ORIGINAL ARTICLE

The signal transduction of xanthone as a protector on 2-methoxyethanol-induced cardiac cell damage in mice

Ernawati, Reny I'tishom[1], Sri Agus Sudjarwo[2]

Program Study of Doctoral Degree in Medical Science, [1]Department of Medical Biology, Faculty of Medicine, Airlangga University, [2]Department of Pharmacology, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

J. Adv. Pharm. Technol. Res.

ABSTRACT

This research aims at investigating the role of antioxidant of xanthone on 2-methoxyethanol (2-ME)-induced cardiac cell damage in mice. Forty mice were grouped into: (1) The control group (mice were given with distilled water), (2) the ME group (mice were given with 2-ME 200 mg/kg BW orally), and (3) the treatment group (mice were given of xanthone with doses 60 mg, 120 mg, 240 mg/kg BW orally and were also given 2-ME 200 mg/kg BW). Their blood samples were taken to measure the level of lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB). Heart tissues were also taken to determine the malondialdehyde (MDA), histological findings of heart damage, and the immunohistochemical of the expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx). The administration of 2-ME resulted in a significant increase level of the LDH, CK-MB, MDA, and a decrease in SOD and GPx expression were compared with the control group. The 2-ME also induced loss of the normal structure of heart cells and necrosis. However, treatment with the xanthone, only dose 240 mg/kg BW significantly decrease the level of LDH, CK-MB, MDA, and increase SOD, GPx expression. The xanthone 240 mg/kg BW also demonstrated significantly improved heart cell damage. From the results, it is concluded that the xanthone are a potent antioxidant in against 2-ME-induced cardiac toxicity in mice, through increasing SOD and GPx expression, and also inhibiting LDH, CK-MB and MDA.

Key words: 2-methoxyethanol, antioxidant, cardiac cell, creatinine kinase-MB, lactate dehydrogenase, malondialdehyde, xanthone

INTRODUCTION

Ethylene glycol monomethyl ether (2-methoxyethanol [2-ME]) regarded as a potent occupational toxin and its toxicological manifestations are well known in humans. The main sources of 2-ME toxicity are of industrial products, including paints, inks, hydraulic fluids, and jet fuels.[1] It has been reported that ME toxicity can interfere the physiological, biochemical, and behavioral in human and animal, which, including disorders of cardiovascular, brain, hematopoietic, renal, reproductive and hepatic system producing serious toxicity,[2-4] while the likely effect of toxicity on the cardiovascular system hasn't been precisely proven.

Address for correspondence:
Prof. Sri Agus Sudjarwo,
Department of Pharmacology, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia.
E-mail: ags158@yahoo.com

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/japtr.JAPTR_57_19

How to cite this article: Ernawati, I'tishom R, Sudjarwo SA. The signal transduction of xanthone as a protector on 2-methoxyethanol-induced cardiac cell damage in mice. J Adv Pharm Technol Res 2019;10:184-9.

Creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) are the indicative serum marker enzymes of cardiotoxicity. This enzymes escape via the injured tissues, and they are the most effective marker of cardiotoxicity because of their tissue selectivity and serum catalytic activity.[5,6]

The 2-ME mechanism induced toxicity and this occurs once there is unevenness between the formation of reactive oxygen species (ROS) and the glutathione (GSH), Catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) in biological organelles.[7-9]

A current research has proven that the ROS like superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) play an essential function in 2-ME-induced cellular toxicity.[10,11] Furthermore, ROS are very reactive to membrane lipids, protein, and are primarily responsible for causing stress injuries and rapid cellular damage. Malondialdehyde (MDA) is a trivial product of lipid peroxidation and can be used as a marker for cell injury. A rise in MDA levels depicts antioxidant failure and the development of free radicals improves lipid peroxidation thereby resulting in cellular toxicity.[5,6,12] The MDA level is the direct evidence of tissue injury processes caused by free radicals. Several researchers have proven that antioxidants can prevent or inhibit the oxidation of molecules by the ROS in tissue or cellular organisms, scavenge free radicals which can weaken harmful effects of ROS.[13,14]

