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Effects of Moringa Leaves on Blood Glucose and Fatty Liver: An Experimental Animal Study

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ABSTRACT

Background: Moringa has been widely used in health care for its various biological activities. This study investigated the effects of moringa leaves on reducing blood glucose (GLU) levels and improving nonalcoholic fatty liver disease (NAFLD).

Methods: Twenty 4-week-old db/db mice and matched db/m mice were randomly assigned to a blank control group or a moringa treatment group. The mice were administered either deionized water or a moringa leaf solution via oral gavage. Blood GLU, body weight, and other physiological indices were monitored throughout the study. At week 8, all mice were anesthetized, and blood and liver tissue were collected for analysis.

Results: Compared with db/m mice, db/db mice exhibited elevated blood GLU, liver lipid accumulation, and oxidative stress. In db/db mice, moringa leaf treatment significantly reduced blood GLU, pathological hepatic changes, triglyceride content, inflammatory cytokine levels, and malondialdehyde content while increasing liver catalase, glutathione peroxidase, and total superoxide dismutase activities. Moreover, moringa leaves significantly lowered interleukin (IL)-6, IL-10, and IL-17 levels in db/db mice ($P < 0.05$).

Conclusion: Moringa leaves demonstrated significant effects in reducing blood GLU and alleviating liver cell damage caused by diabetes mellitus. These findings imply that moringa leaves may be a promising nutritional supplement or drug candidate for improving blood GLU and managing NAFLD.

Keywords: Moringa Leaf; Blood Glucose; Nonalcoholic Fatty Liver Disease; Liver; Oxidative Damage

Abbreviations: GLU: Glucose; NAFLD: Nonalcoholic Fatty Liver Disease; IL: Interleukin; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: Triglyceride; TC: Total Cholesterol; BUN: Blood Urea Nitrogen; T-SOD: Total Superoxide Dismutase; GSH-Px: Glutathione Peroxidase; T-AOC: Total Antioxidant Capacity; MDA: Malondialdehyde

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases, with a global prevalence of approximately 25% [1]. The prevalence of NAFLD in Shanghai, Guangzhou, Hong Kong, and other regions of China is approximately 15% [2]. Worldwide, the prevalence of NAFLD continues to rise each year, with onset occurring at increasingly younger ages [3]. Studies have shown that as a metabolic stress-induced liver injury, NAFLD is significantly associated with insulin resistance. For example, approximately 70% of patients with type 2 diabetes mellitus also have NAFLD; in patients with both diabetes mellitus and obesity, the incidence of NAFLD can be as high as 100% [4]. Patients with diabetes mellitus and NAFLD are at significantly higher risk of developing nonalcoholic steatohepatitis and advanced liver fibrosis [5,6]. NAFLD is also closely linked to metabolic syndrome. Reportedly, the detection rate of fatty liver in patients with

metabolic syndrome is approximately 48% [7]. In addition, NAFLD increases the risk of cardiovascular disease [8]. At present, there is no specific therapeutic drug for NAFLD. However, some plant constituents appear to improve liver fat metabolism, and it is expected that effective anti-NAFLD ingredients will be identified.

Moringa (Moringa oleifera) is a perennial tropical deciduous tree that is widely distributed in tropical marine climate regions such as Africa, Arabia, and Southeast Asia. It is commonly known as the horseradish tree because of its pungent rhizome. The roots, stems, leaves, flowers, and seeds of moringa are rich in medicinal and nutritional compounds and serve as high-quality raw materials for health foods [9-11]. Studies have shown that moringa leaves possess various bioactivities, including lowering blood GLU, antitumor effects, antibacterial activity, and free radical scavenging [12-14]. However, there are no reports on the use of moringa leaves to improve NAFLD. In this study, Zucker diabetic fatty db/db mice were used as a model of NAFLD and were given an aqueous solution of moringa leaf powder (hereinafter referred to as moringa leaves) as an intervention. The aim was to observe the effects of moringa leaves and explore their mechanisms, thus providing data that support the potential of moringa in treating NAFLD.

Materials and Methods

Materials

Twenty specific pathogen-free–grade 6- to 8-week-old male C57BL/KsJ db/db mice and 20 control db/m mice were purchased from the Institute of Biomedicine of Nanjing University (license number: SCXK(Su)2010-0001) and housed in laboratory animal barrier facilities at the Guangzhou Center for Disease Control and Prevention. Complete high-fat feed was obtained from the Guangdong Medical Laboratory Animal Center. During the experiment, mice had free access to food and water and were maintained under 12-hour light/dark cycles, with an ambient temperature of 22°C ± 2°C and humidity levels of 40–70%. A Roche Accu-Chek Performa glucometer and Accu-Chek Performa test strips, 1% pentobarbital sodium solution, and assay kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), free fatty acids, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (TC) were purchased from Mindray Bio-Medical Electronics Co., Ltd. (Shenzhen, China). Moringa leaf powder was extracted by Guangzhou Zeli Pharmaceutical Technology Co., Ltd. (Guangzhou, China) using its proprietary patented technology: "high-efficiency, high-pressure differential and low-temperature continuous extraction, separation and concentration technology and complete equipment (HHLSE)."

