Effects of an n-butanol extract from the stem of *Tinospora crispa* on blood pressure and heart rate in anesthetized rats

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**Abstract**

Ethnopharmacological relevance: *Tinospora crispa* has been used in folkloric medicine for control of blood pressure, as an antipyretic, for cooling down the body temperature and for maintaining good health.

**Aim of the study:** To investigate the effects and mechanisms of action of an n-butanol extract from the stems of *Tinospora crispa* (*T. crispa* extract) on blood pressure and heart rate in anesthetized rats.

**Materials and methods:** Air-dried stems of *T. crispa* were extracted with water, followed by partitioned extract with chloroform, ethyl acetate, and finally by n-butanol. The n-butanol soluble part was evaporated under reduced pressure and lyophilization to obtain a crude dried powder (*T. crispa* extract). The effects and mechanisms of the *T. crispa* extract on blood pressure and heart rate were studied in anesthetized normal and reserpinized rats in vivo in the presence of different antagonists.

**Results:** *T. crispa* extract (1–100 mg/kg, i.v.) caused a decrease in mean arterial blood pressure (MAP) and this effect was inhibited by propranolol, phentolamine, atenolol and/or the β2-antagonist ICI-118,551, but not by acetylcholine or hexamethonium. In reserpinized rats, the *T. crispa* extract had a dual effect: reduction in hypotensive activity, followed by a small increase in blood pressure. The decrease in MAP in reserpinized rat was slightly potentiated by phentolamine, but inhibited by propranolol or ICI-118,551 only if atenolol and phentolamine were also present. The increase in MAP was potentiated by propranolol and ICI-118,551, but was not inhibited by phentolamine. The *T. crispa* extract had a dual effect on heart rate in the normal rat: a small transient decrease, followed by an increase in heart rate. The positive chronotropic effect of *T. crispa* extract was inhibited by propanolol, phentolamine and atenolol, but not by ICI-118,551, atropine or hexamethonium. Reserpine potentiated the positive chronotropic effect of the *T. crispa* extract and this effect was inhibited by propranolol, atenolol and ICI-118,551, but not by phentolamine.

**Conclusions:** From these results we suggest that *T. crispa* extract possesses at least three different cardiovascular-active components that act directly via (1) β2-adrenergic receptors to cause a decrease in blood pressure, and β1- and β2-adrenergic receptors to cause an increase in heart rate, (2) α-adrenergic receptors to cause an increase in blood pressure and heart rate, and (3) a non-adrenergic and non-cholinergic pathway to cause a decrease in MAP and heart rate. These findings provide scientific support for the tradition of using this plant to modify the actions of the human cardiovascular system.

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1. Introduction

*Tinospora crispa* (L.) Miers ex Hook. f. & Thoms (*Tinospora rumphii* Boerl or *Tinospora tuberculata* Beumee), Thai name: Borapet, belongs to the family Menispermaceae. It is found in primary rainforests or mixed deciduous forests throughout a large part of Asia and Africa (Pathak et al., 1995), including all parts of Thailand, Malaysia and Indonesia. In Thai traditional medicine, a decoction from the stems of *Tinospora crispa* has been used as an antipyretic, for treating internal inflammations, reducing thirst, increasing appetite, cooling down the body temperature and for maintaining good health (Kongsaktrakoon et al., 1994; Dweck and Cavin, 2006). In Indonesia (Borneo) it has been used to treat diabetes, hypertension, and lumbago (Dweck and Cavin, 2006). However, scientific
investigations to test these therapeutic claims are very scarce. Mokkhasmit et al. (1971) conducted a pharmacological screening of some Thai medicinal plants and found that a crude alcohol extract from stems of *Tinospora crispa* caused an increase in blood pressure with a decrease in heart rate in anesthetized dogs. Later Kongkathip et al. (2002) isolated cycloeucalenone and cycloeucalenol from its crude hexane and chloroform extracts respectively, and found that only cycloeucalenol had an effect on the force of atrial contraction by causing an increased contraction of the right atrium, but a decreased contraction of the left atrium.

