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## Preliminary Investigation on the Antinociceptive Properties of Haruan (*Channa striatus*) Fillet Extracted with Various Solvent Systems

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**Abstract:** The present study was conducted with an aim of establishing the best organic solvent system that can yielded the *Channa striatus* extract with highest antinociceptive activity and also to determine the polarity of the respective bioactive compound using the acetic acid- induced abdominal constriction test. Briefly, the *Channa striatus* fillet were extracted separately with methanol, ethanol, chloroform, chloroform:methanol and distilled water overnight. This procedure is repeated three times and each of the supernatant obtained was pooled together and then subjected to the evaporation process. The extracts obtained was prepared in the doses of 0.5, 1.0, 2.0, 4.0 and 8.0 g kg<sup>-1</sup> and administered subcutaneously (SC) into mice. Thirty min after the administration of extracts, 0.6% acetic acid was administered intraperitoneally (IP). All solutions were administered in the volume of 10 mL kg<sup>-1</sup>. From the data obtained, it can be concluded that the bioactive compounds to be of two types, the polar compound that is water-soluble and the non-polar compound that is fat-soluble. These two compounds might act alone, as in DH<sub>2</sub>O and chloroform extracts or act together, as in CM, methanol and ethanol extracts. Furthermore, the ability of this compound(s) to exhibit the antinociceptive activity in all the three types of solvents also suggested the presence of peptide compound. However, further investigation need to be carried out in order to isolate and identify the respective compound. In addition, methanol and chloroform:methanol were found to be the best solvents for further isolation of the respective bioactive compound of *Channa striatus*.

**Key words:** Antinociceptive, *Channa striatus*, abdominal constriction test, organic solvents, polar and non-polar compounds

### INTRODUCTION

Animal extracts have been used for the treatment of different ailments since antiquity. Previous studies have reported the presence of various pharmacological properties and activities, such as antinociceptive<sup>[1-3]</sup>, antimicrobial<sup>[4]</sup> and wound healing, in the animal preparations, such as frogs, spiders, snails and sea cucumber. The discovery of new compounds of animal origin that have the above mentioned activities is of considerable interest in view of the ever increasing frequency of the side effects caused by the used of various chemically and synthetically produced drugs that might sooner or later harm human life<sup>[6]</sup>.

This research describes the study of *Channa striatus* (Channidae, common name: Haruan, Snakehead fish)

a freshwater, air-breather and carnivorous fish indigenous to many tropical and subtropical countries of South America, Africa and Asia, including Malaysia<sup>[7,8]</sup>. It has been widely used in traditional medicine in a believed that it promotes wound healing<sup>[9]</sup>. In addition, it is popularly used in the hospitals by patients in the post-operative period in the belief that it promotes wound healing, especially in mothers who had a caesarian operation and alleviates post-operative pain and discomfort<sup>[10]</sup>. Previous studies have revealed the potential of *Channa striatus* as an antinociceptive agent<sup>[11]</sup> and later the bioactive compound responsible for the respective activity was found to withstand extreme temperature and pH<sup>[12]</sup>. The objectives of this study was to establish the best organic solvent system that can yielded the *Channa striatus* extract with highest antinociceptive activity and also to

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determine the polarity of the respective bioactive compound.

## MATERIALS AND METHODS

**Experimental animals:** Balb-C mice (25-30 g; 5-7 weeks old), obtained from the Institute of Medical Research (IMR, Kuala Lumpur, Malaysia), were used in this study. The animals were and kept under room temperature ( $27\pm 2^\circ\text{C}$ ; 70-80% humidity; 12 h light/darkness cycle) in the Animal Holding Unit, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia for at least 48 h before used. Food and water were supplied *ad libitum*. At all times the mice were handled in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals<sup>[13]</sup>. All experiments were conducted between 09.30 and 18.30 h to minimize the effects of environmental changes.

**Chemicals:** Aspirin (Bayer, Singapore) was prepared in the dose of  $10\text{ mg kg}^{-1}$  and used as a standard peripheral antinociceptive drug. All solvent, namely methanol (Merck, Germany), ethanol (Scharlau, Spain), chloroform (Merck, Germany), chloroform:methanol and distilled water ( $\text{DH}_2\text{O}$ ), used were of analytical grade. The  $\text{DH}_2\text{O}$  was prepared using the Bibby Sterlin Ltd. (UK) W 4000 water distiller.