Grouping of Experimental Mice

After 1 week of adaptive feeding, the mice were randomly divided into a blank control group and a moringa leaf group according to body weight, with 10 C57BL/KsJ db/db mice and 10 db/m mice in each group. The blank control group received deionized water, while the moringa group received an aqueous solution of moringa leaf powder (200 mg/kg body weight) by oral gavage once daily for 8 consecutive weeks. The general condition of the mice in each group was observed and recorded. Body weight was measured every 2 weeks, and blood GLU and lipid levels were assessed using orbital venous blood samples.

Detection of Serum Biochemical Indices

After the mice had been weighed, they were anesthetized with 1% pentobarbital sodium, and fasting blood samples were collected from the abdominal vein. Samples were analyzed for ALT, AST, TG, TC, HDL-C, LDL-C, creatinine, blood urea nitrogen (BUN), and GLU using an automatic blood biochemical analyzer.

Detection of Oxidative Stress Indices

Liver tissue was homogenized and centrifuged, and the supernatant was collected to measure catalase, total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) using kits from Nanjing Jiancheng Bioengineering Institute, following the manufacturer's instructions.

Organ Coefficients and Histopathological Analysis

To calculate organ coefficients, the heart, liver, spleen, lung, kidney, and brain were dissected, rinsed with phosphate-buffered saline, blotted dry with filter paper, and weighed. Organs were fixed in 4% paraformaldehyde solution for 48 hours, then embedded in paraffin and sectioned at 5-μm thickness. The sections were stained with hematoxylin–eosin and examined under a light microscope to assess the pathological changes in each tissue.

Oil Red O Staining

Frozen liver tissue was sectioned and stained with diluted oil red O solution for 10–15 minutes, protected from light, sealed, and differentiated with 60% ethanol until the interstitial space appeared clear under the microscope. Sections were then counterstained with Mayer's hematoxylin, mounted, and examined for liver fat content. Image-Pro Plus 6.0 software was used for semiquantitative analysis. The optical density of red lipid droplets was measured in each field of view, and the mean optical density from five fields was calculated.

Detection of Multiple Cytokines Using Merck Milliplex Multiplex Assays

Plasma samples were collected and analyzed using a MAGPIX multiplexing instrument to detect the cytokines IL-2, IL-4, IL-6, IL-10, and IL-17. Data were processed using MILLIPLEX Analyst V5.1 software. All samples were tested in triplicate, and results were analyzed by variance analysis and correlation analysis using GraphPad Prism 9.

Statistical Analysis

All data are expressed as $\bar{x} \pm s$. Statistical analysis was performed using SPSS 29.0 software. Analysis of variance was used to assess differences between groups, with the least significant difference method applied for post hoc comparisons.

Results

Effects of Moringa Leaf on the Growth of Mice

The mice in the db/m control and moringa groups appeared in good condition, with active behavior and shiny fur. By contrast, the mice in the db/db control group showed signs of polydipsia, polyuria, polyphagia, and dull fur. Mice in the db/db moringa group also exhibited polydipsia, polyuria, and polyphagia, but to a lesser extent, and their fur was noticeably shinier. Compared with the db/m mice, the body weight of db/db mice increased rapidly during the first week and reached approximately twice that of the db/m control group. Analysis showed that moringa leaf treatment had no significant effect on body weight or growth trends in either the db/m or db/db mice ($P > 0.05$), as shown in Table 1.

Table 1: Effects of moringa leaves on the growth of mice.

Group	db/db		db/m	
Weight	Control Group	Moringa Leaf Group	Control Group	Moringa Leaf Group
Initial (g)	30.74±1.19**	31.37±1.32**	17.36±0.71	17.36±0.76
Week 2 (g)	35.61±1.31##	36.52±1.52##	20.94±0.71	17.13±0.76
Week 4 (g)	42.24±2.63##	43.70±2.43##	23.20±0.89	20.78±0.68
Week 6 (g)	44.39±3.17##	46.23±2.62##	23.71±0.41	22.49±0.82
Week 8 (g)	44.24±4.65##	45.68±2.07##	24.99±3.82	23.81±0.85

Note: *means being compared with the mice in the db/m control group, # means being compared with the mice in the db/db group, the same below.

