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RESEARCH ARTICLE

# The Effect of Extra Virgin Olive Oil To Increase Endogeneous Antioxidant and PlGF In Preeclampsia Rat Model

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## Abstract

Background: Preeclampsia is a special disease in pregnancy with oxidative stress as one of its pathogenesis pathways, suspected that it can interfere the process of angiogenesis and negatively impacts the fetus and maternal wellbeing. Objective: This study aimed to determine effect of EVOO as a strong exogenous antioxidant to increase SOD activity and GSH level in accordance with improvement on PlGF level as an angiogenesis factor in preeclampsia rat model. Method: This research consisted of five groups; negative control, positive control (preeclampsia rat model), doses 1, 2, and 3 that were preeclampsia rats given EVOO in 3 different doses (0.5 mL/day, 1 mL/day and 2 mL/day respectively). Blood pressure measurements were carried out at the 12th, 15th, and 19th day of pregnancy. After sacrificed, placentas were collected to determine SOD activity and GSH level either maternal plasma to determine PlGF level. Results: Result of this study showed that there was a reduction of GSH (p=0.02) and PlGF (p=0.233) level between negative and positive control group meanwhile SOD activity seem increased (p=0.049). There were significant differences among positive control and dose 1 in SOD activity (p=0.008), dose 3 in GSH level (p=0.000), both dose 1 (p=0.017) and 2 (p=0.000) in PlGF level. Conclussion: Administration of EVOO increased SOD and GSH in all three doses groups, while there was a decrease in PlGF at dose 3. EVOO had been shown to be a potential antioxidant in preeclampsia through increased SOD activity and GSH level, at once modulate angiogenesis through increased PlGF level.

**Keywords:** *Preeclampsia, EVOO, SOD, GSH, PlGF, Antioxidant, Oxidative stress, Angiogenesis.*

## Introduction

Preeclampsia defined as new onset of hypertension with or without proteinuria after 20 weeks gestation. Preeclampsia as one of the main causes of maternal death in the world is a pathological condition in pregnancy which described as a disease of theories. Various pathophysiologies involved in the process of preeclampsia, such as angiogenesis, oxidative stress, genetic and immunological-inflammation are mentioned as trigger for preeclampsia.

The combination of various precipitating factors above is responsible for the appearance of clinical symptoms that are typical in preeclampsia such as hypertension and proteinuria [1].

In preeclampsia, defect of trophoblast invasion as the main process of placental formation disturb spiral artery remodeling. This causes intermittent arterial blood flow resulting period of recurrent ischemia-reperfusion which causes decrease plasental blood flow and lead to hypoxia.

Hypoxia trigger oxidative stress characterized by changes in various markers and oxidative damage [2]. Imbalance between ROS and endogenous antioxidants in preeclampsia resulting oxidative stress

characterized by increase oxidative stress markers (Malondialdehyde/MDA) and decrease endogenous antioxidant activities such as Superoxide Dismutase (SOD) and Glutathione (GSH)[3]. Besides oxidative stress, another factor involved in pathophysiology of preeclampsia is angiogenesis factor such as Placental Growth Factor (PlGF).

PlGF is responsible for the process of angiogenesis and vasculogenesis because of its role in stimulating mitogenesis and endothelial cell migration for new blood vessel formation [4]. In preeclampsia, failure of trophoblast invasion results in abnormal production of vasculogenic and proangiogenic factors such as Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF).

VEGF activity is mediated by its receptors, VEFGR-1 or Flt-1 expressed by the surface of endothelial cell. The function of proangiogenic factors is influenced by antiangiogenic factors such as sFlt-1 and sEng [4].The hypoxic condition in preeclampsia cause sFlt-1 being produced in high concentrations. SFlt-1 binds to PlGF which causes disruption of the angiogenesis process by reducing the bioavailability of PlGF to its receptors (Flt-1).sFlt-1 functions as a scavenger in the circulation and tissues that can bind PlGF to prevent PlGF from binding to its membrane receptors in the endothelium.

Therefore, preeclampsia showed increase in sFlt-1 production accompanied by decrease in PlGF [4,6].This imbalance of pro-angiogenic and anti-angiogenic factors resulting systemic vascular dysfunction. Systemic vascular dysfunction is a mechanism responsible for clinical manifestations that appear in preeclampsia such as hypertension, proteinuria, glomerular endotheliosis, coagulation disorders, liver function disorders (HELLP syndrome) and cerebral edema resulting in neurological disorders (eclampsia) [7,5].

Extra Virgin Olive Oil (EVOO) is the first oil produced by olives. EVOO is widely used in Mediterranean countries because of its important benefits in health. The diet pattern of Mediterranean countries containing EVOO is associated with a reduced risk of chronic degenerative diseases and increased life expectancy. One of the active ingredients in EVOO which is polyphenol has many functions as antioxidants, anti-inflammatory and antimicrobial. Through its function as an antioxidant, polyphenol in EVOO play potential role reducing ROS production, inhibiting NADPH oxidase to produce superoxide and hydrogen peroxide, free radical scavenger include hydrogen peroxide replacing the role of GSH, and increasing total plasma antioxidant activity including SOD and GSH [8,10].