The chemical constituents of *T. crispa* extracts have been extensively studied since 1983. They have been identified as the terpenoids and terpenoid glycosides: borapetol A, borapetol B, borapetoside A, B, C, D, E, F and tinocrisiposide, the bitter components of *Tinospora crispa*; alkaloids: N-formylannonain, N-formylanondine, N-formylnornuciferine, N-acetyl nornuciferine, and others; β-sitosterol, picrotein, tinotubride, N-trans-feruloyl tyramine, N-cis-feruloyl tyramine, cycloeucalenol, cycloeucalenone and cis-clerodane-type furanoditerpenoids (Bisset and Nwaiwu, 1983; Fukuda et al., 1983, 1985, 1986; Pathak et al., 1995; Martin et al., 1996; Cavin et al., 1998; Kongkathip et al., 2002; Choudhary et al., 2010). However, pharmacological studies on most of these substances have not yet been seriously investigated.

The effects of *Tinospora crispa* on the cardiovascular system mentioned above, led us to carry out a preliminary study of an n-butanol fraction (*T. crispa* extract) that had been extracted from the stem decoction on blood pressure and heart rate in anesthetized rats. We found that the *T. crispa* extract (10–30 mg/kg, n=3) caused a decrease in mean arterial blood pressure with an increase in heart rate in a dose-dependent manner. Thus, in the present study, we aimed to investigate the effects of the *T. crispa* extract on the cardiovascular system and elucidate the mechanisms involved in its hypotensive and positive chronotropic effects. Studies were performed with anesthetized rats in vivo using pharmacological methods. We have explored the possibilities that the active component(s) interact via the peripheral adrenergic receptors and/or muscarinic cholinergic receptors of the cardiovascular system, or perhaps via central mechanisms. The dose–response relationships of the *T. crispa* extract on blood pressure and heart rate in normal rats were studied before and after blocking the autonomic receptors with adrenergic receptor antagonists, and a muscarinic cholinergic antagonist or a ganglion blocking agent, respectively. In order to investigate whether its action is on a presynaptic site, the effects of the *T. crispa* extract were studied in reserpine-primed rats where the store of norepinephrine at the sympathetic nerve terminals and of the catecholamine and epinephrine at the adrenal medulla had been depleted (Temma et al., 1977; Weiner, 1985; Taesotikul et al., 1998).

2. Materials and methods

2.1. Plant material

Stems of *Tinospora crispa* (10 kg) were collected from Phangnga Province, Thailand. Botanical identification of the plant was carried out by Prof. Poungpen Sirirugsa, Department of Biology, Prince of Songkla University, Thailand, and a voucher specimen of the plant material has been deposited there.

2.2. Preparation of *T. crispa* extract

Air-dried stems of *Tinospora crispa* (10 kg) were simmered in hot filtered water for a period of 3 h. The clear solution was collected and heated at 50 °C to reduce the volume to 30%. The concentrated solution was partitioned extracted with chloroform, followed by ethyl acetate, and finally by n-butanol. The n-butanol soluble part was evaporated under reduced pressure, and the residue was lyophilized to obtain 105.3 g of a crude brown powder (*T. crispa* extract, yield about 0.011%).

The *T. crispa* extract as well as known catecholamines and derivatives: epinephrine, norepinephrine, pseudoephedrine and tyramine were analyzed by High Performance Liquid Chromatography (HPLC) in order to obtain a chemical profile. 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, 150 mm × 3.9 mm i.d.; Waters), with a gradient of MeOH: H₂O+0.05% of trifluoroacetic acid (5:95 → 100:0). The flow rate was 1 ml/min; the UV traces were measured at 210 and 254 nm and the UV spectra (DAD) were recorded between 190 and 500 nm. The HPLC chromatograms together with the corresponding UV spectra of the *T. crispa* extract and catecholamine and its derivative are shown in Fig. 1. The UV spectra of peaks 4 and peak 6 show the same pattern as that of the epinephrine with a difference in retention times. The UV spectra of peaks 8, 9 and 10 show the same patterns as that of borapetoside, the bitter component of an extract of *Tinospora crispa* (Cavin et al., 1998).

