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# Antidiabetic Effect of Noni Fruit and Moringa Leaves Extract on Blood Glucose Level, Hepatic and Pancreatic Histological Features of Diabetic Mice

# Efek Antidiabetes Ekstrak Buah Mengkudu dan Daun Kelor Terhadap Kadar Glukosa Darah, Gambaran Histologis Hepatik dan Pankreas Tikus Diabetes

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**ABSTRACT**

One of the chronic diseases with high blood glucose levels is a characteristic of Diabetes Mellitus. Utilization of herbal ingredients needs to be developed to reduce blood sugar levels in diabetes, and one of them is by using moringa leaf extract and noni fruit. This study aimed to analyze the effect of a combination of moringa leaf extract and noni fruit on blood glucose levels and tissue cytology in diabetic mice. The study was a randomized pretest-posttest control group design using 28 male mice divided into seven groups. The data analysis showed the lowest decrease in blood glucose levels in the group of diabetic mice given noni fruit and moringa leaf extract at a dose of 150mg/20g BW/oral/day for two weeks. The results of histological examination of liver and pancreas tissue showed no tissue is damaged.

**Kata kunci:**

Diabetes mellitus, noni extract, moringa leaves, blood glucose, tissue histology

Salah satu penyakit kronis dengan kadar glukosa darah tinggi merupakan ciri Diabetes Melitus. Pemanfaatan bahan herbal perlu dikembangkan untuk menurunkan kadar glukosa darah pada diabetesi dan salah satunya dengan menggunakan ekstrak daun kelor dan buah mengkudu. Tujuan penelitian ini adalah untuk menganalisis efek pemberian kombinasi ekstrak daun kelor dan buah mengkudu terhadap kadar glukosa darah dan gambaran sitologi jaringan pada mencit diabetik. Penelitian berjenis *randomized pre-test post-test control group design* dengan menggunakan 28 ekor mencit jantan yang dibagi menjadi 7 kelompok. Hasil analisis data menunjukkan bahwa terdapat penurunan kadar glukosa darah terendah pada kelompok mencit diabetik yang diberikan ekstrak buah mengkudu dan daun kelor dengan dosis 150mg/20g BB/oral/hari selama 2 minggu, serta dari hasil pemeriksaan histologi jaringan hepar dan pancreas menunjukkan tidak ada jaringan yang mengalami kerusakan.

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## INTRODUCTION

One of the diseases in the world whose prevalence is increasing every year is Diabetes Mellitus. WHO predictions regarding the number of diabetics in Indonesia are around 21.3 million in 2030, and Indonesia ranks fourth in the world. The use of sulfonylurea drugs in diabetics, if the diet is too strict or consumes the wrong dose, can result in hypoglycemia and potentially impair liver or kidney function in the elderly. The side effects of using the drug are an increase of 2 kg in body weight (Defirson & Azizah, 2021). Alternative medicine is a safe and inexpensive option due to the impact of the prolonged economic crisis, including the Covid 19 pandemic. The presence of phytochemicals in 300mg of moringa leaves boiled, washed, then boiled in 3 cups of water = 450ml, for 15 minutes until the boiled water becomes 1 cup = 150ml, shown through alkaloids and steroids/triterpenoids, has a strong effect on blood glucose levels. The mechanism of reducing blood glucose levels is terpenoid compounds in herbs stimulating pancreatic ß cells to secrete insulin (Syamra & Indrawati, 2017). The content of alkaloids, flavonoids, tannins, saponins, and steroids is also found in the ethanol extract of noni leaves. The results of Wulandari's (2022) research showed that administration of ethanol extract of noni leaves at doses of 250, 500, and 750 mg/g BW had the effect of lowering blood glucose levels, although the adequate amount was unknown in diabetic animal models induced by streptozotocin (Wulandari & Lakiu, 2022).

