The Protective Effect of Xanthone via Malondialdehyde and Superoxide DismutaseExpression on Mice Sertoli cell Induced by 2-Methoxyethanol

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Abstract

The antioxidants can be used for protective in oxidative stress that is one of the important mechanisms of 2-methoxyethanol (2-ME)-induced testis toxicity. The current study was carried out to evaluate The protective effect of xanthone via Malondialdehyde (MDA) and Superoxide Dismutase (SOD) expression on mice Sertoli cell induced by 2-Methoxyethanolon the Sertoli cell number induced by 2-ME in mice. The study used 35 male mice divided into 5 groups: control group (mice were given daily with water purified by distillation); 2-ME group (mice were given daily with 2-ME 200 mg/kg BW orally once in a day for 35 days); and the treatment group (mice were given the xanthone 60 mg, 120 mg, and 240 mg/kg BW orally once in a day for 38 days, and on the 3th day, were given 2-ME 200 mg/kg BW one hour after the xanthone administration). After 38 days, the testis tissues were collected to evaluate the histological of Sertoli cell number, and also evaluated the immunohistochemical of MDA and SOD expression on Sertoli cell. The result showed that 2-ME administration significantly increased MDA expression, and decreased both SOD expression and the number of Sertoli cells. However, the treatment of xanthone significantly decreased MDA expression, and increased the expression of SOD of the Sertoli cell in the immunohistochemical. Xanthone also significantly increased the Sertoli cell number in histopathological evaluation. In conclusion, From the results of this study demonstrated that xanthone is able to protect Sertoli cell number in mice treated with 2-ME through decreasing MDA and increasing SOD

Keywords: *Xanthone, MDA, SOD, Sertoli cell*

Introduction

2-Methoxyethanol has been reported to be used in paints, coatings, 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 very toxic metabolite in the body of animals and humans[3]. It has been reported that 2-ME and its metabolites, 2-MAA, can cause disturbances in the testes and spermatozoa so can occur infertility[4,5,6].

The oxidative stress is an important mechanisms of 2-ME-induced testis damages via Reactive oxygen species (ROS) generation[5,6].Oxidative stress focuses the attention of worldwide researchers for its damaging effects on the body and also responsible for the death of a cell. Oxidative stress can occur when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the cells[7]. It has been reported 2-ME-induced overproduction of Reactive oxygen species (ROS) or free radicals such as superoxide ion (O_2-), Hydroxyl radical (OH^-) and Nitric oxide (NO) and consequently enhance lipid peroxidation, impairment of antioxidant enzymes activities,

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such as superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) [8,9]. In addition, free radicals are highly reactive to membrane lipids, protein, DNA of spermatozoa cells, Leydig cells and Sertoli cells in the testes so occur oxidative damage in the cell membrane lipids, protein molecules, and DNA that can produce Malondialdehyde (MDA) [7,8].

Studies have revealed that antioxidants possess the ability of both preventing and curing the damage caused by the toxic effects of 2-ME that causes the generation of free radicals in the body[4,9]. It has been reported that antioxidants derived from plants such as Tribulus terrestris, Withania somnifera, Mucuna pruriens; Garcinia kola, and Garcinia mangostana can be used as protectors for spermatozoa and testicular cell damage due to 2-ME exposure[5,10,11,12]. Several studies have proven the pharmacological activity of xanthone which one of the active compounds contained in Garcinia mangostana as an antioxidant[13]. Xanthones are a natural chemical substance that is classified as polyphenolic compounds. Xanthones have an antioxidant effect because xanthones have a hydroxil group (OH^-) that effectively binds to free radicals in the body[6,14,15]. The xanthones have a very strong antioxidant effect, therefore is needed research to prove that SOD expression have important role on xanthone to protect sertoli cells number due to exposure to 2-ME.

