Jurnal Impresi Indonesia (JII)
p-ISSN: 2828-1284 e-ISSN: 2810-062x
website: https: //rivierapublishing.id/jii/index.php/
Vol.5. No.2. 2026

The Effect of Soybean Ethanol Extract in Tnf-A Levels of Huvecs Culture Induced by Preeclampsia Plasma

Luh Citrarasmi Dara Mestika*, Wiwit Indriyani Aslina, Putu Eva Yuni Dyantari, Ni Putu Citra Laksmi, Sumiati
Universitas Pendidikan Ganesha, Indonesia
Email: citrarasmidara@gmail.com

Keywords
huvecs, preeclampsia, soybean ethanol extract, tnf-α

ABSTRACT
The main characteristics of preeclampsia are endothelial dysfunction and inflammatory response of the immune system. Oxidative stress contributes to the pathogenesis of preeclampsia by increasing tumor necrosis factor-α (TNF-α) due to increased reactive oxygen species (ROS). Isoflavones are known to be able to prevent endothelial dysfunction due to increased ROS and proinflammatory cytokines. This study aims to determine the effect of soybean extract on TNF-α associated with preeclampsia using the endothelial cell culture method. The study using in vitro design with human umbilical vein endothelial cells (HUVECs) cultures sample induced by normotensive plasma and preeclampsia plasma and then given various doses of soybean ethanol extract. The TNF-α level of each observation group was determined by the enzyme-linked immunosorbent assay (ELISA) procedure. Statistical analysis were performed with One-Way ANOVA test using SPSS Version 25. Statistical analysis showed a significant difference ($p < 0.05$) in TNF-α levels of HUVECs cultures exposed to preeclampsia plasma. Compared to the group exposed only to preeclampsia plasma (223.975±5.222ng/mL), the model given a dose of 35 ppm soybean ethanol extract showed a significant decrease in TNF-α levels (178.350±10.093ng/mL), similar to the control group exposed to normal plasma (185.993±16.393ng/mL). This study results indicate that the bioactive compounds in soybean ethanol extract assumed can reduce TNF-α levels in HUVECs cultures induced by preeclampsia plasma and have a potential future therapy for preeclampsia.

INTRODUCTION

Preeclampsia remains a leading cause of maternal and neonatal morbidity and mortality worldwide, affecting 2–10% of pregnancies and contributing to an estimated 50,000–60,000 maternal deaths annually (Al-Jameil, 2013; Costa & Cecatti, 2018; Eiland et al., 2012). In Indonesia, it is the second leading cause of maternal death after postpartum hemorrhage (Akbar, 2020; Say et al., 2014). The pathogenesis of preeclampsia is multifactorial, but its main characteristics are widely recognized as endothelial dysfunction and an exaggerated systemic inflammatory response (Rana et al., 2019). This dysfunction involves an imbalance between vasoconstrictors and vasodilators, leading to hypertension and tissue hypoxia. Hypoxia, in turn, activates leukocytes and promotes the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), primarily through the nuclear factor-kappa B (NF-κB) pathway (Susianto et al., 2009). TNF-α is a key regulator of inflammation and a potent mediator of endothelial cell dysfunction, playing a central role in the systemic manifestations of preeclampsia (Alijotas-Reig et al., 2017) (Calleja-Agius et al., 2012).

Research by Rahardjo et al. demonstrated that increased levels of the pro-inflammatory cytokine TNF-α occurred concomitantly with increased levels of the transcription factor NF-

κB in monocyte cell cultures exposed to preeclamptic plasma (Rahardjo et al., 2014). Another study by Ambarwati et al. stated that TNF-α levels were significantly increased in the group of HUVECs induced by preeclamptic plasma compared to the group induced by normotensive plasma (Ambarwati et al., 2017). This suggests that the plasma of preeclamptic women can stimulate an inflammatory response in cell cultures—both monocyte cells and HUVECs—because preeclamptic plasma contains several circulating factors produced by the placenta, such as syncytiotrophoblastic microparticles (STBM) and reactive oxygen species (ROS), which are capable of stimulating monocytes and endothelium to increase the production of pro-inflammatory cytokines, one of which is TNF-α (C. W. G. Redman & Sargent, 2008).

