The effects of *Phaleria macrocarpa* (Scheff.) boerl extract on tumor necrosis factor (TNF-α) level in Preeclampsia-induced HUVEC culture

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Abstract. Preeclampsia is a major cause in both maternal and perinatal mortality and morbidity. The etiopathogenesis of preeclampsia remains unclear but endothelial dysfunction plays important role. Stress oxidative in placenta produces free radicals such as superoxide anions (O_2^-) and H_2O_2, trophoblast debris, pro-inflammatory cytokines, and antiangiogenic factors which are thought to cause vascular endothelial dysfunction and causing excessive maternal inflammatory responses. Tumor Necrosis Factor-α (TNF-α) is considered as one of the potentially specific markers for preeclampsia. HUVEC (Human Umbilical Vein Endothelial Cell) culture is an in vitro model widely used to study the pathogenesis of preeclampsia. *Phaleria macrocarpa* (Scheff.) Boerl also known as Mahkota Dewa is widely used as an anti-inflammation and antioxidant. This study aimed to determine the effects of *Phaleria macrocarpa* (Scheff.) Boerl Extract on inflammation in endothelial cells by measuring the TNF-α level in preeclampsia-induced HUVEC. Our results showed the *Phaleria macrocarpa*'s extract reduce TNF-α level siginificantly at concentration of 7.813 μg/mL. *Phaleria macrocarpa*'s extract at concentration of 62.5 μg/mL reduce TNF-α level to normal level. Thus, *Phaleria macrocarpa*'s extract might be used as agent to overcome endothelial dysfunction in preeclampsia.

1. Introduction

Preeclampsia is one of the leading causes of maternal morbidity and mortality worldwide. It is estimated that maternal deaths worldwide are around 500,000 annually and about 10% - 15% are due to preeclampsia and eclampsia [1]. In 2006 WHO reported that 16% of maternal deaths in developed countries due to hypertension in pregnancy, higher than due to bleeding of 13%, abortion of 8% and sepsis of 2% [2].

Although there have been many studies but the etiopathogenesis of preeclampsia is still not fully elucidated but it is believed to be a multifactor. Thus preeclampsia is called the 'disease of theories' [3].

Endothelial dysfunction plays an important role in the pathophysiology of preeclampsia. Under normal circumstances, endothelial cells maintain vascular integrity, regulating blood pressure, preventing intravascular coagulation, and regulating vascular smooth muscle tone by producing various substances including nitric oxide (NO), endothelin, prostacyclin and thromboxane [4]. Endothelial dysfunction occurs due to cytotoxic factors in the circulation

such as superoxide anions (O_2^-) and H_2O_2, trophoblast debris, pro-inflammatory cytokines, metabolic factors, and anti-angiogenic factors produced by oxidative stressed placenta and causing excessive maternal inflammatory responses [5].

Tumor Necrosis Factor-α (TNF-α) is considered as one of the potentially specific markers for preeclampsia. In endothelial cells TNF-α causes endothelial dysfunction by increasing oxidation of low-density lipoprotein (LDL), inhibiting eNOS enzymes causing NO levels decrease and increasing free radical production by xanthine oxidase enzyme, then binds to endothelial cells and produces an O_2^- anion in endothelial cells [6-7].

TNF-α levels in preeclampsia placenta were significantly higher than normal pregnancies with ELISA method and allegedly TNF-α increases oxidative stress by stimulating the formation of ROS [3]. Udenze et al. [8] found that TNF-α level in preeclampsia serum is significantly higher than in the serum of normal pregnancies (44.80 pg/mL vs 8.15 pg/mL).

Preeclampsia treatment will only be successful and rational if based on understanding the disease pathophysiology. In an attempt to determine the pathophysiology of a disease, in vitro model research is considered the best and most effective way [9]. HUVEC (Human Umbilical Vein Endothelial Cell) cell line culture and trophoblast cell line is an in vitro model widely used to study the pathogenesis of preeclampsia.