Traditional medicine carried out by the community is related to diabetes by utilizing herbs, including Moringa leaves and noni (Widiastuti et al., 2022). In Indonesia, Moringa leaves are commonly consumed as a vegetable with a taste that is less desirable to the public. Moringa leaves contain a variety of polyphenols, apart from quercetin-3-glucoside (Q3G), which lowers blood glucose levels. The leaves also contain flavonoids which can minimize diabetes complications (Kononenko et al., 2020).

Noni fruit, also known as *pace*, is a fruit with many benefits, containing serotonin, proxeronine and proxeroninase substances, scopoletin, rutin, quercetin, vitamin C, diacetyl asperuloside acid (DAA), kaempferol and secondary metabolites of flavonoids, tannins, saponins, alkaloids, steroids act as metabolites in repairing cell damage. With the existence of this enzyme, it is hoped that damage to pancreatic beta cells in DM sufferers can be overcome by re-functioning the pancreatic tissue and producing adequate insulin (Ghorbani, 2019).

With the above facts about the benefits of noni fruit and moringa leaves, the authors are interested in examining the effects of noni fruit and moringa leaf extracts on tissue histology and blood glucose levels in diabetic mice.

## METHOD

The design of this study was experimental: randomized pretest-posttest control group design.

The sample in this research is *Mus musculus* (mouse), male, healthy, adult, 2-3 months old, 20-40 gram body weight, taken by random allocation, injected with *streptozotocin* (STZ) so that it becomes an animal model of diabetes mellitus—the procedure of measuring blood glucose level using POCT and making histological tissue with Hematoxylin Eosin Staining.

Sample size: There are seven groups, so the minimum number in each group is four individuals, and the total sample is 28. This research was conducted at the Biochemistry Laboratory, Faculty of Medicine, Airlangga University, Surabaya. This research had ethical approval from the Medical Research Ethical Committee of Politeknik Kesehatan Kementerian Kesehatan Surabaya with reference number: EA/315/KEPK-Poltekkes_Sby/V/2020.

### Manufacturing of Diabetic Animal Models

During the acclimatization period of 1 week, the mice were fed ad libitum with a standard diet. The health of experimental animals is shown by monitoring body weight, hair loss, clear eyes, slimy noses, diarrhea, and motoric activity. Experimental animals were grouped into seven treatment groups, each consisting of 4 mice fed high in carbohydrates, moisture 12%, protein 20%, fat 4%, crude fiber 4%, Calcium 12%, Phosphorus 0.7% for two weeks, and drinking water ad libitum.

Making mice into a diabetic condition began with 28 *Mus musculus being* fasted for ± 4 hours, then measured body weight and blood glucose levels were. After the wound had dried, *Mus musculus* was injected intraperitoneally with 55 mg/kg BW streptozotocin *(STZ)* solution, then *Mus musculus* was given a 10% dextrose drink libitum and left in the cage for two days. On the third day, after the mice fasted for ± 4 hours, blood glucose levels and body weight were measured again—the procedure of measuring blood glucose levels using POCT and making histological tissue

with Hematoxylin Eosin Staining. The research was carried out by dividing randomly into seven treatment groups with four replications, consisting of 4 control and three treatment groups. The group consists of:

1. Pre-Test Group (diabetic mice)
2. Placebo control group (diabetic mice given water to drink)
3. The positive control group was given anti-DM drugs (provide a method of preparation diabetic mice were given metformin 195mg/kg body weight).
4. Negative control (normal mice given noni extract and moringa leaves)
5. Treatment group (1) diabetic mice were given noni fruit extract and Moringa leaves (composition 1:1) 150mg/200g BW/oral/day
6. Treatment group (2) Diabetic mice were given noni fruit extract and Moringa leaves (composition 2:1) 150mg/200g BW/oral/day
7. Treatment group (3) diabetic mice were given noni fruit extract and moringa leaves (composition 1:2) 150mg/200g body weight/oral/day

The mice ate and drank ad libitum for two weeks during the study period. At the end of the observation, hepatic and pancreatic tissue histology was examined, and blood glucose levels were measured.

