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Antinociceptive and Anti-inflammatory Activities of *Tinospora crispa* in Various Animal Models

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Abstract: The present study was carried out to determine the antinociceptive and anti-inflammatory activities of the ethanol extract of *Tinospora crispa* stem. The antinociceptive activity was measured using the writhing and hot plate tests, while, the anti-inflammatory was measured using the carrageenan-induced paw edema test. The air-dried stems of *L. japonica* were soaked in ethanol (1:5; w/v) at the temperature of 48°C for 48 h. The supernatant collected was evaporated under reduced pressure and kept at -80°C until used. Prior to each experiment, the extract was dissolved in distilled water (dH_2O) to prepare the doses of 30, 100 and 300 mg kg^{-1}. The extract administered intraperitoneally exhibited significant ($p<0.05$) antinociceptive and anti-inflammatory activities in a dose-dependent manner in all assays used.

Key words: *Tinospora crispa*, ethanol extract, antinociceptive activity, anti-inflammatory activity

INTRODUCTION

Tinosprora crispa L., or '*Akar patawali*' as it known to the Malays, is a plant belonging to the family Menispermaceae. *T. crispa* can be found distributed from the southwestern part of China to Southeast Asia, including Malaysia. It is widely used in the traditional medicinal practice of peoples living in Malaysia, Indonesia and Thailand to treat ailments like fever, hyperglycemia, wounds, intestinal worms and skin infections. Other than that, *T. crispa* is also used to treat tooth and stomach aches, coughs, asthma and pleurisy (Nik Najib *et al*., 1999).

Scientifically, *T. crispa* has been demonstrated to possess antibacterial (Zakaria *et al*., 2006), antifilarial, antimalarial, antipyretic (Kongkathip *et al*., 2002) and antihyperglycaemic effects. The extract of *T. crispa* have also been reported to suppress the synthesis and release of nitric oxide, which is known to take part in various physiological processes within the body, including in the pain and inflammation processes. In term of the chemical constituents successfully isolated from various parts of *T. crispa*, the plant contained quaternary alkaloids (including berberine (Bisset and Nawaiwu, 1984), borapetol A and B, borapetoside A and B, tinocrisposide, *N*-formylanondine, *N*-formylnornuciferine, *N*-acetyl nornuciferine, γ-sitosterol, picrotein and tinotubride. In addition, Kongkathip *et al*. (2002) have also isolated 2 new triterpenes, cycloeucalenol and cycloeucalenone from *T. crispa*.

Based on the traditional uses of *T. crispa*, which include for the treatment of pain-related ailments, the present study was carried out to determine the antinociceptive and anti-inflammatory activities of *T. crispa* stems using various animal models.

MATERIALS AND METHODS

Plant material and preparation of its aqueous extract: *Tinospora crispa* stems were collected around Banting, Selangor, Malaysia, washed under running water, cut into small pieces and sun-dried for 4 days before being grinded into powder form. The dried powder (447 g) was mixed with absolute ethanol (1:5; w/v), placed in water bath at 50°C for 72 h and then filtered using Whatman No. 1 to obtain the supernatant. The collected supernatant was then evaporated to dryness at 50°C under reduced pressure and the crude dried extract obtains, labelled as ETCS, was kept at 4°C until use. Prior to use, the ETCS was emulsified using 0.1% Tween-80 in normal saline to the doses of 30, 100 and 300 mg kg^{-1} body weight for administration into the animals.

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Preparation of drugs: Ibuprofen (100 mg kg^{+}) (Sigma, USA), acetylsalicylic acid (ASA; 10 mg kg^{-1}) (Sigma, USA) and morphine (5 mg kg^{-1}) (Sigma, Germany) were used as reference drugs and prepared by dissolving them in distilled H_2O.

Experimental animals: Male Balb-C mice (25-30 g; 5-7 weeks) and Sprague-Dawley rats (180-200 g; 8-10 weeks old), obtained from the Animal Source Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, were used in this study. All of the animals were kept under room temperature (27±2°C; 70-80% humidity; 12 h light/darkness cycle) in the Animal Holding Unit, Faculty of Medical and Health Sciences, UPM for at least 48 h before use. Food and water were supplied *ad libitum* up to the beginning of the experiments. At all times the mice and rats were cared for in accordance with current UPM principles and guidelines for the care of laboratory animals and the UPM ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann (1983).

