reduction of systemic blood pressure in both groups of anaesthetized rats (Fig 1, 2, 3 and 4). The blood pressure rapidly fell followed the administration and sustained for 0.5 minutes. In addition, The *in vitro* studies showed a reduced in heart rate and force of atrial contraction in response to royal jelly administration (Fig 11 and 12). On the other hand, the *in vivo* experiment was no significant changes of heart rate (Fig 5). No change in heart rate was detected in intact study which maybe the result of some compensatory mechanisms function to maintain blood pressure in normal level. 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 (Fig 8) as previous study in isolated vessels preparation (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 (Fig 6 and 7) and acetylcholine (data not shown) in the both groups of anaesthetized rats. *In vitro* studies, all effects of royal jelly were significantly blocked by the cholinergic blocking agent such as: effect of vasodilatory (Fig 9), negative chronotropic (Fig 11) and negative inotropic (Fig 12) response in normal and SZT-diabetic rats. The results suggested that the hypotensive effect of royal jelly may be mainly mediated via mascarinic cholinergic receptors. The result of hypotension in this study emphatically established by the effect of acetylcholine that consisted in royal jelly (Dayan, 1960).

This study showed that at the time of IDDM, Acetylcholine or royal jelly elicited less reduction of blood pressure in diabetic rats than normal rats (Fig 1, 2, 3 and 4). Interestingly, the vasodilatory response to royal jelly was significantly attenuated in STZ-diabetic rats (Fig 8) Furthermore, the attenuated vasodilatoy response was emphatically found in without endothelial preparation, in particular diabetic rats. (Fig 10). The attenuated endothelium-dependent relaxation which was same previous studies was produced by acetylcholine in aortic rings from diabetic rats (Oyama, 1986). The relaxations induced by acetylcholine in aortic strips were significantly attenuated in genitically diabetic rats, compared with those from age-matched control vessels of normal Wistar rats (Miyata, 1992). Isolated ring segments of diabetic dog femoral artery exhibited a decreased endothelium-dependent relaxation to acetylcholine as compared with controls Sarioglu 1993). Endothelium-mediated vasodilation was impaired in SZT-diabetic rats (Brands, 1996). Diminished vasodilation in diabetic dogs was due to a defect in endothelial nitric oxide production and action. Vasodilating prostanoids do not

sufficiently compensate this defect. (Koltai, 1997). Recently, Miranda et al. demonstrated that acetylcholine induced a concentration-related endothelium-mediated relaxation of carotid artery from control rabbits that was significantly higher with respect to that obtained in diabetic animals (Miranda, 2000).

The vasodilating function of endothelial cells is particular impotence for the physiological function of the vessel wall. Furchgott et.al. (Furchgott, 1980; Furchgott, 1984) had discovered endothelium-derived relaxing factor (EDRF). It had been established that similar to nitrovasodilators–EDRF-mediated vasodilatation was associated with increased levels of cyclic GMP and activatation of cyclic GMP kinase activety in smooth muscle cells (Fiscus, 1983; Rapoport, 1983; Ignarro, 1984), and that EDRF could directly stimulate purified soluble guanylyl cyclase (Forstermann 1986; Ignarro, 1986). In 1987 it was concluded that nitric oxide (NO) can account for the biological activity of EDRF (Ignarro,1987, Palmer, 1987), and analogous to the macrophage system, L-arginine was established as a substrate for EDRF/NO synthesis in endothelial cells (Palmer, 1988; Schmidt, 1988). Nitric oxide synthetase III (NOS III or eNOS), which is one of three isozyme of nitric oxide synthetase (NOS) as being responsible for NO synthesis is mainly expressed in endothelial cells. It is produced by the vascular endothelium under basal conditions and its production is stimulated by a variety of receptor agonists as well as the shear stress produced by the flowing blood. NO released by endothelial cells is a major endogenous vasodilator system counterbalancing the vasoconstriction produced by the sympathetic nervous system and the rennin-angiotensin system. A study in rabbits indicated that the counterregulation against peripheral vasodilatation by NO accounts for 69% of the basal norepinephrine release (Halbrugge, 1991), thus demonstrating the powerful nature of the endothelial NO system.

In addition to NO, endothelial cells also release prostaglandins with vasodilatative action, such as prostacyclin (epoprostenol). Prostacyclin induces vasodilatation and inhibits platelet aggregation (Guerra, 1989). It is possible that the endothelium secretes additional vasodilating substances that not yet been identified (Haller, 1997). Damage or excessively activated endothelial cells can also secrete vasoconstricting factors (Jensen, 1989; Lorenzi, 1991), of which the recently discovered endothelin-1 (ET-1) is the best

known (Yanagisawa, 1988; Panza, 1990). In addition to its strong vasoconstrictive properties, ET-1 potentiates the constrictive effects of other vasoactive agonist (Yang, 1990). Thromboxane is an other vasoconstrictor that can be released by damaged endothelial cells (Strano, 1991).

How endothelial cells damage contributes, under pathological conditions, to vascular disease can be illustrated in diabetes mellitus, where hyperglycemia contributes to the cellular process occurring in the vessel wall. Patients with diabetes mellitus show pronounced changes in endothelial cell function, and these have been demonstrated in both *in vivo* studies and *in vitro* experiments (Yamouchi, 1990; Lorenzi, 1991). Enlarged endothelial cells with dense inclusions on electron microscopic examination, as signs of the degenerative change, are seen in the small vessels of patients with diabetes mellitus (Williams, 1980). Recently, the smaller cardiac myocytes observed in patients with diabetes and streptozotocin rats are related to the decrease in F-actin in myocytes (Kawaguchi, 1999)

Damage to endothelial cells initially affects the vessel wall by secreting vasodilating substances, in particular NO. Several study groups have shown that vasodilatation is disturbed in animal models of diabetes mellitus, probably because of a decrease in the release of vasodilating substance from damaged epithelial cells (Meraji, 1987, Tesfamariam, 1990) Production of the vasodilating prostacyclin by endothelial cells is similarly disturbed. It was shown *in vitro* (Yamouchi, 1990) that high glucose concentrations stimulate the secretion of the vasoconstricting ET-1 by aortic endothelial cells. Increased circulating levels of ET-1 have been observed in patients with diabetes mellitus, indicating that the endothelial cells are damaged. Growth factors such as platelet-derived growth factor (PDGF), which are secreted by stimulated endothelial cells, also have constrictor action (Berk, 1986; Pober, 1990). In patients with diabetes mellitus, the ratio of vasodilatiing to vasoconstricting substances in the vessel wall is thus markedly shafted toward those with a vasoconstricting action (Panza, 1990).

Diabetic mellitus is associated with the development of vascular complication leading to occlusive arterial disease. A hallmark of diabetic vascular disease is endothelial dysfunction. Reduced endothelium-mediated vasodilation has been found in arteries from

diabetic animals and humans (Luscher, 1993). Bucala et al. (Bucala, 1991) demonstrated that advanced glycosylation producted quench nitric oxide activeity in vitro and in vivo. Acceleration of the advanced glycosylation process *in vivo* result in a time-dependent impairment in endothelium-dependent ralaxation. Decreased NO activity can be caused by impaired endothelial production of NO, due to uncoupling of the NOS substrate L-arginine, or a decreased availability of one or more cofactors essential for NOS function. Uncoupling of receptor-mediated signal transduction is suggested by experiments demonstrating that endothelium-dependent (NO-mediated) vasodilation to receptor agonist such as acetylcholine was impaired in diabetic arteries, where responses to the receptor-independent calcium ionophore A23187 remained largely unimpaired (Pieper, 1995). Plasma concentrations of basic amino acids (e.g. L-arginine, L-lysine and L-histadine) were reduced in diabetes, and arterial preparations from diabetic rats showed impaired endothelium-mediated vasodilation. Pretreatment with L-arginine potentiated the relaxation to acetylcholine in diabetic rings. Thus, decreased L-arginine concentrations and/or a defect in the utilization of L-arginine by NOS III may contribute to the endothelial dysfunction seen in diabetes (Pieper, 1995 ; Li, 2000).

