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Original Article

ANDALIMAN FRUIT EXTRACT *(ZANTHOXYLUM ACANTOPHODIUM)* AND IT'S EFFECT ON PREECLAMPSIA AS ANTI-INFLAMMATORY

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ABSTRACT

Objective: This study is the first study to test the effect of andaliman on preeclampsia. This study aims to discover whether andaliman fruit extract *(Zanthoxylum acanthopodium DC)* affects the level of TNF α and IL-6 in preeclamptic LPS Induces mice

Methods: This study uses analytical research with quasi-experimental research design in laboratory rats (*Micetus Norvegicus)* pregnant females given andaliman extract *(zantoxylumacantophodium)* at doses of 100 mg and 200 mg per day. The study design allowed the researchers to measure the effect of treatment (intervention) in the experimental group by way of comparing the experimental group and control group. This design allows the researcher to determine the extent or extent of the change. The treatment of all samples was carried out simultaneously and during the treatment, it was observed using the type of *Postest Only Control Group.*

Results: Andaliman has been shown to reduce TNF-α levels in preeclampsia mice. The mean TNF-α in the K-, P1, P2 and K^+ groups was 84.4; 90.1; 95.1; 109.7 (P<0.001). Andaliman has been shown to reduce IL-6 levels in preeclampsia rats. The mean IL-6 in the K-, P1, P2 and K+groups was 16.7; 67.5; 18.8; 21.1 (P<0.001).

Conclusion: This study proves that there are anti-inflammatory effects possessed by the extract of Andaliman (*Zanthoxylum acanthopodium),* thus showing a decrease in proinflammatory cytokine levels, namely TNF-α, IL6. This study also has a good clinical outcome after administering Andaliman extract (*Zanthoxylum acanthopodium)*, where there are improvements in blood pressure, cystole-diastole, MAP and decreased urinary protein in research subjects with preeclampsia.

Keywords: Andaliman, *(Zanthoxylumacantophodium, TNF-a, IL-6,,* Preeclampsia

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INTRODUCTION

One indicator a country's health status is the maternal mortality rate (MMR). According to the *World Health Organization*, the global maternal mortality rate in the world amounted to 289,000 in 2013. The World Health Organization (WHO) in 2013 estimated that around the world every day around 800 women die from complications of pregnancy, childbirth, and the puerperium [1]. About 99% of maternal deaths occur in developing countries, including in Indonesia. The ratio of maternal deaths in developing countries is around 240 per 100,000 live births and in developed countries around 16 per 100,000 live births [2].

Hypertension in pregnancy is one of the causes of maternal death and ranks third in the cause of maternal death in Indonesia. Hypertension in pregnancy occurs in about 10% of all pregnant women throughout the world. Hypertension in pregnancy accounts for more than 60,000 maternal deaths worldwide each year [3, 4].

Preeclampsia is a special pregnancy syndrome that can affect the entire organ system. The criteria for preeclampsia is hypertension that arises after 20 w' gestation with or without proteinuria. Preeclampsia occurs in about 2-8% of all pregnancies [4]. According to data from the *National Vital Statistics Report,* preeclampsia occurs in 1 per 2000 births. The frequency of preeclampsia in Indonesia is around 3-10% [5]. In Indonesia, severe preeclampsia and eclampsia are ranked third in the causes of maternal mortality in Indonesia, as many as 1.5 percent-25 percent while infant deaths range from 45 percent to 50 percent [6].

Andaliman *(Zantoxylumacantophodium)* is a species of wild plant known in North Sumatra and is widely used in typical Batak dishes. This plant is used to eliminate the smell of fish and raw meat due to its strong odor. This plant has been reported to have anti-inflammatory and antioxidant activity [7]. Several studies have been conducted on the benefits of Andaliman fruit, including preservatives, medicinal ingredients and supplements, and vegetable pesticides. Currently Andaliman extract is widely used as a food supplement because it is believed to increase endurance. The contents in Andaliman such as neolignan, alkaloids, geranyl acetate, amides, and benzoid have high potential for the treatment of chronic inflammatory diseases [8]. In Yanti's study of macrophages induced by lipopolysaccharides, triggering the release of inflammatory cytokines, dose-dependent andaliman administration (2.5 and 10 µg/ml) appeared to inhibit the expression of TNF-α, modulate the expression of IL-6, reduce levels of protein and mRNA of COX-2, and inhibit the expression of protein and mRNA activity of MMP-9 in macrophages compared with a control group that examined gelatin zimografi, w estern blot, and RT-PCR. This causes the induction blockage of inflammatory enzymes so that the oxidative stress process is inhibited [9].

