Moringa Leaf Powder (Moringa oleifera) Decrease of Inflammation Plasma Cytokine of Pregnant Rats with Diabetes Mellitus

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Abstract

BACKGROUND: Preeclampsia is a major obstetric problem worldwide, especially in developing countries that can cause maternal and fetal morbidity and mortality that affect 2–10% of pregnancies worldwide. More than 4 million pregnant women each year develop preeclampsia and cause 15–20% of maternal deaths worldwide. There are several high-risk factors associated with preeclampsia. Diabetes mellitus is one of the risk factors for preeclampsia. Preeclampsia has been shown to have higher levels of Th1 (pro-inflammatory) products and lower levels of Th2 (anti-inflammatory) products compared to normal pregnancy in blood serum. Interleukin (IL) 1, IL-2, IL-8, tumor necrosis factor (TNF)-alpha, and interferon gamma are Th1 cytokines or pro-inflammatory cytokines that can induce inflammatory reactions and are associated with pregnancy complications such as repeated abortion, preterm labor, rupture of membranes, preeclampsia, and stunted fetal growth. In contrast, Th2 cytokines such as transforming growth factor-β (TGF-β), IL-4, IL-5, IL-6, and IL-10 are associated with normal pregnancy.

AIM: This study aims to prove the effect of giving Moringa leaf powder (M. oleifera) to decrease blood levels, so this study will analyze the effect of Moringa oleifera leaf powder on blood level of IL-6, IL-10, TNF-α and TGF-β.

METHODS: A total of 30 rats were checked for sugar levels on day 4 after being induced by alloxan for 18 days to see if the rats were already in a hyperglycemic state. Then, they were divided into six groups, each group contained five pregnant white rats. Group 1 was the negative group (without being given alloxan), Group 2 was positive (given alloxan 150 mg/day/kg BW), and Groups 3, 4, 5, and 6 were given alloxan 150 mg/day/kg BW and each given a dose of 100 Moringa leaf powder; 200, 400, and 800 mg/day/kg body weight. After alloxan was induced and given a dose of Moringa leaf powder according to each group, blood samples were taken to separate the serum and continued with the ELISA method to calculate the levels of IL-6, IL-10, TNF-α, and TGF-β.

RESULTS: The administration of Moringa leaf powder at a dose of 800 mg/day/kg BW was able to increase the levels of IL-10 in the positive control Group 4 by 7.211 mIU/ml and reduced levels of IL-6 in the 8th dose group by 2.112 mIU/ml, was able to increase TGF-β levels in the dose Group 4 by giving Moringa leaf powder as much as 800 mg/day/kg BW, amounted to 1049.066 mIU/ml, and decreased TNF-α levels in the dose Group 4 by giving Moringa leaf powder as much as 800 mg/day/kg BW 800 mIU/ml.

CONCLUSION: The administration of Moringa leaf powder was able to increase the levels of IL-10 in the positive control Group, reduced levels of IL-6, was able to increase TGF-β levels, and decreased TNF-α levels.

Introduction

Maternal mortality rate (MMR) in Indonesia is still quite high compared to other countries. The MMR is useful for describing the level of awareness of healthy living behavior, nutritional status and maternal health, environmental health conditions, and the level of health services, especially for pregnant women, during childbirth and the postpartum period [1]. According to the World Health Organization (WHO) in 2016, there was a very high MMR in the world, around 830 women died from complications of pregnancy or childbirth worldwide every day. It is estimated that in 2015, around 303,000 women died during and after pregnancy and childbirth. According to the WHO in 2011, hypertensive disorders of pregnancy affect approximately 10% of pregnant women worldwide. In Africa and Asia, nearly one-tenth of all maternal deaths are associated with hypertensive disorders of pregnancy, while a quarter of maternal deaths in Latin America are associated with these complications.

