Original Article

Moringa oleifera decrease blood sugar level and blood pressure in pregnant diabetic rats

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ABSTRACT

It has been known that 10% of pregnancy will undergone diabetes and increases the risk of hypertension, premature birth, intrauterine growth disorders, perinatal death, kidney and liver failure, pre-postpartum hemorrhage, and maternal death. Many treatment regimen had been use however still there were no satisfied result. Recently, Moringa oleifera was popular as miracle tree that had many health properties. Many paper had report its anti-cancer properties, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, antioxidant, antidiabetic, hepatoprotective, antibacterial, antifungal, antiatherosclerotic, heart and blood circulation stimulant. Hence we conduct this research to dig the role of moringa oleifera in diabetic pregnant rat. We used 30 pregnant rats divided into 6 groups of treatment, i.e two control group and others were treatment group of different doses of Moringa powder at 100, 200, 400 and 800 mg/day/kg. Alloxan 150 mg/day/kg was used to induce diabetic animal model. Blood glucose was measure using glucostick methods and blood pressure was measure using the tail-cuff method using CODA blood pressure analyzer. In this study, it can be concluded that the administration of Moringa leaf powder (Moringa oleifera) at a dose of 200 mg/day/kg BW decreased glucose levels and decreased blood pressure at a dose of 400 mg/day/kg BW on in diabetic pregnant animal model.

Keywords: Diabetes, Pregnancy, Hypertension, Moringa oleifera, Blood pressure

Introduction

Until now, diabetes mellitus (DM) is still the highest non communicable disease in Indonesia [1, 2]. Diabetes mellitus is a condition where the concentration of glucose in the blood is chronically higher (hyperglycemia) than normal due to insulin deficiency or ineffective insulin function [3]. This disease was not only attacked the old age group but nowadays the incidence is mostly happen on young and productive age groups, including pregnant women. In normal conditions, blood sugar levels in the body are regulated by the hormone insulin. In pregnancy, a woman's body has hormonal changes that can cause the body to become less responsive to insulin. For some women, conditions like this make blood sugar increase dramatically. There are 2 terms in diabetes when pregnant, namely Gestational Diabetes Mellitus (GDM) and PreGestational Diabetes Mellitus (PGDM). Gestational Diabetes Mellitus (GDM) is diabetes that occurs during pregnancy, whereas before pregnancy the mother does not have diabetes. PreGestational Diabetes Mellitus (PGDM) is diabetes that occurs in pregnant women with a previous history of diabetes, both type 1 and type 2 diabetes mellitus.

Pregnancy is a diabetogenic condition characterized by insulin resistance and increased pancreatic-cell response and hyperinsulinemia as compensation. Insulin resistance generally begins in the second trimester of pregnancy and continues throughout the remainder of the pregnancy. Insulin sensitivity during pregnancy can decrease by up to 80%. Hormones secreted by the placenta, such as progesterone, cortisol, human placental lactogen (hPL), prolactin, and growth hormone, are factors that play an important role in the state of insulin resistance during pregnancy.

Progesterone and estrogen can affect insulin resistance directly or indirectly. HPL levels increase with increasing gestational age, this hormone works like growth hormone, which increases

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lipolysis. Lipolysis causes increased levels of free fatty acids circulating in the blood, which in turn can cause insulin resistance in peripheral tissues. Fetal growth depends on maternal plasma glucose levels. The presence of insulin resistance causes high maternal plasma glucose levels, which will then diffuse into the fetal bloodstream through the placenta. Mothers who suffer from gestational diabetes have higher levels of insulin resistance than normal pregnancies and are not compensated by adequate insulin secretion [4].

In pregnant women, there are metabolic changes, one of which is glucose metabolism. In early pregnancy, there is hyperplasia of pancreatic cells which is the impact of the increase in the hormones estrogen and progesterone in the mother. This condition causes high insulin levels in early pregnancy. In the second and third trimesters, the presence of factors from the fetoplacental causes a decrease in insulin sensitivity of the mother. Because the fetus needs glucose transport because it is not able to form glucose, so the fetus needs a supply from the mother. Therefore, in the body of the mother, there is an increase in gluconeogenesis. The pathophysiology of gestational diabetes is divided into two: 1) the role of the fetoplacental unit and 2) the role of adipose tissue [5].

