

# Transforming Growth Factor Expression (TGF- $\beta$ ) Correlate with Serum Level of Malondialdehyde (MDA) after EVOO Administration in Preclinical Rat Models of Preeclampsia

Syafuruddin Ilyas<sup>1,2</sup>, Salomo Hutahaean<sup>1</sup>, dan Evi Irianti<sup>1</sup>

<sup>1</sup>Departement of Biology, Faculty Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia.

<sup>2</sup>Featured Center for Science and Technology "Stem Cell," Universitas Sumatera Utara, Medan. Dr. Masyur Street, No. 4, Kampus USU, Padang Bulan, Medan, Indonesia.

Email: [syafuruddinilyas2013@gmail.com](mailto:syafuruddinilyas2013@gmail.com)

**Abstract.** Preeclampsia can cause cell death either apoptosis or necrosis. One cause is the disturbance of the emergence of malondialdehyde (MDA). Very few reports on the role of Transforming Growth Factor Expression (TGF- $\beta$ ) in the remodeling process of placental cells and their association with serum MDA content. Research of true experiment with complete randomized design (CRD) with five treatment groups. The first group, preeclampsia negative control (T0). The second group, preeclamptic rats model (T1). The third group, preeclamptic rats model+EVOO 0.45g/kg-Body Weight/day (T2). The fourth group, preeclamptic rats model+EVOO 0.90g/kg-BW/day (T3). The fifth group, preeclamptic rats model+EVOO 1.8 g/kg-BW/day (T4). The results showed a significant effect of EVOO on TGF- $\beta$  expression in preeclampsia rats, meaning that there was a role of TGF- $\beta$  against pre-eclampsia placenta remodeling. There was a positive and strong relationship ( $r=0.494$ ) as well as a very significant relationship ( $p<0.01$ ) between TGF- $\beta$  and the serum MDA.

**Keywords:** Apoptosis, Reactive Oxygene Species (ROS)

## 1. Introduction

Preeclampsia is a complication of pregnancy characterized by increased of blood pressure (hypertension) and signs of organ damage, such as kidney damage indicated by increased levels of protein in the urine (proteinuria). In humans, the symptoms of preeclampsia usually appear at the age of gestation entering the week 20 or later (most often between 24 and 26 weeks of pregnancy until shortly after birth) Preeclampsia is not realized by pregnant women can develop into eclampsia, a serious medical threat to maternal safety conditions pregnant and their fetus.

Preeclampsia is a medical complication of pregnancy and occurs in about 5-8% of pregnancies causing high maternal and fetal morbidity or mortality [1,2]. In 2013 hypertension in pregnancy is the second cause of AKI in Indonesia as much as 27.1% [3].

Caused of preeclampsia unclear because preeclampsia is "the disease of theories" [4]. The results of several factors that may cause preeclampsia include maternal adaptation imbalances that lead to systemic inflammatory reactions and endothelial dysfunction [5,6]. Endothelial dysfunction is a condition where there is no balance of mediator products by the endothelium to regulate vascular tone, platelet aggregation, coagulation, fibrinolysis. The occurrence of endothelial dysfunction is characterized by increased endothelin (ET-1) [7]. ET-1 is a peptide of 21 amino acids as a potent



vasoconstrictor, and the main isoform produced from human endothelial cells causes vasoconstriction and causes hypertension and proteinuria which is a symptom of preeclampsia [8].

Until now there is no proper therapy to deal with preeclampsia, considering preeclampsia is a serious health problem to be addressed, so the need for prevention of this disease. One such prevention effort is the provision of antioxidants such as a combination of vitamin C and vitamin E supplementation. They were able to decreased the incidence of preeclampsia significantly [9, 10].

Extra virgin olive oil (EVOO), contains antioxidants that are tocopherol (vitamin E). The process of purifying olive oil produces some components, including hydroxytyrosol, and target tyrosols. Hydroxytyrosol has been shown to be effective in increasing plasma antioxidant activity as well as a dilator to the oxidation of LDL (lipid density low). Tyrosol and other phenolic antioxidants can bind LDL so that can delay the process of atherosclerosis.

