Hypotensive Effect of n-butanol Extract from Stem of *Salacia chinensis* in Rats

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Abstract

Dried stems of *Salacia chinensis* were chopped and boiled with filtered water. The clear solution was collected and partition extracted with n-butanol. The n-butanol fraction (*S. chinensis* extract) was evaporated and followed by lyophilization. The *S. chinensis* extract was investigated for hypotensive activity in anesthetized female rats in estrus, and for vasodilator activities on isolated thoracic aortic rings in vitro. Results showed that intravenous injection of *S. chinensis* extract (4-120 mg/kg) caused a decrease in mean arterial blood pressure and heart rate of anesthetized rats in a dose-dependent manner. These effects were not blocked by atropine, a muscarinic receptor antagonist or propranolol, a β-adrenergic receptor antagonist. For the in vitro preparation, the *S. chinensis* extract (0.01-0.3 mg/ml) caused vasodilatation of the thoracic aortic rings pre-constricted with phenylephrine in a dose-dependent manner. These effects persisted in the presence of atropine, propranolol or both of atropine and propranolol. However, when N\(^{\text{G}}\)-nitro-L-arginine, a nitric oxide synthase inhibitor, was added or removal of vascular endothelium, the vasodilator activity of the *S. chinensis* extract on the thoracic aortic ring disappeared. These results suggest that the n-butanol extract from stems of *Salacia chinensis* possesses a hypotensive effect. The mechanism involved may be an indirect effect by stimulated release of nitric oxide from vascular endothelial cells and causes vasodilatation.

INTRODUCTION

*Salacia chinensis* L., or Khampangjed chan in Thai, belongs to the family Celastraceae. It is widely distributed in China, India and Thailand. Stems of *Salacia chinensis*, in combination with some other plants, were used in Thai traditional medicine for “washing out” dirty blood, cardiotonic or blood tonic, and for anti-diabetes (Farnsworth and Bunyapraphatsara, 1992). In China, a decoction of the root part of the plant is taken for anti-menorrhea and anti-diabetes (Duke and Ayensu, 1985). In India, dried roots of the plant are the major ingredient in an Ayurvedic drug which is used for anti-diabetic (Krishnan and Rangaswami, 1967a), lithontriptic and antilipemic agents (Rajurkar and Pardeshi, 1997). Chemical constituents of both the roots and the stems of *Salacia chinensis* have only been studied by Krishnan and Rangaswami to date (1967a and b). They found that the major constituent is proanthocyanidinin (condensed tannin) which is composed of 0.03% leucopelargonidin and 0.2% polyprenoid. These major constituents possess antioxidant (Plumb et al., 1998) and cardioprotective (Sato et al., 1999) properties. However, no study has been done on the pharmacologic activities of this plant extract on the cardiovascular system. Thus, it is of particular interest to evaluate cardiovascular effects of the crude extract of stem parts of *Salacia chinensis* and to elucidate possible mechanisms which might be involved.
MATERIALS AND METHODS

Plant Material and Extraction

Dried stems of *Salacia chinensis* Linn. (10 kg) were chopped into small pieces and simmered in filtered water for a period of 6 hours three times. Clear solution was collected and heated at 50°C to reduce the volume to 50%. The concentrated solution was partition extracted with water-saturated n-butanol. The n-butanol phase was collected and evaporated to dryness in vacuo and lyophilized to obtain a dark brown powder (46.8 g) of *S. chinensis* extract. The *S. chinensis* extract was chemically characterized by HPLC (Hewlett Packard Series 1100), using Waters symmetry C-18, 3.9x150 mm analytical column (Waters Corporation, USA), eluated with gradient concentration of MeOH and H2O in 0.05% TFA at a flow rate of 1 ml/min. The *S. chinensis* extract HPLC fingerprint chromatograms of three different UV wavelengths (254, 210 and 310 nm) are shown in Fig. 1. Analysis of three inorganic ions by IPC-AES analysis method found that the *S. chinensis* extract contained (ppm) Na⁺, 12.83; K⁺, 73.49 and Ca²⁺, 1.89. The relative quantity of each inorganic ion in the *S. chinensis* extract powder was calculated to obtain the same amounts of NaCl, KCl and CaCl₂ to dissolve in distilled water (Electrolyte) for using as a vehicle control in the in vivo study.

