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Foreword

It is quite a commendable feat that within a short span of time since its last issue, the University Publication Centre (UPENA) of UiTM Pulau Pinang has produced its sixth volume of the Esteem Academic Journal UiTM Pulau Pinang. Of course, this issue would not come into fruition if not for the firm commitment and close cooperation of all the relevant parties involved.

First and foremost, I would like to extend my thanks to Associate Professor Mohd Zaki Abdullah, Director of UiTM Pulau Pinang, Associate Professor Dr Mohamad Abdullah Hemdi, Deputy Director of Academic Affairs and Associate Professor Ir. Damanhuri Jamalludin, Deputy Director of Research, Industry Linkages, Development & Maintenance for offering their continuous and untiring support. They were the driving force behind the successful publication of this journal. Time and again they rendered invaluable advice on how to address the problems that UPENA encountered in the publication of this academic journal.

UPENA highly appreciates the comments and expertise proffered by the panel of external reviewers when articles in this journal were sent to them for blind reviews. Likewise, UPENA also salutes the dedicated panel of language editors for their time in editing the authors' manuscripts.

However, all the assistance tendered would have been a futile effort if there were no authors willing to submit their articles for publication. This journal comprises articles on the social sciences and technology disciplines. I am proud to state that there is no shortage of writers from Penang and the response from them in these two disciplines has been overwhelming.

Lastly, I would like to urge more lecturers to submit their articles to UPENA. Authors' contributions of articles in this refereed journal help to disseminate and share knowledge with readers. It also helps to elevate the status of UiTM Pulau Pinang in research writing. In return, the authors gain recognition from the wider audience and also consideration for promotion in their career. It is a win-win situation for both parties. So lecturers, what are you waiting for? Put on your thinking caps and start contributing your research articles to UPENA.

Liaw Shun Chone
Chief Editor
ESTEEM Vol. 6, No. 2, 2010
(Social Sciences and Technology)

The Effect of *Tinospora crispa* Extracts on the Contraction of Isolated Atrium and Aorta of Rats

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ABSTRACT

In the present study, the cardiovascular effect of Tinospora crispa extracts on the isolated paced left atrium, spontaneously beating right atrium and the descending aorta of rats were studied. The stems of T. crispa were serially extracted with petroleum ether, followed by chloroform, methanol and lastly water. The chloroform extract caused the strongest inhibition on the inotropic effect of isoprenaline (ISP) to the left atrium. The inhibition was non-competetive since the maximum of the log dose-response curve of the atrium to ISP was reduced. However, none of the extracts (0.2 – 1.0 mg/mL) significantly influenced the inotropic activity of the right atrium to ISP. All the extracts studied also non-competetively inhibited the noradrenaline (NA)-induced rat aortic strips contraction and the chloroform extract was again found to be the most potent extract. The chloroform extract was therefore fractionated and the effect of the fractions on the NA-induced rat aortic strips contraction was examined. CF3 fraction was found to be the most active fraction and was further fractionated. The fractions were tested again on the NA-induced rat aortic strips contraction. However, all fractions showed almost the same effect where 0.50 mg/mL fractions reduced the maximum response by about 60-80%.

Keywords: *Tinospora crispa, left atrium, right atrium, aorta, anti-hypertensive, isoprenaline, noradrenaline*

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Introduction

Throughout most of South-East Asia, *Tinospora* species are widely acclaimed source of remedies for many different complaints. *Tinospora crispa* Miers (Menispermaceae) which is recognised by its slender tubercled stems, is well known in their use for treating skin complaints, malaria, bacterial abscess, high blood pressure and diabetes (Burkill, 1935; Fasihuddin and Hasmah, 1993). The plant is commonly known as seruntum in Malaysia and ratnawali in Brunei (Goh et al., 1995). There has been a steady interest in the chemical nature of the active principles contained in *Tinospora* species. They appeared to be protoberberine alkaloids and related bases (Lian and Feng, 1982). Although few pharmacological studies have been reported (Shahimi and Mohsein, 1982; Hamdan and Ashcroft, 1989; Hamdan et al., 1989), this plant has not been subjected to systematic pharmacological anti-hypertensive evaluation to support its use. Through the years, very few experiments were conducted to identify the compound responsible for anti-hypertension and the mechanism of action in *T. crispa* (Kongkathip et al., 2002; Yokozawa et al., 2001). The present study, therefore, was carried out to evaluate the effect of *T. crispa* plant extract on the inotropic activity of isolated left and right atria of the heart and vasodilating activity on the aorta of rats, which are among the mechanisms of actions involved in lowering blood pressure.

