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RESEARCH ARTICLE

The Effect of Extract Ciplukan (*Physalis angulata L.*) to Placental Vessels, Placental Weight and Fetal Birth Weight in Preeclampsia Rat Model

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Abstract

Background: Preeclampsia, which is one of the specific diseases of pregnancy at the age of 20 weeks. Preeclampsia is due to the vascular abnormalities of placenta that can cause chronic hypoxia of the placenta and the fetus's nutritional disorder, causing impaired function of the placenta, and fetal development during pregnancy. *Physalis angulata L.* contains antioxidants, and anti-inflammatory effects in improving placental function and preventing nutritional disorders in the fetus. The study aims to determine the effect of *Physalis angulata L.* against the number of placental blood vessels (capillaries), the weight of placenta and birth weight fetus on the preeclampsia rat model. Methods: The research is an experimental laboratory study conducted in vivo design consisting of five groups; Negative control group, positive control group (preeclampsia rat model), preeclampsia rat treatment group with therapeutic dose 1, dose 2, and dose 3 (500mg/kg/BB; 1500mg/kg/BB; 2500mg/kg/BB). Counting the number of capillaries with the H&E stain method, the placenta weight and the fetus weight is measured immediately using analytical balances. Data analysis using One-way Annova test. Result: The results showed significant difference of capillaries p value < 0.05 (p = 0,000), the placenta weight p value < 0.05 (0.025), and the birth fetal weight p value < 0.05 (0.002). Conclusion: *Physalis angulata L.* can be a potential antioxidant in preeclampsia through increased eNOS and NO level which can improve the function of the placenta in preeclampsia rats so that the number of placenta capillaries increased, while the placenta weight and the fetal weight increased.

Keyword: *Preeclampsia, Placenta Vessels (capillaries), Placenta Weight, Fetal Weight, eNOS, NO*

Introduction

Preeclampsia is characterized by hypertension and new onset proteinuria at the age of pregnancy of more than 20 weeks, and almost every country is the cause of the morbidity and mortality of the high mother and fetus [1]. The incidence of preeclampsia varies from 2% to 10% of pregnancies worldwide. The World Health Organization estimates that the incidence of preeclampsia is seven times higher in developing countries (2.8% of live births) than in developed countries 0.4% [2].

Factors believed to be the pathogenesis of preeclampsia were a disorder of placental abnormalities and endothelial dysfunction [3] in preeclampsia, there was a decrease in placental perfusion utero, hypovolemia, vasospasm, and the damage of the placenta vascular endothelial cells [4]. Ischemia of uteroplacenta causes hypoxia due to reduced blood flow at the placental implant site, and results in the release of free radicals resulting in impaired function and

development of the placenta and fetal nutritional disorders so that frequent fetal growth delays that can end with low birth weight [5-7].Complex mechanisms involving eNOS pathways, oxidative stress, reduced bioavailability Nitric Oxide (NO) can increase the oxidative damage of microvascular and support abnormal placental perfusion, by contributing to reduce placental blood flow and increase the resistance in the circulation of pregnant women that can be the findings of the characteristic in the placenta preeclampsia [8-10].

Herbal plants in the traditional medicine system became one of the therapeutic options of the last few years. The chemical content contained in herbal plants has the function to prevent and treat pregnancy with preeclampsia. One of the medicinal herbs of the plant (*Physalis angulata L.*) contains steroids, tannins, saponins and flavonoids [11, 12].

Based on the background, it is assumed that the preeclampsia of the free radical increase of oxidative stress leads to excessive cell apoptosis and placental endothelial dysfunction. Hypoxia due to damage to the endothelial cells of the placenta may lead to increased capillaries, weight loss of placenta and birth weight of the fetus.

Material and Method

Animal Model

This research is a laboratory research conducted in vivo with the research design of Post Test Only Control Group. The population in this study is a pregnant Wistar strain rat.

There are five replication for each group [13]. The negative control group is a normal pregnant rat; Positive control is preeclampsia pregnant rat (preeclampsia rat model); and the treatment groups 1, 2, and 3 are preeclampsia rats that were given three different doses (500 mg/kgBW; 1500 mg/kgBW; 2500 mg/kgBW) of the extract *Physalis angulata L.*, respectively. In the morning after marriage, each animal is examined to see a vaginal plug. The presence of vaginal plugs is assumed as the first day of pregnancy [14]. This study was conducted in the University of Brawijaya Bioscience Laboratory, Physiology and Biomolecular Laboratory of Biochemistry, Faculty of Medicine at Brawijaya University Malang.