Pharmacological Studies of *S. chinensis* Extract

The methods employed in this study were approved by the Prince of Songkla University Animal Care and Use Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals. Female Wistar rats in estrus, weighing 180-250 g were used in the study. The animals were housed at 25°C on a 10 h dark and 14 h light cycle. All rats were allowed access to food and drinking water ad libitum.

For the in vivo experiments, the rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). A polyethylene catheter was cannulated through the right common carotid artery and connected to a pressure transducer and polygraph for monitoring blood pressure and heart rate of the rats. Another polyethylene tube cannulated through the left jugular vein for Electrolyte or drug injections. The animal was then equilibrated for 1 h. The dose-response relationship to equivalent concentration of the above ions (Electrolyte) or the *S. chinensis* extract (4-120 mg/kg) was determined by injection of the drug through left jugular vein before and after pretreatment the animals with (0.6 mg/kg, i.v.) atropine or propranolol.

For the in vitro experiments, the rats were killed by decapitation with a guillotine. Two adjacent rings from the thoracic aorta were cut, and the endothelium removed from one by mechanical disruption 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 contractile force signals were recorded on a polygraph. The organ bath contained Kreb’s Henseleit solution of the following composition (mM): NaCl, 118.3; KCl, 4.7; MgSO₄, 0.45; KH₂PO₄, 1.18; NaHCO₃, 25; CaCl₂, 1.9; Na₂EDTA, 0.024; ascorbic acid, 0.09 and glucose, 11.66, maintained at 37°C, and continuously bubbled with 95% O₂ and 5% CO₂. The tissues were then equilibrated for 60 min under the resting tension 1.0 g. The Kreb’s solution was replaced every 10-20 min.

After equilibration, the presence of the functional endothelium of the thoracic aortic rings were tested as follows. The aortic rings were preconstricted with 3x10⁻⁶ M phenylephrine for 5-8 min (by which time the response had reached a plateau) and dilator responses to 10⁻⁵ M acetylcholine recorded. Eighty to ninety percent vasodilatation to acetylcholine occurred with the endothelium-intact thoracic aorta rings.

After 40 min re-equilibration, cumulative dilator responses to *S. chinensis* extract on the thoracic aortic ring preconstricted with phenylephrine (3x10⁻⁶ M for endothelium-intact aortic ring and 3x10⁻⁷ M for endothelium-denuded aortic) were studied. Following several washings, only the thoracic aortic rings with endothelium-intact were pre-incubated with atropine (10⁻⁶M) for 40 min, and then the cumulative dilator responses to *S. chinensis* extract were performed in the presence of atropine. Following several
washings, the cumulative dilator responses to \textit{S. chinensis} extract were repeated after pre-incubation of the aortic ring with $10^{-7}$ M atropine.

Using the same protocol as above, another three sets of thoracic aortic rings, the cumulative dilator responses to \textit{S. chinensis} extract were performed in the presence of (1) $10^{-8}$ and $10^{-7}$ M propranolol, (2) a combination of atropine ($10^{-7}$ M) and propranolol ($10^{-7}$ M) and after addition of $3 \times 10^{-4}$ M N\textsuperscript{G}-nitro-L-arginine (LNA), or (3) only $3 \times 10^{-4}$ M (LNA).

**Drugs**

The following drugs were used: pentobarbital sodium, atropine sulfate, phenylephrine chloride, DL-propranolol hydrochloride, N\textsuperscript{G}-nitro-L-arginine and acetylcholine chloride which were obtained from Sigma, USA.

