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Molecular Size of the Bio-active Components from Haruan *Channa striatus* Extract

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Abstract: Extracts of an indigenous tropical carnivorous and air breathing fish haruan *Channa striatus* was shown to have antinociceptive activity in mice and the aim was to study tinvestigateto, at the molecular size of the bioactive compound(s), for isolation and identification. The aqueous portion of crude extract in chloroform-methanol 2: v/v was subjected to filtration by Millipore Ultrafree-CL low binding cellulose filter 5,000; 10,000 and 30,000 Dalton (Nominal Molecules Weight Limit or NMWL) and centrifuged for 10 min at 5,000 rpm. Series of 0, 25, 50 and 100% dilutions of the filtered solutions in distilled water were purified through preparative HPLC. The fraction collected between 1-3 min was dried under vacuum yielding 30 mg to be reconstituted in distilled water to a concentration of 0.0005, 0.005, 0.05 and 0.5 mg mL^{-1} in distilled water which were then used for abdominal constriction tests in mice according to method described by Mat Jais. The non-filtered, the 5,000 and 10,000 NMWL samples produced similar HPLC traces. The results indicated that the bioactive compound is less than 5,000 NMWL and finally, the fraction 1 at retention times 1 to 3 min, of the HPLC purified sample was also produced inhibition in the constriction test.

Key words: Bio-active components, molecular size, *Channa striatus*, extract

INTRODUCTION

Haruan, *Channa striatus*, an indigenous to Malaysia is a tropical freshwater species, carnivorous and air breathing fish. This fish is being consumed for protein and to induce wound healing in post-operative as well as after giving birth, especially among caesarean mothers. Subsequently, the Haruan based cream is also effective for exfoliation dermatitis, such as psoriasis, eczema and ichthyosis. The Haruan extracts anti-nociceptive activity in mice was recognized by the Society of Anaesthesiologist and the extract has provided a 100% block on peritoneal pain receptor and enhanced morphine (Mat Jais *et al*., 1997). It was reported that the *Channa striatus,* contains all the essential amino and fatty acids for wound healing (Mat Jais *et al*., 1994, 1998) and the bioactive compound in Haruan extract is stable to pH 6-8 and temperature up to 100°C. During cooking, it was digested by enzymes α-amylase protease and lipase (Dambisya *et al*., 1999). These are the basis of our analysis to identify the bioactive compound(s) in haruan's extract and to determine the molecular size of the identified compound(s).

MATERIALS AND METHODS

Fresh midline fillet of Haruan, *C. striatus*, is homogenised in chloroform:methanol 2:1 v/v and the aqueous portion was siphoned out as stock and 2 mL aliquot of this is placed in 5,000, 10,000 and 30,000 NMWL (Nominal Molecule Weight Limit), Millipore Ultrafree-CL low binding cellulose filter and centrifuged for 10 min at 5,000 rpm. Series of 0, 25, 50 and 100% of the 30,000 NMWL filtered samples were prepared in distilled water for the constriction test, followed by 5, 10 and 25% of the 10,000 and 5,000 NMWL samples. Subsequently, 1 mL of the non-filtered, 5,000 and 10,000 NMWL filtered samples were purified through an analytical column with multiple injections and collection of material Hewlett Packard HPLC at 1 mL min^{-1}, 205 nm, 5% CH_3OH/H_2O for 20 min followed by 100% CH_3OH. The fraction collected between 1-3 min was dried under vacuum yielding 30 mg of extracts, reconstituted in distilled water to a concentration of 0.0005, 0.005, 0.05 and 0.5 mg mL^{-1} in distilled water and used in abdominal constriction test in mice according to method described by Mat Jais *et al.* (1997). Acetic Acid (0.6%) was used to induce pain in mice through intra peritoneal injection in a volume of 10 mL kg^{-1} 30 min after subcutaneous administration of 0.3 mL of saline as control and the haruan extracts. The activity was calculated as percentage of inhibition of the abdominal constriction or writhing. All the evaluating of the chemically induced peripheral antinociceptive activity was carried out in Male Balb-C Mice (25-30 g, 5-7 weeks) obtained from Animal Source Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). All 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 Medicine and Health Sciences, UPM for at least 48 hr before experimentations, where water and food were given *ad libitum*. The mice were divided into groups of 7 per group (n = 7).

