EFFECTS OF RED FRUIT (*Pandanus conoideus Lam*) OIL ON MALONDIALDEHYDE LEVEL AND SPERMATOZOA QUALITY IN MICE (*Mus musculus*) EXPOSED TO MONOSODIUM GLUTAMATE

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh pemberian minyak buah merah (Pandanus conoideus Lam) terhadap kadar MDA dan kualitas spermatozoa pada mencit (Mus musculus) yang dipapar MSG. Kualitas meliputi motilitas, viabilitas, konsentrasi, dan morfologi spermatozoa. Penelitian dilakukan dengan desain studi eksperimental randomized post-test only control group. Subyek penelitian ini adalah mencit (Mus musculus) sejumlah 25 ekor, dibagi menjadi 5 (5 ekor mencit per kelompok). K-: Kelompok dengan pemberian distilled water selama 35 hari. K+: Kelompok dengan pemberian MSG 4 mg/g BB selama 21 hari. P1, P2, dan P3: Kelompok perlakuan pemberian MSG 4 mg/g BB selama 21 hari + minyak buah merah 0,02; 0,04; 0,08 ml/g BB pada hari ke 22-35. Hasil penelitian menunjukkan rerata morfologi spermatozoa kelompok K-, K+, P1, P2, P3 secara berurut-urut sebagai berikut: 0,86; 0,56; 0,67; 0,61; dan 0,87 (%). Konsentrasi spermatozoa secara berurut-urut sebagai berikut: 21; 10; 15; 32,8; dan 19 (107 sel/ml). Viabilitas spermatozoa secara berurut-urut sebagai berikut: 0,64; 0,14; 0,24; P2: 0,36; 0,68 (%). Kadar MDA secara berurut-urut sebagai berikut:0,29; 0,60; 0,35; 0,23; 0,19 (nm). Sebagai simpulan, kadar MDA testis yang dipapar MSG dan diberi minyak buah merah lebih rendah daripada kadar MDA mencit yang dipapar MSG dan tanpa diberi minyak buah merah. Kualitas spermatozoa pada mencit yang dipapar MSG dan diberi minyak buah merah lebih tinggi daripada mencit yang dipapar MSG dan tanpa diberi minyak buah merah. ***(FMI 2018;54:84-88)***

Kata kunci*: Minyak buah merah; MSG; spermatozoa; kadar MDA; kualitas spermatozoa*

ABSTRACT

This study aimed to determine the effects of red fruit (Pandanus conoideus Lam) oil on MDA levels and spermatozoa quality in mice (Mus musculus) exposed to MSG. The quality includes motility, viability, concentration, and morphology of spermatozoa. This experimental study used randomized post-test only control group design. The subjects of this study were 25 mice (Mus musculus), divided into 5 groups (5 mice per group). K- group received distilled water for 35 days. K+ group received 4 mg/g BW MSG for 21 days. P1, P2, and P3 treatment groups received 4 mg/g BW MSG for 21 days and 0.02; 0.04; 0.08 ml/g BW red fruit oil, respectively, from day 22 to 35. The results showed that mean spermatozoa morphology in K-, K+, P1, P2, P3 groups were as follows: 0.86; 0.56; 0.67; 0.61; and 0.87 (%). The spermatozoa concentrations were sequentially as follows: 21; 10; 15; 32,8,19 (107 cells/ml). The spermatozoa's vitalities were as follows: 0,64; 0,14; 0,24; P2: 0.36; 0.68 (%). MDA levels were respectively: 0.29; 0.60; 0.35; 0.23; and 0.19 (nm). As a conclusion, testicular MDA levels in mice exposed to MSG and given with red fruit oil were lower than those in mice exposed to MSG without receiving red fruit oil. The quality of spermatozoa in mice exposed to MSG and receiving red fruit oil was higher than that of mice exposed to MSG without being given with red fruit oil. ***(FMI 2018;54:84-88)***

Keywords*: Red fruit oil; MSG; spermatozoa; MDA levels; quality of spermatozoa*

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INTRODUCTION

Infertility is one of the problems of reproductive health. The prevalence of infertility is also increasing, and not only can be experienced by women, but also by men. Male factor is the major cause of all cases of infertility (Fritz & Speroff 2011). Lifestyle changes and technological advances make individuals increasingly consume fast food. Finally, they depend on food preserved using chemicals, such as flavoring, which mostly consists of monosodium glutamate (MSG). Monosodium glutamate (MSG) is one of the cytotoxic substances that will be metabolized by the body and react to form free radicals. One of its effects is that it can be cytotoxic to male reproductive system which can lead to decreased quality of spermatozoa and increased levels of testicular malondialdehyde (MDA) (Hayati 2011). Radical compounds (Reactive Oxygen Species, ROS) produced from metabolism of toxic substances can decrease the availability of antioxidant reserves of the body and oxidative stress (Birben et al 2012). The condition of the body experiencing oxidative stress

causes the antioxidants not able to prevent free radicals optimally, increases the formation of ROS and decreases the levels of endogenous antioxidants, resulting in disruption in the cycle of spermatozoa formation that affects its quality (Aitken 2008).

