Effect of Evoo on Mda, Adma and no Level in Rattus Norvegicus Pre-Eclampsia Model

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WORD COUNT	3368	TIME SUBMITTED	02-NOV-2021 10:02AM
		PAPER ID	78362587

Effect of Evoo on Mda, Adma and no Level in Rattus Norvegicus Pre-Eclampsia Model

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Abstract

Background: Preeclampsia is one of the three main factors causing maternal death. In Preeclamcia found an increase in MDA, ADMA and decreased NO. How to treat preeclampsia until now is to end the pregnancy. EVOO has a fatty acid profile (FA) with a high ratio of monounsaturated fatty acids (MUFA) or monounsaturated fatty acids and rich antioxidant content.

Objective: To determine the difference in MDA, ADMA and NO level with the effect of giving EVOO to the experimental group and comparing it with the control group.

Method: The method of this research was carried out in vivo using the experimental animal rat (Rattus norvegicus) wistar strain. In this study consisted of 5 treatment groups. Negative control group (healthy pregnant rats), positive control (pregnant rats with preeclampsia), treatment 1, treatment 2 and treatment 3 where the group treated were preeclampsia pregnant rats who were given EVOO with various doses (0.5 cc, 1 cc and 2 cc).

Results: The results One way ANOVA test showed on MDA levels obtained significant differences (*p-value =0,000*) in the five treatment groups. ADMA levels obtained significant differences (*p-value =0,000*) in the five treatment groups. NOlevels obtained significant differences (*p-value =0,015*) in the five treatment groups.LSD test shows that the dose of EVOO is most effective in decreasing MDA, ADMA and NOlevels in dose 2cc.

Conclusion: EVOO can decrease MDA, ADMA and increase NOlevelsin rats model preeclampsia.

Keywords: *MDA, ADMA, NO, Preeclampsia, EVOO.*

Introduction

Pre eclampsia is a disorder in pregnancy that can cause increased mortality and morbidity in the mother and fetus. Preeclampsia can result in mental retardation in children and can also result in prematurity labor, IUGR and stillbirth caused by damage to the placenta which causes a reduced supply of food and oxygen to the fetus(1).In the world it is estimated that pregnancy complications include hypertension and preeclampsia in the amount of 5-10%. As many as 200 women die every day due to preexlampsia. Women in developing countries have a risk of dying from preeclampsia 300 times greater than developed countries(2)(3).

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In preeclampsia, the cytotropoblast fails to differentiate into an endothelial phenotype, so that invasion of the spiral arteries becomes more superficial, blood vessels remain stiff and narrow[4][5][6]. This condition can cause ischemia or hypoxia in the placenta. Hypoxia causes tissue or cell damage and endothelial dysfunction due to the presence of free radicals that cause lipid peroxidase. Lipid peroxidase is most common in cell membranes because unsaturated fatty acids and proteins are constituent components of cell membranes. Measurements of lipid peroxidation levels can be measured through the final product, namely Malondialdehyde (MDA). MDA is known to increase in the plasma of women with preeclampsia[7]. This condition describes oxidative stress. In conditions of oxidative stress an increase in superoxide and peroxynitrite production. inpreeclampsia this increase will induce activation of Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and its ligand Oxidized low-density lipoprotein (oxLDL)[8]. Activation of both NADPH oxidase positive feedback loops to re-produce superoxide and aggravate oxidative stress conditions. In addition, an increase in oxLDL causes an increase in L-Arginine which has an effect on increasing ADMA. Both of them trigger eNOSencoupling which inhibits NO synthesis.Reduced amount of NO results in vasoconstriction [9][10].The decrease in NO causes an imbalance in the synthesis of ET-1, so that ET-1 has increased. Decreasing NO as a vascular vasodilator and increasing ET-1 causes blood vessels of preeclampsia to experience vasoconstriction. This condition reflects endothelial dysfunction that triggers damage to the glomerular filtration barrier causing proteinuria, hypertension, HELLP syndrome, cerebral or visual disorders and edema. All of these conditions lead to the clinical manifestation of preeclampsia[11][12].One alternative that might be used in additional therapy or support for preeclampsia is the provision of Extra virgin olive oil or commonly called EVOO. Extra virgin olive oil is one type of oil that comes from the first juice of olives[12].