Herbs or medicinal plants have been used traditionally as alternative medicine since ancient times. *Phaleria macrocarpa* (Scheff.) Boerl also known as Mahkota dewa belongs to the *Thymelaceae* family, that originated from Papua province, is very popular in Indonesia used in the treatment of various diseases such as cancer, hemorrhoids, diabetes mellitus, hypertension, and others [10-12]. The phenol and flavonoid compounds in the extract of *Phaleria macrocarpa* have high antioxidant and anti-inflammatory activity [12-13].

The aim of this study is to determine the effects of *Phaleria macrocarpa* (Scheff.) Boerl Extract on Tumor Necrosis Factor – Alpha (TNF- α)Level in preeclampsia-induced Human Umbilical Vein Endothelial Cell (HUVEC).

2. Method

Serum samples used were obtained from women at >20 - 42 weeks of gestational age, which were diagnosed preeclampsia at Dr. Hasan Sadikin General Hospital. Research subjects have fulfilled inclusion and exclusion criteria.

2.1. Cell Culture

HUVEC cell line ATCC CRL-1730 obtained from American Type Collection Culture. HUVEC cell line was growth into tissue culture flask (25 cm^2) containing RPMI 1640 media, 20% (v/v) FBS qualified (fetal bovine serum) supplementation, 10% endothelial supplement, 1% Penicillin G - Streptomycin solution stabilized, and 1% antimycotic Fungizone Amphotericin B and 1% gentamicin. The cells were then incubated at 37^OC and 5% CO_2 (v/v). Culture medium is replaced every 2 - 3 days. Then cells are passaged every seven days until reach 80-90% confluence.

2.2. Phaleria macrocarpa's Extract

Phaleria macrocarpa (Scheff.) Boerl was obtained from the Research Institute for Industrial Plants at Manoko, Lembang, and West Java, Indonesia. The plant species was identified by the laboratory of Plant Taxonomy staff at Herbarium Bogoriense, Bogor, Indonesia.

2.3. Measurement of TNF- α Level

As many as $6x10^5$ cells/mL induced with normal and preeclampsia serum, were placed into 60-well plate, then incubated at 37^OC and 5% CO_2 (v/v). Each well then was washed with 37^OC PBS 3-4 times. Furthermore, various concentrations of Phaleria macrocarpa's extract ((0,977; 1,953; 3,906; 7,813; 15,625; 31,25; 62,5; 125; and 250 μg/mL) were added into each well, then incubated for 24 and 72 hours 37^OC and 5% CO_2 (v/v). Each well then was washed with 37^OC once for five minutes. Transfer the cells into centrifugation tube using 1.5 mL pipette. Centrifuged at 1.500 rpm for 10 minutes at 4°C. Use the supernatant as a sample for the ELISA method measurement, then the rest of the sample can be stored at -80 °C.

2.4. Data Analysis

Data was analyzed with repeated ANOVA (analysis of variance) test and followed by Bonferroni test as post hoc comparison test

3. Results and Discussion

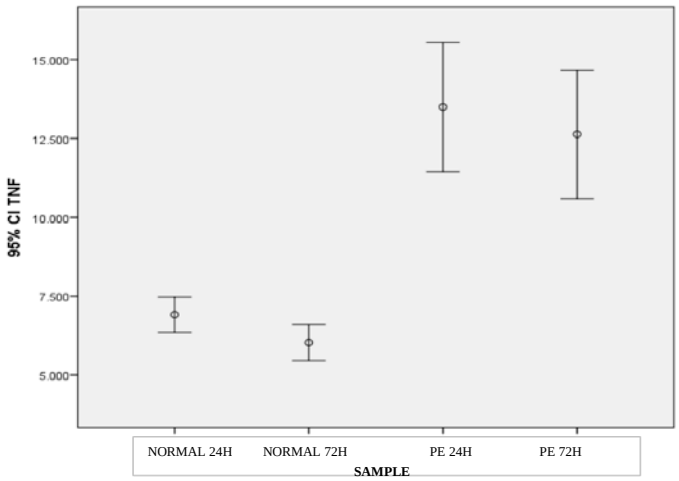

Figure 1.TNF-α levels in normal and preeclampsia-induced HUVEC based on incubation time.

As shown in figure 1 TNF-α level in preeclampsia HUVEC culture model is higher than normal pregnancy HUVEC culture model. The TNF-α level at 72 hours incubation time was lower than the 24 hours incubation time in both normal and preeclampsia models.