**Statistical Analysis**

Vasodilator activities by the \textit{S. chinensis} extract of the thoracic aorta were calculated as a percentage of the induced tension which existed at the start of a relaxation-effect experiment.

Other data are expressed as means $\pm$ s.e. mean of 6-7 experiments (n=6-7), and tests of significance made using 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**

Intravenous injection of the \textit{S. chinensis} extract (4-120 mg/kg), but not of the Electrolyte, caused a decrease in mean arterial blood pressure and heart rate in anesthetized female rats in a dose-dependent manner (Fig. 2). At 120 mg/kg, the highest dosage, the hypotensive effect lasted for $24.2 \pm 3.6$ min and the negative chronotropic effect lasted for $16.5 \pm 4.8$ min. However, at this highest dosage, 2-3 out of 6 rats from each group died after injection. The hypotensive and negative chronotropic effects of the \textit{S. chinensis} extract were not modified by pretreatment of the animals with atropine, a muscarinic receptor antagonist or propranolol, a $\beta$-adrenergic receptor antagonist (Fig. 3).

The \textit{S. chinensis} extract caused vasodilatation of the endothelium-intact thoracic rings pre-constricted with phenylephrine (Fig. 4) in a dose-dependent manner. Complete relaxation was seen at the concentration of $0.3$ mg/ml of the extract. The vasodilator effect of the \textit{S. chinensis} extract was not significantly changed by pretreatment of the blood vessels with atropine, propranolol or a combination of these two antagonists. However, this effect was negated by pre-incubation of the blood vessels with LNA independently whether atropine or propranolol was present or not, or by removal of the vascular endothelium.

**DISCUSSION**

In the Thai Herbal medicine, traditional use of \textit{Salacia chinensis} was prepared as a decoction, which contains both organic and inorganic substances. The present study aims to investigate the hypotensive activity of the organic part of the \textit{S. chinensis} decoction. Thus, the decoction or the concentrated water soluble part of the \textit{S. chinensis} was partition extracted with n-butanol, a very polar water insoluble organic solvent. The n-butanol soluble part was collected, evaporated and lyophilized respectively to obtain the n-butanol extract of \textit{S. chinensis}, which contained little amount of Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+}.

The present study demonstrates that n-butanol extract of \textit{S. chinensis} exerts a hypotensive and negative chronotropic effect in anesthetized rats in estrus. These effects would be solely produced by the active organic substances, not by the Electrolyte, present in the \textit{S. chinensis} extract. Since the electrolyte solution which contained the same amount of those three ions, Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+}, as the \textit{S. chinensis} extract did not show any effects on arterial blood pressure or heart rate in the rats. The dosages of 4-40 mg/kg of the \textit{S. chinensis} extract produced a definite decrease in mean arterial blood pressure and heart rate without toxicological symptoms. The highest dosage, 120 mg/kg, however,
exhibited lethal episodes to about 50% of each group. The reason for this may be an extreme decrease in blood pressure and/or heart rate, or may be produced by some other types of toxicity by any toxic substances present in the *S. chinensis* extract. However, further studies were needed to clarify these possibilities. The hypotensive and negative chronotropic effects of the *S. chinensis* extract were not modified by pre-treatment of the animals with atropine, a muscarinic receptor antagonist or propranolol, a β-adrenergic receptor antagonist. The dosage of atropine or propranolol used were the same as reported by Hopkins and Hodgson (1998). These results suggest that it is unlikely that the hypotensive and negative chronotropic effects of the *S. chinensis* extract were due to the active substances acting through the muscarinic receptors or β-adrenergic receptors in the cardiovascular system.

