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Evaluation of the Aphrodisiac Potential of Rice Field Eel, *Monopterus albus* Extracts in Male Mice

(Penilaian Potensi Afrodisiak Ekstrak Belut Sawah, *Monopterus albus* ke atas Tikus Jantan)

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ABSTRACT

Rice field eel, Monopterus albus*, is widely consumed by the local people as a source of traditional medicine. Therefore, this study was conducted to evaluate the* M. albus *aqueous and lipid extracts as an aphrodisiac based on the mounting behaviour and mating assessment. The doses used for both extracts were 50, 100 and 200 mg/kg. Negative control mice received 0.9% saline while positive control received 5 mg/kg Sildenafil citrate or Viagra®. The observation was carried out for 1 h after administration of the respective doses of the extracts. Male mice treated with the different doses of lipid extract showed substantial mounting behaviour. Meanwhile, only male mice treated with 200 mg/kg aqueous extract displayed aphrodisiac property which was similar to 200 mg/kg lipid dose. As both extracts produced higher number of mounting than Sildenafil citrate at 1st h of observation, this indicated that they are as competent as Sildenafil citrate. In conclusion, this study provides evidence that* M. albus *could be used as an alternative source for male sexual activity.*

Keywords: Aphrodisiac; mating assessment; Monopterus albus*; mounting behaviour; rice field eel*

ABSTRAK

Belut sawah, Monopterus albus, *digunakan secara meluas oleh penduduk tempatan sebagai sumber perubatan tradisi. Oleh itu, kajian ini dijalankan untuk menilai potensi ekstrak air dan lipid sebagai afrodisiak berdasarkan penilaian tingkah laku seks dan mengawan. Dos yang digunakan untuk kedua-dua ekstrak adalah 50, 100 dan 200 mg/kg. Tikus kawalan negatif menerima 0.9% larutan garam manakala kawalan positif menerima 5 mg/kg Sildenafil sitrat atau Viagra®. Pemerhatian ke atas tikus telah dijalankan selama satu jam selepas setiap dos ekstrak diberikan. Tikus jantan yang dirawat dengan setiap dos yang berbeza bagi ekstrak lipid menunjukkan peningkatan dalam bilangan memanjat ke atas tikus betina. Sementara itu, hanya tikus jantan yang dirawat dengan dos 200 mg/kg ekstrak air menunjukkan kesan afrodisiak sebagaimana tikus jantan yang dirawat dengan 200 mg/kg ekstrak lipid. Oleh kerana kedua-dua ekstrak menghasilkan bilangan memanjat yang lebih tinggi daripada Sildenafil sitrat pada jam pertama pemerhatian, ini menunjukkan bahawa kedua-dua ekstrak tersebut setanding dengan Sildenafil sitrat. Kesimpulannya, kajian ini membuktikan bahawa* M. albus *boleh digunakan sebagai sumber alternatif untuk aktiviti seks jantan.*

Kata kunci: Afrodisiak; belut sawah; Monopterus albus*; penilaian mengawan; tingkah laku seks*

INTRODUCTION

Rice field eel, *Monopterus albus*, inhabits freshwater areas in Malaysia and been consumed as delicacies. It is also used in traditional medicine to treat asthma, impotency and healing. The rice field eel is rich in protein, iron, calcium (Rahman et al. 2012) and free fatty acids including arachidonic acid (AA), docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) (Razak et al. 2001). DHA and EPA help improve the flexibility of red blood cells, immune system and reduce inflammation (Schwartz & Kloner 2011).

