Evaluation of the Onset and Offset Time of Aqueous Supernatant of Haruan
(Channa striatus) Fillet Antinociception Administered Subcutaneously in Mice

1,2 Z.A. Zakaria, 2,3 M.R. Sulaiman, 3,4 M.N. Somchit and 2,4 A.M. Mat Jais
1School of Biotechnology and Life Sciences, Universiti Industri Selangor,
Jalan Zirkon A7/A, Seksyen 7,40000 Shah Alam, Selangor, Malaysia
2Department of Biomedical Sciences, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3Laboratory of Pharmaceutical and Neutriceutical, Institute of BioScience,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract: The onset and offset time of aqueous supernatant of haruan (Channa striatus) fillet (ASH) antinociception was evaluated using the abdominal constriction test. The ASH, extracted using chloroform methanol (2:1; v:v) overnight and then evaporated to remove the residual solvent, was prepared in the concentration of 10, 25 and 100% and administered subcutaneously (SC) at four different set of time (0, 5, 30 and 60 min). At the end of the respective time, acetic acid (0.6%) was administered intraperitoneally (IP) and the number of abdominal constrictions was then calculated. ASH was found to exhibit a concentration-dependent antinociception. The onset time of antinociception was found to be between 0 to 5 min depending on the concentration of ASH used with the highest and lowest concentrations of ASH produced antinociception 0 and 5 min after their administration, respectively. This activity, which increased with increase in time of ASH administration, was found to reach the highest level after 30 min of ASH administration regardless of the concentration used. The offset time, on the other hand, does not depend on the concentration of ASH used. The three respective concentrations of ASH antinociception were found to diminish completely after 60 min of their administration. Interestingly, it was also found that ASH produced activity comparable to that of acetylsalicylic acid (ASA).

Key words: Antinociception, haruan (Channa striatus), abdominal constriction test.

INTRODUCTION

The continuing quest to find a cure for pain, together with the unwanted side effects reported on many antinociceptive agents, has lead scientists to other fascinating places. They are now turning to natural product from plants as well as marine and aquatic or terrestrial animals as a new source for pain relieving agent. The potential of haruan (Channa striatus) as an antinociceptive agent have been demonstrated recently by Mat Jais et al. It was demonstrated that the fillet and mucus extracts of haruan exhibited a dose-dependent antinociception. Furthermore, Mat Jais et al. also suggested that both extracts act mainly through the peripheral nervous system rather than central nervous system since they only showed their effects on their own when assessed by the abdominal constriction test but not the tail-flick test. However, up to date there is no attempt to establish the onset and offset time for both extracts antinociception. The present study was undertaken to determine the onset and offset time for the Aqueous Supernatant of Haruan (ASH) antinociception.

MATERIALS AND METHODS

Preparation of fresh haruan fillet: Six month old (50-400 g) fresh haruan fish were used through the study. Briefly, precleaned live fish was weighed and slaughtered. The fish fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain maximum amount of flesh without any backbone.

Preparation of ASH and acetylsalicylic acid (ASA) solutions: The ASH was prepared using the chloroform:methanol (CM) (2:1; v:v) system as described by Benkendorff et al. with slight modifications. Briefly,
the fillet obtained was mixed with CM in the ratio of 1:2 (w:v) and left overnight (24 h). After 24 h, the supernatant obtained was collected and left for another 30 min to settle down into two distinct layers of supernatant. The upper layer, which is an aqueous supernatant (ASH), was collected and then evaporated to remove the methanol residues. On the other hand, the lower layer, which is the chloroform:methanol layer, was stored at 4°C for future studies. The evaporated ASH, which is considered as the stock solution with 100% concentration, used in this study together with 10 and 25% concentration ASHs, DH3O prepared by diluting the stock solution in distilled water for this study. Acetylsalicylic acid (ASA) (Bayer, Singapore) prepared in the dose of 10 mg kg⁻¹ by dissolving it in DH3O, was used as the positive control group.

Experimental animals: Male Balb-C mice (25-30 g; 5-7 weeks old) were kept under room temperature (27±2°C; 70-80% humidity; 12 h light/darkness cycle) for at least 48 h before used. Food and water were supplied ad libitum. All the animals 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[9]. Experiments were conducted between 09.30 and 18.30 h to minimize the effects of environmental changes. All mice were equally divided into 20 groups of 7 mice and further divided into 5 subgroups each for treatment with DH3O, ASA (10 mg kg⁻¹) or ASH (10, 25 and 100% concentration), subcutaneously (SC). The mice were then left to rest at four respective set of time (0, 5, 30 and 60 min) before administration of acetic acid (J.T. Baker, USA) intraperitoneally (IP) (0.6%, v/v). All the solutions were administered in the volume of 10 mL kg⁻¹ of mice.

Antinociceptive Assay: The abdominal constriction test[8] was used to study the onset time and offset time of ASH antinociception. Briefly, acetic acid was administered IP 30 min after the SC administration of DH3O, ASA or ASH. The abdominal constriction response resulting from injection of acetic acid, which consists of a contraction of the abdominal together with a stretching of the hind limbs[8], was counted cumulatively over the period of 25 min, 5 min following the acetic acid administration. Antinociception was indicated by the reduction in the mean of the number of abdominal constrictions in the test groups compared to the control group.

Statistical analysis: The results are presented as Mean±SEM. One-way ANOVA test was used to analyze and compare the data, with p<0.05 as the limit of significance.