The role of antioxidant activity or inhibition of the production of free radicals plays an important role in the prevention of such oxidative damage. Hence, it has been claimed that protective agents against free radicals like antioxidants, might be effective prevention for oxidative stress induce tissue damage.[8,10,15] Thus, it is believed that antioxidant should be one of the important components for treatment of ME poisoning. There is an increasing interest toward the use of naturally occurring phytochemicals with cardioprotective and antioxidant activity in 2-ME intoxication therapy.

Based on the aforementioned statement, numerous researches have been conducted with different natural product that contain antioxidant properties to examine their probable protective effects in 2-ME caused tissue damage. Among those *Mucuna pruriens*, *Withania somnifera,* and *Garcinia mangostana* have been proven to have protective tasks against 2-ME intoxication.[7,16,17] *G. mangostana L* is one of the medicinal plant which also shown antioxidant activity. Phytochemical studies of *G. mangostana* show that this plant contains xanthones.[18] The ethnopharmacological views of xanthone suggest remarkable properties such as antioxidants, antitumor, anti-inflammatory, analgesic, antiviral activities, cardioprotective effects, antifungal, antiallergy, antibacterial, antituberculosis, and immunomodulation.[18-20] Xanthone has been shown to have strong antioxidant activity.[21] Therefore, the aim of this study was to prove that the SOD and GPx have role important on xanthone in protected 2-ME-induced cardiac damage in mice.

MATERIALS AND METHODS

Experimental animal

The experimental animals used were male mice, and they each weighed between 25 and 30 g (2.5–3 months). They were gotten from the Veterinary Farm, Surabaya, Indonesia for experimental use. The mice were kept in plastic cages with a constant temperature of 26°C ± 2°C and 12 h rotation of light and dark cycles. The mice drank tap water that contained *ad libitum,* and they ate economical rat food. This study was reviewed by the Ethical Clearance Committee for preclinical research, Faculty of Medicine, Airlangga University and obtained ethical clearance under No. 178/FK/1/2018. Date: March 14, 2019.

Experimental design

The sample used 40 male mice were divided into five groups: control group (mice were given daily with distilled water); 2-ME group (mice were given with 2-ME, orally, at a dose of 200 mg/kg BW for the 35 consecutive days), and the treatment group (mice were given the xanthone 60 mg; 120 mg; 240 mg/kg BW orally once in a day for 40 days and on 5th day, were given with 2-ME, orally, at a dose of 200 mg/kg BW 1 h after the xanthone). On day 40, the mice blood samples were taken by cardiac puncture to be measured levels of LDH and CK-MB. Furthermore, mice were sacrificed, and cardiac tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediamine tetraacetic acid. The supernatant was separated by centrifugation at 1000 g for 20 min at 4°C. The supernatant was used for the analyses of MDA. The cardiac tissues were also fixed in a 10% neutral-buffered formalin solution for immunohistochemical evaluation of the expression of SOD and GPx and histopathological evaluation of the cardiac damage.

Measurement of lactate dehydrogenase and creatine kinase-MB fraction

The serum was examined for the existence of various enzymes linked to myocardial infarction like LDH and CK-MB fraction.[5] All analyses were conducted with commercially available kits based on the references via the experimenter.

Measurement of malondialdehyde

MDA was observed in the supernatant of homogenate cardiac tissue using the thiobarbituric acid (TBA) technique. The concentration of MDA was acquired to be 532 nm by the absorbance coefficient of MDA-TBA complex.[5] MDA is expressed as nanomoles MDA/g tissue.

Histopathological examination of heart damage

Tissues extracted from the heart was placed in 10% neutral-buffered formalin solution, inserted in paraffin, and utilized for histopathological analyzes with hematoxylin and eosin stain.