MATERIALS AND METHODS

Rresearch has been conducted checks of blood pressure, MAP, proteinuria, levels of TNF-α, IL-6, with the research design quasi-experimental laboratory rat/mice (*Rattus norvegicus*) pregnant female who administered the extract andaliman *(Zantoxylumacantophodium)* at a dose of 100 mg and 200 mg per day with the *ELISA* method for 4 groups of rats, each group consisting of 6 subjects so that the number of samples needed was 24 animals. The research conducted in July-October 2019. The research sample is part of the study population that meets the inclusion and exclusion criteria by using the Postest Only Control Group Design.

RESULTS

The experimental animals used in this study were 24 white Wistar white mice (*Rattus norvegicus sp)* 10-week-old females who met the inclusion and exclusion criteria. Then female and male *Rattus Norvegicus* mice were put together in one cage and kept for one night at a ratio of 1: 1. The diagnosis of pregnancy is obtained by the presence of vaginal spermatozoa/vaginal plugs and is counted as pregnancy day 0. *Pregnant* female *Rattus Norvegicus* rats are grouped into 4 groups randomly. On the 1st day of pregnancy, all the samples are divided into four groups, namely: ((1) Group 1, negative control (normal), pregnant mice were not given any treatment in general, given excessive eating and drinking (ad libitum) in their cages. (2) Group 2, the treatment group is pregnant mice given LPS injection on day 5 of pregnancy on the basis of day 8 of pregnancy trophoblast invasion was started, in order to become a model of preeclampsia mice but were not given andaliman (Zanthoxylum acanthopodium). (3) Group 3, the treatment group ie pregnant mice given LPS injection on day 5 of pregnancy on the basis of day 8 of pregnancy Trophoblast invasion was started, in order to become a preeclampsia model mice and given andaliman (Zanthoxylum acanthopodium) at a dose of 200 mg once daily. for 15 d. (4) Group 4, the treatment group namely pregnant mice given LPS injection on day 5 of pregnancy on the basis of day 8 of pregnancy trophoblast invasion was started, in order to become a model of preeclampsia mice and given andaliman (Zanthoxylum acanthopodium) at a dose of 800 mg once dailyfor 15 d.

Table 1: Trial group distribution

Group	F	%
Control-(K-)	6	25.0
Control+(K+)	6	25.0
Andaliman 200 (P1)	6	25.0
Andaliman 800 (P2)	6	25.0
Total	24	100.0

In groups 2, 3, and 4 on the 5th day of pregnancy the intravenous LPS injection was given to the tail to become a model of preeclampsia. After that, monitoring of systolic blood pressure in mice in the morning (8:00 to 10:00) was evaluated every three days. Providing andaliman (Zanthoxylum acanthopodium) in groups 3 and 4 was given immediately after an increase in systolic blood pressure in mice. Providing andaliman (Zanthoxylum acanthopodium) orally administered according to the dose of each group every day until the 15th day with the amount of fluid that was given was still within the recommended maximum volume of fluid in mice as much as 5 ml/kg. The procedure of feeding the mice is done first by immobilizing the mice by gently holding the loose skin area between the neck and back of the back so that the rat does not feel threatened. Then the distance between the oral cavity and the xyphoideus process is measured as the length of the gavage to be entered. *Oral gavage is* then connected to a syringe containing andaliman at the dose of each treatment group, then inserted from the left side of the mice mouth between the front teeth with diastema following the hard palate. When the gavage needle reaches the back of the mouth, the rats head is slightly bent back and pressure is applied to the gavage so that the esophageal position is straight with the stomach. Gavage must be moved without pressure by following the force of gravity along the esophagus to the measured length. If gavage enters the respiratory tract, mice will have difficulty breathing. If the mark is not present, a slow solution can be injected to prevent regurgitation. After administration of the extract is complete, the gavage needle is removed slowly in the opposite direction to the direction of the insertion of the gavage needle [10]. On the 16th day, terminations were made to the four groups.

