

Cardiovascular Effects of an *n*-Butanol Extract from Fresh Fruits of *Randia siamensis*

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Randia siamensis is used in Thai folkloric medicine for inducing abortion and controlling blood pressure. The present study investigated the cardiovascular effects of an *R. siamensis* fruit extract, and mechanisms involved in anesthetized normal and reserpinized rats. *R. siamensis* (0.4—12 mg/kg) i.v. increased the mean arterial pressure (MAP) and heart rate. Both effects were significantly inhibited by phentolamine (2 mg/kg, i.v.) or propranolol (0.6 mg/kg, i.v.). The combination of phentolamine and propranolol, or reserpine pretreatment, inhibited the positive chronotropic effect with a slight decrease in the MAP. *In vitro*, *R. siamensis* (0.001—0.3 mg/ml) increased the rate of beating of the right atrium and the strength of the electrical field-stimulated contraction of the left atrium, both effects were inhibited by propranolol, or with reserpine pretreated rats. *R. siamensis* (0.01—3 mg/ml) produced a contraction of isolated thoracic aorta, which was potentiated by *N*^G-nitro-L-arginine (LNA), or by removal of the vascular endothelium, but inhibited by phentolamine, or reserpine. *R. siamensis* (0.3—3 mg/ml) caused a relaxation of phenylephrine-precontracted aortic rings, which was potentiated with reserpine pretreatment, and abolished after removal of the vascular endothelium, or in the presence of LNA. These results suggest that *R. siamensis* extract exerts both hypertensive and positive chronotropic effects via the α - and β -adrenergic receptors of blood vessels and the heart, due to release of endogenous catecholamines, likely from nerve ending and adrenal medulla. The hypotensive activity results from the release of nitric oxide causing dilatation of the blood vessels. The present data support the folkloric therapeutic uses of this plant.

Key words *Randia siamensis*; pseudoginsenoside; hypertension; hypotension; heart; blood pressure

Randia siamensis (*R. siamensis*) is a climbing tree of the subfamily Gardennieae, family Rubiaceae. It is widely distributed in tropical and sub-tropical regions, especially in Asia and Africa.¹⁾ Craib²⁾ reported that *R. siamensis* CRAIB is synonymous with *R. longiflora* HOOK f., *R. uncata* RIDL., *Griffithia siamensis* MIQ. and *Weberia siamensis* KURZ. It can be found in all parts of Thailand and Burma, especially in humid regions. This plant is known by various local names in Thailand: Khat khao, Khet khao, Khat khao naam and Khat khao thuea. All parts of *R. siamensis* have been used in Thai folkloric medicine. The fruits have been used for inducing abortion and as an emmenagogue or hematinic, the leaves are claimed to control blood pressure, the root is used for antipyretic and antiscurvy activities, the flowers have been used to stop nosebleeds, and the stem part is used as a hematinic.³⁾ However, scientific investigations to test these therapeutic claims are very scarce. Aukkanibutra⁴⁾ identified the chemical constituents of fruits of the plant. She found that dried fruit of *R. siamensis* contains: ursolic acid, pseudoginsenoside-RT₁, pseudoginsenoside-RP₁ and an unidentified saponin. Later, Reanmongkol *et al.*⁵⁾ reported that pseudoginsenoside-RP₁, the minor component of the plant fruit, has an antinociceptive activity similar to that of aspirin, a peripherally acting analgesic. However, the activity of the purified compound was much less potent than that of the crude extract. Jansakul *et al.*⁶⁾ tested the activities of the pseudoginsenoside-RT₁, isolated by Aukkanibutra,⁴⁾ on the cardiovascular system and found that the pseudoginsenoside-RT₁ (4—32 mg/kg) caused a decrease in mean arterial blood pressure, with an increased heart rate in a dose-dependent manner in anesthetized rats. However, the mechanisms for these effects have not yet been elucidated due to the limited availability of

the substance. This led us to return to study the cardiovascular effects of the crude extract from fresh fruits of *R. siamensis* (*R. siamensis* extract). We found that the crude extract (at 4—12 mg/kg) caused an increase in both the mean arterial blood pressure and heart rate in a dose-dependent manner. The effect was more potent than that of the saponin pseudoginsenoside-RT₁. It is possible that the fresh fruit of *R. siamensis* contains some other substances, besides pseudoginsenoside-RT₁, that have effects on the cardiovascular system. In the present study, we aimed to investigate the cardiovascular profile of the *R. siamensis* extract in the rat, as well as to assess the mechanisms involved, and compare the results with those produced by norepinephrine, isoproterenol and epinephrine.

MATERIALS AND METHODS

Plant Material Fresh fruits of *R. siamensis* were collected in Songkhla Province, Thailand. Authentication was achieved by comparison with herbarium specimens in the Department of Biology Herbarium, Faculty of Science, Prince of Songkla University, Thailand, where a voucher specimen of plant material has been deposited.

Preparation of *Randia siamensis* Extract Fresh and unripe fruits of *R. siamensis* (5 kg) were blended and macerated three times with 80% methanol (10 l) during a week period. The methanol extract was filtered and evaporated under reduced pressure, yielding 200.5 g of the MeOH extract. The methanol extract was dissolved in water, and a liquid/liquid partition was undertaken with chloroform, followed by *n*-butanol. The *n*-butanol phase was evaporated under reduced pressure, and the residue was lyophilized to obtain a crude