Extra virgin olive oil is more than just monounsaturated fat because it contains high amounts of antioxidants[13].The antioxidants possessed by EVOO are known to have a way of working to help protect cells from oxidative damage caused by free radicals. The antioxidants in EVOO belong to the preventive group of non-enzymatic antioxidants by damaging reactive oxygen formation[14]. The antioxidants contained in EVOO have the role of delaying the oxidation process. In this case, the main antioxidant that inhibits the oxidation process at EVOO is OP (Olive Phenols), which acts as a chain breaker by donating hydrogen radicals to alkylperoxyl radicals produced by lipid oxidation and the formation of stable derivatives during reaction[13].

Material and Method

The research design used in this study was post test only control group design. This research was conducted in vivo using experimental animals rats (Rattusnorvegicus) wistar strain. This study used 20 pregnant rat and randoccy divided into 5 groups. The negative control group consisted of normal pregnant rat. The positive control group was pregnant rat injected with L-NAME intraperitoneally without EVOO. The treatment group was pregnant rat injected with L-NAME intraperitoneally and given EVOO at a dose of 0.5 cc, 1 cc and 2 cc. Determination of the EVOO dose is based on the average daily consumption of the Mediterranean community converted to rat[15].

Pregnant rat were randoccy placed in 5 groups consisting of negative control group, positive control group and three treatment groups. Each group contains 4 pregnant rats. Intraperitoneal injection of L-NAME with a dose of 125 mg L-NAME / kilogram of body weight was given to rats with 13-19 days of gestation[16]. Preeclampsia rat model can be made by injection of L-NAME intraperitoneally used dose of 125 mg/ Kilogram of body weigt was injected to rats with 13 days of gestation until 19 days of gestation[12]. The Blood presure was measured on the 12, 15 and 19day of gestation using Tail Cuff methode with Kent Scientific CODA. This research has been approved by ethics committee Faculty Of Medicine Brawijaya University No. 73 / EC / KEPK-82/02/2019.

Measurement of MDA level was performed with spectrofometryMDA KIT (No catalog K739-100). Measurement of ADMA level was performed with elizaADMA KIT (No catalog E-EL-R0480). Meansurement of NO concentration was performed with colorimetry NO kit a wavelength op 550 nm (No catalog Elabscience E-BC-K036). Data was analyzed statistically with ANOVA.

Findings: Figure 1 shows that the preeclampsia rat that were no treated with EVOO had a higher MDA levels compared to the preeclampsia rat model treated with EVOO.

