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Analgesic activity of three *Channa spp.* fish extracts

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SUMMARY

In the present study, three Malaysian *Channa spp.* fish *Channa striatus*, *Channa micropeltes* and *Channa lucius* were assessed for their analgesic activity. Distilled water and mixture of chloroform : methanol were used for extraction. The analgesic or antinociceptive activity was investigated by abdominal writhing and hot plate test. The water extract of *Channa striatus* and the chloroform : methanol extract *Channa lucius* produced potent antinociceptive effect when assessed with the abdominal writhing test. The activity was compared to morphine where the activity of the extracts was less potent than the opioid. In the hote plate test, water extract of Channa striatus revealed significant activity and chloroform:methanol extract of Channa micropeltes had moderate activity. However, these activities were statistically lower than morphine. Collectively, this study also showed that *Channa striatus* extract was more potent analgesic agent when compared to the other closely related snakehead *Channa micropeltes* and *Channa lucius*.

Key words: Channa striatus; Channa micropeltes; Channa lucius; Aanalgesic; Pain

INTRODUCTION

Channa (C.) striatus (haruan), *C. micropeltes* (toman) and *C. lucius* (bujuk) are snakehead fish belongs to Channidae family indigenous to many tropical and subtropical countries including Malaysia. There are fresh water, air breather and carnivorous fish which are a valuable source of protein in most Asia Pacific countries (Mohsin and Ambak, 1983). *C. striatus* has been studied and has been reported to possess antinociceptive, anti-eczema (Mat Jais *et al.*, 1997) and wound healing properties (Mat Jais *et al.*, 1994; Baie and Sheikh, 2000). In Malaysia, *C. striatus* is consumed as a remedy to help promoting healing after surgical intervention, childbirth or trauma (Mat Jais *et al.*, 1998). *C. striatus* contains all the essential amino acid and fatty acids required for wound healing (Mat Jais *et al.*, 1994, 1998).

Recently, we reported that all three *C. spp.* fish having anti-inflammatory property (Somchit *et al.*, 2004). *C. striatus* has been studied extensively for its pharmacological properties. However, to our knowledge, no other study has been reported on the other pharmacological benefits of the two closely related *C. micropeltes and C. lucius*. Therefore, the aim of this study are to access the water and chloroform:methanol extracts of *C. striatus*, *C. micropeltes* and *C. lucius* on anti-

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nociceptive activities by using abdominal constriction or writhing test and hot plate test.

MATERIALS AND METHODS

Preparation of fish extracts

Adult *C. striatus* (1.0 - 2.0 kg), *C. micropeltes* (2.0 - 2.3 kg) and *C. lucius* (0.7 - 1.2 kg) were caught from the wild in Kuala Terengganu district (East Coast of Malaysia) and were verified by the Terengganu Fisheries Department, Ministry of Agriculture, Malaysia. The whole fish fillet extract was prepared in two methods of using distilled water or chloroform : methanol as solvent as described by Mat Jais *et al.* (1997).

Antinociceptive assay

One hundred and sixty eight male Balb/C mice weighing 20 - 35 g were used for the antinociceptive activity tests. Mice were housed in plastic cages with wood shaving as bedding in group of 6 (28 ± 2°C, 12 h light dark cycle) for 1 week prior to experiments. Food and water were available *ad libitum*.

The abdorminal writing test was performed as described by Mat Jais *et al.* (1997) and Zakaria *et al.* (2004a). Mice (n = 6 / group) were pretreated with water or chloroform : methanol extracts of either *C. striatus, C. micropletes or C. lucius* (15 mg/kg and 30 mg/kg) subcutaneously (s.c) 30 min before intraperitoneal injection of acetic acid 0.6% (v/v). Morphine (0.6 mg/kg) was used as reference drugs and was administered 30 min before acetic acid injection. Controls animals received a similar volume of saline solution (10 ml/kg).

