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Effects of Papua Ant Nests (*Myrmecodia pendens*) on Level of sFlt-1, PIGF, MDA and NO in Preeclampsia-induced HUVEC Cell Line

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Abstract : Preeclampsia remains major cause in both maternal and perinatal mortality and morbidity. Preeclampsia causes endothelial dysfunction due to imbalance of proangiogenic and antiangiogenic indicated by increased soluble fms-like tyrosine kinase-1 (sFlt-1) and decreased placental growth factor (PlGF), also followed by oxidative stress indicated by decreased malondialdehyde (MDA) and increased nitric oxide (NO). Antioxidant is believed in preventing such mechanism. Herbal antioxidant is widely used in Indonesia, one of which is ant nests from Papua. Ant nests used is formed in water fraction and known containing antioxidant compounds such as flavonoid, tannin, terpenoid and alkaloid. This study aims to analyze therapeutic effects of ant nests on level of sFlt-1, PIGF, MDA and NO in preeclampsia-induced HUVEC cell line. Measurement of level of sFlt-1 and PlGF was performed with ELISA. Measurement of level of MDA was performed with thiobarbituric acid-reactive substances (TBARS), and level of NO was performed with NWLSS™ Nitric Oxide Assay. Data was analyzed statistically with ANOVA and Duncan test. Level of sFlt-1 and MDA in preeclampsia-induced HUVEC ATCC CRL 1730 cell line were decreased whereas level of PlGF and NO in preeclampsia-induced HUVEC ATCC CRL 1730 cell line were increased, after exposure of ant nest water fraction on concentration 31,25 ug/ml. **Conclusion:** Ant nest water fraction has therapeutic effects on preeclampsia. Further studies regarding development of ant nests in prevention of preeclampsia are encouraged.
Keywords: ant nests, sFlt-1, PIGF, MDA, NO.

Introduction

Preeclampsia remains major cause in both maternal and perinatal mortality and morbidity. *National Vital Statistics Reports* reported increased preeclampsia onset was approximately 40%.[1] According to *Survei Demografi Kesehatan Indonesia* (SKDI) in 2013, number of maternal mortality in Indonesia was significantly increased approximately 359 per 100.000 living birth compared to SDKI in 2007. Preeclampsia contributed to maternal mortality about 23%, makes it the second cause of maternal mortality in Indonesia. [2] In Dr. Hasan Sadikin Hospital, Bandung, preeclampsia was reported in 3.5% cases and eclampsia in 2.8% cases in 2006,

while in 2008-2010 preeclampsia was reported in 4.0-10.4% and eclampsia in 2.3-4.3%.[3-5] Therefore, studies regarding underlying mechanism of preeclampsia are conducted.

Endothelial dysfunction is a major point in preeclampsia and associated with alteration of angiogenics and antiangiogenics balance, placental metabolism, placental inflammation mediator, and v*ery low density lipoprotein* (VDRL) level.[6] Alteration in preeclampsia occurs in presence of excess antiangiogenic, *soluble fms-like tyrosine kinase-1* (sFlt-1).[7] sFlt-1 is endogenic antiangiogenic protein produced by placenta which undergo ischemic and neutralize proangiogenic protein, *Vascular Endothelial Growth Factor* (VEGF) and *Placental growth factor* (PlGF).[8] sFlt-1 is expressed by placenta as response of hypoxia, and increased before its clinical symptoms emerged (early trimester). The presence of sFlt-1 was further observed to diagnose preeclampsia in laboratory before hypertension and proteinuria.[9,10]

In preeclamptic patients, altered endothelial vessels decrease nitric oxide (NO) phosphorylation synthesis in endothelial cells due to increased sFlt-1. Decreased oxide phosphorylation synthesis affects NO bioavailability produced by endothel, that promotes alteration in cardiovascular homeostasis.[11-13] NO is a free radical molecule which is reactive in a short time, but modifies the activity of trombocit in endothelial cells to undergo adhesion, aggregation, and releasing reaction. Decreased NO worsens hypoxia in preeclampsia.[14,15] Hypoxia causes production of *reactive oxygen species* (ROS) such as superoxidation (O_2), hydroxyl radical (OH) and hidrogen peroxide (H_2O_2) in preeclampsia following disturbance in regulation of prooxidant and antioxidant.[16] Decreased enzymatic production of prooxidant and increased lipid peroxide caused by free radical *malondialdehyde* (MDA), are also detected. The presence of MDA is therefore used as a marker of endothelial dysfunction on molecular level. [17,18-22]

Increased free radical molecules is documented in preeclamptic patients which is associated with decreased cellular antioxidant. Antioxidant is responsible in reducing damage through scavenging mechanism of radical cluster, as well as preventing oxidative stress through metal binding mechanism.[23,24] It is naturally produced by cell or obtained from outside such as foods. Decreased level of *Nicotinamide Adenine Dinucleotide Phospahte* (NADPH) occurs in preeclampsia, and theoretically prevented by antioxidant exposure from outside that provides additional protective effects and synergistic with intracellular enzyme as internal antioxidant. [25-27] It has been reported that herbal medicine possess antioxidant properties which is known safe for patients. [28,29]