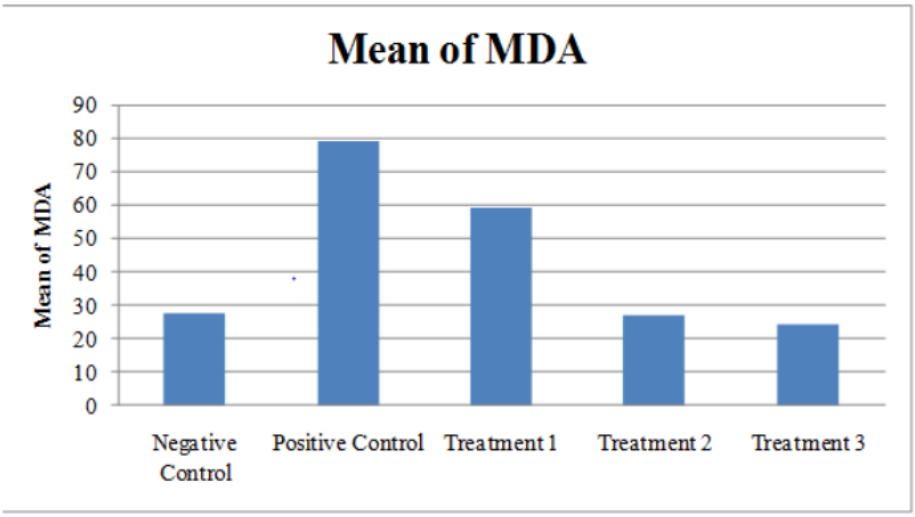
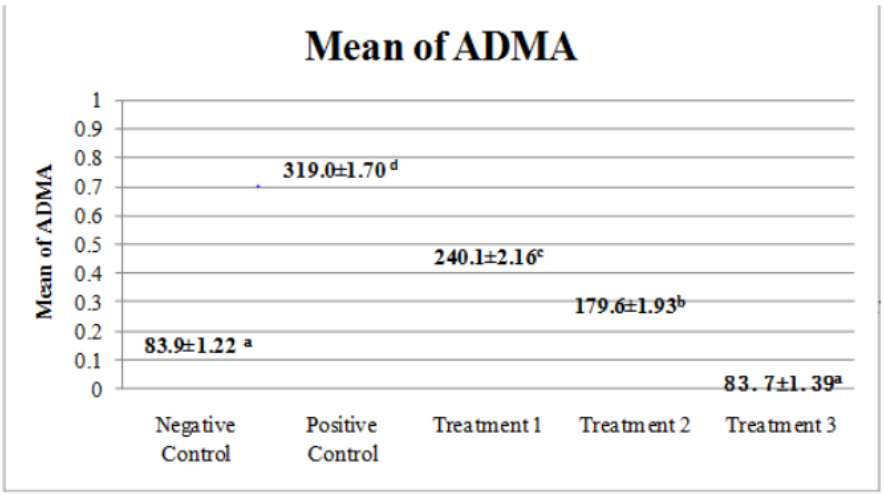


Figure 1: Comparison of mean of MDA of negative control, positive control, treatment 1, treatment 2 and treatment 3.

Note: Negative control is a normal pregnant rat. Positive control is a pregnant rat model of preeclampsia. Treatment 1 was a preeclampsia model rat that was given EVOO 0.5 cc. Treatment 2 was a preeclampsia model rat that was given EVOO 1 cc. Treatment 3 was a preeclampsia model rat that was given EVOO 2 cc.

A significant decrease in MDA levels was observed in the preeclampsia rat administered EVOO at 1 cc/day and 2 cc/day doses. EVOO 0.5 cc/day doesdid not appear to significantly decrease MDA levelin the preeclampsia rat model. The data showed that the levels of MDA in the treated group at 1 cc/day dose did not differ significantly from the mean in the normal pregnant rat group. This means that the optimum dose of EVOO to reduce MDA levels in the preeclampsia rat model was 1 cc/day.



A significant decrease in ADMA levels was observed in the preeclampsia rat

Figure 2: Comparison of mean of ADMA of negative control, positive control, treatment 1, treatment 2 and treatment 3.

Figure 2 shows that the preeclampsia rat that were no treated with EVOO had a higher ADMA levels compared to the preeclampsia rat model treated with EVOO.

Note: Negative control is a normal pregnant rat. Positive control is a pregnant rat model of preeclampsia. Treatment 1 was a preeclampsia model rat that was given EVOO 0.5 cc. Treatment 2 was a preeclampsia model rat that was given EVOO 1 cc. Treatment 3 was a preeclampsia model rat that was given EVOO 2 cc.

ADMA levels appeared to decrease in the treatment 1, 2, and 3 groups when compared to the positive control group. Decreased ADMA levels along with the increase in the EVOO dose given. So the third dose of EVOO was able to reduce ADMA levels in rat preeclampsia model. While the EVOO dose which is considered the fastest able to reduce ADMA levels is a dose of 2 cc/ day, because the average level of ADMA in the dose 3 group is the closest to the average value of ADMA in the negative control group.

