ORIGINAL ARTICLE

Cardiovascular effects of *Tacca integrifolia* Ker-Gawl. extract in rats

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Abstract

Kitjaroennirut, N., Jansakul, C., and Sawangchote, P.
Cardiovascular effects of *Tacca integrifolia* Ker-Gawl. extract in rats
Songklanakarin J. Sci. Technol., 2005, 27(2) : 281-289

Rhizome of *Tacca integrifolia*, a Thai folk medicinal herb, has been used for controlling blood pressure and improving sexual function in humans. However, the biological activities of this herb on the cardiovascular system have not yet been documented. In the present study, we investigated the cardiovascular effects of methanolic extract from the rhizome of this herb (Tacca extract). In the *in vivo* study, intravenous injection of the Tacca extract (0.04-40 mg/kg) caused a decrease in both mean arterial blood pressure and heart rate of anesthetized rats (Nembutal sodium, 60 mg/kg, i.p.) in a dose dependent manner. Pretreatment of the animals with muscarinic receptor antagonist, atropine (1 mg/kg, i.v.), significantly reduced the hypotensive and the negative chronotropic activities of the Tacca extract.

In the *in vitro* preparation, the Tacca extract (0.001-3 mg/ml) caused a decrease in both force and rate of spontaneous contraction of isolated atria in a dose dependent manner. These effects were reduced by pre-incubation of the atria with atropine (10^{-7} or 10^{-6} M). For isolated blood vessels, the Tacca extract (0.003-3 mg/ml) caused vasodilation of endothelium-intact thoracic aortic rings pre-constricted with phenylephrine (3×

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Received, 5 July 2004 Accepted, 11 September 2004

10^{-6} M). This effect disappeared after pre-incubation of blood vessels with atropine (10^{-6} M) or with N^{ω}-nitro-L-arginine (3×10^{-4} M), or by removing the vascular endothelium.

The results obtained suggest that the hypotensive and negative chronotropic effects of the Tacca extract in the rat are due to the active components acting via the muscarinic receptors at the blood vessel to cause vasodilatation by stimulating the release of nitric oxide, as well as on the muscarinic receptors at the atria to cause the decrease of both rate and force of the atrial contraction.

Key words : *Tacca integrifolia*, blood pressure, vasodilation, nitric oxide, acetylcholine receptors

บทคัดย่อ

นงเยาว์ กิจเจริญนิรุตม์ ฉวีวรรณ จั่นสกุล และ ประกาศ สว่างโชติ

ผลของสารสกัดจากว่านนางครวญ (*Tacca integrifolia* Ker-Gawl.) ต่อหัวใจและหลอดเลือดในหนูแร็ท

ว. สงขลานครินทร์ วทท. 2548 27(2) : 281-289

เหง้าของว่านนางครวญ (*Tacca integrifolia*) เป็นสมุนไพรไทยชนิดหนึ่ง ใช้สำหรับควบคุมความดันโลหิตและเสริมสร้างสมรรถภาพทางเพศ อย่างไรก็ตาม ยังไม่มีรายงานทางวิทยาศาสตร์เพื่อพิสูจน์สรรพคุณดังกล่าวนี้ ในการศึกษาครั้งนี้จึงได้ศึกษาผลต่อหัวใจและหลอดเลือดของสารสกัดจากว่านนางครวญด้วยเมธานอล การศึกษาแบบ *in vivo* พบว่าการให้สารสกัดจากว่านนางครวญ (0.04-40 มก./กก.) เข้าทางหลอดเลือดดำของหนูแร็ทที่สลบด้วย Nembutal sodium (60 มก./กก., i.p.) มีผลทำให้ความดันเลือดเฉลี่ยและอัตราการเต้นของหัวใจลดลง แปรตามความเข้มข้นของสารสกัดที่ให้ การให้ atropine (1 มก./กก., i.v.) แก่สัตว์ทดลองก่อนการฉีดสารสกัดจากว่านนางครวญ มีผลทำให้การลดความดันเลือดและการลดอัตราการเต้นของหัวใจโดยสารสกัดจากว่านนางครวญ ลดน้อยลงอย่างมีนัยสำคัญทางสถิติ