The locals believe the rice field eel could act as energy booster and has aphrodisiac properties. Aphrodisiac is the word derived from the Greek word 'Aphrodite', which mean goddess of sexual, love and beauty. An aphrodisiac is defined as an agent of food or drug that arouses sexual desires (Singh et al. 2013). Men are always seeking aphrodisiac to increase their sexual performance and as an early prevention from erectile dysfunction (ED). The disorder will affect the quality of life in men. ED is defined as the total inability to achieve or maintain erections sufficient for sexual performance. The disorder is highly age-dependent, as the combined prevalence of moderate to complete ED increases from approximately 22% at age 40, to 49% by age 70. ED is expected to exceed 300 million men by the year 2025 (Jackson et al. 2005). In modern medication of ED, the oral prescribed medication of the popular pill, Viagra (Sildenafil citrate) is effective. However, in some men, it is reported that the drug is not compatible and less than 70% of men that used with Sildenafil have certain side effects. Oral testosterone can reduce ED in some men with low levels of natural testosterone, but it is often ineffective and may cause liver damage (Singh et al. 2013).

Therefore, there is the need to produce aphrodisiacs from natural resources that are effective and safe to be consumed. It has been discovered that many plants resources have aphrodisiac value such as *Eurycoma longifolia* (Ang et al. 1997), *Asparagus racemosus* (Ramachandran et al. 2004) and *Myristica fragrans* (Tajuddin Ahmad et al. 2005). Lately, scientists have discovered that marine resources also have the potential to act as an aphrodisiac agent, this include *Holothuria scabra* (sandfish) (Nurjanah et al. 2008), *Crassotrea iredalei* (oyster) (Ridzwan et al. 2013) and *Aplysia dactylomela* (sea slug) (Ridzwan et al. 2014). Previous studies have shown that these marine resources contain a high level of steroid, which can enhance the sexual performance in men. In 1969, Chan and Philips discovered that *M. albus* also contained sex steroid, which is commonly found in most other vertebrate animals. Thus, the study of *M. albus* as an aphrodisiac was conducted in this study in order to evaluate the aphrodisiac properties in animal models.

Materials and Methods

SAMPLE COLLECTION

Fresh samples of *M. albus* were purchased from the local market. Prior to analysis, the internal organs of *M. albus* were removed and washed to remove the residual blood. Fish fillet was obtained by cutting the eel lengthwise along the backbone to obtain maximum amount of flesh including the backbone. The fillet was then chopped into small pieces. The samples were kept at -40°C in a deep freezer until further use.

CHEMICALS PREPARATION

Viagra® or Sildenafil citrate (5 mg/kg) was dissolved in dimethyl sulfoxide (DMSO) prior to treatment. Preparation of 10% DMSO solution was performed by dissolving 10 g of DMSO in 100 mL distilled water. The solution was mixed thoroughly before used. For the preparation of 10% Tween 80 solution, 10 g of Tween 80 was dissolved in 1000 mL of distilled water. Next, 0.9% normal saline solution was prepared by dissolving 0.9 g of NaCl into 100 mL of distilled water. Each solution was mixed thoroughly and kept in a reagent bottle until used. Concentrated chloroform and methanol were used as lipid extract media.

Sample Preparation

AQUEOUS EXTRACTION

Aqueous extraction was conducted according to the method described by Ridzwan et al. (2013) with slight modifications. About 100 g of chopped samples were weighed after being defrosted under running tap water. The samples were rinsed again with distilled water to remove all traces of dirt and debris. The samples were then blended together with distilled water using electrical blender at a ratio of 1:4. Next, the mixture was filtered using a gauze pad and then re-filtered by using Whatman No. 1 filter paper. The filtered extract was then kept at -80°C in a freezer for 24 h before being freeze dried. After freeze drying, the powder of *M. albus* was obtained and diluted with normal saline prior to treatment.

