



## The Use of *Moringa Oleifera* Powder to Prevent Pancreatic Organ Cell Damage in Pregnant Mice with Diabetes Mellitus

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### ABSTRACT

**Introduction:** Diseases that accompany pregnancy, such as diabetes mellitus, affect preeclampsia. This disease is a hereditary disorder characterized by reduced circulating insulin, high blood sugar concentrations, and reduced glycogenesis. This study tried to determine the effect of giving Moringa leaf powder (*Moringa oleifera*) in preventing pancreatic organ cell damage in pregnant rats with diabetes mellitus.

**Method:** A total of 30 pregnant white rats, which were divided into 6 groups, were examined for their sugar levels on day 4 after being induced by alloxan for 18 days to ensure that they were already in a hyperglycemic state.

**Result:** The results showed that the administration of Moringa leaf powder at a dose of 800 mg/day/kg BW could reduce pancreatic cell apoptosis, approaching the negative control group.

**Conclusion:** In conclusion, the moringa leaf powder is able to improve the clinical pathological condition of pregnancy, due to inhibition of apoptosis and repair of pancreatic Langerhans cells.

**KEYWORDS:** Diabetes Mellitus, Moringa Leaf Powder, Pancreatic Apoptosis.

### 1. INTRODUCTION

Diabetes mellitus (DM) is a chronic degenerative disease characterized by the patient's blood sugar level being above the normal average. It is a group of metabolic diseases associated with the dysfunction or failure of several organs of the body. There are some causes of diabetes mellitus, such as pancreatic beta cell destruction, insulin secretion disorder, genetic defects, exocrine pancreas disease (pancreatitis), endocrinopathy, infections due to drugs or chemicals, and other genetic syndromes.

This current research focuses on pancreatic damage as a cause of diabetes mellitus. Pancreas is a very long organ located retroperitoneally in the upper abdomen, in front of lumbar vertebrae I and II. The pancreas produces two glands namely endocrine glands and exocrine glands. The exocrine portion produces digestive enzymes. Together with alkaline fluids, they are excreted into the small intestine via the exocrine duct. Excretion is carried out in response to a small intestinal hormone called secretin. The endocrine portion of the pancreas is made up of millions of cells that form discrete clusters known as the islets of Langerhans. The islets of Langerhans vary in shape and size, located between the cells of the exocrine part of the pancreas.

According to the American Diabetes Association (ADA) in 2012, there are two major groups of diabetes mellitus when viewed clinically. The first is type-1 diabetes, insulin-dependent diabetes mellitus (IDDM), which is characterized by permanent damage to the pancreas due to autoimmune mechanisms. The second is type-2 diabetes, non-insulin-dependent diabetes mellitus (NIDDM), which is characterized by pancreatic and insulin dysfunction. Thus, permanent damage to the pancreas is the basic mechanism of all types of DM<sup>1</sup>.

In Boland et al.'s research<sup>3</sup>, it was found that the reduced number of pancreatic beta cells occurred not because of the reduced formation (neogenesis) but because of the imbalance between neogenesis and cell death (apoptosis). Apoptosis is a biological mechanism which is one type of programmed cell death<sup>8</sup>. Apoptosis is used by multicellular organisms to get rid of cells that are no longer needed by the body<sup>8</sup>.

In diabetes mellitus, the speed of the apoptotic process increases, which is caused by some mechanisms including genetic differences and the levels of oxidative stress in cells<sup>4</sup>. In addition, elevated levels of excess glucose can directly induce apoptosis in human pancreatic beta cells. This is caused by damage to pancreatic  $\beta$ -cells so that they cannot produce insulin or due to insulin resistance. This pancreatic damage can be caused by increased free radical compounds due to increased blood sugar levels in DM cases<sup>16</sup>.