Material and Methods

Experimental animals

Male BALB/c mice weighing approximately 25-30 g (2-2.5 months) were obtained from Gadjah Mada University, Yogyakarta, Indonesia for experimental purpose. They were housed in plastic cages in an air-conditioned room with a temperature maintained at 26 ± 2 oC and 12 h alternates light and dark cycles. The rats were given *ad libitum* with tap water and fed with standard commercial rat chow.

Experimental design

The research used 35 male mice divided into 5 groups: negative control (mice weregiven daily with water purified by distillation); positive control (mice were given daily with2-ME 200 mg/kg BW orally once in a day for 35 days); and the treatment group (mice weregiven the xanthone 60 mg, 120 mg, and 240 mg/kg BW orally once in a day for 38 days, andon the 3th day, were given 2-ME 200 mg/kg BW one hour after the xanthone administration).After 38 days, the testis tissues were collected to evaluate the histological of Sertoli cell number, and also evaluated SOD expression on leydig cell.

Immunohistochemical examination

For immunohistochemical studies, a LSAB System HRP (Dako, Carpinteria, CA), anti MDA and anti SOD monoclonal antibody (Abcam International, USA) were used. In brief[16], the sections were deparaffinized, after hydrated with decreasing alcohol concentrations and washed three times for 3 min each time in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for heat-induced epitope retrieval; the sections were boiled in citrate buffer (pH 6 or 9) in a microwave oven for . The sections were preincubated with 0.3% hydrogen peroxide in PBS and later incubated with MDA and SODantibody (1 : 100) by 90 min at room temperature. Slices were washed two times with PBS for 2 min followed by incubation with a secondary biotinylated antisera and then immersed in avidin–biotin peroxidase complex (LSAB System HRP, Dako, Carpinteria, CA) for 20 min at room temperature. The immune reaction resulted in the oxidation of the 3,3-diaminobenzidine by peroxidase (Liquid DAB, Dako, Carpinteria, CA) into an insoluble brown precipitate. Counterstaining with hematoxylin was performed after immunostaining.

Histopathological examination:

The tissue of testis was fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination with hematoxylin and eosin (H&E) stain.

Statistical Analysis

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

Results

Table 1 and Figure 1 showed the results of xanthone on the expression of MDA in against 2-ME-decreased Sertoli cell number. The administration of 2-ME on mice caused a significant increase in the expression of MDA in Sertoli cell number compared to the control group. Dose dependent of xanthone decrease the expression of MDA in Sertoli cell number.

Table 1: Protective effect of xanthone on MDA expression in against 2-ME-decreased Sertoli cell number

Group	MDA in Sertoli (Mean ± SD)
Control group	2.87a ± 0.28
2-ME group	4.79b ± 0.37
Xanthone 60 mg/kg BW	4.69b ± 0.36
Xanthone 120 mg/kg BW	4.56c ± 0.65
Xanthone 240 mg/kg BW	3.54c ± 0.49

[a,b,c]Different superscript within each column differ significantly($P < 0.05$)

