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Article

Xanthone Protective Effects Against Spermatozoa Morphology of Mice Induced by 2-Methoxyethanol (2-ME)

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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 morphology 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, next sperm mice will conducted inspection morphology . The results showed that the administration of *2-ME* could influence abnormality spermatozoa morphology . However, *xanthone* treatment significantly shows _ enhancement normality spermatozoa morphology . Conclusion From the results of this study indicate that *xanthones* are able to increase spermatozoa morphology 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 *methoxyaldehyde (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.

III. METHODS

A Male B albB / 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 diet. 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.

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 morphology is normal or abnormal.

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 morphology is normal or abnormal.

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 .

Statistic analysis:

Data are presented as means ± standard deviation. Oneway 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).

IV. RESULT

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

1.1 Effect of *Xanthones* on Spermatozoa Morphology of Mice Induced by 2 - *Methoxyethanol*

Data on the results of *xanthone administration* on the percentage of normal morphology of spermatozoa in mice induced *by 2-ME* can be seen in Figure 1, while the results of the mean and standard deviation of the percentage of normal morphology of spermatozoa can be seen in Table 1.1 Normality test results using *Saphiro-Wilk* the test of the percentage of normal spermatozoa morphology data obtained P value> 0.05 which indicates the data is normally distributed , and the homogeneity test using *Levene 's test test* the percentage of normal spermatozoa morphology data obtained P value> 0.05 which indicates that the data is homogeneous (Table 1.1)

results of the ANOVA test showed that there was a significant difference in the average percentage of normal spermatozoa morphology between groups (negative control group, positive control group, *xanthones* at a dose of 60 mg /kg BW, 120 mg /kg BW and 240 mg /kg BW). The test was continued with the *LSD test* , the results of which can be seen in table 1 1.1. The *LSD* test showed a significant difference in the percentage of normal spermatozoa morphology between the negative control group and the positive control group at p<0.05. These results indicate that the administration of *2-ME* can reduce the percentage of normal morphology of spermatozoa in mice. The results of the examination of the percentage of abnormal spermatozoa morphology due to the administration of *2-ME* found many spermatozoa that did not have a head, small head, broken neck, broken tail and coiled or folded tail as shown in Figure 1.1 .

Table 1 .1 Effect of *xanthones* on normal morphology of mouse spermatozoa induced by 2 - *Methoxyethanol*

Group	Sample Size	Normal Morphology (%) (X ± SD)	median	Min-Max	Saphiro-Wilk test (P)	lavender-test (P)
K negative	7	64.0 ^{a} ± 5.2	64.5	57 - 72	0.925	
Positive K	7	33.8 ^{b} ± 4.1	34	28 - 40	0.941	
Xanthones 60mg/kg BW	7	36.0 ^{b} ± 4.0	35.5	30 - 41	0.749	0.160
Xanthones 120 mg /kg BW	7	51.0 ^{c} ± 9.1	53.5	41 - 66	0.532	
Xanthones 240 mg /kg BW	7	48.3c ± $^{8.6}$	52.5	40 - 65	0.208	

Different superscripts in the same column indicate a significant difference

The administration of *xanthones* with different doses can inhibit the decrease in the percentage of normal spermatozoa morphology, namely that many spermatozoa have a head shape like hooks, large necks and straight tails in

mice induced by 2 -*ME* as shown in Figure 1.1.

