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Modulation of Endothelin-1 and Placental Microvascular Diameter by Extra Virgin Olive Oil in Preeclampsia Rat Model

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Abstract

Introduction: *Preeclampsia is linked to abnormal placental vasoconstriction and elevated endothelin-1 (ET-1). Polyphenols in Extra Virgin Olive Oil (EVOO) has antioxidant and vasoprotective properties, but its impact on ET-1 and placental microvasculature in preeclampsia is unclear.* ***Objective:*** *Investigate whether EVOO can attenuate hypertension and improve placental vascular health in preeclampsia.* ***Method:*** *20 pregnant rats randomly assigned to five groups: normal (K−), preeclampsia model (K+), and three EVOO-treated groups (D1, D2, D3) receiving 0.5, 1, and 2 mL/day, respectively. Preeclampsia was induced with L-NAME, blood pressure was monitored on gestational days 12, 15, and 19. Placental ET-1 expression and microvessel diameter were assessed on day 19 using immunohistochemistry.* ***Result and Discussion****: EVOO treatment normalized blood pressure by gestational day 19. ET-1 expression increased with EVOO dose suggesting a role in vascular remodelling. Microvessel diameter improved in D1 and D2 but slightly decreased in D3.* ***Conclusions:*** *EVOO mitigates hypertension and enhances placental microvasculature in preeclampsia, with optimal effects at moderate doses.*

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Introduction

Preeclampsia is a hypertension-related condition occurring during pregnancy, usually appearing after 20 weeks of gestation. It is characterized by the abrupt development of hypertension accompanied by dysfunction in multiple organs. Affecting approximately 2–8% of pregnancies worldwide, it remains a significant contributor to maternal and perinatal morbidity and mortality. Despite its multifactorial origins, primary pathological characteristics comprise abnormal placentation, systemic endothelial dysfunction, oxidative stress, and uteroplacental ischemia (Overton et al., 2022; Bisson et al., 2023).

Endothelin 1 (ET 1) is a potent vasoconstrictive peptide synthesized mainly by vascular endothelial cells and placental trophoblasts. ET 1 exerts its actions via ETA receptors (promoting vasoconstriction, inflammation, and oxidative stress) and ETB receptors (involved in clearance or vasodilatory nitric oxide release) . In preeclampsia, both circulating and placental ET 1 levels are elevated, correlating with disease severity, and contribute to maternal hypertension, placental hypoperfusion, and endothelial injury (Opichka et al., 2021).

Recent studies using human placental samples and animal models have provided further mechanistic insights. For instance, ET 1 significantly potentiates vasoconstrictive contractions in preeclamptic chorionic plate veins, via both ETA and ETB receptors, even in the presence of decreased vascular calcium channel activity (CaV1.2). Elevated expression of contractile smooth muscle marker Caldesmon 1 (CALD1) was also observed, indicating a switch toward a contractile phenotype in preeclamptic placental vessels (Su et al., 2024). Correspondingly, in rat models of placental ischemia such as the Reduced Uterine Perfusion Pressure (RUPP) model, ET 1 upregulation is accompanied by narrowed microvascular diameter, impaired remodelling of spiral arteries, and reduced uteroplacental perfusion. These alterations mirror clinical features of preeclampsia and can be ameliorated by ETA receptor antagonism (Saleh et al., 2016).

Polyphenolic compounds in Extra Virgin Olive Oil (EVOO) (e.g. hydroxytyrosol, oleuropein, oleocanthal) known for their antioxidant, anti-inflammatory, and endothelial modulating properties (D'Agostino et al., 2021). Moreover, the intake of olive oil is linked to a reduced occurrence of gestational diabetes mellitus, preeclampsia, and cardiovascular risk. Research on fetal outcomes indicates that consuming olive oil is associated with a lower likelihood of having infants that are small or large for their gestational age. In general, consuming olive oil offers protective benefits for pregnancy results (Cortez-Ribeiro et al., 2023).

Recent preclinical investigations in rat models of pregnancy complications have demonstrated that EVOO supplementation can modulate key angiogenic markers, including increased endothelial nitric oxide synthase (eNOS), elevated VEGF, and decreased soluble fms like tyrosine kinase 1 (sFlt 1) — markers of improved placental vascularization and endothelial function (Silvani et al., 2020) . However, no study has specifically evaluated the effect of EVOO on placental ET 1 expression or the microanatomy of placental vascular diameter in models of preeclampsia.