การศึกษาแบบ *in vitro* พบว่าสารสกัดจากว่านนางครวญ (0.001-3 มก./มล.) มีผลทำให้อัตราและความแรงในการหดตัวได้เองของกล้ามเนื้อ atrium ลดลงแปรตามความเข้มข้นของสารสกัด เมื่อ incubate กล้ามเนื้อ atrium ด้วย atropine (10^{-7} หรือ 10^{-6} M) ก่อนให้สารสกัด มีผลทำให้การลดอัตราและความแรงในการหดตัวได้เองของกล้ามเนื้อ atrium โดยสารสกัดลดน้อยลงอย่างมีนัยสำคัญทางสถิติ สำหรับการศึกษาผลของสารสกัดจากว่านนางครวญต่อหลอดเลือดแดงใหญ่ thoracic aorta พบว่าสารสกัดจากว่านนางครวญ (0.003-3 มก./มล.) มีผลทำให้เกิดการคลายตัวของหลอดเลือดที่ยังคงมีเซลล์เอนโดธีเลียมที่ทำให้ตีบตัวอยู่ก่อนด้วย phenylephrine (3×10^{-6} M) แต่ผลการคลายตัวของหลอดเลือดต่อสารสกัดจากว่านนางครวญจะหายไป ถ้า incubate หลอดเลือดก่อนด้วย atropine (10^{-6} M) หรือ N^{ω}-nitro-L-arginine (3×10^{-4} M) หรือโดยการทำลายเซลล์บุหลอดเลือด

จากผลการทดลองที่ได้แสดงให้เห็นว่า ผลในการลดความดันโลหิตและการลดอัตราการเต้นของหัวใจของสารสกัดจากว่านนางครวญ เป็นผลเนื่องมาจากสารออกฤทธิ์แสดงฤทธิ์ผ่านทาง muscarinic receptor ที่หลอดเลือดทำให้มีการคลายตัวของหลอดเลือดโดยการกระตุ้นให้มีการหลั่งของไนตริกออกไซด์จากเซลล์บุหลอดเลือด และที่ muscarinic receptor ที่หัวใจส่งผลให้ทั้งอัตราและความแรงในการหดตัวของหัวใจลดลง

Tacca integrifolia Ker-Gawl. belongs to the family Taccaceae, which is distributed predominately in tropical regions of Asia (Pengklai, 1993). In China, Tacca species have been used for the treatment of gastric ulcer, enteritis, and hepatitis (Dictionary of Chinese Medicinal Materials, 1977). In Thai herbal medicine, rhizomes of *T. integrifolia* are used for controlling blood pressure and

improving sexual function (Chuakul *et al*., 2000).

A numbers of *Tacca* spp. have been studied for their chemical constituents and their biological activities. For examples, the rhizome of *T. plantaginea* was found to contain several kinds of taccalonolides which are A, B, C, D, E, F, G, H, I, J, K, L, and M (Chen *et al*., 1987, 1988, 1989 and 1997; Shen *et al*., 1991 and 1996). Some other taccanolides (N, R, S, T, U, and V), were also found from root of *T. paxiana* (synonym *T. chanterieri*, Pengklai, 1993), the Vietnamese plant (Mühlbauer *et al*., 2003). Among those taccanolides, the taccalonolide E and A, from the rhizome of *T. chanterieri* were studied for mitotic cell activities, and both have been found to cause an increased density of cellular microtubules in interphase cells and the formation of thick bundles of microtubules similar to the effects of Taxol (Tinley *et al*., 2003). Rhizome of *T. chantrieri* contains diarylheptanoids, seven diarylheptanoid glucosides, saponins, pregnane glycosides, chantriolide A and B and two withanolide glucosides (Yokosuka *et al*., 2002 and 2003). The diarylheptanoids and diarylheptanoid glucosides have cytotoxic activities against HL-60 human promyelocytic leukemia cells, HSC-2 human oral squamous carcinoma cells and normal human gingival fibroblasts. (Yokosuka *et al*., 2002).