Red fruit (*Pandanus conoideus Lam*) is one of Indonesia's native plants which contains much antioxidants, such as carotene and tocopherol. Until now, there has been no study on the effects of red fruit oil on MDA levels and quality of spermatozoa in mice exposed to MSG. This study aimed to prove the difference of MDA levels and spermatozoa quality in mice receiving MSG and red fruit oil. This study aimed to determine the effect of red fruit oil (*Pandanus conoideus Lam*) on MDA levels and spermatozoa quality in mice (*Mus musculus*) exposed to MSG. The quality includes the viability, concentration, and morphology of spermatozoa.

MATERIALS AND METHODS

This was an experimental study using post-test only group design. Experimental animal were 25 mice (*Mus musculus*), divided into 5 groups (5 mice per group). The negative control group (K-) was given with distilled water for 35 days, the positive control group (K+) was given with 4 mg/g BW MSG for 21 days, and the treatment groups (P1, P2, P3) were given with 4 mg/g BW MSG for 21 days and 0.02 ml/g BW; 0.04 ml/g BW; 0.08 ml/g BW red fruit oil on day 22-35.

This study was conducted at Laboratory of Experimental Animal and Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The study was conducted in September, 2016. At the end of the study, all mice were sacrificed, the epididymis and vas deferns were taken for spermatozoa quality (viability, morphology, and concentration) examination and then examined by light microscope and taken for examination of testicular MDA by spectrophotometer to observe the MDA levels.

Data obtained were MDA levels and spermatozoa quality of the mice in each treatment group. The normality distribution of the data was tested using Kolmogorov-Smirnov test. To observe the homogeneity of variance, the homogeneity test was performed. If the variation was homogeneous, one-way ANOVA test was conducted. To observe the mean and differences between pair treatments, LSD (Least Significant Difference) test was carried out. If abnormal data was obtained, Kruskal Wallis test was done. To observe the differences, the Mann-Whitney test was conducted.

RESULTS

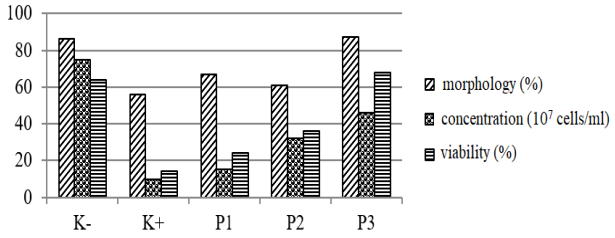

Fig. 1. Spermatozoa quality of mice in control and treatment groups. Note: K-: administration of distilled water, K+: 4 mg/g BW MSG, P1: 4 mg/g BW MSG + 0.02 ml/g BW red fruit oil, P2: 4 mg/g BW MSG + 0.04 ml/g BW red fruit oil, and P3: 4 mg/g BW MSG + 0.08 ml/g BW red fruit oil.

Fig. 1 shows that the morphology of spermatozoa in positive control group (K+) and treatment group (P1 and P2) decreases compared to negative control group (K-). The morphology of normal spermatozoa in treatment group (P3) increases compared to negative control group (K-). Spermatozoa concentration in group K+ decreases compared to group K-. Spermatozoa concentrations in the three treatment groups (P1, P2, and P3) decrease compared to the K-group, but increase compared to the (K+) group. The viability of spermatozoa in the positive control group (K+) decreases compared to the negative control group (K-). The viability of spermatozoa in P1 and P2 decreases compared to negative control group (K-), but P3 increases compared to the positive control group (K+).

