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Effect of Squid Ink Extract on Testis Histological Parameters in D-Galactose-Induced Aging Mice

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ABSTRACT: Squid ink is among the marine-derived substance that is known to have many bioactive properties such as antioxidant, anticancer activity, antimicrobial activity, and anti-inflammatory activity. Through its antioxidant properties squid ink was suggested to have therapeutic potential for reproductive damages. This study is an attempt to evaluate whether squid ink has therapeutic benefits against age-related decreases in testicular histology parameters 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 all test mice were sacrificed. To assess testicular histology parameters left testis from each mouse was fixed in Bouin's solution. Transverse sections of the organs were cut at 5 µm and stained with hematoxylin and eosin and examined under a light microscope. The results, squid ink extract at a dose of 100 ml/kg body weight significantly increase testis histological parameter values in the D-galactose-induced aging mouse model. However, the recovery rate of the seminiferous tubule epithelium thickness, number of spermatogonia, primary spermatocytes and spermatids is still far below the values of normal mice. Thus, it can be concluded that squid ink has the potential to prevent age-related testis histological damages in mice but is less effective in restoring them.

Key words: aging, d-galactose, squid ink, reproductive damages, testicular histology

I. INTRODUCTION

In all living organisms, especially in human, aging is a part of natural progression characterized by decline in physiological function, followed by dysfunction and finally death [1]. The progressive decline in physiological function is due to degradation of the biosynthetic and cellular repair mechanisms. Furthermore, changes in physiological function have a wider impact, one of which is decreased reproductive function [2]. Even though aging is considered a purely chronological phenomenon, but because each individual experiences a different aging process, many scientists believe that the biological aging can be used as a therapeutic target [3].

To study anti-aging therapeutic interventions, researchers need models [4]. Currently, the age-related decline in cellular, tissue and organ function has been successfully modeled by administering D-galactose to animals. In beagle dogs the D-galactose aging model exhibited significant similarities with the naturally aging model in physiological and histopathological aspects [5]. In rats (Rattus novergicus) induction of D-galactose able to accelerate aging of skeletal muscles and suitable model for penile erectile dysfunction assessment in male [6,7].

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 [8]. 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 [9].

In order to look for natural ingredients that can be used in the aging therapy in current study the D-galactose-induced aging male mouse models were used to evaluate therapeutic effects of squid ink on testicular histopathology damages.

Squid ink, as known from many previous reports has many bioactive properties such as

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antioxidant, anticancer activity, antimicrobial activity, anti-inflammatory activity [10]. Chemicals screening studies revealed that squid ink contains a large amount of proteins, minerals, lipids and carbohydrates, melanin, glycosaminoglycans, various metals (Copper, Cadmium) and a variety of melanogenic enzymes, including tyrosine [11, 12]. On male mice, as revealed by Gu et al. (2017), squid ink has therapeutic potential for sexual function disorders through its antioxidant properties [13].

II. MATERIALS AND METHOD

2.1 Squid ink extraction

The squid ink used in this study was extracted from ink sacs of fresh squid (Loligo sp) purchased from fishermen at the Fish Auction Market, Bandar Lampung City, Indonesia. The ink sacs of 500 squid with an average mantle length of 5cm were separated from the squid's body then dissected and squeezed to remove the ink. 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.

2.2 Test animal and experimental design

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 not given D-galactose induction and neither squid ink (normal control, C_0). Group-2 was the group that received D-galactose induction but not squid ink (negative control, C-). Group-3 was the mice that received D-galactose induction and squid ink (SI) at the dose of 40 ml/kg body weight (SI- 40). Group 4 was a group of mice that were given induction of D-galactose and squid ink at a dose of 100 ml/kg body weight (SI-100).

2.3 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 squid 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 were sacrificed to have their testicles dissected

2.4 Testis histological studies

To assess the thickness of seminiferous tubule epithelium, the number of spermatogonia, spermatocyte as and spermatids left testis from each mouse was fixed in Bouin's solution. Transverse sections of the organs were cut at 5 μm and stained with hematoxylin and eosin and examined under a light microscope. The thickness of seminiferous tubule epithelium was determined using occulometer. Spermatogonia, permatocytes and spermatids counts were done at 10 seminiferous tubules of each experimental unit and then averaged.

2.5 Data analysis

The data of epithelium thickness, number of spermatogonia, primary spermatocytes and spermatids, presented as the mean ± SEM (standard error of the mean), were analysed using one-way ANOVA (analysis of variance) and the LSD (least significant difference) test were applied in the post hoc test.

III. RESULTS AND DISCUSSION

The impact of D-galactose induction and administration of squid ink extract on the testicular histology parameters of test mice can be seen in the cross-sectional photo of the testicular seminiferous tubules shown in Figure 1. Furthermore, the average values of seminiferous tubule epithelium thickness, number of spermatogonia, primary spermatocytes and spermatids of test mice according to the treatment given are presented in Table 1.