For *T. integrifolia* (synonym *T. aspera*, Pengklai, 1993), its rhizome contains ochratoxin A (Roy and Kumar, 1993), amino acids (Tiwari and Tripathi, 1980), n-triacontanol, castanogenin, betulinic acid, quercetin-3-α-arabinoside, and taccalin (Tripathi and Tiwari, 1981). However, no study has been reported on the cardiovascular activities of this plant. Thus, it is of interest to study whether the methanolic extract from *T. integrifolia* has any effects on blood pressure and heart rate of anesthetized rats, and which mechanisms would be involved for those activities.

Materials and Methods

1. Preparation of crude methanolic extract of *Tacca integrifolia* Ker-Gawl.

Tacca integrifolia was collected from the forest in Thepha District, Songkhla Province, southern part of Thailand. The specimen was identified and was deposited at the Prince of Songkla University Herbarium (Collecting No. 2541-01).

Fresh rhizomes (1.6 kg) of *T. integrifolia* were chopped into small pieces and were immersed in 100% methanol for three days. The clear methanol extract was collected and evaporated to dryness *in vacuo*, the residue was lyophilized to obtain a brown powder crude methanolic *Tacca integrifolia* extract (Tacca extract, 65 gm). Analysis of three ions by the inductively-coupled plasma atomic emission spectrometer (PERKIN-ELMER, Optima 4300 DV) found that the Tacca extract contained Ca^{2+} 11.66, K^+ 524.85, and Na^+ 3.36 ppm. The relative quantity of each inorganic ion in the Tacca extract powder was calculated to obtain the same amount of NaCl, KCl, and $CaCl_2$ to dissolve in distilled water for using as a vehicle control in the *in vivo* study.

2. Pharmacological studies of the methanolic Tacca extract

In vivo preparation

Male Wistar rats (250-350 g) were anesthetized with Pentobarbital sodium (Nembutal sodium®, 60 mg/kg, i.p.). An endotracheal tube (PE No. 50) was inserted into the rat's trachea for prevention of airway obstruction. For the actual experiment, two polyethylene catheters were used. One was cannulated through the right common carotid artery and connected to a pressure transducer (Model Stathum P23XL) and a Grass® polygraph (Model 7D) for monitoring blood pressure and heart rate. Another polyethylene tube was cannulated through the left jugular vein for drug injection.

Effects of Tacca extract on blood pressure and heart rate

After equilibration of the animals for 40 minutes, the dose-response relationship to the Tacca extract (0.04-40 mg/kg) or to the vehicle was determined. In another set of animals, after dissection and 40 minutes equilibration, the dose-response relationship to the Tacca extract was studied after 20 minutes intravenous injection of

atropine (1 mg/kg).

***In vitro* preparation**

The rats were decapitated with a guillotine. Both the left and the right atria were excised and mounted immediately in a 20 ml organ bath. For thoracic aorta, two adjacent rings were cut. In one ring, endothelium was removed mechanically by gently rubbing the intimal surface with a stainless steel rod, using the method of Jansakul *et al*., 1989. The thoracic aortic rings were placed in organ baths and attached to isometric force transducers and the signals were recorded on a polygraph. The organ bath contained Krebs-Henseleit solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4 7H_2O$ 0.45, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 11.66, Na_2EDTA 0.024, and ascorbic acid 0.09. This solution was maintained at 37ºC and continuously bubbled with 95% O_2 and 5% CO_2.

Prior to addition of drugs, tissues were equilibrated for 45 minutes under resting tension of 1.0 g for both atria and thoracic aortic rings. The Krebs solution was replaced every 10-20 minutes.

After equilibration, the presence of functional endothelium of the thoracic aortic ring was assessed in all preparations as follows: the thoracic aortic ring was pre-constricted with 3×10^{-6} M phenylephrine until the response had plateaued (5-8 min), and dilator responses to 3×10^{-5} M acetylcholine were recorded. Eighty to ninety percent vasodilatation to acetylcholine occurred with the endothelium-intact thoracic aortic rings.