Other studies have suggested that (6R)-5,6,7,8-tetrahydrobiopterin (BH_4) availability may also play a role in the regulation of NO production by diabetic endothelium, because 6-methyl-5,6,7,8-tetrahydrobiopterin improved the impaired endothelium-dependent vasodilation in arteries from diabetic animals (Peiper, 1997). In addition, hyperglycemia also stimulated the production of advanced glycosylated end-products (Brownlee, 1988), enhanced the polyol pathway (Greene, 1987), and activated protein kinase C (King, 1996). These conditions may lead to increased oxidative stress. Reactive oxygen species rapidly inactivate NO, leading to the formation of $ONOO^-$.$ONOO^-$ is a toxic oxidant capable of damaging many biological molecules. Endothelial dysfunction in forearm resistance vessels of patients with diabetes mellitus could be improved by administration of the antioxidant vitamin C, supporting the hypothesis that NO inactivation by oxygen-derived free radicals also contributes to abnormal vascular reactivity in diabetes (Williams, 1996).

More recent studies strongly point to a decisive role of protein kinase C pathway for vascular complications in diabetic mellitus (Ishii, 1998). Incubation of vascular tissue with high concentrations of glucose has been shown to cause a strong increase in intracellular diacylglycerol levels, which ultimately lead to protein kinase C activation. Endothelial dysfunction caused by incubation of vascular tissue with high concentrations of glucose could be corrected with protein kinase C inhibitor (Tesfamariam, 1991). These *in vitro* observations were supported by *in vivo* studies demonstrating that therapy with protein kinase C inhibitors ameliorated vascular complications in diabetic rats (Ishii, 1996). The mechanisms underlying protein kinase C-mediated endothelial dysfunction remain poorly understood. *In vitro* experiments have shown that protein kinase C-dependent phosphorylation of the NOS III gene can reduce NOS III activity (Hirata, 1995). Incubation of cultured endothelial cells with high concentrations of glucose increased the expression of the NOS III gene, but also increased O_2^- production, leading to more rapid inactivation of NO (Cosentino, 1997). Thus, the endothelial dysfunction seen in diabetic blood vessels seems to be a multifactorial event involveing both reduced NOS III activity and accelerated inactivation of NO. Reduced NO availability may not only be of relevance to the development of vascular complications in diabetes, but may also interfere with insulin-mediated post-prandial glucose disposal and possibly contribute to the development of insulin-resistance (Honing, 1998).

In conclusion, the present investigation demonstrated that royal jelly caused a dose dependent and reversible reduction in blood pressure in normal and diabetic anaesthetized rats. This effect was mainly mediated through mascarinic cholinergic rather than nicotinic cholinergic receptors. The cause of hypotensive effect should be action of acetylcholine in royal jelly. Especially, royal jelly maybe directly stimulate vascular endothelial cells. The stimulated endothelial cells secrete nitric oxide (NO), which relaxes smooth muscle cells of blood vessel as acetylcholine activity. Diminished vasodilation was produced by royal jelly in diabetic rats was due to a defect in endothelial dysfunction. It is probably because of a decrease in the release of vasodilating substance from damaged epithelial cells.

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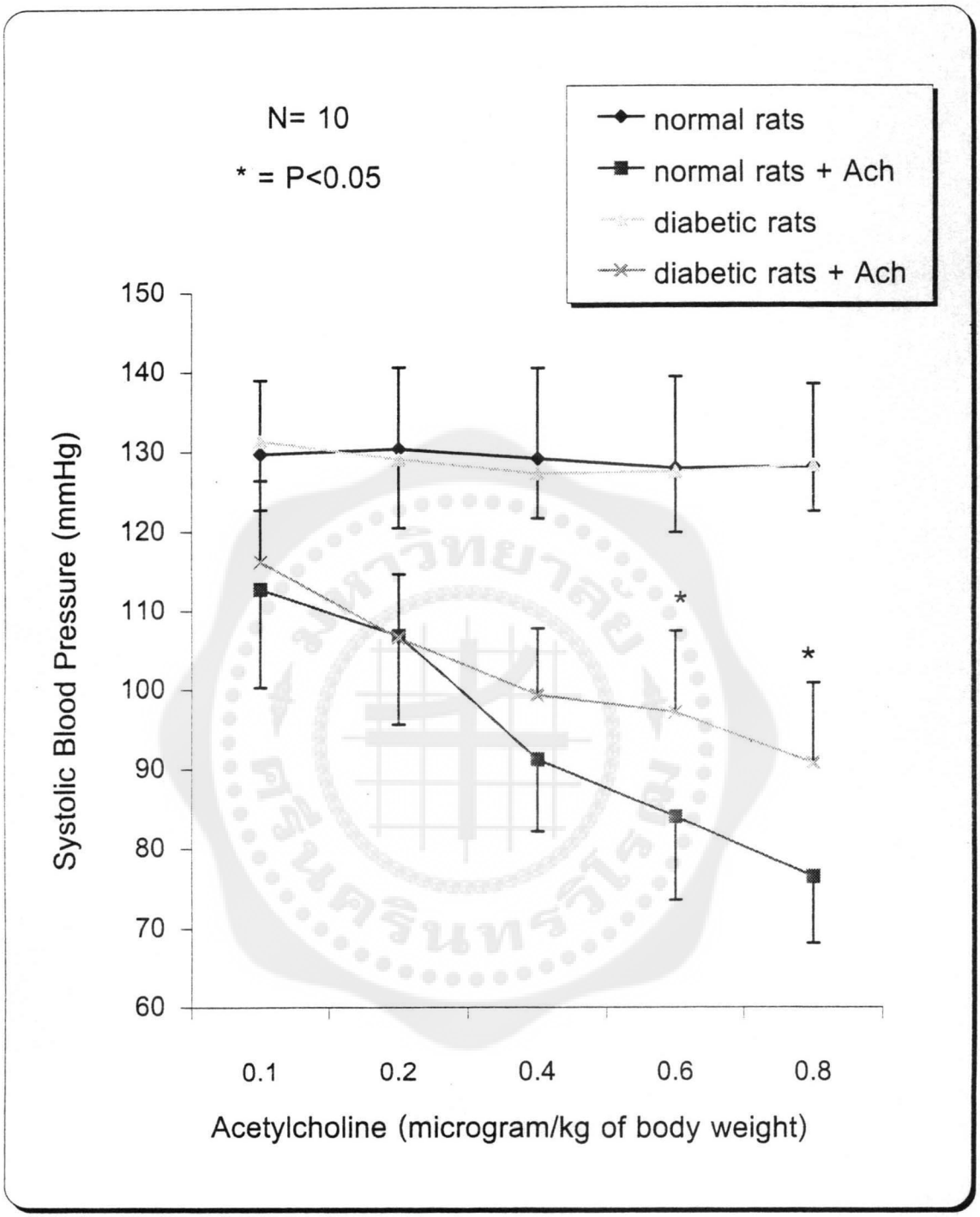

Figure 1 The effect of various doses of acetylcholine (Ach) intravenous injection on systolic blood pressure in anaesthetized diabetic rats (n=10) at 30 sec after injection. Symbol with line graphs represent mean $\pm$ SD. * Significant compared with the group of acetylcholine administration in normal rats $P<0.05$.