Vascular endothelial cells are in direct contact with the bloodstream, making them ideal sensors for circulating factors and increasing their susceptibility to damaging agents. Endothelial cell activation induced by inflammatory substances increases leukocyte adhesion due to enhanced expression of adhesion molecules. Leukocytes such as neutrophils adhere to endothelial cells through these adhesion molecules and increase the production of ROS, causing oxidative stress (Lamarca, 2012). The elevated production of the pro-inflammatory cytokine TNF-α occurs concomitantly with increased oxidative stress caused by ROS. Phosphorylation of the IκB kinase (IKK) complex by ROS leads to activation of the transcription factor NF-κB, which enters the nucleus and subsequently increases the transcription of TNF-α (Aouache et al., 2018) (Tenório et al., 2019).

Since the balance between pro-oxidants and antioxidants regulates ROS production, an imbalance due to excessive ROS leads to vascular dysfunction and endothelial barrier impairment, mainly through reduced nitric oxide (NO) bioavailability (Losso, 2022). Flavonoids and their derivatives are known to exert strong anti-inflammatory and antioxidant effects by reducing pro-inflammatory cytokine levels and ROS production in human, animal, and cell culture models (Al-Khayri et al., 2022; Di Gesso et al., 2015; Losso, 2022). One of the flavonoid-rich natural food sources is soybean (Glycine max), which contains isoflavones—compounds that act as antioxidants and anti-inflammatory agents (Astuti, 2008; Hu et al., 2020).

Administration of isoflavones has been reported to reduce the risk of cardiovascular disorders associated with inflammation by downregulating TNF-α at the endothelial level through inhibition of the NF-κB transcription system (Yu et al., 2016) (Droke et al., 2007), as well as preventing oxidative stress by acting as a ROS scavenger. However, evidence regarding the effect of soybean ethanol extract as a therapeutic agent in preeclampsia remains limited. To date, no further studies have examined soybean-based interventions targeting endothelial inflammatory reactions as a causal mechanism of preeclampsia (Tohalifah et al., 2020). This knowledge gap inspired the current research, which was designed to determine the anti-inflammatory effect of soybean extract on NF-κB and TNF-α levels associated with preeclampsia in an endothelial cell culture model.

Therefore, this study was conducted to investigate the anti-inflammatory effect of soybean ethanol extract on TNF-α levels in HUVEC cultures induced by preeclamptic plasma. The primary aim was to determine whether the extract could modulate this key inflammatory cytokine, potentially by interfering with the NF-κB pathway. By addressing this gap, the study

seeks to contribute to the development of novel nutritional or adjunctive therapeutic strategies for preeclampsia, focusing on alleviating endothelial inflammation and dysfunction.

METHOD

Soybean ethanol extract

Anjasmoro cultivar soybean was obtained from Balai Penelitian Tanaman Aneka Kacang dan Umbi (BALITKABI) Malang Regency, East Java. Two kilograms of soybeans are processed into simplicia. Extraction of soybean simplicia was performed by maceration method using 96% ethanol solvent with a ratio of 1:10 overnight, then the solvent was evaporated using a rotary evaporator. This process produced 40 grams condensed extract from a total of 500 grams of extracted simplicia. Screening for phytochemical compounds was performed to determine the bioactive compounds in the ethanol extract of soybeans.

Plasma Isolation

Normotensive plasma was taken from healthy pregnant women at 37 weeks' gestation and preeclampsia plasma was taken from preeclampsia-diagnosed pregnant women at 36 weeks' gestation. Informed consent was given prior to taking the samples. Blood samples were taken using a 3.2% citrate vacutainer with aseptic technique, then centrifuged at 1000g for 10 minutes. Plasma samples were aliquoted into eppendorf tubes and stored in a -40^{o}C refrigerator prior to use.

Umbilical cord sample isolation

Newborn's umbilical cord sample were collected from vaginal delivery of healthy pregnant women, with informed consent before. Umbilical cord was cut 15-20 cm long and immediately put into the cord solution as soon as the placenta was born. Endothelial cells (HUVECs) isolation and culture performed at maximum of 8 hours after sample was collected.