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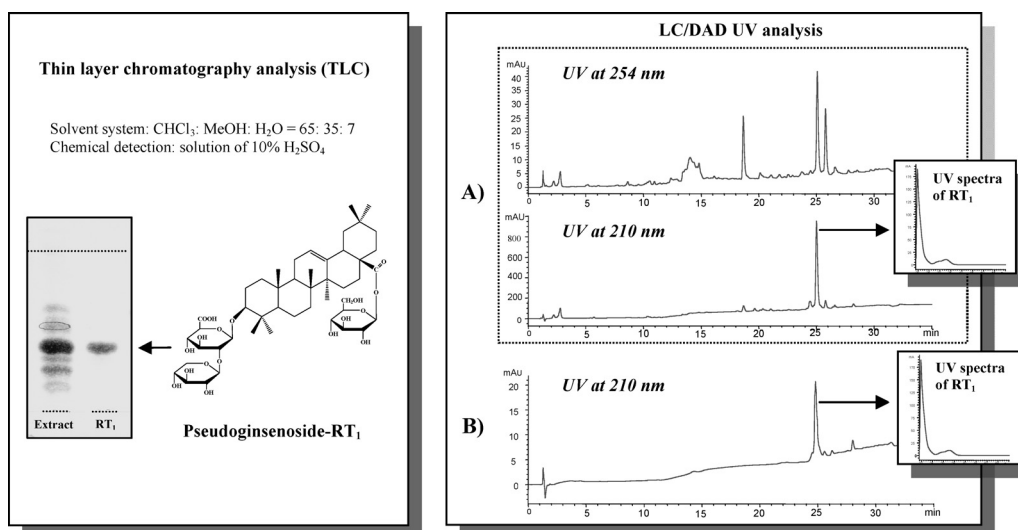


Fig. 1. TLC and LC/UV DAD Analysis of the *n*-Butanol Extract of *R. siamensis* (A) and the Standard Saponin Pseudoginsenoside-RT₁ (B)

yellowish powder, the *R. siamensis* *n*-butanol extract (*R. siamensis* extract, 126.2 g).

The *R. siamensis* extract was analysed by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) in order to obtain its chemical profile. TLC analysis was carried out on precoated Kieselgel 60 F₂₅₄ (0.25 mm, Merck, Darmstadt, Germany) plate, using a mixture of CHCl₃:MeOH:H₂O=65:35:7 as a mobile phase. The spots were visualized by spraying the plate with 10% H₂SO₄ solution, followed by heating. Analytical HPLC was carried out on a HP 1100 system equipped with a photodiode array detector (Agilent Technologies). The extract was analyzed on a Symmetry[®] C₁₈ column (5 μm, 3.9×150 mm i.d.; Waters), with MeOH:H₂O+0.5% of trifluoroacetic acid in gradient mode (20:80→100:0). The flow rate was 1 ml/min; the UV traces were measured at 210 and 254 nm and UV spectra (DAD) were recorded between 200 and 500 nm.

Pure saponin pseudoginsenoside-RT₁, previously described in *R. siamensis*, was used as reference for the compound.⁶⁾ The presence of pseudoginsenoside-RT₁ in the extracts was confirmed by both analyses (Fig. 1). The TLC analysis showed the presence of a major compound with the same *R_f* of the standard. The LC/DAD UV analysis confirmed this hypothesis, the major compound of the extract had the same retention time and the same UV spectrum of the standard. The analysis of the *R. siamensis* as in agreement with the literature indicated the presence of saponins. On the other hand the LC/DAD UV analysis at 254 nm showed the presence of other two major compounds (Fig. 1A). The UV spectra of these compounds presented with absorptions around 260–345 nm typical UV data of flavonoids.⁷⁾

Pharmacological Studies of the *R. siamensis* Extract.
Effect on Blood Pressure and Heart Rate *in Vivo* Adult female Wistar rats in estrus (220–270 g) were supplied from the Animal House, Faculty of Science, Prince of Songkla University. They were maintained in controlled environmental conditions (24–26 °C) with a 12 h light/dark cycle and allowed access to standard food and tap water *ad libitum*. Preparation of animals followed the Prince of Songkla University guidelines for the approved Care and Use of Experi-

mental Animals.

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The tracheal tube was cannulated with a polyethylene tube to facilitate spontaneous respiration. The systemic blood pressure was recorded from the right common carotid artery *via* an arterial cannula connected to a pressure transducer (P23 ID, Gould Statham Instrument, Hato Rey, Puerto Rico), and the heart rate was recorded using a tachograph driven by the blood pressure wave, which were connected to a Grass polygraph (Model 7D, Grass Instrument, Quincy, MA, U.S.A.). The animal was then equilibrated for at least 40 min before the experiment was started. After the period of equilibration, the dose–response relationships to norepinephrine, isoproterenol, epinephrine, or *R. siamensis* extract were determined by intravenous injection into left jugular vein of a volume not exceeding 0.1 ml for each dose and flushed in with 0.1 ml saline. Each rat was used for only one agonist.

With other sets of animals, after equilibration of the animal for 40 min, propranolol (0.6 mg/kg), phentolamine (2 mg/kg), or both propranolol and phentolamine were injected through the left jugular vein. After 20 min re-equilibration, the dose–response relationships to norepinephrine, isoproterenol, epinephrine, or *R. siamensis* extract were determined.

With another set of animals, the rat was treated with reserpine at a dose of 5 mg/kg, i.p., once a day, starting two days before the experiment. Thereafter the dose–response relationship to *R. siamensis* extract was determined using the same protocol as above.

***In Vitro* Preparation** A normal or reserpinized rat was killed by decapitation with a guillotine. The thorax was opened, the heart was rapidly removed and placed in Krebs Heinseleit solution saturated with carbogen (95% O₂+5% CO₂) at 37 °C, and allowed to beat for a few seconds to expel intra-atrial chamber blood. Both the left and the right atria were excised from the ventricles, and were then separated. The right atrium was mounted in a 20-ml organ bath, one end was fixed at the bottom and the other end connected to a force-displacement transducer (FT03C) connected to a Grass polygraph, under a basal tension of 0.8 g. The rate of sponta-

neous atrial contraction was recorded using a tachograph. The atrium was allowed to equilibrate for 50 min with changes in Krebs Heinseleit solution every 15 min. Then, the concentration–response relationship to *R. siamensis* extract was determined before and after pre-incubation (at least 40 min for each concentration of the antagonist) with (10^{-8} , 10^{-7} M) propranolol and/or atropine.