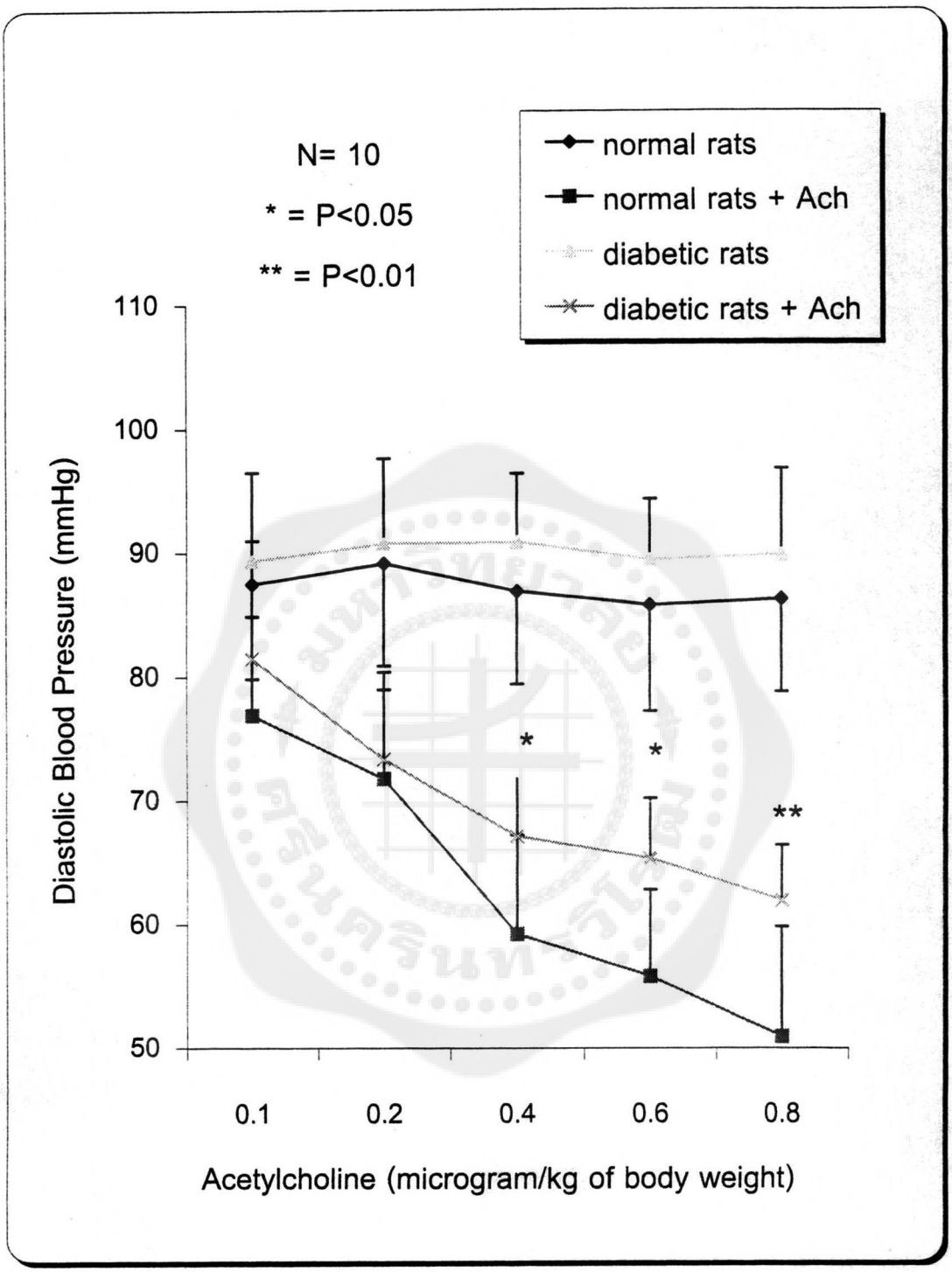

Figure 2 The effect of various doses of acetylcholine (Ach) intravenous injection on diastolic blood pressure in anaesthetized diabetic rats (n=10) at 30 sec after injection. Symbol with line graphs represent mean ± SD. * and ** Significant compared with the group of acetylcholine administration in normal rats $P<0.05$ and $P<0.01$, respectively.

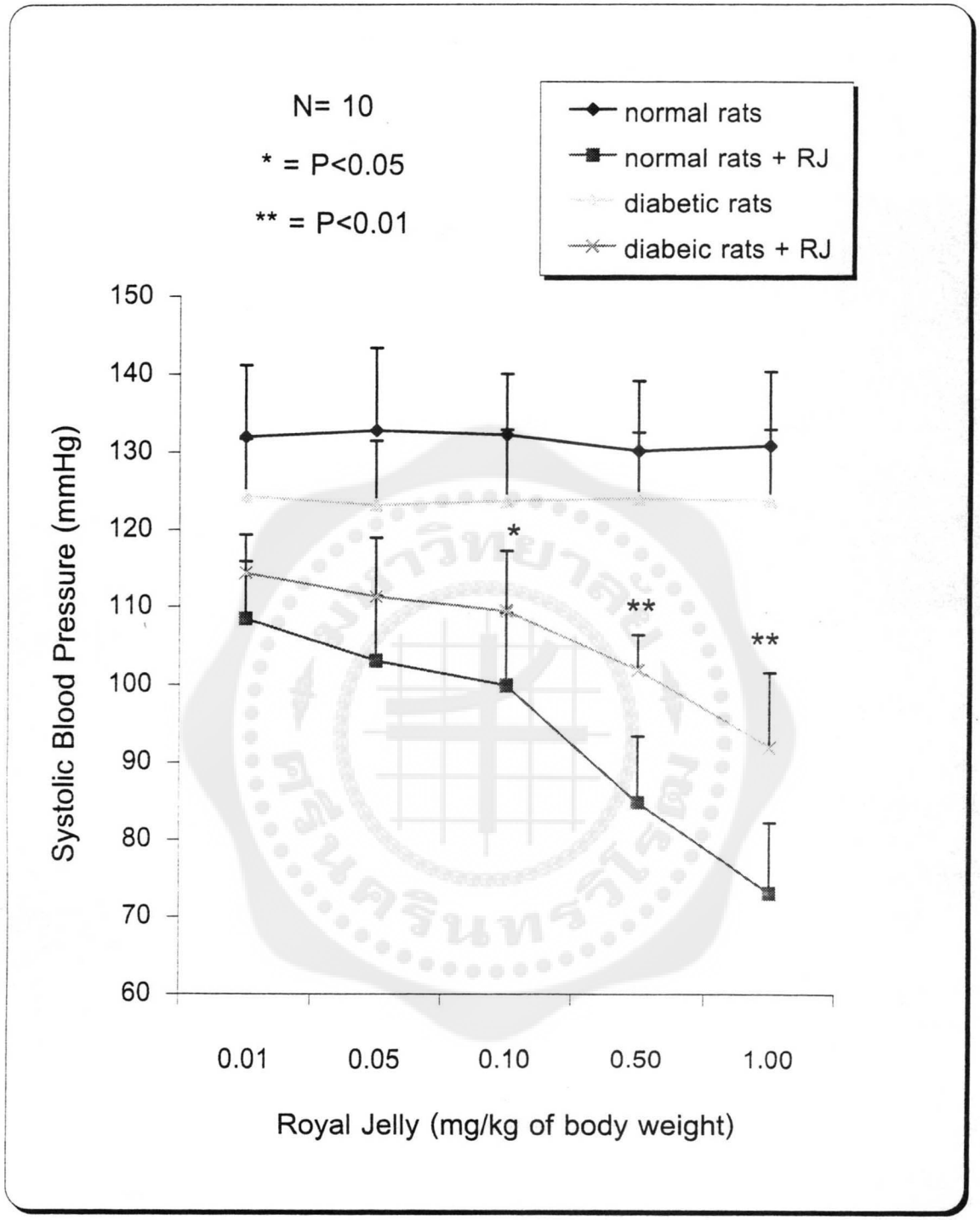

Figure 3 The effect of various doses of royal jelly (RJ) intravenous injection on systolic blood pressure in anaesthetized diabetic rats (n=10) at 30 sec after injection. Symbol with line graphs represent mean ± SD. * and ** Significant compared with the group of acetylcholine administration in normal rats P<0.05 and P<0.01, respectively.