Effects of Tacca extract on rate and force of contraction of isolated atria

After 45 min equilibration, cumulative dose-response to the Tacca extract on the rate and force of spontaneous atrial contraction was studied. Following several washings and re-equilibration for 45 minutes, the atria were pre-incubated with atropine (10^{-7} or 10^{-6} M) for 30 minutes, after which the cumulative dose-response relationship to the Tacca extract was repeated.

Effects of Tacca extract on thoracic aortic rings *in vitro*

After 45 minutes re-equilibration, the cumulative dose-response to the Tacca extract of endothelium-intact or denuded thoracic aortic rings pre-constricted with 3×10^{-6} M phenylephrine was studied. Following several washings, only the thoracic aortic rings with endothelium-intact were exposed to atropine (10^{-6} M) or N^{ω}-nitro-L-arginine (L-NA), the nitric oxide synthase inhibitor (3×10^{-4} M), for 40 minutes, then cumulative dilator responses to the Tacca extract of the thoracic aortic ring pre-constricted with phenylephrine were again assessed.

Drugs

The following drugs were used: phenylephrine chloride, N^{ω}-nitro-L-arginine (L-NA), atropine sulphate and acetylcholine chloride, all

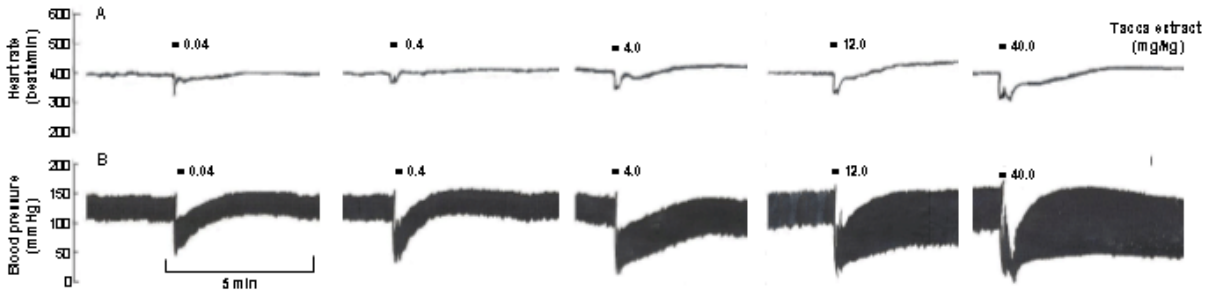

Figure 1. Tracing recorded by a polygraph of different doses (mg/kg) of methanolic extract from *Tacca integrifolia* on heart rate (panel A) and on mean arterial blood pressure (panel B) in an anesthetized rat.

obtained from Sigma, U.S.A. All drugs, and the Tacca extract were dissolved in distilled water, when used in *in vivo* and *in vitro* studies.

Statistical analysis

The changes in blood pressure and heart rate were recognized as the difference between the steady pressure before and the lowest pressure after injection. The blood pressure were recorded in mmHg as systolic pressure (SP) and diastolic pressure (DP) and were expressed as mean arterial pressure (MAP), which was calculated as DP + 1/3(SP-DP). Relaxation responses were expressed as a percentage of the induced tension which existed at the start of a relaxant concentration-effect experiment. All results are expressed as means ± SEM of 6-8 experiments (n=6-8). Student's paired or unpaired t-tests or one-way ANOVA were used for statistical analysis. In all cases, a p-value of 0.05 or less was considered statistically significant.

