

Original Research Article

Effects of Loligo Squid Ink on Sexual Arousal Parameters in D-Galactose-Induced Aging Male Mice

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Abstract: Squid ink is known to contain many bioactive properties such as antioxidant, anticancer activity, antimicrobial activity, and anti-inflammatory activity. Through its antioxidant properties squid was suggested to have therapeutic potential for sexual function disorders. This study is an attempt to evaluate whether squid ink has therapeutic benefits against age-related decreases in sexual arousal in D-galactose-induced aging male mice. Four groups of male mice were treated as follows. Group-1 received only standar food but no D-galactose induction nor squid ink given. Group-2 received D-galactose induction but not squid ink. Group-3 and group-4 received D-galactose induction and squid ink at the dose of 40 and 100 ml/kg body weight respectively. After 35 days of treatment, the test mice were mated with estrous virgin females to determine their levels of sexual arousal. The results, Loligo squid ink extract at a dose of 100 ml/kg body weight significantly enhances sexual arousal parameter values in the D-galactose-induced aging mouse model. However, the recovery rate did not achieve nor surpassed the parameter values of sexual arousal in normal mice. It suggests that Loligo squid ink has the potential to prevent damage to sexual function in males but is less effective in restoring it.

Keywords: Aging, d-galactose, squid ink, sexual function, sexual arousal.

INTRODUCTION

Aging is a part of natural progression in the human life characterized by degradation of the biosynthetic and cellular repair mechanisms. Endocrine function impairment is one of the age-related cellular functions degradation in question. The decrease of thyroxin hormone secretion, for example, results in overall decreased metabolic activity. Furthermore, changes in metabolism have a wider impact, one of which is decreased sexual function [1]. Currently, the age-related decline in cellular, tissue and organ function has been successfully mimicked using animals by administering D-galactose. In beagle dogs the D-galactose aging model exhibited significant similarities with the naturally aging model in physiological and histopathological aspects [2]. In rats (*Rattus norvegicus*) induction of D-galactose able to accelerate aging of skeletal muscles and suitable model for penile erectile dysfunction assessment in male [3, 4]. By using the D-galactose-induced animal aging model enable researchers to study anti-aging therapeutic interventions [5]. Regarding sexual function in D-galactose-induced aging model, there have been several reports on its therapeutic studies. Wang *et al.* (2018) indicated that in D-galactose-induced aging mouse models administration of ginsenoside Rg1 extracted from *Panax ginseng* showed a protection effect on testes due to antioxidant properties of the substance [6]. Next, still regarding the benefits of *Panax ginseng*, Zhang *et al.* (2021) reported that in the D-galactose aging mice, the administration of ginseng stem-leaf saponins ameliorated testosterone level and reproductive damages [7]. In current study D-galactose-induced aging male mouse models were used to evaluate therapeutic effects of Loligo squid ink on male sexual behavior based on the sexual arousal parameters. Squid ink, as indicated in many previous reports has many bioactive properties such as antioxidant, anticancer activity, antimicrobial activity, anti-inflammatory activity [8]. Chemicals screening studies revealed that squid ink contains a large amount of melanin, proteins, lipids, glycosaminoglycans, various metals (Copper, Cadmium) and a variety of melanogenic enzymes, including tyrosine [9]. Experiments on male mice carried out by Gu *et al.* (2017) revealed that squid ink has therapeutic potential for sexual function disorders through its antioxidant properties [10].

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MATERIALS AND METHODS

Squid ink extraction

The squid (*Loligo* sp) used in this study were purchased fresh from fishermen at the Fish Auction Market, Bandar Lampung City, Indonesia. A total of 500 squid with an average mantle length of 5 cm were sampled to collect their ink sacs. The ink sacs were then dissected and squeezed. The sac fragments and ink are collected in a bottle, diluted with distilled water then filtered. The filtrate was stored at 4°C and used as stock.

Test animal and experimental design

By using a Completely Randomized Design (CRD) 24 male mice (*Mus musculus* L.) aged 12 weeks with a weight range of 30-40 grams were grouped into four (6 individuals each). Group-1 was a group of mice that were only kept according to maintenance standards but neither D-galactose induction nor squid ink given (normal control, C₀). Group-2 was the group that received D-galactose induction but not squid ink (negative control, C⁻). Next, group-3 was the mice that received D-galactose induction and *Loligo* squid ink (LSI) at the dose of 40 ml/kg body weight (LSI- 40). Finally, group 4 was a group of mice that were given induction of D-galactose and *Loligo* squid ink at a dose of 100 ml/kg body weight (LSI-100).

D-galactose induction and squid ink administration

Each male mouse in groups 2, 3 and 4 was induced with D-galactose at a dose of 150 mg/kg BW via peritoneal injection. The induction was carried out 3 times at the beginning of the 1st, 3rd and 5th weeks. Next, group 3 and 4 mice were given 40 and 100 ml/kg body weight of squid ink extract, respectively. The *Loligo* ink was given every day starting at the beginning of the 3rd week until the end of the 5th week (35 days). After 35 days, all mice in all groups were prepared for mating trials with estrous virgin females.

