THE EFFECT OF MORINGA LEAF EXTRACT (MORINGA OLEIFERA L) AGAINST MOTILITY OF SPERMATOZOA MICE EXPOSED TO MONOSODIUM GLUTAMATE

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ABSTRACT

Objective: *Excessive use of MSG in everyday life can cause infertility to men. Exposure to MSG can cause cells to experience oxidative stress which subsequently triggers the generation of free radicals. Free radicals can be resisted with antioxidants.* ***Material & Methods:*** *This research was a laboratory experimental test with a post-test only control group design using male mice as the experimental object. The statistical test used the One Way ANOVA test.* ***Results:*** *In this research, 5 male mice were assigned into 4 groups and 1 control group. Using the One Way ANOVA test, it was found that there were significant differences in ≥ 2 treatment groups in the motility group of spermatozoa with active movement ($p < 0.001$) and the motility group of spermatozoa with weak movement ($p = 0.036$). The two groups showed significant differences. The Post hoc LSD test in the motility group with active movement showed significant differences between the control group with MSG treatment ($p < 0.001$) and the treatment group given Moringa leaves extract of 600 mg/kgW ($p < 0.001$) and 1200 mg/kgW ($p < 0.001$).* ***Conclusion:*** *Moringa leaves extract can effect the improvement of the motility of spermatozoa exposed to monosodium glutamate at a given dose of 300 mg/kgW and 600 mg/kgW.*

Keywords: *Spermatozoa motility, moringa leaves extract, monosodium glutamate, free radical.*

ABSTRAK

Tujuan: *Paparan MSG dapat menyebabkan sel mengalami kondisi stress oksidatif. Stress oksidatif memicu timbulnya radikal bebas. Radikal bebas dapat ditangkal dengan antioksidan. Daun Kelor (Moringa oleifera L) merupakan antioksidan eksogen yang didalamnya terdapat flavonoid.* ***Bahan & Cara:*** *Penelitian ini merupakan uji eksperimental laboratorik dengan rancangan post test only control group design yang menggunakan mencit jantan sebagai objek percobaan. Uji statistik menggunakan uji One Way ANOVA.* ***Hasil:*** *Pada penelitian ini menggunakan 5 ekor mencit jantan yang dibagi menjadi 4 kelompok dan 1 kelompok kontrol dengan menggunakan uji One Way ANOVA didapatkan perbedaan signifikan pada ≥ 2 kelompok perlakuan dalam kelompok motilitas spermatozoa dengan pergerakan aktif ($p < 0.001$) dan kelompok motilitas spermatozoa dengan pergerakan lemah ($p = 0.036$). Pada kedua kelompok menunjukkan perbedaan signifikan. Uji post hoc LSD pada kelompok motilitas dengan pergerakan aktif menunjukkan perbedaan signifikan antara kelompok perlakuan Kontrol dengan perlakuan MSG ($p < 0.001$) dan perlakuan yang diberi ekstrak daun kelor 600 mg/kgBB ($p < 0.001$) dan 1200 mL/kgBB ($p < 0.001$).* ***Simpulan:*** *Ekstrak daun kelor memiliki pengaruh terhadap perbaikan motilitas spermatozoa yang dipapar monosodium glutamat pada dosis pemberian 300 mg/kgBB dan 600 mg/kgBB.*

Kata Kunci: *Motilitas spermatozoa, ekstrak daun kelor, monosodium glutamat, radikal bebas.*

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INTRODUCTION

The process of making food at this time cannot be separated from the presence of additives. One food additive that is often used is MSG.[1] In terms of the reproductive system, excessive use of MSG can lead to infertility in a man. Infertility can occur because exposure to MSG can trigger free radicals.[2-3] Increased oxidative stress can cause interference with spermatogenesis. Spermatogenesis disorders itself can affect the products from the sperm produced. Poor quality sperm is one of the

factors causing a person to experience infertility.[4]