Effects of Moringa Leaves on the Organ Coefficients of Mice

The organ coefficients of the liver, kidney, heart, lung, brain, and testis were all significantly lower in db/db mice than in db/m mice ($P < 0.05$). While there were no significant differences in organ coefficients between the db/m moringa group and the db/m control group ($P > 0.05$), the liver and spleen coefficients in the db/db moringa group were significantly lower than those in the db/db control group ($P < 0.05$), as shown in Table 2.

Table 2: Effects of moringa leaves on organ coefficients.

Item	db/db		db/m	
	Control Group	Moringa Leaf Group	Control Group	Moringa Leaf Group
Liver (%)	5.21 ± 0.33*	4.75 ± 0.40*#	6.99 ± 1.1	6.42 ± 1.67
Kidney (%)	1.11 ± 0.13#	1.14 ± 0.13	1.22 ± 0.13	1.31 ± 0.15
Heart (%)	0.31 ± 0.07#	0.29 ± 0.04#	0.41 ± 0.05	0.42 ± 0.05
Lung (%)	0.32 ± 0.05#	0.36 ± 0.05	0.47 ± 0.09	0.44 ± 0.06
Spleen (%)	0.24 ± 0.22	0.18 ± 0.05*	0.22 ± 0.07	0.21 ± 0.08
Brain (%)	0.61 ± 0.48#	0.49 ± 0.55#	0.69 ± 0.57	0.74 ± 0.43
Testis (%)	0.38 ± 0.10#	0.39 ± 0.10#	0.54 ± 0.11	0.56 ± 0.06

Effects of Moringa Leaves on Serum Biochemical Indices in Mice

Serum analysis showed that levels of ALT, AST, blood GLU, TG, BUN, HDL-C, and LDL-C were significantly higher in db/db mice than in db/m mice ($P < 0.05$). Serum levels of ALT, AST, blood GLU, and BUN were significantly lower in the db/db moringa group than in the db/db control group ($P < 0.05$), whereas HDL-C and LDL-C levels did not significantly differ between the two groups ($P > 0.05$), as shown in Table 3.

Table 3: Effects of moringa leaves on serum biochemical indices.

Item	db/db		db/m	
	Control Group	Moringa Leaf Group	Control Group	Moringa Leaf Group
ALT(IU/L)	170.06 ± 20.83**	68.84 ± 23.05*#	38.2 ± 8.02	20.9 ± 6.03
AST(IU/L)	218.2 ± 43.73##	88.8 ± 36.01**#	41 ± 11.5	52.8 ± 5.71
CHOL (mmol/L)	3.23 ± 0.44	2.98 ± 0.19	3.04 ± 0.51	2.96 ± 0.19
CREA (μmol/L)	30.66 ± 6.32	28.52 ± 3.13	30.54 ± 4.89	31.74 ± 3.79
GLU (mmol/L)	37.26 ± 1.10##	21.95 ± 1.86*##	7.75 ± 1.09	7.89 ± 1.56
TG (mmol/L)	3.07 ± 0.30#	2.94 ± 0.24#	1.02 ± 0.12	1.03 ± 0.17
BUN (mmol/L)	27.56 ± 2.91#	15.25 ± 2.10**#	5.65 ± 0.44	5.71 ± 1.47
HDL (mmol/L)	2.26 ± 0.12#	2.52 ± 0.52#	1.42 ± 0.58	1.52 ± 0.49
LDL (mmol/L)	2.26 ± 0.06#	2.12 ± 0.51#	1.52 ± 0.32	1.86 ± 0.86

Effects of Moringa Leaves on Blood GLU in db/db Mice

The blood GLU monitoring results showed that compared with db/m mice, db/db mice exhibited significantly higher blood GLU, reaching 20 mmol/L by the second week and remaining elevated throughout the experiment. Blood GLU levels were lower in the db/db moringa group than in the db/db control group. Additionally, the analysis showed that moringa leaves had no significant effect on blood GLU levels in db/m mice (Figure 1A).

Pathological Analysis of Mouse Liver

Histopathological analysis revealed that db/db mice exhibited prominent vacuolation and inflammatory cell infiltration in the liver. Treatment with moringa leaves reduced both lipid vacuole formation and inflammatory infiltration (Figure 1B). Oil red O staining further indicated that TG accumulation in the liver was more pronounced in the db/db mice, and the moringa leaf intervention effectively reduced this accumulation, as shown in Figure 1C.

