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The Effects of Extra Virgin Olive Oil on Lox-1 and COX-2 in High Fat Diet Rats

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ABSTRACT

Background: High fat diet (HFD) is a diet containing large amounts of fat consistently. The increase dietary fat and cholesterol have a key role in development of health problems. Extra virgin olive oil associated with prevention of LDL oxidation, beneficial changes in lipid ratios and low risk for Coronary Heart Disease.

Objective: To determine the anti-inflammatory and anti-oxidant effect of extra virgin olive oil extract to blood levels of COX-2 and LOX-1 in rats induced by high fat diet.

Method: This research is used randomized posttest only control group design. Thirty Wistar rats were divided into five groups: group of control (-) which received normal diet and group of control (+) which received HFD without EVOO treatment and three HFD groups treated by EVOO 1 mL/kg BW/day, 2 mL/kg BW/day and 3 mL/kg BW/day orally for 2 months. The blood was collected from rat eyes and serum was collected after centrifugation. COX-2 and LOX-1 concentrations were measured by the enzyme linked immunosorbent assay (ELISA).

Results: The result showed higher COX-2 concentrations in groups treated with EVOO than control (-) group. COX-2 serum levels of negative control where significantly lower than those of rats treated with 2 ml/kg/day ($p = 0.047$) and 3 ml/kg/day EVOO ($p = 0.014$). The COX-2 serum levels of group received 1 ml/kg/day were significantly lower than those of rats received 3 ml/kg/day EVOO ($p = 0.027$). LOX-1 concentrations showed no significant deference among all groups ($p = 0.570$).

Conclusion: It's demonstrated in this study that extra virgin olive oil extract has only minor anti-inflammatory and antioxidant effect in rats with high fat diet.

Keywords: EVOO, HFD, LOX-1, COX-2.

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INTRODUCTION

High fat diet is a diet which contains more than 35 percent of calories from fat consistently. The increase dietary fat and cholesterol becomes a key role inthe development of health problems. According to Health Organizations reports, the risk factors of high fat diet (cardiovascular disease, atherosclerosis) are the causes of more deaths than all other combined causes that become the pre-eminent health problem worldwide especially in developed countries. Atherosclerosis has been related to the immune reactivity-mediated disruption of the vascular system, characterized by inflammation and dysfunction of the lining of the involved blood vessels and to build cholesterol, lipids and cellular debris.

LOX-1, a receptor for ox-LDL, is present primarily on endothelial cells. Ox-LDL stimulates the overlying endothelial cells (ECs) to produce adhesion molecules, chemotactic proteins such as monocyte chemoattractant protein-1(MCP-1). These pro-inflammatory molecules recruit monocytes and lymphocytes to the sub-endothelial region of the vessel wall. Cyclooxygenase (COX), the key enzyme required for the conversion of arachidonic acid to prostaglandins, has an important role in biology and disease. COX-2 is normally unexpressed in most cells or tissues, but is expressed primarily induced in response to inflammatory stimuli by mitogens, growth factors, and cytokines. COX-2 has pro-inflammatory and anti-inflammatory effects. Macrophages are an abundant source of COX-2 enzymatic products, while nuclear factor-kappaB (NF-κB) is a major transcription factor involved in regulation of COX-2 gene expression. Extravirgin olive oil (EVOO) is source of high monounsaturated fat in Mediterranean diet.EVOO is considered the best, least processed Olive oil. EVOO which has phenolic compound was associated with decreased LDL cholesterol, increased HDL cholesterol, low risk for CHD. The aim of the present study was to observe the effect extra virgin olive oil on high fat diet (HFD) Wistar rats by measuring blood levels of COX-2 and LOX-1.

MATERIALS AND METHODS

Design of the Study

An experimental animal study using randomized posttest only control group design was performed from January to March 2014. The animal care was in the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) at Gadjah Mada University while and the blood examination for biomarker (LOX-1 and COX-2) measurement was done in the biochemistry department in the same University.