The left atrium was mounted between two platinum electrodes approximately 10 mm apart (left and right) under a basal tension of 0.5 g. The atrial tension was recorded by means of a Grass force-displacement transducer connected to a Grass polygraph. The tissues were equilibrated for 50 min. After the equilibration, the left atrium was stimulated with several trains at 3 Hz, 5 ms pulse duration with a 1 V increase in voltage for each step until the threshold voltage (3–5 V) was reached. Preparations were allowed to equilibrate for another 15 min, then a 10–20% suprathreshold voltage (5–7 V) was started, allowing 5 min of continuous contraction, followed by a cumulative challenge with *R. siamensis* extract before and after pre-incubation (at least 40 min for each concentration of the antagonist) with propranolol (10^{-8} , 10^{-7} M). Each concentration was left for 2–3 min by which time the response reached a plateau. Inotropic responses of the drugs were calculated just before starting the construction of the concentration–response curves to the test agents.

Immediately after an excision of the heart, the thoracic aorta was removed and dissected free of connective tissue and fat. Two adjacent rings of 4–5 mm in length 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.*⁸⁾ The aortic rings were suspended horizontally between two stainless steel hooks in a 20-ml organ bath containing Krebs solution. One of the hooks was fixed to the bottom and the other was connected to a force displacement transducer connected to a Grass polygraph for the recording of changes in isometric tension. Prior to addition of drugs, tissues were equilibrated for 60 min under a resting tension of 1 g. The Krebs solution was replaced every 15 min.

After equilibration, the presence of a functional endothelium of the thoracic aortic rings was assessed in all preparations as follows: the thoracic aortic ring was preconstricted with 3×10^{-6} M phenylephrine until the response had plateau (5–8 min), and the dilatory response to 3×10^{-6} M acetylcholine was recorded. The experiment was continued only when there was no dilatory response for the endothelium-denuded thoracic aortic rings, and at least 80% vasodilatation to acetylcholine for the endothelium-intact thoracic aortic rings.

After equilibration, the thoracic aorta was challenged with 3×10^{-6} M norepinephrine for 10 min, by which time the maximal response had plateaued. Then followed several washings, and re-equilibration for 30 min, a cumulative concentration–response relationship of the thoracic aorta to the *R. siamensis* extract was obtained. Effects of nitric oxide or phentolamine on the thoracic constrictor response of *R. siamensis* extract were studied using another two sets of the thoracic aorta: one without endothelium, and the other one with intact endothelium, thoracic aortic rings that had been pre-incubated with N^G -nitro-L-arginine (3×10^{-4} M) for 40 min. Then the concentration–response relationship of the thoracic

aortic rings to an *R. siamensis* extract was obtained before and after being pre-incubated (40 min for each concentration) with phentolamine (10^{-8} , 10^{-7} M). Using the same protocol, a cumulative concentration–response relationship to phenylephrine of the thoracic aortic rings was obtained in the absence or presence of N^G -nitro-L-arginine.

Dilator responses of the thoracic aorta to the *R. siamensis* extract were also studied using endothelium-intact, endothelium-denuded, and reserpinized thoracic aortic rings. After 40 min equilibration, the aortic rings were preconstricted with 3×10^{-6} M phenylephrine for 5–8 min (plateau reached), and the concentration–response relationship to *R. siamensis* extract was determined.

The organ bath contained Krebs Henseleit solution of the following composition (mM) NaCl 118.3, KCl 4.7, CaCl_2 1.9, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.45, KH_2PO_4 1.18, NaHCO_3 25.0, glucose 11.66, Na_2EDTA 0.024 and ascorbic acid 0.09, maintained at 37°C, and continuously bubbled with 95% O_2 and CO_2 .

Drugs The following drugs were used: acetylcholine chloride, atropine sulphate, (–)-epinephrine bitartrate, (±)-isoproterenol hydrochloride, phentolamine hydrochloride, phenylephrine hydrochloride, (–)-norepinephrine bitartrate, propranolol hydrochloride, reserpine, and N^G -nitro-L-arginine (LNA). All drugs were purchased from Sigma, U.S.A. *R. siamensis* extract and LNA were dissolved in distilled water, the remainder were dissolved in a solution containing NaCl 9 g/l, NaH_2PO_4 0.19 g/l and ascorbic acid 0.03 g/l.

Data Analysis Data are expressed as means \pm S.E.M. of 6 experiments ($n=6$). Vasoconstrictions are expressed as means \pm S.E.M. of percentage contraction of the maximal response obtained from 3×10^{-6} M norepinephrine. Vasorelaxations are expressed as means \pm S.E.M. of percentage relaxation from 3×10^{-6} M phenylephrine precontraction levels. Tests of significance were made using the Student's paired or unpaired *t*-test or one way ANOVA. In all cases, a *p* value of 0.05 or less was considered statistically significant.

RESULTS

Effects of Propranolol and/or Phentolamine on Mean Arterial Blood Pressure and Heart Rate of Anesthetized Rats to Norepinephrine, Isoproterenol, Epinephrine, or *R. siamensis* Extract As shown in Figs. 2A and B, norepinephrine, a non-specific α -adrenergic receptor agonist, caused an increase in mean arterial blood pressure (MAP) in a dose-dependent manner and a decreased heart rate but only at a very high dose. In the presence of phentolamine (2 mg/kg), a non-specific α -adrenergic receptor antagonist, the dose–response curves of the norepinephrine on both MAP and heart rate were shifted to the right. Figures 2C and D shows that isoproterenol, a non specific β -adrenergic receptor agonist, caused a dose-dependent decrease in MAP with an increase in heart rate. In the presence of propranolol (0.6 mg/kg, i.v.), a non-specific β -adrenergic receptor blocking agent, both the increase in MAP and the increase in heart rate to isoproterenol were significantly inhibited. Small doses (10^{-5} – 10^{-4} mg/kg) of epinephrine caused a decrease, while at higher doses (10^{-3} – 3×10^{-3} mg/kg) an increase in MAP was found. All dosages caused an increase in heart rate in a dose-dependent manner. Both the hypotensive and the positive chronotropic effects of epinephrine were inhibited by