**Animal material:** Throughout the study, 6 month old (50-400 g) *Channa striatus* were used. They were cultured from Pontian, Malaysia and transferred to Universiti Putra Malaysia (UPM), Malaysia and acclimatized for at least 3 days prior to experiments.

**Preparation of freeze-dried *Channa striatus* fillet:** Precleaned live fish was weighed and then slaughtered. The fish fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain maximum amount of flesh without any backbone<sup>[11]</sup>. Their fillets were cut into small portions and placed into a sterile plastic bag (15x30 cm). The bag was sealed and transferred to a freezer at  $-80^\circ\text{C}$  for 24 h. At the end of 24 h, the bag was removed from the freezer and was cut to open its sealer. The opened bag containing haruan fillet was transferred immediately into the freeze-dryer bottles for freeze-drying procedure using the freeze-dryer equipment (Virtis 254110, USA). This process was done for 48 h for the purpose of removing all the water inside the fillet. At the end of 48 h, the dried fillet was obtained and removed from the freeze dryer bottles. The weight of the freeze-dried fillet (FDF) was then recorded.

**Preparation of *Channa striatus* extracts:** The FDF obtained were treated separately with various organic solvents, namely, methanol, ethanol, chloroform, CM (2:1; v/v) and  $\text{DH}_2\text{O}$ , to extract the solvent-soluble bioactive compound according to their polarity. Briefly, the FDF were homogenized for 5 min in absolute methanol, in the ratio of 1:5 (w/v), in a conical flask and then stirred for 24 h to mix it thoroughly. At the end of 24 h, the supernatant was collected by filtering the mixture solution through the filter paper (Whatman<sup>®</sup> No. 1; UK). This process was repeated three times, using fresh solvent on each occasion, until it is quite satisfy that the entire bioactive compound, which dissolved in methanol, has been extracted. The satisfaction is based on the changes of color of the crude methanol supernatant. The supernatant, which is yellowish when extracted for the first time, indicated the present of methanol-soluble compounds while the supernatant, which is crystal clear when extracted for the third time, indicated that there are no more methanol-soluble compounds present in it. The same procedure was carried out on the new set of FDF using the other solvents as mentioned earlier.

Except for water-based extract, all the respective solvent-soluble layers obtained from each stage were pooled together into one container and then filtered again before they are subjected to the evaporation process. The evaporation process was carried out using a rotary vacuum evaporator (Heidolph VV2011 and Heidolph WB2001, Germany). The weights of each solvent extract obtained were recorded and stored at  $4^\circ\text{C}$  until used. As for the  $\text{DH}_2\text{O}$  extract, it was not evaporated by means of rotary evaporator since the evaporator can only evaporate solvent with boiling point up to  $95^\circ\text{C}$ . Water, which is known to have boiling point of  $100^\circ\text{C}$ , was removed from the extract by heating the water-based extract by using heater (Thermolyne, USA). This process of heating was carried out with regard that the antinociceptive property of *Channa striatus* was not destroyed even after cooking under pressure at  $120^\circ\text{C}$ <sup>[11]</sup>. The methanol, ethanol, chloroform, CM and  $\text{DH}_2\text{O}$  extracts obtained were then dissolved in  $\text{DH}_2\text{O}$  to the doses of 0.5, 1.0, 2.0, 4.0 and  $8.0\text{ g kg}^{-1}$ .

**Experimental procedure:** Three hundred and sixty mice were equally divided into 5 groups for treatment with methanol-, ethanol-, chloroform-, CM- and water-extract, respectively. From each groups, the animals were further divided into 6 subgroups; the first subgroup was used as a control group (receiving only  $\text{DH}_2\text{O}$ ) and the other five subgroups were treated with different doses of the respective extracts ( $0.5, 1.0, 2.0, 4.0$  and  $8.0\text{ g kg}^{-1}$ ). All of the solutions were administered subcutaneously (SC) in the volume of  $10\text{ mL kg}^{-1}$ , 30 min before the acetic acid

administration. Administration of acetic acid marked the final stage at which evaluation of the respective extract antinociceptive activity was carried out.

