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The Antinociceptive Effects of Water Extracts from Sea Cucumbers *Holothuria leucospilota* Brandt, *Bohadschia marmorata vitiensis* Jaeger and Coelomic Fluid from *Stichopus hermanii*

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Abstract: Sea cucumber or "gamat", as it is locally known is one of the traditional medicine that has been used until today. It is believed to help accelerate wound healing, especially after giving birth and also help to relief post-surgery pain. This experiment is to study the antinociceptive effect of water extract from sea cucumbers *Holothuria leucospilota* Brandt, *Bohadschia marmorata vitiensis* Jaeger and also the antinociceptive effect of coelomic fluid from *Stichopus hermanii* in mice. Two methods were used, i.e. writhing test and tail-flick test, in comparison to morphine sulphate and normal saline as positive and negative control, respectively. In the writhing test, the group of mice treated subcutaneously with control normal saline did not show a significant ($p<0.05$) inhibition effect. Mice group treated subcutaneously with extract and coelomic fluid of sea cucumbers (50, 75 and 100 mg kg^{-1}) and also morphine sulphate (0.8 mg kg^{-1}) were found to inhibit abdominal contraction induced by acetic acid (0.6%). The tail-flick test using water bath (50°C) showed a weak antinociceptive effect at the dose of 100 and 200 mg kg^{-1}, but was not statistically significant ($p < 0.05$). This effect could be seen 15 min after intraperitoneal administration of extracts or morphine sulphate (5 mg kg^{-1}) into the mice. However, when tested statistically, both methods showed no dose-response relationship for the doses used. Hence, this study suggests the potential of water extract from *H. leucospilota* and *B. marmorata vitiensis* and the coelomic fluid from *S. hermanii* as alternative analgesic drug sources in the future.

Key words: Antinociceptives, extracts, coelomic fluid, sea cucumber

INTRODUCTION

Sea cucumbers are locally known as "bat" or "balat" among the ethnics of Sabah. In the Peninsular of Malaysia, they are referred to as "gamat", a name given to one particular species which is widely used in traditional medicine. However, other species appear to have similar medicinal properties (Ridzwan *et al*., 1995). Like haruan, *Channa stratus* (Mat Jais *et al.,* 1997), sea cucumbers promote wound healing (Ridzwan *et al*., 1990) whereas their extracts being antibacteria (Ridzwan *et al.,* 1995; Noah *et al.,* 2001) and contain antioxidant properties (Hawa *et al*., 1999) and fatty acids (Fredalina *et al*., 1999). Relief from pain and irritation is one of the reported benefits in those taking sea cucumbers based remedies for the treatment of skin conditions such as eczema and arthritis. However, despite the widespread use of preparations from sea cucumbers for medicinal purposes, there have been hardly any study to establish the scientific basis for its claimed benefits, especially, the analgestic tendency of sea cucumbers, hence the present study. Water extracts of *Holothuria leucospilota Brandt* and *Bohadschia marmorata vitiensis* Jaeger and coelomic fluid *Stichopus hermanii* were investigated for their antinociceptive effects in mice with a view to establishing whether or not the reported pain-relieving activities of sea cucumbers have a scientific basis.

MATERIALS AND METHODS

Animals: Male ICR albino mice (25-35 g) were used in accordance with the Institute Medical Research (IMR), Malaysia guidelines on animal experimentation. The animals were obtained from IMR and kept in the animal house for at least 72 h before used. Food and water were supplied *ad libitum*. Each mice was used only once (Filho *et al*., 1997). All experiments were performed during the period between 9:30 h and 13:00 h.

Preparation of the extracts: The sea cucumbers *Holothuria leucospilota, Bohadschia marmorata vitiensis* and *Stichopus hermanii* were collected from coastal areas of the Peninsular Malaysia. Identification of species was based on Ridzwan (1993). Except for *S. hermanii* in which the coelomic fluid was obtained immediately, the samples from other species were kept in labelled plastic bags before they were stored in a freezer at -80°C.