Results

Effects of Tacca extract on blood pressure and heart rate *in vivo* preparation

The effects of the Tacca extract on arterial blood pressure and heart rate is shown in Figure 1 and the changes in mean arterial blood pressure (MAP) and heart rate (H.R.) is shown in Figure 2. Basal mean arterial pressure and heart rate of anesthetized rats both of the control and the experimental groups are similar (vehicle control group, MAP=152.85±7.81 mm Hg, H.R.= 470.71 ±12.65 beats/min, n = 7; experiment group, MAP =144.92±5.6 mm Hg, H.R.= 455.0±10.62 beats/min, n = 8). The Tacca extract (0.04-40.00 mg/kg) caused a decrease in mean arterial blood pressure and heart rate in anesthetized rats in a consistent dose-dependent manner, while the vehicle, which has the same ion-concentration as those of the Tacca extract, did not have significant effects on blood pressure or heart rate. The lowest dose of the Tacca extract (0.04 mg/kg) caused a decrease in mean blood pressure and heart rate by 51.14±2.71 mmHg and 28.75±4.18 beats/min, respectively, whereas the highest dose (40 mg/kg) caused decrease in blood pressure and heart rate by 112.92±5.72 mmHg and 86.88±13.78 beats/min, respectively. The highest dose caused cardiac arrest after injection of the Tacca extract for 1-2 min. The incidence of cardiac arrest of the rats was 25%. The data from these animals were excluded.

Figure 3 shows the effects of the Tacca extract on mean arterial blood pressure and heart rate after blocking the muscarinic receptors with atropine. Blocking the muscarinic receptors by atropine significantly decreased the lowering effect of blood pressure and heart rate by the Tacca extract.

Effects of Tacca extract on rate and force of isolated atrial contraction *in vitro*

The effects of the Tacca extract on force and rate of spontaneous contraction of isolated atria are shown in Figures 4 and 5. The Tacca extract caused a dose-dependent decrease in rate and force of atrial contraction. Pre-incubation of the atria with the muscarinic antagonist, atropine, caused a decrease in the negative chronotropic and inotropic effects of atrial contraction by the Tacca extract (Figure 5).

Effects of Tacca extract on thoracic aortic rings *in vitro*

As shown in Figure 6, Tacca extract caused vasodilatation of the endothelium-intact thoracic aortic ring pre-constricted with phenylephrine in a dose-dependent manner. This effect disappeared after pre-incubation of the endothelium-intact aortic rings with atropine, L-NA, or by removal of the vascular endothelium.

Discussion

The Tacca extract caused a decrease in both mean arterial blood pressure and heart rate of anesthetized rats in a dose-dependent manner. Pretreatment of the animal with atropine, the muscarinic receptor antagonist, significantly reduced the hypotensive and negative chronotropic activities of the extract. This finding suggests a possible involvement of the Tacca extract on the

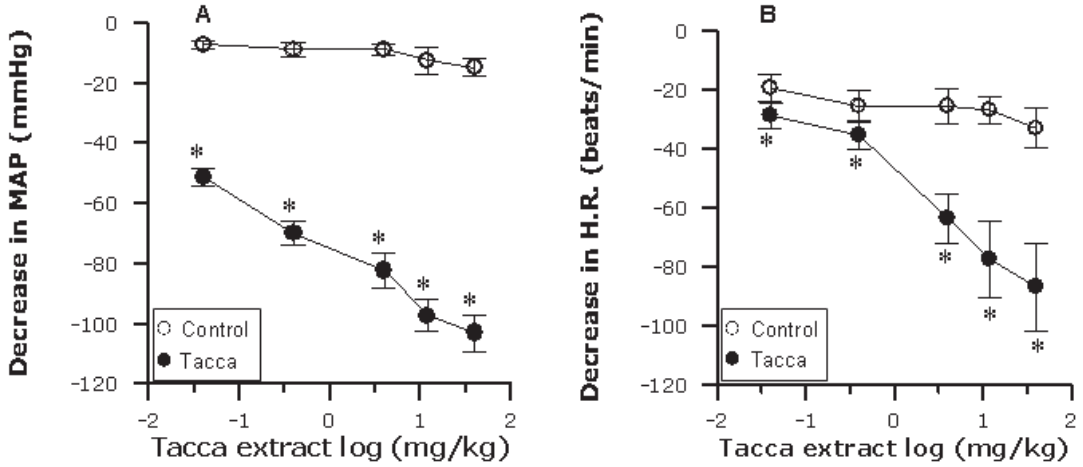

Figure 2. Effects of methanolic extract from *Tacca integrifolia* on mean arterial blood pressure (A) and heart rate (B) in anesthetized rats compared to the vehicle (control). Each point represents the mean±SEM of data from 6-8 experiments.
***Statistically significant lower than those of control group (p-value < 0.05).**