Mating trials

For the sexual behavior test in this experiment, 24 estrous virgin female mice were prepared as mates for the test male mice. Mating behavior test was carried out in an open round plastic tray with a diameter of 40 cm and height 25 cm. To begin the test, both male and females were put into a tray in two separated room partitioned by a cardboard (Fig.1). Before being met (the partition was removed) the two mice were allowed to adapt the tray environment for at least 5 min. Starting from the partition board being removed mating activities of the mice then observed and recorded for 10 min. Throughout the experiment, videotaping was performed to observe the sexual arousal parameters: courtship latency, mounting latency and mounting frequency. Courtship latency is the time from when the partition board was removed until the male displayed the first courtship action. Mounting latency is the time from when the cardboard was removed until a first mounting action was shown by the test males. Mounting frequency was the total number of attempts made by the male to ride on the female's back.

Data analysis

The data, sexual arousal parameters, presented as the mean \pm SEM (standard error of the mean), were analysed using one-way ANOVA (analysis of variance). Where a significant difference was detected by ANOVA, the treated groups were then compared with each other and the control group using the LSD (Least Significant Difference) test.

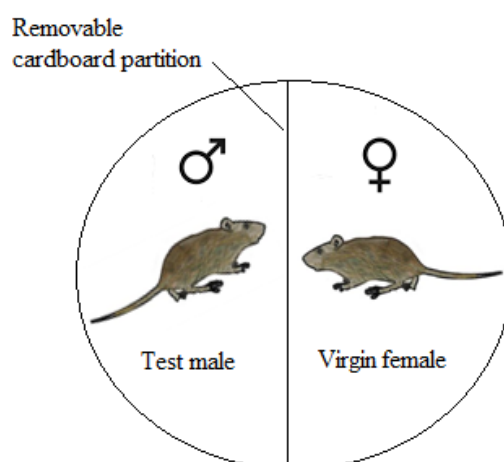


Figure 1: Diagram showing the position of the animal in an open tray for sexual behavior tests

RESULTS AND DISCUSSION

Analysis of video recordings of mice mating activity yielded parameter values for sexual arousal in test male mice as presented in Table 1. The meaning of the sexual arousal parameter values is as follows: the shorter the courtship and mounting latency and the higher the riding frequency, the higher the sexual arousal of the mouse.

Table 1: Sexual arousal parameters of male mice after given different treatments

Treatment	Courtship latency in sec (mean \pm SE)	Mounting latency in sec (mean \pm SE)	Mounting frequency
Normal control (C ₀)	308.00 \pm 3.969 ^a	498.50 \pm 14.830 ^a	7.50 \pm 1.104 ^a
Negative control (C-)	329.17 \pm 3.970 ^c	596.83 \pm 14.831 ^c	0.00 \pm 1.105 ^d
LSI 40 ml/kgBW	326.50 \pm 3.971 ^c	585.17 \pm 14.832 ^c	2.83 \pm 1.106 ^c
LSI 100 ml/kgBW	315.83 \pm 3.972 ^b	545.33 \pm 14.833 ^b	5.17 \pm 1.10 ^b

*Values followed by the same super scripts are not different statistically at $\alpha < 0.05$; C₀: test mice received only water and food; C-: test mice induced with D-galactose without squid ink; LSI: mice induced with D-galactose and squid ink.

Based on the data in Table 1 it is clear that all parameter values of sexual arousal in D-galactose-induced aging male mice significantly decrease compared to that of normal ones (C₀). Administration of Loligo squid ink extract at a dose of 100 ml/kg body weight significantly enhances sexual arousal parameter values in the D-galactose-induced aging mouse model. However, the recovery rate did not achieve nor surpassed the parameter values of sexual arousal in normal mice. Squid ink factors that are thought to reduce damages of sexual functions in D-galactose-induced aging mice is the antioxidant properties. As indicated by Le *et al.* (2015) that in cyclophosphamide-induced male mice treated with squid ink polysaccharides, the resulting reproductive damages due to oxidative stress in testes can be ameliorated [11]. Glycosaminoglycans, a type of the polysaccharides revealed to reduce oxidative damage induced by copper in human fibroblast cultures [12]. Another factor that can be associated to antioxidant properties of squid ink is the presence of taurine [13]. Taurine as reported by Thirupathi *et al.* (2020) is known to be efficacious in reversing oxidative damages and restoring muscle function in overuse of exercised muscle [14]. As previously mentioned, squid ink extract not only contains active compounds that are antioxidants, but also contains many substances that trigger oxidative stress such as: tyrosine, heavy metals of copper and cadmium. Copper, as mentioned above, can be used to induce oxidative stress in cultured human fibroblasts [12, 15]. Whereas cadmium (and lead), in the pathogenesis of the lead- and cadmium-induced pathotoxicity also cause oxidative stress in human and animals [16] It is very likely the presence of such molecules that trigger oxidative stress causing Loligo squid ink unable to reverse age-related sexual function damage in D-galactose-induced aging male mice in this study.

CONCLUSION

Administration of squid ink extract at a highest dose (100 ml/kg body weight) significantly restored sexual arousal parameter values in the D-galactose-induced aging male mice. However, the recovery rate of the sexual arousal parameters is still far below the values of normal mice. Thus, it can be concluded that Loligo squid ink has the potential to prevent damage to sexual function in males but is less effective in restoring it.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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