2.3. Pharmacological studies of the *T. crispa* extract on blood pressure and heart rate

Adult female Wistar rats in estrus (220–280 g) were supplied from the Animal House, Faculty of Science, Prince of Songkla University. They were maintained in a controlled environment (24–26 °C), with a 12 h light/dark cycle and allowed access to standard food and tap water ad libitum. The preparation of the animals followed the Prince of Songkla University guidelines for the approved Care and Use of Experimental 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 was connected to a Grass polygraph (Model 7D, Grass Instrument, Quincy, MA). 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 *T. crispa* extract were determined by intravenous injection of a volume not exceeding 0.1 ml for each dose into the left jugular vein and flushed in with 0.1 ml saline. Each rat was used only once.

Using sets of animals that had been separated, after equilibration of the animal for 40 min, atenolol (2 mg/kg, Hu et al., 1996; Dabire et al., 1998), propranolol (0.6 mg/kg, Ferrari et al., 1987), phentolamine, (2 mg/kg, Tung et al., 1982), ICI-118,551 (0.01 mg/kg, Alvarez-Guerra et al., 1997), atropine (0.6 mg/kg, Ferrari et al., 1987) or hexamethonium chloride (10 mg/kg, Dabire et al., 1998) alone or in various combination, were first injected through the left jugular vein. After 30 min re-equilibration, the dose–response relationship to the *T. crispa* extract was again determined.

With other sets of animals, rats were pretreated with reserpine at a dose of 5 mg/kg, i.p., once a day, starting two days before the experiment. Thereafter the dose–response relationships to *T. crispa* extract were determined in the absence or presence of atenolol, propranolol, phentolamine and/or ICI-118,551 using the same protocol as above.
Fig. 1. HPLC chromatogram with the UV spectra of *T. crispa* extract and some known catecholamines: epinephrine, tyramine, hordenine and pseudoephedrine. Detection was at 210 and 254 nm.
2.4. Drugs

The following drugs were used: acetylcholine chloride, atropine sulphate, atenolol, hexamethonium chloride, isoproterenol, phentolamine hydrochloride, phenylephrine hydrochloride, propranolol hydrochloride, pseudoeedrine, reserpine and tyramine were purchased from Sigma, USA, hordenine was purchased from ChromaDex, ICI-118,551 was purchased from Tocris Bioscience, UK. Atenolol, ICI-118,551 and the *T. crispa* extract were dissolved in distilled water, reserpine was dissolved in 10% DMSO, and the remainder were dissolved in a solution containing NaCl 9 g/l, NaH₂PO₄ 0.19 g/l and ascorbic acid 0.03 g/l.

2.5. Data analysis

Data are expressed as means ± S.E.M. (n = 6 animals). The mean arterial blood pressure (MAP) was calculated as diastolic + [(systolic – diastolic)/3]. Tests of significance were made using the Student’s unpaired t-test. In all cases, a p value of 0.05 or less was considered statistically significant.

3. Results

The basal mean arterial blood pressure (MAP) and heart rate of anesthetized normal and reserpinized rats before and after blocking with the adrenergic or muscarinic receptor antagonists, or with the ganglion blocking agent are shown in Table 1. *T. crispa* extract (1–10 mg/kg, i.v.) caused a decrease in the MAP in anesthetized rats in a dose-dependent manner. This effect was significantly inhibited by propranolol (0.6 mg/kg), a non-specific β-adrenergic receptor antagonist or phentolamine (2 mg/kg), a non-specific α-adrenergic receptor antagonist. When propranolol and phentolamine were given at the same time, the *T. crispa* extract caused no further reduction in blood pressure was found (Fig. 2a, c and e). ICI-118,551 (0.01 mg/kg), a specific β₁-adrenergic receptor antagonist, significantly inhibited the hypotensive effect of the *T. crispa* extract. In contrast, atenolol (2 mg/kg), a specific β₁-adrenergic receptor antagonist, slightly inhibited the hypotensive effect of the *T. crispa* extract. When phentolamine and atenolol were given together, the hypotensive effects of the *T. crispa* extract were restored to the same level as that of the control group. However, when ICI-118,551 was also given together with phentolamine and atenolol, the hypotensive effect of the *T. crispa* extract was again inhibited (Fig. 3g). Atropine (0.6 mg/kg), a muscarinic cholinergic receptor antagonist, or hexamethonium chloride (10 mg/kg), a ganglion blocking agent, did not significantly modify the dose–response curves of the *T. crispa* extract (Fig. 4a and c).