The *S. chinensis* extract caused a dose-dependent vasodilatation in the endothelium-intact thoracic aortic rings pre-constricted with phenylephrine. This result suggests that the hypotensive activity of the *S. chinensis* extract may be caused by a decrease in peripheral vascular resistance due to the dilatation of the peripheral blood vessels. However, further study would need to clarify whether this hypotensive activity is solely caused by vasodilatation. In the present study we also investigated the mechanisms involved in the vasodilator activity of the *S. chinensis* extract. As shown in the results section, the vasodilator activity of the *S. chinensis* extract was not modified by pre-incubation of the blood vessel with atropine or propranolol or a combination of these two antagonists (10⁻⁸ and 10⁻⁷ M of atropine and propranolol caused a significantly rightward shift of the dose-response curves to acetylcholine and isoproterenol respectively, data not shown). These results confirmed that the vasodilator effect of the *S. chinensis* extract was not acting via the muscarinic or β-adrenergic receptors in the cardiovascular system. As shown in Fig. 4d, the vasodilatation of the *S. chinensis* extract on the endothelium-intact thoracic aortic rings disappeared after removal of the vascular endothelium. This finding suggests that the vasodilator activity of the *S. chinensis* extract on the thoracic aortic rings is endothelium-dependent. Thus, the vasodilatation of the *S. chinensis* extract may be an indirect effect by stimulated release of some vasodilator substances from the vascular endothelium, such as prostacyclin, endothelium hyperpolarizing factor or nitric oxide (Mombouli and Vanhoutte, 1997; Vanhoutte and Mombouli, 1996). In order to prove this possibility, the thoracic aortic rings were pre-incubated with LNA, a specific nitric oxide synthase inhibitor (Dubbin et al., 1990; Salerno et al., 1997; Woodman and Dusting, 1991) before obtaining the dose-response curve to the *S. chinensis* extract. As shown in Fig. 4c and 4d no vasodilatation occurred for the thoracic aortic rings pre-inbubated with LNA whether atropine and propranolol were presented or not. These results suggest that the vasodilatation of the *S. chinensis* extract is an indirect effect caused by stimulated release of nitric oxide from the vascular endothelium which then caused vasodilatation. It is unlikely that prostacyclin or EDHF contributed for this vasodilator activity of the *S. chinensis* extract. The reason is that if prostacyclin or EDHF play a part, a certain degree of vasodilation would persist after blocking the nitric oxide synthase of the blood vessel by the LNA.

In conclusion, the n-butanol extract of *S. chinensis* possesses hypotensive and negative chronotropic effects. These cardiovascular responses may well account for the beneficial effects of *S. chinensis* in traditional medicines. However, the n-butanol extract of *S. chinensis* is a mixture of many pharmacologically active components, and further study for the isolation of pure compounds to determine their chemical structure and evaluation of their pharmacological actions are required.

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Literature Cited
Figures

Fig. 1. HPLC fingerprint chromatograms of three different UV wavelengths: 254 nm (a), 210 nm (b) and 310 nm (c) of the *S. chinensis* extract.

Fig. 2. Effects of *S. chinensis* extract and Electrolyte on mean arterial blood pressure (a) and heart rate (b) in anesthetized rats. Each point represents the mean ± s.e. mean of 6 experiments (n=6). * significantly lower than those with Electrolyte injection.
Fig. 3. Effects of atropine (Atro, a and b) or propranolol (Propra, c and d) on the decrease in mean arterial blood pressure and heart rate produced by *S. chinensis* extract in anesthetized rats. Each point represents the mean ± s.e. mean of 6-7 experiments (n=6-7).
Fig. 4. Effects of atropine (Atro, a), propranolol (Propra, b), atropine and propranolol (Atro+Propra, c), N\textsuperscript{G}-nitro-L-arginine (LNA) or the endothelium (d) on the vasodilator activity of the *S. chinensis* extract on the thoracic aortic rings pre-constricted with phenylephrine (3x10\textsuperscript{-6} M). Each point represent the mean ± s.e. mean of 6 experiments (n=6). * significantly lower than those with LNA or those without endothelium (No endo).