Preeclampsia is a major obstetric problem worldwide, especially in developing countries that can cause maternal and fetal morbidity and mortality that affect 2–10% of pregnancies worldwide. Preeclampsia was defined as gestational hypertension with proteinuria after 20 weeks of gestation. More than 4 million pregnant women each year develop preeclampsia and cause 15–20% of maternal deaths worldwide. There are five high-risk factors associated with preeclampsia. The maternal death of preeclampsia is related to five high-risk factors, i.e. history of hypertension, advanced age, high blood lipids, body mass index, and history of diabetes mellitus. Diabetes mellitus is one of the risk factors...
for preeclampsia [2]. One of the mechanisms involved in the mechanism of preeclampsia is an exaggerated systemic inflammatory response due to the decomposition of one or more of the maternal immune systems. During pregnancy, there is an increased immune response to inflammation. Preeclampsia has been shown to have higher levels of Th1 (pro-inflammatory) products and lower levels of Th2 (anti-inflammatory) products compared to normal pregnancy in blood serum [3]. T-helper (Th) has an important role in regulating immune cells with the production of certain cytokines. Cytokines play an important role in pregnancy processes such as ovulation, implantation, placentaion, and delivery. Preeclampsia-related cytokines can be divided into two, pro-inflammatory cytokinins and anti-inflammatory cytokinins. Granulocyte macrophage colony-stimulating factor, interleukin (IL) 3, IL-10, and transforming growth factor-β (TGF-β) are anti-inflammatory cytokines that are associated with successful pregnancy. TGF-β is synthesized by various normal cells and platelets and affects the activation of macrophages, proliferation of fibroblasts, synthesis of connective tissue fibers and their matrix, local angiogenesis, healing, and regulation of T-lymphocytes. Therefore, TGF-β plays a very important role in counteracting these effects. Hence TGF-β will act as protective agent in detrimental effect of inflammation in pre eclampsia [4].

Cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) appear to have detrimental effects. IL-1, IL-2, IL-6, IL-8, TNF-α, and IFN-γ are Th1 cytokines or pro-inflammatory cytokines that can induce inflammatory reactions and are associated with pregnancy complications such as repeated abortions, preterm labor, ruptured membranes, preeclampsia, and stunted fetal growth. TNF-α is the main mediator in the inflammatory process because it has pleiotropic properties that make it a strong pro-inflammatory cytokine.

Recently, research and clinical trials on the function of Moringa as a drug are starting to develop even though the benefits and efficacy are not widely known. The latest discovery is the function of Moringa leaves as a pharmacological agent, namely, antimicrobial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, antidiabetic, and pro-implantation [5]. One of the high contents of Moringa (Moringa oleifera) leaves is flavonoid compounds. Flavonoids will block free radicals in pancreatic Langerhans cells. Flavonoid act as antioxidant via neutralizing free radical before its damaging body cells. There have not been many studies on the effect of using Moringa leaf powder on the levels of IL-6 and IL-10 as well as TNF-α and TGF-β in pregnant rats with diabetes mellitus, so this study aims to prove the effect of giving Moringa leaf powder (M. oleifera) to decrease blood levels, so this study will answer the effect of Moringa oleifera leaf powder on blood level of IL-6, IL-10, TNF-α and TGF-β.

### Materials and Methods

This study was an experimental study using white rats in experimental diabetic conditions in alloxan administration for 18 days. The selection of research objects for grouping and giving treatment used the RAL method (completely randomized design), this was because the experimental animals, ration materials, experimental sites, and other research materials were homogeneous. The research design for each treatment carried out in this study followed the procedures carried out by previous researchers Krisnadi [6]. The study used a rat model with preeclampsia pregnant conditions due to alloxan induction. To obtain the same gestational age (homogeneous), 30 female white rats were synchronized with their estrus cycle by treating white rats with Leeboth, Pheromone, and Whitten effect before mating white rats, to increase the success of pregnancy and get pregnant rats with the same gestational age, the synchronization method is used first.

a. Leeboth effect: Isolation of female rats, collected by female rats (separated from male rats) for 2 weeks to condition the unestrus cycle

b. Pheromone effect: Female rats were exposed to cages given the husks of male rat urine to stimulate their lust cycle and condition the estrus cycle

c. Whitten effect: Within 72 h after treatment, female white rats will be in estrus condition.