When the animals had been pretreated with reserpine, a dual effect of the *T. crispa* extract on the blood pressure was found: a reduction in hypotensive activity, followed by a small increase in blood pressure (Fig. S2, Supplementary data). For the first phase of the response (hypotensive activity) phentolamine caused a slight enhancement of the hypotensive activity, whereas propranolol caused a further increase in the hypertensive effect, which was abolished when ICI-118,551 was also given together with atenolol and phentolamine (Fig. 6e and g).

For the second phase of the response the increase in blood pressure (hypertensive effect) was inhibited by phentolamine, whereas propranolol caused a further increase in the hypertensive effect.
Fig. 2. Effects of propranolol (prop) and/or phentolamine (phento) on the decrease in the mean arterial blood pressure (MAP, left), and the positive chronotropic effect (increase in HR, right) of T. crispa extract in anesthetized rats. Each point represents a mean±S.E.M. of 6 animals. *Significantly higher than the control group, $p<0.05$, †significantly higher than the one with propranolol and/or phentolamine, $p<0.05$.

and this potentiation was not significantly inhibited by additional injection of phentolamine, except that the highest concentration of the T. crispa extract (Fig. 7a). ICI-118,551 significantly enhanced the hypertensive activity, whereas atenolol tended to cause a slight inhibitory effect on the increase in blood pressure ($p=0.08$, Fig. 7b). However, when phentolamine was also given with atenolol there was a significant inhibitory effect (Fig. 7c), and the inhibitory effect disappeared when ICI-118,551 was also given (Fig. 7d).

The T. crispa extract had a dual effect on the heart rate: a transient decrease followed by an increase in the heart rate in a dose-dependent manner (Figs. S1 and S2, Supplementary data). The positive chronotropic effect of the T. crispa extract was significantly inhibited by propranolol and phentolamine, but not by ICI-118,551, atropine or hexamethonium (Figs. 2–4). Atenolol significantly inhibited the positive chronotropic effect of T. crispa extract, and a further inhibitory effect was found when phentolamine was also given, but no further modification was obtained when ICI-118,551 was also given.

Reserpine potentiated the positive chronotropic effect of the T. crispa extract, and this effect was significantly inhibited by propranolol, ICI-118,551 and atenolol, but not by phentolamine (Figs. 5 and 6). When phentolamine was given together with atenolol no further inhibition was found (maximal responses: 27±6.96 bpm). However, when ICI-118,551 was given together
Fig. 3. Effects of atenolol (atenol), phentolamine (phento) and/or ICI-118,551 (ICI) on the decrease in the mean arterial blood pressure (MAP, left), and the positive chronotropic effect (increase in HR, right) of *T. crispa* extract in anesthetized rats. Each point represents a mean ± S.E.M. of 6 animals. *Significantly higher than the control group, $p<0.05$, †significantly higher than the ones in the presence of atenolol alone, or in combination with phentolamine and ICI-118,551, $p<0.05$. 

Normal Rat

**A**
- *T. crispa* extract
- $\pm$ ICI (0.01 mg/kg)

**B**
- *T. crispa* extract
- $\pm$ ICI (0.01 mg/kg)

**C**
- *T. crispa* extract
- $\pm$ Atenol (2 mg/kg)

**D**
- *T. crispa* extract
- $\pm$ Atenol (2 mg/kg)

**E**
- *T. crispa* extract
- $\pm$ Atenol + phento

**F**
- *T. crispa* extract
- $\pm$ Atenol + phento

**G**
- *T. crispa* extract
- $\pm$ Atenol + phento + ICI

**H**
- *T. crispa* extract
- $\pm$ Atenol + phento + ICI
with both phenotolamine and atenolol, a further inhibition of the positive chronotropic effect of the *T. crispa* extract was obtained (maximal responses: $11.43 \pm 4.22$ bpm, Fig. 6f and h). The transient decrease in heart rate of the *T. crispa* extract is dose-dependent and this effect was not significantly modified by atropine or hexamethonium (Fig. 8).