The material used is *streptozotocin* (STZ), Dextrose 10%, Metformin, *pellet* starter feed, aqua dest, Moringa leaves, and noni fruit. Analytical balance, scale, mouse cage, oral probe, glucometer, Erlenmeyer flask, filter paper, *rotary evaporator, water bath*, scalpel, object glass, mortar.

**Preparation of Moringa Leaf Extract**

Moringa leaves are cleaned with water, drying temperature, and time until the water content is less than 10%, followed by blending and sifting to obtain a size of 40 mesh. Extraction using the maceration method requires 300g of Moringa leaf powder to be included in 96% ethanol with a ratio of 1:5 (w/v) in the macerator. Maceration was carried out for three days in a closed container while stirring. After three days, the pulp was filtered and soaked using 96% ethanol with a new ratio of 1:5 (w/v) 3 times. The extraction results are filtered and then concentrated with *a rotary vacuum evaporator* at 40$^{o}$ C (Jusnita & Syurya, 2019)

**Noni Extract Manufacture**

Washing and peeling ripe noni fruit is done before the extraction process begins, then sliced thinly, dried at 45$^{o}$C in an oven for 48 hours, then crushed with a blender and sifted until smooth. The extraction method used is maceration, starting with weighing 150grams of noni fruit powder with an analytical balance. Samples were put in 3 different Erlenmeyer flasks, with a type of solvent @150mL consisting of n-hexane, distilled water, and acetone. Erlenmeyer covered it with aluminum foil for 24 hours while shaking it using a *shaker*. The soaking results were filtered using a *Buchner funnel* to obtain the filtrate. The residue from the first extraction was added with solvents with a volume of 150 mL each, namely: n-hexane, distilled water, and acetone, then covered with aluminum foil for 24 hours while shaking using a *shaker*. Filtering is done with a Buchner funnel to obtain the filtrate. The combined filtrate from extractions 1 and 2 is then concentrated using *a rotary evaporator* until thick at 50$^{o}$ C (Hardani et al., 2020).

**RESULT**

The average blood glucose level after being induced with a dose of STZ of 55mg/kg body weight intraperitoneally increased overall; this indicates the success of the process of making diabetic animal models. The results of the research data are in the following table:

**Table 1. Blood Glucose Levels of Mice in Each Group Using POCT**

| Groups | Average blood glucose level (mg/dL) | | |
|---|---|---|---|
| | Pre Test | After being given STZ 55 mg/kg body weight intraperitoneally | Post Test |
| Pre Test group (Diabetic mice) | 55,5 | 138 | 152,5 |
| Placebo control group (Diabetic mice given water to drink) | 57,25 | 140 | 155,25 |
| The positive control group was given anti-DM drugs (diabetic mice were given Metformin 195mg/kg body weight) | 67 | 140 | 56,7 |
| Negative control (normal mice given noni extract and moringa leaves) | 66,25 | - | 70 |
| Treatment group (1) Diabetic mice were given noni fruit extract and Moringa leaves (composition 1:1) 150mg/200g BW/oral/day | 53,5 | 140,75 | 127 |
| Treatment group (2) Diabetic mice were given noni fruit extract and Moringa leaves (composition 2:1) 150mg/200g BW/oral/day | 71,25 | 149,75 | 102,75 |
| Treatment group (3) diabetic mice were given noni fruit extract and moringa leaves (composition 1:2) 150mg/200g BW/oral/day | 62,25 | 150,25 | 67,25 |

Table 1 shows that the average pre-test blood glucose level was 62.29mg/dL, while the blood glucose level after STZ and examined on day 3 had an average of 143.13mg/dL. This shows an increase in glucose levels due to the administration of streptozotocin.

