Modulation of Paraoxonase Activity (PON)-1 by Xanthone in Sub Chronic Exposure of Organophosphate : Antioxidant in Dichorvos Intoxicity

Titin Andri Wihastuti1, Djanggan Sargowo2, Teuku Heriansyah3, Grace Rahmawati4 & Yuni Hendrati Sulfia4

1Department of Biomedical, Faculty of Medicine, Brawijaya University, Malang, Indonesia
2Department of Cardiology, Faculty of Medicine, Brawijaya University, Malang, Indonesia
3Department of Cardiology, Faculty of Medicine, Syiah Kuala University, Aceh, Indonesia
4Department of Medical Education Programs, Faculty of Medicine, Brawijaya University, Malang, Indonesia

Correspondence and requests for materials should be addressed to T. A. Wihastuti (diktitin@yahoo.com)

Received 10 January 2015 / Received in revised form 4 June 2015
Accepted 11 June 2015
DOI 10.1007/s13530-015-0232-2
©The Korean Society of Environmental Risk Assessment and Health Science and Springer 2015

Abstract
This research aims to find out the levels of xanthone in mangosteen pericarp extract (MPE) and to prove the ability of xanthone in modulating the activity of paraoxonase-1 (PON-1), reduce oxidized-low density lipoprotein (ox-LDL) and increase the levels of acetylcholinesterase (AChE) serum in animal model with organophosphates subchronic and subcutaneous exposure. This research is a true experimental laboratory with in vivo approach to post-test with control group, using 25 animal models of Wistar strain of Rattus novergicus, were exposed to dichlorvos as organophosphate (2 mg/kgBW/day) for 21 days. Those animal models are divided into no exposure group, organophosphate exposure group, and organophosphate exposure plus administration of xanthone groups. The parameters (levels of PON-1, ox-LDL and AChE measured by ELISA Test. The results showed that administration of xanthone significantly increased the levels of AChE, decreased levels of ox-LDL and PON-1.

Keywords: PON-1, ox-LDL, AChE, Mangosteen pericarp extract, Xanthone, Organophosphat

Introduction
Organophosphate is effective and acceptable to protect crops from pests and have a significant contribution in improving agricultural productivity and crop. Organophosphate is a special chemical substance designed to control pests, weeds or plant diseases1,2. In medical treatment, some organophosphate are used on myasthenia gravis, glaucoma, plasticizer, stabilizer in lubrication and hydraulic oil3,4.

Accumulation of organophosphate pesticide residues in humans can cause both acute and chronic poisoning. Organophosphate intoxication is associated with increased lipid peroxidation, decreased levels of glutathione and increased oxidative stress5,6. The role of organophosphates as free radicals are to increase the modification of LDL process become oxidized-LDL. Ox-LDL is one of the main sources of the atherosclerotic process through the formation of ROS (Reactive Oxygen Species) which activates NF-κB as proinflammatory transcription factor and increases the expression of TNF-α. NF-κB stimulates adhesion of proteins ICAM-1 and TNF-α cytokines. ROS, cytokines of inflammation, adhesion molecule (ICAM-1 and VCAM-1), Ox-LDL scavenger receptor which is recognized by the macrophages, could initiate the formation of foam cells as the initiation of atherosclerosis and cause improvement of vascular inflammation7. The accumulation of ox-LDL in the blood will increase the incidence of cardiovascular disease.

On the other hand, the use of organophosphate also have a negative impact because these compounds inhibit AChE, resulting in an increasing accumulation of acetylcholine (ACh) which can increase neural activity followed by symptoms such as headache, nausea, vomiting, shortness of breath, muscle spasms and can lead to paralysis. Factors affecting organophosphate intoxication, are the dose, toxicity, long periods of exposure and its route of entry into the body8. The high prevalence of poisoning seen from the results of cholinesterase activity measurements in the blood, may become an indicator of high exposure of organophosphate9.