Table 2: Differences in IL-6 levels

	Mean	Median	SE	SD	95% CI	*p*
K-	16.7	16.7	3.5	8.5	7.8-25.7	<0,001
K+	67.5	69.9	3.5	8.5	58.6-76.4	
P1	18.8	21.5	3.5	8.5	9.9-27.7	
P2	21.1	23.8	3.4	8.3	12.4-29.9	

The mean levels of IL-6 showed a high of 67, 5 in the group of rats with preeclampsia, whereas in the group of normal mice got pregnant premises mean levels of IL-6 at 16.7. In the group of mice that received the treatment showed that the average levels of IL-6 in group P1 is 18, 8 and P2 group of 21.1. This shows a decrease in the average level of IL-6 in the administration of exalactandaliman. Based on the analysis, it was found that there were differences in the mean levels of IL-6 in the study group (*p<0.001).*

Table 3: Differences in TNF-α levels

	Mean	Median	SE	SD	95% CI	*p*
K-	84.4	85.8	3.3	8.1	76.0-92.9	<0,001
K+	109.7	111.1	3.3	8.0	101.3-118.1	
P1	90.1	91.4	3.2	7.9	81.8-98.4	
P2	95.1	96.4	3.3	8.0	86.6-103.5	

The mean TNF-α value was highest in the preeclampsia (K+) group of 109.7, whereas in the normal pregnant group (K-) the TNF-α value was 84.4. In the treatment group P1, the mean TNF-α levels were 90, 1 and 95.1 in the P2 group. This shows that the administration of andaliman extract reduced the average TNF-α level in preeclampsia mice both at a dose of 200 mg and a dose of 800 mg. Based on the analysis, it was found that there were differences in the mean TNF-α levels in the study group (*p<0.001).*

DISCUSSION

TNF-α induces the occurrence of oxidative damage due to destabilizing the flow of electrons inside the mitochondria causing release Oxidative free radicals and the formation of peroxides that make cell damage endothelial. TNF-α stimulates the production of angiotensin II in the reproductive tract women and IL-6 regulate levels of angiotensin II type 1 receptors in the muscles vascular

plain. The high TNF-α will induce an acute response to stimulates the liver to produce reactive protein C (CRP), a marker inflammation and sensitive tissue damage [11, 12]. TNF-α contributes to the placental invasion abnormalities, endothelial cell damage, and oxidative stress. TNF-α can stimulate IL-6 and IL-6 production can inhibit TNF-α release. TNF-α concentration significantly higher in the first and second trimesters in women who have a high risk of preeclampsia [13-15]. Based on Yanti *et al.*, andaliman extract can reduce TNF-levels α. Andaliman actively mRNA blocks from TNF-α. During the inflammatory process, Macrophages play an important role in the immune response through protein production proinflammation. TNF-α is a proinflammatory cytokine produced as a result of systemic inflammation process in PE. Anti-inflammatory activity from andaliman actively suppress TNF-α levels [16] This shows that administration of andaliman extract (Zanthoxylum acanthopodium) can reduce serum TNF-α levels in preeclampsia mice. The author has not found other studies that prove or refuting the results of this study [17]. IL-6 is a glycosylated protein with the molecular size of 21-28 kDa and has a four-stranded spindle structure that produces four strands length includes A, B, C, D. IL-6 has three places to bind to the receptors are IL-6R, gp130, and Ig-like domain gp130 [18]. The IL-6 gene is located in the short arm of chromosome 7 (7q21) [19]. IL-6 increases the permeability of endothelial cells by changing shape cells and rearrange intracellular actin fibers. IL-6 can also decrease prostacyclin synthesis (PG12) by inhibiting the enzyme cyclooxygenase. IL-6 can increase the thromboxane A2 ratio to prostacyclin and that seen in preeclampsia. IL-6 can also stimulate growth factors for platelet derivatives that are also seen in preeclampsia. Oxygen free radicals can induce IL-6 synthesis by endothelium. That can be cause endothelial damage and later reduce nitric oxide synthesis and disturbing the balance of prostaglandins [19]. Some the study found high levels of IL-6 mRNA and protein in decidual cells free of leukocytes in patients with preeclampsia. Cell human endometrial endometrium has recently been known to be capable of phagocytosis apoptotic trophoblasts and then secrete proinflammatory cytokines IL-6. Thing Such may include one mechanism that contributes to.

Inflammatory response was seen in placenta preeclampsia. IL-10 is known is a strong suppressor of proinflammatory cytokine secretions such as TNF-α and IL-6 but in preeclampsia insufficiency of IL-10 production occurs proinflammatory cytokines are produced uncontrollably. At the placental level, cytokines IL-6 along with TNF-α appear to induce abnormal apoptosis and excessive and necrotic death from trophoblast cells. After unraveling things, this will induce endothelial activation. IL-6 activates the renin system angiotensin [18]. This shows that administration of andaliman extract (Zanthoxylumacanthopodium) can reduce serum IL-6 levels in preeclampsia mice

CONCLUSION

Andaliman has been shown to reduce TNF-α levels in preeclampsia rats.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that this research was conducted without any commercial of financial relationship that could be seen as a potential conflict of interest

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