EFFECTS OF LYCOPENE ON SPERMATOZOA MORPHOLOGY IN Balb/C MICE EXPOSED TO 2-METHOXYETHANOL (2-ME)

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ABSTRAK

Senyawa 2-methoxyethanol (2-ME) adalah salah satu bahan toksik yang berpotensi merusak organ reproduksi jantan. Senyawa ini dapat masuk ke dalam tubuh melalui berbagai cara yaitu kontak langsung melalui kulit, pernafasan dan pencernaan. Senyawa ini dapat menyebabkan stress oksidasi pada spermatozoa yang merupakan penyebab utama disfungsi spermatozoa. Likopen dikenal juga sebagai pigmen merah yang merupakan antioksidan golongan karoten. Likopen mempunyai aktivitas antioksidan dua kali lebih kuat dibandingkan beta karoten dan sepuluh kali lipat lebih kuat dibandingkan vitamin E. Sehingga, reaksi likopen sebagai antioksidan di dalam tubuh lebih baik daripada vitamin A, C, E, maupun mineral lainnya. Penelitian ini dilakukan untuk mengetahui pengaruh likopen terhadap morfologi spermatozoa mencit yang terpapar 2-ME. Penelitian dilakukan pada 30 ekor mencit, dibagi dalam 5 kelompok. Kelompok K- adalah kelompok kontrol tanpa pemberian 2-Methoxyethanol dan likopen, kelompok K+ adalah kelompok dengan pemberian 2-Methoxyethanol 200 mg/KgBB hari 1-5, dan kelompok P1, P2 dan P3 adalah kelompok-kelompok dengan pemberian 2-Methoxyethanol 200 mg/KgBB hari 1-5 dan likopen dosis (5, 10 dan 20 mg/kgBB) hari 6-35. 2-ME diberikan secara intraperitoneal dan likopen diberikan personde. Pada hari ke 36, mencit dikorbankan dan diambil epididimis dan vas deferen untuk pemeriksaan morfologi spermatozoa. Pengamatan dilakukan menggunakan mikroskop dengan pembesaran 1000x. Hasil penelitian menunjukkan ada perbedaan signifikan. Pada morfologi spermatozoa, pemberian likopen meningkatkan persentase morfologi normal spermatozoa mencit yang terpapar 2-ME. ***(FMI 2017;53:264-266)***

Kata kunci*: 2-Methoxyethanol; likopen; morfologi spermatozoa*

ABSTRACT

The compound of 2-methoxyethanol (2-ME) is one of toxic materials that potentially damage male reproductive organs. This compound can enter the body by various means of direct contact through the skin, respiratory and digestive tract. This compound can cause oxidative stress in spermatozoa which is the main cause of spermatozoa dysfunction. Lycopene, also known as red pigment, is an antioxidant class of carotene. Lycopene has antioxidant activity twice stronger than beta carotene and ten times stronger than vitamin E. Thus, the lycopene reaction as an antioxidant in the body is better than vitamin A, C, E, and other minerals. This study was conducted to determine the effects of lycopene on spermatozoa morphology in mice exposed to 2-ME. The study was conducted on 30 mice, divided into 5 groups. The K-group was the control group without 2-methoxyethanol and lycopene, the K + group was the group with 200 mg/kg BW 2-methoxyethanol on days 1-5, and the P1, P2 and P3 groups were the ones with 200 mg/kg BW 2-methoxyethanol on days 1-5 and lycopene doses of 5, 10 and 20 mg/kg BW on days 6-35. 2-ME was given intraperito-neally and lycopene was given per sonde. On day 36, the mice were sacrificed and the epididymis and vas deferens were removed for morphological examination of spermatozoa. The observations were performed using a microscope with 1000x magnification. The results showed that there was significant difference. In the morphology of spermatozoa, the administration of lycopene increases normal morphological percentage of spermatozoa in mice exposed to 2-ME. ***(FMI 2017;53:264-266)***

