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RESEARCH ARTICLE

The Effect of *Moringa oleifera* Leaf Infusion on Reducing Blood Glucose and Total Cholesterol Levels in Mice (*Mus musculus*) with Diabetic Ulcers

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ABSTRACT

Background: Diabetes mellitus (DM) is a chronic disease in the form of metabolic disorders, characterized by blood glucose levels (BGL) that exceed normal limits and are accompanied by disorders of carbohydrate, lipid, and protein metabolism, as a result of abnormalities in insulin secretion. Diabetics are known to have slow wound healing. Moringa oleifera leaves contain flavonoid compounds, which can reduce glucose absorption.

Objective: This study aims to determine the effect of Moringa leaf infusion on blood sugar levels, glucose tolerance, cholesterol levels, body weight, and speed of wound healing in mice models.

Method: A total of 25 male mice aged 2-3 months weighing 25-30 grams were divided into 5 experimental groups: negative control, positive control, 10% Moringa infusion, 20% infusion, and 30% infusion. The negative control was not given any treatment. Positive controls were induced by a high-fat diet and PTU and given sucrose for 3 weeks. The infusion group was the same as the positive control, but at the end of each week, 10%, 20%, and 30% Moringa leaf infusion were given successively. Moringa leaf intervention was given for 3 weeks after feed induction, and observations were made during that range. A 1 cm long wound was made on the back after feeding induction.

Result: Moringa leaf infusion had a significant effect on reducing the levels of each variable:

- *A dose of 10% on body weight.*
- *A dose of 30% on BGL (KGD).*
- *A dose of 20% on BGL with glucose loading.*
- *A dose of 20% on cholesterol.*
- *A dose of 20% on wound closure. This was proven based on the results of statistical tests.*

Conclusion: Moringa leaf infusion can reduce blood sugar levels, blood sugar levels with glucose loading, cholesterol levels, body weight, and accelerate wound healing in mice induced by sucrose, high-fat diet, and PTU.

Key words: Diabetes mellitus, Moringa oleifera, mice

INTRODUCTION

Diabetes mellitus (DM) is a disease characterized by higher-than-normal blood glucose (BGL) levels, accompanied by disturbances in carbohydrate, lipid, and protein metabolism, due to abnormalities in insulin secretion. (1)

Based on the description above, this study was conducted to determine the effect of moringa leaf infusion on reducing BGL, BGL with glucose loading, total cholesterol, body weight, and wound healing in male mice.

According to the 10th edition of the Diabetes Atlas published by the International Diabetes Federation (IDF) in December 2021, it was reported that 1 in 10 adults worldwide is diagnosed with type 2 diabetes. It is also predicted that the number of DM sufferers will increase from 537 million in 2021 to 786 million in 2045. (2)

One plant that is quite well known among Indonesians as a blood glucose and cholesterol lowering agent is the moringa plant. The flavonoid content in moringa can regenerate pancreatic beta cells, thereby reducing blood glucose. Flavonoids in moringa can also inhibit pancreatic cholesterol esterase activity, thereby reducing plasma cholesterol concentrations. (3,4). Based on the description above, this study was conducted to determine the effect of moringa leaf infusion on reducing blood glucose, blood glucose with glucose loading, total cholesterol, body weight, and wound healing in male mice.

METHOD

This was a laboratory experimental study with a posttest-only controlled group design on male mice with hyperglycemia and hypercholesterolemia. The study was conducted in the Animal House Laboratory, Faculty of Medicine, Methodist University of Indonesia, from April to June 2022.

The tools and materials used in this study included animal cages, gloves, animal feeders, glucose meters, cholesterol meters, rulers, stirring rods, sieves, containers and stoves for making infusions, scales, syringes, probes, glass beakers, 25 male mice, moringa leaf infusion, sawdust, standard feed, high-fat feed, sucrose, PTU, tap water, and label paper.

Procedure

Making Moringa Leaf Infusion

Fresh moringa leaves were dried at room temperature for 4 days, then ground using a grinder. Ten grams of the dried moringa leaf powder was placed in a saucepan and 100 mL of distilled water was added. The pot was placed inside a larger pot filled with water and heated at 90ºC for 15 minutes. The infusion was filtered while hot using a flannel cloth. If the volume was less than 100 mL, warm water was added to the infusion residue until the volume reached 100 mL. The 20% and 30% infusions were prepared in the same manner, using 20 grams and 30 grams of moringa leaf powder, respectively.