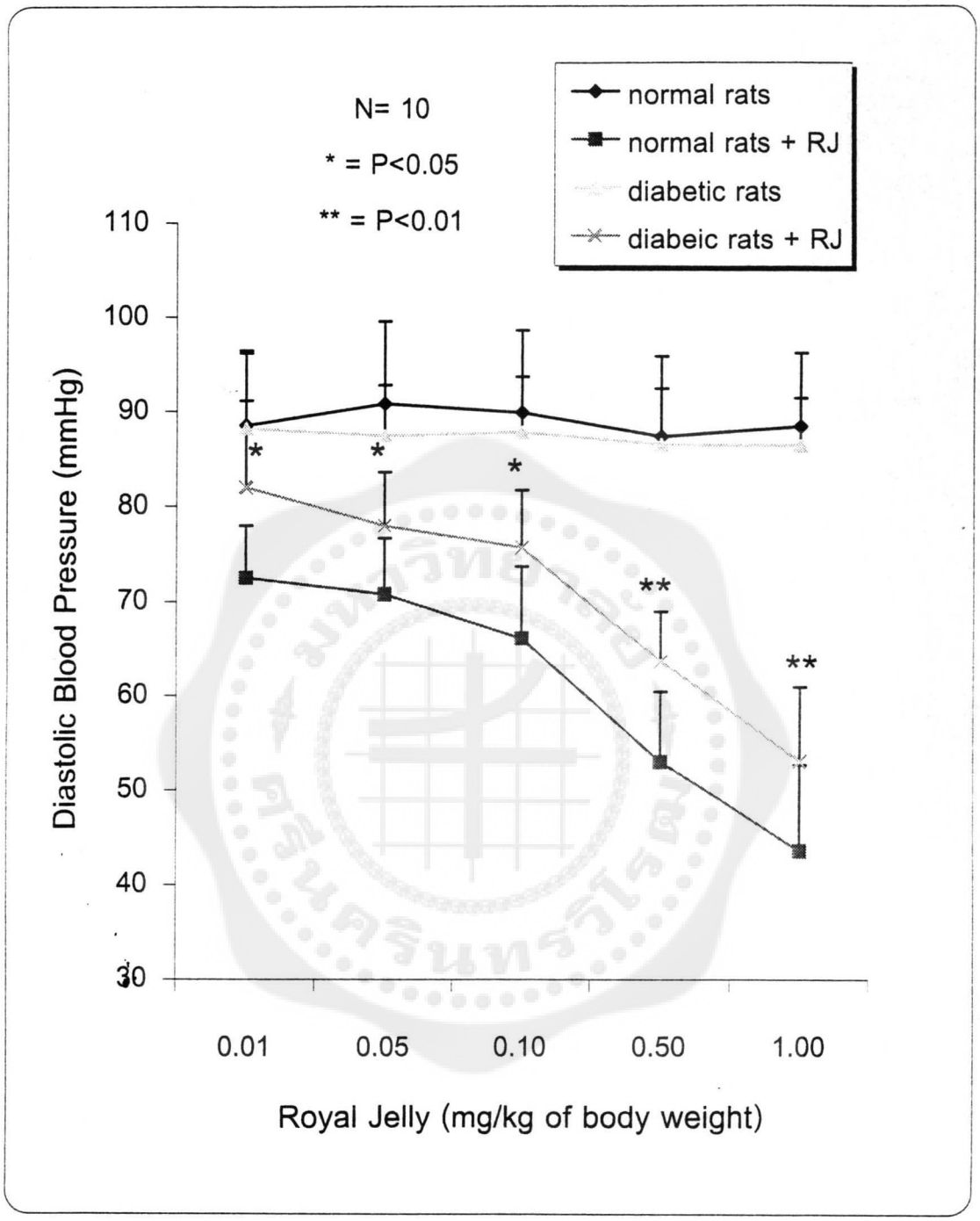

Figure 4 The effect of various doses of royal jelly (RJ) intravenous injection on diastolic blood pressure in anaesthetized diabetic rats (n=10) at 30 sec after injection. Symbol with line graphs represent mean ± SD. * and ** Significant compared with normal rats that administrated acetylcholine $P<0.05$ and $P<0.01$, respectively.