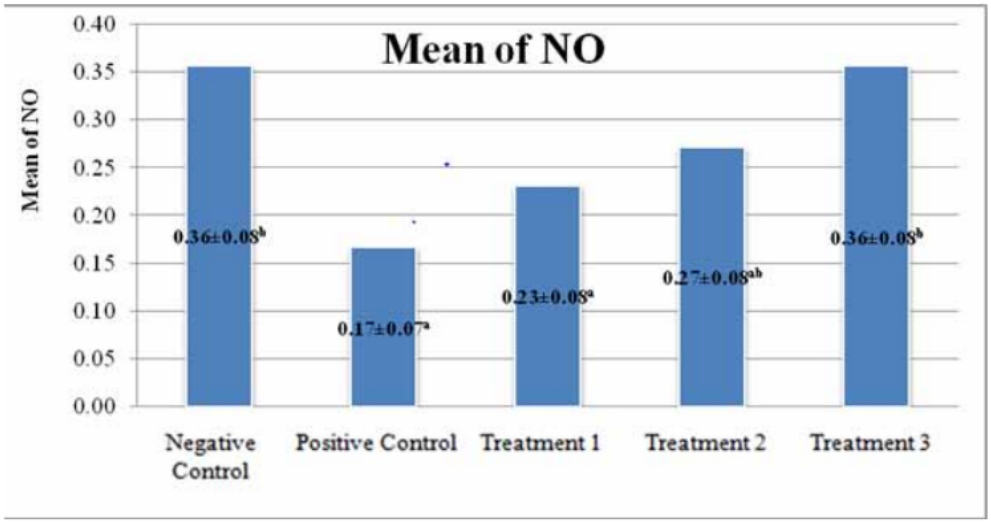


Figure 3:Comparison of mean of ADMA of negative control, positive control, treatment 1, treatment 2 and treatment 3.

Note: Negative control is a normal pregnant rat. Positive control is a pregnant rat model of preeclampsia. Treatment 1 was a preeclampsia model rat that was given EVOO 0.5 cc. Treatment 2 was a preeclampsia model rat that was given EVOO 1 cc. Treatment 3 was a preeclampsia model rat that was given EVOO 2 cc.

In the picture 3 above shows the average histogram of NO levels in normal pregnant wistar rat that were not given anything (negative control), wistar preeclampsia pregnant rat with a given EVOO dose of 0.5 cc/day, dose of 1 cc/day and 2 cc/day. The figure shows the highest NO concentration in the negative control group and the P3 group and the lowest for the NO concentration in the positive control group. This means that preeclamsia exposure in wistar rats resulted in decreased NO concentration. While the mean NO concertation appeared to increase in groups P1, P2 and P3 when compared to the positive control group. Increased NO concentration along with the increase in the EVOO dose given, although this increase did not reach statistical significant. So the third dose of EVOO is able to increase NO concentration in pregnant rat with preeclamsia. While the EVOO dose which is condidered the fastest able to increase NO concentration of group P3.

Table 1: Comparison of mean of MDA levels of preeclampsia rat model without EVOO treatment (positive control) compared to the preeclampsia rat model treated with EVOO

Observation Group MDA	Mean±Standard Deviation	p-value
Positive control	79.13±12.89[a]	0.000<α
Treatment 1	58.88±15.34[a]	
Treatment 2	27.22±19.10[b]	
Treatment 3	24.12±4.799[b]	

Table 2: Comparison of mean of ADMA levels of preeclampsia rat model without EVOO treatment (positive control) compared to the preeclampsia rat model treated with EVOO

Observation Group ADMA	Mean±Standard Deviation	p-value
Positive control	319.0±1.70[d]	0.000<α
Treatment 1	240.1±2.16[c]	
Treatment 2	179.6±1.93[b]	
Treatment 3	83.7±1.39[a]	

Table 3: Comparison of mean of NO levels of pre eclampsia rat model without EVOO treatment (positive control) compared to the preeclampsia rat model treated with EVOO

Observation Group No	Mean±Standard Deviation	p-value
Positive control	0.17±0.07[a]	0.015<ααα
Treatment 1	0.23±0.08[a]	
Treatment 2	0.27±0.08[ab]	
Treatment 3	0.36±0.08[b]	

Discussion

Our study confirmed that MDA and ADMA levels significantly increased and NO levels significantly decreased ($p < 0.05$) in pregnant rat injected with L-NAME compared with normal pregnant rat.