Material and Methods

Identification of Plant Material and Preparation of Extract

The stems of *T. crispa* Miers were collected from Penang Island, Malaysia. This plant had been identified and voucher specimen was deposited in the herbarium of the School of Pharmaceutical Sciences, Universiti Sains Malaysia.

The air-dried ground stems (4.5 kg) was serially extracted with petroleum ether (60-80°C), chloroform, methanol and water. The marc left after each extraction was completely dried from the previous solvent of extraction. The resulting extracts were filtered and the filtrates were evaporated under reduced pressure (Rotavapor, Buchi).

Fractionation of the chloroform extract was carried out using dry-column flash chromatography (Sharp et al., 1989) with various

combination of n-hexane/chloroform followed by chloroform/methanol as the mobile phase.

Pharmacological Evaluation

Isolated Rat Atrium

Male Sprague-Dawley rats weighing 220-250 g were killed by the process of stunning and exsanguinating them. The chest was opened, the heart quickly removed and cleaned of all adhering tissue. The left atrium and the spontaneously beating right atrium were isolated and immersed in a 10 mL tissue bath filled with modified Krebs solution kept at 37 °C and aerated with 95% O_2 and 5% CO_2 (Wohl et al., 1967; Anon, 1970). The Krebs solution had a content of (mM): NaCl (118.93), KCl (4.69), $NaHCO_3$ (25), KH_2PO_4 (1.03), $MgSO_4$ (1.18), glucose (10.09) and $CaCl_2$ (3.15). Precautionary steps were taken to ensure no damages were done on the sinoatrial nodes, the right atrium pacemaker. One end of each atrium was attached to a hook at the bottom of the organ bath and the other end connected to a transducer for isometric recordings by Grass polygraph Model 7D under a resting tension of 1g. The left atrium was suspended in between electrodes to enable electrical stimulation (2 Hz, 5 ms, threshold voltage +50%). The tissues were allowed to stabilize for 150 minutes before cumulative dose response curves of the atria to positive inotropic effect of ISP ((-)-isoproterenol, Sigma Chemical Co., St. Louis MO) were obtained. Subsequently, the preparations were washed with fresh Krebs solution to allow the tissues to relax to base line, incubated for half an hour with Krebs solution containing the desired concentration of extracts before new cumulative dose response curves were constructed. All extracts used were triturated with 0.5% Polyethyleneglycol-400 before being dissolved in the Krebs solution.

Isolated Rat Aorta

The descending aorta of the above rat was carefully excised and placed in Krebs solution at 37 °C and gassed with 95% O_2 and 5% CO_2. The solution had the following composition (mM): NaCl (119), $NaHCO_3$ (25), $MgSO_4$ (1.2), KCl (4.6), KH_2PO_4 (1.2), glucose (12.2) and $CaCl_2$ (1.5). The aorta was cleaned of from connective tissue and cut into spiral strips (approximately 10 mm × 1 mm wide). The strips were then placed

vertically in a 10 mL organ bath chamber containing aerated Krebs solution. Each strip was placed under a resting tension of 1 g and allowed to equilibrate for 60 minutes before control measurements were made. The contractile responses were recorded isometrically by a tension transducer connected to a Polygraph recorder. Contractile responses were induced with NA (± arterenol, Sigma Chemical Co., St. Louis MO) to obtain dose-response curve. The effects of different concentration of extracts on the dose response curve were examined.

Results and Discussion

Isolated Rat Left Atrium

Figure 1 shows that the chloroform extract was the most active extract that inhibited the positive inotropic responses of isolated left atrium to