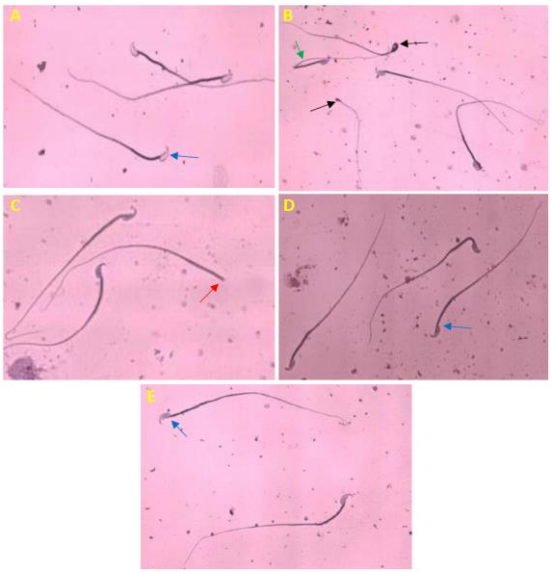

Figure 1.1. Effect of *xanthones* on the morphology of mice spermatozoa induced by 2 - *Methoxyethanol* with normal spermatozoa staining (blue arrows); Small head (black arrow); No head (red arrow) and broken tail (green arrow). Negative Control (A); Positive Control (B); *Xanthones* doses of 60 mg /kg BW (C), 120 mg /kg BW (D) and 240 mg /kg BW (E) (Observation using a light microscope at 400 X magnification).

The administration of *xanthones* at a dose of 120 mg /kg BW and a dose of 240 mg /kg BW could inhibit the decrease in the percentage of normal spermatozoa morphology significantly different with the positive control group and with the negative control group at $p<0.05$, while the dose of 60 mg /kg BW was not significantly different. These results indicate that the administration of *xanthones* at a dose of 120 mg /kg BW and a dose of 240 mg /kg BW is an effective dose in inhibiting the decrease in the percentage of normal spermatozoa morphology in mice induced with *2-ME* , although the inhibition of the decrease in the percentage of normal spermatozoa morphology has not been able to achieved as in the negative control group.

Administration of *xanthones* the dose of 120 mg /kg BW was not significantly different from the dose of 240 mg /kg. These results show that the administration of *xanthones* a dose of 120 mg / kg BW is the optimum dose in inhibiting the decrease in the percentage of normal morphology of spermatozoa in mice induced with *2-ME* .

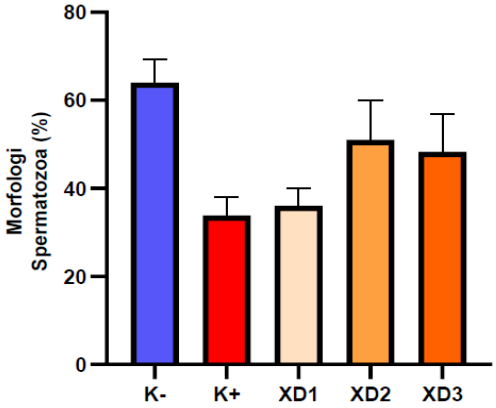

Figure 1.2 . Effect of *xanthones* on the morphology of mice spermatozoa induced with 2 - *methoxyethanol*. Negative Control (K-); Positive Control (K+), *Xanthones* 60 mg /kg BW (XD1), 120 mg /kg BW (XD2) and 240 mg /kg BW (XD3).

The administration of *xanthones* inhibited the decrease in the percentage of normal spermatozoa morphology in mice induced by 2-ME and depended on the dose given. The larger the dose of *xanthones* given, the stronger the resistance to decreasing the percentage of normal morphology of spermatozoa as shown in Figure 1.2.

V. DISCUSSION

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

Morphology 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 morphology is disturbed, it will affect the low functional ability of the spermatozoa. Abnormal spermatozoa morphology has been reported to be associated with spermatozoa *DNA fragmentation* in infertility [7].

Examination of the morphology of spermatozoa is very necessary because with morphological examination can be known the normal and abnormal forms of spermatozoa. Morphology is very influential on the movement and resistance 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 is based on the shape of the spermatozoa, normal spermatozoa have a curved head shape such as hooks (claws), neck, middle and tail without any abnormalities, 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 morphology 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 cells .

Xanthones, especially -mangostin as antioxidants can increase endogenous antioxidants such as *SOD* , *Catalase* and *GPx* which can inhibit the oxidation of *poly*

unsaturated fatty acids (*PUFAs)* Leydig cells so that it can increase the hormone testosterone. Testosterone is very important for the development of spermatozoa shape so that spermatozoa can have a perfect morphology. In the epididymis , spermatozoa undergo a series of morphological changes, these changes in the epididymis are highly dependent on testosterone, so low testosterone levels can cause impaired spermatozoa formation.

