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Article

Effect of Xanthone Administration on The Number of Sertoli Cells In Mice Induced By 2-Methoxyethanol

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A B S T R A C T

Antioxidants can be used to protect against oxidative stress which is one of the important mechanisms of 2-methoxyethanol (2-ME) -induced testicular toxicity . This research was conducted to find out normal and abnormal motility of spermatozoa cells in mice induced by 2- Methoxyethanol . The study used 35 male mice which were divided into 5 groups, namely: the control group (the mice were given purified water every day by distillation); the 2-ME group (the mice were given 2-ME 200 mg /kg BW daily orally once a day for 35 days); and the treatment group (the mice were given xanthones 60 mg , 120 mg , and 240 mg /kg BW orally once a day for 38 days, and on the 3rd day, 2-ME 200 mg /kg BW were given one hour after. Xanthones were given). After 38 days, the next sperm mice will conduct inspection motility. The results showed that the administration of 2-ME could influence abnormality spermatozoa motility. However, xanthone treatment significantly shows _ enhancement normality spermatozoa motility . Conclusion From the results of this study indicate that xanthones are able to increase spermatozoa motility in mice that were given 2-ME.

I. INTRODUCTION

2-Methoxyethanol has been reported to be used in paints, inks, cleaners, polishes, brake fluids and jet fuels and to find wide application as a solvent [1,2]. *2-ME* can be oxidized by Alcohol dehydrogenase to *methyl aldehyde (MALD);* and *MALD is* rapidly oxidized by aldehyde dehydrogenase to *2 -methoxyacetic acid (2-MAA)* which is a stable and highly toxic metabolite in animals and humans [3]. It has been reported that *2-ME* and its metabolite, 2-MAA, can cause disturbances in the testes and spermatozoa so that infertility can occur [4,5,6].

Oxidative stress is an important mechanism of *2-ME- induced testicular damage* through the generation of reactive oxygen species (ROS) [5,6]. Oxidative stress has focused researchers around the world for its damaging effects on the body and is also responsible for cell death. Oxidative stress can occur when there is an imbalance between ROS generation and antioxidant capacity in cells [7]. Overproduction of Reactive has been reported oxygen species (ROS) or 2-ME-induced free radicals such as superoxide ions (O 2 -), hydroxyl radicals (OH^-) and Nitric oxide (NO) and consequently increase lipid peroxidation , impaired activity of antioxidant enzymes, such as superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) [8,9]. In addition, free radicals are highly reactive to membrane lipids, proteins, DNA of spermatozoa cells, Leydig cells and Sertoli cells in the testes, resulting in oxidative damage to cell membrane lipids, protein molecules, and DNA which can produce malondialdehyde (MDA). [7,8].

Studies have revealed that antioxidants have the ability to prevent and heal the damage caused by the toxic effects of *2-ME* that lead to the formation of free radicals in the body [4,9]. It has been reported that plant-derived antioxidants such as Tribulus terrestris, Withania somnifera, Mucuna pruriens; Garcinia kola and Garcinia mangostana can be used as a protector of spermatozoa and testicular cell damage due to exposure to *2-ME* [5,10,11,12]. Several studies have proven the pharmacological activity of *xanthones* which is one of the active compounds contained in *Garcinia mangostana* as an antioxidant [13]. *Xanthones* are natural chemicals belonging to polyphenol compounds . *Xanthones* have an antioxidant effect because they have a hydroxyl group (OH^-) which is effective at binding free radicals in the body [6,14,15]*. Xanthones* has a very strong antioxidant effect, therefore research is needed to prove the important role of *xanthones* to fix spermatozoa morphology due to *2-ME* exposure.