Endothelial cell isolation and culture

HUVECs cultures were made at the Biomedical Laboratory, Faculty of Medicine, Brawijaya University using newborn's umbilical cord sample. Endothelial cells isolated from the umbilical vein, then grown in a 25 cm^2 culture flask coated with 0.2% gelatin. The cell culture was incubated in a CO_2 incubator at 37^{o}C until a monolayer was formed. Subcultures were performed on well plates once the cells were 80-90% confluent in the flask. The treatment was given on cell culture with passage one.

Treatment

A negative control group was given normotensive plasma, a positive control group was given preeclamptic plasma alone without soybean ethanol extract, and five treatment groups were treated by preeclamptic plasma and soybean ethanol extract at 17.5 ppm, 35 ppm, 70 ppm, 140 ppm and 280 ppm dosages. After treatment, cultures were incubated for 24 hours then the supernatant was collected for examination.

Analysis of TNF-α levels

TNF-α levels in HUVECs cultures were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. The procedure performed based on the standard procedure of the kit used, which is Human TNF-α ELISA Kit (Bioassay Technology Laboratory, China, Catalog Series No. E0082Hu). Optical density (OD) was measured using a microplate reader with a wavelength of 450 nm then the final result were calculated in ng/mL.

Statistical analysis

Hypothesis testing was performed using the One-Way ANOVA test with statistical software SPSS version 25 (SPSS Inc., Chicago, IL, US). If the results show a significant difference, then proceed with the Tukey's HSD post hoc test.

Ethics

This study has passed an ethical review by the Health Research Ethics Committee of Dr. Saiful Anwar General Hospital Malang, East Java, Indonesia with Ethics No. 400/059/K.3/102.7/2023.

RESULT AND DISCUSSION

Based on the statistical analysis of One-Way ANOVA and Tukey's HSD, there was found a significant difference ($p < 0.05$) in the TNF-α levels of HUVECs cultures. There was a significant difference ($p < 0.05$) in the levels of TNF-α in HUVECs cultures induced by preeclampsia plasma compared to the control group induced by normotensive plasma. Significant differences were also found ($p < 0.05$) in TNF-α levels in the control group induced by normotensive plasma with the control group induced by preeclampsia plasma and the groups treated with 140 ppm and 280 ppm dosages. A significant difference ($p < 0.05$) was also found between the control group induced by preeclampsia plasma and the group induced by preeclampsia plasma and treated to 17.5 ppm and 35 ppm dosages. However, there was no significant difference between the groups induced by preeclampsia plasma given 70 ppm dosage compared to the control group.

Table 1. TNF-α levels examined with ELISA method after the treatment given

Observation Group	Mean±SD (ng/mL)	p-value
HUVECs normotensive	185,993±16,393[a]	
HUVECs preeclamptic (PE)	223,975±5,222[c]	
HUVECs PE + soybean17,5 ppm	191,241±7,205[a,b]	
HUVECs PE + soybean 35 ppm	178,350±10,093[a]	0,000<α
HUVECs PE + soybean 70 ppm	202,782±14,929[a,b,c]	
HUVECs PE + soybean 140 ppm	216,808±11,204[b,c]	
HUVECs PE + soybean 280 ppm	219,579±15,655[c]	

p value < 0.05 means that there is a significant difference; different alphabet means there was a significant difference ($p < 0.05$)

As shown in table 1, compared to the control group of HUVECs induced by preeclampsia plasma (223.975±5.222 ng/mL), the mean value of TNF-α levels was lower in the group induced by preeclampsia plasma and treated 35 ppm dosage of soybean ethanol extract (178.350±10.093 ng/mL). In addition, the mean value of the group induced by preeclampsia plasma with soybean ethanol extract at 35 ppm dosage was also lower than the mean value of the control group induced by normotensive plasma (185.993±16.393 ng/mL).

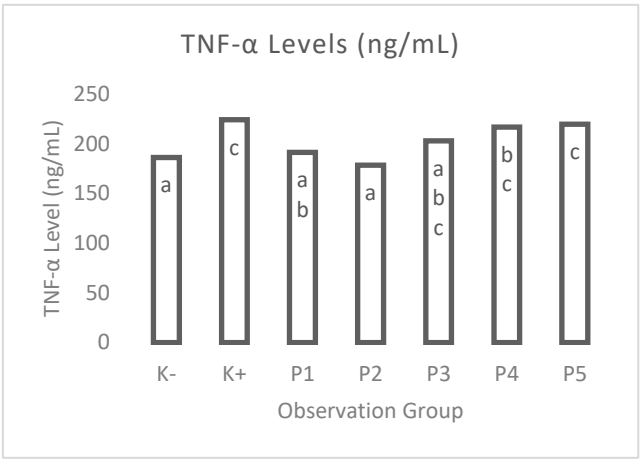

Figure 1. Histogram showing a comparison of TNF-α levels in HUVEC cultures incubated with preeclamptic plasma and various doses of soybean ethanol extract. Identical alphabets indicate no significant difference ($p > 0.05$).