Ant nests (*Myrmecodia pendens*) is reported to contain alkaloid, flavanoid, tanin and terpenoid. Flavanoid is water dissolved and used as antioxidant, antiangiogenic, wide spectrum antimicrobial, antithrombogenic, antiviral, decreases blood cholesterol level and inhibits cell proliferation.[35] The other compound, tannin, is also hydrolyzed and has condensed protein complex and quickly reactive trapping hydroxyl radical cluster. [37,38] Previous studies show ant nests extract to possess antiangiogenic compound.[36] Antiangiogenic (*angiogenesis inhibitor*) inhibits activity of angiogenic factors inducing extracellular matrix enzyme which attacks endothelial vessels walls and inhibits endothelial vessels cells proliferation.[39]

Water fraction of ant nests is considered as food alternative. However, direct exposure of compounds to preeclamptic patients has never been performed due to its clinical test which has never been done. Thus, this study aims to know effects of ant nests extract (ANE) on endothelial damage caused by angiogenesis alteration and oxidative stress in HUVEC cell line with preeclampsia by measuring level of sFlt-1, PIGF, MDA, and NO in culture.

Materials and Method

Preparation of water fraction of ant nests

Ant nests were obtained from Ayawasi village, South Sorong, West Papua. Ant nests were identified in Herbarium, Department of Biology, Faculty of Mathematics and Science, Padjadjaran University, Bandung. Tuber of ant nests was removed and cleaned, stripped into pieces and dried in oven 60°C for 5 hours. 10 g dried ant nests were boiled with 200 ml aquadest for 20 minutes. Boiled water was filtered and left until warm. Boiled water was sterilized with filtration using *syringe filter millipore* 0,22 μm into sterilized bottle.[52]

Cell culture

HUVEC ATCC CRL 1730 cell line was human endothelial cells-derived in *cryopresipitat* form, progenitor free, isolated from amnion blood vessels. Cell culture was done in Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University. Cell culture was divided into 2 steps; thawing and passage.

Resusitation was initiated by growing HUVEC cell line into *tissue culture flask* containing RPMI 1640 medium, incubated to reach *confluent* 90%. Passage was initiated after proliferation and cell invasion 6 x 10^5 cell per cm^2.[57,58]

Measurement of IC_{50} Water fraction of ant nests in HUVEC cell line

Measurement was performed in Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University. Samples were dissolved with aquadest to obtain concentration 100 µg/ml, 1000 µg/ml and 5000 µg/ml.[51] 600 µl aquadest were added into cuvette and added with 3 ml sample. Solution was measured at λ=400-600 nm and absorbance was recorded at λ=517 nm. Samples with variety of serial concentration starting from 100µg/m (multiplied from 0,195 µg/ml to 100 µg/ml); 1000µg/ml (multiplied from 1,953 µg/ml to 1000 µg/ml); and 5000µg/ml (multiplied from 9,766 µg/ml to 2500 µg/ml) in HUVEC ATCC CRL 1730 cell line to measure its toxicity activity. Absorbance was measured with spectrophotometer at λ=517 nm and measured at 24 hour, to measure IC_{50} value from each sample. 600 µl aquadest was used as controls, added into cuvette and added with 3 ml DPPH, whereas negative control 600 µl aquadest were added into cuvette and added with 3 ml HUVEC ATCC CRL 1730 cell without ant nest water fraction. Solution was quantified using spectrophotometer at λ=400-600 nm and absorbance was recorded at λ=517 nm. Control negative value was used as standard in measurement of IC_{50}.[34]

Measurement of level of sFlt-1, PlGF, MDA, and NO

Preeclampsia serum was obtained from Cibabat Hospital, Bandung. Measurement was performed in Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University. Measurement of sFlt-1, PlGF, MDA and NO levels in both normal and preeclampsia-induced HUVEC ATCC CRL 1730 cell line after exposure of variety of ant nests concentration was performed. Measurement of level of sFlt-1 and PlGF was performed with ELISA. Measurement of MDA level was performed with *thiobarbituric acid-reactive substances* (TBARS), and level of NO was performed with NWLSS™ *Nitric Oxide Assay*. Data was analyzed statistically with ANOVA and Duncan test.

Compliance with Ethical Standards

Written informed consent was obtained from all participants. The ethical reviews boards of the Health Research Ethics Committee, Faculty of Medicine Padjadjaran University and Dr. Hasan Sadikin Hospital, Indonesia, approved this study.

Results

Antioxidant compounds in ant nests water fraction

Phytochemical analysis was conducted to confirm antioxindant compounds contained in water fraction of ant nests. Antioxidant compounds measured were tannin, flavanoid, alkaloid and terpenoid (Table 1 and Figure 1). Tannin, flavanoid and alkaloid compounds were present in ANE. Qualitative measurement showed color alteration to red-violet in ant nests water fraction that indicate presence of terpenoid.