**Antinociceptive assay:** The abdominal constriction test was used, as described by Dambisya and Lee<sup>[12]</sup>, to study the antinociceptive activity of *Channa striatus* extract treated with various solvents. The acetic acid (0.6%; v/v) (J.T. Baker, USA), used to induce pain in mice peritoneal cavity, was administered intraperitoneally (IP) in a volume of 10 mL kg<sup>-1</sup>, 30 min after the administration of DH<sub>2</sub>O or extracts, respectively. The abdominal constrictions or writhing response resulting from injection of acetic acid consisting of a contraction of the abdominal together with a stretching of hind limbs<sup>[14]</sup>. The number of abdominal constrictions was counted cumulatively over a period of 30 min following the acetic acid administration and analgesia was calculated as the percentage of antinociception:

$$\frac{\text{Saline control group mean} - \text{test group mean}}{\text{Saline control group mean}} \times 100\%$$

**Statistical analysis:** The Jandel Scientific Sigma Stat's (Version 2.0) statistical programs were used to analyze and compare the data and all values were presented as Mean±SEM. Data from the control-, extracts- and reference drug-treated animals were compared by Analysis of Variance (ANOVA) followed by Tukey Test<sup>[15]</sup>.

## RESULTS

**Preparation of *Channa striatus* FDF:** From 1018.60 g of wet weight fillet of haruan approximately 266.09 g (26.12%) of FDF was obtained after the freeze-drying process.

**Preparation of *Channa striatus* extracts:** From each of the 109.50 g of FDF used, approximately 31.10 g of pale brown methanol extract and 11.19 g of dark brown chloroform extract was obtained while from each of the respective 129.50 g of FDF, approximately 29.84 g of chocolate DH<sub>2</sub>O extract and 10.02 g of dark brown ethanol extract was obtained, respectively. On the other hand, from 30.00 g of FDF used, approximately 11.33 g of dark brown CM extract was obtained. These extracts were then prepared in the doses of 0.5, 1.0, 2.0, 4.0 and 8.0 g kg<sup>-1</sup> before being used for antinociceptive evaluation.

**The antinociceptive activity profiles of various solvents extracts of *Channa striatus*:** There was a highly significant and demonstrable dose-response pattern for each of the respective extracts between the doses of

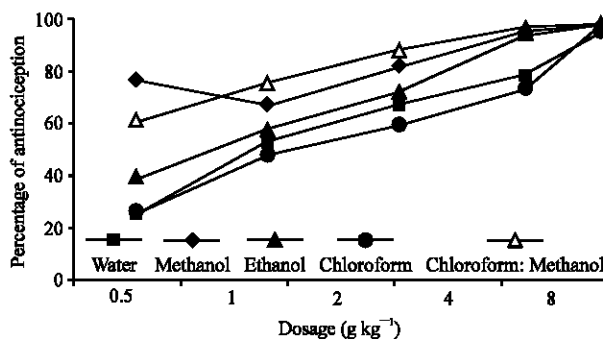


Fig. 1: The antinociceptive profiles of *Channa striatus* fillet extracted with various types of solvents

0.5 to 8.0 g kg<sup>-1</sup>. Except for the chloroform extract, the 1.0 g kg<sup>-1</sup> of the rest of the extracts were found to give approximately more than 50% antinociception. CM extract, followed by methanol extract, was found to give the highest inhibition, which exceeded the 50% inhibitory level even at the lowest dose of 0.5 g kg<sup>-1</sup>. The almost complete inhibition of abdominal constrictions was reached in all extracts at the dose of 8.0 mg kg<sup>-1</sup> with the ethanol extract producing the complete antinociception (100% antinociception). In addition, the data obtained for water and chloroform extracts were found to be insignificant against each other at their respective dose. Interestingly, the antinociceptive activity was found to present in all extracts regardless of the polarity of the solvents used. Except for the ethanol extract, the methanol, water, chloroform and CM extracts were found to produce similar and insignificant effect as the aspirin at the dose of 2.0, 4.0, 1.0 and 1.0 g kg<sup>-1</sup>, respectively (Fig. 1).

## DISCUSSION

Freeze-drying is a process of removing the water contained inside a sample so that an accurate weight of sample can be obtained and used in the solvent extraction procedure. The process is carried out by using freeze-dryer and approximately 100% of water can be removed away from the sample. Furthermore, samples that have been freeze-dried do not lose its biological activity since this process did not involved activation, destruction/denaturation or alteration in the structure of its bioactive compound(s).