Various concepts and theories related to the pathogenesis of preeclampsia have been widely described; however, the main characteristics remain the inflammatory response of the immune system and endothelial dysfunction. An exaggerated inflammatory immune response has been suggested as a major driver contributing to endothelial dysfunction and the clinical syndrome of preeclampsia. Pregnancy is associated with several stimuli that can enhance the inflammatory response, including cellular particles, cytoplasmic proteins, soluble fetal DNA, placental lipid peroxidation products, pro-inflammatory cytokines, soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng), all of which are released into the maternal circulation from syncytiotrophoblast surfaces (C. W. G. Redman et al., 1999; C. W. Redman & Sargent, 2000).

In the present study, TNF-α levels increased significantly in endothelial cells induced by preeclamptic plasma compared with endothelial cells induced by normotensive plasma. This indicates that plasma from preeclamptic pregnant women contains substantial factors capable of increasing the inflammatory response. Research by Baktiyani et al. reported that 2% preeclamptic plasma exposure could significantly increase TNF-α levels in HUVEC cultures (Walsh, 2006). Rahardjo et al. also suggested that plasma from preeclamptic pregnant women contains several factors capable of inducing the expression of pro-inflammatory cytokines, such as STBM, ROS, and various cytokines. Redman and Sargent speculated that STBM in maternal circulation not only directly damages endothelial cells but also stimulates a systemic inflammatory response. STBM activate neutrophils secondarily when interacting with human umbilical endothelial cells in vitro.

Results of the present study, as shown in Table 1, revealed a significant difference ($p < 0.05$) in TNF-α levels among HUVEC cultures induced by normotensive plasma, preeclamptic plasma, and cultures treated with soybean ethanol extract. Various doses of the extract were administered to determine their effect on the pro-inflammatory cytokine TNF-α when exposed to preeclamptic plasma. A significant decrease in TNF-α levels was observed at the 35 ppm dose compared to HUVECs induced by preeclamptic plasma alone. This finding suggests that

even a relatively low dose of soybean extract can reduce pro-inflammatory cytokines, particularly TNF-α, resembling the HUVEC model induced by normotensive plasma.

Stimulation by pathological factors can reduce eNOS expression, consequently decreasing NO synthesis and secretion, while simultaneously stimulating hypoxia-inducible factor 1 (HIF-1) and endothelin-1 (ET-1) production in vascular endothelial cells. In addition, these factors activate the NF-κB signaling pathway in endothelial cells, upregulating the production of pro-inflammatory mediators such as TNF-α, which contribute to endothelial dysfunction. These inflammatory factors also enhance ROS production and adhesion molecule expression, further exacerbating endothelial damage (Baktiyani, 2013; Guber et al., 2018; Liang et al., 2018; Loscalzo, 2018).

These findings align with a study by Yi et al., which demonstrated that TNF-α expression increased in endothelial cells exposed to lipopolysaccharide (LPS), as detected by RT-PCR and ELISA, compared with the control group. Pre-treatment with genistein-3′-sodium sulfonate (GSS), an isoflavone derivative of soybean, inhibited the increased expression of TNF-α. Yi et al. proposed that GSS attenuates TNF-α expression by inactivating the NF-κB signaling pathway, a transcription factor that plays a critical role in initiating the inflammatory response (Yi et al., 2019; J. Zhang et al., 2016; Q. Zhang et al., 2021).

This study also corresponds with research by Ambarwati et al., who found that flavonoids from red pomegranate extract reduced TNF-α levels by suppressing the modulation of pro-inflammatory signaling in HUVEC cultures incubated with preeclamptic plasma, via inhibition of NF-κB activation. Similarly, Chuang et al. demonstrated that isoflavones from Glycine tomentella root extract, a member of the soybean family, produced a 67% inhibition of TNF-α response in TO cells (macrophage-like lines from Atlantic salmon).

