The Role of Extra Virgin Olive Oil in Suppressing Oxidative Stress through Reducing Superoxide Dismutase Activity in Ratus Novergicus Preeclampsia Model

Dwi Norma Retnaningrum*, Wenny Rahmawati
STIKES Widyagama Husada
*Email: dwinorma@widyagamahusada.ac.id

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ABSTRACT

Oxidative stress is a key factor in the pathophysiology of preeclampsia. Preeclampsia is characterized by hypertension and proteinuria. Superoxide Dismutase (SOD) will decrease when reactive oxygen species (ROS) are overproduced. Extra Virgin Olive Oil (EVOO) contains polyphenols and vitamin E, which are known to have strong antioxidant properties. The aim of this study was to assess how EVOO supplementation affects SOD activity in a rat model of preeclampsia (Rattus norvegicus). Pregnant rats were divided into a normal control group, a preeclamptic control group administered with L-NAME, and treatment groups receiving L-NAME and different doses of EVOO in a post-test control group design. Spectrophotometry was used to measure SOD activity. The results showed a significant difference between the SOD activity of the control and treatment groups (P value = 0.002). The optimal EVOO dose returned SOD activity to near normal control levels. In conclusion, EVOO supplementation shows potential as an adjunct therapy to reduce oxidative stress in preeclampsia by increasing SOD activity.

INTRODUCTION

Globally, preeclampsia continues to be a leading cause of maternal and neonatal morbidity and death. After 20 weeks of pregnancy, this hypertensive condition of pregnancy is clinically defined by high blood pressure and proteinuria. Its etiology is closely linked to oxidative stress and endothelial dysfunction. Reactive oxygen species (ROS) overproduction causes vascular damage, lipid peroxidation, and a reduction in the body's natural antioxidant defenses. Superoxide Dismutase (SOD) is a crucial enzyme in the antioxidant defense system, which catalyzes the dismutation of superoxide radicals into less harmful molecules. Reduced SOD activity has been consistently reported in preeclamptic conditions, contributing to the worsening of oxidative stress and vascular impairment (Ives *et al.*, 2020).

Efforts to counteract oxidative

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stress in preeclampsia have increasingly focused on nutritional and natural antioxidant supplementation. High concentrations of phenolic compounds, tocopherols, and monounsaturated fatty acids with potent antioxidant activity are found in extra virgin olive oil (EVOO), a functional dietary component of the Mediterranean diet. Previous studies have demonstrated that EVOO can scavenge free radicals, enhance antioxidant enzyme activity, and improve endothelial function. However, its potential role in modulating oxidative stress during pregnancy, particularly in preeclampsia, remains underexplored (Dwi Norma Retnaningrum, Wenny Rahmawati and Alfima Rahasti, 2021)(Kusuma *et al.*, 2022).

Extra Virgin Olive Oil (EVOO) is the first oil produced from olives. EVOO is widely used in Mediterranean countries due to its important health benefits. Mediterranean diets containing EVOO are associated with a reduced risk of chronic degenerative diseases and increased life expectancy. One of the active ingredients in EVOO is polyphenols, which function as antioxidants, anti-inflammatory agents, and antimicrobials. Through their antioxidant function, polyphenols in EVOO reduce ROS production and play a crucial role as free radical scavengers. They also increase total plasma antioxidant activity, including SOD. Furthermore, the polyphenol content in EVOO also influences angiogenesis through Nuclear Factor Erythroid 2-related Factor-2 (Nrf2), which is associated with increased VEGF (Mart, Rodr and Torre, 2017)(Jimenez-Lopez *et al.*, 2020).

The effect of oral EVOO administration on SOD activity in preeclampsia is currently unknown. This study aims to prove that oral EVOO administration can increase SOD activity in pregnant Rattus norvegicus mice (a model of preeclampsia).

RESEARCH METHODS

Study Design and Subjects

A post-test only control group design was used in this experimental investigation. Replication is the number of experimental units receiving the same treatment under certain conditions. In this study, replication was determined using the formula (Solimun, 2001).

$$p\,(n - 1) \geq 15$$

Description:
p: number of treatment groups

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n: number of replications for each treatment group

If in this study there are 5 treatment groups, then the replication per group is:

$$p\,(n-1) \geq 15$$
$$5n - 5 \geq 15$$
$$5n \geq 20$$
$$n \geq 4$$

Twenty-four pregnant Rattus norvegicus (Wistar strain) were randomly divided into five groups (n = 4 per group) for the study:

1. Normal control group (K-): pregnant rats without intervention.
2. Preeclampsia control group (K+): pregnant rats induced with L-NAME to develop preeclampsia.
3. Treatment group 1 (P1): preeclampsia-induced rats administered with EVOO at a dose of 0.5 mL/day.
4. Treatment group 2 (P2): preeclampsia-induced rats administered with EVOO at a dose of 1 mL/day.
5. Treatment group 3 (P3): preeclampsia-induced rats administered with EVOO at a dose of 2 mL/day.