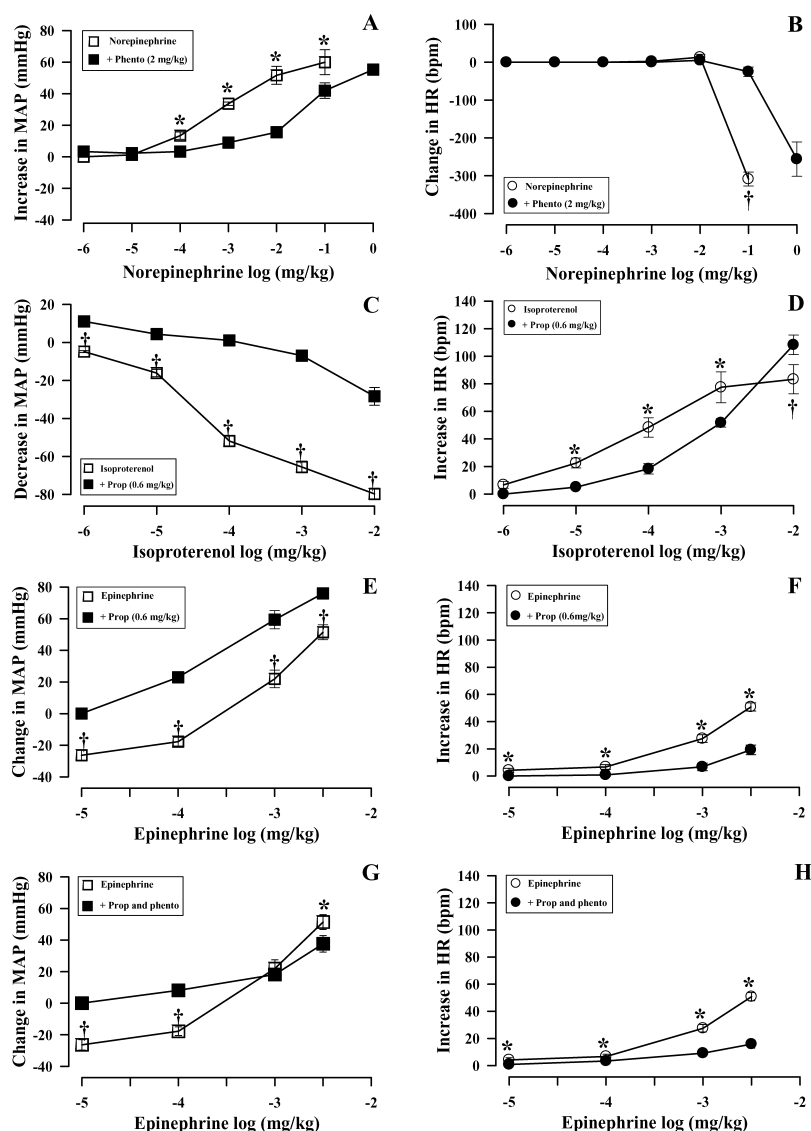


Fig. 2. Effects of Norepinephrine, Isoproterenol or Epinephrine on the Mean Arterial Blood Pressure (MAP, Left) and Heart Rate (HR, Right) before or after Blocking the β -Adrenergic Receptor with Propranolol (Prop, 0.6 mg/kg) or α -Adrenergic Receptor with Phentolamine (Phento, 2 mg/kg) in Anesthetized Rats

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than those in the presence of propranolol and/or phentolamine, $p < 0.05$. † Significantly lower than those in the presence of propranolol and/or phentolamine, $p < 0.05$.

propranolol (Figs. 2E, F). However, when phentolamine was given together with propranolol, the hypertensive effect of the epinephrine on the animals with propranolol was further suppressed (Fig. 2G), whereas there was no further effect on heart rate (Fig. 2H).

The effects of *R. siamensis* extract on the MAP and heart rate are shown in Fig. 3. An intravenous injection of *R. siamensis* extract (0.4–12 mg/kg) into anesthetized rats caused an increase in both the MAP and heart rate in a dose-dependent manner. Propranolol (0.6 mg/kg), caused a rightward parallel shift of the dose–response curve of *R. siamensis* extract on the heart rate, but produced no change on the dose–response curve of the MAP. In the presence of phentolamine (2 mg/kg), however increasing amounts of extract caused a decrease in the MAP, rather than an increase, and reduced the extract-induced increase on the heart rate. When propranolol and phentolamine were given to the animals at the same time, the dose–response curve to *R. siamensis* extract on

heart rate was shifted to the right in a similar way to that caused by propranolol alone, while the mean arterial blood pressure was decreased in a similar manner to that obtained with phentolamine alone.

Effects of Reserpine on the Positive Chronotropic Effect and the Increase in Mean Arterial Blood Pressure of the *R. siamensis* Extract Figure 4 shows the effects of *R. siamensis* extract on the MAP and heart rate of normal and reserpinized rats. *R. siamensis* extract caused an increase in heart rate and the MAP in normal rats. After treatment with reserpine the positive chronotropic effect of the *R. siamensis* extract was almost abolished. In the case of the blood pressure, the *R. siamensis* extract caused a slight decrease in MAP instead of an increase.