Table 1 Measurement of Tannin, Flavanoid and Alkaloid contained in Ant nests

Sample	Mass (g)	Volume (ml)	Concentration (ppm)	In sample (ppm)	Equivalent total (%b/b)	Average
Tannin	0,0509	10	5090	4,481	0,088	0,089
	0,0509	10	5190	4,609	0,089	
Flavanoid	0,0515	10	5150	42,901	0,833	0,848
	0,0510	10	5100	44,030	0,863	
Alkaloid	0,1011	10	10110	73,094	0,723	0,717
	0,1091	10	10910	77,542	0,711	

Inhibition percentage represented by absorbance value was showed by *microplate reader* BIO RAD 680 XR, used as standard to measure IC_{50} of ANE in HUVEC ATCC CRL 1730 cell line. In this study, ant nests water fraction samples were divided into three serial dilutions, 100 µg/ml, 1000 µg/ml and 5000 µg/ml, for each group that consists of ten-fold multiplies. Measurement of ANE was performed with three replications. IC_{50} obtained for ant nests on 500 µg/ml was 52,57% of death percentage and on 250 µg/ml was ≤ 20,44% of death percentage (Figure 2). Determination of ANE activity was conducted by 50% of death percentage (IC_{50}) in HUVEC ATCC CRL 1730 to be less than 500 µg/ml indicating non-toxicity activity of cells (250µg/ml).

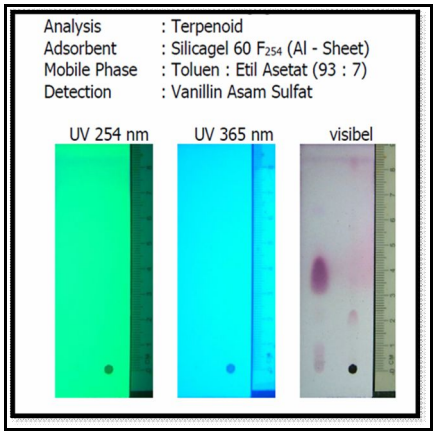

Figure 1 Qualitative measurement of terpenoid in ant nests. Terpenoid spot color: Red-Violet. Rf. Detected Terpenoid: 0,26 ; 0,97. Water fraction of ant nests was examined with phytochemical test to know antioxidant compounds contained. Antioxidant compounds measured were tannin, flavanoid, alkaloid and terpenoid.

Measurement of ANE effects in level of sFlt-1, PlGF, MDA, and NO was performed using ELISA for sFlt-1 and PlGF and *thiobarbituric acid-reactive substances* (TBARS) for MDA, and NWLSS™ *Nitric Oxide Assay* for NO.

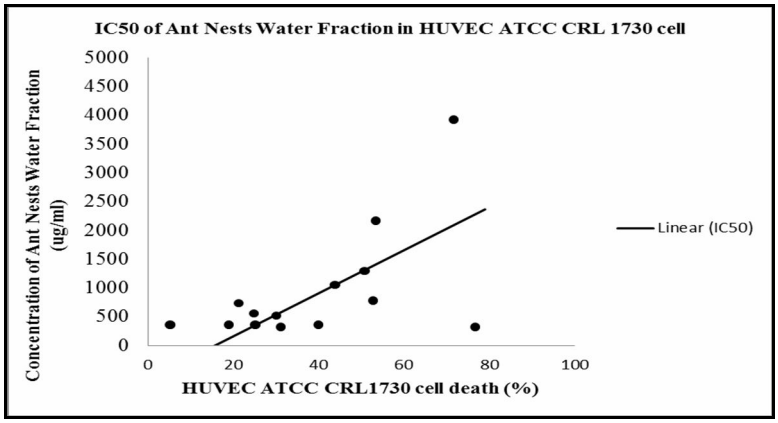

Figure 2 Measurement of IC_{50} in Ant nests water fraction. Measurement of ant nests water fraction was performed with three replications. IC_{50} obtained for ant nests on 500 µg/ml was 52,57% of death percentage and on 250 µg/ml was ≤ 20,44% of death percentage. Determination of ant nests water fraction activity was conducted by death percentage 50% (IC_{50}) in HUVEC ATCC CRL 1730 cell line, which is less than 500 µg/ml indicating non-toxicity activity of cells (250µg/ml).

As shown in Table 2 and 3, there is difference between level of sFlt-1 based on serum used. In preeclampsia serum, sFlt-1 level was higher than that in normal. However, sFlt-1 level in preeclampsia-induced HUVEC significantly decreased following increased ant nests concentration ($p<0,005$). Level of sFlt-1 was significantly lower in range 29,010-31,095 pg/ml in preeclampsia-induced HUVEC ATCC CRL 1730 after exposure of ANE on concentration 62,5 µg/ml that appeared to reach its level in normal-induced HUVEC ATCC CRL 1730.

As shown in Table 4 and 5, level of PlGF were significantly different in each serum used ($p<0,005$). In preeclampsia serum, level of PlGF was lower than that in normal. Level of PlGF was significantly increased following increased ANE concentration approaching normal. Level of PlGF were high in range 5,005-5,647 pg/ml preeclampsia-induced HUVEC ATCC CRL 1730 after exposure of ANE in concentration 125 μg/ml approaching condition in normal serum-induced HUVEC ATCC CRL 1730.