Extraction of sea cucumbers: The animals were thawed from deep freezer, thoroughly washed with distilled water, cut up longitudinally along the bodyline to remove the visceral organs. The remaining body walls were dried on blotting papers before being homogenized to a fine texture. The extracts were prepared according to Yasumoto *et al.* (1967) in which 50 g of the homogenized tissues for each species was placed in a 250 ml conical flask to which 100 ml of each of distilled water in the ratio of 1:2 was added. Each of the flask containing the blended tissues in their corresponding solvents was shaken in a water-bath shaker at 80 rev min^{-1} at 30°C for 4 h. The resulting mixtures were centrifuged at 3,000 rpm for 20 min. The supernatants were then collected before being freeze-dried overnight using a Freeze-dryer (Model Heto FD3, ID 87154). Similarly, the coelomic fluid from *S. hermanii* after being filtered, its supernatant was also being freeze-dried. The powder form of each extract was then stored in labelled sterile bottles at 4°C until being assayed for the present of antinociceptive agents performed on mice.

Preparation of samples: For antinociceptive determination test, 100 mg of distilled water extract from each species of sea cucumbers were solubilized in 1 ml sterile distilled water, respectively.

Drugs: Morphine sulphate (Delta West, USA) was dissolved in a physiological saline solution to such concentrations that requisite doses were administered in volume of 10 mg kg^{-1}. The dose of morphine used in each test was adopted from ED50 values previously established in our lab setting, being 0.8 mg kg^{-1} for the abdominal contraction test and 5 mg kg^{-1} for the tail-flick test (Dambisya and Lee, 1994, 1995). In all experiments, saline was used as the negative control and morphine sulphate as positive control.

Antinociceptive assays: The acetic acid abdominal contraction test (writhing assay) was used with local adaptations as described by Dambisya and Lee (1995), a modification of the method by Collier *et al.* (1968). Acetic acid (0.6%) 10 mg kg^{-1} was administered (i.p.) to induce pain in mice. Each mouse was then placed in a tray for observation. Response to pain was indicated when the abdominal muscle undergone contraction and the hindlegs began to stretch (Filho *et al.*, 1997). This was done 30 min after the animal being administered (s.c.) 10 mg kg^{-1} with water extracts or coelomic fluid extract (50, 75, 100 mg kg^{-1}) or controls (saline or morphine sulphate 0.8 mg kg^{-1}). The number of abdominal contraction (writhing) was counted for 5 min soon after the first contraction started using single blind observation. Analgesia was calculated as the percentage inhibition of the abdominal contractions

$$\frac{\text{Mean saline control group - Mean test group mean}}{\text{Mean saline control group}} \times 100$$

Hot tail-flick test: The hot tail-flick test was used as described in the previous studies on pain (D' Amour and Smith, 1941; Sewell and Spencer, 1976; Cakici *et al.*, 1997). Each mouse was weighed with its tail marked about 2 cm from the distal end. Later, the mouse was placed in a restrainer with its tail immersed in water at 50°C for antinociceptive screening. The screening cut-off time was 5 s. If the reaction exceeded 5 s, the animal would be discarded. The time for screening was recorded as '0' time. The animal was removed from the strainer, then administered (10 mg kg^{-1} i.p.) with either saline or morphine (5 mg kg^{-1}) as control or extract (100 or 200 mg kg^{-1} i.p.), again being kept in the restrainer with its tail immersed in water at 50°C. The time taken for the mouse to flick its tail or its body (sudden jerk) due to pain was recorded in second. The first reading was omitted. Reaction time was recorded based on the average of the next two readings. Time gap for each test was 1 min. The reaction time was taken before the animal being administered '0' min and at 15, 30, 45 and 60 min after administration. The maximum time was fixed at 15 sec to avoid tissues damaged. If the reading exceeded 15 sec, it would be considered as maximum analgesia.

Percentage maximum possible analgesia (MPA) were calculated as follows:

$$MPA = \frac{\text{Reaction time test - Reaction time for saline}}{\text{15 - Reaction time for saline}} \times 100$$

Statistical analysis: The results were presented as mean±S.E.M. The Student's t-test was used to analyze and compare the data with $p<0.05$ as the limit of significance.