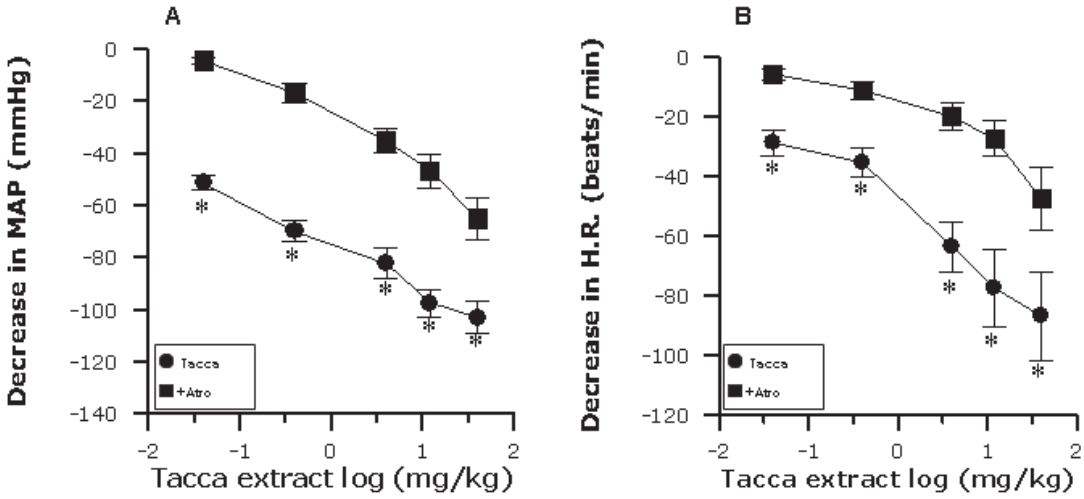

Figure 3. Effects of methanolic extract from *Tacca integrifolia* on mean arterial blood pressure (A) and heart rate (B) in anesthetized rats before (●) and after blocking with atropine (■). Each point represents the mean±SEM of data from 6-8 experiments.
*** Significantly lower than those obtained with atropine (p-value < 0.05).**

muscarinic receptor. The extract may act as a muscarinic agonist, causing directly a decrease of both rate and force of atrial contraction. In order to prove this possibility, the *in vitro* preparations were investigated. The Tacca extract reduced both force and rate of spontaneous contraction of isolated atria in a dose-dependent manner. Pre-incubation of the atria with atropine significantly reduced the negative inotropic and chronotropic effects on the atrial contraction of the extract.