**4. Discussion and conclusions**

The present study demonstrates that a *T. crispa* extract exerts a hypotensive activity, and this supports the traditional use of this plant for treatment of hypertension. The finding that *T. crispa* extract also caused a transient decrease followed by an increase in heart rate indicated that the *T. crispa* extract might contain several active substances (as shown on the HPLC fingerprint in Fig. 1) that might have direct effects on the heart rate. In order to reveal these possibilities as well as to identify the mechanisms that would be responsible for these effects, studies were carried out on both normal and reserpinized rats in vivo in the presence of different receptor antagonists.

**4.1. Effects on blood pressure**

Propranolol or phenotolamine alone or in combination caused only partial inhibition of the hypotensive effect of the *T. crispa* extract. This indicated that the *T. crispa* extract might contain at least 3 different active components: (1) a hypertensive component acting via $\alpha$-adrenergic receptors, (2) hypotensive and hypertensive components that acted via the $\beta$-adrenergic receptors, and (3) a hypotensive component that did not act via the $\alpha$- or $\beta$-adrenergic receptors. In order to confirm that the active component that acted via the $\beta$-adrenergic receptors worked differently between the $\beta_1$- and $\beta_2$-adrenergic receptors, further experiments were carried out in the presence of ICI-118,551 or atenolol alone, or in combinations with phenotolamine. ICI-118,551 significantly inhibited the hypotensive effect of the *T. crispa* extract. Atenolol alone slightly inhibited the hypotensive activity of the *T. crispa* extract, this inhibitory effect disappeared in the presence of phenotolamine, which then reappeared when ICI-118,551 was also given to the same rat. These results suggest that the hypotensive effect of the *T. crispa* extract is most likely due to the active component acting via the $\beta_2$-adrenergic receptors and that the hypertensive effect acts via the $\beta_1$-adrenergic receptors of the cardiovascular system.

The finding that atropine did not modify the dose–response relationship to the *T. crispa* extract, indicated that the hypotensive effect of the *T. crispa* extract is unlikely to act via the muscarinic receptors of the cardiovascular system. Also hexamethonium did not inhibit the hypotensive effect of the *T. crispa* extract, suggesting that the response was a direct effect at the periphery, and not indirect via the central nervous system.

In further experiments we examined whether the adrenergic component of the *T. crispa* extract acted directly at the postsynaptic receptors of the cardiovascular system, or indirectly by a stimulated release of the sympathetic neurotransmitters and the neurohormones. In order to examine these possibilities, the rats
Fig. 5. Effects of reserpine and phentolamine (phento) or propranolol (prop) on the decrease in the mean arterial blood pressure (MAP, left), and the positive chronotropic effect (increase in HR, right) of T. crispa extract in anesthetized rats. Each point represents a mean ± S.E.M. of 6–8 animals. *Significantly higher than their corresponding control group, p < 0.05, †significantly lower than control group, p < 0.05.