*Channa striatus* extract, either fillet or mucus, have been reported to produce a concentration-dependent antinociception when assessed by abdominal constriction test and not the tail-flick test<sup>[11]</sup>. This finding seems to suggest the involvement of peripheral rather than central mechanism in *Channa striatus* antinociception. The ability

of the mucus extract to withstand the effects of enzymes<sup>[16]</sup>, extreme temperature and pH, together with the failure of naloxone (a general opioid antagonist) to block its antinociception, have lead to suggestions that the bioactive compound is a stable and short-chained macromolecule working on the non-opioid receptor system<sup>[12]</sup>. The present study was generally able to prove the presence of two set of bioactive compounds, polar and non-polar compounds, with antinociceptive properties in *Channa striatus* fillet. The polar compound is expected due to the ability of DH<sub>2</sub>O extract to exhibit antinociception while the non-polar compound is expected based on the present of antinociceptive activity in chloroform extract. Furthermore, this study was also able to determine CM, followed by methanol, as the best solvent system for isolation of the respective bioactive compounds, which is CM followed by methanol, based on the respective extract potential to produce highest antinociception.

The order of decreased in polarity of the solvents used were water (DH<sub>2</sub>O), methanol, ethanol and chloroform. Thus, the highest in activity of CM followed by methanol and ethanol extracts is expected since methanol possesses moderately polar activity and place between water (DH<sub>2</sub>O) and ethanol as stated earlier. Methanol is well known to dissolve in both polar and non-polar solvents and is capable of dissolving both the polar and non-polar compounds at the same time while DH<sub>2</sub>O (highly polar solvent) or chloroform (highly non-polar solvent) only dissolved the respective polar or non-polar compound at a time. This statement is supported by our finding that methanol, in combination with chloroform (CM) or alone (as in methanol extract) gave the highest antinociception compared to other extracts. The ability of CM extract, followed by methanol and ethanol extracts, to give the highest antinociceptive activity might be due to the synergistic effect caused by the present of both polar and non-polar antinociceptive compounds in the extract. The ability of CM extract to give the antinociception, in all doses tested, may be explained by the fact that it contains two types of solvents, which differ in their polarity and ability to extract certain type(s) of compound(s). Due to this, the extract might contain various types of compounds, which dissolved in chloroform and methanol separately, but act together when administered subcutaneously into the mice. On the other hand, the different in the results between methanol and ethanol extracts may be explained by the fact that ethanol is less polar than methanol. It is suggested that ethanol might have less ability to extract both type of compounds (water-soluble and oily

compounds) compared to methanol. This activity is lowered in DH<sub>2</sub>O and chloroform extracts since there is only one type of compound present in the respective extract and that no synergistic effect occurs.

The ability of the bioactive compound to produce the activity when extracted with both the polar and non-polar, as well as the moderate polar, solvents might suggest the presence of peptide compounds. This is based on the fact that only peptide has the ability to dissolve in those three types of solvents<sup>[17]</sup>. Their ability to dissolve in all the three types of solvents is due to the present of the different charged, positive and negative, amino acid units in their structure. The basic peptides, which contained more negative charged amino group, often dissolved in water. On the other hand, the acidic peptides, which contained more positive charged amino group, may dissolved in water and acetic acid. The third type of peptide, neutral peptides, which contained equal amount of positive and negative amino group, may require other organic solvents, such as methanol, ethanol, chloroform, isopropanol and acetonitrile. There is also report on the presence of proteinaceous materials during the lipid extraction of fish meal using the Bligh and Dyer, Hot Sandler and Hot Hexane Reflux methods<sup>[18]</sup>, respectively. These methods were known generally to use chloroform, chloroform:methanol and hexane as their solvent system for lipid extraction. All solvents used were expected to extract non-lipid nitrogenous material, which are considered to be proteins. These non-lipid or proteinaceous materials are often 'solubilised' by the polar lipids into the chloroform or hexane phase. Earlier, there was also report on the presence of amino acids in phospholipids<sup>[19]</sup>.

From the data obtained, it can be concluded that the bioactive compounds responsible for haruan antinociceptive activity to be of two types, the polar compound that is water-soluble and the non-polar compound that is fat-soluble. These two compounds might act alone, as in DH<sub>2</sub>O and chloroform extracts, or act together, as in CM, methanol and ethanol extracts. Furthermore, the ability of this compound to exhibit the antinociceptive activity in all the three types of solvents suggested the presence of peptide compound. Thus, it can be concluded that the bioactive compounds responsible for haruan antinociceptive activity might be a complex and stable macromolecule, which might contain protein, lipid or carbohydrate in different combination as in glycoprotein, polypeptide, glycolipid, polysaccharide, or even glycolipoprotein. However, further investigation need to be carried out in order to isolate and identify the respective compound. In addition, methanol and

chloroform:methanol were found to be the best solvents for further isolation of the respective bioactive compound(s) of haruan.

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