II. METHODS

A Male BalB / C mice weighing about 25-30 g (2-2.5 months) were obtained from Gadjah Mada University, Yogyakarta, Indonesia for experimental purposes. They were placed in plastic cages in an air- conditioned room with temperature maintained at 26 ± 2 °C and 12 h alternating light and dark cycles. Mice were fed *ad libitum* with tap water and fed standard commercial mouse diets. This research has been reviewed by the Ethical Committee Clearance for preclinical research , Faculty of Medicine Universitas Airlangga and obtained ethical clearance based on No.183/FK/12/2019.

1. Experimental Animal

This study used 35 male mice which were divided into 5 groups: negative control (the mice were given purified water every day by distillation); positive control (rats were given 2-ME 200 mg /kg BW daily orally once a day for 35 days); and the treatment group (the mice were given xanthones 60 mg, 120 mg , and 240 mg/kg BW orally once a day for 38 days, and on the 3rd day, 2-ME 200 mg /kg BW were given one hour after. Xanthones were given). After 38 days, to find out Spermatozoa motility is normal or abnormal.

2. Experimental Design

This study used 35 male mice which were divided into 5 groups: negative control (the mice were given purified water every day by distillation); positive control (rats were given 2-ME 200 mg /kg BW daily orally once a day for 35 days); and the treatment group (the mice were given xanthones 60 mg, 120 mg, and 240 mg/kg BW orally once a day for 38 days, and on the 3rd day, *2-ME* 200 mg /kg BW were given one hour after. *Xanthones were given*). After 38 days, to find out Spermatozoa motility is normal or abnormal.

3. Examination of mouse spermatozoa cells:

Spermatozoa Cells of mice placed in a filled petri dish with solution copy then conducted Observations using a light microscope at 400X magnification.

4. Statistic analysis:

Data are presented as means ± standard deviation. One Way ANOVA has performed a post hoc test and statistical comparisons between groups were performed with the LSD test using the statistical package program SPSS version 17.0 (SPSS Inc., Chicago, USA).

III. RESULT

This study aims to prove that xanthones at doses of 60, 120 and 240 mg/kg BW can increase spermatozoa motility in mice induced by 2-methoxyethanol (2-ME) 200 mg/kg BW. Data obtained from observations are analyzed through statistical tests related to hypotheses and research results. The presentation of research and analysis results is displayed in the form of diagrams and tables which are arranged and processed according to the research design.

1. Effect of *Xanthones* on the Motility of Mouse Spermatozoa Induced by *2-Methoxyethanol*

Data from the examination of xanthone administration on the percentage of motility in mouse spermatozoa induced with 2-ME. while the results of the mean and standard deviation of spermatozoa motility percentage can be seen in table 1.1. The results of the normality test using the Shapiro-Wilk test on data on the percentage of motility of spermatozoa obtained a P value of> 0.05, which indicates that the data is normally distributed, and in the homogeneity test using the Levene test on the data on the percentage of motility of spermatozoa, the value of P> 0.05 was obtained, which indicates that homogeneous data (Table 1.1).

Table 1.1 Effect of *xanthone* administration on mouse spermatozoa motility induced by *2-Methoxyethanol*

Group	Sample Size	Motilitas Progresif (%) (X±SD)	Median	Min-Max	Saphiro-Wilk test (P)	Lavene-test (P)
K Negatif	7	$71.43^{a} \pm 3.6$	72.5	67 - 78	0.529	
K Positif	7	$42.43^{b} \pm 6.1$	44	36 - 52	0.424	
***Xanthone* 60 mg/kg BB**	7	$44.28^{b} \pm 9.2$	44	32 -56	0.623	0.071
***Xanthone* 120 mg/kg BB**	7	$62.86^{c} \pm 5.8$	60	51 - 69	0.086	
***Xanthone* 240 mg/kg BB**	7	$59.43^{c} \pm 5.4$	59.5	52 - 67	0.660	

Different superscripts in the same column indicate significant differences

The results of the ANOVA test showed that there was a significant difference in mean spermatozoa motility between groups (negative control group, positive control, xanthones at a dose of 60 mg/kg BW, 120 mg/kg BW and 240 mg/kg BW) at $p<0.05$.

