The Effect of Moringa oleifera Extract on Histopathological of Mice Testes Exposed by Monosodium Glutamate

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The Effect of *Moringa oleifera* Extract on Histopathological of Mice Testes Exposed by Monosodium Glutamate

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ABSTRACT

Background: Excessive use of Monosodium Glutamate (MSG) can affect a man's fertility and cause infertility. MSG can become a source of free radicals that can damage cells. Free radicals can be countered with antioxidants. If the number of free radicals in the body exceeds the number of endogenous antioxidants, there will be an imbalance, so exogenous antioxidants are needed to help balance. The leaves of Moringa (*Moringa oleifera L*) are exogenous antioxidants that contain flavonoids. Flavonoids are believed to have 4-5 times greater antioxidant power than vitamins.
Objectives: To determine the effect of moringa leaf extract on the histopathological picture of mice testes exposed to MSG.
Methodology: The study was conducted with a posttest-only control group design. The sample is 25 male mice which were divided into five groups. The control group (K-) was only given standard feed, group (K +) was exposed to MSG 6 g / day, treatment group 1 (P1) was given 6 g / day of MSG exposure + Moringa leaf extract 300 mg/kg BW / day, treatment group 2 (P2) was given MSG exposure of 6 g / day + Moringa leaf extract 600 mg/kg BW / day, treatment group 3 (P3) was given MSG exposure of 6 g / day + Moringa leaf extract 1200 mg/kg BW / day. After 30 days, the mice were terminated. The testes were taken for histopathological observation with hematoxylin-eosin staining and assessed according to the Johnsen score criteria.
Result: In the Shapiro-Wilk test, abnormal results were obtained in the P3 group (MSG 6 g / day + Moringa Extract 1200 mg/kg BW / day) ($p = 0.023$). The P3 group was obtained ($p < 0.05$), so the data was insignificant. In the Kruskal Wallis test, it was found ($p = 0.117$) because the p value> 0.05 showed no significant difference between the seminiferous tubules based on the treatment group.
Conclusion: *Moringa oleifera* leaf extract affects the histopathological picture of the mice testes that were exposed to MSG at doses of 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW

Keywords: Testicular histopathology, *Moringa oleifera* leaf, Monosodium glutamate, Free radicals, Antioxidants

INTRODUCTION

The consumptive lifestyle of today's society is preferred to something fast and instant, and one example is the use of flavorings. Monosodium Glutamate (MSG) is one flavoring we often use. MSG is an effective flavor enhancer that gives a savory and delicious taste, so many people often add MSG to food (Kurtanty,2018). However, excessive use of MSG will have side effects, one of which is infertility (Edward,2010). Budiman (2015) reported a study on 18 mice that were exposed to MSG 6 mg / gW/day for 30 days, proving that there was damage to the histological structure of the testes from the seminiferous tubules, interstitial compartments, spermatogenic cells, Sertoli cells, Leydig cells, and visible erythrocyte cells. This is because MSG is a source of free radicals.

The use of antioxidants can reduce these free radicals. This is because the human body has antioxidants (endogenous antioxidants) to fight free radicals and other reactive oxygen compounds, including MSG. Still, if these free radicals and other reactive oxygen compounds are present excessively, the body needs antioxidants from outside the body (exogenous antioxidants) to fight

them (Budiman,2015). One example of exogenous antioxidants is flavonoids.(Winarsi,2011;Arifin,2018;Parwata,2016).

Flavonoids are secondary metabolites of polyphenols that function as antioxidants. Flavonoids can be found widely in plants (Munhoz,2014). *Moringa oleifera* plant is a natural material with high antioxidant content and is believed to have various benefits (Krisnadi,2015; Hardiyanthi,2015). The Moringa leaves are rich in calories, protein, calcium, potassium, and magnesium nutrients (Gopalakrishnan,2016). Based on phytochemical tests by Pratama (2017), *Moringa oleifera* contains various compounds such as alkaloids, flavonoids, glycosides, terpenoids, and tannins.

