

Screening for Aphrodisiac Property in Local Oyster of *Crassostrea iredalei*

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Abstract: Oyster has been reputed since ancient time to increase sexual performance. This myth has been around for many years and still going strong until today. However, there is no scientific evidence to support the claim. Therefore, this study was conducted to screen the aphrodisiac property in local oyster of *Crassostrea iredalei*. Mounting behaviour and assessment of mating were the parameters tested in this study. Three doses; 50, 100 and 200 mg/kg from each aqueous and ethanol extracts of *C. iredalei*, respectively were administered (i.p.) to male mice for measuring both test parameters. Sildenafil citrate at the dose of 5 mg/kg was used as positive control while physiologic saline solution as negative control. Male mice treated with ethanol extract of *C. iredalei* showed substantial evidences in the mounting behaviour at the dose levels of 50, 100 and 200 mg/kg compared to aqueous extract. However, only mild aphrodisiac effect was showed with aqueous extract at the dose of 50 mg/kg. Besides, all doses of ethanol extract showed no significant differences ($p > 0.05$) after administration compared to the positive control. This indicated the ability of extract to be as competent as Sildenafil citrate at certain dosages even though Sildenafil citrate produced higher activities than the local oyster. No sperm was observable in all groups for the assessment of mating test. Thus, this study provided preliminary evidence regarding the potential aphrodisiac property of the local oysters that could be used as an alternative therapy to restore male sexual activity.

Key words: Aphrodisiac • Extracts • Local Oyster • *Crassostrea iredalei*

INTRODUCTION

Many foods and drinks are claimed to have a reputation for making sex more attainable and pleasurable. One interesting reason that leads to this claim is because many ancient people believed in the so-called "law of similarity" in which objects resembling genitalia may possess sexual powers. One example is oyster. The reputation whether these foods have aphrodisiac effect are associated with people's mind or their psychology [1]. Since oysters are rich in zinc, which is one of the essential minerals, they might have been associated with improving sexual potency in men. Adequate zinc is needed for sperm production and hormone metabolism [2]. Oyster efficacy is yet to be scientifically validated before claiming that oysters have the aphrodisiac effect due to their

pharmacological properties [3]. Therefore, local oysters, *Crassostrea iredalei* cultured in Malaysia was selected in this study for screening their aphrodisiac property.

MATERIALS AND METHODS

Chemical Preparation: Normal saline was prepared by dissolving 9 g of NaCl in 1000 ml distilled water (0.9%) while 10 g of Dimethyl sulfoxide, DMSO being dissolved in distilled water and filled up to 100 ml total volume (10 %). Tween 80 was prepared by dissolving 10 g of Tween 80 in 1000 ml distilled water (1%). All solutions were kept for further used.

Sildenafil citrate obtained from a pharmacy outlet in Kuantan, Pahang was dissolved in DMSO solution before treatment.

Sample Preparation: Samples of *C. iredalei* were obtained from the Centre for Marine and Coastal Studies, School of Biological Science, University Science Malaysia, Penang.

Aqueous Extraction: About 500 g fresh flesh of *C. iredalei* were separated from their shells and washed for 2 to 3 times under running tap water to remove all traces of dirt before rinsing them with distilled water. The flesh was then blended with distilled water in a ratio of 1: 4 by using an electrical blender. Later, the mixture was filtered by using gauze pad and then refiltered by using Whatman filter paper No.1. The filtrate was freeze-dried. The powder obtained was diluted in distilled water prior to treatment.

Ethanol Extraction: Fresh oyster meat of *C. iredalei* obtained by the above procedure was subjected to the same process of cleaning before being extracted with ethanol by using Soxhlet extraction. The ethanol extract was diluted with 10% Tween 80 prior to treatment.

Animals: Twenty four male and 24 female ICR mice weighing from 25 - 35 g body weight were kept in the Animal Research Laboratory, International Islamic University Malaysia (IIUM) and treated in accordance with the IIUM animal ethics. They were conditioned with temperature at 22 °C, relative humidity 60-70% and 12 h light/12 h dark cycle for at least 1 week before used. Food and water were provided *ad libitum*. All 24 ICR male mice were randomly grouped into 8 groups (n = 3).

Treatment: Three groups received aqueous extract of *C. iredalei* at the dose of 50, 100 and 200 mg/kg, respectively while the other three groups received ethanol extract of *C. iredalei* at similar treatment doses. Positive control group received 5 mg/kg Sildenafil citrate while negative control group received normal saline. All treatments were administered intraperitoneally (I.P.).