Immunohistochemical examination of the expression of superoxide dismutase and glutathione peroxidase

The paraffin-inserted heart was sliced into 4 μm parts and firmly fixed on positively charged slides for the expression of caspase-3 immunohistochemistry. This was carried out using peroxidase/anti-peroxidase (PAP) procedure. Methanol consisting of 0.1% H_2O_2 was used for the extensive peroxidase reaction. The four parts were nurtured with natural goat serum to prevent extensive reactions, with some particular antibodies. The tissue parts were then cleaned with phosphate buffer and nurtured with secondary antibodies (1:2000; Sigma, USA). It was cleaned with phosphate buffer and nurtured with the PAP procedure (dilution, 1:200). The peroxidase reaction was carried out using a solution of 3, 3′-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6).

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

Effects of xanthone on 2-methoxyethanol-induced changes in creatinine kinase-MB, lactate dehydrogenase and malondialdehyde

The results of the CK-MB, LDH, and MDA levels in each group are shown in Table 1. The administration of 2-ME on mice caused a significant increase of CK-MB, LDH, and MDA ($P < 0.05$) when compared with the control group. However, the treatment with xanthone in mice only at dose 240 mg/kg BW but not at dose 60 mg/kg and 120 mg/kg BW showed a significant decrease ($P < 0.05$) in CK-MB, LDH, and MDA level as compared to the 2-ME group.

Effects of xanthone on the superoxide dismutase expression in against 2-methoxyethanol-induced cardiotoxicity

Table 2 and Figure 1 showed the results of xanthone on the expression of SOD in against 2-ME-induced cardiac cell toxicity. The administration of 2-ME on mice caused a significant decrease in the expression of SOD of cardiac tissue compared to the control group ($P < 0.05$). The treatment xanthone group at dose 240 mg/kg BW but not at dose 60 mg/kg and 120 mg/kg BW increase cardiac tissue SOD expression in mice induced-2-ME which significantly was different to the 2-ME group ($P < 0.05$).

Effects of xanthone on the glutathione peroxidase expression in against 2-methoxyethanol induce cardiotoxicity

Table 2 and Figure 2 showed the results of xanthone on the expression of GPx in against 2-ME induce cardiac cell toxicity. The administration of 2-ME on mice caused a significant decrease in the expression of GPx of cardiac tissue was compared to the control group ($P < 0.05$). Treatment with xanthone at dose 240 mg/kg BW but not at dose 60 mg/kg and 120 mg/kg BW increase cardiac tissue GPx expression which significantly was different to the 2-ME group ($P < 0.05$).

Effects of xanthone on 2-methoxyethanol induce cardiac cell damage

Histopathological study was conducted using light microscopy. Histological investigation on the control group showed that in a cardiac cell have a normal structure. In the administration of 2-ME in mice showed cardiac cell damage (necrosis). In the treatment with xanthone, the number and morphological integrity of cardiac cells are being maintained. The results show that the cardiotoxic effects of 2-ME were inhibited by xanthone [Figure 3].

DISCUSSION

The CK-MB and LDH are biomarkers measured to evaluate heart function. They can be useful in the early prediction of cardiotoxicity. The serum CK-MB and LDH level are the best markers of cardiotoxicity due to cardiac tissue damage.[5,6] In our results showed that the administration of 2-ME resulted in a significant increase in the level of the LDH and CK-MB was compared with the control group. This suggests that the 2-ME might cause cardiac lipid peroxidation leading to cardiac cell damage followed by the secretion of CK-MB and LDH into the serum.