Irace et al., in their study measuring endothelial function, found that genistein administration for six months significantly improved endothelial function in postmenopausal women with metabolic syndrome (Irace et al., 2013). In parallel, Simao et al. reported a significant increase in NO levels and a corresponding reduction in blood pressure in postmenopausal women who were given konako soy containing isoflavones (Simão et al., 2010).

Figure 1 shows a decrease in TNF-α levels at doses of 17.5 ppm and 35 ppm soybean extract compared with the positive control group that did not receive soybean ethanol extract. However, TNF-α levels increased at higher doses (70 ppm, 140 ppm, and 280 ppm). This phenomenon may be attributable to the IC_{50} value—where increased IC_{50} correlates with reduced antioxidant activity of the extract. Several studies have classified antioxidant strength based on IC_{50} as follows: below 50 ppm is categorized as very strong, 50–100 ppm as strong, above 100 ppm as weak, and greater than 500 ppm as inactive (Asshidiqy et al., 2020; Kusumawati et al., 2021; Nerdy & Manurung, 2018; Simorangkir et al., 2019).

According to the theory of the hormetic effect—a dose–response relationship in which a compound produces adverse biological effects at moderate to high doses but beneficial effects at lower doses (Calabrese et al., 2010)—the observed increase in TNF-α levels at higher extract concentrations may reflect this biphasic effect. As the soybean extract dose increases, its antioxidant inhibitory effect weakens, leading to possible detrimental or even cytotoxic outcomes at excessive concentrations.

CONCLUSION

Administration of soybean ethanol extract to endothelial cell cultures (HUVECs) induced by preeclamptic plasma affected TNF-α levels by downregulating this pro-inflammatory cytokine through inhibition of the NF-κB signaling pathway and a decrease in ROS production. Isoflavone bioactive compounds in soybean are believed to inhibit NF-κB activation and reduce ROS generation, thereby suppressing TNF-α cytokine expression. Thus, soybeans with their isoflavone content may serve as potential future therapeutic candidates for preeclampsia by mitigating endothelial dysfunction and reducing inflammatory responses.

REFERENCES

Akbar, M. I. A. (2020). *Preeclampsia: Biomarkers, imitators and clinical complications* [Universitas Airlangga]. https://repository.unair.ac.id/103033/

Al-Jameil, A. (2013). A brief overview of preeclampsia. *Journal of Clinical Medicine Research*, *6*(1), 1–7. http://www.jocmr.org/index.php/JOCMR/article/view/1682

Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V, Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, *27*(9). https://doi.org/10.3390/molecules27092688

Alijotas-Reig, J., Esteve-Valverde, E., Ferrer-Oliveras, R., Llurba, E., & Gris, J. M. (2017). Tumor Necrosis Factor-Alpha and Pregnancy: Focus on Biologics. *Clinical Reviews in Allergy & Immunology*, *53*(1), 40–53.

Ambarwati, D., Fatmawati, F., Nooryanto, M., Santoso, S., Windu Baktiyani, S. C., & Nurdiana, N. (2017). Punica granatum fruit extract inhibits the production of pro-inflammatory cytokines and angiogenic factors of HUVEC cells induced by plasma from patients with pre-eclampsia. *Clinical Nutrition Experimental*, *15*, 9–14. https://doi.org/10.1016/j.yclnex.2017.06.001

Aouache, R., Biquard, L., Vaiman, D., & Miralles, F. (2018). Oxidative stress in preeclampsia and placental diseases. *International Journal of Molecular Sciences*, *19*(5), 1162. https://doi.org/10.3390/ijms19051162

Asshidiqy, R., Putri, W. D. R., & Maligan, J. M. (2020). Optimasi Elisitasi Suhu dan Waktu Kejut Listrik untuk Meningkatkan Aktivitas Antioksidan dan Kandungan Total Fenol Kacang Kedelai (Glycine max). *Jurnal Keteknikan Pertanian Tropis Dan Biosistem*, *8*(2), 153–160.

Astuti, S. (2008). Isoflavon kedelai dan potensinya sebagai penangkap radikal bebas. *Jurnal Teknologi Industri Dan Hasil Pertanian*, *13*(2), 126–136.