Preeclampsia Induction and the Extract *Physalis angulata L.* Administration

Induction material for preeclampsia uses NOS inhibitors, L-NAME (C7H15N5O4 HCl) from Sigma-Aldrich (Merck KGa, Darmstadt, Germany) [15]. The given L-Name dose is 75 mg/kgBW ranging from 9 days to 18 days in Intraperitoneal [16, 17]. Meanwhile, extract *Physalis angulata L.* administered per-tube oral gavage filler from day 12 to day 18 pregnancy [18].

Clinical Examination

Placenta blood vessels examined using the HE (Hematoxilen Eosin) examination with samples of placenta fixation in a formalin buffer 10%, cut with a thickness of 2-3 mm inserted in the tissue cassette, sectioning with Microtom (in the form of thin ribbon), then the stage of dyeing Haematoxylin. Monitoring of direct blood vessels with a microscope, the number of placenta veins measured with the help of the Image J for Windows software. The weight of the placenta and fetal weight was measured immediately using analytical balance after the rat was sacrificed.

Data Analysis

Data analysis is done using SPSS 25.0. Whereas One-way Anova is used to describe the differences of each group.

Results

Placental Vessels

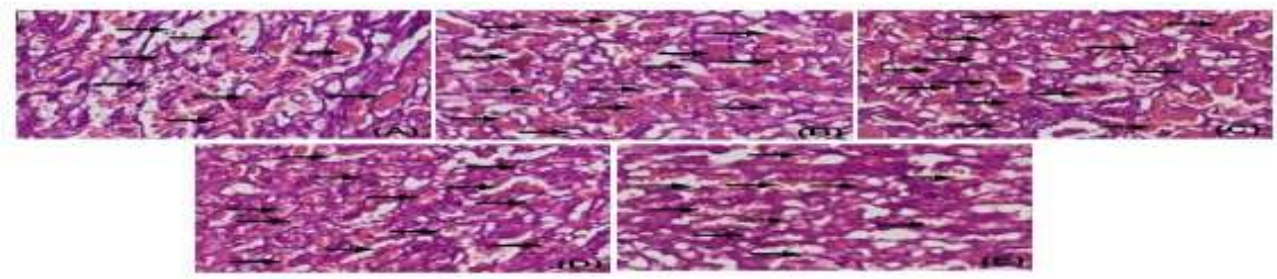

Figure 1: HE placenta rat preeclampsia part of the trophoblast (arrows indicating blood vessels), (a) normal group, (b) preeclampsia group, (c) treatment group dose 1, (d) treatment group dose 2, (e) treatment group dose 3

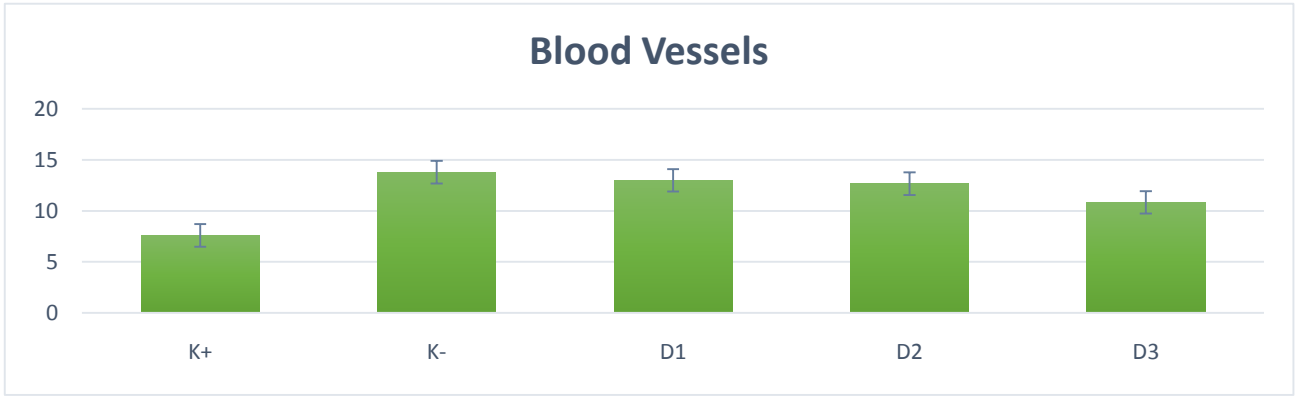

Figure 2: Average value of blood vessels (capillaries) (test One Way Annova performed in each group. The difference is significantly on the number of placenta veins with a result of p value <0.05)

Based on the test results of One Way Annova, there is a significant difference p value < 0.05 (0.000) on the examination of the number of blood vessels. Seen in the above image that the number of placental capillaries increased in rats with induction of preeclampsia, then decreased in number in preeclampsia rats with extract therapy of the trigger leaf ethanol dose 3 (2500 mg/Kg BW/day).