In addition, the polyphenol content in EVOO also affect angiogenesis through Nuclear Factor Erythroid 2-related Factor-2 (Nrf2) which is associated with increased PlGF [11,9]. The purpose of this study is to prove the effect of administering extra virgin olive oil orally to increase SOD activity, GSH and PlGF level of preeclampsia pregnant rat model.

## Materials and Methods

### Animal Model

This research was in vivo laboratory research. The design was a Post Test Only Control Group design. The population in this study was Wistar strain pregnant rats. There were four replications for each group [12]. 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 Extra Virgin Olive Oil (EVOO) in three different doses (0,5 mL/day, 1 mL/day and 2 mL/day) respectively [13].

The next day after mating was assumed to be the day 1 of pregnancy. The sacrifice of rat did in 19 day of pregnancy. The sample used in the study was placenta and plasma. The research was carried out in the Laboratory of Biosains Universitas Brawijaya, Laboratory of Physiology and Laboratory of Biomolecular Biochemistry, Faculty of Medicine Universitas Brawijaya Malang.

### Preeclampsia Induction and EVOO Administration

The material used for induction of preeclampsia was using NOS inhibitors, L-NAME (C7H15N5O4 • HCl) from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany)1 [14]. The L-NAME dose given was 125 mg/kg BW from day 13 to day 18

 

intraperitoneally [15,16].While, the EVOO "B" was given per oral gavage feeding tube from day 1 to day 18 of pregnancy.

### Clinical and Sample Examination

Clinical examination was carried out by using non-invasive blood pressure measurement (CODA®, Kent Scientific Corporation). The measurement available at the Laboratory of Physiology, Faculty of Medicine Universitas Brawijaya. Blood pressure measurements were carried out at the 12, 15, and 19 day of pregnancy.

### SOD Activity Measurement

Measurement of SOD activity using colorimetric method with Elabscience SOD colorimetric assay Kit, catalog number E-BC-K022. The sample used to measured SOD Activity was Plasenta. The prosedure were sample preparation : 0.2 mL of the sample was added with 0.2 mL of Reagent 7, then mixed for 1 minute using vortex and centrifuged at 3500-4000 rpm for 15 minutes. Supernatants were taken for measurement of sample SOD activity.

In addition, 0.2 mL of normal saline was added with 0.2 mL of Reagent 7, then mixed evenly for 1 minute using vortex and centrifuged at 3500-4000 rpm for 15 minutes. Supernatants were taken to measure SOD activity as a control. SOD Activity measurement : 1 mL of Reagent 1 was added with 0.1 mL Reagent 2, 0.1 mL Reagent 3, 0.1 mL Reagent 4 and:0.05 mL supernatant control and 0.05 mL of supernatant sample.

Then incubation for 40 minutes at 37 ° C. After incubating 2 mL of Chromogenic agent, mixed evenly, incubated again for 10 minutes at room temperature, then OD values were read at a wavelength of 550 nm using a 1 cm diameter cuvette. The SOD (U/mgprot) activity is measured by the formula:(OD control-OD sample) / (OD control) : 50% x (Total volume reaction system (mL)) / (Sample volume) : Concentration of protein sample (mgprot/mL)

### GSH Level Measurement

Measurement of GSH Levelin placenta using the colorimetric method with the Elabscience Reduced glutathione (GSH) Assay Kit, Catalog number E-BC-K051. The prosedur of measurement were Sample Preparation: Weigh the tissue in cold condition, add PBS (0.01 M, PH 7.4) with weight ratio (g): volume (mL) = 1:19. Grind the pasenta tissue in cold conditions, centrifuse it at 10000g for 15 minutes. GSH Level meaurement : Take 0.7 mL of the supernatant, add 0.7 mL of reagent 1 and mix, then centrifuse at 4500 g for 10 minutes. Blank tube: add 1 mL of reagent 1 to 5 mL EP tube. Standard tube: add 1 mL of 20 μmol / L GSH standard solution to 5 mL EP tube.

Sample tube: Add 1 mL of supernatant to 5 mL EP tube. Add reagent 2 of 1.25 mL, reagent 3 0.25 mL, reagent 4 0.05 mL on each tube. Mix and wait 15 minutes at room temperature. Prepare a spectrophotometer at 0 with distilled water and measure the OD value of each tube with a wavelength 420 nm. The formula was (OD Sample-OD Blank): (OD Standard-OD Blank) x Concentration of standard ($20 \times 10^{-3}$).

### PlGF Level Measurement

Measurement of PlGF levels using the ELISA method with the Elabscience PlGF assay kit, catalog number E-EL-R0742. The prosedur were wash the plate 2 times before adding standards, samples and controls; Add 100μL standard or sample to each well for 90 minutes at 370C;Add 100μL Biotin-dectection antibody (biotinylated Rat sFlt-1 antibody / biotinylated Rat PlGF antibody) working solution in each well for 60 minutes at 370C;Aspiration and washing three times; Add 100μL of SABC working solution to each well.