In Vitro Effects of Propranolol and/or Atropine on the Positive Chronotropic and Positive Inotropic Effects of the *R. siamensis* Extract *R. siamensis* extract caused an increase in the rate of spontaneous contraction of the right

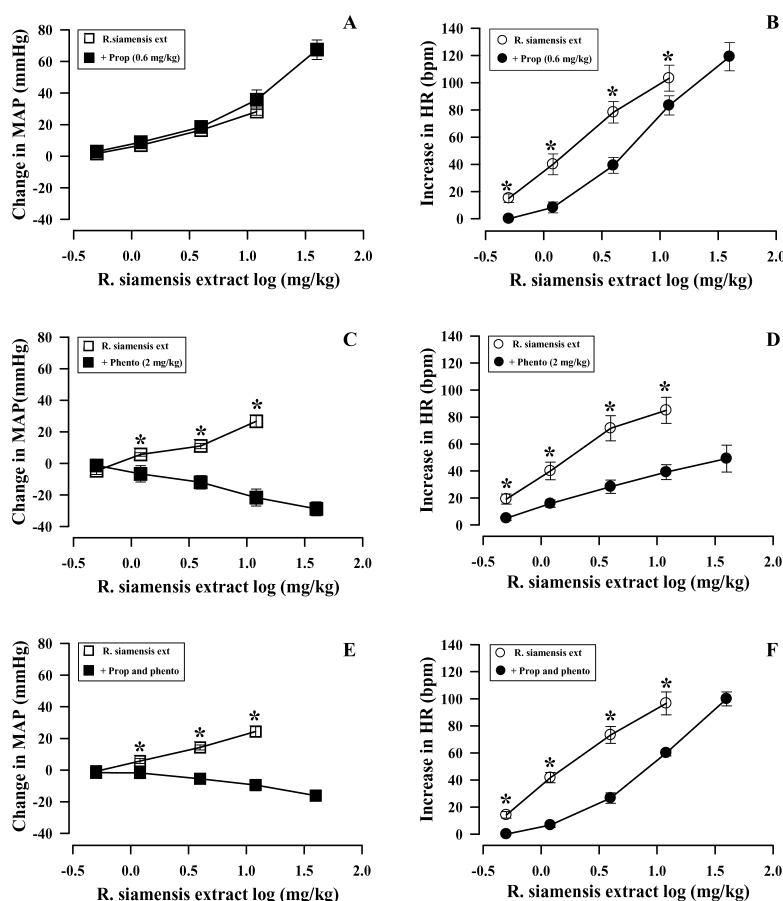


Fig. 3. Effects of Propranolol (Prop) and/or Phentolamine (Phento) on Changes in the Mean Arterial Blood Pressure (MAP, Left), and the Positive Chronotropic Effect (Increase in HR, Right) of *R. siamensis* Extract in Anesthetized Rats

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than those in the presence of propranolol and/or phentolamine, $p < 0.05$.

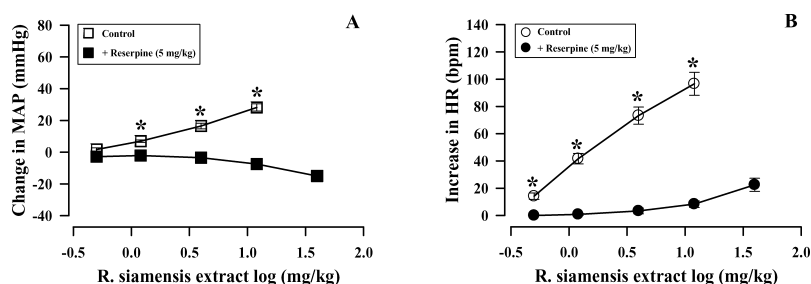


Fig. 4. Effects of the Depletion of Sympathetic Neurotransmitters by Reserpine on the Increase in Mean Arterial Blood Pressure (MAP, Left) and the Positive Chronotropic Effect (Increase in HR, Right) of *R. siamensis* Extract in Anesthetized Rats

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than those in reserpinized rats, $p < 0.05$.

atrium. In the presence of 10^{-8} M propranolol the dose-dependent curve of the increased rate of contraction of the right atrium made a parallel shift to the right with a decrease in its maximal response. At a concentration of 10^{-7} M propranolol, the dose-dependant response of the spontaneous contraction to the extract was almost completely suppressed. 10^{-8} M and 10^{-7} M atropine had no effect on the positive chronotropic effect of the *R. siamensis* extract (Fig. 5B). In addition, when both atropine and propranolol (10^{-8} , 10^{-7} M) were applied together, the responses to the *R. siamensis* extract did not significantly change (Fig. 5C).

R. siamensis extract caused an increase in the strength of the electrical field-stimulated contraction of the left atrium.

Propranolol (10^{-8} M, 10^{-7} M) caused a dose-dependent significant rightward shift of the dose-response curves to the *R. siamensis* extract (Fig. 5D).

However, with reserpinized rats, both these effects of the *R. siamensis* extract were suppressed (Fig. 6).

Effects of the *R. siamensis* Extract and Phenylephrine on Thoracic Aortae in Vitro *R. siamensis* extract caused a slight increase of vasoconstriction of the endothelium-intact thoracic aortic rings (Fig. 7A). When the vascular endothelium was removed, or nitric oxide synthase was blocked by *N*^G-nitro-L-arginine (LNA), the vasoconstriction responses of the thoracic aorta to the *R. siamensis* extract were significantly increased. However, removal of the endothelium