Fertility assessment can be measured, one of the indicators is sperm quality. Assessment of sperm quality can be seen both macroscopically including PH, coagulation, color, viscosity, odor, and volume of semen and microscopic which includes morphology, concentration, viability, and motility. Motility is the ability of the spermatozoa to move. The term motility is applied to things like the movement of the sperm's tail which allows it to swim.[5] Poor sperm motility can affect a person's fertility. Various internal and external factors can affect the quality of spermatozoa. Internal factors, for example, hormones, hormones are a major part of regulating sexual function.[5] In addition, both acute and chronic diseases of the testes in the form of a blockage or absence of the vas deferens can cause azoospermia (no sperm formation).[6] While external factors such as food, foods rich in high lipid content can affect the hormone estradiol which will affect the production of the hormone testosterone directly affecting sperm quality.[7] Apart from that, another external factor is exposure to chemicals. The use of large amounts of chemicals can increase the disruption of spermatogenesis.[8]

Research previously found that administration of MSG orally can affect the fertility level of a man with a decrease in sperm quality such as decrease in sperm motility, normal concentration, and decrease the percentage of normal sperm morphology.[9] MSG contains reactive oxygen compounds or Reactive Oxygen Species (ROS), which are free radicals that cause cell and tissue damage.[4] A free radical can damage spermatozoa DNA and increase apoptosis (cell death).[10] Based on journals from the ministry of health, the average Indonesian consumes around 0.6 g/kgW of MSG.[1] Where if MSG is consumed by people who do not tolerate more than 3 g/day will cause adverse health effects.[11]

Moringa leaves which have the Latin name Moringa oleifera L are known to have a role as a medicinal plant in the medical world. Based on the research that has been done previously that Moringa leaves contain exogenous antioxidants, one of which is flavonoids.[12] Flavonoids are a group that has antioxidant power 4-5 times greater than vitamin C and vitamin E.[13] Flavonoids are believed to be an antidote to free radicals and are contained in Moringa leaves.[14]

OBJECTIVE

Knowing the effect of leaf extract Moringa oleifera L on the sperm motility of mice given monosodium glutamate.

MATERIAL & METHODS

This research is an experimental test with a post-test only control group design that uses 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. The research period is from June to July. Before being given the treatment, 35 male mice were adapted to be penned and given standard feed and drink for one week. Then 35 mice were divided into five groups, each of which consisted of seven male mice that were randomly selected and randomized.

After that, the mice were given treatment in the form of monosodium glutamate using a gastric swab given orally at a dose of 0.02 g/KgBW per day for 30 days. Moringa leaf extract was also administered using the gastric swab method for 30 days, dividing the treatment groups. After 30 days of treatment, termination was carried out by inserting the mice into a jar that had been given a cotton swab containing liquid chloroform/ether so that the mice were not aware after that the mice were killed by means of cervical dislocation. After that, the mice sperm were taken and made preparations.

After the mice were turned off per treatment group, the mice were surgically removed and the 2 cm cauda epididymis was taken into a petri dish given physiological NaCl solution, then massage using a spatula on 2 cm cauda epididymis to get the required sperm and stirred so that it becomes homogeneous so that it makes it easier to examine the sperm cells that have come out of the epididymis are taken using a pipette then placed in a glass object and covered with a glass deck. After that, it is observed under a microscope with a magnification of 100x or 400x. Then seen using a spermatozoa motility microscope using 3 fields of view. Observe what percentage of the motility category is active/straight, moving in place, and not moving. Then record it on the observation sheet.

RESULTS

This study was conducted to determine the effect of Moringa leaf extract on sperm motility of male mice exposed to monosodium glutamate. This research was conducted for 30 days using a sample of 35 male mice aged 6 weeks, weighing 25-30 g, healthy and without anatomical defects which were divided into 5 groups. Research and maintenance of mice were carried out in the laboratory Biology Faculty of Natural Science, Semarang State University. After dividing the groups, then the adaptation was carried out for 7 days for each group. After the adaptation was carried out, the research was carried out on male mice samples according to the grooves. During this time, 3 mice died. However, of the remaining 32 mice, only 25 were used for sperm motility analysis.

Table 1 shows a description of the median and motility range of the spermatozoa. The calculation of the percentage of spermatozoa with active movement showed that the control group had the highest mean motility and the MSG + Moringa leaf extract at a dose of 1200 mg/kgbb had the lowest mean percentage of motility. The percentage of weak movement spermatozoa showed that the MSG group + moringa leaf extract 300 mg/kgbb and the MSG + moringa leaf extract group 600 mg/kgBW and the MSG + moringa leaf extract group of 1200 mg/kgbb had the lowest mean percentage of motility. The calculation of the percentage of spermatozoa without movement showed that the MSG + Moringa leaf extract group of 1200 mg/kgbb had the highest mean percentage of spermatozoa motility and the group giving MSG + Moringa leaf extract 300 mg/kgbb had the lowest mean percentage of motility.