Induction of Preeclampsia

Preeclampsia was induced by administering L-NAME (Nω-nitro-L-arginine methyl ester) at a dose of 125 mg/kgBW orally from gestational day 12 to 19 to inhibit nitric oxide synthase, leading to hypertension and proteinuria(de Alwis *et al.*, 2022).

EVOO Administration

EVOO administration refers to research by Nugraheni (2012) and Irianti et al. (2017). Referring to the Mediterranean diet, the daily requirement of olive oil is 30-50 grams. If converted into a dose based on the rat's body weight, then = 30 x 0.018 = 0.54 ≈ 0.5 grams/day.

Extra Virgin Olive Oil (EVOO) was administered orally by gastric gavage at the designated doses once daily for 8 consecutive days during pregnancy, concomitant with L-NAME administration.

Measurement of SOD Activity

On gestational day 20, blood samples were taken from the retro-orbital plexus under mild anesthetic at the conclusion of the operation. Serum was separated by centrifugation and analyzed for Superoxide Dismutase (SOD) Activity using the Kolorimetri method (Kit Elabscience) according to the manufacturer's instructions. A microplate spectrophotometer was used

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to measure optical density at 450 nm(Aouache *et al.*, 2018).

Statistical Analysis

The Shapiro-Wilk test was used to check for normality, while the Levene test was used to check for homogeneity. One-way ANOVA was used for group comparisons, and the Least Significant Difference (LSD) post hoc test was used for pairwise analysis. The threshold for statistical significance was fixed at $p < 0.05$.

Ethical Clearance Certificate with No:425/KEPKPOLKESMA/2025 was obtained from The Health Research Ethics Committee State Polytechnic Of Health Malang.

RESULTS AND DISCUSSION

Results

Pregnant rats with a preeclampsia model were created by injecting L-NAME at a dose of 125 mg/kgBW from day 13 to 18 of pregnancy, and were also given various doses of EVOO. Changes in maternal body weight were measured on days 1, 10, and 19 of pregnancy, while blood pressure and proteinuria were measured on days 12, 15, and 19 of pregnancy. Systolic blood pressure of pregnant rats at G 12 before L-NAME injection was normal (<140 mmHg), after L-NAME injection (G 15) there was an increase in systolic blood pressure (>140 mmHg) indicating the success of creating a preeclampsia model. At G 19 before surgery, there was a decrease in systolic blood pressure back to normal (<140 mmHg) after EVOO administration. From the recording and measurements, the following results were obtained:

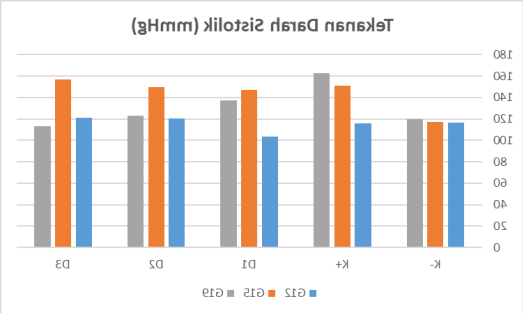

Figure 1. Results of Diastolic Blood Pressure Measurements in Pregnant Rats with Preeclampsia Model Given Extra Virgin Olive Oil.

Description: The average diastolic blood pressure of pregnant rats with preeclampsia model given EVOO was measured on days 12, 15 and 19 of pregnancy in the negative control group (K -), positive control (K +), EVOO dose 1, namely 0.5 ml/day (D1), EVOO dose 2, namely 1 ml/day (D2), and EVOO dose 3, namely 2 ml/day (D3).

Changes in proteinuria in preeclampsia model rats in this study can be observed in full in table 1:

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Table 1. Changes in Proteinuria in Pregnant Rats with Preeclampsia Model Given Extra Virgin Olive Oil

Kelompok	G12	G15	G19
K (-)	Negatif (4)	Negatif (4)	Negatif (4)
K(+)	Negatif (4)	Positif 1 (4)	Positif 1 (2) Positif 2 (2)
D1	Negatif (4)	Positif 1 (3) Positif 2 (1)	Negatif (2) Positif 1 (2)
D2	Negatif (4)	Positif 1 (1) Positif 2 (3)	Negatif (2) Positif 1 (2)
D3	Negatif (4)	Negatif (2) Positif 1 (2)	Negatif (3) Positif 1 (1)

Description: Measurement of proteinuria in pregnant rats with preeclampsia model given EVOO was carried out on days 12, 15 and 19 of pregnancy in the negative control group (K -), positive control (K +), EVOO dose 1, namely 0.5 ml/day (D1), EVOO dose 2, namely 1 ml/day (D2), and EVOO dose 3, namely 2 ml/day (D3).

The results of the proteinuria examination of the negative control group showed negative results at all three measurement times (G 12, G 15 and G 19). In the positive control group, proteinuria was negative at G 12, increased after L-NAME injection (G 15) and worsened at G 19. Meanwhile, the EVOO administration group generally showed negative results at G 12, increased at G 15 after L-NAME exposure, and there were various results at G 19 after EVOO administration (negative, decreased although not yet back to normal, and persisted). In addition, it can also be observed that 2 samples at dose 3 did not experience an increase in proteinuria after L-NAME injection (G 15), however, the diagnosis of preeclampsia in experimental animals can still be established based on increased blood pressure.