After 72 h of being stimulated by pheromone (husk of male rat urine), female rats were mated for one night in pairs (1:1), the next day after mating was considered the 1st day of pregnancy. On the 1st day of pregnancy, alloxan was not given. After pregnancy, alloxan was given for 3 consecutive days as much as 150 mg/day/kg BW according to research [6] that hyperglycemic rats can be produced by injecting 120–150 mg/kgBW. On the 4th day and 18th day after the first alloxan injection, blood glucose analysis (post-test) was carried out for blood hyperglycemic confirmation. Total 30 rats will grouped in 6 groups, each group consist of five rats. Group divided into several treatment, i.e.:

1. K-: Negative control (without alloxan induced)
2. K+: Positive control (induced by alloxan at a dose of 150 mg/day/kg BW) on the 2nd day
after pregnancy/mating for the next 3 days, consecutively using a probe.

3. Dose 1: Induced alloxan at a dose of 150 mg/day/kg BW and given Moringa leaf powder at a dose of 100 mg/day/kg BW after administration of alloxan, for the next 14 days, consecutively.

4. Dose 2: Induced alloxan at a dose of 150 mg/day/kg BW and given Moringa leaf powder at a dose of 200 mg/day/kg BW.

5. Dosage 3: Induced alloxan at a dose of 150 mg/day/kg BW and given Moringa leaf powder at a dose of 400 mg/day/kg BW.

6. Dose 4: Induced alloxan at a dose of 150 mg/day/kg BW and given Moringa leaf powder at a dose of 800 mg/day/kg BW.

After alloxan was induced and given a dose of Moringa leaf powder according to each group, blood samples were taken to separate the serum and continued with the ELISA method to calculate the levels of IL-6, IL-10, TNF-α, and TGF-β.

Results and Discussion

Based on the results, the levels of IL-10 was significantly increased after the administration of Moringa leaf powder at a dose of 800 mg/day/kg BW (Figure 1). On the other side this dose 800 mg/day/kg body weight reduced the levels of IL-6 (Figure 2). In Figure 3, we can see the same dose (800 mg/day/kg BW was significantly increased the level of TGF beta compare to control group P+.

Different result obtain from TNF alpha assay that significantly decreased TNF alpha at lower dose 200 mg/day/kg BW (Figure 4). This is because Moringa leaves contain sitosterol 90 mg/g, total phenolic 8 μg/ml, and flavonoids 27 μg/ml, which are related to antioxidant activity. Moringa leaves have antioxidant activity from phenolic compounds of the flavonoid group. The benefits of flavonoids include protecting cell structure, increasing the effectiveness of Vitamin C, anti-inflammatory, preventing bone loss, and as antibiotics [6].

Flavonoids can prevent damage by free radicals in several ways, one of which is the direct binding of free radicals by donating hydrogen ions from the hydroxyl group (OH) to free radicals (R) so that free radical bonds are formed that are more stable and less reactive. In addition to having antioxidant activity, flavonoids also have anti-inflammatory activity by modulating the production of pro-inflammatory mediators so that they can reduce the production of pro-inflammatory cytokines such as TNF-α and ILs.

The role of the cytokine IL-10 has been extensively studied in infectious and inflammatory processes that occur in non-pregnant women, because of its important ability as an immunosuppressant in response to inflammation. IL-10 is a cytokine that has an important role in pregnancy, namely, regulating the maternal immune response, having anti-inflammatory effects, and effects on the vasculature at the maternal-fetal interface [7]. During pregnancy, IL-10 levels increase in early pregnancy and continue to increase until the third trimester of pregnancy [8]. The role of IL-10 during pregnancy is to suppress maternal immunity to allow acceptance of fetal allograft properties. In addition, the secretion of IL-10 by various maternal and fetal cells has been shown to play a role in maintaining a normal pregnancy process.
Figure 4: Average effect of Moringa leaf powder on tumor necrosis factor-α (TNF-α).

**Conclusion**

The administration of Moringa leaf powder at a dose of 800 mg/day/kg BW was able to increase the levels of IL-10 in the positive control Group 4 by 7.211 mIU/ml and reduced levels of IL-6 in the 8th dose group by giving Moringa leaf powder as much as 800 mg/day/kg BW by 2.112 mIU/ml, was able to increase TGF-β levels in the dose Group 4 by giving Moringa leaf powder as much as 800 mg/day/kg BW, amounted to 1049.066 mIU/ml, and decreased TNF-α levels in the dose Group 4 by giving Moringa leaf powder as much as 800 mg/day/kg BW mIU/ml.

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