All mice were equally divided into 10 groups of 6 mice each (n = 6) and received (*sc*) distilled H_2O, ASA (100 mg kg^{-1}) or morphine (5 mg kg^{-1}), or ETCS (30, 100 and 300 mg kg^{-1}) 30 min prior to subjection to the abdominal constriction or hot plate tests, respectively. On the other hand, all rats were equally divided into 5 groups of 6 rats each (n = 6) for the anti-inflammatory study and received (*sc*) distilled H_2O, 100 mg kg^{-1} ibuprofen or ETCS (30, 100 and 300 mg kg^{-1}), respectively 30 min prior to subjection to the test. All of the test solutions were administered in the volume of 10 mL kg^{-1} body weight.

Antinociceptive assay

Abdominal constriction test: The acetic acid-induced abdominal constriction test which has been slightly modified (Mat Jais *et al*., 2004) was used to evaluate the chemically-induced antinociceptive activity of ETCS. Thirty minutes after treatment with test solutions, each group was administrated 0.6% acetic acid (10 mL kg^{-1}) and the mice were placed in transparent perspex observation boxes. After a 5 min lag period following the administration of acetic acid, the presence of contractions of the abdominal muscle together with stretching of hind limbs (writhing effect) were cumulatively counted for 25 min. The number of writhing and stretching movements was used to express the percentage of analgesia using the following ratio:

$$\text{Percentage of analgesia} = \frac{\text{(Control group mean)} - \text{(Test group mean)}}{\text{Control group mean}} \times 100$$

Hot plate test: The 55°C hot-plate test which has been slightly modified (Sulaiman *et al*., 2004) was used to evaluate the thermally-induced antinociceptive activity of ETCS. Before the experiment was carried out, a group of mice was subjected to the selection process in which the untreated mice were placed on the Ugo Basile 7280 hot plate set at 55±0.2°C. Only those mice with response latency times between 5-8 sec were selected for this study. The selected mice (n = 6) were treated (*ip*) with test solutions 30 min prior to performing to the test. The latency time of response to discomfort, observed when the animal start licking their fore- or hind-paws or jumped when placed on the hot plate, was recorded immediately (0 min) and at 30, 60, 120, 180 and 240 min after the administration of each test solution. The prolongation of latency time was taken as an indicator of antinociceptive activity. A cut-off time of 20 sec was used to prevent excessive tissue damage to the animals.

Anti-inflammatory assay: The procedure used was as described by Winter *et al*. (1962) but with slight modifications. Rats were divided into 5 groups (n = 6) and received (ip) normal saline, ASA (100 mg kg^{-1}), or ETCS (30, 100 and 300 mg kg^{-1}) followed 30 min later by the administration (ipl) of 0.1 ml of 1% carrageenan suspension into the rat's right hind paw. Paw volume was measured before (V_0) and at 1, 2, 3, 4 and 5 h (V_T) following the carrageenan injection using a plethysmometer (Model 7140, Ugo Basile, Italy). The degree of inflammation was quantified by measuring the volume displaced by the paw between the final volume (V_T) and the initial volume (V_0). The percentage of anti-inflammation was calculated using the formula given below:

$$\text{Percentage of anti-inflammation} = \frac{(V_T - V_0)_{\text{Control}} - (V_T - V_0)_{\text{Treated}}}{(V_T - V_0)_{\text{Control}}} \times 100$$

T-Time interval

Statistical analysis: The results are presented as Mean±Standard Error of Mean (S.E.M.). The one-way ANOVA test with Dunnett post-hoc test was used to analyze and compare the data, with $p<0.05$ as the limit of significance.

RESULTS

The antinociceptive profile on the ETCS: The 30, 100 and 300 mg kg^{-1} doses of ETCS significantly ($p<0.05$) reduced the acetic acid-induced writhing response in dose-dependent manner (Table 1). The 300 mg kg^{-1}

Table 1: Antinociceptive activity of ETCS assessed by the acetic acid-induced writhing test in mice

Treatment groups (n = 6)	Writhing response	Inhibition (%)
Saline	36±1.45	-
100 mg kg^{-1} ASA	7±0.71*	81
30 mg kg^{-1} ETCS	15±1.03*#	58
100 mg kg^{-1} ETCS	12±0.83*	67
300 mg kg^{-1} ETCS	3±0.72*#	92

The writhing response was expressed as mean ± S.E.M, *Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group, # Data differs significantly ($p \leq 0.05$) when compared against ASA-treated group

Table 2: Antinociceptive activity of ETCS assessed by the 53°C hot plate test in mice

