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Research Article

The Effects of *Phaleria macrocarpa* (Scheff.) Boerl Extract on Malondialdehyde (MDA) Level in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (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 is one of the leading theories. Increased oxidative stress and lipid peroxides and reduced antioxidants play important role in the pathophysiology of preeclampsia. Malondialdehyde (MDA) is the final product of lipid peroxidation, used as the oxidative stress marker. 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 because of alkaloids, saponins, flavonoids and polyphenols properties. This study aimed to determine the effects of *Phaleria macrocarpa* (Scheff.) Boerl Extract on oxidative stress in endothelial cells by measuring the MDA level in preeclampsia-induced HUVEC. Our results showed the Phaleria macrocarpa's extract reduce MDA level siginificantly at concentration of 3.906 µg/mL. *Phaleria macrocarpa's* extract at concentration of 15.625 µg/mL reduce MDA level to normal level. Thus, *Phaleria macrocarpa's* extract might be used as agent to overcome oxidative stress in preeclampsia.

Keywords: *Phaleria macrocarpa*; Preeclampsia; HUVEC; MDA; Oxidative Stress

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 multifactors. 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.

Oxidative stress is considered as a mediator of endothelial dysfunction in preeclampsia [5]. Pregnancy itself is a state of oxidative stress along with mitochondrial activity and increased production of Reactive oxygen species (ROS) but is offset by increased antioxidants production. Increased oxidative stress and lipid peroxides and reduced antioxidants play a role in the pathophysiology of preeclampsia [6-8]. Wang and Walsh [9] found there is elevated levels of lipid peroxide in preeclampsia placental tissue compared to normal pregnancy placental tissue.

Malondialdehyde (MDA) is the final product of lipid peroxidation, thus it is used as one of the oxidative stress marker. Madazli., *et al.* [10] found that MDA levels in preeclampsia placental and plasma were significantly higher than normal pregnancies (6.06 ± 0.94 nmol/ml vs 4.49 ± 0.71 nmol/ml) and (10.18 ± 1.32 nmol/gr wet weight vs 6.72 ± 1.27 nmol/gr wet weight). Whereas superoxide dismutase (SOD) levels decreased significantly in preeclampsia placental and plasma than normal pregnancies (23,51 ± 2,27 U/ml vs 26,57 ± 1,44 U/ml) and (40,83 ± 9,62 U/gr wet weight vs 50,43 ± 16,87 U/gr wet weight).

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 [11]. 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 [12-14]. The phenol and flavonoid compounds in the extract of *Phaleria macrocarpa* have high antioxidant and anti-inflammatory activity [14,15].

The aim of this study is to determine the effects of *Phaleria macrocarpa* (Scheff.) Boerl Extract on Malondialdehyde (MDA) Level In preeclampsia-induced Human Umbilical Vein Endothelial Cell (HUVEC).

Materials and 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.

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^{0}C 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.

Phaleria macrocarpa's extract

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

Measurement of MDA level

As many as $6x10^5$ cells/mL induced with normal and preeclampsia serum, were placed into 60-well plate, then incubated at 37^{0}C and 5% CO_2 (v/v). Each well then was washed with 37^{0}C 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^{0}C and 5% CO_2 (v/v). Each well then was washed with 37^{0}C 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.

Data analysis

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

Results

As shown in figure 1 MDA levels in preeclampsia HUVEC culture model is higher than normal pregnancy HUVEC culture model. The MDA levels at 72 hours incubation time was lower than the 24 hours incubation time in both normal and preeclampsia models. Figure 2 shows that LC3-II levels in preeclampsia HUVEC culture model is higher than normal pregnancy HUVEC culture model. The LC3-II levels at 72 hours incubation time was higher than the 24 hours incubation time in both normal and preeclampsia models.

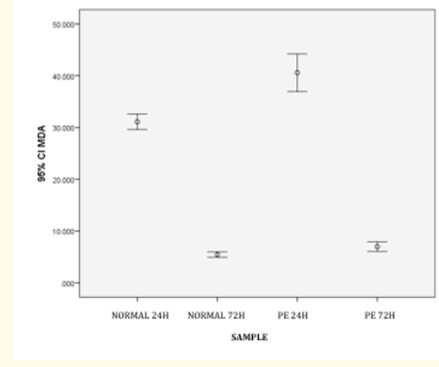

Figure 1: MDA levels in normal and preeclampsia-induced HUVEC based on incubation time.

Table 1 shows MDA 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 shows MDA level decreased in preeclampsia serum-induced HUVEC ATCC CRL 1730 following increased *Phaleria macrocarpa's* extract concentration. MDA level significantly decreased after exposure of *Phaleria macrocarpa's* extract on concentration 3.906 µg/mL. (p<0,05).