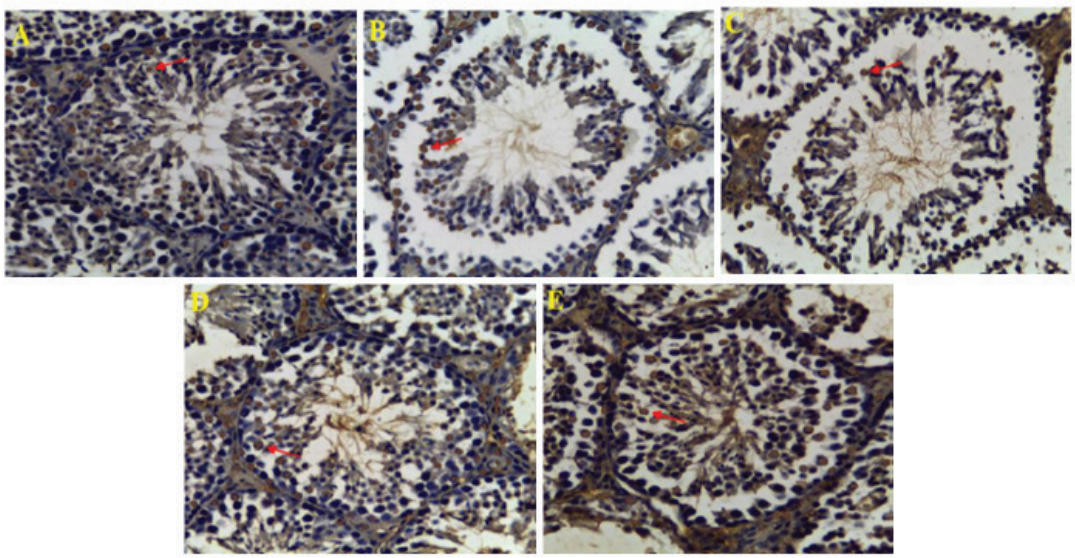

Figure 1: Immunohistochemical study of xanthone on MDA expression (indicated by red arrows) on 2-methoxyethanol-decreased Sertoli cell number. Control group (a); 2-methoxyethanol group (b); mice treated with xanthone 60 mg/kg BW; 120 mg/kg BW, and 240 mg/kg (c-e)

Table 2 showed the results of xanthone in protective 2-ME-decreased Sertoli cell number. The administration of 2-ME on mice caused a significant decrease Sertoli cell number compared to the control group. The treatment xanthone increase Sertoli cell numberin a *dose-dependent manner.*

Table 2: Protective effect of xanthonein against 2-ME-decreased Sertoli cell number

Group	Sertoli cell number (Mean ± SD)
Control group	22.04a±2.47
2-ME group	13.07b±2.36
Xanthone 60 mg/kg BW	12.46b±2.07
Xanthone 120 mg/kg BW	13.56b±2.36
Xanthone 240 mg/kg BW	17.20c±2.03

[a,b,c]Different superscript within each column differ significantly ($P < 0.05$)

Table 3 and Figure 3 showed the results of xanthone on the expression of SOD in protective 2-ME-decreased Sertoli cell number. The administration of 2-ME on mice caused a significant decrease in the expression of SOD and Sertoli cell number compared to the control group. The treatment xanthone increase the expression of SOD and Sertoli cell numberin a *dose-dependent manner***.**

Table 3: Protective effect of xanthone on SOD expression in against 2-ME-decreased Sertoli cell number

Group	SOD in Sertoli cell (Mean ± SD)
Control group	6.51a ± 0.53
2-ME group	3.89b ± 0.30
Xanthone 60 mg/kg BW	3.90b ± 0.34
Xanthone 120 mg/kg BW	3.96c ± 0.26
Xanthone 240 mg/kg BW	4.96c ± 0.39

[a,b,c]Different superscript within each column differ significantly ($P < 0.05$)

