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Punica granatum fruit extract inhibits the production of pro-inflammatory cytokines and angiogenic factors of HUVEC cells induced by plasma from patients with pre-eclampsia

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SUMMARY

This study aims to find out whether the ethanol extract of *Punica granatum* fruit can inhibit pro-inflammatory cytokines (TNF-α and IL-6) and anti-angiogenic factors (sFlt-1 and sEng) of endothelial cells given plasma stimulus of pre-eclamptic patients. When the cells reached confluence, cells would be grouped into five groups (five replicates per group), i.e. endothelial cells exposed to the plasma of normal pregnancy, endothelial cells exposed to the plasma of patients with severe pre-eclampsia, endothelial cells exposed to the plasma of patients of severe pre-eclampsia and given the extract of *P. granatum* in various doses (14 ppm; 28 ppm, and 56 ppm). Once induced, measurement of pro-inflammatory cytokine levels and anti-angiogenic factors would be carried out by using the technique of enzyme-linked immunosorbent assay (ELISA). Phytochemical analysis showed that the extract contains phenolic, flavonoid and tannin. Plasma from patients with pre-eclampsia increased the levels of TNF-α, IL-6, sFlt-1 and sEng significantly compared with normal pregnancy plasma exposure group. The increase in the levels of TNF-α, sFlt-1 and sEng was significantly inhibited by the administration of extract at a dose of

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56 ppm ($P < 0.05$). For IL-6, the increase was significantly inhibited by the extract at doses of 28 and 56 ppm. It is concluded that *P. granatum* fruit extract provides protection to the endothelial cells through suppressing the production of pro-inflammatory cytokines (TNF-α and IL-6) and anti-angiogenic factors (sFlt-1 and sEng) due to plasma stimulus of patients with severe pre-eclampsia.

1. Introduction

Pre-eclampsia (PE) and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) are two specific diseases for pregnant women which are multifactorial and involve genetic components and environmental influences [1]. Symptoms of pre-eclampsia include hypertension and proteinuria after 20 weeks of pregnancy. Pre-eclampsia occurs in 3–5% of pregnancies, as a cause of maternal and fetal morbidity and mortality [2,3]. The pathogenesis of this syndrome is underlain by the mismatch of trophoblast invasion and endothelial cell dysfunction that triggers placental dysregulation [4].

The imbalance between pro- and anti-inflammatory cytokines is also involved in the development of preeclampsia [5]. Pre-eclampsia is characterized by a shift in Th-1-type maternal immune response in the form of the production of pro-inflammatory cytokines [5–7]. Plasma levels of TNF-α were higher in pre-eclamptic pregnancy with early and late onset than normal pregnancy [8]. In addition, the levels of IL-6 increased in severe preeclampsia, but not increased in mild pre-eclampsia [5]. In addition to inflammatory factors, anti-angiogenic factors of soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin are also released in the placenta of pre-eclampsia and is involved in endothelial dysfunction [9,10].

In tropical countries, many plants which can be used as a medicine. The utilization of herbs for pre-eclampsia is still very limited [11–14]. *P. granatum* is a typical Indonesian plant which has been used as a medicine in many areas and is believed to be safe and non-toxic [15]. Anti-inflammatory effects of this plant have been proved in previous studies [16,17], but its anti-angiogenic effects and application for pre-eclampsia have never been performed. Under these conditions, this study is directed to investigate the effects of *P. granatum* extracts in the modulation of pro-inflammatory cytokines (TNF-α and IL-6) and the anti-angiogenic factors (sFlt-1 and sEng) in the cells of human umbilical vascular endothelial cells induced by plasma from patients with pre-eclampsia.

2. Material and method

2.1. Taking off newborn's umbilical cord

The umbilical cord of newborn baby was taken off by caesarean section. Endothelial cell culture was carried out in a range of 12 h after delivery. Before taking off the umbilical cord, bottle containing cord solution had to be prepared stored in −4 °C. Immediately after birth, the umbilical cord was cut along ±10 cm, and was directly put into the cord solution. The umbilical cord was then transferred to the laboratory in cold and sterile conditions.

2.2. Isolation of plasma of normal pregnancy and severe pre-eclampsia

Normal pregnant women or patients with severe pre-eclampsia whose blood would be collected had given a statement of willingness on the consent form. The blood was collected by aseptic technique