

Effects of three local Malaysian *Channa spp.* fish on chronic inflammation

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SUMMARY

Water and chloroform/methanol extracts of the three local Malaysian snakehead fish, *Channa striatus* (striped snakehead), *Channa micropeltes* (giant snakehead) and *Channa lucius* (blotched snakehead) were evaluated for inhibitory activity in chronic inflammation, using cotton pellet granuloma test. Both water extracts of *C. striatus* and *C. micropeltes* showed marked inhibition of the transudative and proliferative components of chronic inflammation (42.9 and 31.2% respectively for *C. striatus*, 35.6 and 26.2% for *C. micropeltes*) when compared to those of mefenamic acid (25.1 and 21.3% respectively) and piroxicam (36.1 and 26.2% respectively). The chloroform/methanol extracts did not exhibit any anti-inflammatory effects. These results indicated that *C. striatus* has more anti-transudative and anti-proliferative activities than the extract of *C. micropeltes*. *C. lucius* extract in contrast, did not inhibit these two components. This present study indicated the beneficial effects of the water extracts of *C. striatus* and *C. micropeltes*, but not *C. lucius* on chronic inflammation.

Key words: *Channa striatus*; *Channa micropeltes*; *Channa lucius*; Chronic inflammation; Granuloma

INTRODUCTION

The family Channidae is an obligate air-breather snakehead fish indigenous to many tropical and sub-tropical countries (Ng and Lim, 1990). These fish are valuable source of protein in most Asia-Pacific regions (Mohsin and Ambak 1983). *Channa striatus* (striped snakehead fish) or Haruan in Malay is one of the favorite fresh water fish. Another two common species of Channidae in Malaysia are *Channa micropeltes* (giant snakehead fish) or 'Toman' and *Channa lucius* (blotched snakehead fish) or 'Bujuk'.

C. striatus has been studied extensively and reported to possess antinociceptive, anti-eczema (Mat Jais *et al.* 1997) and wound healing (Mat Jais *et al.* 1994; Saringat and Sheikh 2000a) properties. Commercial preparations of *C. striatus* are available

especially cream and gel for wound healing (Saringat and Sheikh 2000b). In Malaysia, *C. striatus* is consumed as a remedy to help promoting healing following surgical intervention, childbirth or trauma (Mat Jais *et al.* 1998). However, we are not aware of any previous studies on pharmacological benefits of the other two closely related snakehead fish *C. micropeltes* and *C. lucius*.

Extracts having antinociceptive or analgesic property may possess anti-inflammatory activity (Zakaria *et al.* 2001). In screening new anti-inflammatory compounds especially against the chronic type, cotton pellet granuloma test is widely employed (Billingham and Davis 1979). Therefore, the aims of this current study were to evaluate and compare the properties of extracts of the three-snakehead fish on the chronic inflammation.

MATERIALS AND METHODS

Preparation of fish extracts

Adult *C. striatus* (400-600 g), *C. micropeltes* (1.0-1.3 kg)

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and *C. lucius* (400-600 g) caught from the wild in Kuala Trengganu district (East Coast of Malaysia) were verified by the Trengganu Fisheries Department, Ministry of Agriculture, Malaysia. The whole fish fillet extract was prepared using methods of Mat Jais *et al.* (1997). Briefly, fresh boneless fillet was steamed in a pressurized cooker at 100°C in double distilled water for 60 min. Then, the fillet was discarded, the liquid extract was collected, filtered (Whatman No. 1) and freeze dried for 24 to 48 hours. The extracted material was stored in -20°C prior to use. For the chloroform/methanol extraction, samples were extracted with 2:1 v/v chloroform/methanol according to methods of Mat Jais *et al.* (1998).

Animals

Male Spargue Dawley rats (180 to 200g) were used in accordance with the Ethical Committee, Universiti Putra Malaysia on animal experimentation. Rats were housed in plastic cages with wood shavings as bedding in groups of six at 29±2°C, 12h light dark cycle for 5 days prior to the experiments. Food and water were available *ad libitum*.

Cotton pellet granuloma test

Sterile cotton pellets (30.0±1.0 mg) were surgically implanted into subcutaneous tissue of the dorsal region of the thoracic vertebrae. Extracts of *C. striatus*, *C. micropeltes* and *C. lucius* (10 mg/kg)

suspended in normal saline (n=6 rats/group) were administered intraperitoneally daily for 7 days. Control rats received equivalent volume of normal saline. Two other groups of rats received either 10 mg/kg mefenamic acid or piroxicam (Sigma Chemicals, UK) in the same route for 7 days. On day 8, the rats were sacrificed, the cotton pellets were removed and weighed immediately to obtain the wet weight. The pellets were then dried at 60°C until a constant weight was obtained (dried weight).

Statistical analysis

The results are expressed as mean±s.d. One-way analysis of variance (ANOVA) was performed and sequential differences among the means were calculated at the level of $p \leq 0.05$ using Tukey-Kramer post test analysis.