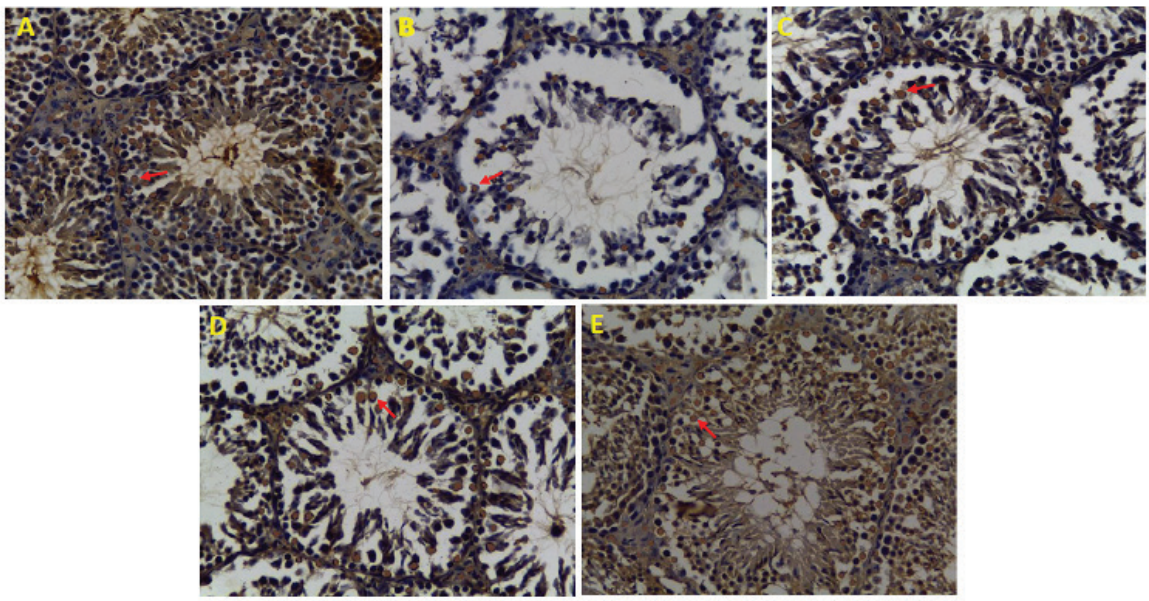

Figure 3: Immunohistochemical study of xanthone on SOD expression (indicated by red arrows) on 2-methoxyethanol-decreased Sertoli cell number. Control group (a); 2-methoxyethanol group (b); mice treated with xanthone 60 mg/kg BW; 120 mg/kg BW, and 240 mg/kg (c-e)

Discussion

2-methoxyethanol was consistently toxic to the male reproductive system in multiple species (mice, rats, guinea-pigs, rabbits and dogs) exposed by all routes of admin-istration (subcutaneous, dermal, oral or inhalation) [1]. Reproductive system is one of the main targets of 2-MEtoxicity following exposure[2]. Therefore, there is a need for further detailedstudies with focus on underlying mechanisms by which 2-MEinduces reproductive dysfunction and male infertility. In the present study, we evaluated the protective mechanism of xanthone against the MDA and SOD expression changes in the Sertoli cell resulting from the administration of 2-ME in mice. The biochemical mechanisms decreased in the Sertoli cell number of 2-ME were studied by measuring the MDA expression and by screening the activities of primary antioxidant enzymes such as SOD expression in immunochemical studies. It also Sertoli cell number was investigated for histopathological studies.