From the results of histological examination of liver and pancreas tissue, the following results were obtained:

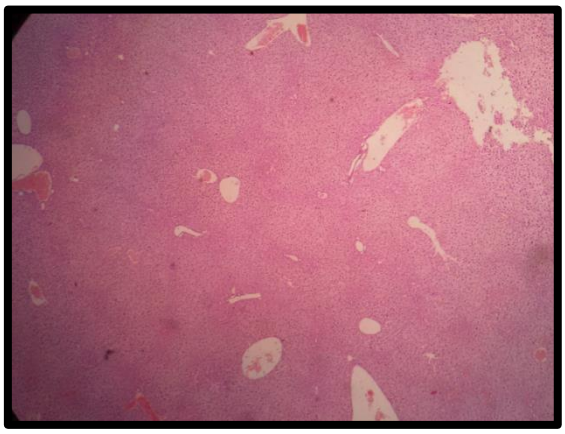

**Figure 1. Liver Tissue Microscopy (100x magnification)**

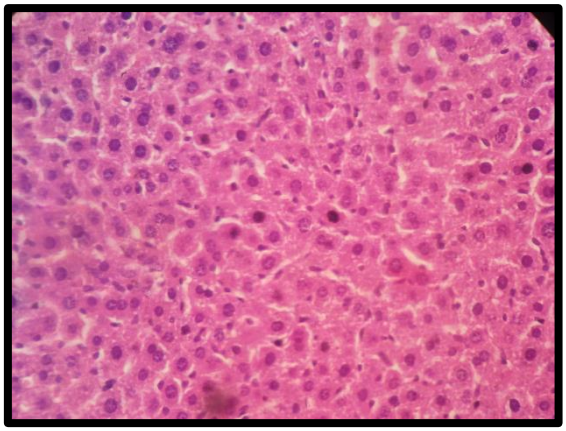

**Figure 2. Liver Tissue Microscopy (400x magnification)**

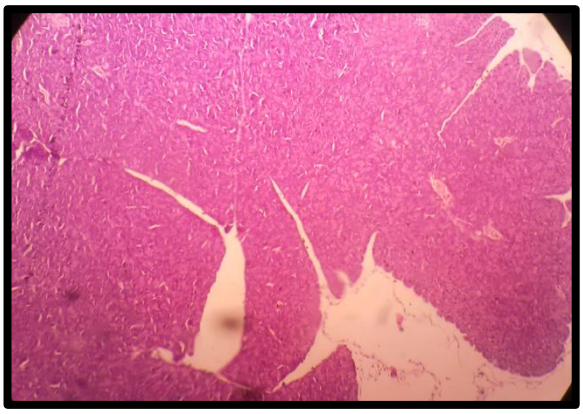

**Figure 3. Pancreas Tissue Microscopy (100x magnification)**

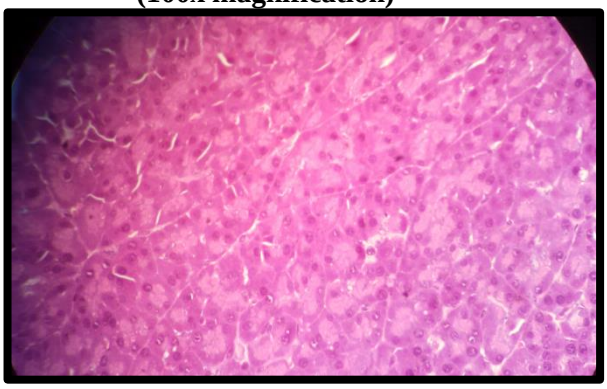

**Figure 4. Pancreas Network (400x magnification)**

From the picture above, it can be seen that there was no damage to the pancreas and liver tissue in the group of diabetic mice given noni fruit extract and Moringa leaves.

## DISCUSSION

Insulin-dependent diabetes mellitus (IDDM)/Type 1 diabetes and non-insulin-dependent diabetes mellitus (NIDDM)/Type 2 diabetes are types of disease characterized by hyperglycemia. Aggressive T lymphocytes due to

autoimmune disorders that infiltrate the pancreas and destroy insulin-producing β cells in the body of IDDM sufferers cause hypoinsulinemia and result in hyperglycemia. Diabetes complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy can occur due to people with diabetes not being appropriately treated.