In Gondo's study<sup>9</sup>, it was found that Moringa leaf powder may result in hypoglycemic and antihyperglycemic activity due to the presence of terpenoids involved in the stimulation of pancreatic beta cells and insulin secretion. In addition, Moringa leaf powder is also assumed to be able to lower the average blood glucose level due to the synergistic effect of the active ingredients contained in it.

One of the high content of Moringa oleifera leaves is flavonoid compounds. As is widely understood, flavonoids will block free radicals in pancreatic Langerhans cells. Flavonoids as antioxidants function to reduce oxidizing agents before damaging body cells. Flavonoids, assisted by Vitamin C contained in Moringa leaves, are able to act as oxidant scavengers that can inhibit oxidation reactions.

Moringa leaves in relation to decreasing blood glucose levels have attracted the attention of many researchers (e.g., Alethea and Ramadhian<sup>1</sup>; Syamra et al.,<sup>16</sup>; Radiansah et al.,<sup>15</sup>, and Munim et al.,<sup>13</sup>. Alethea and Ramadhian<sup>1</sup>, for example, found that Moringa oleifera leaf extract was proven to have antidiabetic and antihyperglycemic effects. Moringa oleifera leaf extract is able to reduce blood sugar levels and reduce HbA1C levels which are indicators of treatment success in diabetes mellitus patients through various mechanisms. Syamra et al.,<sup>16</sup> concluded that administration of Moringa leaves boiled water could decrease blood glucose levels in DM patients. They found that providing Moringa leaves boiled water for 4 days, the patients' blood glucose levels can be significantly decreased. Radiansah et al.<sup>15</sup> in their research on mice found that moringa leaves extract contains alkaloids and steroids that can lower blood sugar levels. He concluded that the concentration of the moringa leaves extract 20% was effective to decrease the blood sugar level of diabetic mice.

Different from those studies, this study focuses on revealing the effect of Moringa Oleifera powder administration to prevent pancreatic organ cell damage in pregnant mice with diabetes mellitus. In addition, this study also revealed an effective dose that can be given to prevent pancreatic organ cell damage in DM pregnant mice.

## 2. RESEARCH METHOD

This study is an experimental study using white mice with experimental DM due to alloxan administration for 18 days. The RAL method (Completely Randomized Design) was applied to select the research objects for grouping and treatment. This is because experimental animals, ration materials, experimental sites and other research materials were homogeneous. The research design of each treatment carried out in this current research followed the procedures carried out by Gondo<sup>9</sup>.

The use of animals in this study has followed the rules set by the Institution of Animal Care Use Committee (IACUC) to ensure that the researcher has animal ethical clearance before conducting research and testing. The principles followed include ethical principles (i.e., respect, beneficiary and justice), 3Rs principles (i.e., Replacement, Reduction, Refinement), and 5F/Freedom (i.e., freedom from hunger and thirst, heat and discomfort, pain, trauma and disease, fear and stress and expressing behavior naturally). Furthermore, this research had got ethical clearance from the Research Ethics Committee, Faculty of Medicine, Universitas Wijaya Kusuma, Surabaya, with Certificate no.: 016/EC/FK-UWKS/21.

This current research used mice with preeclampsia-induced pregnancy condition due to alloxan induction. To obtain the same gestational age (homogeneous), 30 female white mice were synchronized to their estrus cycle by treating them with Leebboth, Pheromone, and Whitten effects. Before the white mice were mated, to increase the success of pregnancy and get pregnant mice with the same gestational age, the synchronization method was used following Gondo<sup>9</sup>. The estrus cycle of female mice was treated in 3 steps, namely:

- a. Lee Both Effect: isolation of female mice. The female mice were separated from the male and grouped for 2 weeks to condition the unestrus cycle.
- b. Pheromone effect: female mice were exposed to cages given the husks of male mice urine to stimulate their lust cycle and to condition the estrus cycle.
- c. Whitten Effect: Within 72 hours after treatment, the female white mice were in oestrus condition.