Placental Weight

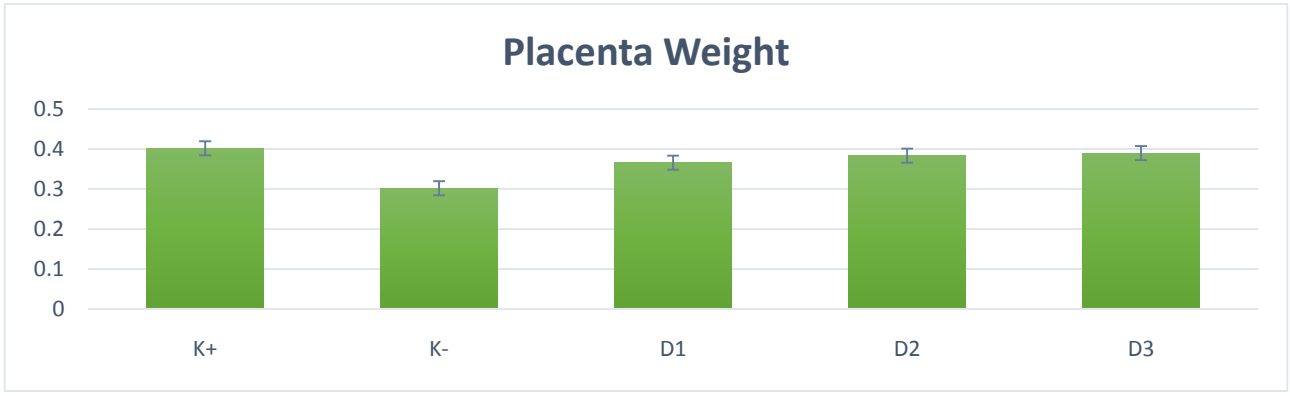

Figure 3: The average weight value of the placenta (test One-way Annova is performed on each group. The difference is significantly on the weight of the placenta with the result p value < 0.05)

Based on the results of the test one-way ANOVA there is a significant difference p value < 0.05 (0.025) on the measurement of placenta weight. Seen in the picture above that the weight of placenta decreased in the preeclampsia pregnant rats compared to normal pregnant rats. Administration of extract therapy in the leaves of ethanol in pregnant rats can increase the weight of good placenta dose 1 (500 mg/Kg BW/day), dose 2 (1500 mg/Kg BW/day), and dose 3 (2500 mg/Kg BW/day). The weight of placenta increased rapidly by using therapeutic dose 3 (2500 mg/Kg BW/day).

Fetal Weight

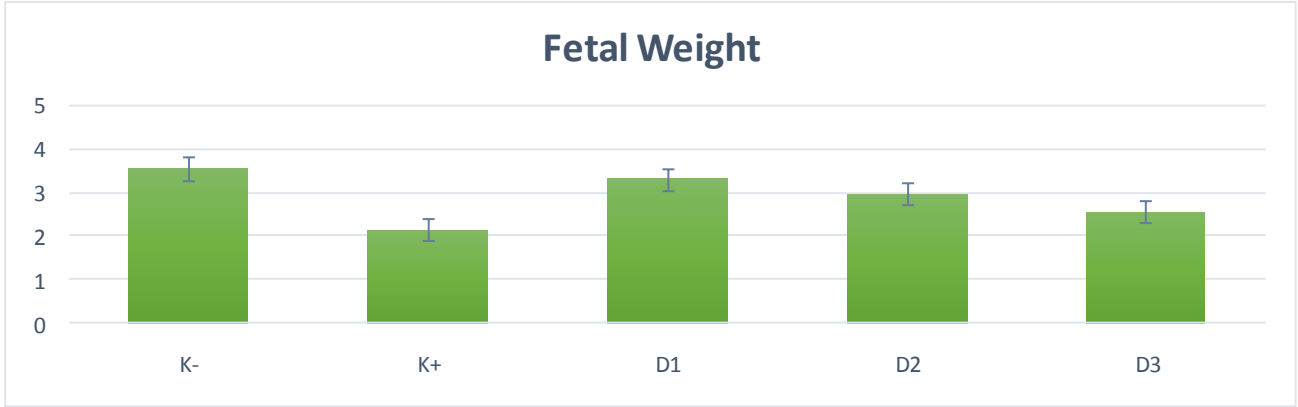

Figure 4: The average weight value of fetus (test One-way Annova is performed on each group. The difference is significantly on weight fetus with a result of p value < 0.05)

Fetal weight measurement on the 19th day. This suggests that there is a significant reduction in fetal weight in preeclampsia when compared to normal mice. Meanwhile, the Anova test also showed significant results (p = 0.002). Statistically, extract *Physalis angulata L.* significantly increases the fetus weight from positive control in dose 1 (500 mg/KgBW/day) and dose 2 (1500 mg/Kg BW/day).