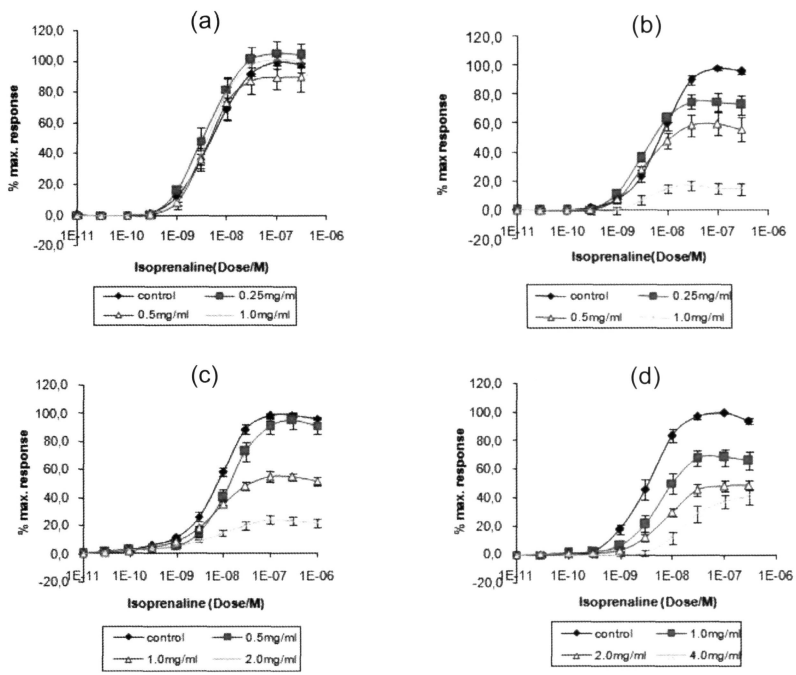

Figure 1: The Effect of *T. crispa* Extracts on the ISP-induced Contraction of Rat Left Atrium of SD Rats: a) Petroleum Ether Extract, b) Chloroform Extract, c) Methanol Extract, d) Water Extract. Graphs were Plotted Based on the Mean ± SEM (n = 6)

ISP. Chloroform extract (1.0 mg/mL) inhibited the maximum response by about 80%. The inhibition of the maximum response and non-parallel shift of the dose-response curve to the right suggested that the extract was not a b-adrenergic blocker. The shape of the curves suggested that the extract behaved as a non-competitive antagonist.

Isolated Rat Right Atrium

Figure 2 indicated that all extracts did not significantly affect the positive inotropic responses of the right atrium to ISP. The effect of higher concentrations of chloroform and methanol extracts (>0.25 mg/mL) could not be studied because they caused the right atrium to stop beating hence the positive inotropic activity of right atrium to isoprenaline could not be obtained. However, the spontaneous beating of the atrium slowly returned

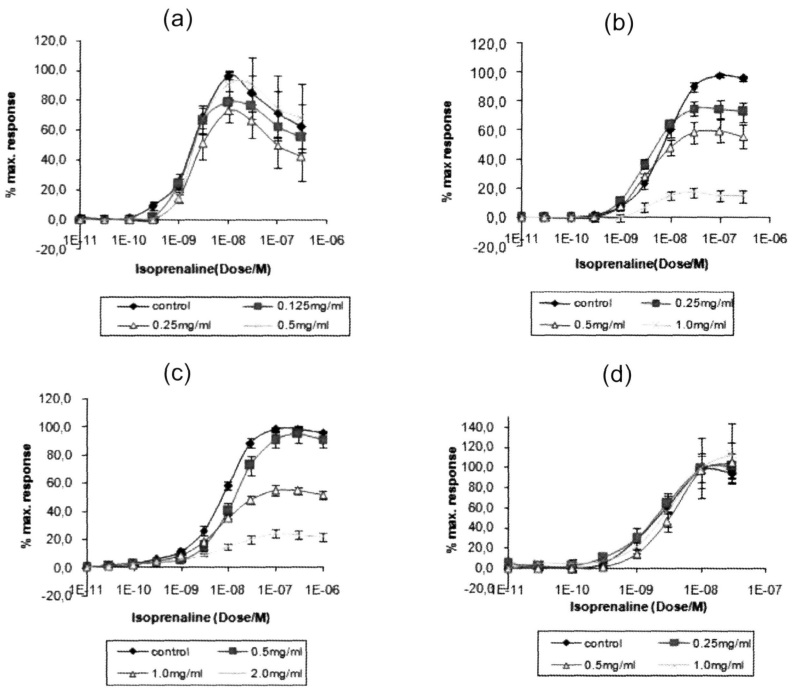

Figure 2: The Effect of the *T. crispa* Extracts on the ISP-induced Contraction of the Isolated Right Atrium of SD Rats: a) Petroleum ether Extract, b) Chloroform Extract, c) Methanol Extract, d) Water Extract. Graphs were Plotted Based on the Mean ± SEM (n = 6)

as the dose of isoprenaline was gradually increased. It suggested that high concentration of *T. crispa* extract inhibited the spontaneous excitation contraction process of the sinoarterial node which maintained the continuous beating of the heart. However the inhibition could be overcome by stronger b-adrenoceptor stimulation of high doses of ISP.

