INTRODUCTION

Sea cucumber, also known as gamat, is a traditional medication believed to have anti-inflammatory and anti-nociceptive properties. Reports claim that gamat has medicinal value in wound healing during the postpartum period.\(^1\)\(^2\) The ability of gamat extract to promote tissue healing was reported by Taiyeb-Ali et al.\(^3\) and the study carried out by Fredalina et al.\(^4\) demonstrated that the high content of eicosapentanoic acid (EPA) in the gamat extract was associated with an ability to initiate tissue healing. There is increasing evidence regarding the anti-inflammatory property of gamat but few reports have investigated its proposed analgesic property. A study carried out in mice demonstrated the anti-nociceptive property of gamat extract using the abdominal contraction test and reported that the anti-nociceptive effect was similar to morphine.\(^5\) This suggests that some species of gamat could have an analgesic property. To date, there are no studies comparing gamat with non-steroidal anti-inflammatory drugs. Therefore, the aim of the present study was to determine the analgesic dose of holothuria extracts and to compare the effects of this dose with an established analgesic agent in an acute pain model. The formalin pain model was utilized because the pain mechanisms in this model are well-characterized: phase 1 is characterized by peripheral stimulation of nociceptors and activation of c-fibres;\(^6\) phase 2 is predominantly characterized by hyperexcitability of dorsal horn neurons.\(^7\) This study presents possible mechanisms of the action of gamat but further research will be required to elucidate these fully.

METHODS AND MATERIALS

The current experimental study was approved by the Research and Animal Ethics Committee, Universiti Sains Malaysia.

Animal preparation

For the first part of the study, 18 Sprague-Dawley male rats (220-300 g) were allocated to three different groups; saline, holothuria 2 mg/kg, and holothuria 4 mg/kg. In further studies, 21 rats were allocated to a saline group, holothuria 4 mg/kg group and dynastat 20 mg/kg group. All rats were supplied by the laboratory of animal research Universiti Sains Malaysia (LARUSM). They were removed from the LARUSM three days before the experiment and...
housed 2-3 rats per cage in the physiology laboratory under standard conditions of light and temperature (22 °C) with free access food and water, and allowed adaptation for a minimum of two days in the laboratory.

Preparation of gamat extracts

Gamat from Holothuria atra and Holothuria edulis were dissected and the internal organs were removed. The tissues were dried in an air oven (Binder BD 115) at 58 °C for two weeks. The dried tissues were blended to produce a powder, which was mixed with petroleum ether and poured into a funnel. The suspension was collected and left to separate into the organic (upper portion) and non-organic (lower portion) components. The organic component was dried for approximately 12 hours using a freeze dry process (Ilshin Lab. Co. Ltd., Yangju-si, Korea). The operating temperature and pressure were -48 °C and 200 atm, respectively. The processes converted the sea cucumber tissues into powder extract that was stored in a refrigerator until used.

Behaviour testing

Dilute formalin (50 μl, 1%) was injected into the plantar aspect of the rat’s right hind paw. In the first part of the study, intraperitoneal holothuria extracts (2 mg/kg or 4 mg/kg) or saline were administered. The doses of holothuria extracts were chosen as earlier observations demonstrated that mice receiving 10 mg/kg of holothuria extracts presented with signs of toxicity including unusual vocalisation, restlessness, paralysis and sedation (unpublished data). After administration of holothuria extracts or saline the rats were subjected to behavioural testing as described by Hayati et al. Behaviour was recorded from the time of formalin injection for one hour. Similarly, rats used in the second part of the study were injected with formalin followed by intraperitoneal administration of saline, holothuria extracts 4 mg/kg or dynastat 20 mg/kg, and behavioural testing was carried out for one hour. Assessment of recorded behaviours and formalin test score quantification were based on the total time spent in four behavioural categories (0 to 3).

Statistical analysis

Pain behaviour scores obtained by the formalin test were analyzed using two-way analysis of variance (ANOVA) with repeated measures with one within-subjects factor (time) and two between-subjects factors (time and drug). Two-way ANOVA was used to analyze the effects of the phase 1 formalin test (mean score at 5 minutes) and phase 2 formalin test (mean of scores from 10 to 60 minutes) with drugs (saline vs. gamat) as the main factors. A post-hoc Scheffe test was performed. A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Effects of holothuria extract on pain behaviour

![Graph](image)