Given the pivotal role of ET 1 in mediating placental vasoconstriction and impaired vessel remodelling in preeclampsia, and the demonstrated vascular benefits of EVOO in related models, a critical gap remains the impact of EVOO on placental ET 1 expression and vascular diameter in preeclampsia models has not been directly assessed.

The present study was designed to fill this gap using a rat model of preeclampsia. It aims to evaluate:

1. Effect of EVOO administration attenuates placental ET 1 expression
2. Effect of EVOO administration restores the diameter of placental microvessels.

By elucidating these effects, this study seeks to clarify the vasoprotective potential of EVOO at the placental level, and inform future nutritional or adjunct pharmacotherapeutic strategies for the management of preeclampsia.

Method

This study involved in vivo laboratory investigation utilizing a Post Test Only Control Group design, consist of five groups with four rats each. The experimental groups consisted of normal pregnant rats group (K-); preeclampsia model group (K+); and dose groups composed of preeclamptic rats treated with EVOO at doses of 0.5 mL/day (D1), 1 mL/day (D2), and 2 mL/day (D3) (Silvani et al., 2020). The first day of pregnancy was defined as the day after mating and all rats were sacrificed on gestational day 19. The study utilized placenta as the sample. The research was conducted at the Bioscience Laboratory, Physiology Laboratory, and Biomolecular Biochemistry Laboratory, Faculty of Medicine, Universitas Brawijaya, Indonesia.

An intraperitoneal injection of L-NAME (C7H15N5O4 HCl) at a dosage of 125 mg/kg body weight was administered from the 13^{th} – 18^{th} day of pregnancy to induce preeclampsia (Silvani et al., 2019). The preeclampsia rat model was established by the elevation of blood pressure more than 140/90 mmHg. Simultaneously, EVOO was administered orally via sonde from the 1^{st} – 18^{th} day of pregnancy. Blood pressure measurements were taken on gestational days 12, 15, and 18 using blood pressure monitoring tool from CODA® by Kent Scientific Corporation.

Placental tissue samples from rats were utilized to assess ET-1 expression and vessel diameter through the immunohistochemistry (IHC) technique. The histopathological preparation included placental tissue sampling, tissue blocking and sectioning, deparaffinization, staining using hematoxylin-eosin, and immunohistochemical staining with primary antibodies against ET-1. This was followed by graded alcohol dehydration, clearing, mounting, and coverslipping. Histological slide interpretation was performed using an Olympus microscope at 400× magnification to assess ET-1 expression and measure the width of microvessel in the placental tissue.

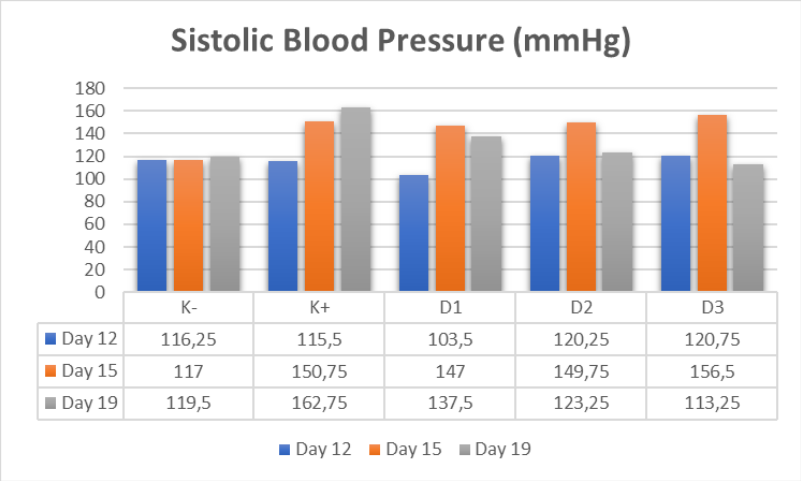
To determine differences among group means, one-way analysis of variance (ANOVA) was conducted following confirmation of data normality via the Shapiro-Wilk test and homogeneity of variances using Levene's test. Upon identifying significant effects, pairwise comparisons were performed using the Tukey HSD post hoc test to elucidate specific group differences. Statistical significance was defined at $p < 0.05$. All analyses were executed using SPSS software version 25.