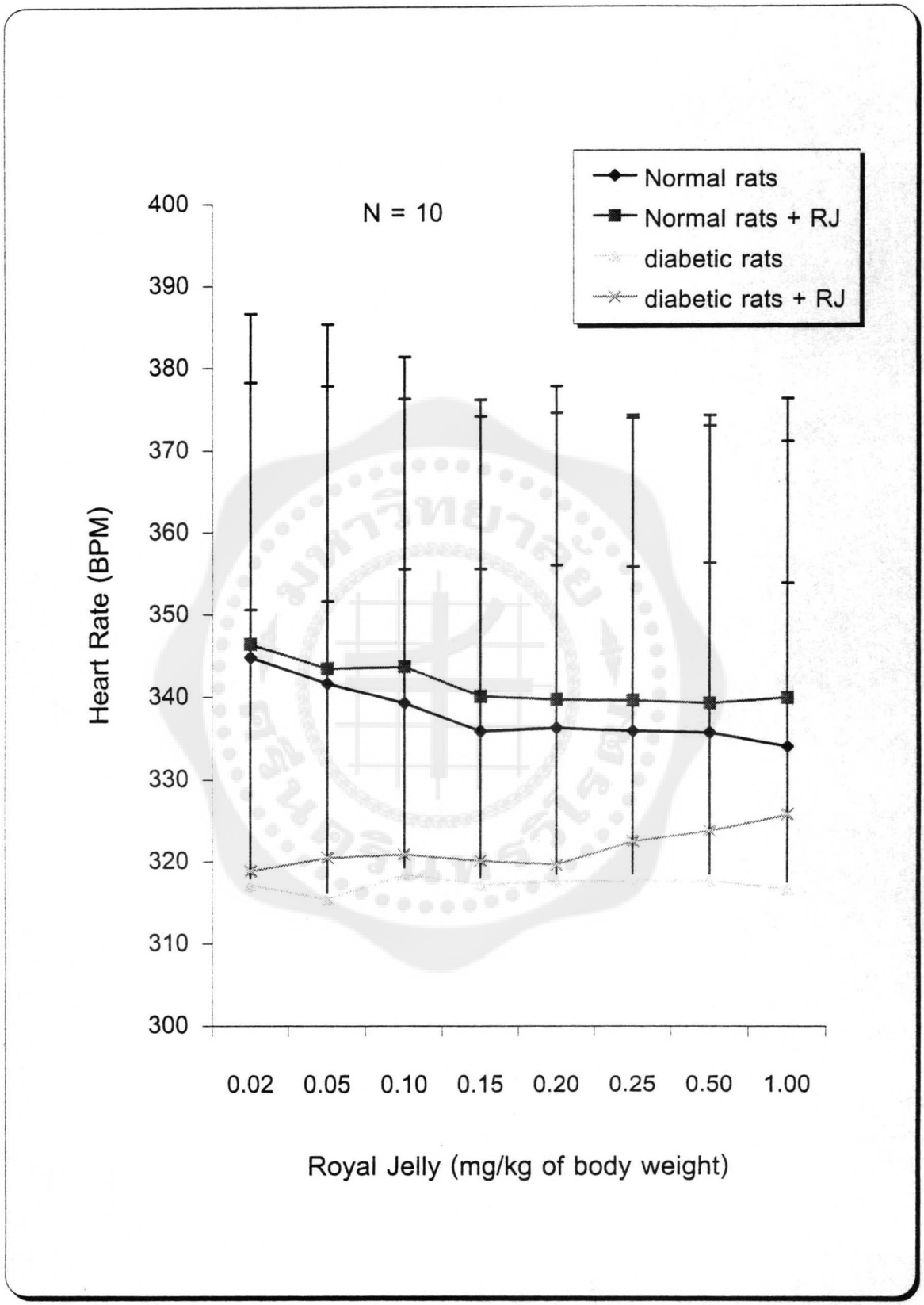

Figure 5 The effect of various doses of royal jelly (RJ) intravenous injection on heart rate in anaesthetized diabetic rats (n=10) at 30 sec after injection. Symbol with line graphs represent mean ± SD.

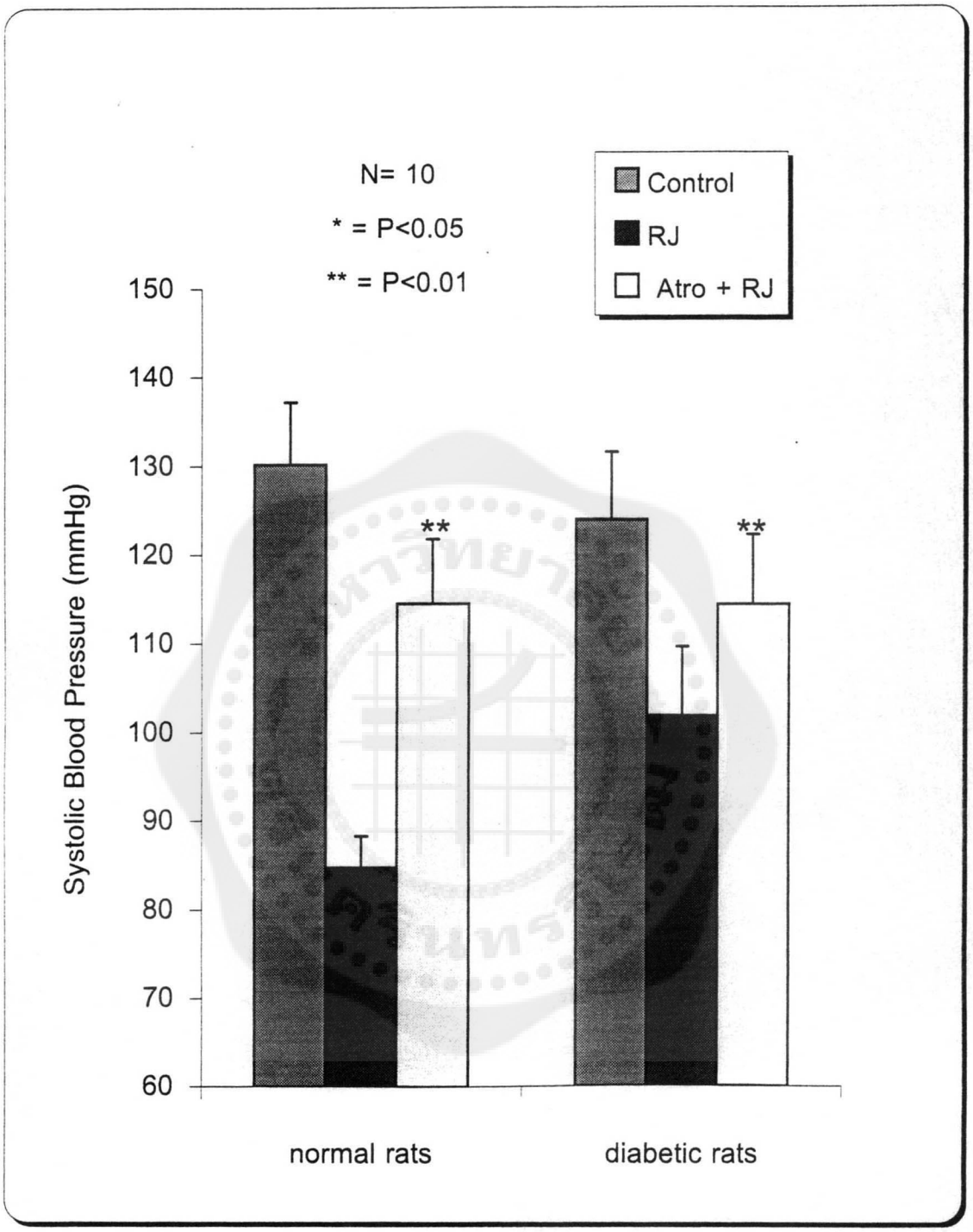

Figure 6 The effect of royal jelly (RJ) 0.5 mg/kg BW intravenous injection on systolic blood pressure before and after atropine (Atro) 0.3 mg/kg BW injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) for 10 animals. ** Significant compared with the group of royal jelly administration. P<0.01

