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Effect of Ethanolic Extract of Avocado (*Persea americana* Mill.) Seed on the Testis Histology (*Mus Musculus* L.) Induced Monosodium Glutamate (MSG)

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Abstrak: Monosodium Glutamat (MSG) merupakan suatu zat yang mampu menghasilkan radikal bebas seperti ROS (*Reactive Oxygen Species*), yang dapat menyebabkan stress oksidatif dalam tubuh dan merusak organ reproduksi seperti testis. Tujuan penelitian ini adalah untuk mengetahui pengaruh ekstrak etanol biji alpukat (*Persea americana* Mill.) terhadap histologi testis mencit (*Mus musculus* L.) yang diberi paparan monosodium glutamat (MSG) Penelitian ini merupakan penelitian eksperimental yang dilakukan pada bulan Oktober 2023 – Januari 2024 di Laboratorium Biologi, Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Padang. Rancangan yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 6 ulangan. Data yang diperoleh dianalisis menggunakan uji ANOVA (*Analisis of varians*) dengan menggunakan aplikasi SPSS 23. Hasil penelitian menunjukkan bahwa pemberian ekstrak etanol biji alpukat menunjukkan pengaruh yang nyata pada diameter testis mencit jantan yang diberi paparan MSG. Namun, tidak menunjukkan pengaruh nyata pada berat testis, diameter tubulus seminiferus dan jumlah sel leydig. Ekstrak etanol bji alpukat dosis 0,84 mg/g BB/hari (P1) mampu memperbaiki diameter testis mencit yang telah dipapar MSG.

Kata kunci: Ekstrak biji alpukat, Histologi testis, Mencit, MSG

Abstract: Monosodium Glutamate (MSG) is a substance that is able to produce free radicals such as ROS (Reactive Oxygen Species), which can cause oxidative stress in the body and damage reproductive organs such as the testes. The purpose of this study was to determine the effect of avocado seed ethanol extract (*Persea americana* Mill.) on the histology of the testes of mice (*Mus musculus* L.) induced monosodium glutamate (MSG). This research is an experimental study that was conducted in October 2023 – January 2024 at Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang. The design used is a Complete Randomized Design (CRD) with 4 treatments and 6 repeats. The data obtained were analyzed using the ANOVA test (Analysis of variance) using the SPSS 23 application. The results showed that the administration of avocado seed ethanol extract showed a noticeable effect on the diameter of the testes of male mice exposed to MSG. However, it showed no noticeable influence on testicular weight, seminiferous tubule

diameter and Leydig cell count. Ethanol extract avocado seed dose 0.84 mg /g body weight / day (P1) was able to improve the diameter of mice testes that had been induced MSG.
Keyword: Avocado seed extract, Histology of testis, Mice, MSG

1. Introduction

Infertility is the failure to achieve pregnancy after having sexual intercourse without contraception for 12 months on a regular basis. According to the World Health Organization there are about 50-80 million infertile couples in the world and about 2 million infertile couples are increasing every year. Infertility occurs in 15% of couples of reproductive ages between the ages of 20 – 45 years, and about 50% is related to abnormalities in men. This is a frightening condition for married couples who are sexually active [1].

Infertility in men is caused by several factors including heavy metals that cause disruption of metabolic processes that produce energy, radiation that can increase body temperature including the testes so that the process of spermatogenesis is disrupted, smoking which causes a decrease in sperm quantity and motility and causes sperm abnormalities, alcohol use that causes a decrease in body testosterone levels, and some drugs that cause male infertility through hormonal disruption, suppressing spermatogenesis, and decreasing sperm motility [2]. In addition, the cause of infertility in men is oxidative stress caused by an increase in reactive oxygen species (ROS) and lipid peroxidase obtained from the environment including consumption patterns of foods containing MSG [3].