Incubate for 30 minutes at 370C; Aspiration and washing five times; Add 90 μL TMB substrate. Incubate for 15-30 minutes at 370C; Add 50 μL Stop Solution. Read OD absorbance at 450 nm directly after added stop solution; Calculate the results. The ELISA test results in the form of optical density (OD). The concentration is calculated based on the value of OD using a standard curve equation.

### Data Analysis

Data analysis using SPSS 25.00.Test used to observe group mean different was One Way Anova Test. Another test used Multiple Comparison Test with LSD.

## Results and Discussions

### Blood Pressure in Preeclampsia Pregnant Rat Model Given EVOO

 

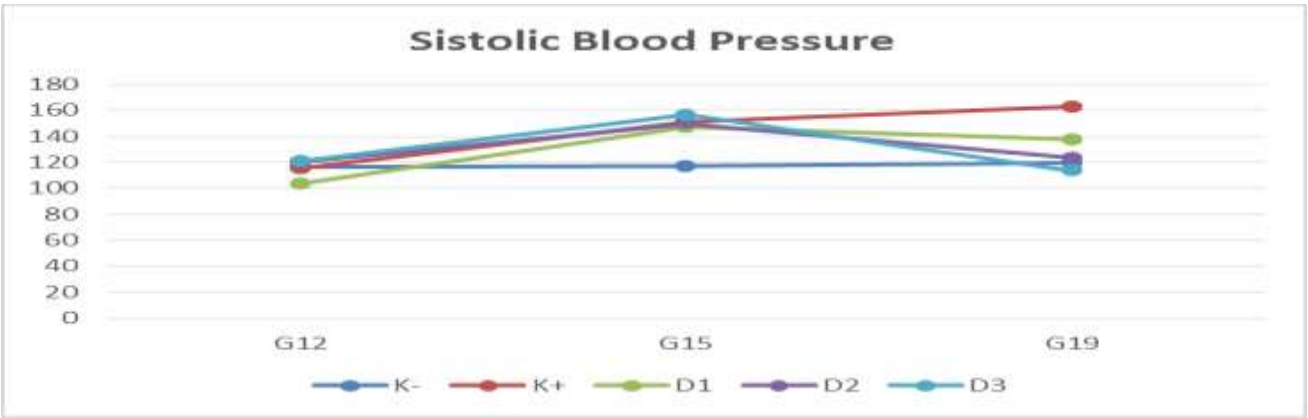


**Fig. 1 Sistolic Blood Pressure in Preeclampsia Pregnant Rat Model Given EVOO**

Average sistolic blood pressure was measured at day 12 of pregnancy (G12), day 15 of pregnancy (G15) and day 19 of pregnancy (G19) on negative control group (K-), positif control group (K+), EVOO 1st dose 0.5 mL/day (D1), EVOO 2nd dose 1 mL/day (D3) and EVOO 3rd dose 2 mL/day (D3). Before L-NAME injection, sistolic blood pressure in all group were under 140 mmHg. There were increase sistolic blood pressures (> 140 mmHg) after L-NAME injection between day 12 and 15 of pregnancy in positive control group, dose 1, dose 2 and dose 3. In day 19 of pregnancy seem decrease after EVOO administration in dose 1, dose 2 and dose 3 groups.