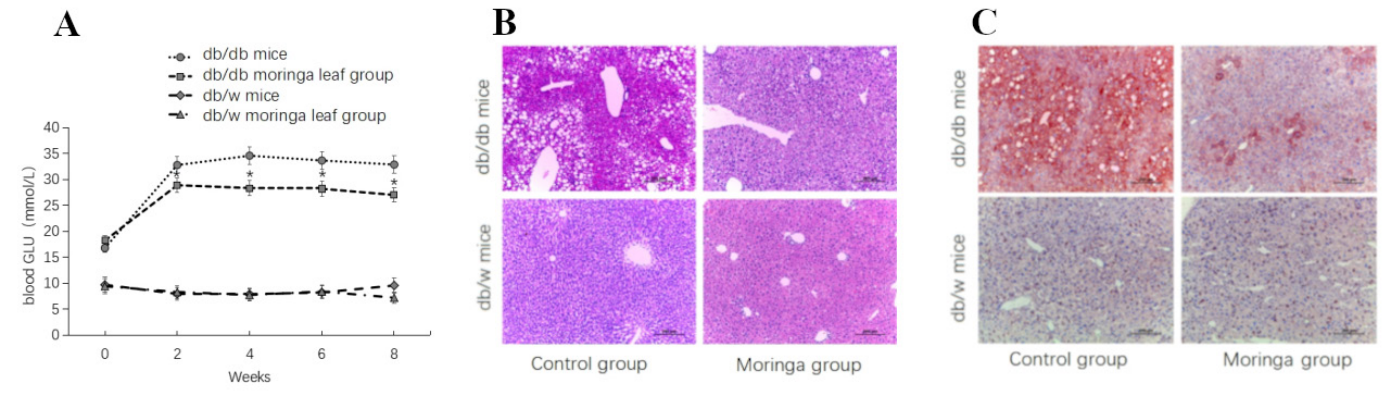

Figure 1: Effects of moringa leaves on blood GLU and fatty liver.
A. Comparison of blood GLU levels among groups.
B. Results of hematoxylin–eosin staining and
C. Oil red O staining of mouse liver.

Liver Oxidative Stress

Oxidative stress is a key factor in the development of steatohepatitis; therefore, we measured oxidative-stress-related indices in the liver, as shown in Table 4. In the db/m mice, moringa leaf treatment significantly increased levels of GSH-Px, T-AOC, and T-SOD ($P < 0.05$). Compared with the db/m control mice, levels of T-SOD and MDA were significantly higher in the db/db mice ($P < 0.05$). In the db/db mice, moringa leaves significantly increased GSH-Px, T-AOC, and T-SOD levels ($P < 0.05$), while reducing MDA levels ($P < 0.05$).

Table 4: Effects of moringa leaves on liver antioxidant function.

Item	db/db		db/m	
	Control Group	Moringa Leaf Group	Control Group	Moringa Leaf Group
CAT (U/mL)	21.6 ± 1.08	68.84 ± 3.44*#	19.98 ± 1	22.3 ± 1.12
GSH-Px (U/mL)	223.6 ± 11.18	425.6 ± 21.28*#	256.3 ± 12.82	299.8 ± 13.49*
T-AOC (U/mL)	212.5 ± 10.63	443.9 ± 22.20*#	256.2 ± 12.81	289.9 ± 14.50*
T-SOD (U/mL)	20.1 ± 1.01#	36.6 ± 1.83*#	13.3 ± 0.67	19.5 ± 0.83*
MDA (nmol/mL)	102.3 ± 5.12##	45.6 ± 2.28**#	28.6 ± 1.43	25.9 ± 1.3

Detection of Liver Immune Indices

The results of inflammatory cytokine detection are shown in Table 5. Compared with the db/m control group, levels of IL-6, IL-10, and IL-17 were significantly higher in the mice in the db/db control group ($P < 0.05$). Treatment of db/db mice with moringa leaves significantly reduced IL-6, IL-10, and IL-17 levels ($P < 0.05$).

Table 5: Effects of moringa leaves on inflammatory cytokines.