MSG is one of the substances that can produce free radicals. MSG that enters the body will produce free radicals in the form of reactive oxygen species (ROS) [4]. Free radicals formed will reduce ascorbic acid levels in the testes [5]. Ascorbic acid acts as an antioxidant or reducing agent that has a considerable role in supporting the male reproductive system. Decreased levels of ascorbic acid in the testes can cause impaired spermatogenesis, Leydig cell degeneration, seminiferous tubule degeneration and decreased testosterone production [6].

The results of Siregar's research stated that MSG can result in an increase in free radicals in the body, which will affect the immune system [7]. Imbalance between free radicals and antioxidants will cause oxidative stress that will damage certain organs such as kidneys, reproductive organs and brain tissue [8]. Free radicals in the form of Reactive Oxygen Species (ROS) that enter the body can cause infertility in males and females, reduced testicular weight, and damage to reproductive function [9].

Research conducted by Vinodini et al [10] on male rats with MSG 4 g/kg body weight for 15 days (short-term exposure) and 30 days (long-term exposure) greatly influenced testicular weight which showed a decrease in both experimental groups. Lipid peroxidation levels increased in both groups, while ascorbic acid levels in the testes decreased. Nayanatara et al., also stated that male rats given MSG for 15 days (short-term exposure) and 30 days (long-term exposure) given 4 g / kg intraperitoneal body weight showed the effect in the form of decreased testicular weight, sperm count, ascorbic acid levels, and increased number of damaged or abnormal sperm [11]. To improve the negative effects caused by MSG, a compound containing antioxidants is needed that can improve the structure and function of the testes.

Antioxidants are compounds needed by humans to ward off free radicals in the body. Dietary fibre and antioxidants are two types of components that are very useful in increasing health activities and are able to prevent various diseases [12]. Antioxidants are produced naturally by the human body. However, often these compounds are not enough to protect our bodies so that antioxidant intake from outside the body is needed [13].

Avocado seeds are one of the natural ingredients that contain high antioxidants [14]. People usually only consume avocado flesh, while the seeds are more discarded and become waste so that it can be one of the causes of environmental pollution. Avocado seeds have a high percentage of antioxidants so it can be considered as one of the natural sources of antioxidants such as phenolics, tannins and flavonoids. The use of avocado seeds, apart from being antioxidants can also reduce environmental pollution waste [15].

Wahyuni et al [17] stated that avocado seeds contain phenols that can be used as antioxidants that can protect themselves from free radicals. Phenolic compounds are known as one of the most important parts of plants and have the ability to ward off free radicals. Phenolic compounds are able to act as antioxidants by donating electrons to free radicals [17]. Felistiani's research stated that avocado seed ethanol extract at a dose of 0.84 mg/g BB and 1.68 mg/g BB in mice injected with Staphylococcus aureus had an influence on liver histopathology with the best dose of 0.84 mg/g BB [18]. Avocado seeds contain active compounds that can provide protection to the liver that is damaged by toxins.

2. Research Method

This study was an experimental study using a complete randomized design (CRD). The sample of this study was 24 male mice aged 3-4 months with a body weight of 20-30 grams. The research sample was divided into 4 groups and each group amounted to 6 mice obtained according to calculations using the Federer formula. K-: group of mice without treatment; K +: group of mice with MSG treatment 4 mg/g BB; P1: group of mice with MSG treatment 4 mg/g BB + avocado seed ethanol extract 0.84 mg/g BB; and P2: group of mice with MSG treatment 4 mg/g BB + avocado seed ethanol extract 1.68 mg/g BB. The treatment was given to test animals for 14 days orally.

On the 15th day, a neck dislocation was carried out on the mice and then dissected and taken the testicular organs to weigh the testicles and measure the diameter of the mice's testes. Then the testes will be made into histological preparations using the paraffin method so that measurements can be made of the diameter of the seminiferous tubules and the number of Leydig cells. Data from histological observations of mouse testes were analysed statistically with One Way ANOVA test (Analysis of variance) and DMRT (Duncan's Multiple Range Test).