were pretreated with reserpine to deplete neurotransmitters at the sympathetic nerve terminals and catecholamine and epinephrine at the adrenal medulla. It was expected that in the absence of norepinephrine and epinephrine, in these reserpinized animals, there should be an enhancement of the hypotensive effects of the T. crispa extract if the active component has a direct effect at the post-synaptic adrenergic receptor site due to the supersensitivity of its receptors according to an up-regulation of the receptors to compensate for the depletion of the pre-synaptic adrenergic neurotransmitter (Chess-Williams et al., 1987). On the other hand, these activities would disappear if it worked indirectly by stimulating the release of the pre-synaptic neurotransmitters. In the present study, T. crispa extract produced a dual effect on the blood pressure in the reserpinized rats: a reduction of the hypotensive effect, followed by an increase in the blood pressure. The reduction of the hypotensive effect of the T. crispa extract in reserpinized rats indicated that the adrenergic hypotensive and hypertensive components of the T. crispa extract acted directly at the post-synaptic adrenergic receptors of the cardiovascular system. The reason for this could be that a post-synaptic supersensitivity of the adrenergic receptors due to depletion of the neurotransmitters by reserpine, was partly counteracted by the adrenergic hypertensive component and partly by a hypotensive component of the T. crispa extract acting by a non-adrenergic pathway.
Fig. 6. Effects of atenolol (atenol), phentolamine (phento) and/or ICI-118,551 (ICI) on the decrease in the mean arterial blood pressure (MAP, left), and the positive chronotropic effect (right) of T. crispa extract in anesthetized reserpinized rats. Each point represents a mean ± S.E.M. of 6 animals. *Significantly lower than the control group, p < 0.05.
Phentolamine caused a slight enhancement of the hypotensive activity, whereas propranolol significantly inhibited the hypotensive activity of the *T. crispa* extract in reserpinized rats. This confirmed that the adrenergic component of the *T. crispa* extract acted directly at post-synaptic α-adrenergic receptors to cause hypertension and at β₂-adrenergic receptors, probably via β₂-adrenergic receptors, to cause a hypotension. To reveal this possibility, the reserpinized rats were pretreated with ICI-118,551 and atenolol alone, or in combination with phentolamine. ICI-118,551 caused a slight inhibition of the hypotensive effect of the *T. crispa* extract, whereas atenolol caused a slight enhancement of the hypotensive effect although neither reached statistical significance. However, when the rats were pre-treated with both atenolol and phentolamine, a substantial enhancement of the hypotensive effect was found, and finally when ICI-118,551 was also given, the hypotensive effect of the *T. crispa* extract was restored to the same level as that of the reserpinized control group. These results indicate that the adrenergic component of the *T. crispa* extract acted directly via the α-adrenergic receptors to cause an increase of the blood pressure, as well as via the β₂-adrenergic receptors to cause...
a decrease in the blood pressure. Therefore, the hypotensive effect of the T. crispa extract would result from the concerted effect of the non-adrenergic hypotensive component and the adrenergic hypotensive and the hypertensive components.

Phentolamine significantly inhibited the small increase in blood pressure that occurred during the second phase of the response to T. crispa extract in reserpinized rat, suggesting that this effect might be due to the active component acting via the α-adrenergic receptor of the blood vessel to cause an increase in blood pressure. In contrast ICI-118,551 significantly potentiated the hypertensive effect, whereas atenolol did not significantly inhibit the hypertensive effect except when phentolamine was also given. Furthermore, when ICI-118,551 was also given together with atenolol and phentolamine, the hypertensive effect was restored to the level that was not different from the one without any antagonists. From these results we suggest that the adrenergic hypertensive effect would act via the α-adrenergic receptor and this was slightly attenuated by the hypotensive component that was acting via the β2-adrenergic receptors at the post-synaptic site of the cardiovascular system. Propranolol, a non-specific β-adrenergic receptor antagonist that blocks both β1- and β2-adrenergic receptors, caused a dramatic enhancement of the hypertensive effect of the T. crispa extract in a reserpinized rat and this effect was not significantly inhibited by phentolamine (except at the highest dosage). These results are different from those after being blocked by atenolol, a specific β1-adrenergic receptor together with ICI-118,551, a specific β2-adrenergic receptor. From this we suggest that the potentiation by propranolol indicated that propranolol itself might have some stimulating effect via the central nervous system since propranolol is able to pass through the blood brain barrier. However, further studies are required to clarify this possibility.