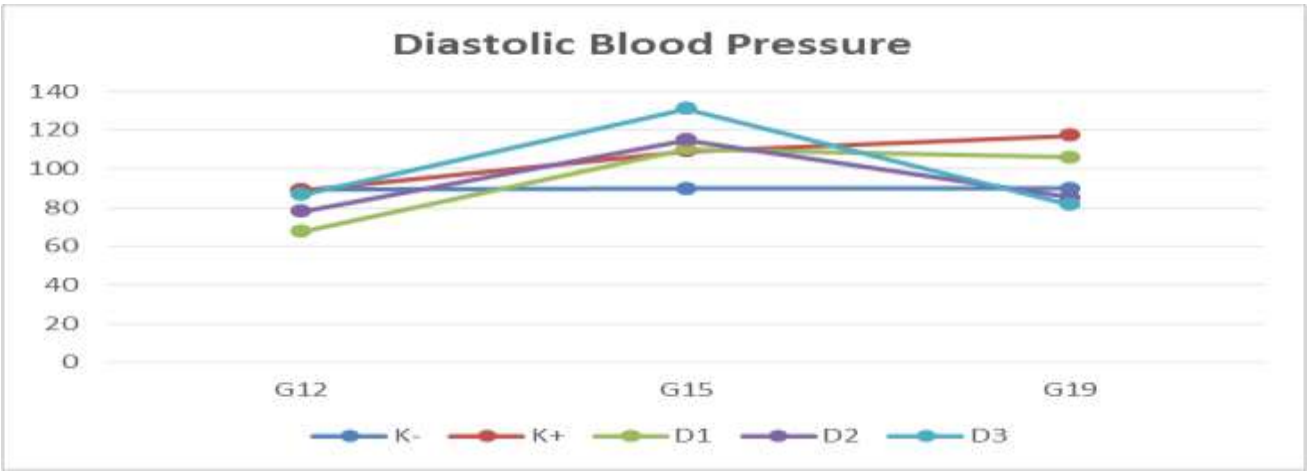


**Fig. 2 Diastolic Blood Pressure in Preeclampsia Pregnant Rat Model Given EVOO**

Average diastolic blood pressure was measured at day 12 of pregnancy (G12), day 15 of pregnancy (G15) and day 19 of pregnancy (G19) on negative control group (K-), positif control group (K+), EVOO 1st dose 0.5 mL/day (D1), EVOO 2nd dose 1 mL/day (D3) and EVOO 3rd dose 2 mL/day (D3) Before L-NAME injection, diastolic blood pressure in all group were under 90 mmHg. There was increase diastolic blood pressure (> 90 mmHg) after L-NAME injection between day 12 and 15 of pregnancy in positive control group, dose 1, dose 2 and dose 3. In day 19 of pregnancy seem decrease after EVOO administration in dose 1, dose 2 and dose 3 groups.

## Effect of EVOO on SOD Activity Preeclampsia Rat Model

Data showed significant higher SOD activity in K (+) compared to K (-) group. Meanwhile, by giving EVOO doses 1 (D1) SOD activity seems decreases significantly than posit if control. In accordance with addition EVOO doses, SOD activities tend to increase in D2 and D3 (Table 1).

**Table 1: Effect of Extra Virgin Olive Oil on SOD Activities Preeclampsia Pregnant Rat Model**

| Groups | Mean SOD Activities (U/mgprot ± SD) | Shapiro Wilk Test (p value) | Levene Test (p value) | One Way ANOVA (p value) |
|---|---|---|---|---|
| K (-) | 11.68 ± 1.58 | 0.366 | 0.859 | 0.002 |
| K (+) | 14.76 ± 1.54 | 0.177 | | |
| D1 | 10.39 ± 1.52 | 0.561 | | |
| D2 | 14.22 ± 1.92 | 0.973 | | |
| D3 | 17.49 ± 3.12 | 0.724 | | |

SOD activities (U/mgprot) in preeclampsia pregnant rat model given EVOO were measured at 19 day of pregnancy in negative control group (K -), positive control group (K +), EVOO 1st dose 0.5 mL/day (D1), EVOO 2nd dose 1 mL/day (D3) and EVOO 3rd dose 2 mL/day (D3). If p value > 0.05 on Shapiro Wilk dan Levene Test mean datas were normally distributed and homogenous. If p value < 0.05 on One Way ANOVA mean there were significant differences in SOD activities between groups. The multiple comparison LSD test showed that there was significant difference between negative control and posit if control (p=0.049), dose 3 (p=0.001); posit if control and dose 1 (p=0.008).

Increased SOD activity (Gohil et al 2011) was reported in pregnant women with preeclampsia 32-36 weeks gestational age. In the study it was also known that a significant increase in MDA in preeclampsia compared to normal pregnancies, so that the excessive lipid peroxidation process was suspected during the preeclampsia process. Increased SOD activity as a form of adaptive response of the body to fight oxidative stress characterized by increased MDA. This condition is supported by the finding of an increase in SOD gene expression in preeclampsia placenta compared with normal placenta [17,18].

Sheena's (2012) and Sultana et al (2016) study stated different things, there was a significant decrease in SOD activity in the preeclampsia group compared to the group of normal pregnant women in the third trimester of pregnancy[19, 20]. Pandey et al (2013) conducted a study of 66 patients 28-38 weeks' gestation showing a significant decrease in SOD activity in preeclampsia compared to normal pregnancies (p <0.001) [21]. The increase in excessive free radical activity in the body of pregnant women with preeclampsia causes inactivation of the enzyme system which results in a decrease in SOD activity [19, 20].

Although there are differences in SOD activity in the various studies above, there is always a similarity in pregnancy with preeclampsia, namely an increase in MDA levels [22, 19, 21, 20]. Therefore it was concluded that the increase in SOD activity and MDA levels in the positive control group compared to negative controls reflected an increase in SOD activity as compensation for an increase in lipid peroxidation. The differences in SOD activity in some of the studies above may be due to differences in gestational age when samples were taken. There is an increase in oxidative stress along with the progress of gestational age especially in preeclampsia. Pregnancies that have the potential to develop preeclampsia show increased oxidative stress at gestational age above 16-20 weeks, maternal plasma MDA trimester 1 and 2 higher than normal pregnancies while SOD decreases in 2nd, 3rd trimester and in umbilicus blood [17].

The administration of EVOO in various doses resulted in a decrease in SOD activity in group 1 but SOD activity increased along with the addition of EVOO doses. A decrease in SOD activity was also seen in the Lopez et al study, SOD activity decreased in subjects who received EVOO intake on their daily diet menu. This is because the content of the EVOO polyphenols acts as a scavanger ROS which functions to reduce the production of superoxide anions [23, 8]. SOD is widely known as an enzymatic antioxidant that is responsible for carrying out superoxide anion dismutase to H2O2 (hydropen peroxide) and O2 [24].