3. Results and Discussion

The results of research on the effect of avocado seed ethanol extract (*Persea americana* Mill.) on the histology of the testes of mice (*Mus musculus*) exposed to monosodium glutamate (MSG) can be seen in the figure 1 below.

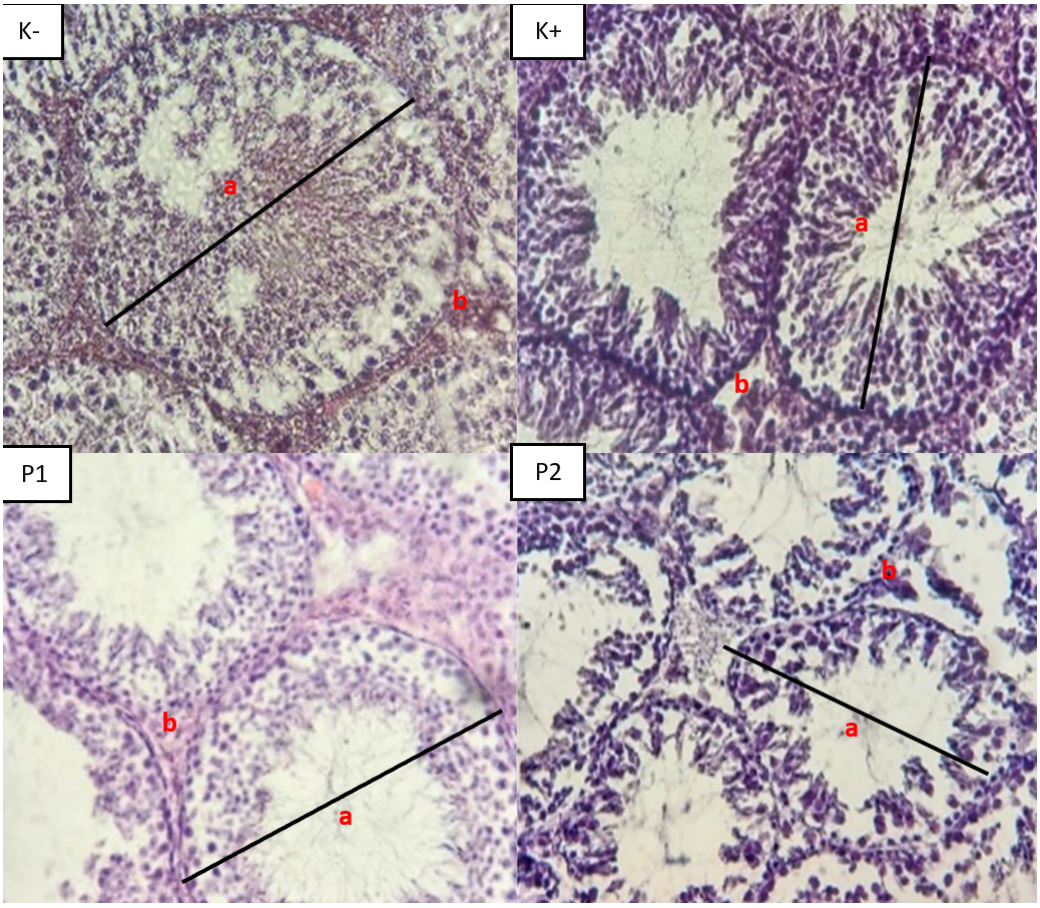

Figure 1. The seminiferous tubules of the mice testes treated with MSG and avocado seed ethanol extract (400x magnification) a: lumen; b: Leydig cell

Overall, it can be seen from the Figure 1 shows that seminiferous tubules exposed to MSG (K+) have decreased in diameter and number of Leydig cells. However, there was an increase diameter and number of Leydig cells in P1 treatment and a decrease diameter and number of Leydig cells in P2.