RESULTS

Abdominal contraction response: Acetic acid (0.6%) administered intraperitoneally induced pain in mice indicated by stretching response of the abdomen. Mean abdominal contraction for mice treated with saline (s.c.) as a negative control for a duration of 5 min was 21.30±0.42 (Table 1). Meanwhile, mice treated with morphine 0.8 mg kg^{-1} (as positive control) showed the antinociceptive effect with a significantly ($p < 0.05$) lower rate of abdominal contraction, i.e. 11.40±1.18. However, when water extract of *H. leucospilota* was administered, there

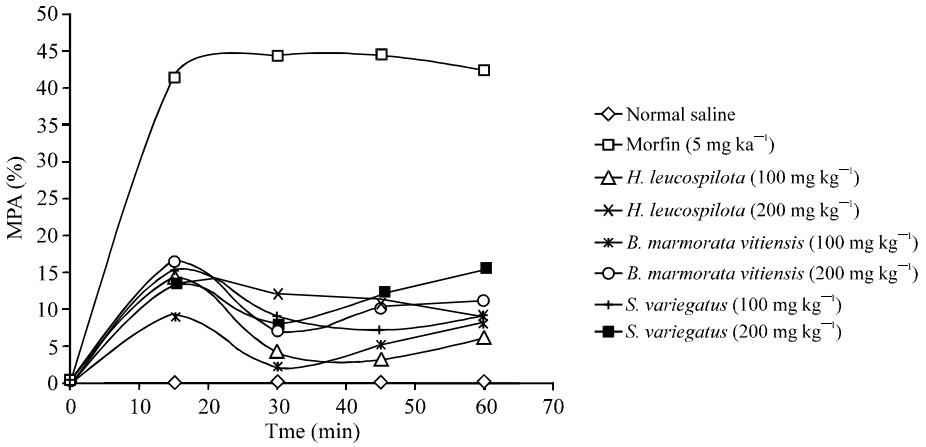

Fig. 1: Maximun Possible Analgesia, MPA (%) representing the effects of water extracts *Holothuria leucospilota* and *Bohadschia marmorata vitiensis* and coelomic fluid of *Stichopus hermanii* compared to morphine sulphate (positive control) and normal saline (negative control) administered into mice (i.p.)

seemed to be increased in percentages of inhibition of contraction, 17.84, 34.74 and 45.54% with the respective doses 50, 75 and 100 mg kg^{-1}. But, when compared to the positive control (morphine treated), these inhibitions were not significant but significant ($p < 0.05$) to the negative control (saline treated). Similar patterns were observed when the induced pain mice were treated with either water extract of *B. marmorata vitiensis* or coelomic fluid *S. hermanii*. For *B. marmorata vitiensis* percentages of inhibition were 23.00, 32.86 and 39.91%, with doses 50, 75 and 100 mg kg^{-1}, respectively. For coelomic fluid *S. hermanii* percentages inhibition were 34.74, 40.85 and 43.66%. Like *H. leucospilota*, all results were significant when compared to the negative control but insignificant to the positive control.

Hot tail-flick test: Hot tail-flick test involved the usage of water bath (50°C) with duration of observation 60 min. Some antinociceptive activity effects were observed in mice treated (i.p.) with water extract of *H. leucospilota* and *B. marmorata vitiensis* and coelomic fluid *S. hermanii*. Mice treated (i.p.) with normal saline (negative control) during 60 min observation did not show any significant effect on tail-flick. But, the animals treated with morphine, 5 mg kg^{-1} i.p. (positive control) seemed to have some antinociceptive effect with maximum effect at 30 minutes and gradually decreased towards 60 min (Table 2). Duration of tail-flick test in morphine-treated animals was significant compared to the saline treated animals. The antinociceptive effects of saline, morphine, water extracts and coelomic fluid of sea cucumbers could be seen by using Maximum Possible Analgesia (MPA) value (Fig. 1). Observation for 60 min in mice treated with water extract *H. leucospilota* at a dose either 100 or 200 mg kg^{-1} i.p. did not give any significant effect compared to the negative control but significant ($p < 0.05$) to the positive control. The MPA value reached it peak at 15 min. For *B. marmorata vitiensis*, only dose of 200 mg kg^{-1} showed some significant difference compared to saline treated mice. But both doses, 100 and 200 mg kg^{-1} gave significant results to the positive control (morphine) (Table 2). Again, the MPA value for the extract reached its peak at 15 min after administration. With reference to MPA value (Fig. 1) mice treated with coelomic fluid *S. hermanii* showed peak value at 15 min after administrating the extract, declined at 30 min and then again increased at 60 min. However, both doses of coelomic fluid (100 and 200 mg kg^{-1}) have no significant effect on the duration of tail-flicks subjected to hot water bath in mice.

