

Research Article

The Effect of Extra Virgin Olive Oil (EVOO) on Fetal Birth Weight in Preeclampsia Rat Model

Pengaruh Extra Virgin Olive Oil (EVOO) terhadap Berat Badan Janin pada Tikus Model Preeklamsia

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ABSTRACT

Preeclampsia, as one of the most common pregnancy-specific diseases, causes high maternal-fetal morbidity and mortality in almost every country. Placental vascular abnormalities in preeclamptic women can cause chronic hypoxia and impaired fetal nutrition, so fetal growth retardation often occurs. EVOO has strong antioxidant effect is assumed to prevent nutritional disorders in the fetus. This study aimed to determine the effect of EVOO on fetal birth weight in a preeclampsia rat model. This research was laboratory research conducted in vivo with a Post Test Only Control Group design which consisted of five groups; negative control group, positive control group (pre-eclampsia rat model), dose 1, 2, and 3 groups that were preeclampsia rats given EVOO in 3 different doses (0.5 mL/day, 1 mL/day and 2 mL/day respectively). Blood pressure and proteinuria measurements were carried out at the 12, 15 and 19 day of pregnancy. After sacrificed, fetal weight was measured immediately using analytical balance. The result of this study showed that there was a significant reduction of fetal weight between negative control and positive control group ($p=0.020$), meanwhile no significant differences among positive control, dose 1 and dose 2 group ($p=0.90$ and $p=0.142$) but statistically significant to dose 3 group ($p=0.005$). EVOO administration increases fetal weight in doses group by its AA and DHA in Long-Chain Poly Unsaturated Fatty Acids (LCPUFA) within. The optimal dose of EVOO to increase fetal weight is 2 mL/day.

Keywords: Extra virgin olive oil, fetal birth weight, preeclampsia

ABSTRAK

Preeklamsia, sebagai salah satu penyakit spesifik kehamilan yang paling umum, menyebabkan morbiditas dan mortalitas ibu-janin yang tinggi di hampir setiap negara. Kelainan vaskular plasenta pada wanita preeklamsia dapat menyebabkan hipoksia kronis dan gangguan nutrisi janin, sehingga sering terjadi retardasi pertumbuhan janin. EVOO memiliki antioksidan kuat, dan efek antiinflamasi diasumsikan untuk mencegah gangguan gizi pada janin. Penelitian ini bertujuan untuk mengetahui pengaruh EVOO terhadap berat lahir janin dalam model tikus preeklamsia. Penelitian ini adalah penelitian laboratorium yang dilakukan secara in vivo dengan desain penelitian *Post Test Only Control Group* yang terdiri dari lima kelompok; kelompok kontrol negatif, kelompok kontrol positif (model tikus pre-eklamsia), dosis 1, 2, dan 3 yang merupakan kelompok perlakuan yang diberi EVOO dalam 3 dosis berbeda (0,5 mL/day, 1 mL/day, dan 2 mL/day). Pengukuran tekanan darah dan proteinuria dilakukan pada hari ke 12, 15, dan 19. Setelah dibedah, berat janin diukur segera dengan menggunakan timbangan analitik. Hasil penelitian ini menunjukkan bahwa ada penurunan berat janin yang signifikan antara kontrol negatif dan kelompok kontrol positif ($p=0,020$), sedangkan tidak ada perbedaan yang signifikan antara kelompok kontrol positif, dosis 1 dan dosis 2 ($p=0,90$ dan $p=0,142$) tetapi signifikan secara statistik antara kelompok kontrol positif dengan kelompok dosis 3 ($p=0,005$). Pemberian EVOO meningkatkan berat janin dalam kelompok dosis melalui AA dan DHA dalam asam lemak tak jenuh rantai panjang (*long-chain polyunsaturated Fatty Acids*). Dosis optimal EVOO untuk meningkatkan berat janin adalah dosis 2 mL/hari.