LIPID EXTRACTION

Lipid extraction was carried out according to the method of Bligh and Dyer (1959), thought with slight modifications. Firstly, the chopped samples (50 g) of *M. albus* were blended in an electrical blender with 100 mL of distilled water. The tissues were then homogenized with a mixture of 100 mL methanol and 50 mL chloroform (2:1). The mixture was stirred for 2 min before 50 mL chloroform was added to the mixture. The homogenate was stirred with a spatula for an additional 30 s. After that, the homogenates were transferred into 50 mL centrifuge tubes and centrifuged at 3300 rpm for 5 min at 20°C. The supernatant obtained was collected, and filtered through Whatman No. 1 filter paper. The chloroform was used to rinse the remainder. Meanwhile, the pellet were discarded and stored at -20°C. The filtrates were combined and transferred into a separatory funnel. It was allowed to settle so as to separate into organic and aqueous layers. The bottom chloroform layer containing lipids was transferred into another beaker and approximately 3 g of anhydrous sodium sulphate was added to remove any remaining water. The solution was then filtered again through a filter paper. Next, butylated hydroxytoluene (BHT) was added to the lipid solution as an antioxidant (Kinsella et al. 1977). The solvent containing the extracted compound was removed by rotary evaporator at 40°C. Lastly, the extract was dried in a fume cupboard to yield the final extract in the form of wax-like substance, and which was then stored at 4°C. The extract was diluted with 10% Tween 80 prior to treatment.

ANIMALS

Thirty-two healthy male and 64 female ICR mice showing brisk sexual activity were selected for this study. The male mice were grouped into eight, each consisting of four mice and kept in separate cages, receiving standard pellet and water *ad libitum*. All mice were maintained at room temperature 22 ± 2°C, relative humidity 60% - 70% and 12 h of light and dark cycle for one week, before used.

TREATMENT

The first three group of mice received 50, 100 and 200 mg/kg aqueous extract while the next three groups received lipid extract of *M. albus* at similar treatment doses, respectively. Group 7 being negative control (normal saline, 0.9%) and Group 8 being the positive control (Sildenafil citrate, 5 mg/kg). All doses were administered intraperitoneally (*i.p.*).

MOUNTING BEHAVIOUR TEST

The mounting behaviour test was carried out 1 h after the administration of the respective dose between 09:00 and 14:00 h at room temperature (26-28°C). One hour after the administration (*i.p.*) of the lipid extracts, a male mouse was allowed to adapt in a cage for 15 min prior to introducing a female receptive mouse. Their mounting behaviours were observed continuously for 3 h and recorded at 1st, 2nd and 3rd h. The mating cage was cleaned thoroughly after used with ethanol to mask the odour left by the animals.

MATING ASSESSMENT

The assessment of mating was carried out in accordance with the method of Ridzwan et al. (2013). In this test, three groups of four male mice each received intraperitoneally (*i.p.*) 50, 100 and 200 mg/kg lipid extract of *M. albus*, respectively, for six consecutive days. Meanwhile, two other groups of mice were given 5 mg/kg Sildenafil citrate and 0.9% normal saline, respectively. On day 6th, each mouse was then placed in a separate mating cage whereby, two female receptive mice were introduced, that is 1:2 ratio. The next morning, the observation of copulatory plugs was conducted on each of female mouse used to determine the successful of mating.

STATISTICAL ANALYSIS

The data was expressed as mean ± standard error mean (S.E.M). The significance of differences for the treatment groups and control groups was assessed using one way analysis of variance (ANOVA) with post-hoc test. Values with $p \leq 0.05$ were considered as significance.

RESULTS

EFFECTS OF *M. ALBUS* EXTRACTS ON MOUNTING BEHAVIOUR

The effect of different doses of aqueous and lipid extracts of *M. albus* on the mounting behaviour of mice are presented in Table 1. Statistical analysis using ANOVA with post-hoc test was analysed to compare the aqueous and lipid extracts of *M. albus* with negative control (normal saline) and positive control (Sildenafil citrate). Positive control displayed significant difference ($p \leq 0.05$) on mounting behavior at 1st h and 2nd h but not at 3rd h. The number of mounting for the dose 200 mg/kg aqueous extract at 1st h was higher compared to the positive control. The number of mounting, however decreased at 3rd h. The doses 50 and 100 mg/kg aqueous extract showed no significant difference ($p \geq 0.05$) at all hours compared to the negative control.