Table 2 Effects of variety of ant nests water fraction concentration and type of serum used in sFlt-1 level

Concentration (μg/ml)	sFlt-1 serum(pg/ml)	
	Normal	Preeclampsia
Control 1	8,813 (0,565)*	33,32 (0,764)
Control 2	9,926 (0,565)	32,845 (1,364)
31,25	38,284 (1,697)	30,202 (0,908)
62,5	34,057 (1,353)	30,053 (1,474)
125	30,3185 (1,767)	27,219 (0,608)
250	26,034 (1,428)	24,208 (2,175)

Note: *) average and deviation standard
Control 1: serum
Control 2: serum+HUVEC

Table 3 Effects of Variety of Concentration of Ant nests water fraction on sFlt-1 level in normal and preeclampsia serum

Control Variable 2n	Preeclampsia serum			
	Ant nests concentration (μg/ml)			
	31,25	62,5	125	250
sFlt-1 (pg/ml)				
X (SD) 9,92(0,56)	30,202(0,908)	30,053(1,474)	27,219(0,608)	24,208(2,175)
Range (9,53-10,33)	(29,560-30,844)	(29,010-31,095)	(26,789-27,649)	(22,670-25,746)
A	B	B	BC	C

Note: Control 2n: normal serum+ HUVEC

Average followed by different alphabet showed significant difference ($\rho<0,05$), according to Duncan test.(A, B, C) indicates different effects of concentration on level of sFlt-1

MDA level significantly decreased in range -2,230- (-1,870)μM in preeclampsia-induced HUVEC ATCC CRL 1730 after treatment of ANE on concentration 125 μg/ml that almost reach its level in normal-induced HUVEC ATCC CRL 1730. MDA decreased following increased ant nests water fraction on concentration 125 μg/ml that close to its level in normal-induced HUVEC ATCC CRL 1730 (Table 6 and 7) .

Table 4 Effects of variety of ant nests water fraction concentration and type of serum used level of PlGF

Concentration (μg/ml)	Serum PlGF (pg/ml)	
	Normal	Preeclampsia
Control 1	6,235 (0,671)*	1,725 (0,077)
Control 2	6,357 (0,744)	1,750 (0,283)
31,25	4,191 (0,742)	3,242 (0,629)
62,5	7,674 (0,495)	5,326 (0,454)
125	8,261 (0,622)	8,345 (0,024)
250	9,822 (0,282)	7,373 (0,733)

Note: *) average and deviation standard
Control 1: serum
Control 2: serum+HUVEC

Table 5 Effects of variety of ant nests water fraction concentration and type of serum used level of PlGF

Control Variable2n	Preeclampsia serum			
	Ant nests concentration (µg/ml)			
	31,25	62,5	125	250
PlGF (pg/ml)				
X (SD) 6,35(0,744)	3,242(0,629)	5,326(0,454)	8,345(0,025)	7,372(0,733)
Range (5,830-6,883)	(2,797-3,687)	(5,005-5,647)	(8,328-8,363)	(6,854-7,891)
AB	A	B	C	C

Note: Control 2n: normal serum+ HUVEC

Average followed by different alphabet showed significant difference (ρ<0,05), according to Duncan test.(A, B, C) indicates different effects of concentration on level of PlGF

NO level significantly increased in range 0,231-0,267 ppm in preeclampsia-induced HUVEC ATCC CRL 1730 after exposure of ANE on concentration 125 µg/ml approaching condition in normal-induced HUVEC ATCC CRL 1730 (Table 8 and 9). Level of NO was increased following increased concentration approaching normal condition.

Table 6 Effects of variety of ant nests water fraction concentration and type of serum used in level of malondialdehyde (MDA)

Concentration (µg/ml)	MDA serum (µM)	
	Normal	Preeclampsia
Control 1	2,028 (0,035)*	12,549 (1,359)
Control 2	3,333 (0,396)	13,586 (1,293)
31,25	15,560 (4,313)	8,320 (0,325)
62,5	6,920 (1,131)	5,570 (0,764)
125	2,290 (0,396)	-2,050 (0,255)
250	-6,860 (1,131)	-7,66 (1,345)

Note: *) average and deviation standard
Control 1: serum
Control 2: serum+HUVEC

Table 7Effects of variety of ant nests water fraction concentration and type of serum used in level of malondialdehyde (MDA)

Control Variable 2n	Serum Preeklamsi			
	Ant nests concentration (µg/ml)			
	31,25	62,5	125	250
MDA (µM)				
X (SD) 3,333(0,396)	8,320(0,325)	5,570(0,764)	-2,050(0,255)	-7,66(1,343)
Range (3,053-3,613)	(8,090-8,550)	(5,030-6,110)	(-2,230- -1,870)	(-8,610- - 6,710)
A	B	C	D	E

Note: Control 2n: normal serum+ HUVEC

Average followed by different alphabet showed significant difference (ρ<0,05), according to Duncan test.(A, B, C) indicates different effects of concentration on level of MDA

Tabel 8 Effects of variety of ant nests water fraction concentration and type of serum used in level of NO