DISCUSSION

The results showed that all the water extracts *H. leucospilota* and *B. marmorata vitiensis* and coelomic fluid of *S. hermanii* have dose-dependent antinociceptive effects as assessed by the abdominal contraction test. Thus, indicating the presence of antinociceptive agents in the extracts and coelomic fluid. However, there was no demonstrable effects in the tail-flick test. The abdominal contraction test is very sensitive and can detect antinociceptive effects of compounds/dose levels that maybe inactive in the tail-flick assay (Collier *et al*., 1986). The abdominal contraction response is thought to involve, in part, local peritoneal receptors (Bentley *et al*., 1983) while the tail-flick response is essentially a spinal reflex. These differences in sensitivity of the two tests and the mechanisms involved may explain the apparent lack of

Table 1: The effects of water extracts *Holothuria leucospilota* and *Bohadschia marmorata vitiensis* and coelomic fluid *Stichopus hermanii* 30 min after their administration (s.c.) on acetic acid (0.6%) induced abdominal contraction in mice. (n=10)

Treatment	No. of abdominal contraction	Inhibition (%)
Control (saline)	21.30±0.42	0
Morphine sulphate	11.40±1.18	46.48
Holothuria leucospilota		
50 mg kg^{-1}	17.50±1.33*	17.84
75 mg kg^{-1}	13.90±1.14	34.74
100 mg kg^{-1}	11.60±0.97	45.54
Bohadschia marmorata vitiensis		
50 mg kg^{-1}	16.40±0.64*	23.00
75 mg kg^{-1}	14.30±1.57	32.86
100 mg kg^{-1}	12.80±2.03	39.91
Stichopus hermanii		
50 mg kg^{-1}	13.90±1.05	34.74
75 mg kg^{-1}	12.60±1.19	40.85
100 mg kg^{-1}	12.00±1.69	43.66

*significant ($p < 0.05$) compared to morphine (0.8 mg kg^{-1}). (n = 10)

Table 2: Effects of administration (i.p.) normal saline, morphine sulphate (5 mg kg^{-1}), water extracts from *Holothuria leucospilota* and *Bohadschia marmorata vitiensis* and coelomic fluid *Stichopus hermanii* on mice using tail-flick test. Normal saline and morphine sulphate being negative and positive control, respectively

	Tail-flick±S.E.M (s)				
Treatment	0 min	15 min	30 min	45 min	60 min
Normal saline	1.95±0.45	3.29±0.37	3.78±0.39	3.49±0.23	3.21±0.36
Morphine sulphate	1.85±0.38	8.12±0.80	8.70±1.00	8.61±1.16	8.21±1.17
Holothuria leucospilota					
100 mg kg^{-1}	2.04±0.35	4.89±0.47	4.25±0.25	3.83±0.21	3.92±0.27
200 mg kg^{-1}	1.94±0.42	4.81±0.40	5.07±0.50	4.75±0.44	4.29±0.26
Bohadschia marmorata vitiensis					
100 mg kg^{-1}	1.78±0.30	4.31±0.41	4.01±0.31	4.09±0.22	4.20±0.47
200 mg kg^{-1}	1.87±0.30	5.19±0.40	4.55±0.23	4.62±0.41	4.45±0.30
Stichopus hermanii					
100 mg kg^{-1}	1.77±0.28	5.03±0.56	4.76±0.37	4.28±0.24	4.26±0.40
200 mg kg^{-1}	2.19±0.40	4.82±0.23	4.63±0.34	4.91±0.46	4.95±0.37

n = 10

effect of these effects in the tail-flick test. Nevertheless, the finding of antinociceptive action in one test and the indication using water extract and coelomic fluid for the tail-flick test, seem to support the common observation among the local as well as the ethnics in Malaysia that utilizing sea cucumbers offers pain relief, especially, in arthritis and burning. In conclusion, the antinociceptive agents found in the extracts and coelomic fluid seemed to be local in reaction.

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