Kata Kunci: Berat badan janin, extra virgin olive oil, preeklamsia

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INTRODUCTION

Preeclampsia is one of the most common pregnancy-specific diseases, characterized by hypertension and proteinuria in pregnancies over 20 weeks, and is a cause of high maternal and fetal morbidity and mortality in almost every country (1). The incidence of preeclampsia varies between 2% to 10% of pregnancies worldwide. The World Health Organization estimates the incidence of preeclampsia to be seven times higher in developing countries (2.8% of live births) than in developed countries 0.4% (2). Preeclampsia is caused by many factors, including immunological, epigenetic, genetic, biochemical, environmental, and inflammatory factors as a basis for preeclampsia (3). There have been many in-depth studies related to the causes and pathogenesis of preeclampsia, but the exact cause is still unknown. Placental abnormalities and endothelial dysfunction have been proven by many researchers as the underlying cause of preeclampsia. The causes of endothelial dysfunction are also unclear, and many hypotheses are proposed to explain this (4).

Low birth weight and preeclampsia are pregnancy and child birth that have a negative impact on possible influence on future health status (5). Preeclampsia is associated with a high number of cases of small babies during pregnancy and perinatal deaths worldwide (6). In preeclampsia, there is a decrease in utero placental perfusion, hypovolemia, vasospasm, and damage to placental vascular endothelial cells (7). Uteroplacental ischemia results in hypoxia due to reduced blood flow at the placental implant site, and results in the release of free radicals. Oxidative stress can cause AT1-AA to induce activation of NADPH in endothelin 1 (ET-1) so that it can increase reactive oxygen species (ROS). The excessive amount of ROS causes an increase in p38 MAPK, which will reduce catalytic activity resulting in abnormal placentation. Trophoblast invasion failure and spiral artery remodeling result in hard and rigid arteries making it difficult to not even be able to vasodilate, this causes the blood supply to the placenta decreases resulting in hypoxia or placental ischemia (8,9).

Placental vascular abnormalities in preeclamptic women can cause chronic hypoxia or uteroplacental ischemia and impaired fetal nutrition so that fetal growth retardation often occurs that can end in low birth weight (LBW) (10). Antioxidants are chemically interpreted as electron donor compounds. The work of antioxidants is through a chain reaction breaker mechanism. Antioxidants that are found in nature have been studied for various diseases (11). Antioxidant activity as a free radical scavenger, a reducing agent, and reducing the formation of singlet oxygen and electron donors are some of the biological effects of phenolic compounds (12). The content of phenolic compounds from extra virgin olive oil (EVOO) is very effective in counteracting free radicals (13). According to Calabriso *et al.*, extra virgin olive oil (EVOO) is an external natural ingredient that has anti-antioxidant properties as well as ROS neutralizers (14).

Based on the background, it is assumed that in preeclampsia an increase in free radicals triggers oxidative stress, thus causing excessive apoptosis of trophoblast cells and endothelial dysfunction. Hypoxia as a result of placental endothelial cell damage can cause nutritional disturbances in the fetus and result in low birth weight. Extra virgin olive oil (EVOO) as a powerful antioxidant is

assumed to prevent complication of preeclampsia by binding to existing oxidants, thereby reducing free radicals and preventing nutritional disorders in the fetus. To prove this assumption, a study will be conducted on the effect of extra virgin olive oil (EVOO) on birth weight in a preeclampsia rat model. Researchers used *Rattus norvegicus* experimental animals because they are able to adapt in laboratory environment well, classified as benign, easy to maintain, and their metabolic functions are similar to humans, so it is expected to be used as a comparison of preeclampsia in humans.

METHOD

Animal Model

This research was laboratory research conducted in vivo with a Post Test Only Control Group research design. The population in this study was pregnant Wistar strain rats. There were four replications for each group (15). The negative control group was normal pregnant rats; the positive control was pregnant pre-eclampsia rats (pre-eclampsia rat model); and the treatment group 1, 2, and 3 were preeclampsia rat given three different doses (0,5 mL/day, 1 mL/day and 2 mL/day) of Extra Virgin Olive Oil (EVOO), respectively (16). The morning after mating, each animal was examined for the presence of a vaginal plug. The presence of a vaginal plug was assumed to be the day 1 of pregnancy. The research was carried out in the Laboratory of Bioscience Universitas Brawijaya, Laboratory of Physiology and Laboratory of Biomolecular Biochemistry, Faculty of Medicine Universitas Brawijaya Malang.

