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The Role of Glutathione Peroxidase Expression in the Protective Effect of Xanthone on Mice Sertoli cell Number Induced by 2- methoxyethanol

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Abstract

Toxicity effects of 2-methoxyethanol on testicular tissues may be mediated by oxidative damage. Xanthone as antioxidant can protect against such oxidative stress. In the current study, we evaluated the role of Glutathione Peroxidase (GPx) expressionin protective activity of xanthone against the oxidative stress changes on 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 GPx expression on Sertoli cell. The result showed that 2-ME administration significantly decreased both GPx expression and the number of Sertoli cells. However, the administration of xanthone significantly increased the expression of GPx of the Sertoli cell in the immunohistochemical. Xanthone significantly increased the Sertoli cell number in histopathological evaluation. In conclusion, our research indicates that xanthone is able to protect Sertoli cell number in mice treated with 2-ME through increasing GPx

Keywords: Xanthone, Glutathione Peroxidase, Sertoli cell

Introduction

2-Methoxyethanol (2-ME) is a glycol ether compound found in various industrial products including paints, inks, varnishes, nail polishes, hydraulic fluids, plastic materials, aircraft fuels and the food industry [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 mechanism of action of 2-ME in causing the decrease in quality and quantity of spermatozoa cells in the epididymis, and testicular damage through Oxidative stress [4,5,6]. Oxidative stress can occur due to an increase in Reactive Oxygen Species (ROS) which includes superoxide (O2-), hydroxyl radical (OH-), hydrogen peroxides (H2O2) and decreased endogenous antioxidants including Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GPx). The imbalance between ROS and antioxidants will cause oxidation of lipid, protein, and 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].

Some researchers report that administration of antioxidants can inhibit cell damage due to 2-ME exposure to spermatozoa and testes [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 GPx expression have important role on xanthone to protect Leydig 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. 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.

Experimental design: The research used 35 male mice divided into 5 groups: negative control (mice were given daily with water purified by distillation); positive control (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 leydig cell number, and also evaluated GPx expression on leydig cell.

Immunohistochemical examination : For immunohistochemical studies, a LSAB System HRP (Dako, Carpinteria, CA), anti GPx 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 GPx antibody (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 in protective 2ME decreased Sertoli cell number. The administration of 2ME on mice caused a significant decrease Sertoli cell number compared to the control group. The treatment xanthone increase Sertoli cell number in a dose-dependent manner**.**

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

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

$^{a, b, c}$ Different superscript within each column differ significantly ($P < 0.05$)

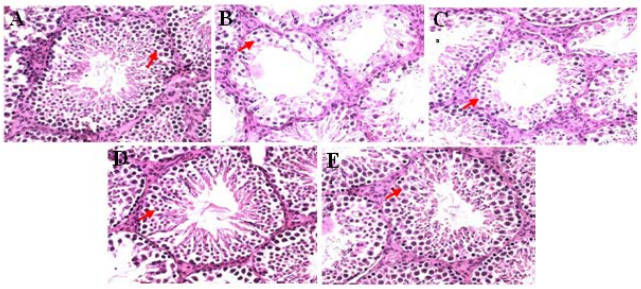

Figure 1: Histopathological study of xanthone in against 2ME-decreased Sertoli cell number (indicated by red arrows). Control group (a); 2methoxyethanol group (b); mice treated with xanthone 60 mg/kg BW; 120 mg/kg BW, and 240 mg/kg (ce)

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

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

Group	GPx in Sertoli cell (Mean ± SD)
Control group	5.54[a] ± 0.59
2-ME group	3.30[b] ± 0.37
Xanthone 60 mg/kg BW	3.41[b] ± 0.49
Xanthone 120 mg/kg BW	3.87[c] ± 0.45
Xanthone 240 mg/kg BW	4.11[c] ± 0.34

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

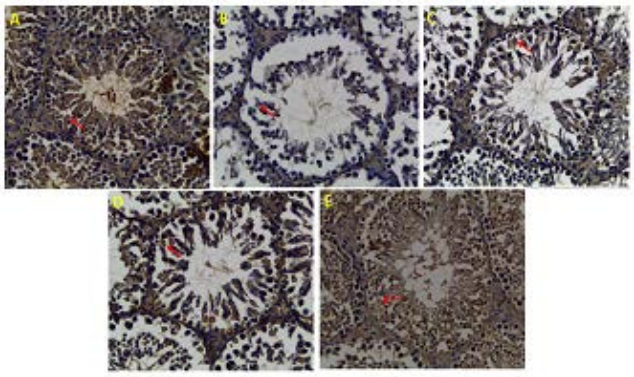

Figure 2: Immunohistochemical study of xanthone on GPx expression (indicated by red arrows) on 2 methoxyethanol decreased Sertoli cell number. Control group (a); 2methoxyethanol group (b); mice treated with xanthone 60 mg/kg BW; 120 mg/kg BW, and 240 mg/kg (ce)

Discussion

2-ME may induce oxidative stress leading to the generation of free radicals and alteration in oxygen free radical scavenging enzyme system or antioxidant such as Catalase, SOD and GPx [6,17,18]. In the current study, we evaluated the protective mechanism of xanthone against the oxidative stress 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 screening the activities of primary antioxidant enzymes such as GPx expression in immunochemical studies. It also Sertoli cell number was investigated for histopathological studies.

The present study showed that 2-ME administration significantly decreased GPx and number of the Sertoli cell. 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 causes inhibition of GPx activity inducing oxidative damage in testicular cell [4,5,6]. 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 [6]. 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 [19]. 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 [20]. 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].

GPx are important antioxidant enzymes. The enzyme GPx plays a vital role in protection from oxidant damage produced by ROS. 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]. 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 GPx have been used to assess oxidative stress in cells [17,18]. In the present study, the activity of GPx in sertoli cell number was decreased by 2-ME treatment. This decreased GPx 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 GPx 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 [5,6,18].

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 xanthone 240 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 both SOD and GPx expression

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