In contrast, all treated doses for lipid extracts (50, 100 and 200 mg/kg) showed significant difference ($p \leq 0.05$) on mounting behaviour as compared to negative control. Lipid extract of 100 and 200 mg/kg showed higher number of mounting than positive control at 1st h and 2nd h, but decreased at 3rd h.

The effects of *M. albus* extracts on behaviour of male mice within 3 h observation were recorded (Table 2). The evaluation recorded includes three different parameters: physiologically active or not; self-exploratory behaviour towards female mouse which involved licking and sniffing of female genital organs; and general behaviour which comprised of climbing.

EFFECT OF *M. ALBUS* ON THE MATING ASSESSMENT

Mating assessment of all treated doses of *M. albus* aqueous and lipid extracts was conducted early in the morning on day 7th. Observation of copulatory plug was carried out to confirm the successful of mating. Only female mice in positive control and all treated doses of lipid extract showed positive results as indicated by the presence of copulatory plugs (Figure 1).

DISCUSSION

MOUNTING BEHAVIOUR

The present study showed that aqueous and lipid extract of *M. albus* possessed aphrodisiac property, though the lipid extract was observed to be more effective as compared to the aqueous extract. As shown in Table 1, it was observed that all treated doses of lipid extract significantly ($p \leq 0.05$) enhanced the sexual *libido* in the normal male mice. Although all the doses of aqueous extract indicated some mounting, only the dose 200 mg/kg showed significant

TABLE 1. Effect of *Monopterus albus* aqueous and lipid extracts on the number of mounting in male mice. All values are represented mean ± S.E.M, $n = 4$. * $p \leq 0.05$ considered significant as compared to negative control

Group	Dose (mg/kg)	Number of Mounting		
		1 h	2 h	3 h
Normal saline	Negative control	4.00 ± 2.16	2.25 ± 0.75	2.25 ± 1.03
Sildenafil citrate	5	22.00 ± 4.06*	11.25 ± 1.65*	5.50 ± 2.60
Aqueous extract	50	2.00 ± 0.71	1.75 ± 1.03	2.50 ± 1.55
Aqueous extract	100	10.00 ± 2.08	2.50 ± 1.90	4.00 ± 2.16
Aqueous extract	200	28.25 ± 7.62*	11.75 ± 3.09*	2.00 ± 1.35
Lipid extract	50	18.25 ± 4.37*	8.50 ± 3.12	1.00 ± 0.41
Lipid extract	100	24.00 ± 4.45*	11.75 ± 1.50*	3.00 ± 0.91
Lipid extract	200	25.25 ± 5.12*	12.00 ± 3.54*	5.25 ± 2.39

TABLE 2. Summary of mounting behaviour of male mice in all treated groups over 3 h of observation

Group	Dose (mg/kg)	Observation
Saline	Negative Control	Less active, pursues and sniffs female's genital organ, tries to mount but easily discouraged
Sildenafil citrate	5	Very active, grooming and sniffing of genitals and mounts (frequency 22 for the 1st h) with an aggressive manner
Aqueous extract	50	Less active, less interaction, tries to mount but easily discouraged
Aqueous extract	100	Active, grooming and sniffing of genitals, mounts with an aggressive manner but easily discouraged
Aqueous extract	200	Very active, grooming and sniffing of genitals and mounts (frequency 28 for the 1st h) with an aggressive manner
Lipid extract	50	Active, grooming and sniffing of genitals mounts (frequency 18 for the 1st h) with an aggressive manner but easily discouraged
Lipid extract	100	Very active, grooming and sniffing of genitals and mounts (frequency 24 for the 1st h) with an aggressive manner
Lipid extract	200	Very active, grooming and sniffing of genitals and mounts (frequency 25 for the 1st h) with an aggressive manner