## 2. Methods

Animal Preparation Try Rats in adaptation to the environment for seven days with feeding in the form of basal rations in all rats. Plastic place of rats having a diameter of 80 cm with a height of 40 cm [11,15,13]. Basal ration composition is prepared based on standard AOAC (2005) that contains carbohydrates, proteins, fats, minerals, vitamins. An animal model of rat (*Rattus norvegicus*) from Universitas Gadjah Mada Yogyakarta with age of 10 weeks and body weight between 150-250 gram which has been approved by Ethical Research Commission of Animal by No. 068/KEPH-FMIPA/2017.

### 2.1 Research design

The study uscaous ofed a pure experimental method with Completely Randomized Design consisting of 5 treatment groups. (T0) of preeclampsia control, (T2) of preeclamptic rats + EVOO 0.45 g / kg BW / day, (T3) rats preeclampsia + EVOO 0.90 g / kg BW / day, T4) mice preeclampsia + EVOO 1.8 g / kg BW / day.

### 2.2 Measurement of MDA Serum Level

Malondialdehyde content with TBARs method. Working procedures are carried out according to the standards set by the local laboratory. Mice blood samples as much as 2 ml, filled into a tube that already contains two drops of EDTA for subsequent centrifugation. After centrifugation at a speed of 3000 rpm, for 30 minutes taken plasmanya. Then prepare the necessary reagents, among others (1). SDS 200  $\mu$ l, (2). EDTA 50  $\mu$ l, (3). BHT 50  $\mu$ l, and (4). Acetate 1500  $\mu$ l. All the ingredients/solutions are inserted into the tube (which inside the tube already contains the plasma) and then vortexed for 10 seconds, then centrifuged at 300 rpm for 10 minutes. After that, the supernatant is taken away; the deposit is removed, then put into a tube that is already available, then add 1500  $\mu$ l of TBA solution. In the meantime prepare six standard tubes (the contents therein are the four solutions mentioned above without mixing with the sample plasma), also prepare 1 blank tube (its contents aquabidest 700  $\mu$ l). Then the standard tube and blanks are added TBA 700  $\mu$ l, the two tubes are vortexed for 10 seconds. When finished, all the tubes are covered with cotton or gauze that is plastered, then inserted into the water bath at 940C for 1 hour. Then cool for 10 minutes, centrifuged for 10 minutes (except standard bloom) and reads the result on the "SmartSpecTMPlus" brand spectrophotometry from BioRad at 532nm wavelength. The way to read it is to take each sample with 1500  $\mu$ l pipette then put it into a cuvette (try the cuvette glass when the reading is not blurry, always cleaned every time you replace the sample by rinsing with aquabidest 2 times, because the glassiness affects the reading result) to the spectrophotometry one by one from the sample.

### 2.3 Observation of TGF- $\beta$ Expression with Immunohistochemistry (IHC)

This observation was performed on the placenta portion by immunohistochemical staining to determine the expression of TGF- $\beta$ . The expression of TGF- $\beta$  expression in placental cells was quantitatively observed using the Olympus BX51 microscope at 400  $\times$  magnification. The intensity of 4: 0 discordable expression when no expression on cells; 1 = when the staining is weak; 2 = when medium dyeing; 3 = when staining is strong. The pored-colored cell suspension: 0 when there is no expression on the cell; 1 = when <33% expression on cell; 2 = when 33-66% expression on cell; 3 =

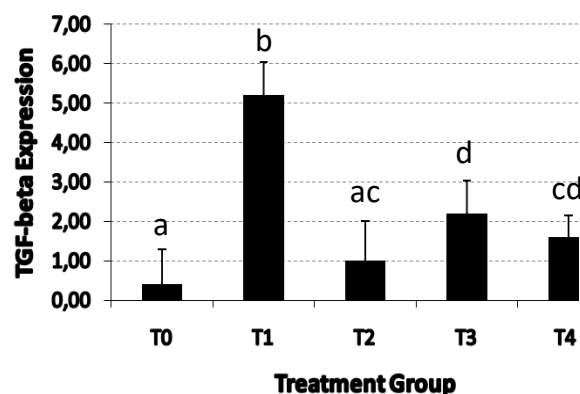
when > 66% expression on cell. Calculated on 100 cells in hot spot area. Then summed up when the score 0-1 = negative, 2 = positive weak, 3-4 = moderate positive, 5-6 = strong positive [14,15,16,17].