Antioxidant agents are considered to be capable in
stimulating PON-1 activities. Paraoxonase (PON)-1 is an enzyme in the blood serum which is linked to high density lipoprotein (HDL) secreted by liver. PON-1 status related to environment factor and the increased risk of cardio vascular disease (CVD). One of the antioxidants which has those potency, was xanthone contained in mangosteen pericarp. Xanthone is an antioxidant from polyphenol component which cannot be synthesized in human body. Because of that, polyphenol consumption from diet is very important to do. Xanthone have derivations, namely 3-isomangosteen, α-mangostin, γ-mangostin, garcinone A, garcinone B, C, D, E, maclurin and mangostenol.

Based on the explanation above, This study aims to find out the levels of xanthone in mangosteen pericarp extract and to conduct the ability of xanthone contained in mangosteen pericarp extract in modulating PON-1 activity, as efforts to protect cardiovascular system from sub chronic exposure of organophosphate.

### Results

Based on Table 1, we can conclude that the level of xanthone on crude mangosteen pericarp extract was higher in the outer part than in the inner part. Therefore, we used extract of outer part of mangosteen pericarp as prophylaxis and treatment for dichlorvos intoxication. Table 2 gives the results of the analysis of the studied parameters using the one-way ANOVA (p < 0.050) and post hoc LSD tests. The data of each parameters should be normally distributed and homogeneity, before we did the one-way ANOVA test. So we used Kolmogorov-Smirnov test to observe whether the data were normally distributed or not. Then, we continued with Levene test to observe the homogeneity of the data of each parameters. The parameters were measured in all treatment groups to observe the advantages of the administration of xanthone. The significance of the administration of xanthone was determined after the p values taken from the measurements of the parameters were compared with the standard p value of the ANOVA test.

### The Average Level of Paraoxonase-1 (PON-1)

Based on the diagram in Figure 1, it is known that the highest average level of PON-1 is in the PC group (2.8935 nmol/L) and the lowest average level is in the NC group (1.23075 nmol/L). The results of Kolmogorov-Smirnov test and Levene test showed that the data of ox-LDL levels in each groups were normally distributed and homogeneity. Statistical analysis using ANOVA showed that the administration of dichlorvos (2 mg/kgBW/day) subcutaneously for 21 days had an obvious effect in the significant increased of PON-1 levels (p = 0.037, p < 0.05). Post Hoc Test results using LSD showed that there is significant difference (p < 0.05) of PON-1 levels between the PC and NC group.

The decreased level of PON-1 seen in the group treated with xanthone. Statistical analysis using ANOVA

### Table 1. Level of xanthone on crude mangosteen pericarp extract measured by HPLC method.

<table>
<thead>
<tr>
<th>Xanthone</th>
<th>Outer pericarp</th>
<th>4591 μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner pericarp</td>
<td>3911 μg/g</td>
</tr>
</tbody>
</table>

### Table 2. Measurement and analysis of parameters by ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Level (mean ± standard deviation)</th>
<th>PON-1 (nmol/L) (p value = 0.037*)</th>
<th>AChE (nmol/L) (p value = 0.006*)</th>
<th>ox-LDL (μg/L) (p value = 0.024*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorvos exposure</td>
<td>2.8935 ± 0.31425</td>
<td>45.75 ± 26.2587</td>
<td>3.5205 ± 0.21465</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>1.23075 ± 0.1122</td>
<td>99.5625 ± 9.35142</td>
<td>2.51325 ± 0.508188</td>
<td></td>
</tr>
<tr>
<td>Xanthone 70 mg</td>
<td>2.286 ± 0.32871</td>
<td>40.27275 ± 13.02612</td>
<td>1.7055 ± 0.0671184</td>
<td></td>
</tr>
<tr>
<td>Xanthone 140 mg</td>
<td>1.84275 ± 0.31</td>
<td>85.25025 ± 30.44256</td>
<td>1.6365 ± 0.569646</td>
<td></td>
</tr>
<tr>
<td>Xanthone 210 mg</td>
<td>2.14475 ± 0.442892</td>
<td>57.5625 ± 22.542624</td>
<td>2.238 ± 0.56418</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 indicates the significant difference.