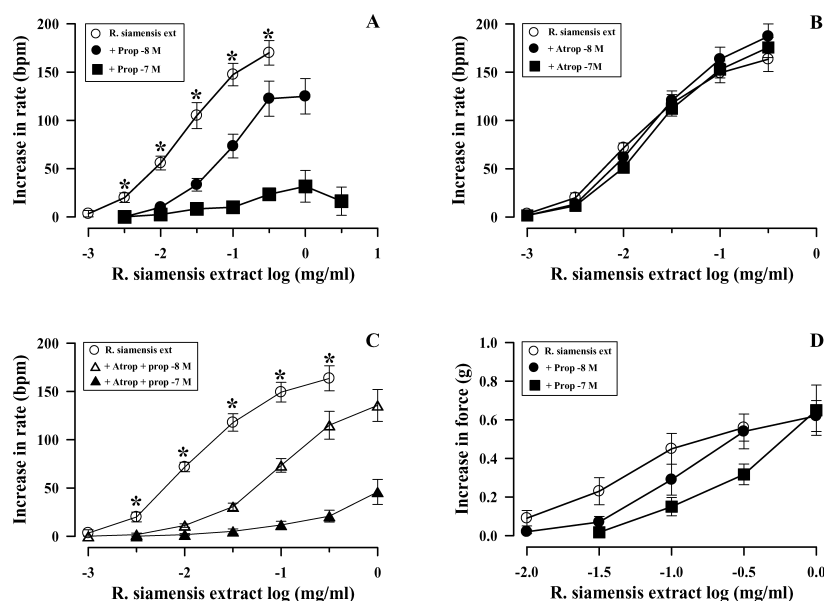


Fig. 5. Effects of Propranolol (Prop, A, C, D) and/or Atropine (Atrop, B, C) on the Positive Chronotropic Effects of Spontaneous Contraction of the Right Atrium (A—C) or on the Positive Inotropic Effects of the Electrical Field-Stimulated Contraction of the Left Atrium (D) to *R. siamensis* Extract

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than those in the presence of propranolol and/or atropine, $p < 0.05$.

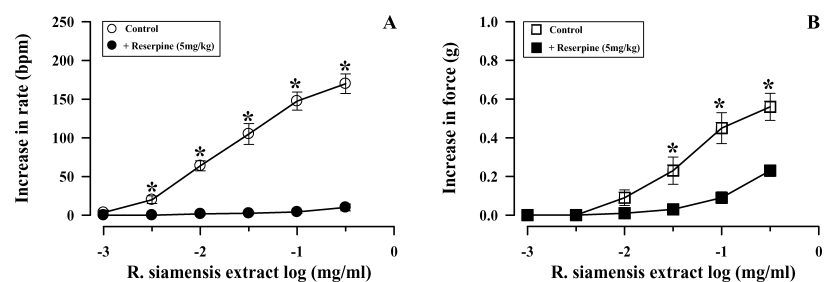


Fig. 6. Effects of the Depletion of the Sympathetic Neurotransmitters by Reserpine on the Positive Chronotropic Effect of Spontaneous Contraction of the Right Atrium (A) or on the Positive Inotropic Effect of the Electrical Field-Stimulated Contraction of the Left Atrium (B) to *R. siamensis* Extract

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than those of reserpinized tissues, $p < 0.05$.

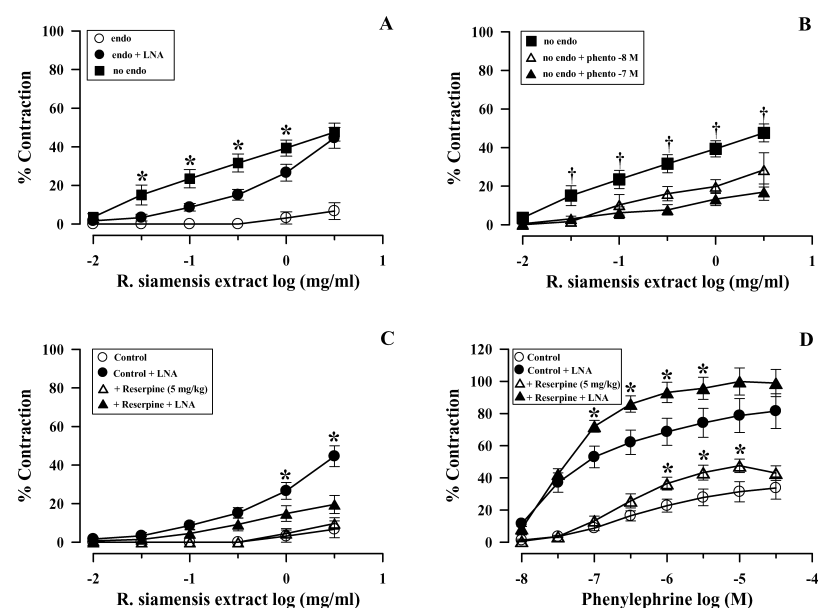


Fig. 7. Effects of N^G -Nitro-L-arginine (LNA, 3×10^{-4} M) or Removal of Vascular Endothelium (A), Phentolamine (B), or LNA and/or Reserpine Induced Depletion of the Sympathetic Neurotransmitters (C) on the Contractile Response of the Thoracic Aortic Rings to *R. siamensis* Extract or to Phenylephrine

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly higher than the ones with endothelium, or with LNA (A, D), or the other three groups (C), $p < 0.05$. † Significantly higher than the ones in the presence of phentolamine, $p < 0.05$.

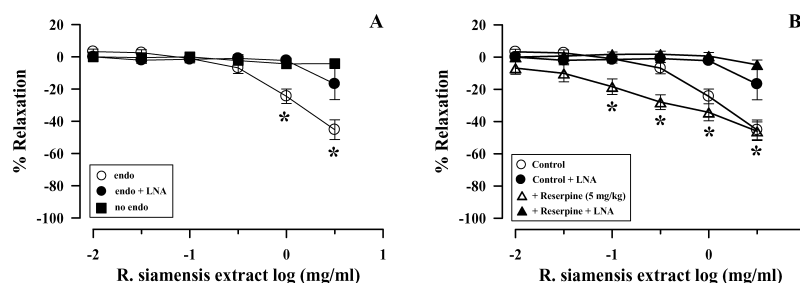


Fig. 8. Effects of N^G -Nitro-L-arginine (LNA, 3×10^{-4} M), Removal of Vascular Endothelium (A), and/or the Depletion of the Sympathetic Neurotransmitters by Reserpine (B) on the Dilator Response (Precontracted with 3×10^{-6} M Phenylephrine) of the Thoracic Aortic Rings to *R. siamensis* Extract

Each point represents mean \pm S.E.M. of 6 experiments. * Significantly lower than those without endothelium, with LNA, or control groups, $p < 0.05$.

caused a bigger response than did treatment with LNA. These effects were significantly inhibited by either phentolamine (Fig. 7B), or reserpine treatment (Fig. 7C).