The abdominal constrictions indicated by contraction of abdominal muscle together with a stretching of hind limbs (Collier *et al.*, 1968; Santos *et al.*, 1995). The number of abdominal constrictions was cumulatively counted over a period of 30 min. The antinociceptive activity was expressed as percentage (Zakaria *et al.*, 2004a).

The hot plate test was employed according to the method described by Eddy and Leimback (1953). Animals were placed on an Ugo Basile hot plate maintained at 52 ± 0.5°C. Mice with baseline latencies less than 5 s were eliminate before start the experiment. The cut-off time for hot plate latencies was set at 20 s to avoid tissue injury. Animals (n = 6 / group) were pretreated with water or chloroform : methanol extracts at 15 and 30 mg/kg, 30 min before experiment. Morphine (5 mg/kg) was used as a reference drug. Control animals received the equivalent volume of saline solution (10 ml/kg). The response latency recorded by either licking of the hind paws, shaking or jumps off from the surface (Mino *et al.*, 2002). The response of latency was determined at the time 0 and after 30, 60, 90, 120, 150, 180 and 210 min intraperitoneal administration of extracts and morphine.

Statistical analysis

The results were presented as mean ± S.E.M. Statistical significance was determined by analysis of variance and subsequent Duncan's multiple range test ($P < 0.05$) for antinociceptive tests. The analysis was performed using SPSS Statistical version 11.5 Software.

RESULTS

Abdominal writhing test

The inhibitory effect of water and chloroform : methanol extracts of three local *C. spp.* in the writhing test is shown in Table 1. Morphine as a reference drug showed significant inhibition by 99.25% was statistically more potent compared all three local *C. spp.* extracts. The groups treated with the water and chloroform : methanol extract of three local *C. spp.* exhibited a significant reduction in the number of writhing compared to control.

In comparison between water extracts of three local *C. spp.* at dose 15 and 30 mg/kg, it was found that *C. striatus* extract (15 and 30 mg/kg) and *C. micropeltes* extract (15 mg/kg) produced more potent inhibition by 59.28%, 57.63% and 47.45%,

Table 1. The effect of the water and chloroform:methanol extracts of three *C. spp.* on writhing test in mice

Groups	Dose (mg/kg)	Number of constrictions	Inhibition (%)
Control	-	111.33 ± 10.50[a]	-
C. striatus (DH_20)	15	47.17 ± 4.13[d]	57.63
C. striatus (DH_20)	30	45.33 ± 3.93[d]	59.28
C. striatus (C : M)	15	75.50 ± 10.45[b,c]	32.18
C. striatus (C : M)	30	65.17 ± 7.04[b,c,d]	41.46
C. micropeltes (DH_20)	15	58.50 ± 6.05[c,d]	47.45
C. micropeltes (DH_20)	30	80.83 ± 8.42[b]	27.40
C. micropeltes (C : M)	15	81.5 ± 3.34[b]	26.79
C. micropeltes (C : M)	30	56.33 ± 6.35[c,d]	49.40
C. lucius (DH_20)	15	77.00 ± 6.26[b,c]	30.84
C. lucius (DH_20)	30	86.00 ± 9.18[b]	22.75
C. lucius (C : M)	15	43.83 ± 1.92[d]	60.63
C. lucius (C : M)	30	57.67 ± 9.14[c,d]	49.20
Morphine	0.6	0.83 ± 0.40[e]	99.25

Values are (mean ± S.E.M.) (n = 6 / group). [a,b,c,d]Values within the same column with different superscript differ ($P < 0.05$).

respectively compared to *C. micropeltes* extract (30 mg/kg) and *C. lucius* extract (15 and 30 mg/kg).