Baktiyani, S. C. W. (2013). Pengaruh pemberian kombinasi NAC dengan vitamin C dan E terhadap stres oksidatif pada HUVECs dipapar plasma eklampsia. *Jurnal Kedokteran Brawijaya*, *23*(3), 144–150. https://jkb.ub.ac.id/index.php/jkb/article/view/300

Calabrese, V., Cornelius, C., Trovato-Salinaro, A., Cambria, M., Locascio, M., & Rienzo, L. (2010). The Hormetic Role of Dietary Antioxidants in Free Radical-Related Diseases. *Current Pharmaceutical Design*, *16*(7), 877–883.

Calleja-Agius, J., Muttukrishna, S., & Jauniaux, E. (2012). The role of tumor necrosis factor receptors in pregnancy with normal and adverse outcome. *International Journal of Interferon Cytokine Mediators*, *4*(1), 1–15.

Costa, M. L., & Cecatti, J. G. (2018). *Preeclampsia in 2018: Revisiting concepts, physiopathology, and prediction*. *2018*.

Di Gesso, J. L., Kerr, J. S., Zhang, Q., Raheem, S., Yalamanchili, S. K., & O'Hagan, D. (2015). Flavonoid metabolites reduce tumor necrosis factor-α secretion to a greater extent than their precursor compounds in human THP-1 monocytes. *Molecular Nutrition & Food*

Research, *59*(6), 1143–1154. https://doi.org/10.1002/mnfr.201400799

Droke, E. A., Hager, K. A., Lerner, M. R., Lightfoot, S. A., Stoecker, B. J., & Brackett, D. J. (2007). Soy isoflavones avert chronic inflammation-induced bone loss and vascular disease. *Journal of Inflammation*, *4*, 1–12.

Eiland, E., Nzerue, C., & Faulkner, M. (2012). Preeclampsia 2012. *Journal of Pregnancy*, *2012*, 1–7. https://doi.org/10.1155/2012/586578

Guber, S., Ebrahimian, T., Heidari, M., Eliopoulos, N., & Lehoux, S. (2018). Endothelial nitric oxide synthase overexpressing human early outgrowth cells inhibit coronary artery smooth muscle cell migration through paracrine functions. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-017-18848-z

Hu, C., Wong, W. T., Wu, R., & Lai, W. F. (2020). Biochemistry and use of soybean isoflavones in functional food development. *Critical Reviews in Food Science and Nutrition*, *60*(12), 2098–2112. https://doi.org/10.1080/10408398.2019.1630598

Irace, C., Marini, H., Bitto, A., Altavilla, D., Polito, F., & Adamo, E. B. (2013). Genistein and endothelial function in postmenopausal women with metabolic syndrome. *European Journal of Clinical Investigation*, *43*(10), 1025–1031. https://doi.org/10.1111/eci.12140

Kusumawati, A. H., Farhamzah, F., Alkandahri, M. Y., Sadino, A., Agustina, L. S., & Apriana, S. D. (2021). Antioxidant Activity and Sun Protection Factor of Black Glutinous Rice (Oryza sativa var. glutinosa). *Tropical Journal of Natural Product Research*, *5*(11), 1958–1961.

Lamarca, B. (2012). Endothelial dysfunction: An important mediator in the pathophysiology of hypertension during pre-eclampsia. *Minerva Ginecologica*, *64*(4), 309–320. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3796355/

Liang, X., Hou, X., Yang, Y., Liu, H., Guo, R., & Yang, Z. (2018). The feedback loop of "EMMPRIN/NF-κB" worsens atherosclerotic plaque via suppressing autophagy in macrophage. *Journal of Molecular and Cellular Cardiology*, *114*, 129–140. https://linkinghub.elsevier.com/retrieve/pii/S0022282817303437

Loscalzo, J. (2018). Nitric oxide signaling and atherothrombosis redux: Evidence from experiments of nature and implications for therapy. *Circulation*, *137*(3), 233–236.

Losso, J. N. (2022). The potential of dietary bioactive compounds against SARS-CoV-2 and COVID-19-induced endothelial dysfunction. *Molecules*, *27*(5).

Nerdy, N., & Manurung, K. (2018). Spectrophotometric method for antioxidant activity test and total phenolic determination of red dragon fruit leaves and white dragon fruit leaves. *Rasayan Journal of Chemistry*, *11*(3), 1183–1192.