Abbreviations: PON-1, paraoxonase-1; AChE, acetylcholinesterase; ox-LDL, oxidized low density lipoprotein; ANOVA, analysis of variance
showed that the administration of xanthone had significant effect ($p = 0.037$, $p < 0.05$) in the decreased level of PON-1. Post Hoc Test result using LSD showed that there was significant difference ($p < 0.05$) of the PON-1 levels between the treatment groups which were given xanthone in various doses. The results indicate that different doses of xanthone used in the treatment groups (xanthone 70 mg, xanthone 140 mg, xanthone 210 mg) has impacted significantly in the decreased of PON-1 levels in rat blood serum.

The Average Level of AchE

Based on the diagram in Figure 2, it is known that the highest average level of AChE was found in NC group (99.5625 nmol/L). Whereas the lowest average level of AChE was found in the xanthone 70 mg group (40.27275 nmol/L). AChE level in the xanthone 70 mg group is lower than AChE level in the PC group is due to biological factors that we can not control, such as the metabolism process in each rats’ body. But the difference of AChE level in PC and xanthone 70 mg group is not significant, based on Post Hoc test using LSD. The results of Kolmogorov-Smirnov test and Levene test showed that the data of AChE levels in each groups were normally distributed and homogeneity. Statistical analysis using ANOVA showed that the administration of diclorvos (2 mg/kgBW/day) subcutaneously for 21 days, significantly ($p = 0.006$, $p < 0.05$) decreased AChE levels. Post Hoc Test result using LSD showed that there was significant difference ($p < 0.05$) of AChE levels between PC and NC group.

The lower level of AChE was seen in the group treated with xanthone compared to NC, but those AChE level was higher if it was compared to PC. Statistical analysis using ANOVA showed that the administration of xanthone contained in MPE have significant effects ($p = 0.006$, $p < 0.05$) in the increased of AChE levels in rat blood sera compared to PC group. The AChE levels in treatment groups were less than AChE level in NC group because the exposure of dichlorvos still had a strong effect in suppressing AChE levels, even though we had given antioxidants. But at least, after administration of xanthone, the AChE levels in xanthone 140 mg and 210 mg groups increased and almost reach the normal level in the NC group. Post Hoc Test result using LSD showed that there was significant difference ($p < 0.05$) of AChE levels between the treatment groups (xanthone 70 mg, 140 mg and 210 mg) which were given with xanthone at various doses. The results indicate that different doses of xanthone used in the treatment groups has impacted the increased of AChE levels in rat blood serum, and the effect is significant.

The Average Level of Ox-LDL on Every Group

Based on Figure 3, the highest average level of Ox-LDL was found in the PC group (3.5205 μg/L) and the lowest average level was found in the xanthone 140 mg group (1.6365 μg/L). The results of Kolmogorov-Smirnov test and Levene test showed that the data of ox-LDL levels in each groups were normally distributed and homogeneity. Statistical analysis using ANOVA showed that the administration of diclorvos (2 mg/kgBW/day) subcutaneously for 21 days, significantly ($p = 0.024$, $p < 0.05$) increased ox-LDL levels. The decreased levels of ox-LDL were visible in the group treated with xanthone. Statistical analysis using ANOVA showed that the administration of xanthone have significant effects ($p = 0.024$, $p < 0.05$) in the increased levels of ox-LDL. Post Hoc Test result using LSD showed that there was significant difference ($p < 0.05$) of ox-LDL levels, between the PC, NC groups, various doses of xanthone (70 mg, 140 mg and 210 mg) groups given in the treatment groups.
**Discussion**

Dichlorvos extensively stored in fat and can cause dichlorvos intoxication. Dichlorvos components are generally lipophilic that can cross the blood brain barrier and blood vessel barrier. These conditions increase the risk of cardiovascular disease. According to Agency for Toxic Substances and Disease Registry (ATSDR), dichlorvos can induce myocard necrosis. Cardio manifestations which can occur are sinus bradycardia, sinus tachycardia, hypertension, hypotension and the disturbances in cardiac rhythm and contractility. The other research also shows that dichlorvos intoxication is associated with the decreased levels of glutathione, increase lipid peroxidation and oxidative stress.