Mounting Behaviour: Each male mouse from the respective groups was then placed separately in an open lid cage. An hour after administration of the extracts, a female mouse was introduced into the cage. Male and female mice remain paired for 3 hrs. The number of mounts was recorded during a 15 min observation period at the start of each hour. All experiments were performed during a light cycle from 0900 to 1400. (*A mount is operationally defined as the male assuming the copulatory position*

but, failing to achieve intromission. Intromission was defined as the male's penis entering the vagina in association with thrusting behaviour.)

Assessment of Mating: In this test, 5 male mice received 50, 100, 200 mg/kg ethanol extract of *C. iredalei*, 5 mg/kg Sildenafil citrate and normal saline, respectively. All doses were administered (I.P.) for 6 consecutive days. On the 6th day, the drug was administered at 1700 during day cycle and each male mouse was placed in a separate cage. After an hour, 3 females were admitted into each cage. On next morning (0700–0800), the vaginal smear for each female mouse was examined under a microscope to observe the presence of sperms. The number of females with sperms were recorded as positive for each treatment. (*Vagina smear was performed by swabbing the exterior part of vagina using sterile cotton swab. Then, the cotton swab was rolled on two microscope slides. First slide was left unstained and second slide was stained with Giemsa staining. All slides were then observed for the presence of sperms under the microscope.*)

Statistical Analysis: In this study, the data recorded were expressed as mean ± standard deviation (SD). All data were statistically analysed by using the Repeated Measures (Mixed Between-Within Subjects ANOVA) and Independent T-test. The significant difference is detected when *P* value is less than 0.05 ($p < 0.05$).

RESULTS

Effect on Mounting Behaviour: The effects of different doses of *C. iredalei* for aqueous extract on mounting behaviour of male mice are presented in Figure 1. For aqueous extract dose 50 mg/kg, 1 h after the treatment, displayed essentially mounting behaviour. The number of mount increased after 1 h treatment but, decreased after 2 hrs. However, at higher doses of the extract, 100 mg/kg and 200 mg/kg, there were no mounting recorded. For the positive control, although the amount of mounting decreased from 1st h of the drug administration, it was still higher than the extract even after 3 hrs. Meanwhile, the amount of mounting for the negative control remained lower than the aqueous extract.

The effects of *C. iredalei* ethanol extract on mounting behaviour of male mice are presented in Figure 2. Except for dose 100 mg/kg which showed highest mounting at 2 hrs compared to the positive control and even until 3 h after the administration, other

Table 1: A summary result of aphrodisiac screening using normal saline, 5-mg/kg Sildenafil citrate, aqueous extract of *C. iredalei* (50, 100, & 200 mg/kg) and ethanol extract of *C. iredalei* (50, 100, & 200-mg/kg) on mounting behaviour of male mice. [Each value is a mean \pm standard deviation of 3 mice weighing 25-35 g

| Group | Dose (mg/kg) | Number of mounting | | |
|---------------------------------------|--------------|--------------------|--------------------|------------------|
| | | 1 h | 2 hrs | 3 hrs |
| Normal saline | - | 4.33 \pm 2.08 | 1.33 \pm 0.58 | 0.00 \pm 0.00 |
| Sildenafil citrate | 5 | 12.33 \pm 1.53* | 8.33 \pm 3.51* | 3.33 \pm 0.58* |
| Aqueous extract of <i>C. iredalei</i> | 50 | 4.67 \pm 2.08* | 5.67 \pm 4.04 | 2.00 \pm 2.00 |
| | 100 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| | 200 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| Ethanol extract of <i>C. iredalei</i> | 50 | 8.67 \pm 1.53** | 6.33 \pm 1.53* | 0.33 \pm 0.58* |
| | 100 | 10.00 \pm 2.00* | 15.67 \pm 2.08** | 3.00 \pm 2.65 |
| | 200 | 7.33 \pm 3.79 | 1.67 \pm 0.58* | 0 \pm 0 |

*Statistically significant from negative control ($p < 0.05$). **Statistically significant from positive control ($p < 0.05$).

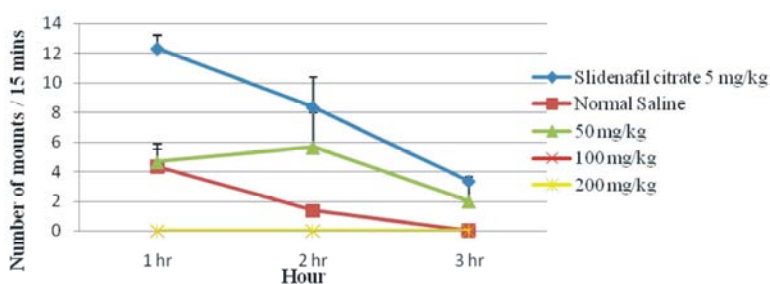


Fig. 1: Effects of different doses of *C. iredalei* (aqueous extract) on number of mounting of male mice over 3 hours duration. Mean value, n=3