Rahardjo, B., Widjajanto, E., Sujuti, H., & Keman, K. (2014). Different levels of IL-1α, IL-6, TNF-α, NF-κB and PPAR-γ in monocyte cultures exposed by plasma preeclampsia and normotensive pregnancy. *Pregnancy Hypertension*, *4*, 187–193.

Rana, S., Lemoine, E., Granger, J., & Karumanchi, S. A. (2019). Preeclampsia: Pathophysiology, challenges, and perspectives. *Circulation Research*, *124*(7), 1094–1112.

Redman, C. W. G., Sacks, G. P., & Sargent, I. L. (1999). Preeclampsia: An excessive maternal inflammatory response to pregnancy. *American Journal of Obstetrics and Gynecology*, *180*(2 I), 499–506.

Redman, C. W. G., & Sargent, I. L. (2008). Circulating microparticles in normal pregnancy and pre-eclampsia. *Placenta*, *29*, 73–77.

Redman, C. W., & Sargent, I. L. (2000). Placental debris, oxidative stress and pre-eclampsia. *Placenta*, *21*(7), 597–602. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10985960&query_hl=11&itool=pubmed_docsum

Say, L., Chou, D., Gemmill, A., Tunçalp, Ö., Moller, A. B., Daniels, J., & al., et. (2014).

Global causes of maternal death: A WHO systematic analysis. *Lancet Global Health*, *2*(6), 1–11.

Simão, A. N. C., Lozovoy, M. A. B., Simão, T. N. C., Dichi, J. B., Matsuo, T., & Dichi, I. (2010). Nitric oxide enhancement and blood pressure decrease in patients with metabolic syndrome using soy protein or fish oil. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *54*(6), 540–545.

Simorangkir, M., Nainggolan, B., & Silaban, S. (2019). Antioxidant activity of vacuum column chromatography fractions of ethanol extract of sarang banua (Clerodendrum fragrans vent willd) leaves. *Journal of Physics: Conference Series*, *1374*(1), 6–11.

Susianto, I. A., Suharsono, & Hadijono, S. (2009). Kadar TNF-α, IL-6 dan Trofoblas pada Preeklampsia - Eklampsia. *Media Medika Indonesia*, *43*(4), 166–174.

Tenório, M. B., Ferreira, R. C., Moura, F. A., Bueno, N. B., De Oliveira, A. C. M., & Goulart, M. O. F. (2019). Cross-talk between oxidative stress and inflammation in preeclampsia. *Oxidative Medicine and Cellular Longevity*, *2019*. https://doi.org/10.1155/2019/3754893

Tohalifah, A. A., Barlianto, W., Wardani, D. S., & Luthfia, T. (2020). *Literature Review: Potensi Genistein dari Ekstrak Kacang Kedelai (Glycine max) untuk Menurunkan Mediator Inflamasi (TNF-α) sebagai Kandidat Terapi Adjuvan pada Preeklampsia*. Universitas Brawijaya.

Walsh, S. W. (2006). What causes endothelial cell activation in preeclamptic women? *American Journal of Pathology*, *169*(4), 1104–1106. http://dx.doi.org/10.2353/ajpath.2006.060713

Yi, L., Zhou, Z., Zheng, Y., Chang, M., Huang, X., & Guo, F. (2019). Suppressive Effects of GSS on Lipopolysaccharide-Induced Endothelial Cell Injury and ALI via TNF-α and IL-6. *Mediators of Inflammation*, *2019*. https://doi.org/10.1155/2019/1549796

Yu, J., Bi, X., Yu, B., & Chen, D. (2016). Isoflavones: Anti-inflammatory benefit and possible caveats. *Nutrients*, *8*(6), 1–16.

Zhang, J., Wang, X., Vikash, V., Ye, Q., Wu, D., & Liu, Y. (2016). ROS and ROS-Mediated Cellular Signaling. *Oxidative Medicine and Cellular Longevity*, *2016*. https://doi.org/10.1155/2016/4391098

Zhang, Q., Liu, J., Duan, H., Li, R., Peng, W., & Wu, C. (2021). Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. *Journal of Advanced Research*, *34*, 43–63. https://doi.org/10.1016/j.jare.2021.06.023