Table 1 shows the results of the spermatozoa normality test using the Shapiro - Wilk test on active movement, weak movement, and no movement. It was found that inactive movement and weak movement, data were normally distributed ($p > 0.05$) and in the group without movement, data were not normally distributed ($p < 0.05$). Therefore, the next step is to test the hypothesis in the form of the One Way Anova test with post hoc LSD follow-up test. Post hoc LSD test has been carried out in the analysis of further data on spermatozoa motility with active movement. In the analysis, it was found that there was a significant difference between two or more groups ($p < 0.05$). There were significant differences in the following groups:

1) Control Group with MSG Group
2) MSG group with group 300 mg/kgW
3) Control group with group 600 mg/kgW
4) MSG group with group 600 mg/kgW
5) Group 300 mg / KgBB with 600 mg/kgW
6) Control Group with 1200 mg/kgW
7) Group 300 mg / KgBB with 1200 mg/kgW
8) Group 600 mg / KgBW with group 1200 mg/kgW

Post hoc LSD test has been carried out for further data analysis of spermatozoa motility with weak movement. In the analysis, it was found that there was a significant difference between two or more groups ($p<0.05$). There were significant differences in the following groups:

1) MSG group with group 600 mg/kgW
2) MSG group with group 1200 mg/kgW

DISCUSSION

The ethanol extract of 70% Moringa leaves was made by the maceration method. The solvent used in maceration is 70% ethanol which aims to attract all the chemical components in Moringa leaves because ethanol is a universal solvent that can

Table 1. Description of Mean Percentage of Spermatozoa Motility

Group	N	Progressive Movement (%)	Non-Progressive Movement (%)
Kontrol	5	44.67 (3.80)	32.67 (4.94)
MSG	5	11.33 (7.67)	22.00 (13.25)
MSG + 300 mg/kgW Moringa leaf extract	5	41.34 (5.06)	38.00 (3.80)
MSG + 600 mg/kgW Moringa leaf extract	5	26.67 (4.08)	36.67 (4.08)
MSG + 1200 mg/kgW Moringa leaf extract	5	5.33 (3.80)	27.33 (10.91)

attract compounds that are soluble in non-polar to polar solvents.[15] Moringa leaves contain antioxidant compounds including tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids, also contain vitamin C. Flavonoids are generally more soluble in water or polar solvents because they have sugar group bonds. Flavonoids in the form of the active compound can be extracted with 70% ethanol.[16]

In this study, spermatozoa motility was assessed by dividing them into three groups: active, weak, immobile. In this study, it was found that in the presentation of spermatozoa motility data with active movement and weak movement the treatment group was given Moringa leaf extract at a dose of 1200 mg/kgW per day and given MSG had the lowest average percentage among other treatment groups, but in the presentation of motility data spermatozoa without movement, it was found that the MSG treatment group had the highest percentage among other treatment groups.

In the motility of spermatozoa with active movement, it was found that there was a significant difference between the group that was only exposed to MSG and the group that was exposed to MSG and additional Moringa leaf extract with multilevel dose. Further analysis of active movement using the test post hoc LSD showed that the dosage of Moringa leaves 300 mg/kgW and 600 mg/kgW had a more protective effect on the sperm of male mice exposed to MSG than other doses. In assessing the motility of spermatozoa with weak movement, it was also found that there were significant differences between groups exposed to MSG and additional Moringa leaf extract with stratified doses. Where the analysis results in the number 0.036 is said to be significant data because $p < 0.05$. Further analysis of weak movements using the test post hoc LSD showed that the dosage of Moringa leaves 300 mg/kgW had a more protective effect on the sperm of male mice exposed to MSG compared to other doses.