Result and Discussions

1. Result

Blood pressure measurements showed that in positive control group that did not receive EVOO administration, blood pressure measurements showed a progressive increase from day 12 to day 15, and continued to rise through day 19. Meanwhile, administration of EVOO at three different doses (D1, D2, and D3) over 18 days exerted a potential antihypertensive effect, as shown by decreases in systolic as well as diastolic blood pressure on day 19. In all dosage groups, systolic blood pressure demonstrated an increase between gestational days 12 and 15, followed by a decrease into normotensive

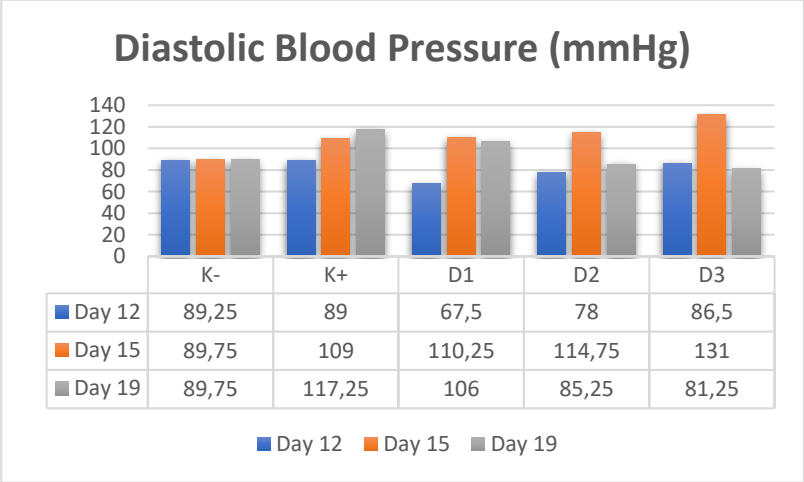
on day 19. Diastolic pressure in this group exhibited a similar trend, with a peak on day 15 and a reduction into normotensive by day 19. In general, the use of EVOO was linked to a decrease in blood pressure, showing reductions in systolic as well as diastolic measurements by the conclusion of the treatment period.



	K-	K+	D1	D2	D3
Day 12	116,25	115,5	103,5	120,25	120,75
Day 15	117	150,75	147	149,75	156,5
Day 19	119,5	162,75	137,5	123,25	113,25

Source: Primary Data 2025

Figure 1 Mean of Sistolic Blood Pressure Measurement of Preeclampsia Rat Models Administered Extra Virgin Olive Oil (mmHg)



	K-	K+	D1	D2	D3
Day 12	89,25	89	67,5	78	86,5
Day 15	89,75	109	110,25	114,75	131
Day 19	89,75	117,25	106	85,25	81,25

Source: Primary Data 2025

Figure 2 Mean of Diastolic Blood Pressure Measurement of Preeclampsia Rat Models Administered Extra Virgin Olive Oil (mmHg)

Results showed that ET-1 expression was lower in (K+) group than (K−) group. Administration of EVOO appeared to increase ET-1 expression in a dose-dependent manner across groups D1, D2, and D3. The Shapiro-Wilk and Levene's tests produced p-values greater than 0.05, confirming that the data satisfied the assumptions of normality and homogeneity of variances, respectively. One-way ANOVA demonstrated a statistically significant difference in microvessel diameter among the groups ($p < 0.05$). Subsequent Tukey's HSD post hoc analysis revealed significant differences between the following group comparisons: negative control vs. D1, D2, and D3; positive control vs. D1, D2, and D3; and between D1 and D3.

Table 1
Mean of ET-1 Expression of Preeclampsia Rat Models Administered Extra Virgin Olive Oil

Group	N	Mean ET-1 expression	Shapiro-Wilk test	Levene test	ANOVA (p value)
K (-)	4	11.25 ± 1.71	p > 0.05	p > 0.05	0.000
K (+)	4	7.50 ± 3.11			
D1	4	17.25 ± 1.71			
D2	4	20.00 ± 1.83			
D3	4	24.75 ± 2.50			

Source: Primary Data 2025

The results showed a reduction in microvessel diameter in the positive control group (K+) compared to the negative control group (K-). Administration of EVOO resulted in an increase in microvessel diameter at doses D1 and D2, followed by a decrease at dose D3. Assumptions of normality and homogeneity of variance were confirmed by Shapiro-Wilk and Levene's tests, respectively, both yielding p-values > 0.05. One-way ANOVA revealed a statistically significant difference in microvessel diameter across the groups ($p < 0.05$). Post hoc analysis using Tukey's HSD revealed significant differences between the following group comparisons: negative control vs. D1, D2, and D3; and positive control vs. D1, D2, and D3.