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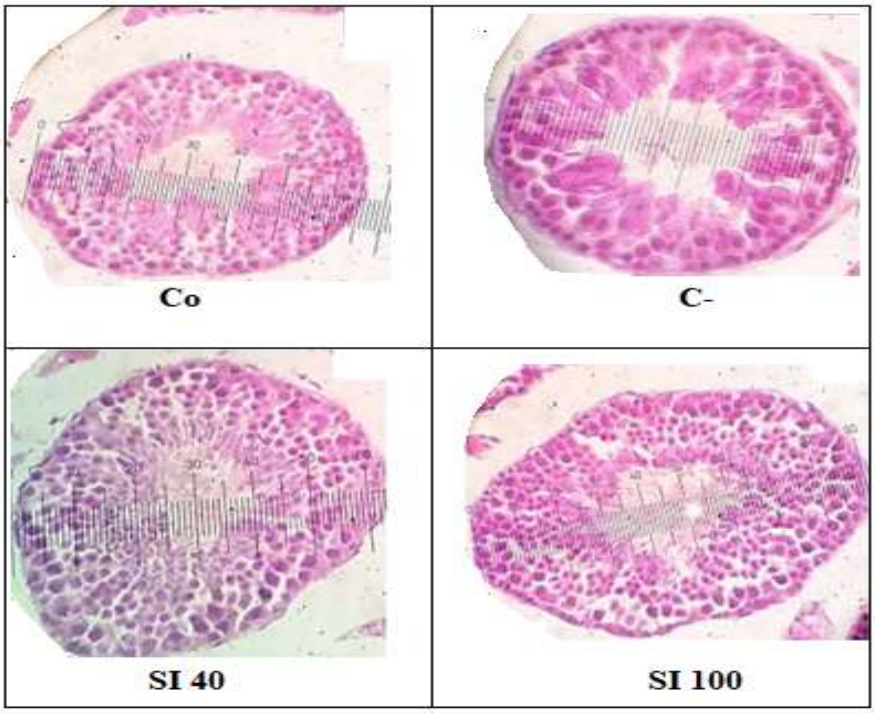

Figure 1 Cross section of histological incision of testicular seminferous tubule of test mice. C_0: test mice received only water and food; C-: test mice induced with D-galactose without squid ink; SI 40 and SI 100: mice induced with D-galactose and treated with squid ink at a dose of 40 and 100 ml/kg BW respectively.

Table 1 Histological parameter values of mice by different treatments

Treatment	Thickness of seminiferous tubule epithelium (µm)	Number of Spermatogonia	Number of spermatocytes I	Number of Spermatids
Normal control (C_0)	63.83 ± 1.16^a	74.00 ± 1.09^a	153.84 ± 1.54^a	174.33 ± 1.36^a
Negative control (C-)	43.17 ± 1.32^d	43.00 ± 1.54^d	102.60 ± 1.42^d	114.83 ± 2.63^d
SI 40 ml/kgBW	53.67 ± 1.21^c	53.83 ± 1.32^c	105.60 ± 1.37^c	125.50 ± 1.04^c
SI 100 ml/kgBW	57.17 ± 1.72^b	62.67 ± 1.75^b	127.00 ± 1.24^b	140.67 ± 2.06^b

*) Values followed by the same super scripts are not different statistically at $\alpha<0.05$; C_0: test mice received only water and food; C-: test mice induced with D-galactose without squid ink; SI 40 and SI 100: mice induced with D-galactose and treated with squid ink at a dose of 40 and 100 ml/kg BW respectively.

Based on the photomicrographs of the testicular seminiferous tubule shown in Figure 1 and parameter values presented in Table 1 it can be assumed that testicular damages in D-galactose-induced aging male mice significantly decrease compared to that of normal ones (C_0). Administration of squid ink extract at a dose of 100 ml/kg body weight significantly enhances seminiferous tubule epithelium thickness, number of spermatogonia, primary spermatocytes and spermatids in the D-galactose-induced aging mouse model. However, the recovery levels did not match nor surpassed the parameter values of testicular histological damages in normal mice.

Factors that are thought to contribute in reducing testicular histological damages in D-galactose-induced aging mice are the antioxidant properties of the squid ink given. As mentioned earlier that squid ink is containing glycosaminoglycans a type of polysaccharides. Administration of glycosaminoglycans was revealed to reduce oxidative damage induced by copper in human fibroblast cultures [14]. In mice, administration of squid ink to males exposed to cyclophosphamide is known to effectively prevent

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testes and sperm of mice from oxidative damages and ameliorate other reproductive damages [15, 16].

Another factor that can be associated to antioxidant properties of squid ink is the presence of taurine [17]. Taurine as indicated by Thirupathi et al. (2020) is known to be efficacious in reversing oxidative damages and restoring muscle function in overuse of exercised muscle [18]. In rats suffered from iron deficiency anemia, administration of squid ink extracted from Sepiotheutis lessoniana known to improve blood parameters such as red blood cell count, haemoglobin and hematocrit [19]. Administration of squid ink melanin-Fe to mice suffered from iron deficiency anemia revealed to significantly remitted iron deficiency anemia symptoms [20].

However, unfortunately, it is a fact that squid ink extract not only contains antioxidant biochemicals but also 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 [14, 21]. Whereas cadmium (and lead), in the pathogenesis of the lead- and cadmium-induced pathotoxicity also cause oxidative stress in human and animals [22]. It is very likely the presence of such molecules that trigger oxidative stress causing squid ink unable to reverse age-related testicular damage in D-galactose-induced aging male mice in present study.

IV. CONCLUSION

Administration of squid ink extract at a highest dose (100 ml/kg body weight) significantly restored testicular histology damage parameter values in the D-galactose-induced aging male mice. However, the recovery rate of the seminiferous tubule epithelium thickness, number of spermatogonia, primary spermatocytes and spermatids is still far below the values of normal mice. Thus, it can be concluded that squid ink has the potential to prevent age-related testis histological damages in mice but is less effective in restoring them.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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