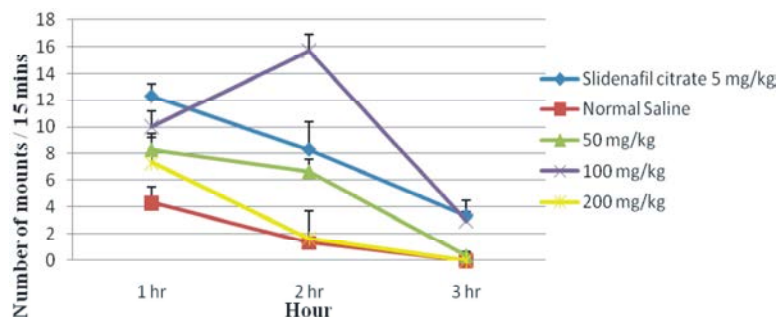


Fig. 2: Effects of different doses of *C. iredalei* (ethanol extract) on number of mounting of male mice over 3 hours duration. Value are mean, n=3

doses 50 and 200 mg/kg seemed to have lower numbers of mounting. However, these numbers were higher than the negative control. The mounting for the controls as well as all the treated groups decreased at 3 hrs after female mice were introduced into the cages.

Statistical analysis (Table 1) from Mixed between-within subjects ANOVA showed there was a statistically significant interaction between time and dose ($p < 0.05$). Statistically significant difference interaction was also observed between time and extract ($p < 0.05$). In addition, statistically significant difference was recorded for main effect for time ($p < 0.05$). The main effect of comparing between extract was statistically significant ($p < 0.05$).

Independent T-test was then conducted to compare treated group (50 mg/kg, 100 mg/kg and 200 mg/kg of aqueous and ethanol extracts of *C. iredalei* and Sildenafil citrate (5 mg/kg) with negative control group. Sildenafil citrate 5 mg/kg exhibited a statistical significant difference ($p < 0.05$) on mounting behaviour at 1 h, 2 hrs and 3 hrs when compared to negative control group. Aqueous extract of *C. iredalei* of 50 mg/kg was not statistically significant ($p < 0.05$) on mounting behaviour at 1 h, 2 hrs and 3 hrs when compared to negative control group. On the other hand, ethanol extract of *C. iredalei* of 50 mg/kg and 100 mg/kg, exhibited significant difference ($p < 0.05$) on mounting behaviour at 1 h and 2 hrs but, not

at 3 hrs when compared to negative control group. Meanwhile, ethanol extract of *C. iredalei* of 200 mg/kg exhibited statistically significant difference ($p<0.05$) on mounting behavior only at 2 hrs. When compared to positive control, *C. iredalei* aqueous extract 50 mg/kg only displayed significant difference ($p< 0.05$) on mounting behavior at 1 h. For ethanol extract 50 mg/kg, significant differences ($p<0.05$) on mounting behavior were observed at 1 h and 3 hrs while no significant difference exhibited at 2 hrs. Meanwhile, ethanol extract 100 mg/kg and 200 mg/kg exhibited significant differences ($p<0.05$) at 2 hrs and 3 hrs, respectively.

Effect on the Mating of Mice: Daily administration of *C. iredalei* (ethanol extract) for 6 days to male mice showed negative result in the mating performance of the mice.

DISCUSSION AND CONCLUSION

Although this oyster (*C. iredalei*) is consumed as a healthy food [1], augmentation of male sexual performance by this mollusc in the ethno medical practice is still hidden. Individual who consumed oyster has a firm belief in that oyster could boost *libido* and excellent for men reproductive health and endurance [4]. However, the claim is merely based on consumers' opinions. Scientific evidences in support of the aphrodisiac activities and reproductive functions are lacking and no study has been made to verify the claim using *C. iredalei*. Based on this information, therefore, sexual behaviour of mice on mounting behavior and assessment of mating were studied to screen the aphrodisiac property of *C. iredalei*. Mounting behaviour or mounting frequency was taken as a first parameter in this screening since it was widely used in almost aphrodisiac studies [5-9].

Generally sexual behaviour in mice like mounting is enhanced by elevated testosterone levels and changes in neurotransmitter levels or their action in the cells by specific drugs. This could increase sexual desire indicated by an elevated number of mounting [6]. Besides, mounting behaviour in male mice may also be influenced by several other external factors such as sounds, diet, lighting and population density and female pheromone [10].