The results of Shapiro Wilks analysis showed that normal morphology of mice had normal data distribution and homogeneous data variance ($p>0.05$). The results of One-Way Anova test showed p value of 0.001, so there was significant differences in normal spermatozoa morphology between groups K-, K +, P1, P2, and P3. The results of Krusskal-Wallis test showed p value of 0.00, indicating that there was significant difference of spermatozoa concentration between groups K-, K+, P1, P2, and P3. Mann-Whitney test showed that the difference of spermatozoa concentrations was significant with $p<0.05$ in K- group with K+, P1, P2, and P3 groups as well as K+ group with P2 and P3 groups, group P1 with group P3, and group P2 with group P3. The results of Krusskal-Wallis test showed p value of 0.001, indicating that there was significant difference in spermatozoa viability. Analysis of spermatozoa concentration data by using Mann-Whitney test showed that the difference in spermatozoa viability was significant with $p<0.05$ in K- group with K+, P1, and P2 groups. group K + with P2, P3, group

P1 with P2, and group P2 with P3. Viable and non-viable spermatozoa, as well as normal and abnormal morphology are presented in Fig. 2.

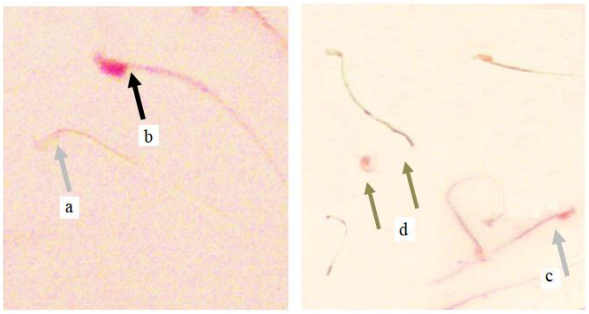

Fig. 2. Viable (a) and non-viable (b) spermatozoa, and spermatozoa with normal (c) and abnormal (d) morphology.

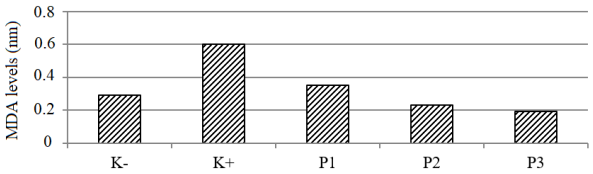

Fig. 3. Mean MDA (nm) of mice spermatozoa in control and treatment groups. Note: K-: distilled water, K+: 4 mg/g BW MSG, P1: 4 mg/g BW MSG + 0.02 ml/g BW red fruit oil, P2: 4 mg/g BW MSG + 0.04 ml/g BW red fruit oil, P3: 4 mg/g BW MSG + 0.08 ml/g BW red fruit oil.

MDA level of the testes in K+ group increased compared to that in K- group. Testicular MDA levels in the three treatment groups (P1, P2, and P3) decreased when compared to K+ group. This means that the administration of red fruit oil can decrease the level of testicular MDA levels exposed to MSG. The results of Kruskal-Wallis test showed that $p<0.05$, indicating that there was significant difference of testicular MDA level between K-, K+, P1, P2, and P3 groups. Analysis of MDA data by using Mann-Whitney test showed that testicular MDA levels between groups were significantly different with $p<0.05$ in K- group with K+ and P3 groups, K+ group with P1, P2, P3 groups, and P1 group with P3 group.

DISCUSSION

The results of this study indicated that the level of testicular MDA in positive control group increased compared to that in negative control group. Analysis results showed that there were significant differences. Malondialdehyde (MDA) is one of the highly reactive products due to ROS reaction to unsaturated lipids spermatozoa cell membranes which cause the formation of lipid peroxide, i.e. malondialdehyde (MDA). Malondialdehyde (MDA) is highly toxic and contributes to the damage of spermatozoa. Lipid peroxidation reactions result in increased membrane fluidity, impaired membrane integrity and inactivation of membrane bonds with enzymes and receptors (Sudatri et al 2011). MSG exposure can stimulate lipid peroxidation in the testes, indicating that there has been a state of oxidative stress due to ROS formation and decreased antioxidant defense systems.

The administration of 0.02 ml/g BW, 0.04 ml/g BW, and 0.08 ml/g BW red fruit oil was able to decrease testicular MDA level of mice exposed to MSG when compared to the positive control group. There was a significant difference between the three treatment groups and negative control group. Decreased levels of malondialdehyde (MDA) of the testes was due to the antioxidant content found in red fruit oil which are betacarotene and tocopherol. Red fruit has high content of antioxidants, consisting of carotenoids and tocopherol, as well as unsaturated fatty acids.