After 72 hours of pheromone stimulation (husk of male mice urine), female mice were mated for one night in pairs (1:1). The next day after mating was considered the 1st day of pregnancy<sup>9</sup>. On the 1st day of pregnancy, alloxan was not yet given. After pregnancy, alloxan was given for 3 consecutive days as much as 150 mg/day/kg BW. According to Gondo's study<sup>9</sup>, hyperglycemic mice can be produced by injecting 120-150 mg/kgBW. On the 4th day, the first alloxan was given and then on the 18th day the blood glucose analysis was performed (post-test). It was known that there were some white mice that increased their



blood glucose. Sample randomization was carried out in each treatment group and control group, positive and negative. At this stage, as many as 30 pregnant female mice were grouped into 6 groups. Each group contains 5 mice, namely:

K- : Negative control (without alloxan induced).

K+ : Positive control induced by alloxan at a dose of 150 mg/day/kg BW on day 2 after pregnancy/mating for the next 3 three 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 alloxan administration, for the next 14 days, in a row.

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

DNA fragmentation is one of the characteristics of apoptosis. In this current research, DNA fragmentation was examined by the TUNEL (Terminal deoxynucleotidyl Transferase mediated dUTP Nick End Labeling) method. The TUNEL reagent consists of a terminal transferase enzyme used to recognize the 3'OH ends (nick end) generated by DNA fragmentation and fluorescein-dUTP to visualize the 3'OH ends observed using a fluorescence microscope. To visualize the comparison of apoptotic cells with non-apoptotic cells in one observation field, a double staining method was used using TUNEL reagent which was counterstained with methylene Green. TUNEL detects apoptotic cells and gives a brown color, while methylene Green detects nonapoptotic cells and gives a green color<sup>9</sup>.

### 3. RESULTS AND DISCUSSION

#### Results

The result of analysis shows that there was a decrease in TUNEL expression after being given Moringa leaf powder with an average dose of 800 mg/day/kg body weight in group 4, as can be seen in Table 1 below.

Table 1. Results of observations of white mice's trophoblast cells in the control group and treatment group

Group	Inti Apoptosis	
	N	Mean
Negative Control Group	5	5.00
Positive Control Group	5	20.00
Treatment Group 1	5	15.44
Treatment Group 2	5	8.66
Treatment Group 3	5	7.75
Treatment Group 4	5	6.75

Based on the multiple comparison test with the LSD test, it was found that the levels of TUNEL were significantly different in each negative control group, positive control group, and the treatment groups given different doses of Moringa leaf powder. In the treatment group with a dose of 100 mg/day/kg BW of Moringa leaf powder, the TUNEL expression was 15.44. In the treatment group with a dose of 200 mg/day/kgBW, the TUNEL expression was 8.66. In the treatment group with a dose of 400 mg/day/kg BW, the TUNEL expression was 7.75. Meanwhile, in the treatment group with a dose of 800 mg/day/kg BW, the TUNEL expression was 6.75.

#### Discussion

The occurrence of apoptosis was measured by the TUNEL staining method using the Transferase (TdT) Mediated dUTPNick End Labeling (TUNEL) kit with the brand 'Enogen' Lot number 20160302. The occurrence of apoptosis in trophoblasts was seen by the brown color in the nucleus; while pancreatic langerhas cells that did not undergo apoptosis, their nuclei were observed to be purple/green in color. Apoptotic expression of pancreatic Langerhas cells was measured using "Image J" software. In this study, pregnant mice were induced by 150 mg/day/kg BW of alloxan for 3 consecutive days. In this study, an



increase in the expression of apoptosis was also found. DNA fragmentation that occurs in the process of apoptosis lasts for several hours, before finally undergoing phagocytosis. By using the labeling technique for fragmented DNA (TUNEL) (Figure 1), the average results of the calculation of apoptosis in the Normal and Treatment groups were obtained. It is known that there is a significant difference in the mean number of pancreatic cells undergoing apoptosis in the control group versus the treatment group.