Table 1. The effect of avocado seed ethanol extract on testicular weight, testicular diameter, seminiferous tubule diameter and number of leydig cells induced MSG

Treatment	Testicular Weight of Mice (grams)	Testicular Diameter (mm)	Diameter of Seminiferous Tubules (µm)	Number of Leydig Cells
K-	0,10 ± 0,01	6,25 ± 0,11[a]	17,36 ± 0,68[a]	357 ± 0,68[a]
K+	0,10 ± 0,01	6,17 ± 0,18[a]	13,59 ± 1,50[b]	284 ± 22,13[bc]
P1	0,11 ± 0,02	6,77 ± 0,13[b]	14,37 ± 0,57[b]	330 ± 42,20[ab]
P2	0,10 ± 0,01	6,42 ± 0,10[c]	13,38 ± 2,67[b]	250 ± 58,93[c]

Remarks: Superscript differences in the same column show real differences ($p<0,05$) on DMRT test, while data without superscript in the same column shows results that are not significantly different ($p>0,05$). Data is presented in averages ± SD.

Based on Table 1 above, the results of measuring the weight of mice testicles in the treatment of avocado seed ethanol extract and MSG showed relatively similar results. The average testicular weight of negative control treatment (K-) mice was 0.10 ± 0.01 grams. MSG 4 mg/g BB (K+) treated mice had an average testicle weight of 0.10 ± 0.01 grams, MSG 4 mg/g BB + avocado seed extract 0.84 mg/g BB/day (P1) the average testicle weight was 0.11 ± 0.02 grams, and MSG 4 mg/g BB + avocado seed extract 1.68 mg/g BB/day (P2) the average testicle weight was 0.10 ± 0.01 grams. The results of statistical tests of mouse testicular weight showed that there was no noticeable difference ($p > 0.05$).

The highest average testicle diameter was obtained from MSG treatment 4 mg/g BB + avocado seed extract 0.84 mg/g BB/day (P1) which was 6.77 ± 0.13 mm and the lowest in MSG treatment 4 mg/g BB (K +) which was 6.17 ± 018 mm (Table 1). The treatment of K+ is not significantly different from K-, but it differs markedly from P1 and P2. Treatment 1 (P1) differs markedly from P2, K+ and K- while P2 differs markedly from P1, K+ and K-.

The mean diameter of the seminiferous tubules of negative control treatment (K-) showed the highest value of 17.36 ± 0.68 µm. MSG 4 mg/g BB (K+) treated mice have an average seminiferous tubule diameter of 13.59 ± 1.50 µm, lower than P1 but higher than the diameter value at P2 (Table 1).

The diameter of the tubules in the negative control treatment (K-) mice differed markedly from K+, P1, and P2. However, the K+ group did not show significantly different results from P1 and P2. This showed that the best mouse

seminiferous tubule diameter was found in the negative control group and decreased seminferus tubule diameter values in the MSG-exposed mice group, both those given avocado seed extract (P1, P2) and those not given avocado seed extract (K+). There was an increase in the diameter of the seminiferous tubules in the P1 group compared to K+ but showed a decrease in values in P2.

The mean number of negative control treatment (K-) leydig cells showed the highest value of 357 ± 0.68. Mice treated with MSG 4 mg/g BB (K+) the number of leydig cells was 284 ± 22.13 lower than P1 but higher when compared to the number of P2 leydig cells (Table 1). The average number of the highest leydig cells treated K- differed markedly from K+ and P2, but did not differ markedly from P1 while the average number of leydig cells lowest treated P2 differed markedly from K- and P1, but did not differ markedly from K+. The treatment of K+ differs markedly from K-, but not markedly differs from P1 and P2. The P1 treatment is significantly different from the P2 treatment, but not the real difference from K- and K+. The number of mice leydig cells decreased after exposure to MSG but increased again in the treatment of avocado seed ethanol extract 0.84 mg/g BB/day (P1) and showed a decrease in value in the P2 group.

In this study there was a decrease in testicular weight in the administration of MSG 4 mg/g BB (K +) and there was an increase again after being given a dose of avocado seed extract 0.84 mg/g BB (P1) when compared to K +. This showed that ethanol extract of avocado seeds had no effect on the weight of testicles that had been exposed to MSG.