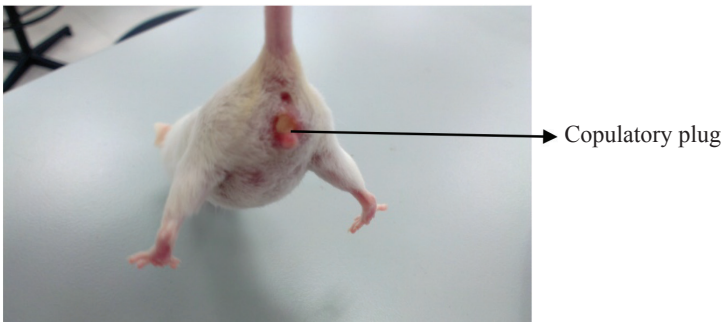

FIGURE 1. Copulatory plug in a female mouse after overnight mating with a male mouse

number of mounting ($p \leq 0.05$) as compared to the negative control. Thus, suggesting that higher dose of aqueous extract may contained high level of bioactive compound. The results complied with the dose-response principles; where generally larger doses would produce larger effects. Higher dose levels may increase the testosterone concentration in blood and produced higher number of mounting, giving the same effects of Sildenafil citrate (Robbins 1996). From the observation, male mice treated with lower dose of aqueous extract showed less interest towards the female mice even though the female mice were in estrus phase.

It was observed the pattern of mounting was highest during the 1st h, but subsequently decreased during the next 2nd and 3rd h. Similar patterns occurred in the positive control group. The lipid extract and Sildenafil may have certain common compound which could improve the number of mounting in mice. According to Chan and Philips (1969), the extract of *M. albus* contained sex steroid hormone. This had been proven by the results that we had obtained for lipid extract of *M. albus*.

The first phase of the study indicated that lipid extract could promote sexual activity. According to Sandroni (2001) and Yakubu et al. (2008), lipid is able to cross the blood brain barrier and consequently increases pituitary hormones concentration at the limbic system thus, stimulating the production of steroid hormone, testosterone. Therefore, it was presumed that due to the probable presence of steroids in the lipid extracts of *M. albus*, an increase of sexual performance was observed in the male mice (Ridzwan et al. 2013). Furthermore, it was also noted that the number of mountings in all groups tend to decrease during the 3 h observation for all the extracts and positive control groups. This was probably due to the effect of testosterone hormone. As reported by Robbins (1996), high testosterone levels will result in higher *libido*. The response will start to reduce when the hormone concentration has reached its optimum. Since the results using the extracts gave similar pattern of mounting behaviour, there is possibility they contained steroid hormone. Therefore, *M. albus* lipid and aqueous extracts may have aphrodisiac properties, which have similar mode of actions as Sildenafil citrate, an anabolic steroid.

MATING ASSESSMENT

After six days of daily administration of *M. albus* extracts, there were positive results in mating performance for all

treated groups of lipid extract as well as the positive control group as indicated by the presence of copulatory plug. Daily administration of lipid extract at various doses could increase serum testosterone which might be responsible for the marked effect on mating assessment in the male mice. According to Razak et al. (2001), lipid extracts of *M. albus* contained omega-3 fatty acids which was proven to have beneficial effect on the sexual function and had the ability to protect testicular tissues due to its antioxidant properties (Saravana Kumar et al. 2011). Thus, this study suggested that lipid extract could enhance mating performance in normal male mice.

CONCLUSION

In conclusion, *M. albus* extract may possesses aphrodisiac properties, though they are mainly present in lipid extract of *M. albus*. Lipid extract of *M. albus* showed substantial evidences in sexual behaviour of male mice at dose 50, 100 and 200 mg/kg as compared to the aqueous extracts. Nevertheless, mild aphrodisiac effect was observed at lower dose (50 and 100 mg/kg) of *M. albus* aqueous extract. At higher dose (200 mg/kg) of aqueous and lipid extracts, similar patterns of aphrodisiac properties to those of the positive control group (Sildenafil citrate) were exhibited by the treatment group. This indicated that both extracts have the ability to be as competent as Sildenafil citrate. Therefore, *M. albus* could be used as one of the alternative therapy to restore male sexual activities.

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