Preeclampsia Induction and EVOO Administration

The induction material for preeclampsia was using NOS inhibitors, L-NAME (C₇H₁₅N₅O₄ HCl) from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) (17). The L-Name dose given was 125 mg/kg BW starting from day 13 to day 18 intraperitoneally (18-19). While, the EVOO "B" was given per oral gavage feeding tube from day 1 to day 18 of pregnancy.

Clinical Examination

Clinical examination was carried out by using non-invasive blood pressure measurement (CODA[®], Kent Scientific Corporation) available at the Laboratory of Physiology, Faculty of Medicine Universitas Brawijaya, and proteinurin with dipstick examination (URISCAN[®] 3 GPH strips). Blood pressure and urine protein measurements were carried out at the 12, 16, and 19 day of pregnancy. The presence of vaginal plug in the morning after mating was determined as day 1 of pregnancy. Fetal weight was measured immediately using analytical balance after the rats were sacrificed.

Data Analysis

The data analysis was conducted using SPSS 25.0. The normality test was done using Shapiro-wilk, and test homogeneity of variance was using Lavene statistic. Independent t-test was used to compare the negative and the positive control groups, while one-way ANOVA was used to describe the differences of each group.

RESULTS

Blood Pressure Examination

The success of making preeclampsia rat model could be

seen from the increasing systolic blood pressure (shown in Figure 1) and the increasing diastolic blood pressure (shown in Figure 2) after being given L-NAME (N (ω)-nitro-L-arginine methyl ester). Blood pressure checks were performed on day 12 of pregnancy, day 15 of pregnancy (after L-NAME administration), and day 19 of pregnancy (before surgery).

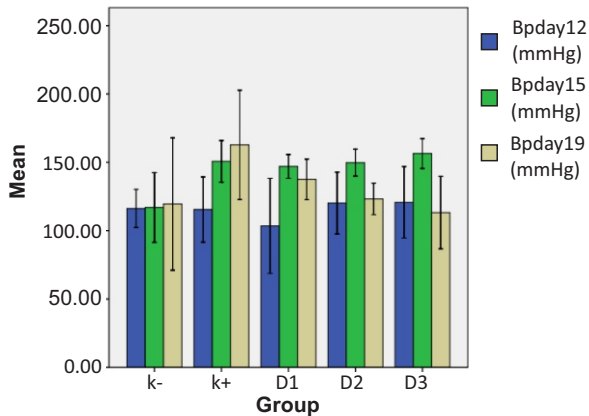


Figure 1. Blood Pressure (Systolic)

Note: Negative control (K-) normal pregnant rat, positive control (K+) preeclampsia rat model without EVOO, preeclampsia rat model were given EVOO 0.5 mL / day dose 1 (D1), EVOO 1 mL / day dose 2 (D2), EVOO 2 mL / day dose 3 (D3).

In the negative control group, there was no difference in systolic blood pressure between groups (Figure 1). In the positive control group there was an increase in systolic blood pressure from day 12 to day 15 and day 19 of pregnancy; in D1 group, there was an increase in systolic blood pressure on the day of systole from day 12 to day 15 of pregnancy then decreased slightly in day 19 of pregnancy; in D2 group, there was an increase in systolic blood pressure on the day of systole from day 12 to day 15 of pregnancy, then in day 19 of pregnancy decreased as same as day 12 of pregnancy; in group 3, there was an increase in systolic blood pressure from day 12 to day 15 of pregnancy, in day 19 of pregnancy decreased more than day 12 of pregnancy.

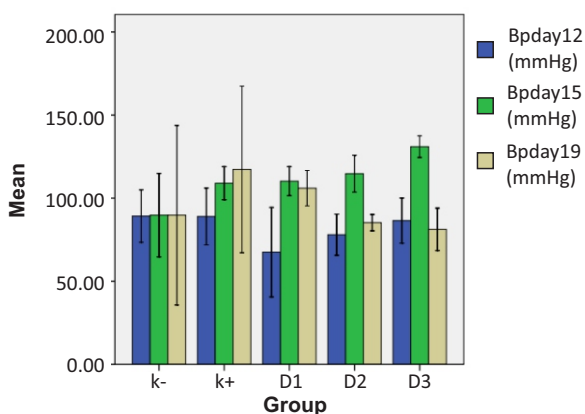


Figure 2. Blood Pressure (Diastolic)

Note: Negative control (K-) normal pregnant rat, positive control (K+) preeclampsia rat model without EVOO, preeclampsia rat model were given EVOO 0.5 mL / day dose 1 (D1), EVOO 1 mL / day dose 2 (D2), EVOO 2 mL / day dose 3 (D3).