According to Christsetiani the weight of the testicle is influenced by several factors such as the number of sex cells and tissue that supports the testicle. Many tissues support the growth, size, and weight of the testicles, including connective tissue that occupies most of the testicles, blood volume, and correlates with the animal's own body weight. The weight of the testicles in this study was not much different from the weight of the testicles of mice in general, which ranged from 0.08-0.1 grams. This is thought to occur due to the provision of MSG and avocado seed ethanol extract given in a short time [19].

Tjondronegoro states that testicular reduction can occur if the obstruction of hypothalamic function is carried out for a long time. So maybe if the administration of MSG and avocado seed ethanol extract is done for a long time, the more noticeable the difference in testicular weight in treatment [20].

Observation of testicular diameter showed a marked difference between positive control (K+) with P1 and P2. In K + there is a decrease in testicular diameter when compared to K- due to the adverse influence of free radicals in monosodium glutamate (MSG). Free radicals accumulate in the testicles, causing oxidative stress [8]. The decrease in testicular diameter is thought to be caused by reduced testosterone secretion due to inhibition of leydig cell development caused by decreased LH secretion. Reduced hormones testosterone and FSH are thought to cause atrophy in the seminiferous tubules, as a result of which the diameter of the testes becomes reduced [21].

In P1 and P2 treatment there is an increase in testicular diameter. This showed that avocado seed ethanol extract was able to increase the diameter of the testicles of mice that had been exposed to MSG. The increase in testicular diameter may be due to an increase in the number of cells and tissues that support the testicle. Tissues that support the growth and size of the testes, including connective tissue that occupies most of the testicles, blood volume, and correlates with the body weight of the animal itself [19].

In the seminiferous tubule diameter data (Table 1) there was a decrease in the K+ group when compared to K-. The decrease in diameter of the seminiferous tubules is thought to be due to the inhibition of LH secretion in the anterior pituitary which functions to stimulate growth and the number of leydig cells to produce testosterone decreases and inhibits leydig cells to produce the hormone testosterone. Reduced levels of testosterone and LH hormones are thought to cause seminiferous tubule atrophy [22].

In the P1 group treated with MSG and avocado seed ethanol extract 0.84 mg / g BB showed an increase in seminiferous tubule diameter when compared to the K + group. However, statistically this does not show a

noticeable difference. This shows that avocado seed ethanol extract has not been able to maintain the seminiferous tubules optimally in mice that have been exposed to MSG.

The data on the number of leydig cells (Table 1) shows that in the K+ group there is a decrease in the number of leydig cells when compared to K-. The K+ statistical test also showed a marked difference with the K- group. The K + group given MSG treatment is suspected to have an increase in Ca2 + in intracellular leydig, an increase in Ca2 + will cause disruption of mitochondria and activate ATPase enzymes, phospholipases, endonuclease, and proteases so that there is a disruption in ATP synthesis and disruption of cell membrane permeability so that it will eventually cause death in leydig cells [23]. As a result of cell death, it results in a decrease in the number of leydig cells in the testes of mice.

The P1 and P2 groups showed different averages where P1 was higher than K+ while P2 was lower than P1 and K+, but statistically P1 and P2 did not show significantly different results from the K+ group. Groups P1 and P2 are groups with MSG and avocado seed ethanol extract with different doses. However, the results did not show a noticeable difference when compared to K+ given MSG. Based on existing data, this shows that avocado seed extract given to test animals has not been able to maintain testicular leydig cells optimally from damage caused by MSG.

4. Conclusion

Ethanol extract of avocado seeds showed a effect on the diameter of the testes of mice induced MSG. However, it showed no noticeable influence on testicular weight, seminiferous tubule diameter and leydig cell count. Ethanol extract of avocado seed dose 0.84 mg / g body weight / day (P1) was able to improve the diameter of mice testes that had been induced MSG.

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