Isolation of the bioactive compound(s) with antinociceptive activity in mice, from the fish Haruan, *Channa striata*

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Introduction

A freshwater fish species haruan, *Channa striata* (Bloch 1793 ; syn. *Channa striatus*), is carnivorous and air breathing. The fish is indigenous to Malaysia and extracts are commonly used to induce post-operative wound healing, especially women following caesarean births. Haruan-based cream is also effective for exfoliation in dermatitis, such as sclerosis, psoriasis, eczema and ichthyosis. The product was awarded a bronze medal at the Annual MINDEX/INNOTEX meeting Kuala Lumpur in 1996. Furthermore, the antinociceptive activity of Haruan extracts in mice was recognised by the Society of Anaesthesiologists as The Most Original Paper Commendation Award, at the Annual Scientific Meeting in Singapore on the 17th April 1997. We have recently demonstrated that the extract provided 100 % inhibition of peritoneal pain receptors and enhanced morphine responses (Mat Jais *et al.*, 1997).

The Haruan fish contains all the essential amino and fatty acids required for wound healing (Mat Jais *et al.*, 1994; 1998). We have previously shown that the bioactive compound in haruan extract is stable to pH 6 to 8, not destroyed by high temperature (100°C) and is not digested by the enzymes α-amylase, protease and lipase (Dambisya *et al.*, 1999).

Materials and methods

A Fresh midline fillet of haruan, *C. striata*, Figure 1, was homogenised in chloroform:methanol 2:1 v/v and the aqueous portion retained. A 2 ml aliquot of the aqueous was filtered through either 5,000, 10,000 and 30,000 dalton (Nominal Molecular Weight Limit) Millipore Ultrafree-CL low binding cellulose filter and centrifuged for 10 min at 5,000 rpm. A Series of 0, 25, 50 and 100 % dilutions of these filtered solutions were prepared in distilled water. Subsequently, 1 ml of the 5,000 and 10,000 dalton filtered solutions were purified by preparative HPLC using a LiChrospher 100 RP-18 column (5 mm, 125 mm x 4 mm, Hewlett Packard, Germany) at 1 ml/min and detected by UV at 205 nm. The mobile phase used 5 % CH_3OH/H_20 for 20 min, followed by gradient elution to 100 % CH_3OH.

The fraction collected between 1-3 minutes was dried under vacuum yielding 30 mg of extract, reconstitute in distilled water to a 0.0005, 0.005, 0.05 and 0.5 mg/ml in distilled water and then used in abdominal constriction tests in mice according to the method described (Mat Jais *et al.*, 1997). Acetic acid (0.6 %) used to induce pain in mice peritoneal cavity, was administered by an intra peritoneal injection in a volume of 10 ml/kg 30 after the subcutaneous administration of 0.3 ml of saline control and the Haruan extracts. The activity was calculated as the percentage inhibition of abdominal constrictions or writhing (Mat Jais *et al.*, 1997).

Results and discussion

Each of the 25, 50 and 100 % of the 5,000, 10,000 and 30,000 dalton filtered samples (see Figure 2 for the 30,000 dalton samples), as well as the HPLC fraction (Rt = 1 – 3 min) produced inhibition in constriction (Table 1). These activities were dose dependent and significantly different at $P < 0.01$. The lower concentrations had produced somewhat inconsistent activities (Figure 3), except the 5,000 dalton sample where the activity was clearly a dose dependent and significantly different at $P < 0.01$.

We have previously reported that the bioactive compound(s) is thought to be a polar macromolecule (Mat Jais *et al.*, 1994). The present findings confirm the molecule is more than likely less than 5,000 daltons. It remains a possibility that the compound(s) is a protein, perhaps a short peptide. It is unlikely however, to be a lipid such as a fatty acid given that fact that it is present in the aqueous fraction of the extraction.

To date the identificaty of the bioactive compound(s) responsible for

the antinociceptive activity remains to be determined and further analyses are currently in progress.