In this study, it was observed that the aqueous extract of *C. iredalei* existed in mounting behaviour only at dose 50 mg/kg. No mounting was observed at higher doses, 100 mg/kg and 200 mg/kg within the 3 hrs duration (Figure 1). This result did not comply with the dose-

response principle where generally, larger doses would produced larger effects. However, this observation might be due to certain particular drug which enhanced the response at lower dose and showed no observable effect at higher dose. According to Irdawaty [11], at any higher dose, it would impair the response. On the other hand, the results on mounting behaviour of ethanol extract of *C. iredalei* have shown a substantial number of mounting for all the three doses (50 mg/kg, 100 mg/kg and 200 mg/kg) (Figure 2). Mounting behaviour of male mice administered with ethanol extract at 50 mg/kg and 100 mg/kg were found to be higher compared to extract at 200 mg/kg over 3 hrs. This is probably because as dose increased, it fails to produce any greater response in which this point is known as the maximal efficacy of the drug. It was also observed that ethanol extract at 50 mg/kg and 200 mg/kg, produced a similar pattern of mounting behaviour with respect to both positive and negative control group. The mounting of control as well as mice treated with ethanol extract at 50 mg/kg and 200 mg/kg were diminished over time. During 3 hrs of the treatment, the bioactive constituents seemed to have undergone metabolic degradation. In addition, there was a statistically significant interaction between time and dose ($p<0.05$) and also between time and extract ($p<0.05$) suggesting that number of mounting differ between doses across the 3 hrs period and there was a difference in the mounting behaviour over time for both extracts. According to Hafez [12], internal factor such as physiological condition of the male mice could also influenced mounting behaviour.

Another finding, both extracts at doses 50 mg/kg (aqueous) and 100 mg/kg (ethanol) increased the number of mounting 2 hrs after their administration. However, the number of mounting decreased at 3 hrs, probably due to the effect of testosterone. As reported by Robbins [13] high testosterone level will result in higher *libido*. When the hormone concentration has reached its optimum, the response will start to reduce. Statistical analysis also showed that there was significant effect at different hour ($p<0.05$). This suggested that there was a significant change or difference in number of mounting across the three time periods. Furthermore, it was found that aqueous extract of *C. iredalei* at dose 50 mg/kg exhibited no statistical difference ($p>0.05$) at 2 hrs and 3 hrs. This gave evidence that the effect of this dose at 2 hrs and 3 hrs on mounting behaviour were similar as the positive control (Sildenafil citrate 5 mg/kg). Meanwhile, for ethanol extract, dose of 50 mg/kg exhibited the identical effect as positive control at 2 hrs while ethanol

extract of 100 mg/kg displayed equal effect as positive control at 1 h and 3 hrs. At 2 hrs, the number of mounting for ethanol extract of dose 100 mg/kg was significantly ($p < 0.05$) higher than positive control. Besides, ethanol extract of 200 mg/kg presented an equal effect on 1 h and 2 hrs as Sildenafil citrate. The number of mounting for aqueous extract at dose 50 mg/kg and ethanol extracts at dose 50 mg/kg and 100 mg/kg showed that they were approaching Sildenafil citrate although did not have the same values. Based on this evidence, administration of *C. iredalei* extracts possessed fundamental value in the aphrodisiac property even though as expected, Sildenafil citrate produced greater activity than *C. iredalei* extracts. This suggested the possibility of a similar mode of action of *C. iredalei* extracts and Sildenafil citrate on mounting behaviour. The standard drug Sildenafil citrate was used as a reference only for quantitative comparison and not for mechanism purpose.

Daily administration of *C. iredalei* (ethanol extract) for 6 days to male mice showed negative result in the mating performance of the mice. For all treated group as well as control group no sperm was presence due to several factors. Firstly, the female mice were not brought into estrous state before they were introduced into a cage for mating. According to several reports [5,6], only female mice in estrous state would allowed overnight mating [9]. Secondly, the most receptive female mice were not selected prior to mating. Thirdly, the negative result might be due to improper vaginal smear technique since the mice were too small to perform the proper swab at the vagina. Swabbed only done on the exterior part around the vaginal to avoid pain. Therefore, it was hard to detect the presence of sperm.

In conclusion, these findings provide pharmacological evidences that *C. iredalei* extract possesses aphrodisiac property. Ethanol extract of *C. iredalei* showed substantial evidences in sexual behaviour of mice at the dose of 50, 100 and 200 mg/kg as compared to aqueous extract. Nevertheless, mild aphrodisiac effect was observed with aqueous extract of *C. iredalei* at lower dose. Besides, the doses of 50, 100 and 200 mg/kg ethanol extract showed no significant differences from the positive control at certain hours after administration of the extract. This indicated the ability of extract to be as competent as Sildenafil citrate at certain dosages and hours even though Sildenafil citrate produces higher activities than the local oyster. The findings from this study also provide preliminary evidences that *C. iredalei* could be used as an alternative therapy to restore male sexual activity.

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