If the superoxide anion has been captured by the EVOO polyphenols, the production of superoxide anions will decrease. Decreasing the amount of superoxide anion radicals resulted in a decrease in endogenous SOD antioxidant activity. This supports the theory that the EVOO polyphenols act as scavanger free radicals while reducing the body's need for certain enzymatic antioxidants and suppress the synthesis of several enzymatic antioxidants due to the amount of free radicals that have decreased in the body [23]. Thus the decrease in SOD activity in this study is the body's adaptive mechanism due to the reduced amount of superoxide anion radical.

The addition of SOD activity to EVOO doses 2 and 3 is likely to occur because one of the benefits of the phenol content in EVOO is increasing total plasma antioxidant activity [8]. The EVOO hydroxythrosol induces an endogenous antioxidant defense mechanism by modulating the transcription factor nuclear factor (Erythroid-Derived 2) -Like 2 (Nrf2) to produce SOD [25, 25, 9]. However, what needs to be considered is an increase in the average SOD activity at dose 2 and dose 3

 

exceeding the average SOD activity of healthy pregnant mice. Because the SOD activity of the positive control group was significantly different from dose 1, it was concluded that the optimal EVOO dose for increasing SOD activity in this study was in the range of doses 1 to 2.

## Effect of EVOO on GSH Level Preeclampsia Rat Model

Data showed significant lower GSH level in K (+) compared to K (-) group. Meanwhile, by giving EVOO doses 1 (D1) GSH level seems decrease than posit if control. In accordance with addition EVOO doses, GSH level tend to increase in D2 and D3 (Table 2).

**Table 2: Effect of Extra Virgin Olive Oil on GSH Level Preeclampsia Pregnant Rat Model**

| Groups | Mean GSH Level (mg/gprot ± SD) | Shapiro Wilk Test (p value) | Levene Test (p value) | One Way ANOVA (p value) |
|---|---|---|---|---|
| K (-) | 0.3412 ± 0.03968 | 0.433 | 0.905 | 0.000 |
| K (+) | 0.2278 ± 0.04114 | | | |
| D1 | 0.2082 ± 0.04221 | | | |
| D2 | 0.2798 ± 0.03186 | | | |
| D3 | 0.4273 ± 0.09014 | | | |

GSH level (mg/gprot) in preeclampsia pregnant rat model given EVOO were measured at 19 day of pregnancy in negative control group (K -), positive control group (K +), EVOO 1st dose 0.5 mL/day (D1), EVOO 2nd dose 1 mL/day (D3) and EVOO 3rd dose 2 mL/day (D3). If p value > 0.05 on Shapiro Wilk danLevene Test mean datas were normally distributed and homogenous. If p value <0.05 on One Way ANOVA mean there were significant differences in GSH levels between groups. The multiple comparison LSD test showed that there was significant difference between negative control and posit if control (p=0.002), dose 1 (p=0.000), dose 3 (p=0.011); posit if control and dose 3 (p=0.000).

The results of this study in accordance with previous studies on placental preeclampsia patients showed a significant or significant reduction in GSH levels in preeclampsia women compared to normal pregnant women [26, 27, 3]. A reduction in GSH synthesis and large use of GSH can damage endothelial function. In the event of a decrease in synthesis, GSH will be activated by signaling pathways related to oxidative stress.

From this study it was concluded that a reduction in GSH would contribute to the occurrence of preeclampsia, one of which is through the abnormal pathways of GSSG and GSH [26]. Glutathione can donate one electron (H +) to another unstable molecule such as ROS. The high oxidative stress in preeclampsia can increase glutathione oxidation of cells and tissues resulting in an increase in oxidized glutathione (GSSG) and a decrease in GSH levels [28]. GSH levels increased with the addition of the EVOO dose and in the dose 3 groups it was seen to increase significantly when compared with the positive control group. This shows the influence of EVOO on the levels of GSH placenta in preeclampsia rats. From previous studies it was known that the EVOO polyphenols act to inhibit NADPH oxidase in producing superoxide and hydrogen peroxide. In addition, EVOO acts as a hydrogen peroxide scavenger so that it can replace the role of GSH [10].

In addition, hydroxytyrosol which is a component of the EVOO polyphenol fraction can increase GSH synthesis by activating nuclear factor (erythroid-derived-2) -like 2 (Nrf2). Nrf2 is a transcription factor and regulator in the antioxidant defense system. Nrf2 induces the expression of gamma-glutamylcysteineligase (GCL) which has the ability to synthesize GSH [29]. This finding is in line with the results of a study of EVOO doses of 2mL/ day which can increase GSH levels assumed because of this function.

## Effect of EVOO on PlGF Level Preeclampsia Rat Model

Data showed lower PlGF level in K (+) compared to K (-) group. Meanwhile, by giving EVOO PlGF level seem increase in dose 1 and 2 then decrease in dose 3 (Table 3).