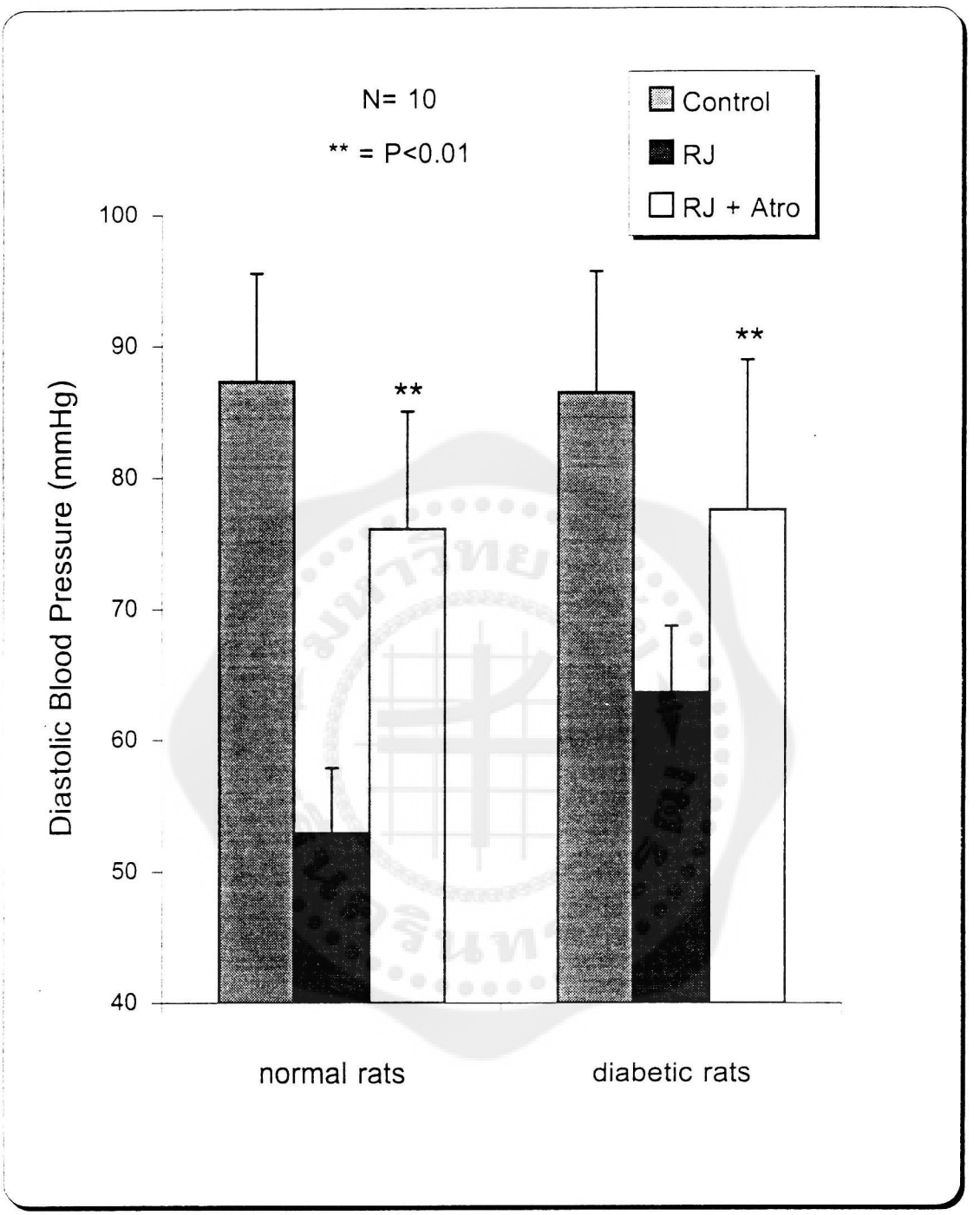

Figure 7 The effect of royal jelly (RJ) 0.5 mg/kg BW intravenous injection on diastolic blood pressure before and after atropine (Atro) 0.3 mg/kg BW injection in anaesthetized rats. Values represent mean ± SD of blood pressure (mmHg) for 10 animals. ** Significant compared with the group of royal jelly administration. $P<0.01$

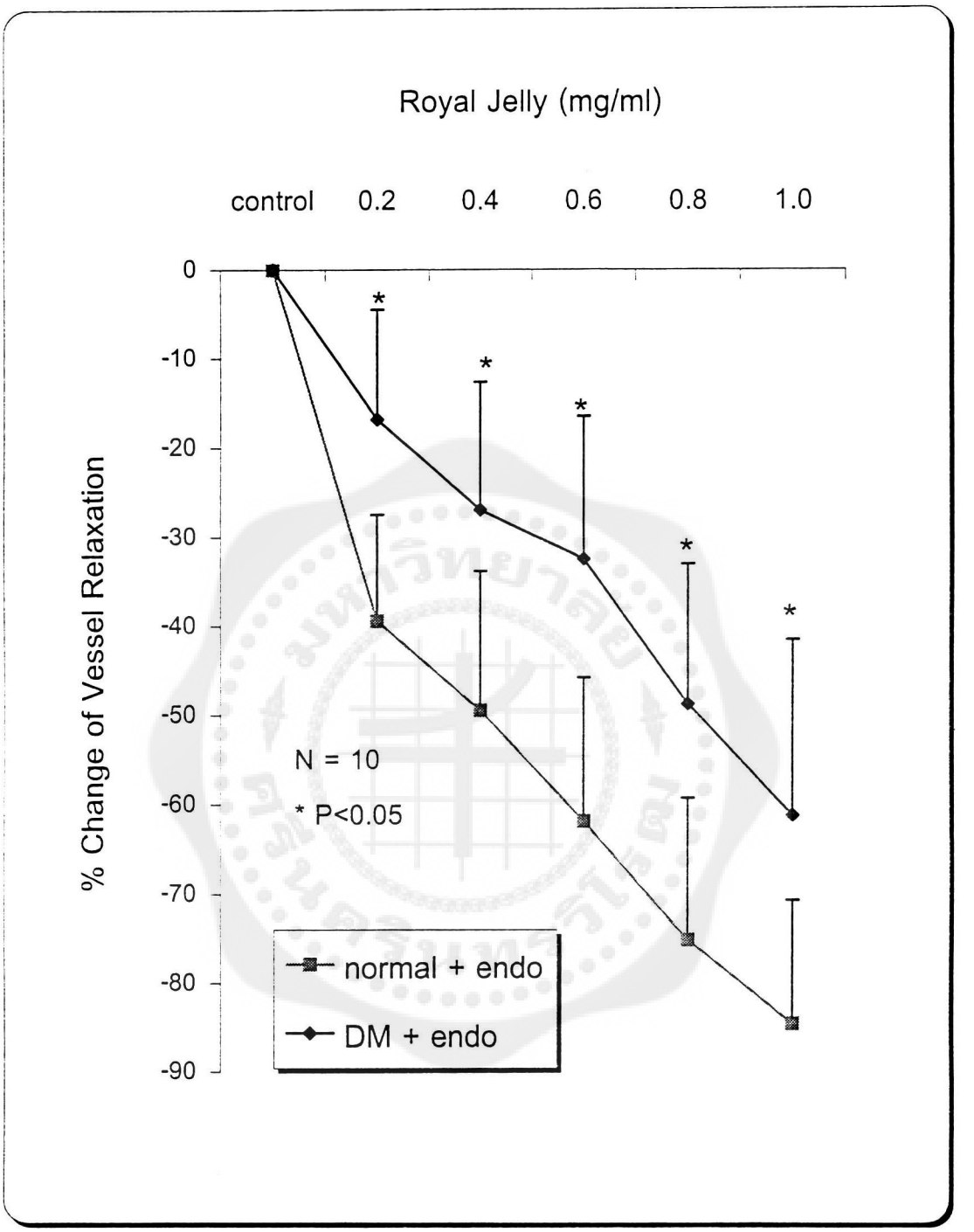

Figure 8 The accumulative dose response curve of royal jelly administration on isolated thoracic aorta relaxation of diabetic rats (DM). Symbol with line graphs represent mean $\pm$ SD.

* Significant compared with the royal jelly administration in each group $P<0.05$.

