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Original Article

Cardiovascular Effects of *Tabernaemontana divaricata* Root Extract in Anesthetized Rats

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ABSTRACT **The present study examined the effect of the crude extract of the root of *T. divaricata* on cardiovascular activity in anesthetized rats and its possible mechanism(s). Male Wistar rats (200-250 g) were used. The effects of intravenous various doses of *T. divaricata* extract (5, 10, 20 and 25 mg/kg BW) and vehicle on blood pressure and heart rate were investigated. The possible mechanism of the hypotensive action of *T. divaricata* root extract was studied by injecting the extract immediately after treatment with phenylephrine (10 μg/ml), norepinephrine (5 μg/ml), and atropine (2 mg/kg), respectively. The role of nitric oxide, an important mediator of blood pressure homeostasis was also tested by L-NAME (50 mg/kg), a nitric oxide inhibitor. The results showed that the extract decreased systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure in dose dependent manner. Also, higher doses of the extract (20 and 25 mg/kg BW) caused significant reduction in the heart rate while lower doses of the extract (5 and 10 mg/kg BW) did not cause any significant changes. Pretreatment with atropine and L-NAME, but not phenylephrine or norepinephrine significantly blocked the hypotensive effect of the extract. These results demonstrated the hypotensive action of *T. divaricata* root extract through muscarinic cholinergic receptors, in addition to the induction of nitric oxide.**

Key words: ***Tabernaemontana divaricata*** **Root Extract, arterial blood pressure, heart rate, hypotensive mechanisms**

INTRODUCTION

The pinwheel jasmine *(Tabernaemontana divaricata*) is native grown in India and Southeast Asia. It is used as traditional medicine in many countries. In Thailand, the root of this plant is used as a neurotonic and analgesic[1]. Plants of the genus *Tabernaemontana*, family Apocynaceae, have a widespread distribution and are known to provide indole alkaloids of intriguing molecular structure[2]. The plants have been reported to have various biological actions such as analgestic effect[1,3], cell proliferation inhibition[4], anti-inflammatory[3], and cytotoxic activity[5]. Recent pharmacological studies have shown that *Tabernaemontana divaricata* (*T. divaricata*) root extract inhibited cortical acethylcholinesterase (AChE) activities and enhanced neuronal activity in the cerebral cortex and also in circulatory system[6]. In addition, many of alkaloids from *Tabernaemontana* have been shown to act on the cardiovascular system[7,8]. However, the effect of the crude extract from the root of *T. divaricata* on cardiovascular activity has not been reported. Therefore, this study examined the cardiovas-

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cular effects of the crude extract from the root of *T. divaricata* on cardiovascular activity in rats in vivo and to assess the possible mechanism(s) of the activity.

MATERIALS AND METHODS

Plant material

T. divaricata was collected from Phitsanulok, Thailand. The voucher specimen (collection no. Changwijit 001) was deposited at a PBM herbarium, Faculty of Pharmaceutical Sciences, Mahidol University, Thailand.

Preparation of plant extraction

Roots of *T. divaricata* (16 kg) were separated from the whole plants and dried under 60 ˚C. The dried materials were ground and macerated with 95% ethanol for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. The ethanolic extract (461 g) was obtained. The HPLC and TLC fingerprints of the chemical constituent of the extracts were recorded.

Animal preparation

Male Wistar rats weighing about 200-250g were used in this study. The animal experiment was conducted according to the guideline of experimental animals by The National Research Council of Thailand (1999). After anesthetized with thiopental (50 mg/kg) intraperitoneally (i.p.) each rat was placed in a supine position. The trachea was cannulated with a polyethylene tube (PE240) to facilitate spontaneous respiration. The femoral vein and artery were clearly defined and cannulated with a polyethylene tube (PE10 and PE50, respectively). The femoral artery was cannulated for the measurements of arterial blood pressure and heart rate via a pressure transducer (Model 1050BP) connected to a PowerLab system (PowerLab/4SP, Model ML750, A.D. Instruments, Australia). The femoral vein was cannulated for experimental drug administration.

Measurement of blood pressure and heart rate

An interval of 15-20 min was allowed for the blood pressure to stabilize before the experiment. The baseline blood pressure parameters were recorded before the extracts were administered. Then systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were measured at 1, 2, 3, 5, 10, 20 and 30 min after the extract injection. HR was continuously calculated from the pressure signal for 15 are and then converted to beats/min by multiplying with four. MAP is a sum of diastolic blood pressure and 1/3 of pulse pressure.