Animals

Thirty male Wistar rats (Rattus novergicus) aged 10 weeks and 150 - 200 grams weight, was obtained from and reared at the Animal Laboratory at Gadjah Mada University Yogyakarta. The animals were divided into five intervention groups, which were fed by the following diets: standard diet (negative control group), high fat diet (positive control group), high fat diet + EVOO 1 mg/kg/day, high fat diet + EVOO 2 mg/kg/day and high fat diet + EVOO 3 mg/kg/day. Before undergoing the interventions, the rats were adapted to laboratory conditions for 7 days. The interventions were conducted for 60 days, after that the rats were sacrificed to take blood through eyes and separating serum by using centrifuge.

Compositions of Animal Food

The standard diet consisted of PARS chicken feed 225 gram (containing water, protein, lipid, fiber, ash, calcium, phosphorus, antibiotics, and coccidiostatics), flour 100 gram, and water 100 ml. total diet 40 gram/rat. The high fat diet was a combination of standard diet (PARS) 200 gram, flour 100 gram, cholesterol 8 gram, cholic acid 0,8 gram, pig oil 40 ml, water 51,2 ml. Total high fat diet 40 gram/ rat.

Measurements of COX-2 and LOX-1

The ELISA assay was performed based on manufacturer guidance. These were in the follow steps: after determining the required number of wells, a standard curve was prepared, samples and standards were added, then the plates were incubated at 37°C for 60 min and then the plates were washed 5 times, after that chromogen solution A and B were added, the plates was incubated at 37°C for 10 min for color development, finally add stop solution and read. Absorbance readings were performed at an optical density (OD) at 450nm in a micro plate reader within 10 min after adding the stop solution, the measurements were in ng/mL.

Statistical analysis

Non-normally distributed data were analyzed using Kruskal-Wallis test followed by the Mann-Whitney test, with significant level or probability value 0,05 ($p = 0,05$) and confidence interval 95% ($\alpha = 0,05$).

Ethical clearance

This study was accorded ethical clearance by the Commission of Medical and Health Research Medical Faculty Diponegoro University with ethical clearance letter under number 176/EC/FK-RSDK/2014.

RESULTS

COX-2 serum concentration: The result of this study showed a higher COX-2 concentrations in groups treated with EVOO than control group (Table 1). Shapiro-Wilk test showed that data of most groups in this study had normal distribution ($p > 0.05$). Except group that received EVOO 1ml/kg/day ($p < 0.05$). COX-2 data in group receive 1 mL/kg/day have not normal by distributed. This therefore COX-2 data were analyzed using Kruskal-Wallis test.

This analysis showed a significant difference of COX-2 levels among groups ($p = 0.046$). Mann-Whitney test indicated that the COX-2 serum levels of negative control where significantly lower than those

of rats treated with 2 ml/kg/day ($p = 0.047$) and 3 ml/kg/day EVOO ($p = 0.014$). The COX-2 serum levels of group received 1 ml/kg/day were significantly lower than those of rats received 3 ml/kg/day EVOO ($p = 0.027$).

Table 1. COX-2 Levels among Groups.

Group	Mean (SD)Ng/ml
Negative control	2.5 ± 0.49
Positive control	2.9 ± 0.69
Treatment 1	3.2 ± 0.16
Treatment 2	3.3 ± 0.47
Treatment 3	3.7± 0.27

Note: Negative control (Standard diet); positive control (HFD); Treatment 1 (HFD + 1ml olive oil); Treatment 2 (HFD + 2ml olive oil); Treatment 3 (HFD + 3ml olive oil). Significant ($p < 0.05$).

LOX-1 serum concentration: Shapiro-Wilk test showed that the data was normally distributed in each groups ($p > 0.05$). The test of homogeneity of variances use Levene's test showed non homogeneity ($p = 0.016$). LOX-1 levels analyzed using Kruskal-Wallis test, were not significantly deferent among all groups ($p = 0.570$).