 

**Table 3: Effect of Extra Virgin Olive Oil on PlGF Level Preeclampsia Pregnant Rat Model**

| Groups | Mean PlGF Level (ng/dL ± SD) | Shapiro Wilk Test (p value) | Levene Test (p value) | One Way ANOVA (p value) |
|---|---|---|---|---|
| K (-) | 0.1785 ± 0.0103 | 0.788 | 0.087 | 0.000 |
| K (+) | 0.1525 ± 0.0064 | 0.499 | | |
| D1 | 0.2089 ± 0.0382 | 0.673 | | |
| D2 | 0.2914 ± 0.0305 | 0.793 | | |
| D3 | 0.1814 ± 0.0430 | 0.301 | | |

PlGF level (ng/dL) in preeclampsia pregnant rat model given EVOO were measured at 19 day of pregnancy in negative control group (K -), positive control group (K +), EVOO 1st dose 0.5 mL/day (D1), EVOO 2nd dose 1 mL/day (D3) and EVOO 3rd dose 2 mL/day (D3). If p value > 0.05 on Shapiro Wilk danLevene Test mean datas were normally distributed and homogenous. If p value < 0.05 on One Way ANOVA mean there were significant differences in PlGF levels between groups. The multiple comparison LSD test showed that there was significant difference between negative control and dose 2 (p=0.000); positif control and dose 1 (p=0.017), dose 2 (p=0.000).

Research related to preeclampsia with experimental animals conducted by Levine RJ et al. (2004), shows results that are in line with this study, that in pre-eclampsia conditions there was a decrease in PlGF [30]. PlGF deficiency in the condition of preeclampsia is possible from a decrease in the expression of PlGF and a decrease in free PlGF which binds to sFlt-1, where sFlt-1 levels are increased in preeclampsia patients. K. Chau et al. (2017) states that even though preeclampsia in the PlGF concentration is identified as low, the normal range of PlGF levels is very wide in healthy pregnancies, making interpretation of cases difficult[31].

Increasing the concentration of PlGF for each patient is also uncertain. So it is important to determine the time and duration of exposure to exogenous PlGF for the treatment of preeclampsia, given the decrease in the concentration of PlGF which is physiological during normal pregnancy. Research conducted by Parchem JG et al. (2018) put forward different ideas from the results of previous studies.

To test whether preeclampsia is a result of an imbalance of angiogenic factors reflected in the sFlt-1 / PlGF ratio, Parchem JG et al. (2018) developed PlGF knockout mice and reported no signs and symptoms of preeclampsia despite an increase in sFlt-1. Then also developed deficient PlGF knockout-COMT model mice and produced a decrease in blood pressure in the mother and an increase in placental glycogen. This study identified the role of PlGF in placental development and supported a complex model for the pathogenesis of preeclampsia beyond the imbalance of angiogenic factors [32]. From the above explanation, it reinforces the assumption that the sFlt-1 / PlGF ratio has been proposed as an index of antiangiogenic activity that reflects changes in both biomarkers and is also a better way to diagnose preeclampsia than just one measure [33].

The positive effects of EVOO on health can be ascertained, and this is related to the composition of special fatty acids (high content of oleic acid, polyunsaturated essential fatty acids (PUFA), low ratio of PU-n-6 / PUFA n-3, and height Bioactive compounds include phenolic, sterol, hydrocarbon (squalene) compounds, vitamins (α- and γ-tocopherol), ß-carotene and other phytosterols [34]. Conducted by Cárdeno A. Et al. (2013) reported phenolic compounds showing broad spectrum bioactive properties, including antioxidants, free radical scavenging, anti-inflammatory effects and chemopreventive effects.

The antioxidants in EVOO can delay the oxidation process. In this case, the main antioxidant that inhibits the oxidation process in EVOO is OP (Olive Phenols), which breaks down the chain by donating hydrogen radicals to alkylperoxyl radicals, which are produced by lipid oxidation and stable derivative formation during the reaction [35]. In this study, there was an increase in the average PlGF level along with the addition of Extra Virgin Olive Oil (EVOO) given to 0.5 mL and 1 mL, but at 2 mL administration it seemed inconsistent.

An increase in anti-angiogenic factors or a decrease in angiogenic factors with higher doses of Extra Virgin Olive Oil (EVOO) is possible due to changes in the content of some antioxidants that have a prooxidant effect.

 

In a study conducted by Maurya DK and Devasagayam TP (2010) reported that oleuropein and hydroxithrosol at EVOO have been reported to have a prooxidative effect, due to inhibitory activity of iron and copper. This reduced metal catalyzes the production of OH radicals via the Fenton reaction [36]. Other studies related to antioxidant and prooxid activity concluded that the ability of food polyphenols to act as antioxidants or prooxides in in vitro and in vivo systems depends on a number of factors such as concentration and structure [37].

## Conclusion

Administration EVOO decreased blood pressure at once increased SOD activity, GSH and PlGF level in preeclampsia rat model. EVOO had been shown to be a potential antioxidant in preeclampsia through increased SOD activity and GSH level, at once modulate angiogenesis through increased PlGF level.