In the treatment groups with doses of 100, 200, 400 and 800 mg/day/kg BW, the TUNEL expression was not statistically significant, but it can be seen that the lowest mean TUNEL expression (6.75) was found in the treatment group with a dose of 800 mg/day/kg BW. This is due to the increased blood glucose levels because of the resistance to insulin action. The occurrence of insulin resistance is caused by the inability of the cells to respond to the increased blood glucose levels so that the levels remain high<sup>8</sup>.

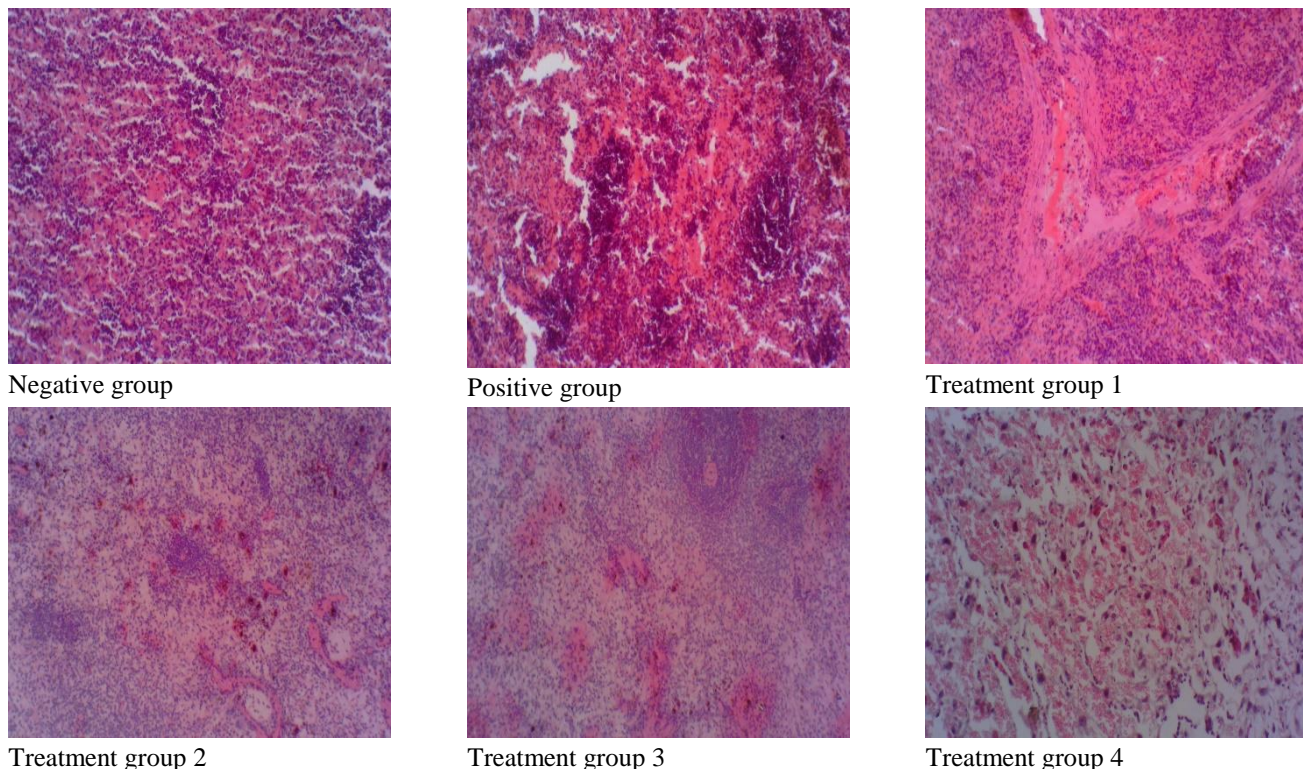
Alloxan is used to induce insulin-dependent DM in test animals because it selectively damages pancreatic beta cells. Alloxan generates diabetes by a mechanism which essentially includes partial degradation of the beta ( $\beta$ ) cells of pancreatic islets and successive compromise in the quality and quantity of insulin produced by the cells<sup>10</sup>. Alloxan-induced diabetes is one of the commonly employed model to induce diabetes mellitus in the experimental animals. Alloxan is selectively toxic to pancreatic beta cells as it specially accrues in the beta cells as glucose analogues<sup>10</sup>. Alloxan acts directly on pancreatic beta cells with its cytotoxic action mediated by Reactive Oxygen Species (ROS) so that it can be used as DM induction. Alloxan can trigger an increase in the production of excess free radicals and cause oxidative stress.