PON-1 prevents the formation of ox-LDL and inactivates phospholipid oxidation which has formed. PON-1 also protects HDL from oxidation. This statement supports the role of PON-1 in atherosclerosis and CVD. PON-1 also metabolizes various drugs through lactonase activity. In addition, a high PON-1 activity may reduce dichlorvos that entered the rat bodies. The evidence of PON-1 activity can be observed from AChE levels measured in rat blood serum. Whereas the result of PON-1 activity on preventing the formation of ox-LDL and inactivating phospholipid oxidation can be observed from ox-LDL levels measured in rat blood serum.

Overall, the average levels of PON-1 in all groups with MPE administration are lower than the PC group and higher than the NC group. This study showed that the administration of MPE had significant effect in decreasing the level of PON-1 compared to PC group that increased PON-1 level. It has proven that the administration of MPE as a prophylaxis of dichlorvos intoxication, affect the body defense mechanism in reducing dichlorvos sub chronic intoxication, so that the body don’t have to produce a lot of PON-1, because PON-1 activity has been assisted by antioxidants. The results indicate that various doses of MPE used in the treatment groups has a definite impact in the decreased of PON-1 levels in rat blood serum. This statement is supported by the result of one-way ANOVA test and post hoc LSD, which showed that the effect of antioxidant contained in MPE, which were given orally, can significantly (p < 0.05) affect the decreased of PON-1 levels in rat blood serum. 140 mg of xanthone is the best dose to assist the activity of PON-1 compared to the other doses of xanthone (70 mg and 210 mg) in this study.

Antioxidants help PON-1 activity after sub chronic intoxication of dichlorvos by preventing oxidative inactivation. As a result, after administration of antioxidants, PON-1 level decreased. Furthermore, the role of antioxidants in diet as a modulator of PON-1 activity requires further research.

At normal environmental conditions, reactive oxygen species (ROS) which was produced are detoxified by antioxidants contained in the human body and there is a balance between ROS and antioxidants. To prevent excessive ROS production, the exogenous antioxidant (xanthone) is given with dose of 70 mg/kgBW/day, 140 mg/kgBW/day and 210 mg/kgBW/day. Xanthone with hydrogen atoms or electrons donation can reduce ROS productions, thereby reduce the activity and modification process of peroxidation or oxidation of LDL. By decreasing LDL oxidation activity, it decreases the formation of ox-LDL and reduce ox-LDL levels in the rat blood serum.

The average levels of ox-LDL in all groups with xanthone administration are lower than the PC group and NC group. This study showed that the administration of xanthone in various dose had definite effects in decreasing the level of ox-LDL compared to PC and NC group which had higher levels of ox-LDL. This statement is supported by the result of one-way ANOVA test, which showed that the effect of xanthone, which were given orally, can significantly (p < 0.05) affect the decreased of ox-LDL levels in rat blood serum. 140 mg of xanthone is the best dose to reduce ox-LDL levels compared to the other doses of xanthone (70 mg and 210 mg) in this study, based on post hoc LSD test. This results indicate that various doses of xanthone used in the treatment groups has impacted the decreased of ox-LDL levels in rat blood serum, and the effect is significant.