#### 2.4 Data analysis

This study used data analysis using quantitative analysis to look at placental histopathology and quantitative histopathology for TGF- $\beta$  expression with ANOVA test to determine the difference between treatments followed by Post Hoc test to find out which treatments gave the best results.

### 3. Results And Discussion

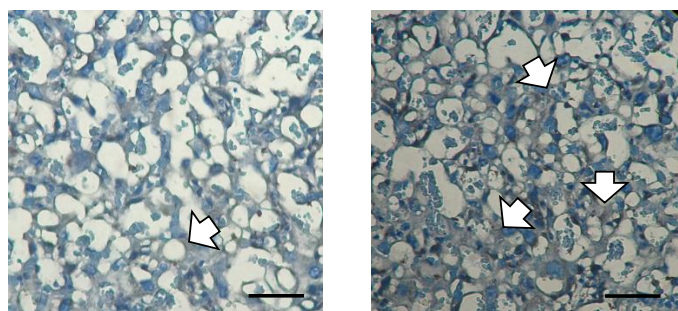
Based on the results of research conducted, the results obtained as shown in Figure 1 below.



**Figure 1.** Graph Bar of TGF- $\beta$  expression on the placenta of mouse model preeclampsia after treatment.

In rats, with preeclampsia, the presence of TGF- $\beta$  expression was significantly greater ( $p < 0.05$ ) compared with the negative control and treatment of EVOO addition. This is due to an increase in oxidants or ROS (Reactive Oxygen Species) due to excessive NaCl content (above physiological/0.9%). The ROS shows the number of unpaired electrons so that they can lie in various cellular metabolic enzymes. As a result, the enzyme does not work and causes cell death either by necrosis or apoptosis [18,19,20,21,22]. Necrotizing cell death results in the release of proinflammatory (inflammatory) signals. As Iranloye [23] points out, that high salt levels can lead to increased Reactive Oxygen Species (ROS). Furthermore, Bertolino et al., [24] and Lebrin, [25] suggest that TGF- $\beta$  is the main cytokine produced abundantly in vascular endothelial cells and trophoblasts. It plays a key role in various physiological processes, including embryonic growth and development, improvement of inflammation and angiogenesis.

EVOO levels in T2, T3 and T4 can significantly lower TGF- $\beta$  expression in placental tissue ( $p < 0.05$ ) compared with T1 or preeclampsia mice without EVOO. This suggests a decrease in TGF- $\beta$  activity as reduced cell damage or cell death (apoptosis or cellular necrosis). The content of EVOO more vitamin E content that serves as an antioxidant that can suppress oxidant activity (ROS) and cause cells to avoid the effects of oxidants or cell damage and cell death and the emergence of proinflammation. As stated Khanduja et al., [26] that vitamin E can inhibit the activity of proinflammatory cytokines in the cells. High doses of vitamin E in the diet may play a role in reducing cytokine lipopolysaccharide and nitric oxide-induced proinflammation.



**Figure 2.** Expression of TGF- $\beta$  in the placenta of preeclamptic model rat in treatment group (T) and control (T), — = 100  $\mu$ m

TGF- $\beta$  expression is seen expressed in the cytoplasm and cytoplasmic membrane. The amount of expression, intensity and percentage of expression seen in the positive control group or preeclampsia without EVOO (Figure 1). This suggests that the activity of the TGF- $\beta$  gene is more stimulated due to the physiological processes of cells that occur due to the administration of a high salt content (NaCl 6%). High salt levels disrupting cell membranes and lead to increased activity of death receptors such as DR (Death Receptor) or Tumor Necrosis Factor (TNF $\alpha$ ). The receptor activity stimulates procaspase 8 to caspase 8 and results in an increase in caspase activity 3 as an apoptotic cell execution. Stimulant receptor stimuli may also increase Bax or Bid expression causing disruption to mitochondrial membranes. Damage to the mitochondrial membrane causes the outtake of the cell cytochrome and joins Apaf-1 and Pro-caspase 9 and forms apoptosome. Apoptosome triggers caspase 3 activity and leads to cell death by fragmentation (apoptosis). Also, cell death through necrosis can also occur due to increased levels of salt in the blood. Increased TGF- $\beta$  can lead to cell death through apoptosis through a set of building information modeling (BIM) cells. As Ramesh et al. [27] find, there is a clear understanding of how TGF- $\beta$  mediates apoptosis through regulatory mechanisms that may be controlled through the level of Bim expression (formerly known as Bcl2L 11).