Toxic effects mechanism of 2-ME are via oxidative damage on the cardiac cell by increasing the generation of ROS which consist mainly of O_2, H_2O_2 and OH^-. Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, altered antioxidant system, DNA damage, altered gene expression, and apoptosis.[13,15] In addition, 2-ME also decreasing endogenous antioxidants which cause significantly to the development of toxic oxidative stress.[8] If these ROS-mediated oxidative stress are not balanced by repair processes, affected cells undergo apoptosis or necrosis.[11]

When the antioxidant failure to inhibit the formation of ROS (free radicals) so can enhance lipid peroxidation and causing cellular toxicity, which produces MDA. In our investigation showed that the administration of 2-ME on mice can cause

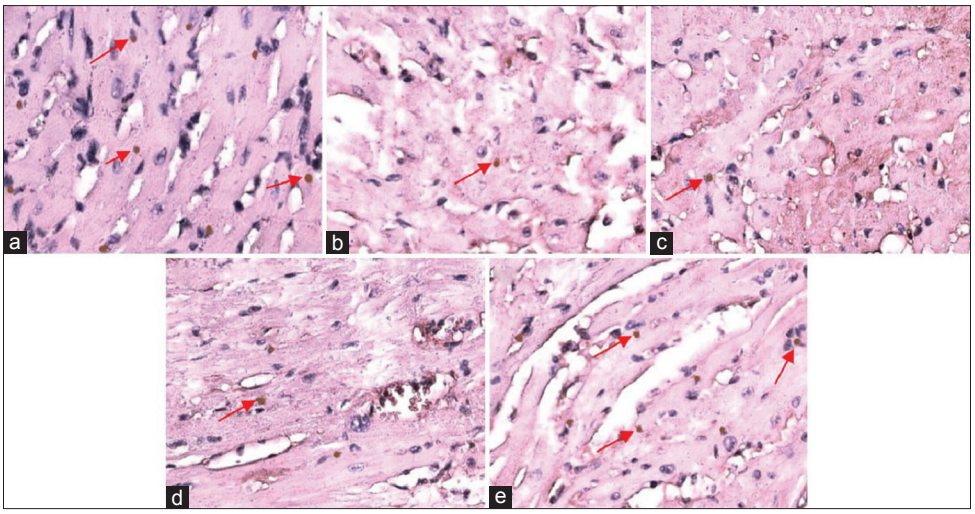

Figure 1: Immunohistochemical study of xanthone on superoxide dismutase expression (indicated by red arrows) of 2-methoxyethanol-induced cardiotoxicity. Control group (a); 2-methoxyethanol group (b); mice treated with xanthone 60 mg/kg BW; 120 mg/kg BW, and 240 mg/kg (c-e)

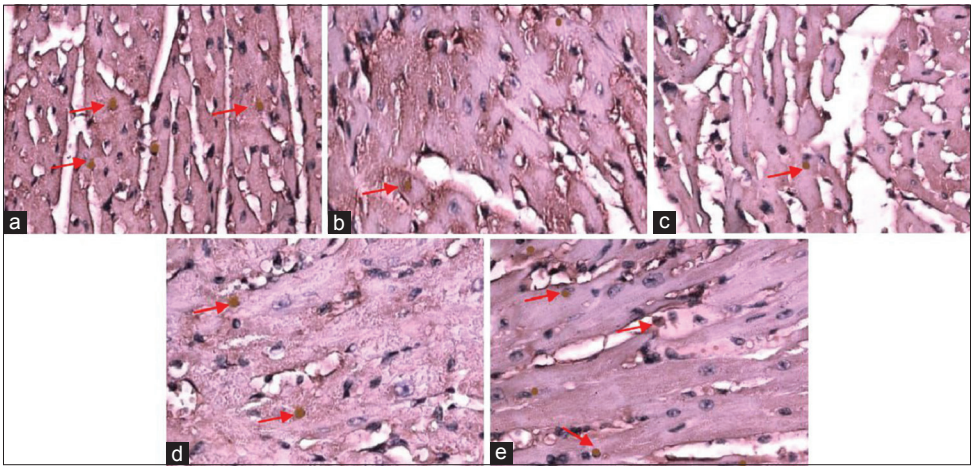

Figure 2: Immunohistochemical study of xanthone on glutathione peroxidase expression (indicated by red arrows) of 2-methoxyethanol-induced cardiotoxicity. Control group (a); 2-methoxyethanol group (b); mice treated with xanthone 0 m6g/kg BW; 120 mg/kg BW, and 240 mg/kg (c-e)

