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# 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 $\beta_2$-antagonist ICI-118,551, but not by atropine 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 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 propranolol, 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) $\beta_2$-adrenergic receptors to cause a decrease in blood pressure, and $\beta_1$- and $\beta_2$-adrenergic receptors to cause an increase in heart rate, (2) $\alpha$-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.


## 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

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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 and F, tinocrisposide, 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 reserpinized 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 partition 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, hordenine, 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® $C_{18}$ column (5 μm, 150 mm × 3.9 mm i.d.; Waters), with a gradient of MeOH: $H_2O$ + 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 peak 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.