This shows that the interpretational injection of L-NAME may cause a significant increase in MDA, ADMAlevels and decrease NO levels in a pre eclampsia rat model. NG-Nitro-L-arginine-methyl ester (L-NAME) works to inhibit NO synthesis by blocking eNOS activity by causing interference with NO signaling in all arteries[17][18][19]. Inhibition of NO synthesis also results in eNOS uncoupling which contributes to the emergence of oxidative stress in vascular tissue[20]. The presence of oxidative stress can damage cell membranes where many cell membranes contain layers of fat which will turn into lipid peroxides which can be measured through the final product called MDA[21]. Increased MDA causes an angiogenic imbalance characterized by an increase in sFlt1 expression associated with a decrease in PlGF or placental growth factor and a decrease in VEGF (vascular endothelial growth factor). This condition describes oxidative stress. In conditions of oxidative stress an increase in superoxide and peroxynitrite production. in preeclampsia this increase will induce activation of Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and its ligand Oxidized low-density lipoprotein (oxLDL). Activation of both NADPH oxidase positive feedback loops to re-produce superoxide and aggravate oxidative stress conditions. In addition, an increase in oxLDLcauses an increase in L-Arginine which has an effect on increasing ADMA. Both of them trigger Enosencoupling which inhibits NO synthesis. Decrease NO prevents interacting with endothelial receptors which will eventually lead to increased ET-1 levels and eventually endothelial dysfunction. ET-1 is a vasoactive peptide which results in a decrease NO vasodilator that plays an important role in the pathophysiology of preeclampsia(11).

This study showed significant differences between MDA, ADMA and NO levels in the preeclampsia rat model without EVOO treatment (positive control) compared to the preeclampsia rat model treated with EVOO with a p-value of 0.000 ($p < 0.05$).

These results indicate that EVOO can decrease MDA and ADMA levels, also increase NO in a rat model of preeclampsia. The antioxidants contained in EVOO have the role of delaying the oxidation process. In this case, the main antioxidant that inhibits the oxidation process at EVOO is OP (Olive Phenols), which acts as a chain breaker by donating hydroxide radicals to alkylperoxyl radicals which are produced by lipid oxidation and stable derivative formation during reaction[13]. The content contained in EVOO is an antioxidant such as phenolic groups such as flavonoids, α-tochoperol (Vitamin E), β-carotene has and EVOO has been shown to have antioxidant activity[12]. Vitamin E itself can fight oxidative stress by preventing lipid peroxidation[19]. Vitamin E can inhibit oxidative stress, by preventing the formation of free radicals through inhibition of the enzyme NADPH oxidase in the placenta and in maternal neutrophils, and can also prevent free radical formation in mitochondria. The antioxidant capacity of vitamin E increases with the presence of

β-carotene. The combination of carotenoids with other antioxidants (Vitamin E) can increase their activity against free radicals[19].

Conclusion

1. Intraperitoneal injection of L-NAME into pregnant rat increased MDA levels.
2. Intraperitoneal injection of L-NAME into pregnant rat increased ADMA levels.
3. Intraperitoneal injection of L-NAME into pregnant rat decreased NOlevels.
4. EVOO decreased MDA levels in a preeclampsia rat model.
5. EVOO decreased ADMA levels in a preeclampsia rat model.
6. EVOO increased NOlevelsin a preeclampsia rat model.

Conflict of Interest: None

Source of Funding: None

Ethical Clearence: This research has been approved by ethics committee Faculty Of Medicine Brawijaya University No. 73 / EC / KEPK-82/02/2019.

Recomendation: The study may be used as a be a reference in supporting therapy in preeclampsia in humans.

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