An irreversible binding of dichlorvos to AChE and then inactivated it, is believed to be the main mechanism of dichlorvos toxicity. The high prevalence of dichlorvos poisoning can be seen from the results of cholinesterase activity measurements in the blood, and may become an indicator of high exposure of dichlorvos. The average levels of AChE in xanthone 140 mg and 210 mg groups are higher than the PC group but lower than NC group. This means that dichlorvos administration was proven to decrease AChE level significantly. The AChE levels in treatment groups were less than AChE level in NC group because the exposure of dichlorvos still had a strong effect in suppressing AChE levels, eventhough we had given antioxidants. But at least, after administration of xanthone, the AChE levels in xanthone 140 mg and 210 mg groups increased and almost reach the normal level in the NC group.

This study showed that the administration of xanthone had a definite effect in increasing the level of AChE compared to PC and NC groups, because xanthone can help PON-1 activity in hydrolysing the active...
metabolites of dichlorvos. This active metabolites of dichlorvos can cause an increase in ROS production and evoke dichlorvos’ side effects. 140 mg of xanthone is the best dose to increase AChE levels compared to the other doses of xanthone (70 mg and 210 mg) in this study, based on post hoc LSD test. This results indicate that various doses of xanthone used in the treatment groups has impacted the increased of AChE levels in rat blood serum, and the effect is significant. So, xanthone contained in MPE definitely can improve the AChE level into a normal level, and help the activity of PON-1 to decrease ROS production after sub chronic exposure of dichlorvos.

Conclusions

Dichlorvos exposure (2 mg/kgBW/day) subcutaneous for 21 days affected the increased levels of ox-LDL and PON-1, and also the decreased levels of acetylcholinesterase (AChE) in animal models of Wistar Strain of *Rattus Novergicus*. The outer part of mango pericarp contain 4591 μg/g of xanthone, is able to modulate the activities of PON-1 in reducing the accumulation of dichlorvos, decreased the ox-LDL levels and increased the AChE levels in rat blood serum. Antioxidant administration has been proven to have an important role in providing body protection against sub chronic exposure of dichlorvos. The most effective dose of xanthone to increase AChE, decrease PON-1 and ox-LDL levels in this study was 140 mg/kg body weight. Future research on Mangosteen is required to further explore the advantages of MPE in the treatment of dichlorvos intoxication.

Materials and Methods

Study Group

25 animal models of Wistar strain of *Rattus novergicus*, 2 months of age weighing about 150-200 g were bought from the breeder in Physiology laboratory, Medical Faculty of Brawijaya University. Those animal models were divided into 5 groups and each group consists of 5 rats. Those animal models are divided into positive control group (PC) (2 mg/kgBB/day subcutaneous injection of dichlorvos), negative control group (NC) (no exposure to dichlorvos), xanthone 70 mg group (2 mg/kgBB/day subcutaneous injection of dichlorvos + 400 mg/kgBW/day dose of MPE), xanthone 140 mg group (2 mg/kgBB/day subcutaneous injection of dichlorvos + 800 mg/kgBW/day dose of MPE), xanthone 210 mg group (2 mg/kgBB/day subcu-

Mangostene Pericarp Extract (MPE)

250 mg of MPE (Laboratory of Pharmacology, Faculty of Medicine, Brawijaya University, Indonesia) was produced from 1 kg of mangosteen pericarp bought in Lumajang, East Java, Indonesia. The extraction processes consist of drying, extraction with ethanol solvent and evaporation. First, mangosteen pericarps were washed, chopped and dried. Then, we put it in a blender until it became powder. 100 g of dry sample were put into an erlenmeyer glass (1 litre) and soaked with ethanol until the volume reached 1000 mL. We mixed it completely for 30 minutes and let it precipitate for one night until we could get the sediment. We took the sediment and put it into 1-liter evaporation flask. Then, we put the evaporation flask in the evaporator. We fully filled the water bath with water. We put all the tools, including a rotary evaporator, heating the water bath (set to 90°C) and connect it with the electricity. We let the ethanol solution separates with the existing active substance in the flask. We waited until the ethanol flow stops dripping in the reservoir flask (approximately 1.5 to 2 hours for 1 flask). We got approximately one-quarter of MPE from the dry natural materials.