Isolated Rat Aorta

Figure 3 shows that the NA-induced contraction of the isolated aortic strips was inhibited by all of the extracts at various concentrations. The chloroform extract was again found to be the most potent extract where 1.0 mg/mL of the extract inhibited the maximum responses by about 85%. Studies on the chloroform fractions showed that all fractions, CF1, CF2, CF3 and CF4 at 1.0 mg/mL significantly depressed the maximum

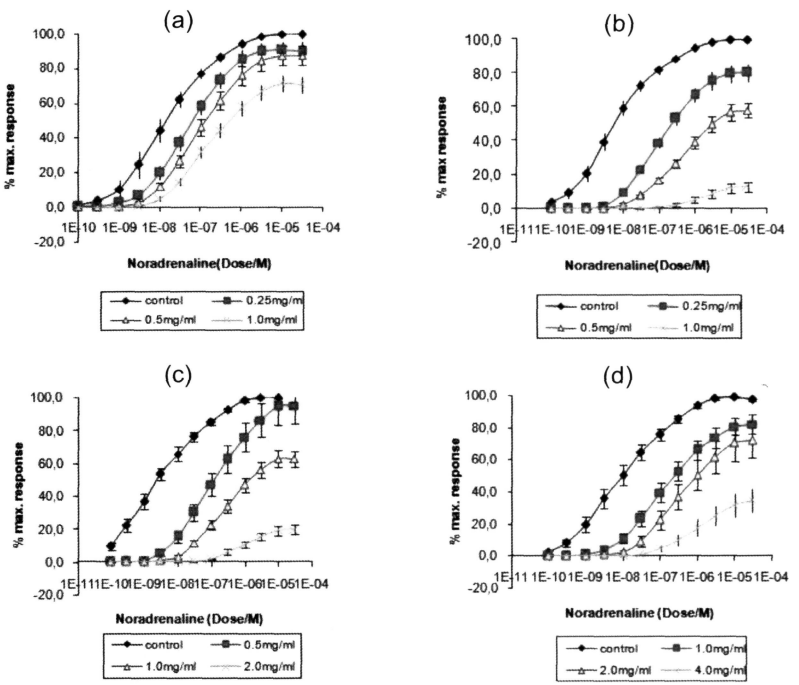

Figure 3: The Effect of *T. crispa* Extracts on the NA-induced Contractions of the Isolated Aortic Strips Preparation; a) Petroleum ether Extract, b) Chloroform Extract, c) Methanol Extract, d) Water Extract. Graphs were Plotted Based on the Mean ± SEM (n = 6)