Hyperglycemia that occurs due to genetic conditions with the contribution of environmental determinants occurs in the body of a patient with NIDDM; although genetically heterogeneous, there is a phenotype that supports the occurrence of diabetes. Insulin resistance in target tissues, especially in the liver, skeletal muscle, and adiposity, is an initial condition for NIDDM. This condition is related to excessive glucose production by the liver and impaired glucose utilization by peripheral tissues. In addition, NIDDM is influenced by ethnicity, degree of obesity, distribution of body fat, sedentary lifestyle, aging, and other accompanying medical conditions.

Diabetic therapy is carried out by (i) diet and exercise, (ii) insulin replacement therapy, and (iii) the use of oral hypoglycemic agents (sulfonylureas and biguanides) (Brunetti,2022). Many oral hypoglycemic agents have been produced, but their use with herbs has begun to be considered because synthetic drugs have effects such as hypoglycemia, weight gain, and drug resistance, so there are still challenges in managing diabetes without side effects. Many studies show that around 80% of the world's population depends on traditional medicines. Ethnobotanical details, antidiabetic and hypoglycemic potential of some plants suspected of having hypoglycemic activity in the presence of saponins.

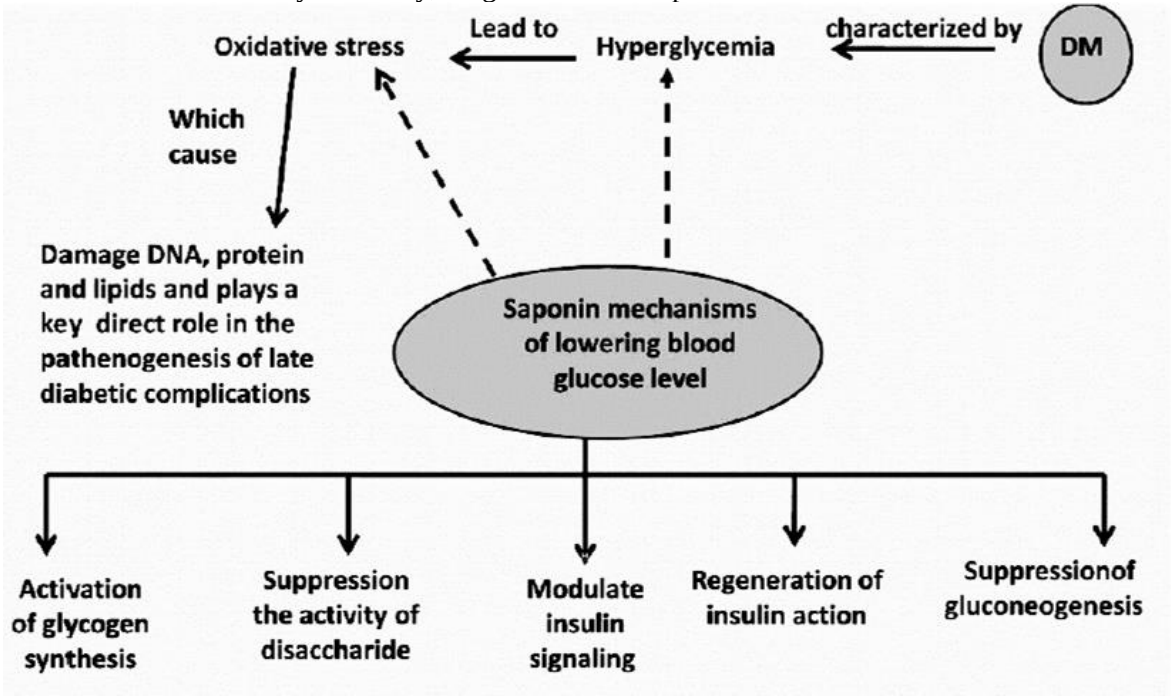


**Figure 5. Mechanism Action of Saponin in Diabetes (El Barky, 2017)**

Evaluation of the saponin constituents of medicinal plants seems to be a logical way to search for diabetes drugs. Indonesian people use many efficacious plants to treat diabetes, so consuming noni fruit and Moringa leaves can solve health problems. The reason for using noni fruit and Moringa leaves is because these plants are easy to find and the price is affordable (Widiastuti et al., 2022; Yanifa et al., 2021)