Reference

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Table I. Antinociceptive activity of Haruan, *Channa striata*, HPLC purified filtered extracts at < 5,000 daltons

Dosage (mg/ml)	Size (n)	N°. of Constriction ± S.E.M	Percentage of Inhibition (%)
Control	9	34.4 ± 0.87	-
0.0005	7	24.43 ± 1.41*	29.07
0.005	9	23.56 ± 1.22*	31.59
0.05	8	21.50 ±1.31*	37.57
0.5	8	18.00 ± 1.63*	47.74

* Significant at P< 0.001 when compared to the control group

Figure 1. Picture of Haruan, *Channa striata*

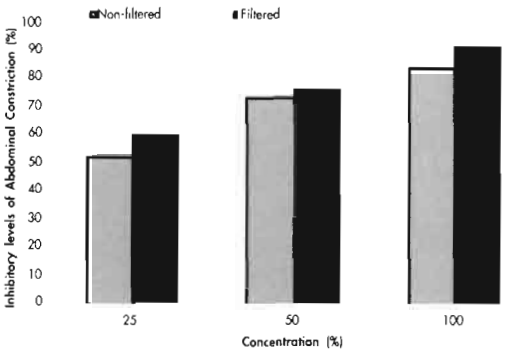

Figure 2. Antinociceptive activity of Haruan, *Channa striata*, non-filtered and filtered extracts at < 30,000 daltons

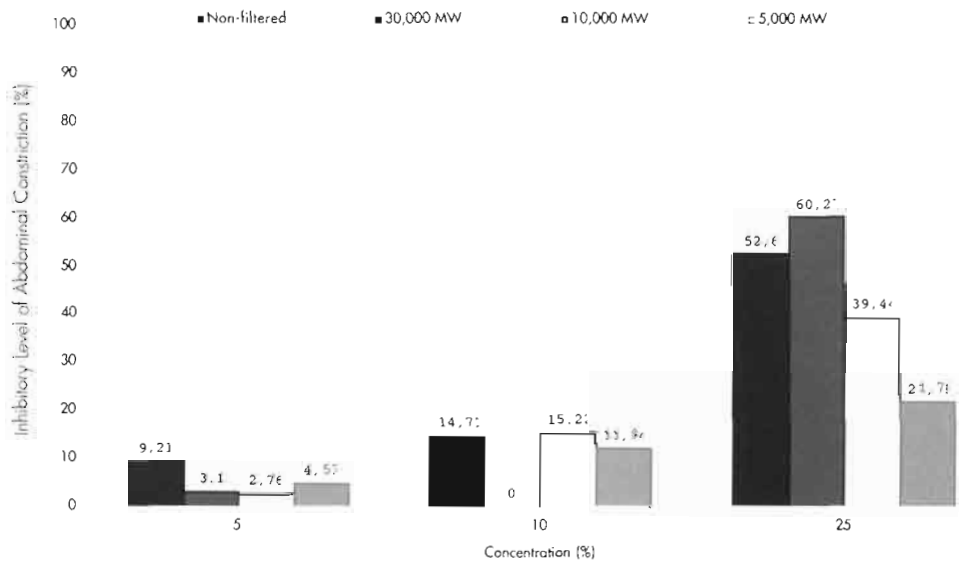

Figure 3. Antinociceptive activity of Haruan, *Channa striata*, non-filtered and filtered extracts at <30,000, <10,000 and < 5,000 daltons

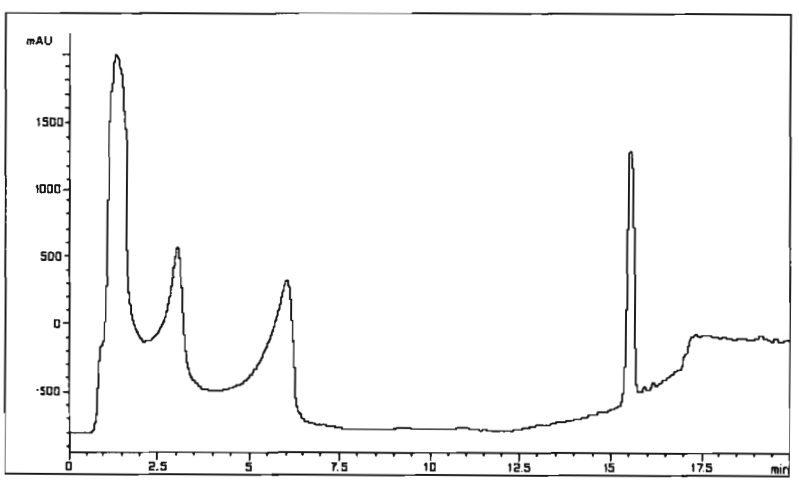

Figure 4. The analytical HPLC trace ofHaruan, *Channa striatus*, of filtered extracts at 5,000 daltons