In comparison between chloroform : methanol extracts of three local *C. spp.* at dose 15 and 30 mg/kg, it was found that *C. lucius* extract (15 and 30 mg/kg) produced potent inhibition by 60.63% and 49.20% respectively, the extract of *C. micropeltes* and *C. striatus* at dose 30 mg/kg exhibited 49.4% and 41.46% respectively when compared to 15 mg/kg of *C. striatus* and *C. micropeltes.*

Comparison was made between water and chloroform : methanol extract of three local *C. spp*, it was found that water extract of *C. striatus* (15 and 30 mg/kg) showed potent inhibition by 59.28% and 57.63% compared chloroform : methanol extract (15 and 30 mg/kg). The chloroform : methanol extract of *C. micropeltes* (30 mg/kg) exhibited 49.90% inhibition compared to water extract of *C. micropeltes* (15 and 30 mg/kg). The chloroform : methanol extract of *C. lucius* (15 and 30 mg/kg) exhibited 60.63% and 49.20% respec-tively compared to the water extract of *C. lucius* (15 and 30 mg/kg).

Hot plate test

Group treated with the chloroform : methanol extract of *C. lucius* (15 and 30 mg/kg), the water extract of *C. lucius* (15 mg/kg) and *C. micropeltes* (15 and 30 mg/kg) respectively failed to change the latency time to licking or jumping when compared to control group. However the water extract of *C. striatus* at dose of 15 and 30 mg/kg showed increased the latency time after 1 and 3 h treatment respectively ($P < 0.05$).

The chloroform : methanol extract of *C. micropeltes* (30 mg/kg) showed increased the latency time only at 90 min treatment ($P < 0.05$). The water extract of *C. lucius* (30 mg/kg) also showed increased the latency time after 3 h treatment ($P < 0.05$). On the other hand, the water and chloroform : methanol extract of three local *C. spp.* failed to promote antinociceptive effect in the hot plate model of analgesia when compared to morphine.

DISCUSSION

The analgesic/antinociceptive activity of three *C. spp.* fish extracts was evaluated using acetic acid induced abdominal writhing and hot plate test. Collier *et al.* (1968) reported that acetic acid acts indirectly by releasing endogenous mediators at

stimulate the nociceptive (pain) effect. The abdominal constriction response is thought to involve in part local peritoneal receptor (Bentley *et al.,* 1983). Abdominal constriction response induced by acetic acid is a very sensitive procedure that enables the detection of peripheral anti-nociceptive activity compounds in laboratory animals. Acetic acid activates a chemosensitive receptor in the abdominal cavity (Matsuda, 1998). Several mediators such as kinin, substance P, acetylcholine and prostaglandins take part in visceral pain model nociception (Jain *et al.,* 1997) and transmission of nociception from the viscera (Cervero *et al.,* 1999). In acetic acid induced abdominal writhing which is the visceral pain model, the processor release of arachidonic acid metabolites via cyclooxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Franzotti *et al.,* 2000). From the results obtained showed that both extract of three local C. *spp.* have antinociceptive effect which were expressed in a dose dependent manner and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolite.

In our previous study, both fillet and mucus extracts have been proved to exhibit antinociceptive activities (Zakaria *et al.,* 2004a,b). The bioactive compound(s) inside the extracts were shown to act either by itself or by enhancing other analgesic agents such as morphine (Mat Jais *et al.,* 1997). Dambisya *et al.* (1999) also reported upon the failure of naloxone, a common opiod antagonist, to block or provide any effects on the antinociceptive activity of haruan mucus extract. Since the haruan extract was only active on abdominal constriction, but not on tail-flick test and the site of the antinociception of morphine is in central nervous system (Bowman and Rand, 1980), they suggested that the bioactive compound(s) in haruan extract is not working on opiod system and may be more active in visceral or peripheral system than central.