The integrity of the spermatozoa nucleus is thought to be the most important determinant of the morphology of the spermatozoa head. *2-ME* can cause oxidative stress which can lead to degradation of chromatin in the nucleus of spermatozoa, so that it can affect and change the morphology of the head. Some investigators suspect that changes in sperm chromatin compaction in infertile men, possibly related to protamine deficiency or sulfhydryl oxidation, may contribute to head morphology damage, thereby influencing the overall spermatozoa morphology assessment. The mechanism of *xanthones* in maintaining the morphology of spermatozoa is thought to be related to the compaction or condensation of chromatin in the nucleus of spermatozoa [5] [6]. The addition of *xanthones* can maintain the chromatin condensation mechanism because *xanthones* can capture free radicals, especially OH^- radicals so that they can inhibit the degradation of chromatin in the nucleus of spermatozoa is will cause the compaction of genetic material in the nucleus to take place well and produce a normal spermatozoa head morphology.

Xanthones e Besides working as an antioxidant, it can also inhibit cytochrome P450 and inhibit *xanthine oxidase* (Hu *et a*) so that *xanthones* are able to inhibit the formation of *ROS* , which can inhibit the oxidation process of lipids, proteins and *DNA* . Therefore, *xanthones* can inhibit oxidative damage to spermatozoa cells which can increase the number of normal morphology of spermatozoa cells.

Administration of *xanthones* can also inhibit protein kinase C through its inhibition of genotoxicity and *DNA* damage [5]. so that it can increase the number of normal morphology of spermatozoa cells in mice that were given *2-ME.*

VI. CONCLUSION

In conclusion, our results show that administration of *xanthones* can improve the normal morphology of mice spermatozoa cells induced by *2-ME* . This is because *xanthones* are antioxidants that can capture free radicals and can increase endogenous antioxidants (*SOD* and *GPx*) so that they can protect Leydig cell damage and can increase testosterone which can inhibit abnormal morphology.

REFERENCES

Adedara IA and Farombi EO. 2014. Influence of kolaviron and vitamin E on ethylene glycol monoethyl ether-induced haematotoxicity and renal apoptosis in rats. *Cell Biochemistry and Function*, vol. 32, no. 10, pp. 31-38

Agarwal A, Saleh R, Bedaiwy MA. 2003. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*. No. 79, pp. 829-843

Agarwal A, Virk G, Ong C, du Plessis SS. 2014. Effect of Oxidative Stress on MaleReproduction. *World Journal Mens Health*, vo. 32, no. 1, pp 1-17

Berndtson WE, Foote RH.1997. Disruption of spermatogenesis in rabbits consuming ethylene glycol monomethyl ether. *Reproductive Toxicology*, vol. 11, no. 1, pp.29-36.

Chin YW., Kinghorn AD. 2008. Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (Garcinia mangostana), apopular botanical dietary supplement. Mini Rev Org Chem. 5:355–364

Ernawati, I'tishom R, Sudjarwo SA. 2019. The signal transduction of xanthone as aprotector on 2-methoxyethanol-induced cardiac cell damage in mice. *Journal of Advanced Pharmaceutical Technology and Research*, no. 10, pp. 184-189

Feradis. 2010. *Bioteknologi Reproduksi pada Ternak*. Bandung: Alfabeta Press

Fritz and Speroff. 2011. *Clinical Gynecologic Endrocrinology and Infertility*. 8th ed. Philadelphia : Lippincott Williams & Wilkins, pp. 579-83.

Hafez ESE. 2000. *Semen Evaluation in Reproduction in Farm Animal. 7th edition*.Philadelphia: Wolters Kluwer Company.

Hayati A, Ernawati, Iswanto M , Maulidyah N, Azzahra EI, Rahmaniyah F,HilmanFAM, Sugiharto, Winarni D. 2017. Sperm quality and testicular structure of Mus musculus after Garcinia mangostana L pericarp extract administration in different polarity of 2-Methoxyethanol. *Journal of Advanced Zoology*, vol. 38, no. 1, pp. 64-78.

Johanson G. 2000. Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Critical Reviews in Toxicology*, no. 30, pp. 307-345.