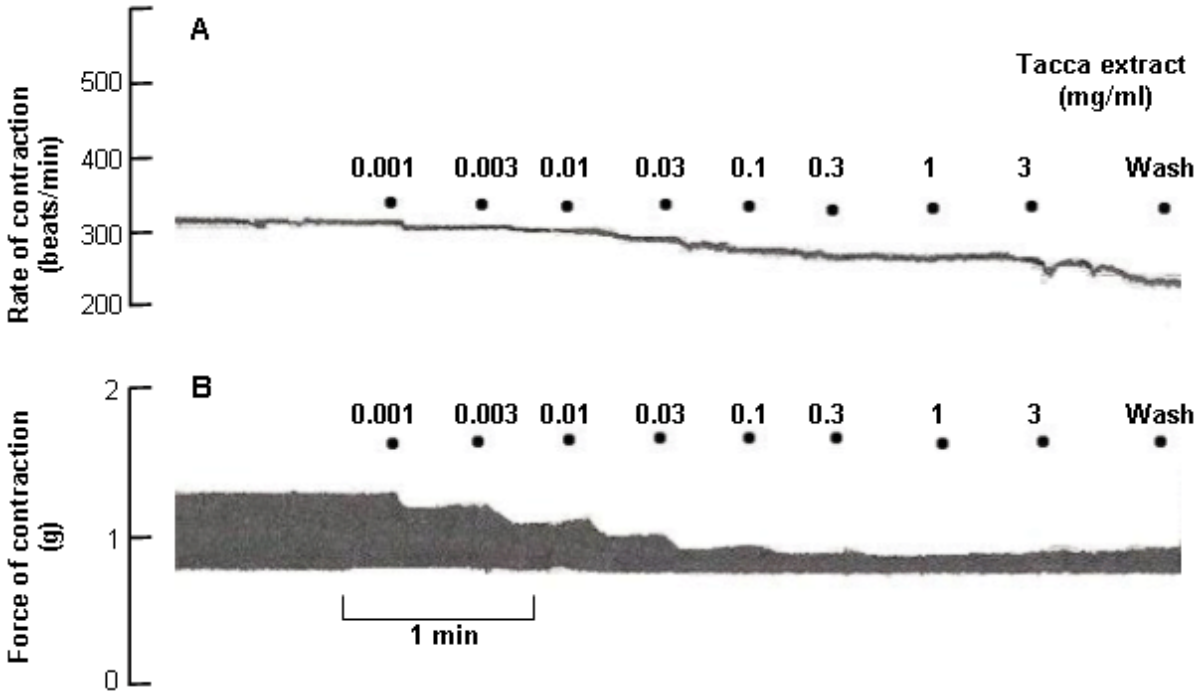

Figure 4. Tracing of rate (panel A) and force (panel B) of spontaneous atrial contraction in responses to cumulative doses of the methanolic extract from *Tacca integrifolia* (Tacca extract, 0.001-3 mg/ml).

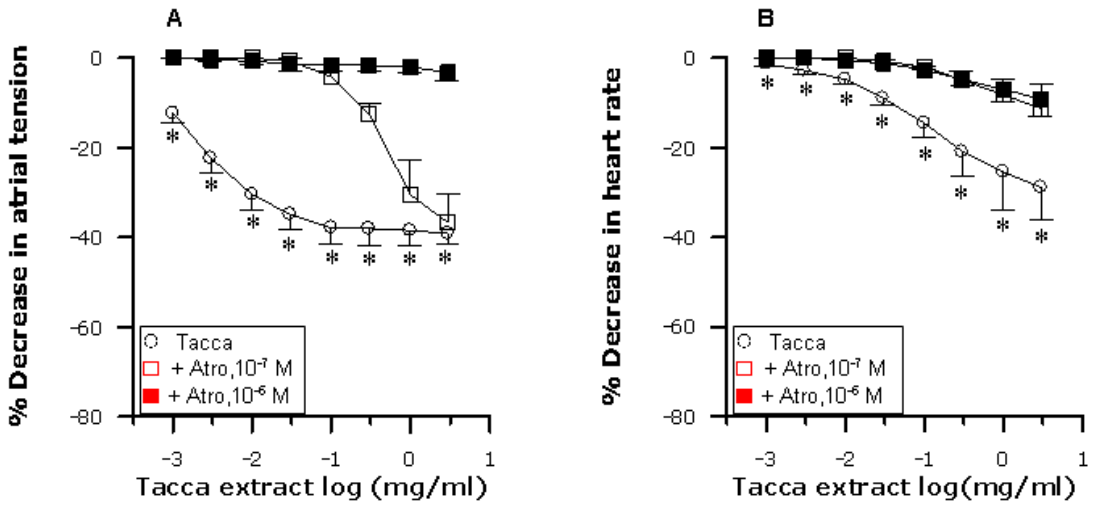

Figure 5. Effects of methanolic extract from *Tacca integrifolia* on force (A) and rate (B) of spontaneous contraction of isolated atria in the absence (○) or presence of atropine (Atro □ 10^{-7} M or ■ 10^{-6} M). Each point represents the mean±SEM of data from 6-7 experiments.
***Significantly lower than those obtained with atropine (p-value < 0.05).**