Concentration (μg/ml)	NO Serum (ppm)	
	Normal	Preeclampsia
Control 1	0,385 (0,100)*	0,0635 (0,0615)
COntrol 2	0,408 (0,125)	0,117 (0,024)
31,25	0,1845 (0,065)	0,150 (0,052)
62,5	0,264 (0,034)	0,203 (0,015)
125	0,273 (0,051)	0,249 (0,025)
250	0,314 (0,066)	0,269 (0,045)

Note: *) average and deviation standard
Control 1: serum
Control 2: serum+HUVEC

Table 9 Effects of variety of ant nests water fraction concentration and type of serum used in level of NO

Control Variable 2n	Preeclampsia serum			
	Ant Nests concentration (μg/ml)			
	31,25	62,5	125	250
NO (ppm)				
X (SD) 0,408(0,125)	0,150 (0,052)	0,203 (0,015)	0,249 (0,025)	0,269 (0,045)
Range (0,319-0,496)	(0,113-0,187)	(0,192-0,214)	(0,231-0,267)	(0,237-0,301)
A	B	B	AB	AB

Note: Control 2n: normal serum+ HUVEC

Average followed by different alphabet showed significant difference ($\rho<0,05$), according to Duncan test.(A, B, C) indicates different effects of concentration on level of NO

Discussion

In this study, ANE used is known to contain compounds such as tannin, alkaloid, flavanoid and terpenoid. There is difference of active compounds obtained which might be caused by different isolation method, technique, and phytochemical test sensitivity. There are no previous studies regarding secondary metabolite of ANE. Lack of information causes antioxidant and antiangiogenic mechanism on preeclampsia remains unclear.

Alteration of angiogenesis on blood vessels and extravillous trophoblast invasion occurred in preeclampsia causes absence of decreasing of placental vascular resistance. It negatively affects placental oxygen supply due to disminished blood vessels and ischemic, that promotes trophoblast villous damage.[40] Ischemic causes time-dependent imbalance of prooxidant and antioxidant, in which higher free radicals are present in longer time. Free radicals produced will reach one point that exceeds its capacity, known as oxidative stress.[41] Non-neutralizing free radicals in preeclamptic patients, generate cell membrane damage, disturbance in cell integrity, endothelial cells lysis, reactivity and increased vascular permeability.[42]

Alteration of angiogenesis balance in preeclampsia is observed through overproduction of sFlt-1.[7,8,44] sFlt-1 is specific protein resulted from response to hypoxia. [43] Placental hypoxia releases free radicals into circulation and can be detected by alteration of NO and MDA level. When hypoxia occurs, placenta releases sFlt-1 expressed before clinical symptoms, hypertension and proteinuria.[7,8,44] Increased sFlt-1 was followed by alteration of pro-oxidant and antioxidant balance indicated by increased MDA and decreased NO. It worsens hypoxia in preeclampsia that later causes severe hypoxia.[9,10]

In preeclamptic patients, increased sFlt-1 causes alteration of other angiogenics, PlGF.[7,44] PlGF in preeclampsia onset is known decreased as result of increased sFlt-1.[45] sFlt-1 causes alteration of endothelial blood vessels by taking place for PlGF receptor leading to decreased NO phosphorylation synthesis in endothelial cells. It changes bioavaibility of NO produced by endothel and leading to alteration of cardiovascular homeostasis which is clinically found in preeclamptic patients. [12-14] PlGF has physiology pattern in pregnancy which is consistently decreased in normal pregnancy in first and second trimester, peaking at 29 to 32 weeks and its level is found consistently low following gestational age. [11-13,43,45,46] It is thought as a result of increased sFlt-1 level starting from gestational age at 33rd week to last weeks of pregnancy. [43,45]

Upregulation of sFlt-1 generate GCM1 degradation that results in decreased PlGF and *metal-responsive transcription factor* (MTF-1) synthesis.[47,48] PlGF is a protein produced by trophoblast, endothelial cells, monosite, and erythroid cells. The presence of sFlt-1 and PlGF was frequently investigated to diagnose preeclampsia in laboratory scale before clinical symptoms.[17,18] Both proteins are expressed by placenta as a response of hypoxia. [9,10]

Hypoxia induces ROS release such as superoxidation (O_2), hydroxyl radical (OH), and hydrogen peroxide. (H_2O_2). Hypoxia generates overproduction of O_2, OH and H_2O_2 reaching its antioxidant capacity. Alteration of prooxidant and antioxidant balance is found in preeclamptic patients which is caused by increased level of MDA. Increased MDA causes its biological function disrupted, endothelial dysfunction, vasoconstriction, frozen blood process and lipid peroxidation process disrupted, biomolecule oxidative damage and DNA damage. Increased MDA level also worsens oxidative stress caused by low level of cellular NO.[16-18,49-51]

The result of present study showed ANE has antioxidant property as in line with previous study done by Dharsono and Soeksmanto. [34, 52] Both only used *n*-hexan and etyl acetate fraction instead of water. Water has higher polarity compared to *n*-hexan and etyl acetate as solvents. However, studies using ant nests has similarity in its antioxidant activity. Ant nests is proved for its antioxidant activity and other active compounds contained such as alkaloid, flavanoid, tanin and terpenoid. Further studies regarding difference of solvents used are encouraged.