The mean serum Superoxide Dismutase (SOD) activity differed significantly among the experimental groups. The normal control group (K–) showed the highest SOD activity, while the preeclampsia control group (K+) exhibited a marked reduction. Administration of EVOO increased SOD activity in a dose-dependent manner. The results are summarized in Table 2.

Table 2. Mean ± SD of SOD Activity in Experimental Groups

Group	*Treatment*	SOD Activity (U/mL, Mean ± SD)
K-	Normal control	15.28 ± 1.21^{a}
K+	Preeclamsia control	7.36 ± 0.84^{c}
P1	Preeclamsia+EVOO 0,5	10.42 ± 1.15^{ab}
P2	Preeclamsia+EVOO 1	12.87 ± 1.34^{a}
P3	Preeclamsia+EVOO 2	14.92 ± 1.08^{a}

One-way ANOVA showed a statistically significant difference in SOD activity between groups ($p < 0.001$). Post hoc LSD tests revealed that:

- K+ had significantly lower SOD activity than all other groups ($p < 0.05$).
- P1, P2, and P3 groups showed significantly higher SOD activity

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compared to K+ ($p < 0.05$).

- The highest dose group (P3) had SOD activity not significantly different from the normal control (K–) ($p > 0.05$).

These findings indicate that EVOO supplementation restored antioxidant enzyme activity, with the 2 mL/day dose demonstrating the most pronounced effect.

Discussion

This study demonstrated that administration of Extra Virgin Olive Oil (EVOO) significantly increased Superoxide Dismutase (SOD) activity in preeclampsia-induced *Rattus norvegicus*. The preeclampsia control group (K+) exhibited markedly reduced SOD activity compared to the normal control, supporting the hypothesis that oxidative stress plays a pivotal role in the pathophysiology of preeclampsia. Excessive production of reactive oxygen species (ROS) in preeclampsia These findings are consistent with previous studies reporting decreased antioxidant enzyme activity in preeclampsia compared to normal pregnancy. These findings are consistent with previous studies reporting decreased antioxidant enzyme activity in preeclampsia compared to normal pregnancy(Bucciantini *et al.*, 2021)(Salsabila *et al.*, 2022).

EVOO supplementation successfully restored SOD activity in a dose-dependent manner. The lowest dose (0.5 mL/day) increased SOD activity moderately, whereas higher doses (1 mL/day and 2 mL/day) showed more substantial improvements. The highest dose group (P3) achieved SOD levels comparable to the normal control group, indicating that EVOO has potent antioxidant properties capable of normalizing oxidative balance in preeclampsia. These results align with earlier reports that EVOO, rich in phenolic compounds, tocopherols, and monounsaturated fatty acids, exerts free radical scavenging activity and enhances the expression and activity of endogenous antioxidant enzymes(Dwi Norma Retnaningrum, Wenny Rahmawati and Alfima Rahasti, 2021).

The mechanism underlying the antioxidant effects of EVOO may involve polyphenolic compounds such as hydroxytyrosol, oleuropein, and tyrosol, which have been shown to upregulate antioxidant pathways and modulate gene expression related to oxidative stress. Moreover, vitamin E (α-tocopherol) contained in EVOO is a

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potent lipid-soluble antioxidant that protects cell membranes from peroxidative damage. By enhancing SOD activity, EVOO contributes to the detoxification of superoxide radicals, thereby reducing endothelial dysfunction, one of the hallmarks of preeclampsia(Kusuma *et al.*, 2022)(Jimenez-Lopez *et al.*, 2020).

These findings have important implications for exploring natural antioxidant therapies in the management of preeclampsia. Nutritional interventions using EVOO may offer a safe and accessible strategy to reduce oxidative stress in pregnant women at risk. However, it is necessary to note that this study was conducted in an animal model, and translation to human clinical settings requires further investigation. Human trials are needed to determine optimal dosage, safety, and efficacy in preventing or ameliorating preeclampsia(Fitriah Ramadhani *et al.*, 2022)(Irianti *et al.*, 2020).

Limitations of the study include the relatively small sample size and the absence of measurement of other oxidative stress biomarkers, such as malondialdehyde (MDA) or glutathione peroxidase (GPx), which could provide a more comprehensive picture of the antioxidant defense system. Future research should include broader biochemical analyses and evaluate long-term outcomes in both animal and human studies(Negishi *et al.*, 2021).

CONCLUSIONS

This study demonstrated that administration of Extra Virgin Olive Oil (EVOO) significantly increased Superoxide Dismutase (SOD) activity in preeclampsia-induced *Rattus norvegicus*. The lowest dose of EVOO (0.5 mL/day) produced a moderate improvement, while higher doses (1 mL/day and 2 mL/day) yielded greater increases in SOD activity. The highest dose restored SOD activity to levels comparable with the normal control group. These findings indicate that EVOO effectively reduces oxidative stress through the enhancement of endogenous antioxidant defense systems. EVOO therefore shows potential as an adjuvant therapy to mitigate oxidative stress in preeclampsia.

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