Table 2
Mean of Diameter Placental Microvascular of Preeclampsia Rat Models Administered Extra Virgin Olive Oil

Group	N	Diameter placental microvascular	Shapiro-Wilk test	Levene test	ANOVA (p value)
K (-)	4	29.00 ± 5.23	p > 0.05	p > 0.05	0.000
K (+)	4	19.75 ± 7.80			
D1	4	40.75 ± 4.35			
D2	4	43.00 ± 4.76			
D3	4	42.25 ± 1.26			

Source: Primary Data 2025

2. Discussion

The present study demonstrated a progressive increase in systolic and diastolic blood pressure in the positive control group from day 12 to day 19, indicating the development of sustained hypertension. In contrast, the groups treated with EVOO (D1, D2, and D3) exhibited a delayed hypertensive response that peaked on day 15, followed by a significant reduction to normotensive levels by day 19. These findings suggest that EVOO administration exerts a blood pressure-lowering effect in a dose-dependent manner. The antihypertensive effect of EVOO observed in this study aligns with prior clinical evidence. A randomized crossover study by Sarapis *et al.* (2020) showed that high-polyphenol EVOO significantly reduced systolic as well as diastolic blood pressure in healthy adults when compared to low-polyphenol olive oil. Furthermore, Vazquez *et al.* (2019) discovered that continuous use of EVOO, which is rich in bioactive compounds from olive fruit and leaves, could be a valuable method for lowering blood pressure. The bioactive components of EVOO, particularly oleuropein and hydroxytyrosol, have been reported to enhance endothelial function, reduce oxidative stress, and increase nitric oxide (NO) bioavailability that its mechanisms contribute to hypotensive effect (George *et al.,* 2019; Massaro *et al.,* 2020; Sarapis *et al.,* 2020).

Endothelin-1 (ET-1), a potent vasoconstrictor, is critically involved in the development of preeclampsia. Interestingly, in this study, ET-1 expression was observed to be lower in the positive control group in comparison to the negative control group, possibly indicating a compensatory downregulation in response to chronic vasoconstriction. Paradoxically, administration of EVOO, particularly at higher doses, was associated with increased ET-1 expression, despite its overall blood pressure-lowering effect. These mechanisms may counterbalance or override the vasoconstrictive effects of ET-1. This unexpected outcome may be explained by the complex interplay between ET-1 and nitric oxide (NO) signaling pathways. Endothelial function can be improved through the action of EVOO-derived polyphenols, especially hydroxytyrosol, which stimulate the upregulation of endothelial nitric oxide synthase (eNOS) through activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway, enhancing NO production, and reducing reactive oxygen species (ROS), which together promote vasodilation and vascular remodelling (Storniolo *et al.,* 2014; Vijakumaran *et al.,* 2023).

Additional findings from this study, previously published, have demonstrated that EVOO administration supports its vascular regulatory potential. Specifically, moderate doses of EVOO (Dose 1 and Dose 2) significantly reduced sFlt-1 levels and increased eNOS expression, partially restoring VEGF and PlGF levels and enhancing antioxidant defenses, including GSH and SOD. In contrast, a higher dose (Dose 3) appeared to reverse these effects, suggesting a possible biphasic response. These results align with previous reports indicating that olive oil polyphenols exert endothelial protective effects by activating the Nrf2/HO-1 and PI3K/Akt pathways, thereby modulating angiogenic signaling and oxidative stress responses (Silvani *et al.,* 2019; Silvani *et al.,* 2020). Taken together, these findings suggest that EVOO may provide angioprotective and antioxidative benefits at optimal doses, while excessive intake could diminish or negate these effects. Furthermore, EVOO administration was shown to modulate ET-1 expression and microvessel diameter in a dose-dependent manner, further supporting its role in vascular regulation.