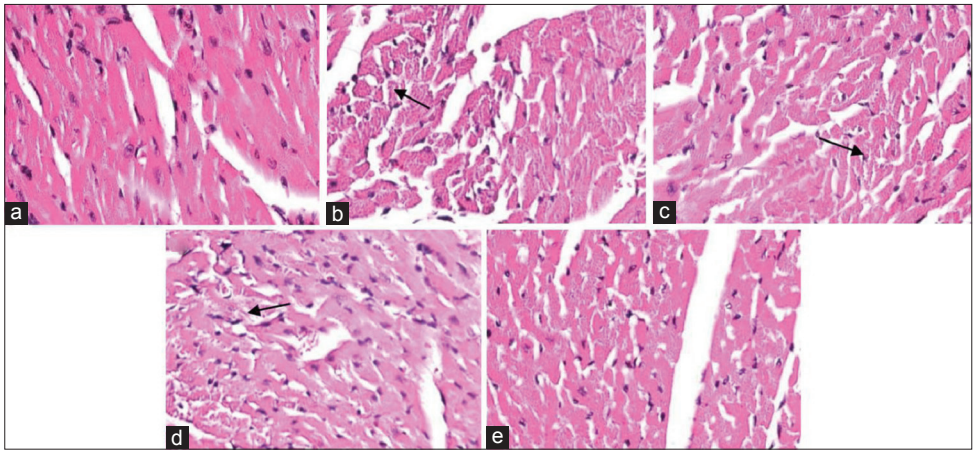

Figure 3: Histological study of pretreatment of xanthone on 2-ME-induced cardiac cell damage. The controls group showed normal morphology of the heart (a). The treatment 2-ME group showed necrosis (indicated by black arrows) (b). Pretreatment xanthone 60 mg/kg BW and 120 mg/kg BW showed necrotic changes (c and d). However, pretreatment xanthone 240 mg/kg showed regeneration on cardiac cells damage (e) H and E, ×400

Table 1: Cardioprotective effect of xanthone on CK-MB, LDH and MDA against 2-ME induce cardiotoxicity

Groups	Means±Standard Deviation		
	CK-MB (IU/L)	LDH (IU/L)	MDA (nmol/mg tissue)
Control group	66.7^{a}±6.35	101.6^{a}±8.41	52.72^{a}±4.18
2-ME group	104.5^{b}±8.53	154.7^{b}±13.42	76.23^{b}±6.16
Xanthone 60 mg/kg BW	98.2^{b}±7.82	148.4^{b}±16.72	73.42^{b}±5.32
Xanthone 120 mg/kg BW	91.7^{b}±6.35	141.7^{b}±9.37	67.79^{b}±4.36
Xanthone 240 mg/kg BW	76.1^{c}±5.78	126.3^{c}±9.63	59.36^{c}±4.48

^{a,b,c}Different superscript within each column indicate significant difference between the means ($P<0.05$)

Table 2: Cardioprotective effect of xanthone on SOD and GPx expression in against 2-ME induce cardiotoxicity

Group	Means±Standard deviation	
	SOD expression	GPx exoression
Control group	9.8^{a}±1.14	8.7^{a}±0.94
2-ME group	3.2^{b}±0.52	2.6^{b}±0.52
Xanthone 60 mg/kg BW	2.9^{b}±0.51	3.3^{b}±0.71
Xanthone 120 mg/kg BW	4.8^{b}±0.62	3.9^{b}±0.87
Xanthone 240 mg/kg BW	7.2^{c}±0.89	6.2^{c}±0.69

^{a,b,c}Different superscript within each column indicate significant difference between the means ($P<0.05$)

a significant increase in cardiac tissue MDA. This result suggests that 2-ME-induced cardiotoxicity on mice can be caused by increasing free radicals.