In the negative control group, there was no difference in diastolic blood pressure between groups (Figure 2). In the positive control group, there was an increase in diastolic blood pressure from 12 to day 15 and day 19 of pregnancy; in D1 group, there was an increase in diastolic blood pressure from day 12 to day 15 of pregnancy then decreased slightly in day 19 of pregnancy; in D2 group, there was an increase in diastolic blood pressure from day 12 to day 15 of pregnancy, then in day 19 of pregnancy seem decreased; in D3 group, there was an increase in diastolic blood pressure from day 12 to day 15 of pregnancy then decreased more than day 12 of pregnancy.

Measurement of Fetal Weight On day 19, surgery and measurement of fetuses' weigh were carried out in each group (Table 1). From the results of the Shapiro Wilk Normality Test, the obtained showed that all data for each group were normal ($p=0.982$; $p=0.066$; $p=0.161$; $p=0.303$; $p=0.063$). The results of the homogeneity of variances test with the Levene statistical results showed that all data were homogeneous ($p=0.064$). The independent t-test results showed that there were significant differences between the negative control group and the positive control group ($p=0.020$). This showed that there was a significant reduction in fetal weight in preeclampsia when compared to normal rats. While, the Anova Test also showed significant results ($p=0.001$). The post hoc test using LSD showed that there was no significant difference between the positive control group and dose 1 and 2 groups ($p=0.90$ and $p=0.142$) but significantly different with dose 3 group ($p=0.005$). Statistically, this means that EVOO significantly increases the fetal weight from positive control in dose 2 mL/day.

Table 1. Fetuses' weight

	Rat 1 (g)	Rat 2 (g)	Rat 3 (g)	Rat 4 (g)	Mean (g \pm SD)	$\bar{x}\Sigma$ fetuses
K-	2.83	1.44	2.42	3.85	2.64 \pm 0.99	7.75
K+	0.82	0.94	1.42	0.81	0.99 \pm 0.29	8
D1	1.36	1.24	1.32	1.36	1.32 \pm 0.057	5.75
D2	2.24	2.33	1.44	1.82	1.96 \pm 0.19	9.25
D3	3.2	2.33	2.37	2.88	2.69 \pm 0.40	8.5

Note: the average of fetal weight was obtained from 4 samples in each group; mean of fetuses weigh from each group shows that positive control group had the lowest mean; the average of total fetuses each group. Fetuses weight is the average of fetuses in each sample with different amounts.

DISCUSSION

Increase Systolic and Diastolic Pressure as Marker of Preeclampsia

Preeclampsia rat model was made by intraperitoneal injection of 125 mg/kg BW L-NAME (19). L-NAME injection in pregnant rat inhibits Nitric Oxide (NO) synthase that produce from converting L-arginine to NO by NOS (nitric oxide synthase). Lack of NO as vasodilator causes blood vessels of preeclampsia in vasoconstriction condition. Vascular vasoconstriction triggers increasing systolic and diastolic pressure in preeclampsia. In this study, systolic and diastolic blood pressure increased, day 12 to 15 of pregnancy both systolic and diastolic in preeclampsia rat model (Figure 1 and 2), in accordance with previous studies (19-21).

Meanwhile, based on the result of this study, after EVOO treatment, systolic and diastolic blood pressures seem to decrease (day 15 to 19 of pregnancy both systolic and diastolic in the preeclampsia rat model, as seen in Figure 1 and 2). Hypertension is related to poor endothelial function caused by vascular oxidative stress and inflammation. Olive oil polyphenol decreases blood pressure in hypertension and improves endothelial function by a mechanism that reduces serum Asymmetric Dimethyl Arginine (ADMA), ox-LDL, plasma C Reactive Protein (CRP) concentration (22).