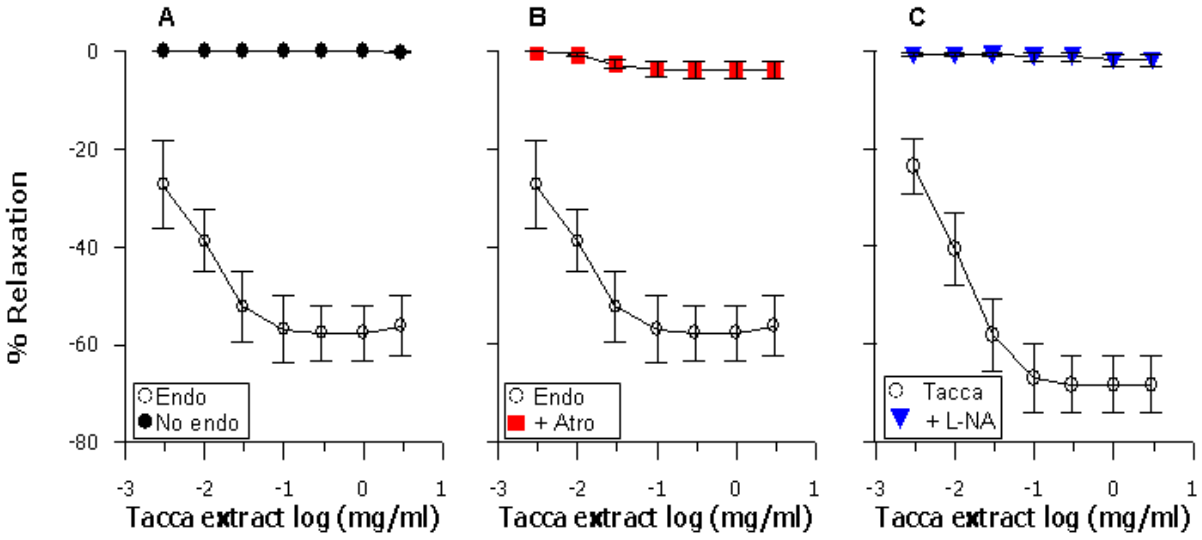

Figure 6. Effects of methanolic extract from *Tacca integrifolia* on thoracic aorta rings with endothelium-intact (○), endothelium-denuded (●), endothelium-intact in the presence of atropine (■) or endothelium-intact in the presence of L-NA (▼) in vitro. Each point represents the mean±SEM of data from 6-7 experiments.

In 1980, Furchgott and Zawadzki demonstrated that the vasodilation of thoracic aorta (*in vitro*) by acetylcholine is mediated via endothelium-derived releasing factors, later known as nitric oxide (Palmer, 1987), and is attenuated after removal of the endothelial cells. Nitric oxide is produced from L-arginine by the enzyme nitric oxide synthase in the endothelial cells that line the inner surface of blood vessels. Inhibition of the activity of nitric oxide synthase causes an increase in animal blood pressure (for review see, Moncada, 1991). Therefore, the hypotensive activity of the Tacca extract may be due to the active component(s) of the Tacca extract acting via the muscarinic receptors at the blood vessels, after which nitric oxide is released. In order to prove this possibility, we performed experiments *in vitro*, using isolated thoracic aortic rings. The Tacca extract caused vasodilation of endotheliam-intact, but not of denuded- thoracic aortic rings pre-constricted with phenylephrine. This result suggests that the vasodilator effect of the Tacca extract is endothelium-dependent. On the other hand, pre-incubating the thoracic aortic rings with atropine, a muscarinic receptor antagonist, or with L-NA, a nitric oxide synthase inhibitor (Moncada, 1997) completely blocked the vasodilator activity of the Tacca extract. These findings suggest that the Tacca extract causes vasodilation by stimulating the release of nitric oxide from the vascular endothelium via muscarinic receptors. In conclusion, Tacca extract exerts a hypotensive and negative chronotropic effects in anesthetized rats. The mechanisms probably involve the active component(s) acting via the muscarinic receptors at the vascular endothelium and causing vasodilatation by stimulated release of nitric oxide, as well as acting via the muscarinic receptors at the atria to cause a decrease both in rate and force of spontaneous atrial contraction.

Acknowledgements

This study was supported mainly by to the Faculty of Science, Prince of Songkla University, Hat Yai, Thailand, and partially by the Department of Physiology, Faculty of Science. The authors would also like to thank to Dr. Alan Geater for proof-reading the manuscript.

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