The unexpected elevation of endothelin-1 (ET-1) observed in the treatment group, despite the well-documented antihypertensive and vasoprotective effects of extra virgin olive oil (EVOO) polyphenols, may not necessarily indicate a pathological response. Instead, it may reflect a transient, localized adaptation associated with endothelial repair or vascular remodeling. While polyphenols are known to downregulate ET-1 expression under conditions of endothelial dysfunction, emerging evidence suggests that their modulatory effects may be context-dependent, permitting a controlled, temporary increase in ET-1 during physiological regeneration processes—without inducing systemic hypertension. This selective regulation likely facilitates vascular homeostasis and adaptation. Therefore, the blood pressure-lowering effects of EVOO are more plausibly attributed to its capacity to enhance nitric oxide (NO) bioavailability through upregulation of endothelial nitric oxide synthase (eNOS) activity and reduction of oxidative stress, rather than through persistent inhibition of ET-1 expression.

The study demonstrated a significant variation in microvessel diameter among treatment groups. The positive control group exhibited reduced vessel diameter, consistent with vasoconstriction and impaired endothelial relaxation. In contrast, EVOO administration at D1 and D2 doses led to a significant increase in microvessel diameter, suggesting enhanced vasodilatory capacity. Interestingly, at the highest dose (D3), vessel diameter decreased again, potentially indicating a threshold effect or hormetic response.

This observation is supported by evidence suggesting that EVOO polyphenols improve endothelial-dependent vasodilation by enhancing NO synthesis and reducing oxidative stress (Liva *et al.,* 2025). The non-linear response at higher doses may reflect a biphasic action of polyphenols, where excessively high concentrations could induce feedback inhibition or mild oxidative stress, attenuating their vasodilatory effect.

The strength of this study lies in the comprehensive statistical validation, including normality and homogeneity tests, ANOVA, and post hoc analyses, ensuring the robustness of the findings. Moreover, the dose-response design allows for insight into the therapeutic window of EVOO's vascular effects.

However, limitations should be acknowledged. The 18-day treatment period may be insufficient to evaluate the long-term impact of EVOO on chronic hypertension. Another notable limitation of this study is the absence of an inter-rater reliability assessment in the histological measurements, particularly in the evaluation of ET-1 expression and microvessel diameter. All histological interpretations were performed by a single observer, which may introduce the risk of observer bias or measurement variability. To enhance the objectivity and reproducibility of histological data, future studies are recommended to involve multiple blinded observers and to perform inter-rater reliability testing. This would ensure greater consistency and credibility in the assessment of histological outcomes.

Conclusion

In conclusion, the administration of EVOO demonstrated significant antihypertensive effects, potentially through modulation of ET-1 expression and enhancement of endothelial vasodilation, as reflected in increased microvessel diameter. These results support the therapeutic potential of EVOO in the dietary management of hypertension. Future studies should include longer treatment durations, molecular assays of endothelial markers, and integration with dietary intervention models to validate these findings further.

Reference

Bisson, C., Dautel, S., Patel, E., Suresh, S., Dauer, P., & Rana, S. (2023). Preeclampsia pathophysiology and adverse outcomes during pregnancy and postpartum. Frontiers in Medicine, 10, 1144170. https://doi.org/10.3389/fmed.2023.1144170

Cortez-Ribeiro, A. C., Meireles, M., Ferro-Lebres, V., & Almeida-de-Souza, J. (2023). Olive oil consumption confers protective effects on maternal-fetal outcomes: A systematic review of the evidence. Nutrition Research, 110, 87–95. https://doi.org/10.1016/j.nutres.2022.12.013

D'Agostino, R., Barberio, L., Gatto, M., Tropea, T., De Luca, M., & Mandalà, M. (2021). Extra virgin olive oil phenols vasodilate rat mesenteric resistance artery via phospholipase C (PLC)-calcium microdomains-potassium channels (BKCa) signals. Biomolecules, 11(2), 137. https://doi.org/10.3390/biom11020137

George, E. S., Marshall, S., Mayr, H. L., Trakman, G. L., Tatucu-Babet, O. A., Lassemillante, A. M., Bramley, A., Reddy, A. J., Forsyth, A., Tierney, A. C., Thomas, C. J., Itsiopoulos, C., & Marx, W. (2019). The effect of high-polyphenol olive oil on cardiovascular risk factors: A systematic review and meta-analysis. Critical Reviews in Food Science and Nutrition, 59(17), 2772–2795. https://doi.org/10.1080/10408398.2018.1470491

Liva, K., Panagiotopoulos, A. A., Foscolou, A., Amerikanou, C., Vitali, A., Zioulis, S., Argyri, K., Panoutsopoulos, G. I., Kaliora, A. C., & Gioxari, A. (2025). High polyphenol extra virgin olive oil and metabolically unhealthy obesity: A scoping review of preclinical data and clinical trials. Clinical Practice, 15(3), 54. https://doi.org/10.3390/clinpract15030054