Based on the background, giving *Moringa oleifera* leaf extract can break the free radical chain. Therefore, it can maintain the body's balance of free radicals and antioxidants to prevent oxidative stress affecting male fertility. To prove this, it is necessary to research the effect of giving *Moringa oleifera* leaf extract on the testicular histopathology of mice exposed to Monosodium Glutamate (MSG).

MATERIALS AND METHOD

Materials

Several mice (Mus musculus) criteria could be used as samples. Inclusion criteria: 1) male sex mice, 2) age 6-8 weeks, 3) weight 25-30 grams. Exclusion criteria: 1) mice are not healthy, hypoactive (weak, inactive), and 2) visible anatomic abnormalities, such as abnormalities in the testes.

Moringa oleifera extract was made by using 1) *Moringa oleifera* leaf, 2) Ethanol 70%, 3) Aquadest

Monosodium glutamate (MSG) was the usual MSG found at the market.

Methods

This research was an experimental test with a posttest-only control group design that used experimental animals as experimental objects. Research, data collection, and data processing were carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) Semarang State University for the treatment of experimental animals and the Satmoko Clinic Semarang and the Anatomical Pathology Laboratory of Dr. Kariadi Semarang Hospital for reading histopathological tissue images. The research period was from June to September 2020.

The sample size was determined based on WHO guidelines on the use of experimental animals. Each treatment group had a minimum of 5 individuals per group. Based on the provisions, the sample used is at least 25 individuals where the sample had met the inclusion criteria, then divided into four treatment groups and one control group using simple random sampling so that the number of samples is 5 per group.

Before the treatment, 25 male mice were adapted to standard feed and drink for one week. Then, the group treatment was carried out for 30 days. The control group (K-) was only given standard feed, group (K +) were exposed to MSG 6 g / day (0.02 g / kg BW), treatment group 1 (P1) was given MSG exposure 6 g / day (0.02 g / kg BW) + Moringa leaf extract 300 mg/kg BW / day, treatment group 2 (P2) was given exposure to MSG 6 g / day (0.02 g / kg BW) + Moringa leaf extract 600 mg/kg BW / day, treatment group 3 (P3) was given exposure MSG 6 g / day (0.02 g / kg BW), + Moringa leaf extract 1200 mg/kg BW / day. The MSG and Moringa leaf was given orally.

After 30 days of treatment, termination was carried out by inserting the mice into a jar that had been given a cotton ball containing liquid chloroform/ether so that the mice were not aware; after that, the mice were killed by using cervical dislocation. The testes were taken for histopathological observations with HE staining and assessed according to the Johnsen score criteria with score criteria of 1-10.

Table 1. Johnsen Score criteria

SCORE	EXPLANATION
10	There is a complete spermatogenesis process.
9	The spermatogenesis process is incomplete, with many late-stage spermatids.
8	Spermatozoa cells <5 per tubule and some late-stage spermatid cells.
7	Lots of early-stage spermatids, no spermatozoa or late-stage spermatids.
6	Multiple early-stage spermatids, no spermatozoa, or late spermatids.
5	Lots of spermatocytes, no spermatozoa or spermatids.
4	Few spermatocytes, no spermatozoa or spermatids.
3	There are only spermatogonia cells.
2	There are only Sertoli cells, no germ epithelial cells.
1	There is no seminiferous epithelium.

Data Analysis

Data analysis using SPSS for Windows. First, mice's testicular microscopic image data were tested for normality using the Shapiro-Wilk test with normal criteria p> 0.05 to see the distribution of normality and homogeneity. If the data is normally distributed and homogeneous, it is continued with the One Way Annova test. However, if the data is not normally distributed and homogeneous, it is followed by the Kruskal-Wallis test.