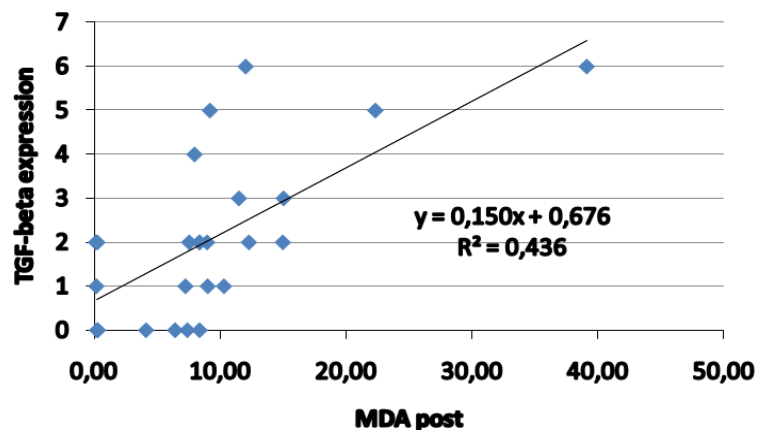
**Table 1.** Correlation between TGF-beta and MDA-post after administration of EVOO on preeclampsia rat.

Spearman's rho	MDA post	Correlation Coefficient	MDA post	TGF-beta
		Sig. (2-tailed)	.	0.640**
		N	25	25
	TGF-beta	Correlation Coefficient	0.640**	1.000
		Sig. (2-tailed)	0.001	.
		N	25	25

\*\*. Correlation is significant at the 0.01 level (2-tailed).

Provision of EVOO to rats of preeclampsia may decrease MDA production produced by placental cells. MDA production signifies the presence of oxidant production by cell activity and as a sign of oxidative stress. By its role that MDA is often used as a biomarker of oxidative stress in clinical and non-clinical investigations. As Khoubnasabjafari et al. [28]) pointed out, MDA is used as a biomarker of oxidative stress in many diseases although it has wide variations in healthy people. According to the evidence gathered, most of the technical issues of MDA measurement have not been resolved and need further investigation, and the role of MDA biomarkers should be reevaluated by experts.

In this study we found a strong and significant relationship ( $r=0.64$  and  $p<0.05$ ) between TGF- $\beta$  expression with post MDA levels in preeclamptic rats given EVOO (Figure 1).



**Figure 3.** Linear graph of serum MDA relationship with TGF- $\beta$  expression in placenta mouse model of preeclampsia.

This suggests the role of TGF- $\beta$  in the process of regulation or the emergence of oxidative stress in rat placenta preeclampsia. By another study by Liu and Leen [29], that TGF- $\beta$  is the most potent pro-fibrogenic cytokine and its expression is elevated in almost all fibrotic diseases. Although signaling via the Smad line is believed to play a central role in TGF- $\beta$  fibrogenesis, emerging evidence suggests that reactive oxygen species (ROS) modulate TGF- $\beta$  signals via different pathways including the Smad pathway. TGF- $\beta$ 1 increases the production of ROS [30] and suppresses antioxidant enzymes, causing a redox imbalance. ROS, in turn, induces TGF- $\beta$ 1 and mediates many of the TGF- $\beta$  fibrogenic effects, forming a vicious circle (see graph flow chart on the right). Here, we review the current knowledge of feedback mechanisms between TGF- $\beta$ 1 and ROS in the development of fibrosis. Therapies targeting TGF- $\beta$ -induced and ROS-dependent cellular signaling are a new approach in the treatment of fibrotic disorders.