RESULTS AND DISCUSSION

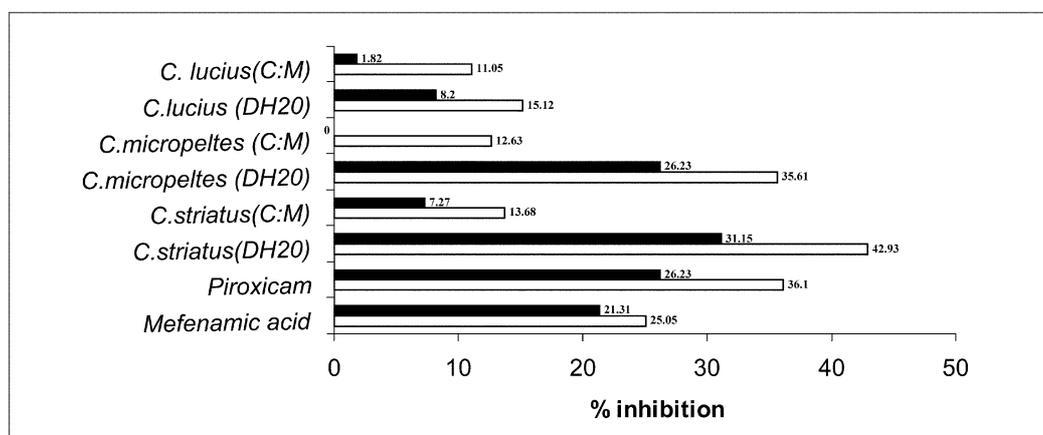
The water extracts of *C. micropeltes* and *C. striatus* reduced significantly the wet weight of the cotton pellet granuloma when compared to that of controls (Table 1). However, the extract of *C. lucius* did not show any activity. Both *C. micropeltes* and *C. striatus* showed similar activity on the granuloma weight as the commercial non-steroidal anti-inflammatory drug, piroxicam. The water extract of *C. striatus* had statistically lower wet granuloma weight when compared to mefenamic acid. The

Table 1. Effect of *Channa spp.* extracts on cotton-pellet granuloma test in rats

Group (n=6)	Wet granuloma weight (mg)	Dry granuloma weight (mg)
DH ₂ O extraction		
Control	205.64±18.89 ^a	61.43±5.92 ^a
<i>C. striatus</i>	117.73±7.78 ^c	42.65±2.08 ^c
<i>C. micropeltes</i>	132.47±4.66 ^b	45.23±0.54 ^b
<i>C. lucius</i>	174.42±13.77 ^a	56.67±5.45 ^a
Chloroform methanol extraction		
Control	190.08±11.66 ^a	55.78±5.97 ^a
<i>C. striatus</i>	164.92±15.05 ^a	51.45±3.58 ^a
<i>C. micropeltes</i>	166.54±11.22 ^a	56.17±3.98 ^a
<i>C. lucius</i>	169.55±9.36 ^a	54.11±2.62 ^a
Mefenamic acid	152.64±8.51 ^b	48.37±3.78 ^b
Piroxicam	131.24±15.98 ^{bc}	45.54±5.38 ^{bc}

Each value represents the Mean±S.D.

^{a-c}Mean with different superscript differ significantly ($p < 0.05$) in the same column



C:M : Chloroform/methanol extraction

Values are Percentage inhibition when compared to respective controls (Refer Table 1).

□ Water extracts; ■ Chloroform/methanol extraction.

Fig. 1. Percentage inhibition of anti-proliferative and anti-transudative portions of chronic inflammation by *Channa* spp. extracts.

cotton pellet granuloma test has been widely used to assess the transudative and proliferative components of chronic inflammation (Syed Ismail *et al.* 1997). The difference between wet and dry weight of the granulomatous cotton pellet is the amount of transudate absorbed during the process of chronic inflammation. In this study *C. striatus* was found to be the most potent inhibitor of the transudative component of chronic inflammation (42.9% inhibition) (Figure 1). The chloroform/methanol extracts of all three fish did not show marked anti-transudative activities (Table 1).

C. micropeltes and *C. striatus* water extracts also produce significantly lower dry granuloma weight when compared to that of controls (Table 1). The dry granuloma weight of the cotton pellet correlates with the amount of the migration of inflammatory cells and the formation of granulomatous tissue (Syed Ismail *et al.* 1997). *C. striatus* was also found to be the most potent inhibitor of the proliferative component of chronic inflammation (31.1% inhibition) (Figure 1) similar to piroxicam and statistically lower than mefenamic acid. The chloroform/methanol extracts and *C. lucius* did not inhibit this component.

As shown in Figure 1, *C. striatus* and *C. micropeltes* showed higher inhibition in the anti-transudative and anti-proliferative (42.9 and 31.2% respectively for *C. striatus*, 35.6 and 26.2% for *C. micropeltes*) when compared to those of mefenamic acid (25.1 and 21.3% respectively) and piroxicam (36.1 and

26.2% respectively). These results indicated that *C. striatus* has more anti-transudative and anti-proliferative activities than the other two extracts of *C. micropeltes* and *C. lucius*.

Monocyte infiltration, fibroblast proliferation and exudation take place in chronic inflammation. This proliferation may spread by proliferation of small vessels or granuloma (Hosseinzadeh *et al.* 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma (cotton pellet), which results from cellular reaction, by inhibiting granulocyte inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides (Onac *et al.* 1996). It is possible that water extracts of *C. striatus* and *C. micropeltes* inhibit monocyte infiltration and fibroblast proliferation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used therapeutic agents. However, the main side effects of NSAIDs include gastric ulcers, hypersensitivities, haematological toxicities, nephrotoxicity and hepatotoxicity (Carson and Rees Willett 1993). Results from the present study indicated the beneficial effects of *C. striatus* and *C. micropeltes*, but not *C. lucius* upon chronic inflammation. Further investigations into acute inflammation and identification of the active compound/s are undergoing.

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