4.2. Effects on heart rate

Propranolol and/or phentolamine inhibited the positive chronotropic effect of the T. crispa extract. This indicated that the component that produced the positive chronotropic effects of the T. crispa extract would probably act via the β1- and α-adrenergic receptors of the heart. Atenolol significantly inhibited the positive chronotropic effect of T. crispa extract, and when phentolamine was also added with atenolol, a further reduction was found. This indicated that the positive chronotropic component was acting via the β1- and α-adrenergic receptors of the cardiovascular system. ICI-118,551 alone, or when given together with atenolol and phentolamine did not significantly modify the positive chronotropic effect of the T. crispa extract to confirm that the positive chronotropic component might not act via the β2-adrenergic receptors.

Atropine or hexamethonium did not modify the dose–response relationship of the T. crispa extract. This indicated that the positive chronotropic effects of the T. crispa extract might not involve the muscarinic receptors of the heart, or may act indirectly via the central nervous system. Reserpine caused a dramatic potentiation of the positive chronotropic effect of the T. crispa extract, indicating that the positive chronotropic effects of the T. crispa extract occurred directly via the post-synaptic receptors. In order to reveal whether the positive chronotropic components of the T. crispa extract acted via the adrenergic receptors, sequential experiments were carried out on the reserpinized rats that had been pretreated with propranolol, phentolamine, ICI-118,551, atenolol, or a combination of these antagonist. Propranolol itself caused a parallel shift of the dose–response curve of the T. crispa extract to the right. This indicated that the positive chronotropic effect was via the β-adrenergic receptors of the heart. Phentolamine did not significantly inhibit the positive chronotropic effect of the T. crispa extract. This indicated that the participation of the α-adrenergic receptors in the positive chronotropic effect of the T. crispa extract was not a direct effect. For the atenolol pretreated reserpinized rats, the positive chronotropic effect of the T. crispa extract was dramatically inhibited. When phentolamine was also given together with atenolol, no further modification of the dose–response curve was found. However, when ICI-118,551 was also given together with atenolol and phentolamine to the same rat, a further reduction in the positive chronotropic effect was found. These results indicate that the positive chronotropic effect of the T. crispa extract would be caused by the active component acting directly via the β1- and β2-adrenergic receptors at the post-synaptic site of the heart.

The finding that atropine or hexamethonium did not significantly modify the transient negative chronotropic dose–response curve of the T. crispa extract indicated that the muscarinic receptors are unlikely to be involved in the negative chronotropic effect of the T. crispa extract, and also this effect would be a direct effect of the active substance acting on the periphery of the cardiovascular system and not via the central nervous system.

4.3. Constituents of Tinospora crispa

Known cardiovascular active substances that have been isolated from stems of Tinospora crispa are cis- and trans-feruloyl tyramine (Fukuda et al., 1983), analogues of tyramine that mediate release of catecholamine from sympathetic nerves (Temma et al., 1991; Khwanchuea et al., 2008). Furthermore, our results demonstrate other unknown substances that are present at the peak 4 and 6 fractions from the HPLC chromatogram of the T. crispa extract (Fig. 1). They have UV spectra pattern similar to that of epinephrine, and might be responsible for these effects. Further studies using bioactive direct fractionation to isolate the active substances responsible for the activity are in progress. 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.

In conclusion, the present study has clearly demonstrated that T. crispa extract exerts a hypotensive activity, as well as both negative and positive chronotropic effects in anesthetized rats. The hypertensive effect was shown only when the store of norepinephrine had been depleted by reserpine. The results imply that the mechanisms responsible for these are associated with the presence of at least three different active components each acting separately via: (1) the β2-adrenergic receptors to cause a decrease in blood pressure, and the β1- and β2-adrenergic receptors of the cardiovascular system resulting in an increase of the heart rate, (2) the α-adrenergic receptors to cause an increase in blood pressure and heart rate, and (3) some other pathway besides the adrenergic or muscarinic receptors to cause a decrease in heart rate and/or decrease in blood pressure. Although the T. crispa extract contains many different substances, some of which produce both synergistic and antagonistic effects on the cardiovascular system and act through different mechanisms, the net effect in the normal rat is for a decrease in blood pressure and increase in heart rate. These findings provide scientific support for the tradition of using this plant to modify the actions of the human cardiovascular system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jep.2010.10.052.

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