**Fig. 1.** Behaviour scores in animals receiving saline (control), holothuria extracts 2 mg/kg (gamat 2) and holothuria extracts 4 mg/kg (gamat 4) ($n = 6$ for all groups). Values are mean ± S.E.M. ($^* P < 0.05; ^*^* P < 0.01$). *Comparison between holothuria 4 mg/kg and control groups. ($P < 0.05, ^*^* P < 0.01$) *Comparison between holothuria 2 mg/kg and control groups.
Formalin produced a typical biphasic pain response in the control group (Fig. 1). The first phase (0 to 5 minutes) demonstrated an increase in the pain behaviour score followed by 5 to 10 minutes of reduced nociceptive behaviour. The second phase of increased pain behaviour was noted from 15 minutes to 60 minutes post formalin injection. The biphasic response was markedly attenuated in the holothuria 4 mg/kg group, signifying inhibited pain behaviour. This attenuation was marked at five and 10 minutes post injection ($P < 0.01$) and was noticeable from 25 to 60 minutes post-formalin injection. Pain behaviour was inhibited in the holothuria 2 mg/kg group compared with control animals in phase 1 ($P < 0.01$) but the behaviour was not significantly different from the control group from 15 to 45 minutes post formalin injection (phase 2) (Fig. 1). There were significant differences in pain behaviour between the groups receiving 2 mg/kg and 4 mg/kg of holothuria extracts from 25 minutes to 45 minutes post formalin injection ($P < 0.05$).

**DISCUSSION**

In the present study, the effects of intraperitoneal administration of holothuria extracts on pain behaviour in an acute pain model were investigated. Two different doses of holothuria extracts were administered and the effects compared. The group receiving 4 mg/kg holothuria extracts demonstrated significantly less pain behaviour than control animals in phase 1 and in the majority of phase 2 ($P < 0.01$), while animals receiving the lower dose of holothuria extracts (2 mg/kg) only had a significant reduction in pain behaviour during phase 1. These results suggest that gamat anti-nociceptive effects are dose dependent. A report by Ridzwan et al. demonstrated the ability of gamat extract to inhibit abdominal contraction in an acetic acid induced writhing test and demonstrated that the percentage of inhibition was higher when a larger dose of gamat was used. However, the doses used in the aforementioned study were substantially greater (50 mg/kg, 75 mg/kg and 100 mg/kg) than those utilized in the present study (2 mg/kg and 4 mg/kg). The difference in the doses used in the two studies could be contributed to a different method of gamat preparation and the type of gamat used.

Holothuria extracts (4 mg/kg) inhibited pain behaviour in both phases. Phase 1 is mediated by peripheral mechanisms and involved in the chemical stimulation...
of nociceptors and activation of C fibers, while central mechanisms are involved in phase 2. The nociceptive behaviour in phase 1 was partly due to the formation of prostaglandin that generates the signs and symptoms of inflammation. The activation of NMDA receptors that lead to hyperexcitable dorsal horn neurons and local inflammatory changes during the second phase are necessary for the full manifestation of the second phase. The inhibition of pain behaviour in phase 1 and phase 2 of the present study suggests that holothuria extract has the ability to modulate the peripheral and central mechanisms governing acute pain and leads to a reduction in pain-related behaviour. The analgesic effects of holothuria extract could be attributed to several possible mechanisms including inhibition of release of prostaglandin, substance P or bradykinin into inflamed peripheral tissue, or inhibition of sensory neuronal firing leading to inhibition of pain transmission. However, further studies are required for a full understanding of the mechanism of action underlying the analgesic effects of holothuria. Dynastat did not inhibit pain behaviour in phase 1 or for the majority of phase 2, having similar effects to saline. These results were not as expected; dynastat has analgesic properties and is used to treat acute pain conditions in humans. However, the present results are consistent with previous reports in which intravenous, spinal and intraperitoneal administration of a cyclooxygenase II inhibitor failed to alter nociceptive behaviour in a formalin test and an acetic acid induced writhing test. The pain behaviour scores in the dynastat group were significantly lower at 55 and 60 minutes post-formalin injection than in the saline group. This suggests the possibility that the onset of action of intraperitoneal dynastat was delayed due to poor or delayed absorption into the circulation. Another probable reason concerns the selective mode of action of dynastat. The action of dynastat, a cyclooxygenase II inhibitor, is predominantly inhibition of prostaglandin synthesis produced by the cyclooxygenase-2 pathway, but it has no inhibitory effects on the cyclooxygenase-1 pathway. Failure of dynastat to suppress pain behaviour in the present study could be partly due to prostaglandin production by the cyclooxygenase-1 pathway.

**CONCLUSION**

This present study has investigated the effects of holothuria extracts on the pain behaviour of rats in the formalin pain model. The results demonstrate that holothuria extracts significantly inhibited the pain behaviour in rats injected with formalin compared with the group receiving saline and that in which dynastat was administered. The anti-nociceptive property of gamat was demonstrated and the results support the potential use of gamat extract as an analgesic in the future. Further studies are required to elucidate the mechanism(s) of action of gamat at the peripheral and central levels.

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**REFERENCES**