Item	db/db		db/m	
(ng/g)	Control Group	Moringa Leaf Group	Control Group	Moringa Leaf Group
IL-2	1456.3 ± 67.8	1356.6 ± 67.8	1425.2 ± 28	1596.6 ± 79.8
IL-4	7715.1 ± 440.5	6593.3 ± 329.7	8810.6 ± 31.5	7968.5 ± 398.4
IL-6	61256.4 ± 3062.8	43265.5 ± 2163.3*	31256.4 ± 0.5	33254.6 ± 1662.7
IL-10	8894.5 ± 444.7	5364.3 ± 268.2**	7894.5 ± 4.9	7854.6 ± 392.7
IL-17	7891.2 ± 394.6	6663.2 ± 333.2*	4891.2 ± 1.1	4587.9 ± 229.4

Discussion

NAFLD is closely associated with the onset and progression of chronic liver diseases such as cirrhosis and hepatocellular carcinoma, and increases the morbidity and mortality related to cardiovascular disease and cancer [4,15]. Spontaneously obese diabetic mice (db/db mice) exhibit significantly increased food intake because of defective leptin receptor expression and are prone to developing metabolic syndrome—including obesity, hyperinsulinemia, hyperlipidemia, hypertension, and impaired GLU tolerance—making them a valuable animal model for studying obesity, diabetes mellitus, and metabolic syndrome [16-19]. In this study, compared with normal mice, the db/db mice had significantly higher body weights and blood GLU levels ($P < 0.01$), elevated serum lipid levels ($P < 0.01$), and significantly increased serum AST and ALT levels ($P < 0.01$). Hematoxylin–eosin staining revealed clear fatty changes in the liver tissue of the db/db mice, with significantly greater lipid deposition than in the normal group ($P < 0.01$), as well as diffuse macrovesicular steatosis in hepatocytes.

The moringa tree is commonly referred to as the "miracle tree" in India. It is a nutrient-dense plant that contains a wide array of bioactive compounds with notable medicinal properties. It is particularly rich in essential vitamins, beta-carotene, and potent antioxidants. Additionally, moringa is a source of various phytochemicals—including flavonoids, phenolic acids, isothiocyanates, glucosinolates, tannins, and saponins—which contribute to its therapeutic potential [20,21]. Moringa exhibits numerous biological activities, such as blood GLU-lowering, lipid-lowering, antitumor, antioxidant, anti-inflammatory, immunoregulatory, and hepatoprotective effects. These health benefits are thought to be due primarily to its content of plant flavonoids, proteins, and fatty acids [22]. Studies have shown that moringa promotes glycogen synthesis. Moringa leaf extract can enhance glycogen synthase activity in diabetic rats, thereby promoting GLU uptake by the liver and muscles and stimulating insulin secretion to reduce blood GLU levels [23,24]. In this study, db/db mice were used as the model. After 4 weeks of feeding, the mice developed hyperglycemia and insulin resistance. The slower increase in blood GLU and elevated insulin levels in the moringa leaf group imply that moringa leaves reduced blood GLU and enhanced insulin secretion.

Our results demonstrate that moringa leaves significantly reduced body weight and liver coefficients ($P < 0.05$), as well as liver and serum TC and TG levels ($P < 0.05$) and serum transaminase levels ($P < 0.05$). Moringa treatment also significantly increased liver SOD activity and total GSH content ($P < 0.01$) while decreasing MDA levels ($P < 0.01$), effectively alleviating hepatic oxidative damage and fat accumulation and improving NAFLD to some extent. Additionally, moringa leaves had no significant effect on the physiological indices of normal

mice. Lipid accumulation and mitochondrial peroxidation in the liver can trigger oxidative stress and inflammatory responses, with inflammatory cytokines being key indicators in the onset and progression of steatohepatitis [25,26]. A previous study found that moringa leaves can alleviate the acute liver injury and abnormal cytokine secretion induced by acetaminophen [26]. The results of the present study show that in the db/db mouse model, levels of IL-6, IL-10, and IL-17 were significantly elevated ($P < 0.05$) and that moringa leaf treatment moderately reduced these levels ($P < 0.05$). These findings imply that moringa leaves may regulate inflammatory responses and oxidative stress by modulating the secretion of inflammatory cytokines.

Conclusion

In diabetic mice, moringa leaves can effectively reduce blood GLU levels, improve fatty liver and inflammation, and enhance the body's AOC. Moreover, moringa leaves showed no significant effects in normal mice. Therefore, moringa leaves may serve as a potential nutritional supplement or therapeutic agent for lowering blood GLU and treating hepatic steatosis. Further research is warranted to clarify their application prospects and therapeutic value.

Author Contributions

Conceptualization: M.S. and Y.Z.; Methodology: M.S. and G.Y.; Data curation: J.T. and J.W.; Formal analysis: M.S. and F.L.; Writing–original draft: M.S.; Writing–review & editing: Y.Z.; Supervision: Y.Z.

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Declaration of Interest

The authors declare no conflicts of interest related to this work.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

This study was approved by the Ethics Committee of the Guangzhou Center for Disease Control and Prevention and was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. All procedures performed were in line with ethical guidelines.

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