The current study showed that 2-ME administration significantly decreased the SOD and increased MDA expression. 2-ME also decreased the Sertoli cell number. 2-ME-decreased Sertoli cell number have been attributed, at least in part, to toxicant-induced oxidative stress. It results suggest that 2-ME stimulates the formation of ROS, thus causing oxidative damage to Sertoli cell resulting in decrease of cell number. Long-term exposure to 2-ME increases MDA or lipid peroxidation and causes inhibition of SOD activity inducing oxidative damage in testicular cell[4,5]. The various toxic effects induced by 2-ME in biological systems have been linked to increased MDA or lipid peroxidation, as an early and sensitive consequences of 2-ME exposure. 2-ME toxicity leads to the generation of free radical damage by two separate pathways, including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA expression as the final products of lipid peroxidation, and the direct depletion of antioxidant reserves[17]. The present investigation resulted in significantly increased of MDA expression in the Sertoli cell of 2-ME-treated mice in comparison to the control. This means that it increased the oxidative stress in the 2-ME-treated mice. Therefore, the significantlylower expression of MDA in the Sertoli cell of xanthone treated groups ascompared with the 2-ME group indicate attenuation of lipidperoxidation. It is known that 2-ME-induced oxidative stress and Sertoli cell damage could be caused by two mechanisms including increased generation of ROS and by causing direct depletion of antioxidant reserves[18]. Intense lipid peroxidation caused by 2-ME exposure may affect the mitochondrial and cytoplasmic membranes, causing more severe oxidative damage in the cell and consequently releasing lipid hydroperoxides into circulation which reflects the induction of oxidative stress[4]. The xanthone, which behaves as a powerful antioxidant and free radical scavenger, can decrease the MDA expression perturbed by 2-ME in mice Sertoli cell, as observed in this study. Treatment of mice with xanthone at a dose of 120 mg/kg BW and 240 mg/kg BW prevented the expression of MDA to rise when the mice were challenged with 2-ME. This means that xanthone minimized the toxic effect of 2-ME via its antioxidant activity. The antioxidant protective mechanism decreases the oxidative stress and scavenges the free radical responsible for the Sertoli cell damage and thus inhibit the lipid peroxidation as measured by MDA expresion. The findings of this study suggest that xanthone could attenuate oxidative stress by decreasing the lipid peroxidation (MDA expression) in the 2-ME-treated Sertoli cell. A similar result has shown that antioxidants derived from plants such as Tribulus terrestris, Withania somnifera, Mucuna pruriens; Garcinia kola, and Garcinia mangostana enhanced theantioxidant status and inhibited lipid peroxidation in rats with2-ME induced testis injury[5,10,11,12].

SOD are important antioxidant enzymes. The enzyme SOD plays a vital role in protection from oxidant damage produced by ROS in terms of dismutation of highly toxic superoxide anion radicals into less toxic hydrogen peroxide, which is then neutralized into oxygen and water by catalase. Further, GPx catalyzes the reduction of lipid peroxides and hydrogen peroxide using glutathione to protect against accumulation of lipid peroxides and other oxidants, thereby preventing oxidant damage[9,16]. The observed reduction in activities of antioxidant defenses demonstrates the failure of the primary antioxidant system to act against 2-ME-induced oxidant stress. Therefore, the activities of SOD has been used to assess oxidative stress in cells[19,20]. In the present study, the activity of SOD in sertoli cell number was decreased by 2-ME treatment. This decreased SOD activities with 2-ME treatment is in agreement with previous studies. This suggested that 2-ME exposure

induced oxidative stress by inhibiting the activity of this antioxidant enzyme. Interestingly, the administration of xanthone increased the activities of SOD in the Sertoli cell of 2-ME-treated mice, which might be due to the ability of xanthone to reduce the accumulation of free radicals. xanthone acts as a scavenger for the oxygen-derived free radicals, thus protecting from Sertoli cell damage[21,22].

The decrease in lipid peroxidation due to xanthone has been attributed to alterations in the antioxidant defense system which includes enzymes such as catalase (CAT), SOD and GPx which normally protect against free radical toxicity. The primary mechanism of action of xanthone may involve the scavenging of free radicals which can inhibit free radical formation[13,21]. It has been found a decrease MDA and an increase in the antioxidant enzyme parameters including SOD, CAT, and GPx in the plasma and tissue such as hearth, testis, and brain of animals that were administered xanthone[5,6,20].

Histopathological results demonstrating structural changes in testis tissue of 2-ME were reported by some researchers. In the present study, histopathological view of testis sections in the 2-ME treated group showed decreasing of the Sertoli cell number, as compared to the control group. The decrearesing were considerably mild in the groups treated with xanthone240 mg/kg.

Conclusion

In conclusion, our results indicate that *xanthone* as antioxidant agent is able to increase Sertoli cell number in mice treated with 2-ME through decreased MDA expression, and increased SOD expression

Ethical Clearance: This study was reviewed by the Ethical Clearance Committee for preclinical research, Faculty of Medicine, Airlangga University and obtained ethical clearance under No.183/FK/12/2019.

Conflicts of Interest: There are no conflicts of interest

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