RESULT AND DISCUSSION

Table 2. Descriptive analysis of the microscopic image of mice seminiferous tubules

Case Summaries

Tubulus seminiferus

Kelompok	N	Mean	Std. Deviation	Median	Minimum	Maximum
Kontrol normal	5	9.5580	.29995	9.4300	9.30	10.00
MSG	5	9.1980	.21253	9.1300	9.00	9.50
MSG + Kelor 300	5	9.5460	.25403	9.6000	9.27	9.90
MSG + Kelor 600	5	9.2940	.55644	9.4700	8.37	9.80
MSG + Kelor 1200	5	9.6660	.19034	9.6000	9.53	10.00
Total	25	9.4524	.35112	9.5000	8.37	10.00

Table 2 shows that the microscopic image of seminiferous tubules in treatment group 3 (MSG + Moringa 1200) has a higher mean value (9.66) than the other four groups. Meanwhile, the lowest average calculation value was found in the K treatment group + MSG exposure (9.19).

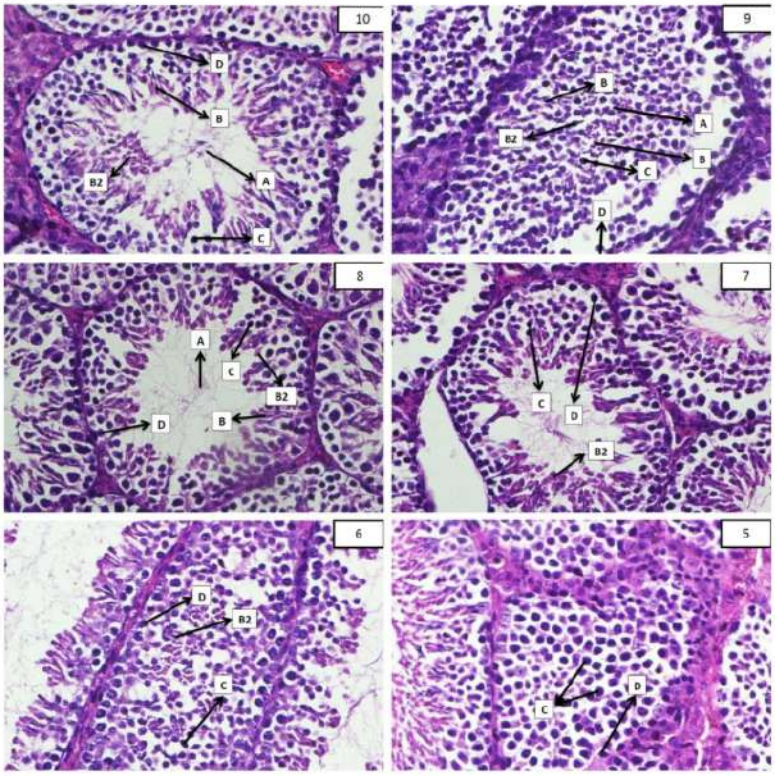

Figure 1. Microscopic of seminiferous tubules in the Johnsen Score criterion assessment of 400x magnification. Score 10, 9, 8, 7, 6, 5. Spermatozoa (A), late stage spermatids (B), early stage spermatids (B2), spermatocytes (C), spermatogonia (D).

The normality test used in this study was the Shapiro-Wilk test because the sample size was less than fifty. In addition, the data was normally distributed if the p-value was> 0.05.

Table 3. Normality test (Saphiro-Wilk test)

Group	Mean ± SD	Median (min-max)	Shapiro-Wilk	
			p	Transf. p
Normal Control	9,56 ± 0,30	9,43 (9,3 – 10)	0,296*	0,306*
MSG	9,20 ± 0,21	9,13 (9 – 9,5)	0,447*	0,456*
MSG + Moringa 300	9,55 ± 0,25	9,6 (9,27 – 9,9)	0,627*	0,632*
MSG + Moringa 600	9,29 ± 0,56	9,47 (8,37 – 9,8)	0,278*	0,225*
MSG + Moringa 1200	9,67 ± 0,19	9,6 (9,53 – 10)	0,023	0,024

Based on the normality test data, it was found that the data were not normally distributed p <0.05 because the MSG + Moringa 1200 group obtained a p-value of 0.023, so a non-parametric test was carried out in the form of the Kruskal Wallis test.