The decrease in blood glucose levels was caused by the activity of secondary metabolites contained in Moringa leaf powder. The administration of Moringa leaf powder to the test mice generated results that were significantly different from the negative control group and not significantly different from the positive control group. It means that Moringa leaf powder has an effect in lowering blood glucose levels. The results of this study are in agreement with the results of previous studies (e.g., Alethea and Ramadhian<sup>1</sup>; Syamra et al.,<sup>16</sup>; Radiansah et al.,<sup>15</sup>, and Munim et al.,<sup>13</sup>) which shows that Moringa leaves are effective for lowering glucose levels in DM patients, either given in powder or solution form.

One of the high contents of Moringa oleifera leaves is flavonoid compounds. Flavonoids block free radicals in  $\beta$  pancreatic Langerhans cells. Flavonoids are antioxidants that function to reduce oxidizing agents before damaging body cells<sup>9</sup>. Flavonoids belong to the class of phenolic compounds with a chemical structure of C6-C3-C6<sup>11,17,8</sup>. The flavonoid framework consists of an aromatic ring A, an aromatic ring B, and a middle ring in the form of an oxygen-containing heterocyclic. A numbering system is used to distinguish the position of carbon around the molecule<sup>4</sup>. Flavonoids act as antioxidants by donating hydrogen atoms or through their ability to chelate metals, in the form of glucosides (containing glucose side chains) or in free forms called aglycones<sup>5</sup>.

Through the histopathological observation, the treatment group 4 with a dose of 800 mg/day/kg BW was the group has the best result, as evidenced from the prepartate picture presented in Figure 1. The picture indicates that the pancreatic tissue is close to normal. A total of 2 subjects fall into category 2, and 3 subjects fall into category 1. On the histopathological description of the islets of Langerhans cells in group P4, namely white mice induced by alloxan and given Moringa leaf powder at a dose of 800 mg/day/kg BW, the level of necrosis is the lightest and most of the islets of Langerhans cells are close to normal. The islets differ substantially in size, but a usual islet is about 50–200  $\mu$ M in diameter<sup>7</sup>.

Picture 1. The histopathological description of the white mice's pancreatic cells



In this histopathological picture, with 400x magnification around the central vein and Kiernan's triangle in 1 field of view, dilated sinuses were found, as well as pancreatic cells undergoing apoptosis. In treatment group 1, 4 apoptotic cells were found; in treatment group 2, there were 3 apoptotic cells; in treatment group 3, there were 2 apoptotic cells; and in treatment group 4, there was 1 apoptotic cell. In the positive control group, there were 4 apoptotic cells, while in the negative control group there were 0 apoptotic cells. From the results of the observations, in the treatment group 4, there was the lowest number of cells that experienced apoptosis in the pancreatic cells of pregnant white mice.

#### 4. CONCLUSION

Giving Moringa leaf powder at a dose of 800 mg/day/kg BW can reduce pancreatic cell apoptosis, approaching that in the negative control group. Moringa leaf powder is able to improve the clinical pathological condition of pregnancy, due to the inhibition of apoptosis and repair of Langerhans Pancreas cells. Moringa leaf powder therapy has also been clinically proven to reduce blood glucose levels in gestational diabetic mice. This condition can be attributed to the repair of Langerhans beta cells in the pancreas due to administration of Moringa leaf powder.

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