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## References

1. De Souza Rugolo LMS, Bentlin MR, Trindade CEP (2011) Preeclampsia: Effect On The Fetus And Newborn. NeoReviews, 12(4):e198-206.
2. Yang X, Guo L, Li H, Chen X, Tong X (2012) Analysis Of The Original Causes Of Placental Oxidative Stress In Normal Pregnancy And Pre-Eclampsia: A Hypothesis. Journal of Maternal-Fetal and Neonatal Medicine, 25(7):884-8.
3. Subandrate, Faisal ME, Anggraini NW (2017) Peranan Stres Oksidatif Pada Preeklampsia. Cermin Dunia Kedokteran, 44(5):353-5.
4. Sánchez-Aranguren LC, Prada CE, Riaño-Medina CE, Lopez M (2014) Endothelial Dysfunction And Preeclampsia: Role of Oxidative Stress. Frontiers in Physiology, 5(OCT):1-11.
5. Hod T, Cerdeira AS, Karumanchi SA (2015) Molecular Mechanisms of Preeclampsia.
6. Gathiram P, Moodley J (2016) Pre-Eclampsia: Its Pathogenesis And Pathophysiolgy. Cardiovascular Journal of Africa [Internet]. 27(2):71-8. Available from: http://cvja.co.za/onlinejournal/vol27/vol27_issue2/#17/z
7. Harapan H, Yeni CM (2015) The Role of MicroRNAs On Angiogenesis And Vascular Pressure In Preeclampsia: The Evidence From Systematic Review. Egyptian Journal of Medical Human Genetics [Internet]. 16(4):313-25. Available from: http://dx.doi.org/10.1016/j.ejmhg.2015.03.006
8. Cicerale S, Lucas LJ, Keast RSJ (2012) Antimicrobial, Antioxidant And Anti-Inflammatory Phenolic Activities In Extra Virgin Olive Oil. Current Opinion in Biotechnology [Internet]. 23(2):129-35. Available from: http://dx.doi.org/10.1016/j.copbio.2011.09.006
9. Martínez-Huélamo M, Rodríguez-Morató J, Boronat A, de la Torre R (2017) Modulation Of Nrf2 By Olive Oil And Wine Polyphenols And Neuroprotection. Antioxidants, 6(4):73.
10. Carnevale R, Nocella C, Cammisotto V, et al (2018) Antioxidant Activity From Extra Virgin Olive Oil Via Inhibition of Hydrogen Peroxide-Mediated NADPH-Oxidase 2 Activation. Nutrition [Internet]. 55-56:36-40. Available from: https://doi.org/10.1016/j.nut.2018.03.045
11. Kweider N, Fragoulis A, Rosen C, et al (2011) Interplay Between Vascular Endothelial Growth Factor (VEGF) And Nuclear Factor Erythroid 2-Related Factor-2 (Nrf2): Implications For Preeclampsia. Journal of Biological Chemistry, 286(50):42863-72.
12. Schwager SJ, Mutschler MA, Federer WT, Scully BT (1993) The Effect Of Linkage On Sample Size Determination For Multiple Trait Selection. Theoretical and Applied Genetics, 86:964-74.
13. Irianti E (2017) Hsp70 Expression Profile In Preeclampsia Model Of Pregnant Rat ( Rattus Norvegicus ) After Giving The EVOO Hsp70 Expression Profile In

 

Preeclampsia Model Of Pregnant Rat (Rattus Norvegicus ) After Giving The EVOO. IOP Conference Series: Materials Science and Engineering Paper, 180:1-6.