The results of this study indicated that there were morphological, viability, and spermatozoa concentration differences between negative control group (K-) and positive control group (K+) exposed to MSG. The K+ group had lower morphology, viability, and spermatozoa concentrations compared with the K- group. MSG has a toxic effect on the male reproductive system that can decrease morphology, viability, and spermatozoa concentration. The effect is toxic because MSG has the capacity to produce ROS and decrease reserves of endogenous antioxidants. This condition results in a state of oxidative stress. The state of oxidative stress can occur at central and testicular levels. At the central level of the hypothalamic pituitary axis, MSG can block the secretion of norepinephrine, suppressing GnRH secretion. Decreased GnRH secretion may interfere with the spermatogenesis process because the levels of the hormone FSH and LH decrease, thus affecting the quantity and quality of spermatozoa produced by the testes.

This study showed that the administration of 4 mg/kg BW MSG for 21 days could increase testicular MDA levels. Another study conducted by Dorostghoal et al (2013), in which 0.1% MSG was given through drinking water for 21 days, showed a significant increase in MDA levels. Radical H2O2 will be formed in peroxisome as a product of ß-oxidation fatty acids. This radical will be neutralized by a catalase that is widely available on peroxisomes so that under normal circumstances it is unlikely that a leak will occur. The produc-

tion of peroxisome radicals can cause oxidative stress, especially in active proliferation (Dröge 2002).

Glutamate is an important neurotransmitter for communication between neurons. Glutamate triggers the NMDA receptor with the effect of opening of the receptor, resulting in the opening of Ca2+ ion canal. The incoming calcium ions will activate enzymes, such as proteases, lipases and endonuclease that can affect the phospholipid that composes the membrane of the cell (Kumar et al 2010). This process is accompanied by the release of radical superoxide (O2*). By superoxide dismutase (SOD), it will be converted to H2O2. In the presence of Fe2+, through the fenton reaction, hydroxy radical (OH*) will be formed, and end with lipid peroxidation, protein peroxidation and DNA damage that causes membrane peroxidation and cell death due to necrosis. When the glutamate level becomes excessive, the Ca2+ channel will remain open so that the chemical reactions that occur will also increase, causing the initiation of cellular damage (Gao et al 2008).

Mechanism of free radical formation begins when MSG is absorbed by the body through inhalation and then into the blood circulation system. If the amount of free radicals or ROS in the body exceeds the endogenous antioxidant capability of glutathione peroxidase, catalase, and superoxide dismutase, the body has no defense mechanisms against oxidative stress (Werdhasari 2014).

Red fruit is one source of natural antioxidants. Antioxidants can provide protection against the negative effects of free radicals that result in severe physical activity. The antioxidant contained in the red fruit has the potential to break the chain of free radicals, decreases strength, and binds heavy metal activity (Rohman et al 2010). Tocopherol is the most potent nonenzymatic antioxidant in the body. The main function of tocopherol is as a chain breaker in PUFA and prevents the propagation reaction of free radicals. Tocopherol is a lipid-radical feeder and in particular binds to PUFA in phospholipid membranes and plasma lipoproteins (Viitala et al 2004). The results of this study showed that red fruit oil in a dose of 0.01 ml/g BW (P3) could be used as a therapy to improve morphology, viability and spermatozoa concentration of mice exposed to MSG.

The mechanism of action of carotenoids as an antioxidant is to extinguish singlet oxygen and then interact with free radicals (Kusmita & Limantara 2008) in three major pathways called electron transfer, hydrogen abstraction, and the addition of radical species. These three reactions cause the charge or free radical does not disappear, so that in a complete reaction one or more molecules remain in a radical state. The beta-carotene radicals formed in the reaction are relatively stable and do not have enough energy to react with other molecules to form new radicals.

In addition to carotenoids, red fruit also contains tocopherol. Tocopherol is a vitamin E compound with a saturated phytyl chain. Tocopherol serves to maintain membrane integrity by working as a carrier of oxygen free radicals, lipid peroxides and singlet oxygen, also protecting oil and carotenoids within the oil from oxidation (Winarsi 2007). Tocopherol (vitamin E) has the ability to stop lipid peroxide by donating one of its hydrogen atoms from the OH group to the radical lipid peroxide, making it less reactive and less damaging (Hariyatmi 2004).

CONCLUSION

MDA levels in MSG-exposed mice receiving red fruit oil was lower than those in mice exposed to MSG without receiving red fruit oil. The quality of spermatozoa in mice exposed to MSG and receiving red fruit oil was higher than that in mice exposed to MSG but without receiving red fruit oil.

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