The test was continued with the LSD test, the results of which can be seen in table 1.1. The LSD test showed a significant difference in the percentage of progressive spermatozoa motility between the negative control group and the positive control group. The negative control where mice were only given 2-ME and xanthone solvent showed the highest progressive spermatozoa motility, whereas the positive control where mice were only given 2-ME showed the lowest progressive spermatozoa motility. These results show that administration of 2-ME can reduce progressive spermatozoa motility in mice. The results of the examination of the percentage of non-progressive motility due to 2-ME administration, found that many spermatozoa cells did not move or moved but slowly.

Giving xanthone can increase the percentage of progressive motility of spermatozoa, namely many spermatozoa that move quickly and straight forward in mice induced with 2 -ME, depending on the dose of xanthones given. Giving xanthones at a dose of 120 mg/kg BW and a dose of 240 mg/kg BW can increase the percentage of progressive motility of spermatozoa which is significantly different from the positive control group or the negative control group at $p<0.05$, while the dose of 60 mg/kg BW is not significantly different . These results indicate that xanthone doses of 120 mg/kg BW and 240 mg/kg BW are effective doses in increasing the percentage of progressive motility of mouse spermatozoa induced by 2-ME, although this increase could not reach the percentage of progressive motility found in the negative control group. . Giving xanthones at a dose of 120 mg/kg BW was not significantly different from a dose of 240 mg/kg. These results show that administration of xanthone at a dose of 120 mg/kg BW is the optimum dose in increasing the percentage of progressive motility of spermatozoa in mice induced with 2-ME.

Administration of xanthone can increase the percentage of progressive motility of mouse spermatozoa induced with 2-ME depending on the dose of xanthones given. The greater the dose of xanthones given, the greater the percentage of progressive motility of spermatozoa as seen in figure 1.1.

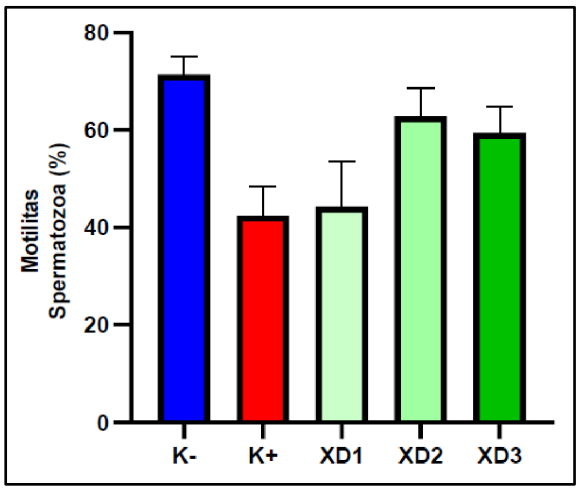

Figure 1.2. Effect of xanthone administration on progressive motility of spermatozoa in mice induced with 2-methoxyethanol. Negative Control (K-); Positive Control (K+), Xanthone dose 60 mg/kg BW (XD1), 120 mg/kg BW (XD2) and 240 mg/kg BW (XD3).

Administration of xanthone can increase the percentage of progressive motility of mouse spermatozoa induced with 2-ME depending on the dose of xanthones given. The greater the dose of xanthones given, the greater the percentage of progressive motility of spermatozoa as seen in Figure 1.2.