Massaro, M., Scoditti, E., Carluccio, M. A., Calabriso, N., Santarpino, G., Verri, T., & De Caterina, R. (2020). Effects of olive oil on blood pressure: Epidemiological, clinical, and mechanistic evidence. Nutrients, 12(6), 1548. https://doi.org/10.3390/nu12061548

Opichka, M. A., Rappelt, M. W., Gutterman, D. D., Grobe, J. L., & McIntosh, J. J. (2021). Vascular dysfunction in preeclampsia. Cells, 10(11), 3055. https://doi.org/10.3390/cells10113055

Overton, E., Tobes, D., & Lee, A. (2022). Preeclampsia diagnosis and management. Best Practice & Research Clinical Anaesthesiology, 36(1), 107–121. https://doi.org/10.1016/j.bpa.2022.02.003

Saleh, L., Verdonk, K., Visser, W., van den Meiracker, A. H., & Danser, A. H. (2016). The emerging role of endothelin-1 in the pathogenesis of pre-eclampsia. Therapeutic Advances in Cardiovascular Disease, 10(5), 282–293. https://doi.org/10.1177/1753944715624853

Sarapis, K., Thomas, C. J., Hoskin, J., George, E. S., Marx, W., Mayr, H. L., Kennedy, G., Pipingas, A., Willcox, J. C., Prendergast, L. A., Itsiopoulos, C., & Moschonis, G. (2020). Effect of high polyphenol extra virgin olive oil on blood pressure and arterial stiffness in healthy Australian adults: A randomized, controlled, cross-over study. Nutrients, 12(8), 2272. https://doi.org/10.3390/nu12082272

Silvani, Y., Lovita, A. N. D., Maharani, A., Wiyasa, I. W. A., Sujuti, H., Ratnawati, R., & Raras, T. Y. M. (2020). Extra virgin olive oil modulates vasodilator enzyme level by repairing angiogenesis function in rat model of preeclampsia. Journal of Family and Reproductive Health, 14(1), 1–6.

Silvani, Y., Maharani, A., & Lovita, A. N. D. (2020). The effect of extra virgin olive oil (EVOO) on fetal birth weight in preeclampsia rat model. Jurnal Kedokteran Brawijaya, 31(1), 13–18.

Silvani, Y., Maharani, A., Lovita, A. N. D., Wiyasa, I. W. A., Sujuti, H., Ratnawati, R., & Raras, T. Y. M. (2019). The effect of extra virgin olive oil to increase endogenous antioxidant and PlGF in preeclampsia rat model. Journal of Global Pharma Technology, 11(Suppl. 4), 231–240.

Storniolo, C. E., Roselló-Catafau, J., Pintó, X., Mitjavila, M. T., & Moreno, J. J. (2014). Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1. Redox Biology, 2, 971–977. https://doi.org/10.1016/j.redox.2014.07.001

Su, H., Li, M., Li, N., Zhang, Y., He, Y., Zhang, Z., Zhang, Y., Gao, Q., Xu, Z., & Tang, J. (2024). Endothelin-1 potentiated constriction in preeclampsia placental veins: Role of ETAR/ETBR/CaV1.2/CALD1. Placenta, 158, 165–174. https://doi.org/10.1016/j.placenta.2024.10.011

Vazquez, A., Sanchez-Rodriguez, E., Vargas, F., Montoro-Molina, S., Romero, M., Espejo-Calvo, J. A., Vilchez, P., Jaramillo, S., Olmo-García, L., Carrasco-Pancorbo, A., de la Torre, R., Fito, M., Covas, M. I., Martínez de Victoria, E., & Mesa, M. D. (2019). Cardioprotective effect of a virgin olive oil enriched with bioactive compounds in spontaneously hypertensive rats. Nutrients, 11(8), 1728. https://doi.org/10.3390/nu11081728

Vijakumaran, U., Shanmugam, J., Heng, J. W., Azman, S. S., Yazid, M. D., Haizum Abdullah, N. A., & Sulaiman, N. (2023). Effects of hydroxytyrosol in endothelial functioning: A comprehensive review. Molecules, 28(4), 1861. https://doi.org/10.3390/molecules28041861