Sucrose Solution Preparation

The sucrose dosage was calculated based on the sucrose dosage for mice, which is 3 g/kg body weight. The sucrose dosage to be used was calculated based on the body weight of each test animal, then dissolved in 0.5 ml of distilled water and administered to each test animal.

Preparation and Treatment of Test Animals: The test animals used were healthy male mice, aged 2-3 months, weighing 25-30 grams. Twenty-five mice were divided into five treatment groups. Before being treated, all mice were acclimatized for 1 week. Then Body weight, blood glucose (BGD), and total cholesterol were measured on the mice to ensure their health. All mice except the negative control group were then given an induction treatment with 3 g/kg of sucrose dissolved in 0.5 ml of distilled water and fed a high-fat diet. This treatment was given for 21 days. On the 21st day, body weight, blood glucose (BGD), and cholesterol were measured to confirm diabetes and hypercholesterolemia. The mice were then wounded on their backs to create a diabetic ulcer.

The mice were then randomly divided into five treatment groups as follows:

K1: Mice received no treatment, only normal food and water in their cages.

K2: Mice were induced with sucrose and a high-fat diet.

K3: Mice were induced with sucrose, high-fat feed, and 10% moringa leaf infusion

K4: Mice were induced with sucrose, high-fat feed, and 20% moringa leaf infusion

K5: Mice were induced with sucrose, high-fat feed, and 30% moringa leaf infusion

The treatments were administered daily for 21 days. Measurements were conducted three times, at the end of each week. Blood glucose (BGD) measurements were taken twice per measurement: one for mice and one for mice with a glucose load, two hours after feeding and induction. Body weight, cholesterol levels, and wound length were measured three times, at the end of each week, after the animals were fed and induced. Measurements were made using A Family Dr. glucometer and lipidometer, a scale, and a ruler were used.

The inclusion criteria for this study were male mice, healthy, 2–3 months old, and weighing 25–30 grams. The exclusion criteria were death of male mice during the study period.

The independent variable in this study was the dose of moringa leaf infusion, and the dependent variables were body weight, blood glucose (BGD), blood glucose with glucose loading, total cholesterol levels, and wound length of the mice, measured using test strips and a ruler.

Data Analysis

The data obtained from the observations were recorded and presented as mean ± standard deviation (mean ± SD). Data normality and homogeneity were tested. If the data were normally distributed and homogeneous, an ANOVA test was performed, followed by a post hoc test if applicable.

If the data were not normally distributed and homogeneous, a Kruskall-Wallis test was performed. All data analysis was performed using SPSS software. In this study, the statistical test decision was taken at a significance level of 5% ($p < 0.05$) which was considered meaningful or significant.

RESULTS

The ANOVA statistical test results showed a significant difference in body weight measurements, followed by a post-hoc Duncan test. A significant difference was found between the 10% infusion group and the positive control group, as seen in Table 1.

The ANOVA test also revealed a significant difference in blood sugar levels. Further

post-hoc Duncan tests showed a significant difference between the negative control and the 10% infusion group, as seen in Table 2.

Table 1 Average body weight of mice ± SD

Group	Mean ± SD	*p value*
Negative control	28.5 ± 2.34[c]	
Positive control	30.86 ± 1.29[d]	
10% Infusion	17.94 ± 0.7[a]	0.001
20% Infusion	24.2 ± 8.34[b]	
30% Infusion	19.78 ± 4.83[a]	

Table 2 Average blood sugar levels of mice ± SD

Group	Mean ± SD	*p value*
Negative control	91.6 ± 2.07[a]	
Positive control	125 ± 9.74[a]	
10% Infusion	173.6 ± 76.97[b]	0.035
20% Infusion	127.4 ± 6.95[a]	
30% Infusion	116.2 ± 29.49a	

The ANOVA statistical test results showed a significant difference in blood glucose measurements with glucose loading. This was followed by a post-hoc Duncan test, as shown in Table 3.
The ANOVA test revealed a significant difference in cholesterol measurements. Further post-hoc Duncan test results showed a significant difference

between the positive control and the other treatment groups, as shown in Table 4.
The ANOVA test revealed a significant difference in wound length measurements in mice. Further post-hoc Duncan test results showed a significant difference between the 20% infusion group and the positive control, as shown in Table 5.