Effects of *T. divaricata* root extract on SBP, DBP, MAP and HR

Various concentrations of vehicles (4% PVP K90 and 1% Tween 80) and *T. divaricata* extract (5, 10, 20 and 25 mg/kg BW) were slowly injected via a cannula inserted into the femoral vein. The maximal volume of injection was 0.5 ml. SBP, DBP, MAP and HR were measured at 1, 2, 3, 5, 10, 20 and 30 min. after the injection. Control blood pressure was recorded before the injection of each dose. Changes in blood pressure were recognized as the difference between the steady state values before and the lowest readings.

Pharmacological studies

The autonomic ganglion transmission, α- and β-adrenergic, muscarinic cholinergic activities on the change of MAP were examined by using their specific antagonists and agonists. Norepinephrine (5 μg/ml, Sigma Co.) and phenylephrine (10 μg/ml, Sigma Co.) acted as α- and β-adrenoceptor agonists, respectively. The cholinergic system was blocked with 2 mg/kg of intravenous injection of atropine (2 mg/kg, Sigma Co.). The role of nitric oxide, an important mediator of blood pressure homeostasis, was also examined with the NO inhibitor L-arginine methyl ester (50 mg/kg L-NAME, Sigma Co.). The possible mechanism interaction between the hypotensive actions of T. divaricata root extract was studied by injecting the extract immediately after treatment with norepinephrine, phenylephrine, atropine, and L-NAME. The changes in MAP induced by drugs and the extract were compared with those before the infusion.

Statistical analysis

Results were shown as means(SEM. One-way ANOVA was used to evaluate the difference of means. Student paired t-test was used to calculate the level of significance different between before and after treatment for comparison made within a group. The statistical differences were considered at the probability level (p-value) of lower than 0.05.

RESULTS

Effects of *T. divaricata* extract on SBP, DBP, MAP and HR

In the control group, an administration of vehicle did not cause any significant changes of SBP, DBP and HR. In anesthetized rats, intravenous administration of T. divaricata extract produced a significant fall in SBP, DBP, and MAP in a dose-dependent manner (Table 1). The significantly decreases of heart rates were also observed after the injection of *T. divaricata* extract at the dose of 20 and 25 mg/kg (Table 1). Injection of the extract resulted in a fall in SBP and DBP reaching its maximal level at 2-3 minutes after the injection, returning to their original level within 30 min (Fig. 1).

The percent of maximal decrease from initial value in SBP, DBP, MAP and HR in each dose after the injection of *T. divaricata* extract was demonstrated in Fig. 2. There was a significant difference in percent of maximal decrease in SBP after the injection of the extract at the dose of 10 (20.65%), 20 (21.63%) and 25 (29.22%) mg/kg as compared to low dose treatment (6.58%). The significant difference in the percent of maximal decrease in DBP and MAP were also observed similar pattern of rise at increasing doses of the extract that were 15.36 and 11.90%, 23.24 and 22.22%, 24.11 and 23.13%, and 26.17 and 27.38%, at the dose of 5, 10, 20, 25 mg/kg, respectively. The percent of maximal decrease in HR produced a significantly increase when increasing doses of the extract at the doses of 20 (13.64%) and 25 (13.25%)

Table 1 Effects of *T. divaricata* extract on SBP, DBP, MAP and HR

Parameter	Control	*T. divaricata* extract			
		5 mg/kg	10 mg/kg	20 mg/kg	25 mg/kg
SBP (mmHg)	122.96 ± 2.54	114.86 ± 2.97	97.57 ± 4.48*,+	96.36 ± 3.94*,+	87.03 ± 4.20*,+
DBP (mmHg)	94.27 ± 4.20	79.79 ± 2.67	72.36 ± 5.07@	71.54 ± 4.78@	69.60 ± 5.68@
MAP (mmHg)	103.83 ± 3.53	91.48 ± 2.53	80.76 ± 4.65*	79.81 ± 4.33*	75.41 ± 5.00*,+
HR (beats/min)	385.60 ±17.14	355.86 ± 2.52	347.25 ±20.53	333.00 ± 6.7✦	334.50 ± 3.78✦

Values are means ± S.E.M of eight observations. * p<0.001 when compared to control. @p<0.01 when compared to control. ✦p<0.05 when compared to control. +p<0.01 when compared to 5 mg/kg of *T. divaricata* extract group.