Discussion

Increase Systolic and Diastolic Pressure as a Preeclampsia Marker

Preeclampsia rat model is made with intraperitoneal injection 75 mg/KgBW/day (17). L-NAME injection in pregnant rats inhibits Nitric Oxide (NO) synthase resulting from the alteration of L-Arginine to NO by NOS (Nitric Oxide Synthase). Lack of NO as a vasodilator causes preeclampsia blood vessels in the condition of vasoconstriction.

Vasoconstriction Triggers Increased Systolic and Diastolic Pressures on Preeclampsia

One of the methods for preeclampsia induction that can be used is L-NG-nitroarginine methyl ester (L-NAME), in line with previous research [18] and evidenced by preliminary studies on the pregnant rat model that L-NAME can increase blood pressure with significant results $p < 0.05$ (0.006) and urine proteins with significant yield $p < 0.05$ (0.000) thus explaining that the L-NAME provides a barrier in the production of NO Syntase (NOS) which increases blood pressure, it raises physiological and pathological characters similar or similar to primary hypertensive disease [16, 17]. In preeclampsia, there is a disturbance of uteroplacenta perfusion which causes the placenta to be in a state of lack of oxygen and cause placental ischemia.

In the condition of placental ischemia, blood flow, oxygen exchange and mother-fetus nutrition is reduced thereby affecting the amount of placental blood vessels, the weight of placenta as well as the growth and development of the fetus [1, 19]. The process of adaptation and compensation of decreased placental vascular function by increasing the number of capillaries in the blood vessels but with capillary size, circulation of the bloodstream is insufficient to adequately meet the needs of the fetus [20].

Disorders of Uteroplasenta perfusion in preeclampsia are evidenced by histological and morphological changes of placenta indicating an increase of blood vessels and the weight of the placenta and lower fetus weight compared to normal pregnancy [21, 22]. In our study, placental blood vessels (capillaries), weight of placenta and weight loss fetus had significant results that the components contained in the plant's leaves were captured. In the rat model of hypertension with induction of deoxycorticosterone acetate (DOCA)-salt, the leaf extract of the trigger re-endothelialization and lowers blood pressure [23].

One of the herbal medicinal plants, *Physalis angulata L.*, contains flavonoids. The content of flavonoids in the leaf extract has antioxidant activity [24, 25]. The extract of the leaves can increase the expression of eNOS and the content of NO [26, 27]. In research extract of *Physalis angulata L.* contains phycopy, a compound metabolite of secondary steroid type. This compound may increase the release of Nitric Oxide (NO) cell endothelial in vitro. In the event of increased release NO, is expected to occur genome effect by increasing the expression NO endothelial synthase (eNOS) and NO induced Synthesis [26], so that the administration of leaf extract in preeclampsia has the potential to improve and increase eNOS and NO level and its antioxidant content can inhibit oxidative stress to prevent nutritional disorders in the fetus.

Some studies have overcome the inhibitory effect of Nitric Oxide on fetal development. Most of the research on this subject is from the 1990-00s. In containing rats, this Nitric Oxide synthesis inhibitor caused fetal growth restriction with reduced cell proliferation due to apoptosis induction, reduced body weight and caused hemorrhagic necrosis of the neonatal back limbs [28, 29].

L-arginine, a precursor of nitric oxide, in rats during pregnancy promoted increased fetal weight may be due to the contribution of Nitric Oxide in increasing the fetus-Maternal circulation by vasodilation and then increasing blood volume and viscosity in fetal-Maternal circulation [30].Extract of the leaves in the picture 3 seen at a dose of 500 mg/KgBW/day, dose 1500 mg/KgBW/day, 2500 mg/KgBW/day showed an increase in

body weight fetus compared to the dose group, this is assumed because the flavonoids contained in the plant can act as antioxidant [24]. Flavonoids play a role in enhancing the parent reproductive function where it can prevent the embryo-received nutritional disorder and content, as a strong antioxidant can prevent preeclampsia complications by binding to existing oxidants, thereby reducing free radicals and preventing nutritional disorders in the fetus [31, 32].

And at a dose of 2500mg/KGBW/day although experienced an increase but this is not significant it was assumed that due to weight loss and length of fetus body influenced by the nutrients received embryos, placenta function in delivering nutrients, and genetic embryos. In previous research the effect of teratogenic extract against weight fetus mice. Especially the compound content of tannin [33]. Tannins are suspected to cause obstructed nutrient absorption, tannin able to bind to proteins and increase the excretion of proteins and amino acids causing the embryos to lack the nutrients needed to do cell division period of organ formation. This leads to an increase in the number of hindered cells and the fetus weight becomes lower [34].

We conclude that preeclampsia rat model leads to increased placental capillaries and low weight of placenta as well as birth weight fetus, while the administration of the leaf extract lowers blood pressure, shows the declining influence of placenta blood vessels, and increases the weight of placenta and weight loss fetus.

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