The biochemical composition of haruan extract (fillet) has an abundance of both free fatty acid and amino acids (Mat Jais *et al.,* 1994) but it is not clear weather or not they form the basis of the analgesic activity demonstrated in the present study. In this current study, water extract of *C. striatus* was found the best extract to give the significant inhibition of writhing response elicited by acetic acid compared to chloroform:methanol extract. However, chloroform : methanol extract of *C. lucius* was found the best extract to give the statistically highest inhibition in writhing response compared to water extract. It can be suggested that bioactive present of two type of compounds shows activity in water and chloroform : methanol extract.

Hot plate test can be a simple and sensitive method for studying analgesic and hyperalgesic reactions in mice. This test measure the nociceptive reactivity to thermal stimuli in mice which is a sensitive acute pain test for detecting opiate analgesia as well as several types of hyperalgesic reaction from spinal origin (Choi *et al.,* 2003). In the present study, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in the hot plate test, a central antinociceptive test (Parkhouse, 1979). While both extract of three local *C. spp.* showed no antinociceptive activity in this test. Thus, these extract may not act via central mechanism, although agents that alter the motor performance of animals without acting on the central nervous system.

Based on the current study, it was found that the water extract of *C. striatus* and the chloroform: methanol extract *C. lucius* produced antinociceptive effect when assessed in chemical models of nociception, acetic acid induced writhing, however both of extract of three local *C. spp.* failed to exhibited effect in a thermal model of nociception, the hot plate test. These results suggested that the antinociceptive action of the extracts is more related to peripheral mechanism than with a central mechanism.

Table 2. The effect of the water and chloroform:methanol extracts of three *Channa spp.* on hot plate latency time in mice