ROS are efficiently detoxified by antioxidant enzymes such as SOD and GPx in normal healthy conditions. It has been reported that antioxidant activity or inhibition of generation of free radicals plays a crucial role in protection against 2-ME-induced toxicity. In the administration of 2-ME, the expression of SOD and GPx of cardiac tissue was significantly decreased compared to the control group. The activities of SOD and GPx have been used to determine oxidative stress in cells. This treatment with 2-ME inhibited SOD, and GPx activities are in agreement with previous studies. This results suggested that administration of 2-ME can cause oxidative stress by decreasing the activity of antioxidant enzyme SOD and GPx. The decrease in the functions of SOD and GPx in 2-ME-induced cardiotoxicity might be as a result of an increased production of reactive oxygen radicals like superoxide and hydrogen peroxide, which in turn causes the prohibition of the functions of these enzymes. The outcomes are the same with the discoveries of Adedara and Farombi (2010) that recorded a huge decrease in SOD, Catalase, and GPx activities and a notable increase in the MDA level in the testis of rats susceptible to 2-ME treatment. The administration of herbal medicine that contains antioxidant properties such as *M. pruriens*, *W. somnifera*, and *G. mangostana* have been proven can be used as a protector on 2-ME intoxication through the increasing SOD and GPx expression, and also inhibiting LDH, CK-MB and MDA.[7]

It has been reported that protective agents against free radicals, such as antioxidants, might be effective preventives for 2-ME toxicity. The natural product can be a better alternative as the antioxidant as a result of their economic costs, availability, and deficiency of undesirable adverse effects.[13,15]

This research concentrated on the protective effect of herbal medicine having antioxidant properties of xanthone for protective in decreasing free radical-induced cardiac cell damage. Phytochemical studies of *G. mangostana* prove that this plant contains xanthones which have very strong antioxidant activity. The administration of the xanthone, only doses 240 mg/kg BW significantly decreased LDH, CK-MB, MDA, and increase expression of SOD and GPx on cardiac cell damage induced by 2-ME. The xanthone 240 mg/kg BW also demonstrated significantly improved heart cell damage. It has been reported that xanthone is a well-known scavenger of free radicals and can greatly prohibit the production of ROS like superoxide anions, H_2O_2, and OH-radical generation.[21] This effect has played an important role in decreased MDA, CK-MB, LDH and increased SOD, GPx expression in against 2-ME-induced cardiotoxicity. It also improved heart cell damage. These result indicated that the cardioprotective effects of xanthone are greatly related to play an essential role in reducing LDH, CK-MB, MDA and raising SOD, GPx expression against 2-ME-induced cardiotoxicity. It also can inhibit cardiac cell injury. The results prove that the cardioprotective consequences of xanthone are hugely linked to their antioxidative activities. Devi and Vijayaraghavan (2007) also reported that xanthone decreased MDA, CK-MB, LDH, and increased SOD, GPx level on isoproterenol-induced myocardial infarct.

CONCLUSION

The outcome of the current research showed that the 2-ME-induced cardiotoxicity can be associated with oxidative stress. The treatment with xanthone can prevent 2-ME-induced cardiac cell damage through increasing antioxidant enzyme (SOD and GPx) and inhibiting MDA, CK-MB, and LDH. Furthermore, protective effects of xanthone can be developed to treat patients with 2-ME-induced testicular toxicity.

Financial support and sponsorship

This work was supported in part by grant from the Educational Fund Management Institution (LPDP), Ministry of Finance, Republic of Indonesia, through Doctoral Program, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

Conflicts of interest

There are no conflicts of interest.

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