RESULTS

The antinociception profiles of ASA and ASH after administration at 0, 5, 30 and 60 min: The duration of action of ASH administered SC was determine to establish the actual time when the extract start to exhibit its activity and the offset time when the activity stop or diminish. The respective DH3O, ASA or ASH was administered at 0, 5, 30 or 60 min prior to acetic acid administration and the results were presented in the histogram (Fig. 1). For the 10 mg kg⁻¹ ASA-treated groups of mice, there were reduction in the number of abdominal constrictions with increased in time. However, this observation is not seen in groups treated with ASA for 60 min before acetic acid administration. The ASA seems to exhibit its activity after 5 min of its administration and tend to give the highest reduction in the number of abdominal constrictions after 30 min of its administration. This activity seems to diminish after 60 min of its administration. The data obtained after treatment with 10% concentration ASH was found to reduce significantly (p<0.05) after 5 min of its administration and this activity is not significantly changed even after 30 min of its administration. The activity was found to diminish after 60 min of its administration. The 25% concentration ASH antinociception was found to appear after 5 min of its administration and disappeared after 60 min of its administration. This activity was found to reach highest peak after 30 min of its administration. The data obtained for groups treated with 100% concentration extracts showed significant antinociception immediately (0 min) after its administration and the activity also tend to disappear 60 min after its administration. This activity was found to reach its highest peak after 30 min of its administration with almost complete inhibition of abdominal constrictions being observed.

Comparison on the ASH activity at the respective time against the control group: Comparisons were also made between the activity of different concentration of ASH at their respective time of administration against the negative and positive control groups and the data were presented as percentage of antinociception. At the time of 0 min following their administration, ASH as well as 10 and 25% concentration ASH were found to produce insignificant activity when compared against the negative control groups and against each other, respectively. The 100% concentration ASH, on the other hand, was found to exhibit significant antinociception immediately after its administration. It is interesting to see significant increased in the antinociception after treatment of mice with 10, 25 and 100% concentration ASH for 5 min.
Fig. 1: The antinociceptive profiles of ASH compared to
the DH2O and aspirin.

The activities of ASH were found to reach their highest
level after 30 min of administration with the 25% concentra-
tion ASH producing approximately 50% of antinocicep-
tion. The 100% concentration ASH, at this
moment, was found to produce almost 90% antinocicep-
tion. Lastly, treatment of mice with the respective compounds for 60 min were found to produce
significant decreased in the percentage of antinocicep-
tion with only the 25 and 100% concentration ASH still
producing an antinociception.

DISCUSSION

In the present study, the duration of antinociception
of ASH was measured to determine the onset and offset
time following its SC administration. From the data
obtained, the ASH was found to produce a concentration-
dependent antinociception as reported by Mat Jais et al.[1]
and this concentration effect seems to influence the onset
but not the offset time of ASH antinociception. Except for
the 100% concentration ASH which exhibited almost
immediate (0 min) antinociception, the 10 and 25% concentra-
tions extracts, were found to exhibit the activity
only after 5 min of their administration. Except for 10% concentra-
tion ASH, all of the compounds antinociception
were found to reach their highest level after 30 min of
administration. The antinociceptive activities of ASH were
found to diminish approximately 60 min after their
administration regardless of their concentrations. The concentra-
tion-dependent antinociception produced by
25% concentration ASH is comparable to that of ASA
after 0, 5 and 30 min of its administration suggesting their
similar efficiency and strength. The activity, which is
significant for both compounds after 60 min of their
administration, lead us to suggest that the ASH are more
easily metabolized and excreted from the body due to its
dlower half-life than ASA which tend to have a higher half-
life. The higher half-life of ASA, which mean that it is
metabolized slower and stay longer in the body, might
explained the ability of ASA to retain its antinociception
after 60 min.

The route of administration that is chosen may have
a profound effect upon the rate and efficiency with which
the drug acts. For example, the rate and efficiency of drug
absorption following SC injection may be greater than,
equal to, or less than that following oral administration
depending on the drug under consideration[10].
Furthermore, after the SC administration, the respective
ASH was disseminated into the system after passage
through capillary or lymphatic endothelium. The presence
of membrane barriers for absorption within the
endothelium of vascular and lymphatic capillaries through
which the respective compounds have to pass by before
they are carried in the blood circulation to the site of
action caused delayed in the time of onset because a lot
of time have to be taken for sufficient amount of
compounds to be absorbed in order to express its
antinociception. This seems to be true for the low
concentration but not the high concentration of ASH. The
reason is that high concentration of ASH tends to provide
enough supply of bioactive compound to produce
antinociception by overcoming the process of rapid
metabolism which is due to its low half-life as mentioned
earlier. On the other hand, the offset time is fast due to
most of the compound injected being metabolized or
excreted from the circulation system before they can
reach the site of action. Thus, the amount of compound
reaching the site of action is expected to be small in
amount which might not be enough to cause significant
antinociception.

Finally, we concluded that the haruan (ASH)
antinociception is concentration-dependent and that
the onset time of antinociception depends on the
concentration of ASH used with the lowest concentra-
tion of ASH (10 and 25%) produced antinociception 5 min after
their administration while the 100% concentration ASH
tend to exhibit the activity almost immediately (0 min) after
its administration. This activity was found to increase
with increase in time of ASH administration and tend to
reach the highest level 30 min after its administration. The
offset time, however, does not depend on the
concentration of ASH used with the three respective
concentrations of ASH antinociception remarkably
diminished after 60 min of their administration.

ACKNOWLEDGEMENT

This study was supported by the Research Grant of
Universiti Industri Selangor, Malaysia (Project Code
Number: 03013; Project Vote Number: 3090303013). The
authors would like to thanks Universiti Putra Malaysia for
the facilities and Mrs. Fatimah Corazon Abdullah for her skillful help in the preparation of the manuscript.

REFERENCES