The research that has been done shows that MSG affects decreasing spermatozoa motility in male mice. Monosodium glutamate causes spermatogenesis disorders.[3] Mechanisms that are anti-fertility, namely prestetiscular, testicular and post-testicular. Spermatogenesis disorders through the testicular mechanism are cytotoxic. Excessive use of MSG can become free radicals and cause a state of oxidative stress. This is characterized by a decrease in testicular weight and an increase in lipid peroxidation levels with the formation of lipid peroxidase levels with the formation of aldehyde compounds, especially MDA and a decrease in testicular ascorbic acid. oxidative stress.[4]

Prolonged ascorbic acid deficiency can lead to a low spermatozoa count, an increase in the number of abnormal spermatozoa and a decrease in sperm motility.[17-18] This is because MSG triggers the emergence of free radicals which are the effect of decreasing ascorbic acid levels in the testes and subsequently causing oxidative stress.[4] Oxidative stress results in lipid peroxidation reactions and the damaged cell membrane organization will reduce the frequency of sperm tail movement. In this study, the results are in line with the findings of a study conducted by Kadir (2011), that the oral administration of MSG at a dose of 250 mg/day, 500 mg/day, and 1 g/day for 15 days, the sperm quality examination showed a decrease in sperm motility.[19] This is due to the ROS mechanism due to decreased antioxidants in the body.

Giving Moringa leaf extract can improve the motility of spermatozoa due to MSG exposure. Because there are differences in results with the control group. The study, it showed that the administration of Moringa leaf extract at a dose of 300 mg/kgW and 600 mg/kgW affected the mean spermatozoa motility of male mice. This result is in line with previous research on the use of Moringa leaves to improve the quality of spermatozoa due to chemical exposure.[20]

Moringa leaves contain many antioxidant compounds that play a role in warding off ROS including tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids, also contain vitamin C.[21] The antioxidant compounds found in Moringa leaves can reduce lipid peroxidation and prevent chain reactions by maintaining antioxidant enzymes such as zeatin, catalase, superoxide dismutase (SOD), and glutathione peroxide.[22] The chain reaction will inhibit the formation of new free radicals to create free radical stability in the body.[22] The antioxidants present in moringa prevent the formation of free radicals so that free radicals are created in the body and their effect on spermatozoa cells can produce ATP which is needed for spermatozoa motility.[23]

At a dose of 1200 mg/kgW Moringa extract showed a decrease in the mean motility of spermatozoa. The decrease in the mean spermatozoa motility of mice is thought to be due to the large

amount of flavonoid compounds that can be estrogenic because they can stimulate the formation of estrogen in the body. High levels of estrogen will provide negative feedback to the anterior pituitary by not releasing FSH and LH.[24] The drop in LH levels leads to impaired secretion of testosterone by Leydig cells. This affects the maturation of spermatozoa in the epididymis.[25]

Anti-fertility compounds from medicinal plants work in 2 ways, namely through cytotoxic effects and through hormonal effects. Moringa leaf ethanol extract is thought to have a cytotoxic effect that causes cell death. One of the mechanisms of apoptotic cell death (programmed cell death).[26] Classes of compounds that can be said to be anti-fertility agents are steroids, alkaloids, flavonoids, and tannins, the four of which are found in Moringa leaves.[27] The mechanism of action of the ethanol extract of Moringa leaves on decreasing sperm quality is unknown. However, some researchers report that spermatozoa immobility is caused by the content of compounds in plants such as cell death, damage to cell membranes and decreased ATP and chromatin damage.[28]

Flavonoids can inhibit the aromatase enzyme, which is an enzyme that catalyzes the conversion of androgens to estrogen which increases the hormone testosterone. Tannins are astringent which causes cell shrinkage, so that they can interfere with membrane permeability because the nutritional needs are disturbed resulting in the death of sperm. Alkaloid class compounds found in Moringa leaves can inhibit the secretion of the reproductive hormone, namely testosterone so that the spermatogenesis process is disrupted and can reduce spermatozoa production.[29]

So it can be concluded that moringa leaves improved sperm motility in mice exposed to monosodium glutamate.

CONCLUSION

This study concludes that there is an effect of Moringa leaf extract on the spermatozoa motility of mice exposed to monosodium glutamate. The effect of Moringa leaf extract can provide a protective effect on exposure to monosodium glutamate in the administration of Moringa leaf extract at a dose of 300 mg/kgW and 600 mg/kgW. Flavonoids contained in Moringa leaf extract can increase the motility of male mice spermatozoa compared to the group given monosodium glutamate.

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