Groups	Dose (mg/kg)	Latency time (seconds)							
		0	30	60	90	120	150	180	210
Control	-	$5.95 \pm 0.13^{a,b}$	6.57 ± 0.32^{a}	6.50 ± 0.30^{a}	6.13 ± 0.19^{a}	$6.98 \pm 0.23^{a,b}$	$7.63 \pm 0.30^{a,b}$	$6.48 \pm 0.42^{a,b}$	6.65 ± 0.27^{a}
Morphine	5	5.73 ± 0.20^{a}	18.12 ± 3.32^{b}	20.47 ± 3.00^{c}	19.83 ± 2.00^{c}	16.80 ± 1.48^{c}	13.75 ± 1.45^{c}	11.27 ± 1.16^{f}	10.40 ± 0.65^{d}
C.striatus (DH$_2$0)	15	$6.85 \pm 0.33^{a,b,c}$	6.15 ± 0.38^{a}	10.28 ± 1.08^{b}	$8.68 \pm 1.14^{a,b}$	$8.67 \pm 0.70^{a,b}$	$8.32 \pm 0.39^{a,b}$	$9.30 \pm 0.50^{d,e,f}$	$8.30 \pm 0.52^{b,c}$
C.striatus (C:M)	15	$6.45 \pm 0.23^{a,b,c}$	6.82 ± 0.61^{a}	6.97 ± 0.45^{a}	6.72 ± 0.61^{a}	$6.85 \pm 0.47^{a,b}$	6.48 ± 0.53^{a}	$6.68 \pm 0.24^{a,b,c}$	6.90 ± 0.20^{a}
C.striatus (DH$_2$0)	30	$6.85 \pm 0.52^{a,b,c}$	7.18 ± 0.76^{a}	7.20 ± 0.92^{a}	$8.40 \pm 1.09^{a,b}$	$8.17 \pm 0.67^{a,b}$	9.53 ± 1.09^{b}	$10.62 \pm 1.02^{e,f}$	10.18 ± 0.65^{d}
C.striatus (C:M)	30	$7.15 \pm 0.48^{b,c}$	7.32 ± 0.64^{a}	$7.88 \pm 0.79^{a,b}$	$8.08 \pm 0.72^{a,b}$	9.18 ± 0.55^{b}	6.85 ± 0.49^{a}	$8.84 \pm 1.14^{b,c,d,e}$	8.77 ± 0.64^{c}
C. micropeltes (DH$_2$0)	15	7.20 ± 0.20^{c}	6.20 ± 0.35^{a}	$7.50 \pm 0.27^{a,b}$	$7.93 \pm 0.64^{a,b}$	$8.58 \pm 0.89^{a,b}$	$7.57 \pm 0.65^{a,b}$	$7.83 \pm 0.89^{a,b,c,d}$	6.47 ± 0.23^{a}
C. micropeltes (C:M)	15	$6.68 \pm 0.36^{a,b,c}$	5.95 ± 0.42^{a}	5.88 ± 0.16^{a}	6.75 ± 0.28^{a}	6.37 ± 0.14^{a}	6.10 ± 0.39^{a}	$6.50 \pm 0.21^{a,b}$	6.33 ± 0.13^{a}
C. micropeltes (DH$_2$0)	30	$6.55 \pm 0.62^{a,b,c}$	5.57 ± 0.18^{a}	5.65 ± 0.33^{a}	6.27 ± 1.12^{a}	$6.75 \pm 1.10^{a,b}$	$8.03 \pm 0.86^{a,b}$	$7.12 \pm 0.50^{a,b,c,d}$	5.98 ± 0.51^{a}
C. micropeltes (C:M)	30	$6.83 \pm 0.36^{a,b,c}$	5.97 ± 0.40^{a}	$7.50 \pm 0.65^{a,b}$	10.52 ± 1.86^{b}	$7.90 \pm 0.76^{a,b}$	$8.28 \pm 0.89^{a,b}$	$7.57 \pm 0.68^{a,b,c,d}$	$7.05 \pm 0.22^{a,b}$
C. lucius (DH$_2$0)	15	$6.77 \pm 0.20^{a,b,c}$	6.82 ± 0.35^{a}	6.73 ± 0.42^{a}	6.87 ± 0.53^{a}	$7.35 \pm 0.49^{a,b}$	$7.12 \pm 0.50^{a,b}$	$6.90 \pm 0.39^{a,b,c}$	6.40 ± 0.16^{a}
C. lucius (C:M)	15	$6.07 \pm 0.31^{a,b,c}$	6.05 ± 0.35^{a}	6.22 ± 0.42^{a}	6.48 ± 0.40^{a}	6.47 ± 0.32^{a}	$7.23 \pm 0.49^{a,b}$	6.07 ± 0.45^{a}	6.53 ± 0.22^{a}
C. lucius (DH$_2$0)	30	$6.50 \pm 0.36^{a,b,c}$	5.60 ± 0.25^{a}	6.57 ± 0.38^{a}	$7.72 \pm 0.81^{a,b}$	$8.13 \pm 0.98^{a,b}$	$7.60 \pm 0.60^{a,b}$	$8.90 \pm 1.05^{c,d,e}$	$8.25 \pm 0.88^{b,c}$
C. lucius (C:M)	30	$6.90 \pm 0.22^{a,b,c}$	5.98 ± 0.40^{a}	5.72 ± 0.43^{a}	6.88 ± 0.79^{a}	$7.00 \pm 0.51^{a,b}$	6.82 ± 0.69^{a}	$6.20 \pm 0.35^{a,b}$	6.30 ± 0.54^{a}

Values are (mean ± S.E.M.). ^{a,b,c,d}Values within the same column with different superscript differ significantly ($P <$ 0.05).

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REFERENCES

Baie SH, Sheikh KA. (2000) The wound healing properties of *Channa striatus*- cetrimide cream-tensile strength measurement. *J. Ethnopharmacol.* **71**, 93-100.

Bentley GA, Newton SH, Starr J. (1983) Studies on the antinociceptive action α-agonist drugs and their interaction with opiod mechnanisms. *Br. J. Pharmacol.* **79**, 125-134.

Bowman WC, Rand NJ. (1980) Textbook of Pharmacology, 2nd edition, Blackwell Science Publication, p. 165.