V. DISCUSSION

Effects of Xanthones on Decreasing Spermatozoa Motility of Yang Mice Induced 2- methoxyethanol

Motility is one of the important factors needed to support the ability of spermatozoa to fertilize. Fertilization will occur when the spermatozoa have a normal shape. Only normal spermatozoa are able to fertilize an egg. Although the number of spermatozoa in a person is normal, if the motility is disturbed, it will affect the low functional ability of the spermatozoa. Abnormal spermatozoa motility has been reported to be associated with infertility [7]. Examination of the motility of spermatozoa is very necessary because with motility movement the normal and abnormal forms of spermatozoa. Motility is very influential on the movement of spermatozoa. Abnormal spermatozoa cannot move properly and do not survive long, so abnormal spermatozoa are rarely able to successfully make a long journey to reach the site of fertilization. To be able to move progressively spermatozoa must have a normal morphology that is adapted to their function. Perfect spermatozoa have normal heads, necks, midsections and tails without any abnormalities so that they can glide forward with perfect movement. If there are abnormalities in the head, neck, middle and tail, it is possible not to move normally [16].

Determination of normal and abnormal spermatozoa have a curved head shape such as hooks (claws), neck, middle and tailwithout any abnormalities, of while abnormal spermatozoa are spermatozoa that have abnormalities in the head, neck, parts of the body. middle and tail like flat heads, small heads, large heads, double heads, large middle parts, short tails, double main tails and there is cytoplasmic residue on the cell membrane. Abnormal shape occurs due to various disturbances in spermatogenesis, especially the timing

of spermiogenesis. The disturbance may be due to hormones, nutrition, drugs, radiation or due to toxic substances [16]. Administration of 2-ME (positive control) can cause a significant decrease in the normal morphology of spermatozoa when compared to negative control (mouse only given solvent 2-ME and xanthone solvents). The administration of xanthones can significantly increase the motility of spermatozoa depending on the dose of xanthones given to mice induced by 2-ME, when compared to the positive control group.

Spermatozoa abnormalities found in this study were small heads, circular tails, double tails, double heads and flat heads. Abnormalities of spermatozoa found are included in the category of primary abnormalities, primary abnormalities are usually caused by disorders of testicular function which include errors in spermatocytogenesis or spermiogenesis, nutritional deficiencies, hormonal imbalances, due to heredity (congenital), due to disease and due to the influence of toxic substances [4]. 2-MAA metabolites of 2-ME compounds can cause oxidative stress due to an increase in the formation of ROS, especially hydroxyl radicals and a decrease in endogenous antioxidants, especially SOD and GPx which can inhibit Leydig cell function so that it can result in abnormally high spermatozoa and coiled tails of spermatozoa [4]. Inhibition of Leydig cell function can reduce testosterone production.

Testosterone is a hormone that plays a role in the process of spermatogenesis if its availability is little it will cause the spermatogenesis process to be disrupted and can result in primary abnormalities, namely abnormalities that occur due to: abnormalities in spermatogenesis such as the head is too large, the head is too large small, double tail and coiled tail [4]. In the epididymis, spermatozoa undergo a series of morphological and functional changes such as size, shape, center, DNA, metabolic patterns and properties of the plasma membrane. Functionally, the epididymis depends on testosterone in the process of these changes, so that if testosterone levels decrease, it causes the formation of abnormal spermatozoa [16]. Decreased levels of the hormone testosterone may cause disturbances in the epididymis so that abnormal spermatozoa are found. This is in accordance with the statement of Gatimel [16]. WHO said that the morphological abnormalities of spermatozoa can be caused by various conditions including hormonal imbalance and nutritional deficiencies. 2-ME can also increase cytochrome P450 and xanthine oxidase which can increase the formation of ROS so that the oxidation process of lipids, proteins and DNA can occur which can cause oxidative damage to spermatozoa cells. Therefore 2-ME can decrease the number of morphologically normal spermatozoa cells [3].

Administration of 2-ME can also increase protein kinase C so that it can reduce the number of normal morphology of spermatozoa

IV. CONCLUSION

In conclusion, our results show that administration of xanthones can improve the normal motility of mice spermatozoa cells induced by 2-ME . This is because xanthones are

antioxidants that can capture free radicals and can increase endogenous antioxidants so that Administration of xanthones can inhibit the decrease in spermatozoa motility in mice (Mus musculus) balb/c induced by 2-Methoxyethanol (2-ME)

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