Table 4. Kruskal Wallis test

Group	Seminiferous Tubules	p
Normal Control	9,43 (9,3 – 10)	0,117

MSG	9,13 (9 – 9,5)
MSG + Moringa 300	9,6 (9,27 – 9,9)
MSG + Moringa 600	9,47 (8,37 – 9,8)
MSG + Moringa 1200	9,6 (9,53 – 10)

The non-parametric Kruskal Wallis test had a p-value of 0.117. Because the p-value > 0.05, there was no significant difference between the seminiferous tubules based on the treatment group. The value could be insignificant from these data, so there was no need for an analysis test.

The results of this study indicate that Moringa leaf extract can protect the histopathological testicular tissue exposed to MSG. This was evidenced by the Johnsen score in the K + group that was exposed to MSG, which obtained a lower Johnsen score which is 9.20 compared to the other three treatment groups, namely the P1, P2, P3 groups (MSG + Moringa for various dosage levels), P1 is 9,55, P2 is 9.29, and P3 is 9.67. In addition, from this score, it can also be proven that giving MSG exposure in the long term can reduce the histopathological quality of the testes.

This is in line with the theory that MSG administration affects the histopathological features of the testes in male mice. MSG, which is a source of free radicals, if the amount in the body exceeds antioxidants, it will turn into Reactive Oxygen Species (ROS), which causes DNA damage and will circulate in the blood throughout the body, including the testes (Susantiningsih,2015; Arief,2018; Christijanti,2011). Monosodium glutamate causes interference in spermatogenesis through three anti-fertility mechanisms: pre-testicular, testicular, and post-testicular. Disorders of spermatogenesis in the pretesticular mechanism by inhibiting spermatogenesis through the hypothalamus, pituitary, and testes. Decreasing LH in serum will reduce intratesticular testosterone, followed by a decrease in FSH, so sperm production is inhibited (Sukmaningsih,2011). According to Das and Ghosh (2010), spermatogenesis disorders occur due to diseases of the hypothalamus, pituitary gland, and adrenal glands, which cause low serum LH and testosterone. If testosterone continuously decreases, it will affect the activity of the LDH enzyme, which functions to convert NADH to NAD + as a lactate-forming material through pyruvate synthesis, which later affects the metabolism of the Sertoli cells so that it will have an impact on spermatogenesis. The function of lactate is as a nutrient intake for spermatozoa in the spermatogenesis process in the Sertoli cells. Inadequate nutritional intake results in poor-quality sperm production (Daiber,2017; Stefani,2015; Schieber,2014; Celino,2011).

Oxidative stress can also cause damage to the mitochondrial membrane and eliminate the potential function of the mitochondrial membrane, resulting in membrane leakage resulting in membrane depolarization, and activation of apoptotic factors, which induce the process of cell damage. This damage is characterized by degenerative changes in the seminiferous tubules, vacuolization of the interstitial, reduced seminiferous basal epithelial cells resulting in the release of the basement membrane, and the formation of hypo-spermatozoa. Damage to the testes will disrupt the sperm spermatogenesis process and can affect the quality of the spermatozoa produced, which can cause infertility problems Akunna,2013,2017; Akingbade,2014).

Andrew's research showed that the administration of MSG doses of 0.04 mg/kg BW and 0.08 mg/kg BW in female Wistar rats experienced changes in the histopathological picture as hypertrophy and degenerative changes (Eweka,2010). In previous studies, it was known that the MSG dose which can cause death 50 % (LD50) is between 15000 - 18000 mg/kg BW in mice and rats (Bera,2017; Husarova,2013).

