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Short Communication

Effects of anti-lipid peroxidation of *Punica granatum* fruit extract in endothelial cells induced by plasma of severe pre-eclamptic patients

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ABSTRACT

Preeclampsia is a pregnancy disorder characterized by hypertension and proteinuria. This disorder involves oxidative stress and changes in endothelial homeostasis. This study was aimed to seek whether an ethanolic extract of *Punica granatum* fruit inhibits 8-iso-PGFα formation and modulates nitric oxide (NO) in endothelial cells induced by plasma from pre-eclamptic patients. Endothelial cells were cultured from human umbilical vein endothelial cells. At confluence, endothelial cells were divided into five groups, which included endothelial cells exposed to 2% plasma from normal pregnancy (NP), endothelial cells exposed to 2% plasma from pre-eclamptic patients (PP), endothelial cells exposed to PP in the presence of ethanolic extract of *P. granatum* (PP + PG) at the following three doses: 14; 28; and 56 ppm. Analysis of 8-iso-PGFα was done by immunoassay technique. Analysis of NO level was done by colorimetric technique. Plasma from PP significantly increased 8-iso-PGFα level compared to cells treated by normal pregnancy plasma. This increase in 8-iso-PGFα was significantly ($p < 0.05$) attenuated by all doses treatments of *P. granatum* extract. The level of NO was insignificant ($p > 0.05$) between groups. *P. granatum* fruit extract protects endothelial cells from oxidative stress induced by plasma from pre-eclamptic patients.

1. Introduction

Pre-eclampsia is a multifactorial disorder which is underlain by a composite of genetic and environmental factors [1]. This disease causes hypertension and proteinuria symptoms which occur after 20 weeks of pregnancy. The pathogenesis of this syndrome is still not fully understood. It is constituted by placental dysregulation triggered by a mismatch of trophoblast invasion and endothelial cell dysfunction [2–4]. One of the important events in the development of pre-eclampsia is the formation of free radicals and oxidative stress [5,6].

Lipid peroxidation is a reaction between reactive oxygen compound and lipid. In particular, the peroxidation reaction between prostaglandin-like compounds and free radicals can form F2-isoprostane [7–9]. In case of pre-eclampsia, F2-isoprostane is found to increase in the placenta and urine [10,11]. This compound is not only a marker of peroxidation, but also a mediator of oxidation injury. One of the effects of the reactivity of F2-isoprostane is triggering vasoconstriction of blood vessels, allegedly through reaction with nitric oxide [12]. NO is a potent vasodilator which has been proven in both *in vivo* and *in vitro* studies; inhibition of NO production triggers pre-eclampsia-like syndrome [13,14], although studies show inconsistent results [15–17].

In order to reduce morbidity and mortality from pre-eclampsia few herbs have been studied, [18–21]. *Punica granatum* is a fruit native to Indonesia with high polyphenol content [22]. In Indonesia and India, this plant is used for it's astringent, anti-parasitic, analgesic, and antipyretic properties [23–25]. The high content of polyphenols underlies the antioxidant properties of this plant [26–28]. Up till now, the application of *P. granatum* plant in pre-eclampsia has not been done. Therefore, this study focuses on analyzing the effects of *P. granatum* against lipid peroxidation

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and changes in NO in endothelial cells exposed to plasma of pre-eclamptic patients.

2. Materials and methods

2.1. Isolation and culture of human umbilical vein endothelial cells (HUVECs)

This was an *in vitro* experimental study using Human Umbilical Vein Endothelial Cells (HUVECs). The technique of isolation and culture of HUVECs was performed in pursuance of the detailed method in a previous study [29]. After having reached confluence, HUVECs were divided into five groups, including control group (group OF HUVECs exposed to plasma isolated from normal pregnancy), group of HUVECs exposed to 2% plasma from pre-eclamptic patients, and group of HUVECs exposed to 2% plasma from pre-eclamptic patients along with the administration of ethanol extracts of *P. granatum* fruit in doses of 14, 28, and 56 ppm.

2.2. Isolation of plasma from severe pre-eclamptic patients

Isolation of plasma from severe pre-eclamptic patients was performed in pursuance of the method in a previous study [29]; this procedure received an approval from the Health Research Ethics Committee, Faculty of Medicine, University of Brawijaya, Malang, Indonesia. The criteria for subject were hypertension occurring after 20 weeks' gestation as assessed by blood pressure ≥140/90 mmHg, increase ≥30/15 mmHg, diastolic ≥90 mmHg measured in a sitting position, cuff with the right size. In addition found uria protein ≥2+ with dipstick examination or ≥300 mg/dL in urine 24 hours. The dose of 2% pre-eclampsia plasma was the optimum dose capable of providing a response to a decrease in endothelial relaxation from blood vessels based on a previous study [29].

2.3. Extraction of P. granatum

P. granatum fruit was obtained from Bandungan Village, Semarang, Central Java, Indonesia. Fruit having been washed clean was then peeled and put in the oven (40 °C–60 °C) to be dried (free water content). After pomegranate fruit was dried, the fruit was mashed using a blender. 100 g of it was then put in an Erlenmeyer flask sized ±1 L and soaked in 900 ml of ethanol. This mixture was deposited for one night until being precipitated. The top layer was taken by filtration. Evaporation was carried out and the results obtained were stored in a freezer.