Streptozotocin at a dose of 55mg/kg body weight intraperitoneally has succeeded in producing a diabetogenic effect on pancreatic β-cells in experimental animals by inhibiting insulin production and secretion, resulting in hyperglycemia, with a marked increase in blood glucose levels from 62.29mg/dL to 143.13mg/dL (129%). STZ enters pancreatic β-cells utilizing the GLUT 2 glucose transporter, resulting in DNA changes. STZ produces *nitric oxide*, which destroys pancreatic beta cells by increasing the activity of the guanylyl cyclase enzyme, forming cGMP, and producing *nitric oxide*. STZ triggers the production of reactive oxygen, which damages pancreatic β cells. The formation of superoxide anions as reactive oxygen compounds in mitochondria will increase xanthine oxidase activity and increase oxidant levels in the body. In addition, STZ is an inhibitor of the Krebs cycle. It reduces the use of oxygen in mitochondria, reducing the amount of ATP in pancreatic β cells. (Saputra et al., 2018).

In the positive control group, diabetic mice were given Metformin as a glucose-lowering agent. Metformin has a mechanism of action by increasing the sensitivity of peripheral and hepatic tissues but does not affect insulin secretion. Metformin has the effect of activating *AMP-activated kinases* and increasing intestinal cells in using glucose, thereby reducing blood glucose levels (Kusuma, 2022).

The results of this study showed that the group with noni extract and Moringa leave 1:2 at a dose of 150mg/20g BW/oral/day for two weeks, and the diabetic group who were given Metformin 195mg/kg BW were also able to reduce blood glucose levels in mice. This can happen because the saponin content in Moringa leaf extract is 81g/kg (Arwani, 2018). Saponins are naturally occurring surface-active glycosides produced by plants, lower marine animals, and some bacteria. Saponins contain glucose, galactose, glucuronic acid, xylose, rhamnose, or methyl pentose, which are glycosidically linked to hydrophobic aglycones (sapogenins), which may be triterpenoid or steroidal. The complex structure of saponins is caused by (a) variations in the structure of the aglycones, (b) the nature of the side chains, and (c) the position of attachment of this group to the aglycones.

Saponins lower blood glucose by inhibiting glucose transport in the digestive tract and stimulating insulin secretion, astringents or chelating agents which can make the minor intestine epithelial membrane contract, thereby reducing the absorption of food essences and lowering blood glucose levels (Wulandari & Lakiu, 2022). Age's research shows that the content of various polyphenols in Moringa leaves lowers glucose levels (Age, 2021). *Morinda citrifolia L.* contains phenolics and flavonoids as antioxidants and absorbs glucose from the digestive tract into the blood vessels. The content of metabolites such as tannins, steroids, alkaloids, flavonoids, saponins, polyphenolics, and triterpenoids in herbs has the potential as an antidiabetic. Antioxidant activity in flavonoids can reduce the action of free radicals as a cause of damage and inhibit damage to pancreatic beta cells resulting in the regeneration of pancreatic beta cells through the process of mitosis. Repairs to pancreatic beta cells will affect increasing insulin production and decreasing blood glucose levels in the body (Yanifa et al., 2021)

## CONCLUSION

Based on the results of the study, it can be concluded that the administration of noni fruit extract (*Morinda citrifolia)* and Moringa leaves (*Moringa oleifera )* with a dose of 150 mg/20 g BW/oral/day for two weeks in diabetic animal models can produce the best decrease in blood glucose levels.

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