In group P, giving Moringa leaf extract could help damage histopathological tissue in male mice testes due to MSG exposure. This is because the results obtained are different from the control group. This study showed that administering Moringa leaf extract at a dose of 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW affected the total Johnsen score. This can be seen with an increase in the Johnsen score in the P group, which was given Moringa leaf extract in stratified doses (P1 is 9.55, P2 is 9.29, P3 is 9.67) compared to the group (K +), which is only exposed to MSG.

The results of this study are in line with the findings of research conducted by Jannah (2018), that the oral administration of Moringa leaf extract at a dose of 100 mg/kg BW, 200 mg/kg BW, 300 mg/kg BW, 400 mg/kg BW and 500 mg. / KgBW for five weeks of testicular histopathological examination of white male rats with Diabetes Mellitus showed an improvement in the

histopathological picture of the testes after giving Moringa leaf extract (Jannah,2018). This is because Moringa leaf extract contains antioxidants that can help prevent damage caused by free radicals.

Moringa leaves contain many antioxidant compounds that play a role in warding off ROS, including tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids, which also contain vitamin C (Krinadi,2015; Gopalakrishnan,2016; Kasolo,2010). Flavonoids are one of the active components of Moringa leaves related to acting as an antioxidant. Some of the main bioactive compounds of its phenolics are flavonoid groups. Most phenolics are antioxidants that neutralize free radicals' oxidation reaction, which can damage cell structure (Cahyani,2017). Flavonoids also protect cells/tissues from oxidative stress due to excessive free radicals by cutting oxidative chain reactions from free radicals or by capturing them so free radicals will not react with cellular components and create free radical stability in the body (Winarsi,2011; Parwata,2016).

Vitamins, macro elements, and microelements can act as antioxidants, protect against damaged DNA, prevent lipid peroxidation and inhibit free radicals chain reactions. For example, the antioxidants in Moringa leaves work by neutralizing free radicals to prevent oxidative damage to most biomolecules and provide significant protection against oxidative damage (Cahyani,2017).

In the P2 group, the administration of moringa leaf extract dosage of 600 mg/kg BW showed a decrease in the results of the Johnsen score compared to the P1 and P3 groups. The reduction in Johnsen's score in the P2 group could be due to the absence of a pre-test on the mice, so it was not known how the quality of the mice was before the study was carried out. Some limitations in research with the posttest-only design type include not being able to assess the degree of function of a sample group before and after treatment, and the degree of change between before and after treatment cannot be determined (Baldwin,2018). Another possibility is the occurrence of hormonal fluctuations in mice during the study, which stress conditions may cause during treatment.

In a study by Moodley (2017), the administration of Moringa leaf extract at a dose of more than 2000 mg/kg BW in mice showed no change in clinical signs or histopathological features (Moodley,2017). In addition, in research conducted by Widowati, it was stated that giving Moringa leaf extract at a dose of More than 4000 mg/kg BW in Wistar rats are included in the practically non-toxic (PNT) material group so that they still show normal conditions in liver and kidney function (Widowati,2014). According to the research that has been done, the dosage of Moringa leaf extract can cause death by up to 50% (LD50) in mice is a dose of 6616.67 mg/kg BW (Osman,2015). So that the dosage of moringa leaf extract of 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW that has been done in this study is a dose that is still safe for consumption.

The research found that Moringa leaves contain antioxidants that can break the free radical chain caused by exposure to monosodium glutamate. So, it can be concluded that Moringa leaves protect the histopathological testicular tissue of mice exposed to monosodium glutamate.

CONCLUSION

There is an effect of Moringa leaf extract on the histopathological picture of the testes of mice exposed to monosodium glutamate. The result of Moringa leaf extract can provide a protective effect on exposure to monosodium glutamate in the provision of Moringa leaf extract at doses of 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW. Furthermore, the flavonoids in Moringa leaf extract can provide histopathological protection of the testes of male mice compared to the group given monosodium glutamate.

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