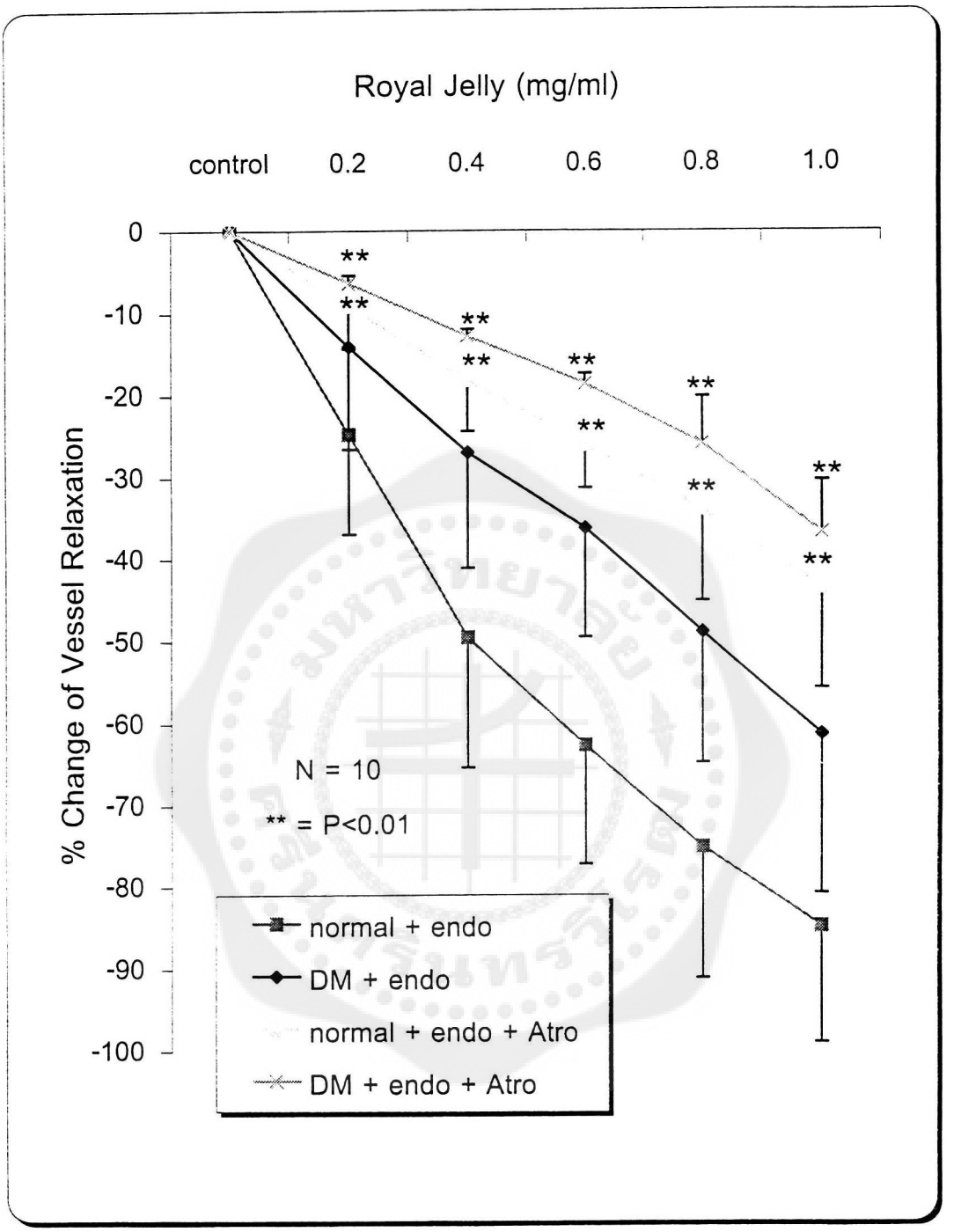

Figure 9 The accumulative dose response curve of royal jelly (RJ) before and after atropine (atro) 10(-6) mol administration on isolated thoracic aorta relaxation of diabetic rats (DM). Symbol with line graphs represent mean ± SD. ** Significant compared with the royal jelly administration in each group P<0.01.

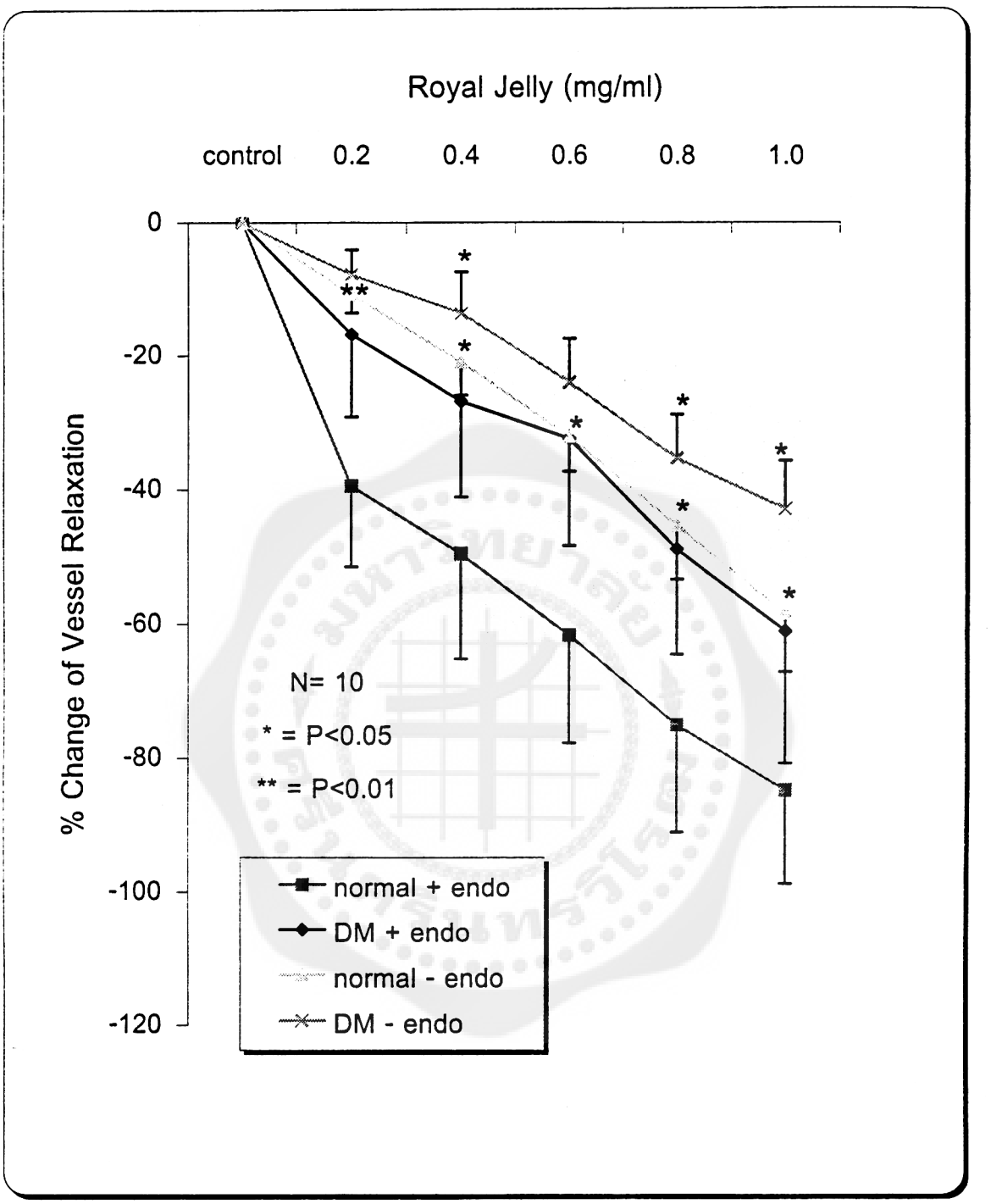

Figure 10 The accumulative dose response curve of royal jelly on isolated thoracic aorta relaxation of diabetic rats (DM) . Symbol with line graphs represent mean ± SD. * and ** Significant compared with the royal jelly administration in intract endothelium of each group P<0.05 and P<0.01, respectively.