One of the factors that influence pregnancy and childbirth complications is diabetes mellitus. Diabetes mellitus causes complications in 10% of pregnancy and increases the risk of hypertension, premature birth, intrauterine growth disorders, perinatal death, kidney and liver failure, antepartum and postpartum hemorrhage, and maternal death. Hypertension in pregnancy is divided into chronic hypertension (pre-pregnancy hypertension), pregnancy-induced hypertension (normal blood pressure before 20 weeks of gestation), pre-eclampsia (accompanied by proteinuria), and eclampsia (accompanied by proteinuria and seizures). Efforts to prevent this disease by itself will reduce morbidity and mortality in pregnant women. For this reason, prevention is needed before pregnancy has occurred. Hypertension before pregnancy is 1-5 percent and continues during pregnancy or can occur with pregnancy. If a normotensive woman experiences pregnancy, then hypertension can occur in 5-7 percent.7,9 prevention can be done during the fertile age woman (WUS) is not pregnant or control the morbidity of hypertension in WUS.

In biomedical research, the use of experimental animals has made an important contribution in developing new concepts and the pathogenesis of many diseases. One of the purposes of using animals in biomedical research is that of animals as substitutes for humans. Human biology is very similar to that of many other animals. In many studies, especially in experimental research using animal subjects, to ensure the accuracy and validity of the data, as well as high statistical power, in addition, for sample size, sample homogeneity is also a major requirement that must be complete. In experimental studies of reproduction such as eclampsia, low birth weight, body weight, and many other pregnancy disorders, animal models of pregnancy with the same gestational age are needed. To have several homogeneous animal models, it is necessary to mate simultaneously [6].

In experimental animals, DM is often caused by giving alloxan which can cause damage to the beta Langerhans pancreatic cells [7]. Alloxan is a chemical compound used to induce diabetes in animal experiments. Alloxan is a hydrophilic compound that is unstable and selectively toxic to the liver and kidneys, but in certain doses, it causes selective destruction of pancreatic beta cells. Pure alloxan is obtained from the oxidation of uric acid by nitric acid. The half-life of alloxan at a pH of 7.4 and a temperature of 370 C is 1.5 minutes and is very easily oxidized [8].

Nowadays, the middle and lower class use alternative medicines in treating diabetes mellitus. One of the traditional medicines that can treat diabetes mellitus and its existence is most commonly found in Indonesia is the Moringa plant is known as Murong (Moringa oleifera) [9].

This research aims to create experimental diabetic conditions in pregnant white rats (Rattus norvegicus) with given alloxan and improve high blood pressure due to diabetes mellitus in pregnant white rats by giving Moringa oleifera leaf powder.

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 Krisnawati, (2013) in the title of the effect of giving Moringa fruit filtrate (moringa oliefera) on blood sugar levels in experimental white rats (Rattus norvegicus) Wistar strain. The study used a rat model with preeclampsia pregnancy 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, 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 [10].

a. Lee Both Effect: isolation of female rats, collected by female rats (separated from male rats) for 2 weeks to condition the estrous 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 hours after treatment, female white rats will be in oestrus condition.

After 72 hours 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 [10]. 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 that

hyperglycemic rats can be produced by injecting 120-150 mg/kg BW. On the 4th day given the first alloxan and then on the 18th-day blood glucose analysis (post-test) was carried out, there were white rats that increased their blood glucose. Randomization of samples in each treatment group, positive and negative control groups. At this stage, 30 pregnant female rats divided into 6 groups :

K-: Negative control (without alloxan-induced)

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

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 alloxone, for the next 14 days, consecutively.

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

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

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

Measurement of blood pressure using the tail-cuff method using a Kent Scientific CODA blood pressure analyzer. The rat was inserted into the holder by holding the tail, the rat condition should be in a calm/basal condition in the holder before the measurement was taken and without stress due to cold or heat, the tail was inserted into the tail hole in the cuff, the cuff was tightened and the rat was ready to be measured [11]. After getting data on systolic and diastolic blood pressure which was measured 3 times, the average value of rat blood pressure was taken.

Results and Discussion

The extract was administered orally every day and the blood pressure was measured from day 0 to day 18. During the treatment, the rats were still induced with alloxan at a dose of 150 mg/day/kg BW, because the physiological conditions of the rats were still normal which could normalize the pathophysiological conditions experienced by the rats, namely hypertension after induction. Measurements were made using a Non-invasive Rat Tail Blood Pressure tool. The parameters measured were diastolic blood pressure (TDD) and systolic blood pressure (TDS). The research data obtained were analyzed by Completely Randomized Design (CRD) using the SPSS program, to determine the hypotensive effect before and after alloxan induction, then the analysis continued with Duncan's test and LSD (Least Significant Difference) test, to see the effect of Moringa leaf powder in reducing glucose levels and high blood pressure in diabetic pregnant rats.

Table 1. Results of blood pressure in pregnant rats treated with moringa leaf powder.