Dichlorvos and MPE are given simultaneously, because we want to discern whether xanthone contained in MPE can be a prophylaxis of dichlorvos’s sub chronic intoxication. The method which was used to label the animal models was simple random sampling. The research was held in physiology and pharmacology laboratories, Faculty of Medicine, Brawijaya University Malang. The research was fully equipped with ethical clearance assessment by the Ethical Committee of Health Research in Faculty of Medicine, Brawijaya University.

Biochemical Test

Level of Xanthone and Alpha Mangosteen on Crude Mangosteen Pericarp Extract Measured by HPLC Method

High Perfoma Liquid Chromatography (HPLC) method was applied to determine xanthone level contained in mangosteen pericarp. Preparation of alpha Mangostin (ChromaDex) and Xanthones (Sigma Aldrich) Standard Solution were dissolved in methanol, 100 mL
of inner and outer mangosteen pericarp Crude extract for each part, which are taken by using pipettes into a 10 mL volumetric flask and add 10 mL of methanol. The solution was filtered using a membrane filter with size of 0.45 μm. High-performance liquid chromatography (HPLC) analysis for the contain of Xanthone and Alpha mangosteen on crude extract of mangosteen pericarp was done at room temperature with a flow rate of 0.5 mL/minute, λ = 337 nm and the ratio methanol : aquabidest for mobile phase was 95 : 5%. The result of HPLC (Waters Systems, MA, USA) test showed that MPE used in this study has xanthone level around 17.5%. So, 400 mg of MPE used in this study contain 70 mg of xanthone, 800 mg of MPE used in this study contain 140 mg of xanthone and 1200 mg of MPE used in this study contain 210 mg of xanthone.

ELISA Test

The levels of PON-1, ox-LDL and AChE in rats blood serum were measured by ELISA test. Three different ELISA kits (BT Lab, Gwang-dong, South Korea) used for measuring each parameter. ELISA’s procedures begin with preparation of ELISA kits. The first ELISA steps for PON-1, AChE and ox-LDL were the addition of the standard, sample diluent and incubated it for 30 minutes at 37°C using Hett Cube 200R (Hettich Lab Technology, Germany). Then, washed it for 5 times using multichannel pipet, added HRP-conjugate reagent and incubated it for 30 minutes at 37°C. Next, washed it for 5 times, added chromogen solution A and B, and then incubated it for 30 minutes at 37°C. After that we added stop solution and read absorbance at 450 nm immediately with ELISA Reader (Tecan Infinite M1000 Microplate Reader, Tecan, MA) within 15 minutes. The last step was the calculation of the results.

Statistical Analysis

Statistical methods used in this study were Kolmorogov-Sirniov for normality test and Levene for homogeneity test. Whereas, for significance test we used One way ANOVA to determined the effects of MPE on PON-1, AChE and ox-LDL in Wistar rats with subchronic exposure to dichlorvos. This analysis was continued by the post hoc test using the LSD method to detect the differences of parameters in each treatment group. Statistical analysis was performed with SPSS software version 20 (IBM Corporation, Armonk, NY, USA).

Abbreviations

Abbreviations: PC, positive control (Dichlorvos Exposure); NC, negative control; SD, standart deviation; PON-1, paraoxonase-1; AChE, acetylcholinesterase; ox-LDL, oxidized low density lipoprotein; MPE, Mangostene Pericarp Extract; CVD, Cardiovascular Disease; HPLC, High-performance liquid chromatography.

Acknowledgements

This work is supported by the scientific Grant of Health Professional Education Quality (HPEQ) program of the Medical Faculty of Brawijaya University, 2013. This work was performed under the benevolence of the Head of the Medical University and with the kind collaboration of the Department of Cardiology, Laboratory of Pharmacology and Laboratory of Physiology, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

References