	Latency of discomfort (sec)					
Treatment groups (n = 6)	0 min	30 min	60 min	120 min	180 min	240 min
Saline	7.24±0.53	7.12±0.71	7.84±0.77	7.03±0.87	7.91±0.68	8.12±0.71
5 mg kg^{-1} morphine	7.72±0.43	16.79±1.97*	17.74±2.13*	19.03±2.05*	18.95±1.77*	18.76±1.33*
30 mg kg^{-1} ETCS	7.31±1.01	7.26±0.76§	10.07±0.48*	11.31±0.81*	10.28±0.26*	10.84±1.73*
100 mg kg^{-1} ETCS	7.69±0.85	9.97±0.44*	9.36±0.58	9.61±0.59*	13.72±1.31*	13.16±1.12*
300 mg kg^{-1} ETCS	7.61±0.64	9.41±0.53*	11.27±0.65*	10.87±1.35*	12.72±0.78*	12.64±1.51*

The latency for licking of the hind paws, shaking or jumping off from the surface was expressed as mean ± S.E.M, *Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group, § Data differs significantly ($p \leq 0.05$) when compared against morphine-treated group

Table 3: Anti-inflammatory activity ETCS assessed by the carrageenan-induced paw edema test in rats

	Mean increase in paw edema±SEM (mL)/Time interval (h)				
Treatment group (n = 6)	1 h	2 h	3 h	4 h	5 h
Saline	0.57±0.05	0.68±0.04	0.81±0.07	0.87±0.07	0.86±0.07
100 mg kg^{-1} ibuprofen	0.11±0.05*	0.19±0.04*	0.16±0.03*	0.14±0.07*	0.10±0.02*
30 mg kg^{-1} ETCS	0.41±0.02*	0.38±0.03*#	0.37±0.02*#	0.30±0.02*#	0.29±0.01*#
100 mg kg^{-1} ETCS	0.13±0.02*#	0.20±0.03*#	0.24±0.04*#	0.26±0.05*#	0.22±0.04*#
300 mg kg^{-1} ETCS	0.11±0.01*	0.15±0.02*	0.17±0.02*	0.16±0.02*#	0.14±0.02*

The volume of hind paw oedema was expressed as mean ± S.E.M, *Data differs significantly ($p \leq 0.05$) when compared against the normal saline-treated group. # Data differs significantly ($p \leq 0.05$) when compared against ASA-treated group

dose of ETCS produced an activity that was approximately equal in strength when compared to the 100 mg kg^{-1} ASA.

The 30, 100 and 300 mg kg^{-1} doses ETCS significantly ($p<0.05$) increase the latency of discomfort that lasted until the end of the experiment (Table 2). The onset of activity for the 100 and 300 mg kg^{-1} doses of ETCS was 30 min while, for the 30 mg kg^{-1} dose of ETCS was 60 min after their administration. However, all doses of ETCS exerted an activity that was lower than that of 5 mg kg^{-1} morphine.

Pre-treatment of ETCS with 5 mg kg^{-1} naloxone: Pre-treatment with 5 mg kg^{-1} naloxone did not cause any significant change in the number of writhing or latency of discomfort of mice treated with 100 mg kg^{-1} ETCS.

The anti-inflammatory profile of the ETCS: All doses of the ETCS significantly ($p<0.05$) inhibited the development of paw edema resulted from the carrageenan administration (Table 3). The activity also occurred in a dose-dependent manner and started after 1 h or their administration and lasted until the end of the experiment. The 100 mg kg^{-1} ASA also shows the same pattern of activity.

DISCUSSION

The present study demonstrated the ability of ETCS to exert antinociceptive and anti-inflammatory activities in various animal models. The ability to inhibit chemically- and thermally-induced nociception indicates the extract's characteristic as strong analgesics (e.g. opioid agonists) (Hunskaar and Hole, 1987; Hosseinzadeh and Younesi, 2002). In addition the ability to inhibit the thermal-induced nociception indicates the extract's central antinociceptive activity (Pini *et al.*, 1997).