Treatment	Systolic 1 (Pre Test)	Systolic 2 (Post Test)	Diastolic 1 (Pre Test)	Diastolic 2 (Post Test)
K-	122,2	102,4	88,4	76,0
K+	211,4	176,8	199,6	164,6
P1	126,2	135,2	92,2	96,6
P2	156,6	146,6	119,2	95,6
P3	199,0	152,0	190,2	120,0
P4	146,4	121,2	117,6	104,0
LSD 5%	22,15	25,52	26,72	23,66

Table 2. The results of the effect of treatment with Moringa powder on glucose levels in pregnant diabetic rats

Treatment	Day4	Day 3	Day 12
K-	93,2	82,4	71,6
K+	89,4	85,8	75,8
P1	110,6	81,2	82,8
P2	87,8	155,4	206,6
P3	73,8	84,0	80,2
P4	83,4	148,0	189,4
LSD 5%	19,29	90,57	137,55

Examination of systolic and diastolic blood pressure was carried out 3 times, and the mean values of systolic and diastolic were taken. The examination was carried out in a basal condition through the tail using a Kent Scientific CODA blood pressure transducer. Differences were found in the systolic blood pressure of experimental animal pregnant rats with diabetes mellitus **(Table 1)**. The results from the study of systolic blood pressure obtained data distribution with homogeneous data distribution normality. Giving alloxan of 150 kg/day/BW rats for 3 consecutive days intravenously, showed an increase in systolic and diastolic blood pressure in pregnant rats [10]. The positive control group after treatment with alloxan in this study also found an increase in systolic blood pressure of 211.4 mmHg compared to the systolic blood pressure negative control group with 122.2 mmHg treatment. In each group, there was a difference in the decrease in systolic blood pressure. The value of systolic blood pressure in the Moringa leaf powder treatment group at a dose of 100 mg/day/kg BW (135.2 mmHg), a dose of 200 mg/day/kg BW (146.6 mmHg), a dose of 400 mg/day/kg BW (152 0.0 mmHg), and at a dose of 800 mg/day/kg body weight (121.2 mmHg) there was a mean decrease in systolic blood pressure and statistically this decrease in mean systolic blood pressure was not different from the negative control group (102.4 mmHg) even though the blood pressure value was higher systolic than the negative control group. The diastolic blood pressure of rats before treatment with alloxan induction obtained the same mean, there was no statistical difference in the blood pressure of rats (the letters were superscribed with the same letter) in the negative control, positive control, and treatment group. After induction with

alloxan and administration of Moringa leaf powder with different doses, there were differences in diastolic blood pressure of pregnant rats with diabetes mellitus. The results obtained from the study of diastolic blood pressure obtained data distribution with homogeneous data distribution normality. In each treatment group with Moringa leaf powder, there were differences in the decrease in diastolic blood pressure. In the Moringa leaf powder group, in the model rats, the mean diastolic blood pressure was different from the negative control group. The value of diastolic blood pressure in the Moringa leaf powder treatment group at a dose of 100 mg/day/kg BW (96.6 mmHg), a dose of 200 mg/day/kg BW (95.6 mmHg), a dose of 400 mg/day/kg BW (120 0.0 mmHg), and at a dose of 800 mg/day/kg BW (104.0 mmHg) there was an average decrease in systolic blood pressure and statistically, this decrease in mean systolic blood pressure was not different from the negative control group (76.0 mmHg) although the pressure value diastolic was higher than the negative control group. The decrease in diastolic pressure was found to be the lowest in the group with moringa leaf powder at a dose of 200 mg/day/kg BW. In the 400 mg/day/kg BW group, the mean diastolic pressure was higher than in the 100, 200, and 800 mg/day/kg BW therapy groups. This can be caused by physiological factors in mice that are not good (can be from the level of stress in rats so that the increase in blood pressure increases), environmental factors, and lack of mineral intake while the intake of alloxan continues to be excessive. Stress conditions can occur through fights between animals in the cage, noise, and room temperature so that it triggers an increase in blood glucose levels. The condition of increased glucose levels in the blood or hyperglycemia is due to a deficiency in insulin production by pancreatic cells and or impaired insulin sensitivity in peripheral tissues so that glucose cannot be used by cells. Hyperglycemia can disrupt oxidative processes in the body or is called oxidative stress.

Illiandri (2013) revealed that Moringa leaves have antidiabetic compounds that play a role in preventing free radicals and regenerating pancreatic beta cells (9). Several research journals reveal that Moringa leaf powder has an antidiabetic effect that can lower blood glucose levels in humans. Dosage of 200 mg/day/kg BW on day 18 gave the greatest effect compared to other doses **(Table 2)**.

Conclusion

Giving Moringa leaf powder (Moringa oleifera) at a dose of 200 mg/day/kg BW decreasing glucose levels and Moringa leaf powder at a dose of 400 mg/day/kg BW decrease blood pressure in pregnant rats with diabetes mellitus.

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Conflict of interest: None

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Ethics statement: None

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