In this study, ant nests were fully boiled in water and it is thought to dissolve antioxidant compounds of ant nests. Ant nests use is considered to dissolve more unidentified water-soluble compounds and they are thought to have better antioxidant activity. Identification of active compounds is crucial in drugs discovery and development. Moreover, interaction between dissolved compounds resulting stronger antioxidant effects might be present, although advanced studies are needed.

Concentration of ant nests water fraction used in this study starting from below 500 µg/ml which starts from 250 µg/ml and its multiple to lower concentration reaching 31,25 µg/ml. Study was continued to measure level of sFlt-1, PlGF, MDA and NO HUVEC ATCC CRL 1730 induced by normal and preeclampsia serum and treated with variety of air ant nests fraction concentration after 24 hours incubation.

Results showed level of sFlt-1 and MDA was decreased in preeclampsia-induced HUVEC ATCC CRL 1730 and its level approached sFlt-1 and MDA level in normal serum-induced HUVEC ATCC CRL 1730 without ant nests water fraction. Moreover, increased level of PlGF and NO in preeclampsia serum-induced HUVEC ATCC CRL 1730 was obtained and its level approached sFlt-1 and MDA level in normal serum-induced HUVEC ATCC CRL 1730 without ant nests water fraction exposure. Data showed significant result statistically for each parameter (sFlt-1, PlGF, MDA dan NO).

Results of ant nests water fraction in normal serum-induced HUVEC ATCC CRL 1730 was weaker than that in preeclampsia-induced HUVEC ATCC CRL 1730. As shown in Table 4.2-4.9, nests water fraction exposure with variety of concentration in normal-induced HUVEC ATCC CRL 1730 was thought increasing level of sFlt-1 and MDA and also decreasing PlGF and NO compared to controls and preeclampsia-induced HUVEC ATCC CRL 1730. It concluded that ant nests water fraction playing different role in normal cells. Ant nests water fraction is considered to have toxic activity in normal cells affecting pathophysiology of normal cells. Although, advanced studies are recommended.

Despite inadequate information in this study, results showed ANE has both antioxidant and antiangiogenic activity in normal and preeclampsia-induced HUVEC ATCC CRL 1730 cell line. It was

described that higher concentration of ant nests water fraction followed by more reduced level of sFlt-1 and MDA, and increased level of PlGF and NO as well. This is in accordance with previous studies by Hamsar dan Mizaton[49] and Dharsono et al.[51] that suggests that ant nests is widely consumed and believed to prevent diseases in presence of high antioxidant.[31-34] It is confirmed that higher concentration of ANE resulted lower occurrence of oxidative stress and hypoxia (Table 4.3-4.6).

The result of present study suggests that ANE contains high antioxidants and antiangiogenic, one of which is flavanoid. Antioxidant in ant nests water fraction plays role as precursor to trap reactive oxygen compounds. It therefore reduces free radicals and maintains cell function including preeclampsia-induced HUVEC ATCC CRL 1730 cell line. Balance of cell function indicated by decreased occurrence of hypoxia directly affecting decreased level of sFlt-1 and MDA, followed by increased level of PlGF and NO. Thus, ant nests water fraction might be used as agent to overcome endothelial dysfunction in preeclampsia. Advanced studies regarding active compounds and optimum concentration of ant nests in embryo are encouraged and it is expected to be tested to preeclamptic patients. Studies measuring ratio average of sFlt-1/PlGF and MDA/NO as a standard in in vivo studies both in experimental animal and humans are also needed.

Conclusion

Level of sFlt-1 and MDA in preeclampsia-induced HUVEC ATCC CRL 1730 cell line was decreased whereas level of PlGF and NO was increased, both after exposure of ant nest water fraction on concentration 31,25 ug/ml.

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References

1. Effendi JS, Permadi W, Hidayat D, Tjahyadi D, Mulyakusumah A, Hermawan M, dkk. *Annual Report* 2010. Bandung: Department of Obstetrics and Gynecology, Faculty of Medicine, Padjadjaran University, dr.Hasan Sadikin Hospital 2011.
2. Effendi JS, Permadi W, Pramatirta AY, Amarullah M, Pribadi A, Johansyah AO, dkk. *Annual Report* 2009. Bandung: Department of Obstetrics and Gynecology, Faculty of Medicine, Padjadjaran University, dr.Hasan Sadikin Hospital 2010
3. Nataprawira DS, Permadi W, Husnisyam H, Hendra R, Barimbing JN, Hermawan M, dkk. *Annual Report* 2008. Bandung: Department of Obstetrics and Gynecology, Faculty of Medicine, Padjadjaran University, dr.Hasan Sadikin Hospital 2009
4. Stepan H, Faber R, Dornhofer N, Huppertz B, Robitzki A, Walther T, New insight onto biology of preeclampsia. *Biol Reprod*. 2006, 74(5), 772-6
5. Noris M, Perico N, Remuzzi G. Mechanisms of disease: preeclampsia. *Nat clin Prac Nephro*., 2005, 5(2), 98-114
6. Cunningham FG, Norman FG, Kenneth JL, Larry CG, John CH, Katharine DW. Williams *Obstetrics*. 22nd editon. New York: Williams and Wilkins; 2005
7. Osol G, Mandala M. Maternal Uterine Vascular Remodeling During Pregnancy. *Physiology*., 2009, 24:58-71
8. Yuan HT, Haig D, Krumanchi SA. Angiogenic factors in the pathogenesis of preeclampsia. *Curr Top Dev Biol*., 2005, 71:297-312
9. Purwosunu Y, Sekizawa A, Farina A, Wibowo N, Koide K, Okazaki S, et al. Evaluation of physiological alterations of placenta through analysis of cell free messenger ribonucleic acid concentrations of angiogenic factor. *Am J Obstet Gynecol*., 2008, 198(1), 124 el-7
10. Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. *Microvasc Res*., 2008, 75(1):1-8
11. Cunningham FG, Norman FG, Kenneth JL, Larry CG, John CH, Katharine DW. Williams *Obstetrics*. 22nd ed. New York: Williams and Wilkins. 2005
12. Robert J, Pregnancy-Related Hypertension, in *Maternal Fetal Medicine Principle and Practice, Creasy R and Iams J*, Editors. 2004, WB Saunders: Philadelphia. p. 859-880