Table 1.TNF-α levels (pg/mL) in preeclampsia and normal serum-induced HUVEC culture model treated with *Phaleria macrocarpa*'s extract in various concentrations incubated for 24 and 72 hours.

Phaleria macrocarpa's extract concentration (μg/mL)	24 H INCUBATION TIME		72 H INCUBATION TIME	
	NP* (Mean ± SD)	PE (Mean ± SD)	NP* (Mean ± SD)	PE (Mean ± SD)
Control	8.718 ± 0.043	18.709 ± 0.007	7.858 ± 0.029	17.848 ± 0.035
0.977	8.218 ± 0.003	18.273 ± 0.051	7.445 ± 0.015	17.395 ± 0.007

1.953	7.886 ± 0.005	17.888 ± 0.001	6.987 ± 0.006	16.778 ± 0.015
3.906	7.382 ± 0.071	15.295 ± 0.087	6.335 ± 0.02	14.666 ± 0.312
7.813	6.814 ± 0.007	14.533 ± 0.000	5.950 ± 0.057	13.763 ± 0.000
15.625	6.009 ± 0.003	12.778 ± 0.000	5.103 ± 0.007	11.492 ± 0.708
31.25	6.000 ± 0.000	10.089 ± 0.000	5.077 ± 0.014	9.325 ± 0.000
62.5	5.994 ± 0.006	8.578 ± 0.000	5.009 ± 0.000	7.668 ± 0.001
125	5.455 ± 0.001	7.654 ± 0.000	4.662 ± 0.000	6.834 ± 0.001
250	5.003 ± 0.000	6.089 ± 0.000	4.101 ± 0.001	5.404 ± 0.000

NP: Normal Pregnancy

Table 1.shows TNF-α levels mean in difference preeclampsia and normal serum-induced HUVEC culture model treated with *Phaleria macrocarpa*'s extract in various concentrations incubated for 24 and 72 hours.

Table 2.TNF-α levels (pg/mL) mean comparison before and after various concentrations of *Phaleria macrocarpa*'s extract treatment at 24 hours and 72 hours incubation time in preeclampsia HUVEC culture model

Phaleria macrocarpa's extract concentration (μg/mL)	24 H INCUBATION TIME		72 H INCUBATION TIME	
	PE (Mean ± SD)	P value*	PE (Mean ± SD)	P value*
Control	18.709 ± 0.007		17.848 ± 0.035	
0.977	18.273 ± 0.051	1.000	17.395 ± 0.007	1.000
1.953	17.888 ± 0.001	0.227	16.778 ± 0.015	0.362
3.906	15.295 ± 0.087	0.470	14.666 ± 0.312	1.000
7.813	14.533 ± 0.000	0.034	13.763 ± 0.000	0.175
15.625	12.778 ± 0.000	0.024	11.492 ± 0.708	1.000
31.25	10.089 ± 0.000	0.017	9.325 ± 0.000	0.082
62.5	8.578 ± 0.000	0.014	7.668 ± 0.001	0.070
125	7.654 ± 0.000	0.013	6.834 ± 0.001	0.065
250	6.089 ± 0.000	0.012	5.404 ± 0.000	0.058

* : statistically significant if $p < 0.05$

Table 2 shows TNF-α level decreased in preeclampsia serum-induced HUVEC ATCC CRL 1730 following increased *Phaleria macrocarpa*'s extract concentration. TNF-α level significantly decreased after exposure of *Phaleria macrocarpa*'s extract on concentration 7.813 µg/mL. ($p<0,05$).