Table 3 Average blood sugar levels with glucose loading of mice ± SD

Group	Mean ± SD	*p value*
Positive control	111 ± 5.78[b]	
10% Infusion	133.6 ± 25.22[b]	0.000
20% Infusion	110.6 ± 21.89[b]	
30% Infusion	116.2 ± 29.49[b]	

Group	Mean ± SD	*p value*
Negative control	91.2 ± 1.78[a]	
Positive control	121 ± 8.71[c]	
10% Infusion	112.4 ± 4.77[b]	0.000
20% Infusion	106 ± 6.04[b]	
30% Infusion	106.6 ± 4.45[b]	

Table 5 Average value of mouse wound length ± SD

Group	Rata-rata ± SD	*p value*
Positive control	0.64 ± 0.11[d]	
10% Infusion	0.62 ± 0.08[d]	0.000
20% Infusion	0.18 ± 0.14[b]	
30% Infusion	0.36 ± 0.11[c]	

The results of the experiment showed weight loss in the group given moringa leaves, but weight gain in both the positive and negative control groups. Significant differences were found between the 10% and 30% infusions compared to the two groups Control.

In the case of a high-fat diet, excess energy is supplied from fat breakdown, leading to storage in adipose tissue, particularly in excess of utilization. This triggers weight gain. Interventions with moringa leaf infusion, which affects the fat metabolism pathway, can reduce weight. (5)
Moringa leaves contain substantial amounts of flavonoids and alkaloids. The antioxidant activity of flavonoids and alkaloids can

regenerate and protect pancreatic cells and stimulate insulin release through the sympathetic nervous system's stimulant effect. Flavonoids have hypoglycemic activity by inhibiting key enzymes involved in the breakdown of carbohydrates into monosaccharides that can be absorbed by the intestines, namely α-amylase and α-glucosidase. Consequently, blood glucose levels decrease. (6)

There are two explanations for the rapid glucose clearance in mice. First, experimental results revealed that a very short and small insulin response is sufficient to clear the exposed glucose. This is because the glucose bolus in mice is cleared through an insulin-independent process. Even with normal insulin levels, induced hyperglycemia can provide a predominant stimulus to increase oral glucose uptake, thereby facilitating glucose uptake throughout the body and exogenous glucose clearance. This mechanism is related to the rapid metabolism of small animals, which is necessary for maintaining body temperature. The second mechanism is related to stress-induced catecholamines. Mice are susceptible to stress because it is associated with a defense mechanism against predators. When stressed, catecholamines are induced, affecting glucose metabolism. Catecholamines can causes increased endogenous glycolysis, inhibits glucose uptake by the liver and muscles, and increases the metabolic rate and glucose transport in brown adipose cells and the heart. (7)

The experimental results show that the cholesterol levels in the negative control group were significantly different compared to the other groups, indicating that the high-fat diet and PTU induction successfully triggered an increase in cholesterol levels. The data showed that the cholesterol levels of mice given 10%, 20%, and 30% Moringa leaf infusions were significantly lower than those in the positive control. However, these cholesterol levels did not reach the levels of the negative control. This indicates that Moringa leaves have an anti-hyperlipidemic effect. Based on the results of a network pharmacology analysis conducted by Sha et al. (2021), there are 219 potential targets of active compounds in M. oleifera and 185 targets of water-soluble active components for the treatment of hyperlipidemia. Furthermore, the potential active components of M. oleifera leaves can also inhibit hyperlipidemia-related metabolic inflammation by modulating the complement and coagulation cascade signaling pathways. (8)

This study found that administering moringa leaf infusion accelerated wound healing, as evidenced by the shorter wound length in the moringa leaf group. This is consistent with previous research by Vijay and Kumar (2012), which found that topical exposure of moringa leaf extract to wounds resulted in faster epithelialization and increased wound contraction. In this study, the oral route was used, and the accelerated wound healing effect was still evident. In vitro, moringa leaves were found to increase fibroblast proliferation and migration in the wound area. Systemically, it is possible that One way moringa leaves can induce accelerated wound healing is by increasing the inflammatory process, angiogenesis, re-epithelialization, and oxidative stress. (9,10)

CONCLUSION

Based on the research results, it can be concluded that the administration of Moringa leaf infusion has a significant effect on the reduction of body weight, blood sugar

levels, blood sugar levels with glucose loading, cholesterol, and wound length in mice.

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