13. VanWijk MJ, Vascular function in preeclampsi. *J Cariovasc Res*, 2000, 47: p. 38-48
14. Shibuya M. Structure and function of VEGF/VEGF receptor system involved in angiogenesis. *Cell Struct Funct*., 2001, 26(1), 25-35
15. Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, et al. Role of PIGF in the intra-and intermolecular cross talk between the VEGF receptors Flt 1 and Flk 1. *Nat amed*., 2003, 9(7), 936-43
16. Geva E, Ginzinger DG, Zalaudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental vascular development: vasculogenic and angigenic (branching and nonbranching) transformation in regulated by vascular endothelial growth factor-A, angopoietin-1 and angiopoetin-2. *J Clin Endocrinol Metab*., 2002, 87(9), 4213-24
17. Purro SA. Hydrogen peroxide (H2O2) level were assayed in the culture media. *Journal of Neurochemistry*., 103(1), 141-4
18. Sayyed A, Sontakke A. Study of lipid peroxidation and antioxidant status in preeclampsia. *JKIMSU*., 2013, 2(2), 69-76
19. Huppertz B, Kadyrov M, John CPK. Apoptosis and its role in the trophoblast. *American Journal of Obstetrics and Gynecology.,* 2006, 195, 29-39
20. Trotter EW, Grant CM. Non-reciprocal regulation of the redox state of the glutathione-glutaredoxin and thioredoxin systems. *EMBO reports*., 2003, 4(2), 184-188
21. Chari S, Gupta M, Ghike S. Correlation of homocysteine and oxidative stress in patients with preeclampsia. *J recent advances in app sci (JRAAS).,* 2011, 26, 1-5
22. Vijayalakshmi, Ambareesha K, Kayalvizhi E, Qairunnisa S, Revathi M, Chandrasekhar M. Effect of antioxidants in preeclampsia women at increased risk. *Int J Med Res Health Sci*., 2013, 2(2), 177-181
23. Leclercq C, Arcella D, Turrini A. Estimates of the theoretical maximum daily intake of erythorbic acid, gallates, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in Italy: a stepwise approach. *Food and Chemical Toxicology*. 2000:38;1075-84
24. Halliwell B. Reactive oxygen species in living system: source, biochemistry and role in human diseases. *Am J Med*., 1991, Suppl.3C, paper 3C-14S
25. Mao D, Che J, Keshen L, Shiyu H, Qi Y, Li Z, Wei Z, Lin L. Association of homocystein, asymmetric dimethylarginine, and nitric oxide with preeclampsia. *Arch Gynecol Obstet.,* 2010, 281, 371-375
26. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. *JAPI*., 2004, 52, 794-804
27. Khatri M. Circulating biomarkers of oxidative stress in normal pregnancy and preeclampsia and efficacy of antioxidant supplementation. *International Journal of Reproduction, Contraception, Obstetrics, and Gynecology.,* 2013, 2(3), 304-310
28. Rukmini MS, Kowsalya R, Pai B, Das P, Perriera J, Nandini M, Asha K. Plasma adenosine deaminase activity and antioxidant status in preeclampsia compared to healthy pregnant and nonpregnant women. *Biomed Res*., 2009, 20(1), 15-20
29. Fiore G, Florio P, Micheli L, Nencini C, Rossi M, Daniela C,et al. Endotelin-1 triggers placental oxidative stress pathways: putative role in preeclampsia. *The Journal of Clinical Endocrinology and Metabolism*., 2005, 90(7), 4205-4210
30. World Health Organization (WHO). *Traditional medicine*. http://www.who.int/topics/ traditional _medicine/en. 2013
31. Hamsar MN, Mizaton HH. Potential of ant-nest plants as an alternative cancer treatment. *J of Pharm Res*., 2012, 5(6), 3063-6
32. Hertiani T, Sasmito E, Sumardi, Ulfah M. Preliminary study on immunomodulatory effect of sarang semut tubers *Myrmecodia tuberosa* and *Myrmecodia pendens*. *Online Journal of Biological Sciences*., 2010, 10(3), 136-41
33. Halliwel B, Gutteridge J. Antioxidant defences: endogenous and diet derived. In: *Free radicals in biology and medicine*. Oxford. Oxford University Press Ed. 4. 2007, 79-80
34. Dharsono HDA, Satari MH, Setiawan N, Dikdik K. Antibacterial Potential of Ant Nests (*Myrmecodia pendens Merr & Perry*) on Lipoteichoic Acid (LTA) of *Enterococcus faecalis* ATCC 29212. [Dissertation]. Padjadjaran University. 2013
35. Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, Leeuwen PAM. Flavanoids: a review of probable mechanism of action and potential applications. *Am J Clin Nutr*., 2001, 74, 418-25
36. Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannin acid. *Arab J Chem*, 2010, 3(1), 43-63