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	37.520 ± 0.358	53.978 ± 0.028	6.906 ± 0.086	10.772 ± 0.006
0.977	32.951 ± 0.068	48.063 ± 0.003	5.947 ± 0.002	7.824 ± 0.001
1.953	31.488 ± 0.564	45.483 ± 0.672	5.847 ± 0.007	7.219 ± 0.001
3.906	31.033 ± 0.062	39.064 ± 0.008	5.450 ± 0.001	6.563 ± 0.003
7.813	29.882 ± 0.013	39.545 ± 0.001	5.232 ± 0.001	6.323 ± 0.003
15.625	29.004 ± 0.003	37.260 ± 0.353	5.083 ± 0.008	6.126 ± 0.001
31.25	28.987 ± 0.006	35.237 ± 0.007	4.901 ± 0.006	5.913 ± 0.003
62.5	28.360 ± 0.494	33.095 ± 0.008	4.611 ± 0.077	5.710 ± 0.002
125	28.010 ± 0.000	31.135 ± 0.000	4.069 ± 0.004	5.508 ± 0.001
250	27.889 ± 0.018	30.566 ± 0.006	3.891 ± 0.002	4.012 ± 0.001

Table 1: MDA levels (pmol/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.

NP: Normal Pregnancy

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	53.978 ± 0.028		10.772 ± 0.006	
0.977	48.063 ± 0.003	0.085	7.824 ± 0.001	0.034
1.953	45.483 ± 0.672	1.000	7.219 ± 0.001	0.032
3.906	39.064 ± 0.008	0.027	6.563 ± 0.003	0.044
7.813	39.545 ± 0.001	0.037	6.323 ± 0.003	0.016
15.625	37.260 ± 0.353	0.009	6.126 ± 0.001	0.022
31.25	35.237 ± 0.007	0.022	5.913 ± 0.003	0.015
62.5	33.095 ± 0.008	0.019	5.710 ± 0.002	0.034
125	31.135 ± 0.000	0.026	5.508 ± 0.001	0.022
250	30.566 ± 0.006	0.019	4.012 ± 0.001	0.017

Table 2. MDA levels (pmol/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.

*: statistically significant if $p < 0.05$

Figure 2 shows that *Phaleria macrocarpa's* extract at concentration of 15.625 µg/mL reduce MDA level in preeclampsia model to normal pregnancy level.

Discussion

This were the first study to evaluate the effects of *Phaleria macrocarpa* (Scheff.) Boerl extract on Malondialdehyde (MDA) level in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (HU-

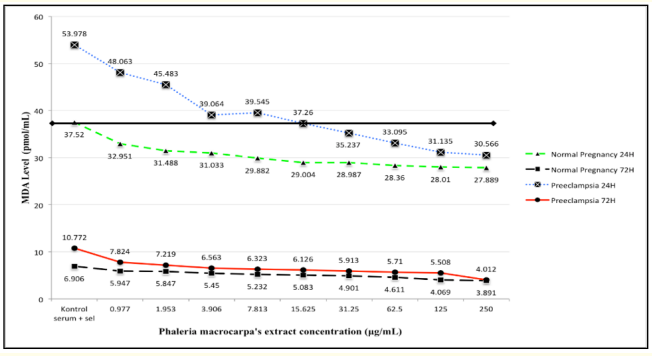

Figure 2: MDA levels in relation with *Phaleria macrocarpa's* extract concentration

VEC). 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 [16,17].

Oxidative stress is considered as a mediator of endothelial dysfunction in preeclampsia [5]. Malondialdehyde (MDA) is the final product of lipid peroxidation, thus it is used as one of the oxidative stress marker. Autophagy can be induced by many overlapping factors such as nutritional deficiencies, growth factors deficiencies, and intracellular stress due to hypoxia. LC3-II is used as a typical marker of autophagosome formation in autophagy.

In this study results showed MDA levels in preeclampsia HUVEC culture model was higher than normal pregnancy HUVEC culture model. MDA level decreased in preeclampsia and normal serum-induced HUVEC ATCC CRL 1730 culture following increased *Phaleria macrocarpa's* extract concentration. MDA level significantly decreased after exposure of *Phaleria macrocarpa's* extract on concentration 3.906 µg/mL. *Phaleria macrocarpa's* extract at concentration of 15.625 µg/mL reduce MDA level to normal level.

The result of present study suggests that *Phaleria macrocarpa's* extract contains anti-oxidant activity proven by decreased level of MDA. It was also described that MDA 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 overcome oxidative stress in preeclampsia. Since the decreased the level of MDA in preeclampsia-induced HUVEC ATCC CRL 1730 culture, further clinical studies regarding the use of *Phaleria macrocarpa's* extract in treatment are encouraged.

Conclusion

The *Phaleria macrocarpa's* extract reduce MDA level siginificantly at concentration of 3.906 µg/mL in preeclampsia-induced HUVEC ATCC CRL 1730 culture. *Phaleria macrocarpa's* extract at concentration of 15.625 µg/mL reduce MDA level to normal level.

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Volume 3 Issue 8 August 2019