ADMA as inhibitor molecule of NO synthase to produce NO, in which hypertension blood vessel in vasoconstriction condition is widely known as consequence lack of NO (23). In oxidative stress, free radical disrupt lipid forming ox-LDL and lead to increase ADMA. Ox-LDL upregulates Protein Arginine Methyltransferases (PRMTs), enzyme catalyzer arginine methylation to form ADMA (24). Anthocyanin as polyphenol antioxidant in EVOO has double bonds and hydroxyl groups that bind free radicals and inhibit LDL oxidation at the initiation and propagation stage, so radical compounds do not have enough energy to react with other lipid molecules (25).

Ox-LDL activates the renin-angiotensin system and angiotensin II to increase blood pressure (26). Olive oil polyphenol reduces ox-LDL because antioxidant compound of olive oil polyphenol, mainly hydroxytyrosol, is responsible for lowering lipoprotein oxidation and disrupting chain oxidation reaction (27). CPR is inducing endothelial dysfunction by declining NO synthase mRNA stability and uncoupling (28). Anti-inflammatory effect of olive oil polyphenol through several molecular pathways including arachidonic acid pathway and nuclear factor-Kb results in decreasing CRP (29,30). Therefore, olive oil polyphenol decreases blood pressure through increasing NO bioavailability due to decreasing ADMA, ox-LDL, and CRP concentration (31).

Fetal Weight in Preeclampsia Rat Model

In our study, the mean of fetal weight in the positive control group was lower than the negative control group (Table 1). In preeclampsia, there was a disruption of uteroplacental perfusion which caused placenta tend to be in a state of lack oxygen and lead to placental ischemia. In condition of placental ischemia, maternal-fetal exchange of oxygen and nutrients reduce so that affect fetal growth and development (1,32). Disruption of uteroplacental perfusion in preeclampsia proved by morphological change in placenta which showed lower placental weight compared to normal pregnancy (33,34). Placental ischemia affects fetal growth by limiting oxygen and negatively influences placental glucose transport as a primary source of energy for the fetus (35). Oxygen, glucose, amino acids, and fatty acids are the main nutrients required for appropriate fetal development and growth. Thus, placental ischemia due to disruption of uteroplacental perfusion affects nutrient transport lead to

lower birth weight (36).

Effect of EVOO in Fetal Birth Weight

This study showed that EVOO can increase fetal weight compared to the positive control group. This is in accordance with the results of previous studies that EVOO administration can increase fetal weight compared with controls (37). The bioactive component found in EVOO includes monounsaturated fatty acids and polyunsaturated fatty acids, tocopherols, and polyphenols (bio-phenols) (38). The long-chain polyunsaturated Fatty Acids (LCPUFA) such as arachidonic acid (AA), timnodonic acid (EPA), arachidonic acid (ARA) and cervonic acid (DHA) are involved in key biological processes, including inflammatory responses, gene expression, and cellular fluidity (39,40). DHA and ARA are very important during pregnancy for fetuses because they form structural constituents of membrane lipids lead to increase fetal weight (40). Former Cross-Sectional human studies have extensively demonstrated lower LCPUFA levels in pregnancy complications like preeclampsia (41,42). Therefore, this study suggests that in preeclampsia EVOO consumption will improve the fetal birth weight.

The use of EVOO is often linked to the Mediterranean diet (MD), whose composition mainly consists of fruits, vegetables, and olive oil (43). Some studies showed that low adherence to the Mediterranean diet (MD) in early pregnancy was significantly associated with a decrease in fetal size, placenta, and birth weight (44,45). There is a lot of evidence regarding the risk of cardiovascular disease and other chronic diseases. The benefits of MD have also been associated with a reduction in oxidative stress through its phenolic components (46-48).

Our study, where early EVOO (early pregnancy) administration was given in late onset preeclampsia which is expected to lead to increased birth weight. This results is also in line with a study that stated (49) there is strong evidence from various disciplines that mention pregnancy humans consist of two opposite periods. During the first trimester, there is little maternal blood flow to the placenta and low oxygen pressure to fetoplacenta, but the uterine gland can provide a lot of nutrient supply. Whereas at the beginning of the second trimester, maternal circulation in the intervillous space gets better, oxygen pressure increases, and hemotropic nutrition becomes dominant.

We conclude that preeclampsia in rat model causes lower birth weight, meanwhile administration of EVOO decreases blood pressure and increase fetal weight.

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