Cervero F, Larid JM. (1999) Visceral Pain. *Lancet* **353**, 2145-2148.

Choi EM, Hwang JK. (2003) Anti-inflammatory and antinociceptive activities of *Piper cubeba, Physalis angulata* and *Rosa hybrida*. *J. Ethnopharmacol.* **89**, 171-175.

Collier HDJ, Dinnin LC, Johnson CA, Schneider C. (1968) The abdominal response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol.* **32**, 295-310.

Dambisya YM, Lee TL, Sathivuu V, Mat Jais AM. (1999) The influence of temperature, pH and naloxone

on the antinociceptive activity of *Channa striatus* (haruan) extracts in mice. *J. Ethnopharmacol.* **66**, 2: 181-186.

Eddy NB, Leimback D. (1953) Synthetic analgesics II Dithienylbutenyl and dithienylbutilamines. *J. Pharmacol. Exp. Ther.* **107**, 385-393.

Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antoniolli AR. (2000) Anti-inflammatory, analgesic activity and acute toxicity of *Sida cardifolia* L. (Malva-branca). *J. Ethnopharmacol.* **72**, 273-278.

Gene RM, Segura L, Adzet T, Marin E, Iglesias J. (1998) Heterotheca inuloides: anti-inflammatory and analgesic effect. *J. Ethnopharmacol.* **60**, 157-160.

Jain SC, Jain R, Sharma RA, Capasso F. (1997) Pharmacological investigation of *Cassia indica*. *J. Ethnopharmacol.* **58**, 135-142.

Matsuda H, Yoshikawa M, Linuma M, Kubo M. (1998) Antinociceptive and Anti-inflammatory Activities of Limonin Isolated from the Fruits of *Evodia rutaecarpa var. bodinieri*. *Planta Med.* **64**, 339-342.

Mat Jais AM. Mc Culloch R, Croft KD. (1994) Fatty acid and amino acid composition of Haruan-potential role in wound healing. *Gen. Pharmacol*, **25**, 947-950.

Mat Jais AM, Matori MF, Kittakoop P, Suwanborirux K. (1998) Fatty acid compositions in mucus and roe of haruan, *Channa striata*, for wound healing, *Gen. Pharmacol*, **30**, 561-563.

Mat Jais AM, Fung R, Bosi E, Platell C, Mccauley MR, Croft K. (1998) Prelimanary evidence on the potential of haruan, *Channa striatus*, for wound healing. *Malays. Appl. Biol.* **27**, 50-51.

Mat Jais MA, Dambisya MY, Lee LT. (1997) Antinociceptive activity of *Channa striatus* (haruan) extracts in mice. *J. Ethnopharmacol.* **57**, 125-135.

Mino J, Avecedo C, Moscatelli V, Ferraro G, Hnatyszyn O. (2002) Antinociceptive effect of the aqueous extract of *Balbisia calycina*. *J. Ethnopharmacol.* **79**, 179-182.

Mohsin AK, Ambak A. (1983) *Freshwater Fishes of Peninsular Malaysia*, pp. 157-161, Universiti Pertanian Malaysia (Publ.), Serdang, Malaysia.

Santos ARS, Cechinel Filho, V Niero, R Calixto JB. (1995) Antinociceptive properties of steroids isolated from *Phyllanthus corcovadensis*. *Planta Med.* **61**, 329-331.

Zakaria ZA, Somchit MN, Sulaiman MR, Mat Jais AM. (2004) Preliminary investigation on the antinociceptive activity of haruan (*Channa striatus*) fillet extract with various solvent system. *Pakistan J. Biol. Sci.* **7**, 1706-1710.

Zakaria ZA, Sulaiman MR, Mat Jais AM, Somchit MN. (2004) Effects of a-amylase, protease and lipase on Haruan (*Channa striatus*) mucus extract antinociceptive activity in mice. *Pakistan J. Biol. Sci.* **7**, 2202-2207.