Although, the exact mechanism of antinociceptive action of the ETCS is not yet determined, it is plausible to suggest the involvement of non-opioid mechanism as part of the mechanism involved, based on the failure of naloxone to block/reverse the extract antinociceptive activity in both assays. Other than that, the involvement of cyclo-oxygenase (COX), at least at the peripheral level, is also suggested based on the ETCS potential to block the acetic-acid-induced nociception (Ballou *et al.*, 2000; Vogel and Vogel, 1997). This suggestion is supported by findings that the abdominal constrictions induced by the acetic acid were due to the release of COX-synthesized prostacyclin (Ballou *et al.*, 2000) and PGE_2 and $PGE_{2\alpha}$ (Vogel and Vogel, 1997), which in turn lead to

inflammatory pain within the peritoneal cavity. The above suggestion is further backed by the extract ability to exert anti-inflammatory activity when assessed by the carrageenan-induced paw edema test (Amanlou *et al*., 2005; Damas *et al*., 1986; Gamache *et al*., 1986).

Based on the previously determined chemical constituents of the *T. crispa* (Bisset and Nawaiwu, 1984; Kongkathip *et al*., 2002), it is plausible to suggest that the observed antinociceptive and anti-inflammatory of ETCS was attributed to the presence and synergistic action of alkaloids and triterpenes. These suggestions were supported by findings described by Hosseinzadeh and Younessi (2002) and Beirith *et al*. (1999).

CONCLUSION

In conclusion, the present study demonstrated the potential of *T. crispa* stem to exert antinociceptive and anti-inflammatory activities and, thus, justify the folklore uses of the plant in treating pain- and inflammation-related ailments.

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REFERENCES

Amanlou, M., F. Dadkhah, A. Salehnia, H. Farsam and A. Dehpour, 2005. An anti-inflammatory and anti-nociceptive effects of hydrochloric extract of *Satureja khuzistanica* Jamzad extract. J. Pharm. Pharmaceut. Sci., 8 (1): 102-106.

Ballou, L.R., R.M. Botting, S. Goorha, J. Zhang and J.R. Vane, 2000. Nociception in cyclooxygenase isozyme-deficient mice. Proc. Natl. Acad. Sci. USA, 97 (18): 10272-10276.

Beirith, A., A.R.S. Santos, J.B. Calixto, S.C. Hess, I. Messana, F. Ferrari and R.A. Yunes, 1999. Study of the antinociceptive action of the ethanolic extract and the triterpene 24-hydroxytormentic acid isolated from the stem bark of *Ocotea suaveolens*. Planta Med., 65: 50-55.

Bisset, B.C. and M.K. Nwaiwu, 1984. Quarternary alakaloids of *Tinospora* sp. Planta Med., 48: 275-279.

Damas, J., G. Remacle-Volon and E. Deflandre, 1986. Further studies of the mechanism of counter irritation by turpentine. Arch. Pharmacol., 332: 196-200.

Gamache, D.A., J.T. Povlishock and E.F. Ellis, 1986. Carrageenan-induced brain inflammation. Characterization of the model. J. Neurosurg., 65: 675-685.

Hosseinzadeh, H. and H.M. Younesi, 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol., 2: 7.

Hunskaar, S. and K. Hole, 1987. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain, 30: 103-104.

Kongkathip, N., P. Dhumma-upakorn, B. Kongkathip, K. Chauvananoraset, P. Sangchomkaco and S. Hatthakitpanichakul, 2002. Study on cardiac contractility of cycloeucalenol and cycloeucalenone isolated from *Tinospora crispa*. J. Ethnopharmacol., 1: 95-99.

Mat Jais, A.M., Y.M. Dambisya and T.L. Lee, 2004. Antinociceptive activity of *Channa striatus* (Haruan) extracts in mice. J. Ethnopharmacol., 57: 125-130.

Nik Najib, N.A.R., T. Furuta, S. Kojima, K. Takane and M.M. Ali, 1999. Antimalarial activity of extracts of Malaysian medicinal plants. J. Ethnopharmacol., 64: 249-254.

Pini, L.A., G. Vitale, A. Ottani and M. Sandrini, 1997. Naloxone-reversible antinociception by paracetamol in the rat. J. Pharmacol. Exp. Ther., 280 (2): 934-940.

Sulaiman, M.R., M.N. Somchit, D.A. Israf, Z. Ahmad and S. Moin, 2004. Antinociceptive effect of *Melastoma malabathricum* ethanolic extract in mice. Fitoterapia, 75: 667-672.

Vogel, H.G. and W.H. Vogel, 1997. Drug discovery and evaluation: Pharmacological assays. Lewisville, J.A. Majors Company, pp: 360-418.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Zakaria, Z.A., A.M. Mat Jais, M.N. Somchit, M.R. Sulaiman and F.O. Faizal, 2006. The *In vitro* Antibacterial Activity of *Tinospora crispa* Extracts. J. Biol. Sci., 6 (2): 398-401.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109-110.