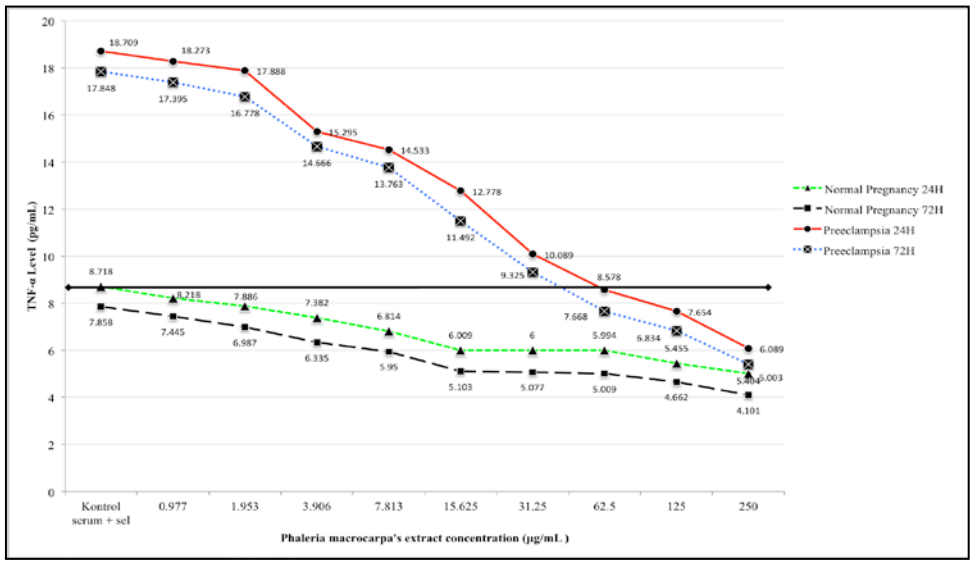

Figure 2.TNF-α level in relation with Phaleria macrocarpa's extract concentration

Figure 2 shows that Phaleria macrocarpa's extract at concentration of 62.5 µg/mL reduce TNF-α level in preeclampsia model to normal pregnancy level.

4. Conclusions

This was the first study to evaluate the effects of *Phaleria macrocarpa* (Scheff.) Boerl extract on Tumor Necrosis Factor – Alpha (TNF- α) level in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (HUVEC). Preeclampsia and eclampsia have been known since ancient times but their pathophysiology is still not clearly understood.

There is compelling evidence that endothelial dysfunction plays a role in the pathophysiology of preeclampsia. A consistent finding is the presence of glomerular endotheliosis in more than 70% of primiparous preeclampsia patients and this glomerular endotheliosis will disappear after delivery.

To date, invitro research using HUVEC has been done a lot recently. Previous invitro research on HUVEC cultures by treating with anti-inflammatory and antioxidant compounds such as curcumin and Papua ant nest (*Myrmecodia pendens*) decrease oxidative stress and inflammation characterized by decreased levels of MDA, and TNF-α. These studies conclude that the Papuan ant nests and curcumin have a therapeutic effect on preeclampsia [14-15].

TNF-α is considered as one of the potentially specific markers for preeclampsia and contributes to the formation of free radicals such as peroxides (H_2O_2), and superoxide (O_2^-) [6]. Autophagy can be induced by many overlapping factors such as nutritional deficiencies, growth factors deficiencies, and intracellular stress due to hypoxia.

In this study results showed TNF- α level in preeclampsia HUVEC culture model was higher than normal pregnancy HUVEC culture model. TNF-α level decreased in preeclampsia and normal serum-induced HUVEC ATCC CRL 1730 culture following increased *Phaleria*

macrocarpa's extract concentration. TNF-α level significantly decreased after exposure of *Phaleria macrocarpa*'s extract on concentration 7.813 μg/mL. Phaleria macrocarpa's extract at concentration of 62.5 μg/mL reduce TNF-α level to normal level.

The result of present study suggests that *Phaleria macrocarpa*'s extract contains anti-inflammatory activity proven by decreased level of TNF-α. It was also described that TNF-α level decreased in preeclampsia and normal serum-induced HUVEC ATCC CRL 1730 following increased *Phaleria macrocarpa*'s extract concentration. Thus, *Phaleria macrocarpa*'s extract might be used as agent to restore endothelial dysfunction in preeclampsia. Since the decreased the level of TNF-α in preeclampsia-induced HUVEC ATCC CRL 1730 culture, further clinical studies regarding the use of *Phaleria macrocarpa*'s extract in treatment are encouraged. The *Phaleria macrocarpa*'s extract reduce TNF-α level siginificantly at concentration of 7.813 μg/mL in preeclampsia-induced HUVEC ATCC CRL 1730 culture.Phaleria macrocarpa's extract at concentration of 62.5 μg/mL reduce TNF-α level to normal level.

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