37. Soeksmanto A, Subroto MA, Wijaya H. Simanjuntak. Anticancer activity test for extracts of sarang semut plant (*Myrmecodia pendens*) to HeLa and MCM-B2 Cells. *Pakistan Journal of Biological Sciences*., 2010, 13(3), 148-151
38. Johnkennedy N, Augustin I, Uduji HI. Alterations in antioxidants enzymes and Malondialdehyde status in preeclampsia. *Asian pasific journal of tropical biomedicine*., 2012, 1691(2), S750-3
39. SKDI. *Maternal Mortality Rate*. In: RI D, editor. Jakarta2007. p. 1-6
40. DeGroot CJM. The role of endothelial cells in preeclampsia. Ed. Den Haag: *Krips repro;* 1995
41. Hung HT, Skepper NJ, Graham J, Burton JG. In vitro ischemia-reperfusion injury in term human placental as a model for oxidative stress in pathological pregnancies. *AMJ Pathology*., 2001, 159, 1031-1043
42. Many A, Hubel AC, Fisher JS, Roberts MJ, Zhou Y. Invasive cytotrophoblasts manifest evidence of oxidative stress in preeclampsia. *AMJ Pathology*., 2000, 158, 321-331
43. Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. *Microvasc Res*. 2008;75(1):1-8
44. Folkman J, Shing Y. Angiogenesis. *J Biol Chem*., 1992, 267, 10931-4
45. Sken, O.; Maquat, L.E.Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA functio, *Genes & Development.,* 2007, 21 (15), 1833
46. Yuditiya Purwosunu, Akihiko Sekizawa, Shiho Okazaki, Antonio Farina, Noroyono Wibowo,el al. Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma. *Am J Obstet Gynecol*., 2009, 200, 386.e1-386.e7
47. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, dkk. Circulating Angiogenic Factors and the Risk of Preeclampsia. *n engl j med*. 2004 february 12, 2004, 350(7)
48. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension.,* 2005, 46, 1243-9
49. Das UN, Elis G, Begin ME, Horrobin DF. Free radical *Bio. J Med.*, 1987, 3:9
50. Candra S, Widodo MA, Soetomo S, I Ketut MG. The level of MDA and the GSH/GSSG ratio in normal pregnancy, heavy preeclampsia and eclampsia at Malang. *Jurnal Kedokteran Brawijaya*, 2007, XXIII(1), 1-5
51. Johnkennedy N, Augustin I, Uduji HI. Alterations in antioxidants enzymes and Malondialdehyde status in preeclampsia. *Asian pasific journal of tropical biomedicine.,* 2012, 1691(2), S750-3
52. Hung HT, Skepper NJ, Graham J, Burton JG. In vitro ischemia-reperfusion injury in term human placental as a model for oxidative stress in pathological pregnancies. *AMJ Pathology*., 2001, 159, 1031-1043
53. Middleton E, Chithan K, Theoharis CT. The effects of plant flavonoids on mammalian cells:implications for inflammation, heart disease, and cancer. department of pharmacology and experimental therapeutics. *Tufts University School of Medicine, Boston, Massachusetts (T.C.T.).,* 2000, 52 (4), 673-751
54. Ferguson-Smith MA. *Placental mRNA in maternal plasma: Prospects for fetal screening.* 2003 [1 Oktober 2013]; Available from: http://www.pnas.org/content/100/8/4360.full.pdf
55. Rodger GM. Hemostatic properties of normal and pertubed vascular cells. *FASEB J.,* 1988, 2, 116-23
56. Candra S. The influence of the combination of NAC with vitamin C and E to oxidative stress on HUVECs exposed with eclampsia plasma. *Jurnal kedokteran brawijaya*, 2007, XXIII(3), 144-51
57. Amaral LM, Pinheiro LC, Guimaraes DA, Ana CTP, Jonas TS, Rafael LP, Jose ETS. Antihypertensive effects of inducible nitric oxide synthase inhibition in experimental preeclampsia. *J Cell Mol. Med*., 2013, XX:1-9
58. Raijmakers MTM and L Poston. The role of oxidative stres in preeclampsia. *Cambridge University.,* 2007, 121-133

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