#### 4. Conclusions

Based on the result of the research, it can be concluded that there is a significant effect of EVOO on TGF- $\beta$  ( $p < 0,05$ ) placenta pre-eclampsia. There was a strong ( $r = 0.494$ ) and significant relationship between TGF- $\beta$  ( $p < 0.01$ ) and post MDA levels.

#### Acknowledgments

The authors highly acknowledge and express our greatest gratitude to the Rector of University of Sumatera Utara that provides the research funding through TALENTA USU program under the contract number 5338/UN5.1.R/PPM/2017, May 22nd 2017, on the fiscal year 2017/2018.

#### Reference

- [1]. Shah DA, and Khalil, RA (2015) Bioactive factors in uteroplacental and systemic circulation link placental ischemia to generalized vascular dysfunction in hypertensive pregnancy and preeclampsia. *Biochemical Pharmacology*, **95**(4), 211–226.
- [2]. WHO (2011) Recommendations for Prevention and Treatment of Preeclampsia and Eclampsia, *WHO Department of Maternal and Child Health*, Geneva, Switzerland
- [3]. Ministry of Health RI (2014) Mother's Day's. InfoDATIN Center for Data and Health Information RI Leong x, Mustafa MR, Jaarin K. (n.d.). *Nigella sativa* and its protective role in oxidative stress and hypertension.
- [4]. Cunningham LBHRS (2010) *Williams Obstetric*. 23 ed. United States of America: The McGraw-Hill Companies, Inc
- [5]. Saucedo R, Valencia J, Manuel L, and Hern M (2014) Early Disturbed Placental Ischemia and Hypoxia Creates Immune Alteration and Vascular Disorder Causing Preeclampsia, 45.
- [6]. Yi KW, Jung SH, Cho GJ et al. (2014) Effects of sflt-1 and alpha 2-macroglobulin on vascular endothelial growth factor-induced endothelin-1 upregulation in human microvascular endothelial cell. **35**:64-69