Phenylephrine caused a dose-dependent increase in vasoconstriction of the endothelium-intact thoracic aortic rings which were potentiated by pre-incubation of the blood vessel with LNA. When the animals were pretreated with reserpine, the contractile responses of the thoracic aortic rings to phenylephrine were enhanced for both the endothelium-intact and LNA-pretreated blood vessels (Fig. 7D).

R. siamensis extract caused relaxation of the endothelium-intact thoracic aortic rings precontracted with phenylephrine (3×10^{-6} M) (Fig. 8A). The sensitivity of this extract induced relaxation was increased in reserpinized rats. However, both these effects disappeared after removal of the vascular endothelium, or after blocking the nitric oxide synthase by LNA (Fig. 8B).

DISCUSSION

The present study demonstrates that an *R. siamensis* extract can exert both hypertensive and tachycardiac activities in anesthetized rats. These effects are similar to those produced by epinephrine, a non-specific α - and β -adrenergic receptor agonist, but not by norepinephrine, a non-specific α -adrenergic receptor agonist with lower β -adrenergic receptor activity, or isoproterenol, a non-specific β -adrenergic receptor agonist. However, small doses of the *R. siamensis* extract did not elicit hypotension, a property of epinephrine. This may be due to the absence of β_2 -adrenoceptor activity in the *R. siamensis* extract so the dilatation of the blood vessels produced by a small dose of epinephrine⁹ does not occur. However, this finding does indicate that the *R. siamensis* extract may act *via* both the α - and β -adrenergic receptors of the cardiovascular system. Further experiments to test this possibility were carried out in rats treated with propranolol, a non-specific β -adrenergic receptor antagonist and/or phentolamine, a non-specific α -adrenergic receptor antagonist¹⁰ before determining the dose-response curve to the *R. siamensis* extract and comparing it with epinephrine. The dose-response curve to the *R. siamensis* extract on MAP was not modified by 0.6 mg/kg propranolol. However 2 mg/kg phentolamine, that caused a rightward shift of the dose-response curve of norepinephrine and epinephrine, not only blocked the hypertensive response, but also caused a hypotension (Fig. 3C). A similar result was found, when the animals were pretreated with both propranolol and phentolamine. These

results indicate that the *R. siamensis* extract most probably acts *via* the α -adrenergic receptors of the vascular system to cause hypertension, whereas it may act through another pathway of the vascular system to cause vasodilatation and hypotension. It is unlikely that the β_2 -adrenergic receptors of the blood vessels play a role in this activity since this effect was not significantly inhibited by propranolol.

Propranolol caused a right shift of the dose-response curve of the positive chronotropic effect of the *R. siamensis* extract, and phentolamine also inhibited this chronotropic effect. However, when the animal was pretreated with both propranolol and phentolamine, the dose-response curve to *R. siamensis* extract there was no additive effect. A similar result was also found for epinephrine. This suggests that the chronotropic effect of α - and β -adrenergic receptor activation is being mediated by similar intracellular pathways, but further investigation is needed to clarify this. In any event the results show that even though there are far fewer α -adrenergic receptor compared to β -adrenergic receptor,¹¹⁻¹³ the positive chronotropic effect of the *R. siamensis* extract is mediated *via* both β - and α -adrenergic receptors.

The positive chronotropic and the hypertensive effects of the *R. siamensis* extract may be due to the active component(s) of the *R. siamensis* extract acting directly on the β - and α -adrenergic receptors or indirectly by stimulating the release of the sympathetic catecholamines. To examine this possibility, the rats were pretreated with reserpine to deplete stores of norepinephrine at the sympathetic nerve terminal and catecholamine and epinephrine at the adrenal medulla.¹⁴⁻¹⁶ The absence of norepinephrine and epinephrine in these reserpinized animals would reduce or abolish the positive chronotropic and hypertensive effects of the *R. siamensis* extract, if the active component acted indirectly by stimulating the release of the catecholamines. As shown in Fig. 4, in reserpinized rats, the positive chronotropic and hypertensive effects of the *R. siamensis* extract disappeared and in addition, hypotension instead of hypertension occurred. These results demonstrate that the positive chronotropic and hypertensive effects of the *R. siamensis* extract on the cardiovascular system is an indirect action occurring *via* the release of the catecholamines, norepinephrine and epinephrine, non-specific adrenergic receptors, at the sympathetic nerve endings and at the adrenal medulla. The finding that *R. siamensis* extract caused hypotension in reserpinized rats, also confirms that the *R. siamensis* extract contains some substances other than those that cause vasodilatation.

The effect of the *R. siamensis* extract on the cardiovascular

system in the *in vivo* experiments indicated that a substance in the extract may act peripherally to the cardiovascular tissues, or centrally through the central nervous system. In order to test whether it acts peripherally, further studies were performed *in vitro* on isolated preparations of atria and thoracic aortae. As shown in Fig. 5, the *R. siamensis* extract caused an increase in both the rate of spontaneous contraction of the right atrium, and the strength of the electrical field-stimulated contraction of the left atrium in a dose-dependent manner. These effects were blocked by propranolol (10^{-8} , 10^{-7} M, Fig. 5A). However, the maximal response to *R. siamensis* extract in the presence of propranolol was significantly reduced. Atropine, a non-specific muscarinic receptor antagonist, did not modify the positive chronotropic activity of the *R. siamensis* extract, because when it was added together with propranolol, no significant suppression was found for the chronotropic response of the *R. siamensis* extract. These results indicate that the *R. siamensis* extract did not contain any other active component that acts *via* the muscarinic receptors of the atrium. The finding that the positive inotropic effect of the *R. siamensis* extract on the left atrium was also blocked by propranolol. This result also confirmed that a component of the *R. siamensis* extract acts peripherally on the β -adrenergic receptors of the atrium.