RESULTS AND DISCUSSION

As expected, the 30,000 NMWL (Fig. 1) filtered samples has produced a dose dependent activity and each of the 25, 50 and 100% concentration of the 5,000, 10,000 and 30,000 Dalton filtered samples and the HPLC fraction (Ht = 1-3 min) also produced inhibition in the abdominal constriction tests. At 5, 10 and 25% dosage of the 30,000, 10,000 and 5,000 NMWL filtered samples has also produced dose dependent activity Table 1 These activities were dose dependent and significantly different at $p<0.01$. Although the lower concentrations had produced somewhat inconsistent activities as in Fig. 1, but the 5,000 Dalton sample the activity was clearly a dose dependent and significantly different at $p<0.01$, Table 2.

From previous works, the bioactive compound(s) were thought to be polar and macromolecule, in the form of a lipoprotein, glycoprotein, glycolipoprotein or even a peptide (Mat Jais *et al*., 1994). In this work, where the less than 5,000 NMWL filtered compound(s) still producing

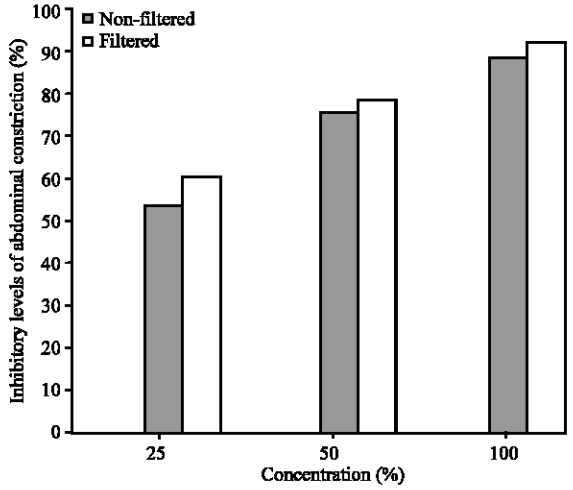

Fig. 1: Antinociceptive activity of Haruan, *Channa striatus*, non-filtered and filtered extracts at 30,000 Dalton. The 25, 50 and 100% concentration of the extracts has increased the percentage of inhibition and they are significantly different at $p<0.001$ and the results is indicating that the bioactive compound is less than 5,000 NMWL. Finally, the fraction 1 at retention times 1 to 3 min, of the HPLC purified sample of both filtered and the nonfiltered extracts had also produced inhibition in the constriction test

Table 1: Antinociceptive activity of Haruan, *Channa striatus*, non-filtered and filtered extracts at 30,000, 10,000 and 5,000 Dalton

Inhibitory levels of abdominal concentration (%)	Non-filtered	30,000 MW	10,000 MW	5,000 MW
5	10	4	3	5
10	13	0	13	10
25	50	58	38	18

Table 2: Antinociceptive activity of Haruan, *Channa striatus*, HPLC puried filtered extracts at <5,000 Dalton

Dosage (mg mL^{-1})	Size (n)	No of Constriction+S.E.M	Percentage of Inhibition
Control	9	34.40+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

significant antinociceptive activity and is very much in line with the suggestion that the active molecule is a small or short chain molecule. Furthermore, the chemical that supposed to come out at first 4 min of the retention time is protein (positive with ninhydrin) of small molecule or short amino acid peptide. However, lipid molecule or a short chain fatty acid should be present due to the facts that the chloroform: methanol solvent is meant to extract lipid or fatty acids. Nevertheless, more analysis is needed, using a much sensitive biochemistry technique to include protein sequencer, mass spectrophotometer and NMR.

CONCLUSIONS

In this study, antinociceptive activity of Haruan extract was investigated. Significant antinociceptive activity was found of components molecular size less than 5,000 NMWL filtered samples. The Haruan extract studied here can be seen as a potential source of useful drugs.

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