Table 2. LOX-1 Levels among Groups.

Group	Mean (SD)Ng/ml
Negative control	1.9 ± 0.31
Positive control	2.3 ± 0 .39
Treatment 1	2.4 ± 0.49
Treatment2	2.4 ± 0 .47
Treatment3	2.4 ± 0.99

Note: Negative control (Standard diet); positive control (HFD); Treatment 1 (HFD + 1ml olive oil); Treatment 2 (HFD + 2ml olive oil); Treatment 3 (HFD + 3ml olive oil). Significant ($p<0.05$).

DISCUSSION

Consumption of HFD together with EVOO in dose of 2 and 3 ml/ kg BW/ day result in significantly higher serum COX-2 levels than those consuming normal diet only. Significantly higher COX-2 levels were found in HFD group receiving EVOO in dose of 3 ml than 1 ml/ kg BW/ day. These findings were unexpected. Several compounds representing those in olive oil were able to impair the AA release, cyclooxygenase-2 (COX-2) expression, and prostaglandin E(2)/ leukotriene B(4) synthesis in PMA-stimulated RAW 264.7.[15] Whole blood in vitro study showed the ability of several phenolic of EVOO in reducing pro-inflammatory cytokines and prostaglandin E2 production.[16] EVOO is considered as fatty acid-rich diets. The long-term ingestion of a diet high fat contained in EVOO results in obesity and insulin resistance but protects endothelial function in rats.[17] Mechanisms involve in increasing COX-2 levels remain unclear and need to be elucidated in rats consuming a combination of EVOO and HFD. The vascular endothelial functions and metabolic syndrome should be further studied in rats consumed a combination of EVOO and HFD.

HFD used in this study did not have significant impact in increasing LOX-1. Previous studies showed that high fat diet increased oxidized LDL that activated LOX-1 to trigger the formation of reactive oxygen compounds to block PI3K.[18] ox-LDL is a crucial parameter in evaluating atherosclerotic disease.[6] Polyphenols are able to prevent LDL oxidation and platelet aggregation and to inhibit LOX and eicosanoid production.[18] Pretreatment of endothelial cells with statins reduces oxLDL-induced LOX-1 expression.[19]oxLDL easily enters into the monocyte–macrophages of the arterial wall and promote the atherosclerotic process.[20] ox-LDL has been shown to upregulate and activation expression of LOX-1.[19] Previous study showed oxidative stress markers decreased linearly with increasing phenolic content of the olive oil, particularly in markers that are directly associated with LDL oxidation.[21]

In this study HFD rats that received oral administration of EVOO extract had no significant different of LOX-1 concentration than those of groups without administration of EVOO extract. LOX-1 released in soluble form (sLOX-1).[22] Serum sLOX-1 is increased at an earlier stage of acute coronary syndrome (ACS).[23] ELISA measured sLOX-1 specifically and sensitively in human serum/plasma and can be used as a diagnostic test for ACS at the earliest stage.[24] HFD rats in this study therefore might not develop ACS yet.

This study is the first evidence that examined the protective effect of EVOO through the events of inflammation by giving the HFD with the treatment at the same time. The results showed that the EVOO might not play role in protecting blood vessels of individual consuming HFD. Further study need to be done to strengthen the recent findings, these are analyzing several variables including the cytokines of vascular wall and foam cells. Studies in humans are warranted due to the complexity of fats intake by human and different absorption of olive oil phenols among human and animal model.

CONCLUSIONS

In short, after following all steps in relation to analyze the data in order to find out the result for answering the research question, the result showed that there is higher COX-2 level in HFD rats treated with 2,3 ml/kg/day EVOO extract was significantly higher than HFD rats treated with 1 ml/kg/day EVOO

and control group. While LOX-1 level in HFD rats treated with extra virgin olive oil extract was not significantly different than HFD rats without extra virgin olive oil extract. It is clearly that extra virgin olive oil extract might have minor anti inflammatory and antioxidant effect in rats.

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