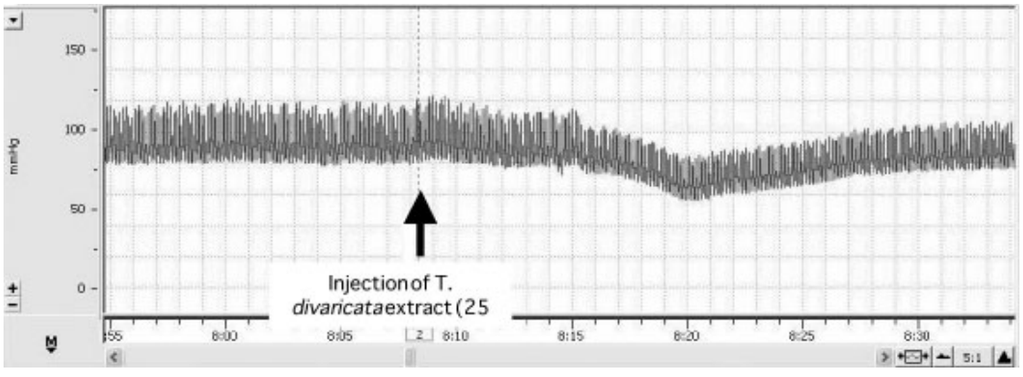

Figure 1 A typical tracing of the hypotensive effect of the root extract of T. divaricata on blood pressure at a dose of 25 mg/kg BW of the extract in anesthetic rat.

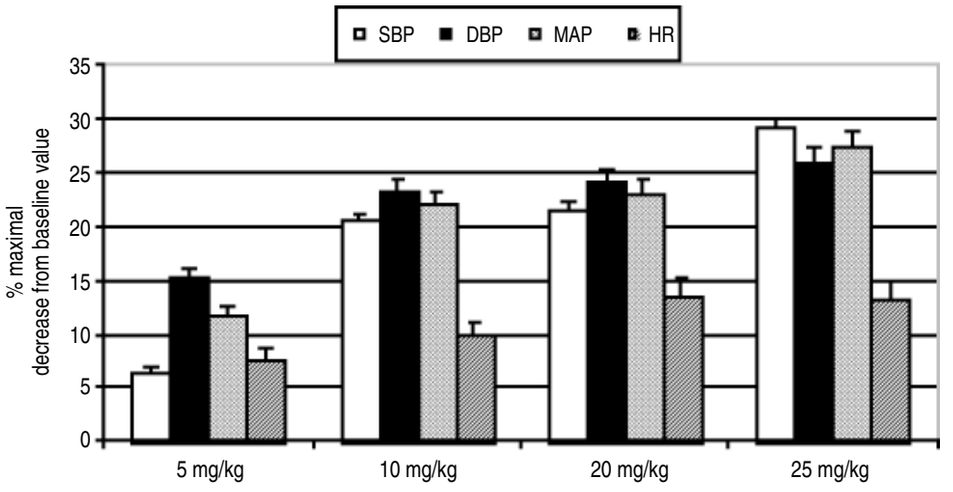

Figure 2 Effects of the root extract of *T. divaricata* on maximal decrease in SBP, DBP, MAP, and HR. Values are means ± S.E.M of eight observations. *p<0.01 when compared to 5 mg/kg of *T. divaricata* extract group. $^{+}$p<0.01 when compared to 10 mg/kg of *T. divaricata* extract group. $^{@}$p<0.05 when compared to 20 mg/kg of *T. divaricata* extract group.