Keywords*: 2-methoxyethanol; lycopene; morphology of spermatozoa*

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INTRODUCTION

Infertility is one of the problems that has become a concern in the society today. There are so many married couples who still need the presence of children in their life because of infertility of one of the couple. Infertility occurs more than 20% of population in Indonesia. From those cases, 40% is in women, 40% is in men and 20% is in both. The cause of male infertility is influenced by many factors, among them is the existence of free radicals (Winarsi 2011). 2-methoxyethanol (2-ME) is one of the metabolites of dimethoxy ethylphthalate (DMEP). DMEP is one of the derivatives of phthalic acid ester (PAE) which is widely used as a plasticizer in plastic making. DMEP compounds that enter the human body will be hydrolyzed into 2-methoxyethanol (2-ME) which is then oxidized by alcohol dehydrogenase to 2-methoxyacetaldehyde (MALD), then converted to meth-

oxyacetic acid (MAA) by aldehydes dehydrogena-se. MAA compounds are toxic and teratogenic (Moslen et al 1995).

This compound is widely used as water-based organic solvent for industrial and household appliances (Starek et al 2010). The 2-ME compound is not found naturally in environment because its presence in nature is the result of industrial and factory activity. The 2-ME compounds may cause decreased motility and morphology of spermatozoa (Hayati et al 2004). The 2-methoxyethanol (2-ME) compound is one of the toxic materials that potentially damage male reproductive organs. This compound can enter the body by various means of direct contact through the skin, respiratory and digestion (Hayati 2007). The accumulation of toxic materials in the environment occurs as industrial and factory increases. The accumulated toxic substances can disrupt human health with various metabolic barriers. 2-ME exposure of 200 mg/kg BW causes damage to seminiferous tubules, decreases spermatogonium and spermatid levels and can damage spermatozoa (Hayati et al 2004).

MATERIALS AND METHODS

This study used 30 mice (*Mus musculus*), divided into 5 groups (6 mice per group). Group K- was a control group without 2-ME and lycopene, K+ group was a group with 200 mg/kg BW 2-ME at day 1-5, group P1 was treated with 200 mg/kg BW 2-ME on days 1-5 + 5 mg/kg BW lycopene on days 6-35, group P2 was a treat-ment group with 200 mg/kg BW 2-ME day 1-5 + 10 mg/kg BW lycopene on days 6-35, and group P3 was a treatment group with 200 mg/kg BW 2-ME day 1-5 + 20 mg/kg BW lycopene on days 6-35. At the end of the study, all mice were sacrificed. Their epididymis and vas deferens organs were taken for morphological examination of spermatozoa. These organs were fused in a PBS solution, then put in CO_2 incubator for 15 minutes and stirred evenly. A smear on the object glass was made. It was emerged in the methanol for 5 minutes and then stained with safranin for 5 minutes, air dried and then washed by dyeing in buffer phosphate solution. Then, it was rinsed with buffer phosphate solution, immersed within crystal violet solution for 5 minutes, dried and rinsed with distilled water, dried again and observed under microscope in 1000 x magnification. The shaped spermatozoa was calculated based on the normality or abnormality until it was 100. The number of normal and abnormal spermatozoa was converted in percent (%).

The resulted data were tested using SPSS. Non parametric test results with One Sample Kolmogrov-Smirnov Test showed that the normal distribution data was Z>0.05. Furthermore, Multivariate Analyze of Variance test with p value<0.05 was performed. The variables with p<0.05 were tested using Post Hoc Fisher's LSD to analyze the significant variance between treatment groups.

RESULTS

The value of KP was lower than that of KN and the values of P1, P2 and P3 were higher than that of KP. Whereas, the values of P1, P2 and P3 were lower than that of KN. This means lycopene administration can improve the morphology of spermatozoa exposed to 2-ME.