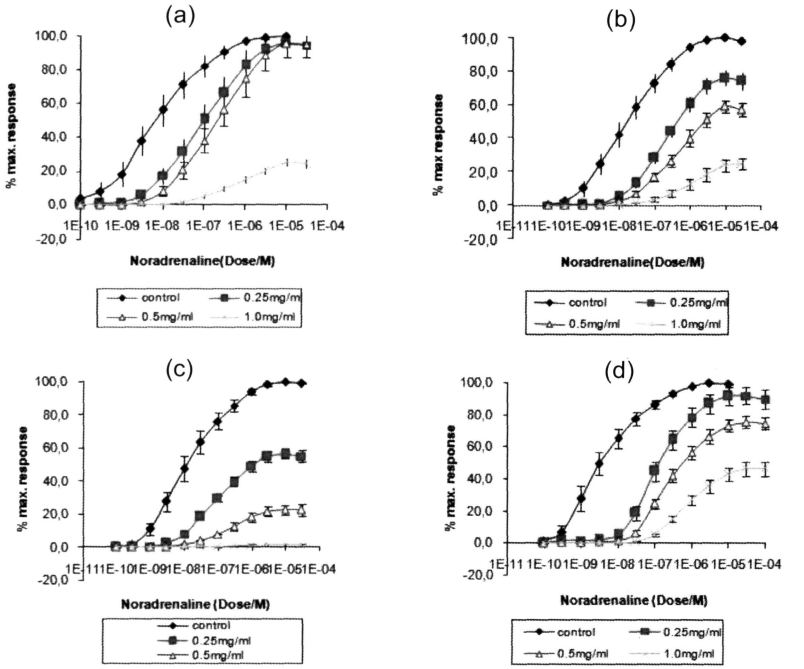

Figure 4: The Effect of the Chloroform Fractions of *T. crispa* on the NA-induced Contractions of the Isolated Aortic Strips Preparation; a) CF1, b) CF2, c) CF3, d) CF4. Graphs were Plotted Based on the Mean ± SEM (n = 6)

response of the dose-response curves. CF3 was found to be the most active, causing an inhibition of about 99% at 1.0 mg/mL (Figure 4).

Further fractionation of CF3 indicated that all fractions (CF3a, CF3b, CF3c and CF3d) showed almost the same effect to the NA-induced rat aortic strips contraction (Figure 5). At a concentration of 0.5 mg/mL, the inhibition was about 60-80%. Similar to the isolated left atrium preparation, all the fractions of *T crispa* caused the dose-response curves of aorta to NA to be shifted to the right with reduce maximum. It suggested that the inhibition of contraction by *T. crispa* extract is not due to competetive α-adrenoceptor blocking activity (e.g. phentolamine). The shape of the curves suggested that the extract had acted as a non-competitive antagonist. The non-competitive antagonism could be either specific to α-adrenoceptor only (as in non-competitive α-adrenoceptor blocker, phenoxybenzamine) or non-specific to all types of receptors (as in non-specific blocker, papaverine).

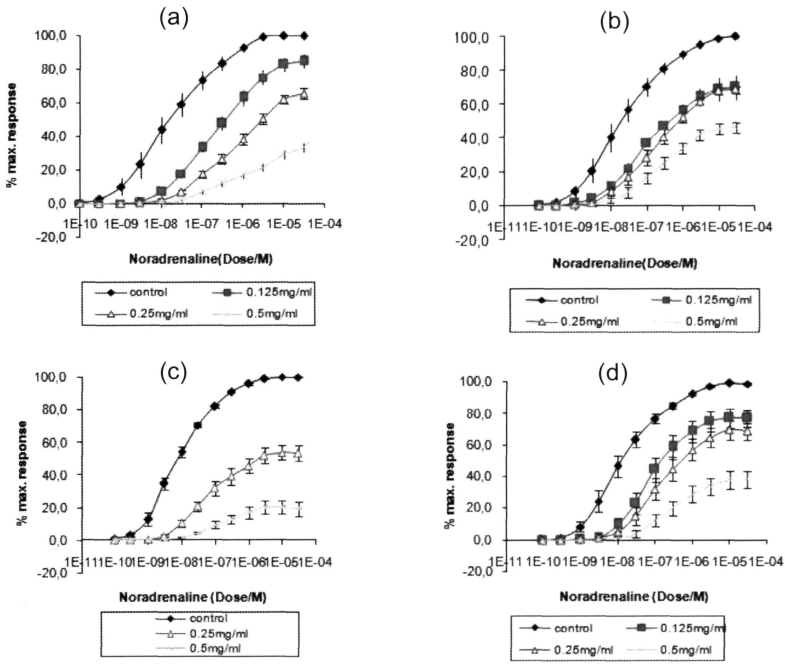

Figure 5: The Effect of CF3 Factions from the Chloroform Extract of *T. crispa* on the NA-induced Contractions of the Isolated Aortic Strips Preparation: a) CF3a, b) CF3b, c) CF3c, d) CF3d. Graphs were Plotted Based on the Mean ± SEM (n = 6)

Conclusion

The present findings suggest that the relatively polar compound(s) of the chloroform extract of *T. crispa* (CF3) possessed negative inotropic and vasodilating activities. Both of these activities could reduce blood pressure which may further explain its use traditionally as an anti-hypertensive traditional medicinal plant. Since the *T. crispa* extract inhibited non-competetively both the β-adrenoceptor stimulation of ISP in left atria and α-adrenoceptor stimulation of NA in aorta, it suggested that the extract did not act as α- or β-adrenoceptor blocker but very likely as a non-specific inhibitor which blocks all type of contraction as the case of papaverine.

Acknowledgement

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