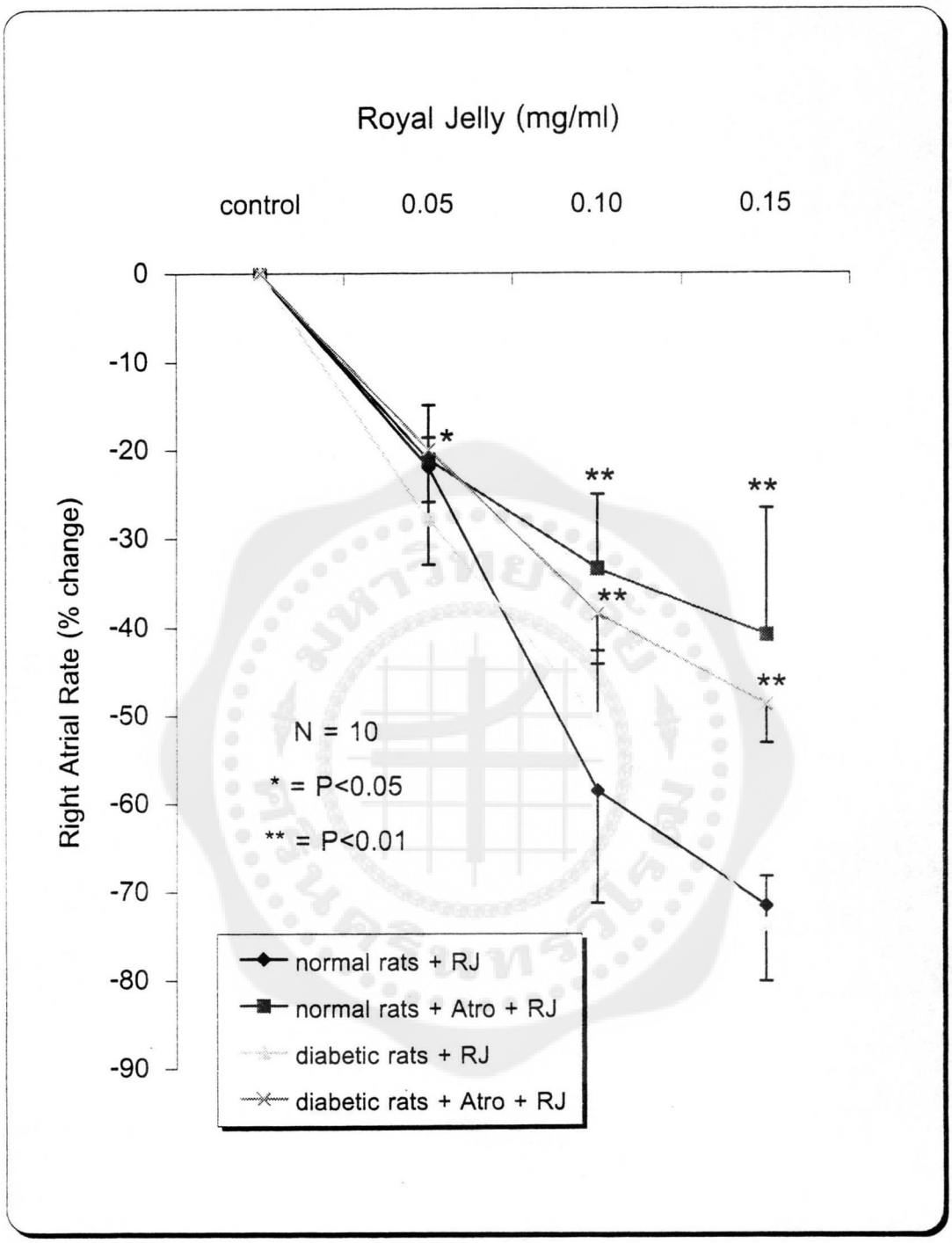

Figure 11 The accumulative dose response curve of royal jelly (RJ) before and after atropine (atro) 10(-6) mol administration on isolated right atrial rate of diabetic rats. Symbol with line graphs represent mean ± SD. * and ** Significant compared with the royal jelly administration in each group P<0.05 and P<0.01, respectively.

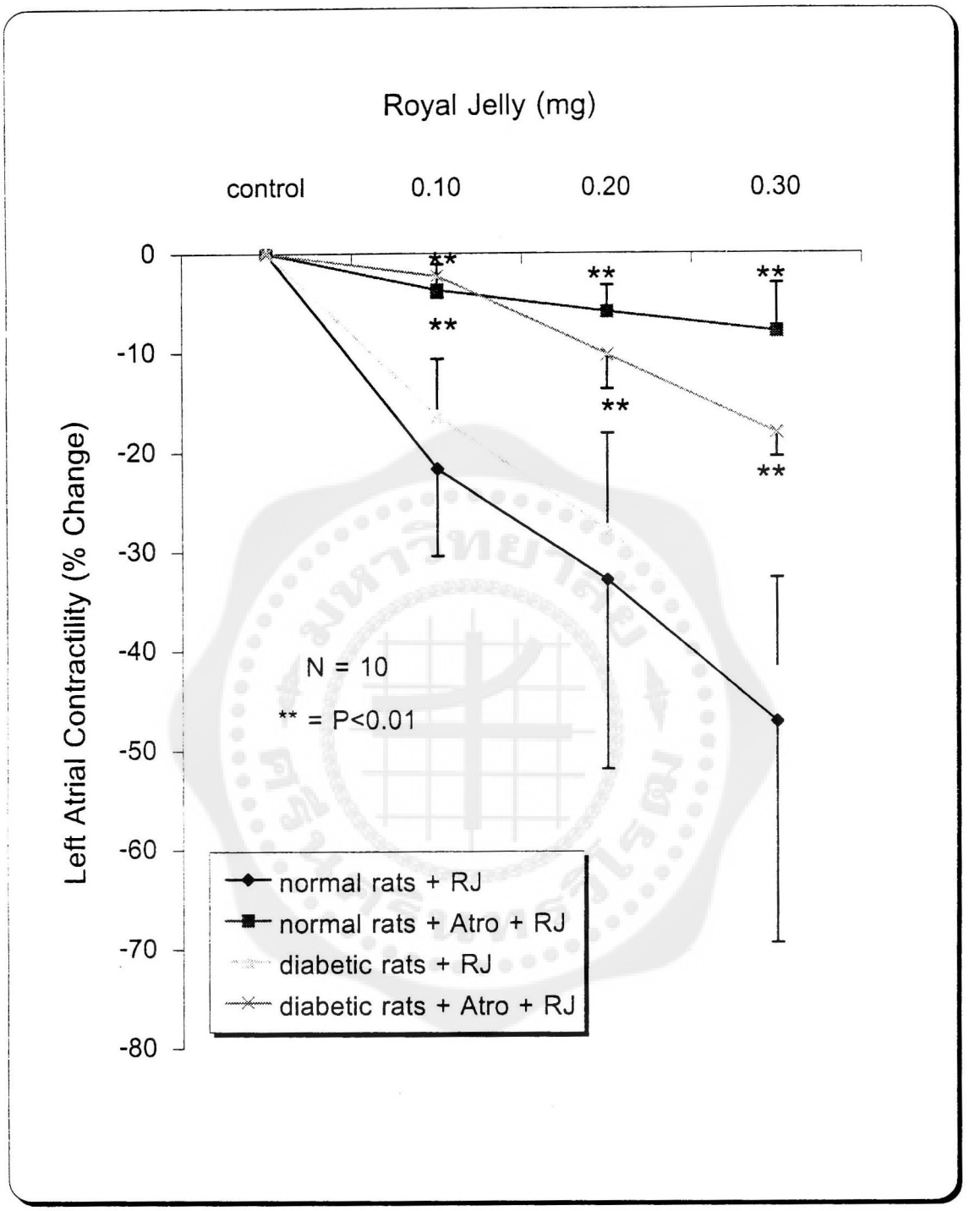

Figure 12 The accumulative dose response curve of royal jelly (RJ) before and after atropine (atro) 10(-6) mol administration on isolated left atrial contractility of diabetic rats. Symbol with line graphs represent mean ± SD. * and ** Significant compared with the royal jelly administration in each group P<0.01.