In the above *in vivo* experiment when rats had been pretreated with reserpine, two days before the experiments, the hypertensive and tachycardiac activities of the *R. siamensis* extract were abolished (Fig. 4). This result indicates an indirect effect of the *R. siamensis* extract on the stimulated release of the sympathetic catecholamine from the sympathetic terminals and the adrenal medulla. In order to confirm this effect on the sympathetic nerve terminals, the dose-response curve to the *R. siamensis* extract on isolated atria was studied using the atria from the rat having been pretreated with reserpine. In this case the positive chronotropic and inotropic effects of the *R. siamensis* extract on isolated atria almost disappeared. These results confirm that the positive chronotropic and inotropic effects of the *R. siamensis* extract are likely to be an indirect effect by stimulating the release of the sympathetic neurotransmitters: norepinephrine from sympathetic nerve terminals to act *via* the β -adrenergic receptors of the atria.

The *R. siamensis* extract caused a contraction of the isolated thoracic aortic rings (Fig. 7). This indicates that the hypertensive effect of the *R. siamensis* extract, at least partly, has a direct effect on the blood vessels. In addition, the constrictor responses to the *R. siamensis* extract were potentiated by blocking the nitric oxide synthase with LNA,^{17–19} or by removal of the vascular endothelium. This result indicates that the *R. siamensis* extract may also stimulate release of nitric oxide from the vascular endothelium to attenuate its vasoconstrictor activity.²⁰ Furthermore, even though the rat thoracic aorta is poorly innervated,^{21–23} when the rat was pretreated with reserpine the constrictor responses of the aorta to phenylephrine were enhanced, whether LNA was present or not. This suggests that the receptor is under substantial influence from the sympathetic innervation, and that catecholamine depletion may cause receptor upregulation.²⁴ Thus, the finding that the vasoconstriction response of the *R. siamensis* extract could be suppressed by phentolamine, or by pretreating the animal with reserpine, also indicated that

the *R. siamensis* extract may act indirectly *via* the α -adrenergic receptor in the blood vessels by stimulating release of norepinephrine.

The *R. siamensis* extract also caused a decrease in the mean arterial blood pressure in anesthetized rats after blocking the α - and β -adrenergic receptors with propranolol and phentolamine respectively. This may be caused by a direct effect of the *R. siamensis* extract on the blood vessels inducing vasodilatation. To test this possibility, we did experiments on the vasodilatation of the *R. siamensis* extract on thoracic aortic rings precontracted with phenylephrine. As shown in Fig. 8, the *R. siamensis* extract induced a dose-dependent vasodilatation. However, the vasodilatory activity of the *R. siamensis* extract may be a direct effect of the active component on the vascular smooth muscle, or *via* an indirect pathway that stimulated the release of the nitric oxide from the endothelium and caused vasodilatation. To test these possibilities, further studies were carried out in denuded-thoracic aortic rings, as well as on endothelium-intact aortic rings in the presence of LNA. It was found that the vasodilatory activity of the *R. siamensis* extract on the thoracic aorta was abolished after removal of the vascular endothelium or by blocking the nitric oxide synthase with LNA. In addition, when the thoracic aortic rings have undergone a depletion of their sympathetic neurotransmitters by pretreating the rats with reserpine, two days before the experiments, there was an enhanced sensitivity of the vasodilatory effect of the *R. siamensis* extract. These results indicate that the vasodilatory activity of the *R. siamensis* extract is an indirect effect of the substance acting by stimulating release of the nitric oxide from the vascular endothelium and causing vasodilatation. This effect was clearly shown when the contractile effect produced by a sympathetic neurotransmitter was abolished by pretreating the animal with reserpine that depletes the sympathetic neurotransmitter of norepinephrine. These results indicate that the hypotensive activity of the *R. siamensis* extract in anesthetized rats after blocking the adrenergic receptors is a direct effect of the *R. siamensis* extract on the blood vessels causing vasodilatation by stimulating release of nitric oxide from the vascular endothelium.

These results differ from our previous report on the effect of the pseudoginsenoside-RT₁ on blood pressure and heart rate, when it was found that the pseudoginsenoside-RT₁ caused a decrease in the mean arterial blood pressure with an increase in heart rate in a dose-dependent manner⁶) similar to those produced by isoproterenol, a non-specific β -adrenergic receptor agonist. Whereas in the present study it was found that the *R. siamensis* extract caused an increase in both MAP and heart rate similar to that of adrenaline, whereas the hypotensive activity was found only when the rats were pretreated with phentolamine or phentolamine plus propranolol. This indicates that the positive chronotropic effect and the hypotensive effect are caused by different substances. The reason for these different results could be that the pseudoginsenoside-RT₁, isolated by Aukkanibutra,⁴ was not 100% pure, and may be contaminated with a positive chronotropic substance. As we know that *R. siamensis* extract contains more than one compound that affects blood pressure and heart rate the reason for these different results could be that the pseudoginsenoside-RT₁, isolated by Aukkanibutra, had different proportions of the active substances than did the

preparation we used in these studies.

In conclusion, the present study has demonstrated that *R. siamensis* extract has both a hypertensive and a positive chronotropic effects on the cardiovascular system, by stimulating the release of endogenous catecholamines, most likely from nerve endings and the adrenal medulla to act directly at the α - and β -adrenergic receptors of the heart and the blood vessels. It also has a hypotensive activity, acting directly on the blood vessels to cause vasodilatation by stimulating the release of nitric oxide. The active substance(s) responsible for the hypertensive and positive chronotropic effects are likely to be different from those causing hypotension, since the hypotensive activity persisted after having depleted the sympathetic neuronal transmitters by reserpine. These findings provide scientific support for the traditional uses of the plant in man: e.g. induced abortion which would be caused by stimulated release of endogenous catecholamine producing uterine contraction, and for controlling blood pressure which would be caused by stimulated release of the nitric oxide from vascular endothelium. However, further study is required to identify the active substance(s) responsible for these activities. This could then provide an opportunity to develop new chemically pure cardiovascular drugs with known properties and further confirm the beneficial effects of the Thai therapeutic uses of this plant.

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