2.4. Analysis of 8-iso-PGFα levels

Levels of 8-iso prostaglandin F2α were analyzed in the medium of HUVECs. Analysis was conducted by Human 8-iso prostaglandin F2α (8-iso-PGFα) (Abcam, catalog number ab 133025). Analysis procedures were performed in accordance with the complete procedures in the kit.

2.5. Analysis of NO levels

Total levels of nitric oxide were analyzed in the medium of HUVECs. The analysis was conducted with a total of nitric oxide and nitrate/nitrite assay (R&D system; catalog KGE001). Analysis procedures were performed in accordance with the complete procedures in the kit.

2.6. Ethics

This study has passed an ethical review and was approved by the Health Research Ethics Committee, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

2.7. Statistical analysis

Levels of 8-iso prostaglandin F2α and NO have been shown in mean ± standard deviation. Differences between treatment groups have been analyzed using one-way ANOVA test with the assistance of SPSS 17.0 statistical package software. If significance in the group was found in the ANOVA test, post hoc test was conducted. Value of $p < 0.05$ was set as a value of statistically significant difference.

3. Results

Table 1 shows the levels of 8-iso-PGFα as a marker of lipid peroxidation in various study groups. The levels of 8-iso-PGFα were significantly higher in the group of HUVEC cells exposed to plasma of pre-eclampsia than the group of HUVECs exposed to normal pregnancy plasma ($p < 0.05$). All three doses of *P. granatum* lower the levels of 8-iso-PGFα significantly compared with the group of HUVECs cell exposed to plasma of pre-eclampsia ($p < 0.05$). Of these three doses, triggering a decline reaching levels of 8-iso-PGFα is in the group of HUVECs exposed to normal pregnancy plasma ($p > 0.05$).

NO levels from various study groups are also shown in Table 1. The NO levels from various study groups showed no significant differences ($p > 0.05$).

4. Discussion

Depending on the levels of free radicals, a variety of redox-sensitive transcription factors will be activated and trigger intracellular biological response [30]. In this study, the levels of 8-iso-PGFα increased significantly in the group of HUVECs exposed to the plasma of pre-eclamptic women compared with the group of HUVECs exposed to normal pregnancy plasma ($p < 0.05$). This indicates that the plasma of patients with pre-eclampsia are pro-oxidative which can trigger lipid peroxidation as one form of oxidative stress in endothelial cells. This is consistent with previous findings that a variety of factors, including free radicals were detected in the maternal circulation of pre-eclamptic patients [29,31–33]. In this study, a variety of active ingredients contained in *P. granatum* extracts could reduce the levels of 8-iso-PGFα significantly, reaching levels comparable to HUVECs exposed to

Table 1
A level of 8-iso-PGFα and NO in endothelial cells induced by plasma pre-eclamptic patients.

Level	NP	PP	PP + *P. granatum*		
			14 ppm	28 ppm	56 ppm
8-iso-PGFα (pg/mL)	63.42 ± 31.43	495.22 ± 110.06[a]	220.40 ± 90.38[ab]	108.28 ± 89.07[b]	200.89 ± 56.00[b]
NO (μmol/L)	40.87 ± 66.17	4.01 ± 0.88	5.04 ± 1.17	5.05 ± 2.3	5.33 ± 0.97

Note: values are presented as mean ± SD; [a]$p < 0.05$; in comparison with the NP group; [b]$p < 0.05$; in comparison with the PP group; NP: plasma from normal pregnancy; PP: plasma from pre-eclamptic patients; NO: nitric oxide; μmol/L: micromol/liter; ppm: parts per million; pg/mL: picogram/milliliter.

plasma from normal pregnancy ($p < 0.05$). This indicates that the antioxidant activity of *P. granatum* reacts with free radicals in the plasma of pre-eclamptic patients thus inhibiting lipid peroxidation. This study is consistent with previous findings that the various contents of *P. granatum* (ellagic acid, gallotannins, and anthocyanins (cyanidin, delphinidin, and pelargonidin glycosides), as well as other flavonoids (quercetin, kaempferol and luteolin glycosides) are antioxidant [26–28,34].

For the NO levels are still contradictory in pre-eclampsia. Low levels of maternal NO were found due to existence of endogenous inhibitor(s) in the production of NO. The other studies found the opposite results [35–37]. In this study, the levels of NO from various study groups showed no significant differences ($p > 0.05$). This indicates that the plasma of normal pregnancy and pre-eclampsia could not modulate the production of NO. Likewise the active ingredients of *P. granatum* could not modulate the production of NO in HUVECs cell exposed to plasma of pre-eclampsia. On the other side, it also becomes a foothold for subsequent studies that the exposure models of plasma of pre-eclamptic patients cannot trigger endothelial dysfunction characterized by decreased levels of NO.

5. Conclusion

It can be concluded that the extract of *P. granatum* was able to inhibit lipid peroxidation in the HUVECs cell model given exposure to plasma of pre-eclamptic patients. Thus, extract of *P. granatum* can be a candidate for herbal antioxidant for pre-eclampsia.

Conflict of interest

None.

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