- [7]. Teixeira BC (2014) *Inflammatory markers, endothelial function and cardiovascular risk*. **13**(2):108–115 21
- [8]. Salaets K, Sliessman, J Speiser R, Anh-Minh T, Wang E, Angerio A (2006) Role of endothelin - 1 in Atherosclerosis Georgetown. *Journal of health Sciences*, **3**(1):1-9
- [9]. Takeuchi T (2001) Regulation of Platelet Aggregation in Vitro by Plasma Adenosine in Preeclampsia. *Gynecol Obstet Invest* **51**(1), 36-39.
- [10]. Satria D, Jansen Silalahi, Ginda Haro, Syafruddin Ilyas (2017) Poppy Anjelisa Z Hsb. Antioxidant and Antiproliferative Activities of an Ethylacetate Fraction of Picria Fel-Terrae Lour. Herbs. *Asian Pac J Cancer Prev*, **18**(2), 399-403.
- [11]. Ilyas, S (2014) Effect of Methanolic *Momordica Charantia* Seed Extract And Depot Medroxyprogesterone Acetate (DMPA) to Quantity and Quality of Rat Sperm. *International Journal of Pharmtech Research*. **6** (6) 1817-1823. 0974-4304.
- [12]. Hasibuan R, Syafruddin Ilyas, Saleha Hanum (2015) Effect of leaf extract Haramonting (*Rhodomyrtus tomentosa*) to lower blood sugar levels in mice induced by alloxan. **88**(6), pp 284-291.
- [13]. Moeloek N, Asmarinah Asmarinah, Nurjati C. Siregar, Syafruddin Ilyas (2008) Testosterone undecanoate and depo medroxyprogesterone acetate induced azoospermia through increased expression of spermatogenic cell caspase 3. **17**(3)
- [14]. Crowe M, Doetschman T, Greenhalgh DG (2000) Delayed Wound Healing in Immunodeficient TGF- $\beta$  Knockout Mice. *The Journal of Investigative Dermatology*. **115**(1).
- [15]. Das RK, Venkatraghavan V, Sheet D, Chakraborty C, Ray AK, Chatterjee J (2010) Evaluation of p63 Expression in Oral Submukus Fibrosis. *Proceedings of 2010 International Conference on Systems in Medicine and Biology*
- [16]. Werner S, Grose R, (2003). Regulation of Wound Healing by Growth Factors and Cytokines. *Physiol Rev*, **83**: 835-870.
- [17]. Yusuf F, Syafruddin Ilyas, Harun AR. Damanik, Fatchiyah (2017) Microbiota Composition, HSP70 and Caspase-3 Expression as Marker for Colorectal Cancer Patients in Aceh, Indonesia. *Acta Med Indones-Indones J Intern Med*. **48**(4).
- [18]. Ilyas S, Hutahaean S, Nursal (2016) Apoptosis overview of cerebellum Purkinje cell in mice (*Mus musculus* L.) after exposure to methanol extract of the seeds of bitter melon (*Momordica charantia*) and DMPA. *International Journal of PharmTech Research*. **9**(9), pp. 444-449.
- [19]. Masfria, Urip Hara hap, Maratua Pandapotan Nasutionl, Syafruddin Ilyas, (2014). Cytotoxic Activity, Proliferation Inhibition and Apoptosis Induction of *Rhaphidophora Pinnata* (L.F.) Schott Chloroform Fraction to MCF-7 Cell Line. **6**(4), pp 1327-1333.
- [20]. Herwanto RY, Jenny Bashiruddin, Syafruddin Ilyas, M. Nadjib Dahlan Lubis (2015) Correlation of Noise Intensity to Heat Shock Response with Hsp 70, p53, Cytochrome C, Caspase 3 expressions and ultrastructure region of *Rattus norvegicus*'s cochlea. **7**(1), pp 80-84
- [21]. Herwanto RY, Syafruddin Ilyas, Rr. Suzy Indharty (2016) HSP70 Gene Expression in Serum and Tissue of Rat Cochlear (*Rattus norvegicus*) Due to Noise Exposure and Heat. **9**(11), pp 58-63.
- [22]. Masyithah C, Sumadio Hadisahputra, Syafruddin Ilyas (2015) Combinational effects of ethylacetate extract of *Zanthoxyluma canthopodium* DC. with doxorubicinon MCF7 breast cancer cells. **7**(4), pp 651-653.
- [23]. Iranloye BO, Gabriel O. Oludare, Ayodele O. Morakinyo, Naomi A. Esume, and Lucy C. Ekeh (2013) Reproductive parameters and oxidative stress status of male rats fed with low and high salt diet. *J Hum Reprod Sci*. Oct-Dec; **6**(4): 267–272.
- [24]. Bertolino P, Deckers M, Lebrin F and ten Dijke P (2005) Transforming growth factor-beta signal transduction in angiogenesis and vascular disorders. *Chest* 128 (6 Suppl): 585S-590S.
- [25]. Lebrin F, Deckers M, Bertolino P and Ten Dijke P (2005) TGF-beta receptor function in the endothelium. *Cardiovasc Res* 65: 599-608.
- [26]. Khanduja KL, Pramod Kumar Avti Surender Kumar, Vandana Pathania, Chander Mohan Pathak (2005) Inhibitory effect of vitamin E on proinflammatory cytokines-and endotoxin-induced nitric oxide release in alveolar macrophages. **76**(23), 22 April 2005, Pages 2669-2680.
- [27]. Ramesh S, Gary M. Wildey, and Philip H. Howe (2009) Transforming growth factor  $\beta$  (TGF $\beta$ )-induced apoptosis: The rise & fall of Bim. *Cell Cycle*. **8**(1): 11–17.

- [28]. Khoubnasabjafari M, Khalil Ansarin, and Abolghasem Jouyban (2015) Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorder. *Bioimpacts*. 2015; **5**(3): 123–127.
- [29]. Liu RM and Leena PD (2015) Reciprocal regulation of TGF- $\beta$  and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biol.* **6**: 565–577
- [30]. Ilyas S, Silvia W. Lestari, Nukman Moeloek, Asmarinah, Nurjati C. Siregar (2013) Induction of Rat Germ Cell Apoptosis by Testosterone Undecanoate and Depot Medroxyprogesterone Acetate and Correlation of Apoptotic Cells with Sperm Concentration. **45**(1).