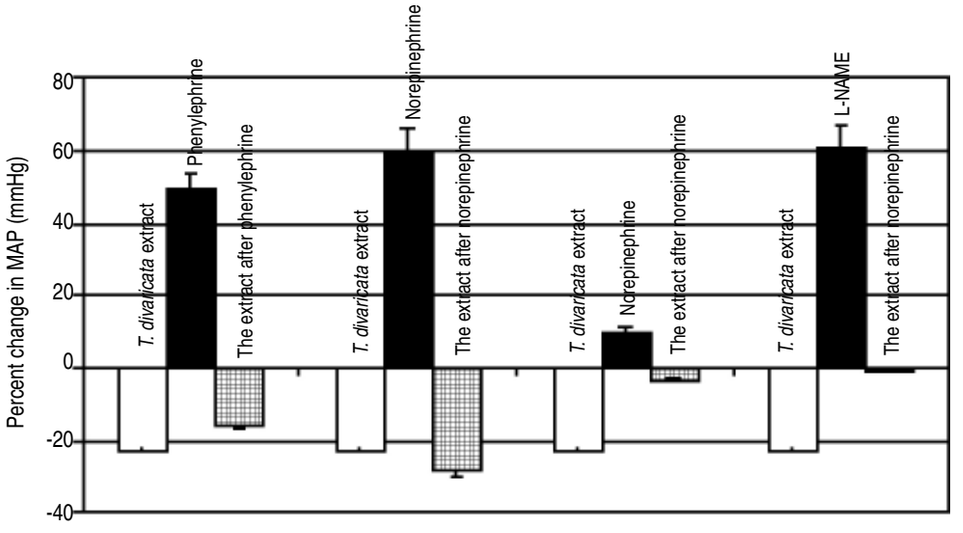

Figure 3 Effects of the autonomic ganglion transmission, α- and β-adrenergic, muscarinic cholinergic activities, and NO inhibitor on the hypotensive action of the root extract of *T. divaricata* in anesthetized rats. Values are means±S.E.M. of five observations. *p<0.001 when compared to those before the drug infusion.

mg/kg (Fig. 2.).

Pharmacological studies

The hypotensive effect of *T. divaricata* root extract was significantly attenuated by atropine and L-NAME at -2.77 and -0.33% in percent change in MAP, respectively. The hypotensive effect of *T. divaricata* root extract was not affected by phenylephrine or norepinephrine which are α- and β-adrenoceptor respectively.

DISCUSSION

The present study demonstrated that intravenous injection of the crude fraction from the root extract of *T. divaricata* produced a dose dependent fall in SBP, DBP,

and MAP. Also, higher doses of the extract (20 and 25 mg/kg BW) caused significant reductions in the heart rate while lower doses of the extract (5 and 10 mg/kg BW) did not cause any significant changes. These findings suggest that the extract produced hypotensive and bradycardiac activities in anesthetized rats. The results are similar to that obtained for the crude alkaloidal fraction from the stem of *Tabernaemontana pandacaqui* which exerted hypotensive and bradycardiac response in anesthetized rats[8]. This suggests that the alkaloid components of *T. divaricata* may play a role in the hypotensive and bradycardiac activities.

The hypotension produced by the extract was blocked by atropine and L-NAME but not by α- and β-adrenoceptor. This indicates that adrenergic mechanisms are not likely to be involved in the hypotension produced by the extract. The attenuation of the response to the extract by atropine suggests that the extract may have a cholinergic agonist action.

Previous study showed that *T. divaricata* root extract inhibited AChE activity in vitro[9,10]. AChE is an esterase critical in the metabolism of acetylcholine (ACh) at central and peripheral synapses. If AChE activity is inhibited, Ach levels should be increased. Recently, Chattipakorn et al. (2007)[6] demonstrated that *T. divaricata* root extract increased neuronal activity in an animal model. They showed that acute intraperitoneal administration of *T. divaricata* root extract with 250, 500 and 1,000 mg/kg inhibited cortical AChE activity and enhanced neuronal activity in the cerebral cortex. This study also demonstrated that *T. divaricata* root extract could inhibit circulating AChE activity within 10, 30, and 60 min after administration. Therefore, the decrease circulating of AChE activity may cause an increase of ACh level in the blood resulting affects on several organs including cardiovascular activity. ACh induced bradycardia and hypotension on cardiac and vascular smooth muscle during parasympathetic stimulation[11]. Hypotensive effect of ACh results from its inhibitory activity on cardiac contraction (negative ionotropic effect), rate of contraction (negative chronotropic effect) and vasorelaxant effects through the release of nitric oxide from the endothelium intact vascular smooth muscle, thus reducing cardiac output and total peripheral resistance which ultimately results in fall in arterial blood pressure[12].

The extract-induced decrease in MAP was almost completely abolished by L-NAME (0.03%). This attenuation was higher magnitude compared to that observed with atropine (2.77%). These findings suggested the involvement of mechanism(s) other than or in addition to activation of muscarinic cholinergic receptors in the extract-induced synthesis/release of nitric oxide (NO). Ach stimulates the muscarinic receptor, which increase intracellular Ca^{2+} in endothelial cells leading to the synthesis/release of NO. NO diffuses into adjacent smooth muscle cells and activates cGMP production to cause relaxation of vascular smooth muscle cells[13,14]. Further study is, however needed to be investigated.

In conclusion, the *T. divaricata* root extract produced hypotensive and bradycardiac activities in anesthetized rats. The hypotensive effect of the extract seems to be mediated through activation of muscarinic cholinergic, in addition to the extract-induced synthesis/release of nitric oxide. The blood pressure lowering effect of *T. divaricata* root extract may provided further evidence to the traditional use of the plant as an anti-hypertensive agent. However, further isolation and purification of the active substances are needed to validate this assertion.

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