14. Amaral TAS, Ognibene DT, Carvalho LCRM, et al (2017) Differential Responses Of Mesenteric Arterial Bed To Vasoactive Substances In L-NAME-Induced Preeclampsia : Role Of Oxidative Stress And Endothelial Dysfunction. Clinical and Experimental Hypertension [Internet]. 00(00):1-10. Available from: https://doi.org/10.1080/10641963.2017.1339073
15. Shu W, Li H, Gong H, et al (2018) Evaluation Of Blood Vessel Injury, Oxidative Stress And Circulating Inflammatory Factors In An L-Name-Induced Preeclampsia-Like Rat Model. Experimental and Therapeutic Medicine, 16(2):585-94.
16. Zhu H, Zhu W, Hu R, Wang H, Ma D, Li X (2016) Hypertension In Pregnancy The Effect Of Pre-Eclampsia-Like Syndrome Induced By L-NAME On Learning And Memory And Hippocampal Glucocorticoid Receptor Expression : A Rat Model. Hypertension in Pregnancy [Internet]. 00(00):18. Available from: http://dx.doi.org/10.1080/10641955.2016.1228957
17. Dsouza V, Rani A, Patil V, et al (2016) Increased Oxidative Stress From Early Pregnancy In Women Who Develop Preeclampsia. Clinical and Experimental Hypertension, 38(2):225-32.
18. Aouache R, Biquard L, Vaiman D, Miralles F (2018) Oxidative Stress In Preeclampsia And Placental Diseases. International Journal of Molecular Sciences, 19: 5.
19. S SP (2012) Compprative Study Of Oxidative Stress In Pregnancy Induced Hypertension Preeclampsia And Eclampsia. International Journal of Biomedical and Advance Research, 3(11):810-4.
20. Lavanya YR, BS (2015) Study Of Oxidative Stress And Uric Acid In Pregnancy Induced Hypertension. International Journal of Biomedical and Advance Research, 6(3):204.
21. Pandey S, Singh B, Agnihotari M, Mahdi AA, George K (2013) Available Online At Http // Www.Ijrdpl.Com Comparative Status Of Oxidant / Antioxidant In Pregnancy Induced Hypertension And Cervical Dysplasia, 2(5):620-5.
22. Gohil JT, Patel PK, Priyanka G (2011) Evaluation Of Oxidative Stress And Antioxidant Defence In Subjects Of Preeclampsia. Journal of Obstetrics and Gynecology of India, 61(6):638-40.
23. Oliveras-López MJ, Molina JJM, Mir MV, Rey EF, Martín F, De la Serrana HLG (2013) Extra Virgin Olive Oil (EVOO) Consumption And Antioxidant Status In Healthy Institutionalized Elderly Humans. Archives of Gerontology and Geriatrics, 57(2):234-42.
24. Winarsi H. Antioksidan Alami Dan Radikal. Kanisius.
25. Chen B, Lu Y, Chen Y, Cheng J (2015) The Role Of Nrf2 In Oxidative Stress-Induced Endothelial Injuries. Journal of Endocrinology, 225(3):R83-99.
26. Jin X, Xu Z, Cao J, et al (2017) Proteomics Analysis Of Human Placenta Reveals Glutathione Metabolism Dysfunction As The Underlying Pathogenesis For Preeclampsia BBA - Proteins And Proteomics Proteomics Analysis Of Human Placenta Reveals Glutathione Metabolism Dysfunction As The Underlying Pathogenesis For Preeclampsia ☆ (July).
27. Taravati A, Tohidi F (2018) Comprehensive Analysis Of Oxidative Stress Markers And Antioxidants Status In Preeclampsia. Taiwanese Journal of Obstetrics and Gynecology [Internet]. 57(6):779-90. Available from: https://doi.org/10.1016/j.tjog.2018.10.002
28. Rani N, Dhingra R, Arya DS, Kalaivani M, Bhatla N, Kumar R (2010) Role Of Oxidative Stress Markers And Antioxidants In The Placenta Of Preeclamptic Patients. Journal of Obstetrics and Gynaecology Research, 36(6):1189-94.
29. Kouka P, Priftis A, Stagos D, et al (2017) Assessment Of The Antioxidant Activity Of An Olive Oil Total Polyphenolic Fraction And Hydroxytyrosol From A Greek Olea Europea Variety In Endothelial Cells And Myoblasts. 2017;(2):703-12.
30. Gaiser R (2005) Circulating Angiogenic Factors And The Risk Of Preeclampsia. Survey of Anesthesiology, 49(1):14-5.

 

31. Chau K, Hennessy A, Makris A (2017) Placental Growth Factor And Pre-Eclampsia. Journal of Human Hypertension [Internet]. 31(12):782-6. Available from: http://dx.doi.org/10.1038/jhh.2017.61

32. Parchem JG, Carmeliet P, Kalluri R, et al (2018) Loss Of Placental Growth Factor Ameliorates Maternal Hypertension And Preeclampsia In Mice. Journal of Clinical Investigation. 128(11):5008-17.

33. Hagmann H, Thadhani R, Benzing T, Karumanchi SA, Stepan H (2012) The Promise Of Angiogenic Markers For The Early Diagnosis And Prediction of Preeclampsia. Clinical Chemistry. 58(5):837-45.

34. Costa I, Moral R, Solanas M, Andreu FJ, Ruiz De Villa MC, Escrich E (2011) High Corn Oil And Extra Virgin Olive Oil Diets And Experimental Mammary Carcinogenesis: Clinicopathological And Immunohistochemical P21Ha-Ras Expression Study. Virchows Archiv., 458(2):141-51.

35. Cárdeno A, Sánchez-Hidalgo M, Alarcón-de-la-Lastra C (2013) An Up-Date Of Olive Oil Phenols In Inflammation And Cancer: Molecular Mechanisms And Clinical Implications. Current Medicinal Chemistry, 20(37):4758-76.

36. Maurya DK, Devasagayam TPA (2010) Antioxidant And Prooxidant Nature Of Hydroxycinnamic Acid Derivatives Ferulic And Caffeic Acids. Food and Chemical Toxicology [Internet]. 48(12):3369-73. Available from: http://dx.doi.org/10.1016/j.fct.2010.09.006

37. Perron NR, García CR, Pinzón JR, Chaur MN, Brumaghim JL (2011) Antioxidant And Prooxidant Effects Of Polyphenol Compounds On Copper-Mediated DNA Damage. Journal of Inorganic Biochemistry [Internet]. 105(5):745-53. Available from: http:// dx.doi. org/ 10. 1016/j.jinorgbio.2011.02.009

 