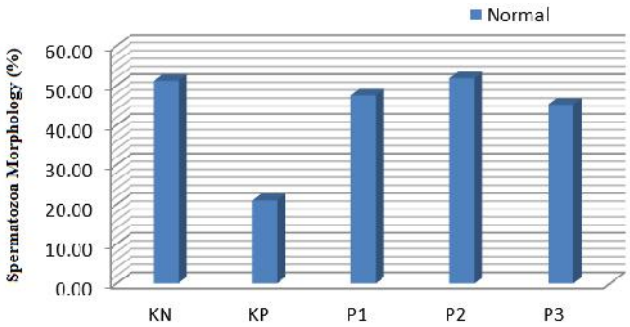

Fig. 1. Effects of lycopene administration on morphological improvement of mice spermatozoa exposed to 2-ME.

Based on Fig. 1, the administration of 10 mg/kg BW lycopene increases the percentage of normal morphology of spermatozoa. However, when the dose was lowered to 5 mg/kg BW and raised to 20 mg/kg BW, the normal percentage decreased, especially when the dose was increased to 20 mg/kg BW. The dose increased the abnormal rate of spermatozoa compared to control group.

Table 1. Mean morphology of mice spermatozoa exposed to 2-ME

Groups	Mean	SD	Min	Max
KN	51.16 [a]	10.64	34	63
KP	21 [b]	10.82	6	37
P1	47.50 [a]	7.06	37	55
P2	51.83 [a]	9.28	42	66
P3	45.16 [a]	6.46	34	52

Anova : p value=0.0001
Note : different superscripts show significantly different value of p<0.05

The value of KP was lower than that of KN and the values of P1, P2 and P3 were higher than KP. Whereas, the values of P1, P2 and P3 were lower than that of KN.

It means that the administration of lycopene may increase the morphology of spermatozoa exposed to 2-ME.

DISCUSSION

The morphology of spermatozoa may increase due to lycopene administration. This condition can occur because the antioxidants contained in lycopene prevent lipid peroxidation and protect the integrity of spermatozoa membrane. Lycopene is an efficient scavenger for single oxygen. When it captures single oxygen, energy is transferred from single oxygen to lycopene molecules, and transformed into tripled energy. Therefore, lycopene makes it possible to be used as a protection against lipid, protein, and DNA oxidation (Matos et al 2000).

The toxic effect occurs because 2-ME can produce ROS and decrease the reserve of endogenous antioxidants, causing a state of oxidative stress. The state of oxidative stress can occur at the central and testicular levels. At the central level, the hypothalamus pituitary axis, lead can block the secretion of norepinephrine, thus suppressing GnRH secretion. Decreased GnRH secretion may interfere with the spermatogenesis process because the levels of FSH and LH hormones decrease, thus affecting the quantity and quality of spermatozoa produced by the testes.

The high rate of disabilities in head, neck or tail that occurs in lycopene doses of 5 and 20 mg/kg BW indicates ineffective antioxidant action in neutralizing ROS. This occurence also raises a suggestion that these doses of lycopene are actually toxic and raise levels of ROS that cannot be tolerated by antioxidants which are physiologically present in spermatozoa. This is in line with statement about a negative correlation between ROS levels and the normal proportion of spermatozoa as well as a positive correlation with the number of spermatozoa with head defect (amorpous, acrosomic damage), mid piece defect, cyto-plasmic retention, tail defect, and sperm deformity index (SDI) scores (Darmawan 2007).

The increasing or decreasing percentage of normal and defective morphology of spermatozoa shows how the effectiveness of antioxidant action contained in lycopene during spermatogenesis process, especially in spermatozoa and spermatozoa maturation process. This condition can also be caused by 2-ME. This statement is supported by the results of previous research which states that exposure to 2-ME 200 mg/kg BW resulted in the destruction of seminiferous tubules, decreased spermatogonium and spermatid levels and damaged spermatozoa morphology (Hayati et al 2004).

CONCLUSION

In the morphology of spermatozoa, the administration of lycopene increases normal morphological percentage of spermatozoa in mice exposed to 2-ME.

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