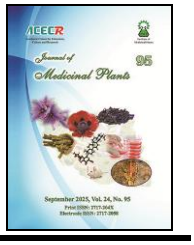
J. Med. Plants 2025; 24(95): 45-59

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Antidiabetic effects of *Morus alba* Linn leaf extract in a high-fat diet and streptozotocin-induced type 2 diabetic mouse model

Ha Thi Thu Nguyen[1], Ngoc Kim Nguyen[1], Hang Thi Thu Dinh[2], Quang Vinh Trinh[2], Quynh Thi Nhu Nguyen[2], Ha Thi Thanh Nguyen[2], Nam Vu[3], Nghia Trong Duong[3], Phong Xuan Pham[4], Van Anh Thi Pham[2,*]

[1]*Department of Traditional Medicine, Hanoi Medical University, Hanoi, Vietnam*
[2]*Department of Pharmacology, Hanoi Medical University, Hanoi, Vietnam*
[3]*Department of Traditional Medicine, National Hospital of Traditional Medicine, Hanoi, Vietnam*
[4]*Department of Traditional Medicine, Military Institute of Traditional Medicine, Hanoi, Vietnam*

ARTICLE INFO

Keywords:
Antioxidant activity
Diabetic mice
High-fat diet
Morus alba Linn
Streptozotocin

ABSTRACT

Background: The rising prevalence of diabetes mellitus underscores the need for safer therapeutic options, including natural products. *Morus alba* Linn leaf extract (MAE), a traditional remedy and the key component of the herbal product DIDALA, has been investigated for its potential antidiabetic properties. **Objective:** This study aimed to investigate the hypoglycemic and lipid-modulating effects of MAE in a type 2 diabetes (T2DM) experimental model induced by a high-fat diet (HFD) and streptozotocin (STZ) in mice. **Methods:** T2DM was induced in male Swiss mice by 8 weeks of an HFD followed by an intraperitoneal injection of STZ at 100 mg/kg. Diabetic mice were orally administered for 14 days with either a low dose MAE (0.8208 g/kg/day), a high dose MAE (2.4624 g/kg/day), or Gliclazide (80 mg/kg/day) as a positive control. Outcomes assessed included fasting blood glucose, lipid profiles (total cholesterol, LDL-cholesterol), liver and pancreatic malondialdehyde (MDA) as a marker of oxidative stress, and histological examination. **Results:** The low dose of MAE significantly reduced fasting blood glucose and improved lipid profile by lowering total cholesterol and LDL-cholesterol, with outcomes comparable to the Gliclazide group. It also reduced liver and pancreatic MDA levels, indicating decreased oxidative stress, and ameliorated histopathological alterations in the liver and pancreas. The high-dose MAE exerted less pronounced effects on glucose levels but still demonstrated some lipid and oxidative stress benefits. **Conclusion:** MAE, particularly at the lower dose, exhibits promising hypoglycemic and lipid-lowering effects in a HFD/STZ-induced T2DM mouse model, supporting its potential as a natural therapeutic candidate for diabetes management. Further studies should explore dose optimization, long-term efficacy, safety, and mechanisms of action.

Abbreviations: AMPK, AMP-activated protein kinase; DM/T2DM, Diabetes mellitus/Type 2 diabetes mellitus; DNJ, 1-Deoxynojirimycin; ELISA, Enzyme-linked immunosorbent assay; FBG, Fasting blood glucose; HDL-C, High-density lipoprotein-cholesterol; H&E, Hematoxylin and eosin; HFD, High-fat diet; LDL-C, Low-density lipoprotein-cholesterol; MAE, *Morus alba* Linn leaf extract; MDA, Malondialdehyde; NFD, Normal-fat diet; ROS, Reactive oxygen species; SD, Standard deviation; STZ, Streptozotocin; TC, Total cholesterol; TG, Triglyceride.

*Corresponding author: phamvananh@hmu.edu.vn

doi: 10.61882/jmp.24.95.45

Received 9 November 2024; Received in revised form 19 May 2025; Accepted 9 August 2025

© 2023. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/)

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

1. Introduction

Type 2 diabetes mellitus (T2DM) constitutes a significant global health burden, leading to chronic hyperglycemia and long-term complications affecting the heart, kidneys, eyes, and nerves. Type 2 diabetes mellitus (T2DM), accounting for over 95 % of diabetes cases, results from insulin resistance and progressive pancreatic *β*-cell dysfunction [1]. T2DM accounts for more than 95 % of diabetes cases and results from insulin resistance coupled with progressive pancreatic *β*-cell dysfunction [1]. The International Diabetes Federation reports a global T2DM prevalence of 10.5 % (536.6 million adults aged 20–79) in 2021, with an estimated rise to 12.2 % (783.2 million) by 2045 [2]. From 2000 to 2019, diabetes-related mortality increased by 70 %, placing DM among the top 10 causes of death worldwide [2]. Effective blood glucose management is, therefore, critical to mitigate complications, yet current treatments pose several challenges.

Persistent hyperglycemia necessitates careful management. Although conventional drugs derived from synthetic processes are widely used for DM treatment, they are often associated with adverse reactions such as hypoglycemia and hepatobiliary disorders [3]. Consequently, the discovery of new therapeutic agents, particularly those sourced from herbs, which offer comparable efficacy with minimized adverse effects, remains a primary objective for ongoing experimental and clinical investigations [4, 5].

To effectively evaluate potential new therapies for T2DM, researchers often utilize animal models that replicate key pathophysiological features of the human condition. The model combining a high-fat diet (HFD) with an intraperitoneal injection of streptozotocin (STZ) at a low dose is widely accepted for this purpose [6]. This approach effectively simulates the key features of human T2DM: the HFD promotes obesity and insulin resistance, while STZ induces partial *β*-cell destruction, thereby impairing insulin secretion [7,8]. This model closely mirrors human T2DM by combining dietary and metabolic stressors with *β*-cell dysfunction, offering advantages over single-agent models due to its pathophysiological similarity and reproducibility [9, 10].

Among herbal remedies, *Morus alba* Linn leaf extract (MAE), derived from the mulberry leaf (Moraceae family), has a long time of use in diabetes management in a traditional way. A key bioactive component of the leaves is 1-Deoxynojirimycin (DNJ), which acts as a potent *α*-glucosidase inhibitor to help lower blood glucose. This primary function is supported by the potent antioxidant such as quercetin and rutin, and anti-inflammatory properties of the extract, making it a multifaceted agent against T2DM [11, 12]. The herbal product DIDALA, with MAE as its main component, aims to support T2DM treatment. However, further rigorous studies are pivotal to comprehensively determine the efficacy of MAE, particularly within products like DIDALA, on blood glucose regulation [13]. Therefore, the primary objective of this research was to evaluate the blood glucose-lowering potential of MAE using an HFD/STZ-induced mouse model of T2DM.

2. Materials and methods

2.1. Plant materials and extract preparation

Leaves of *Morus alba* L. were sourced from MEDIPLANTEX Central Pharmaceutical Joint Stock Company, Vietnam, and authenticated according to the Vietnamese Pharmacopeia, Fifth Edition [14]. Fresh leaves were washed with ultrapure water, boiled twice at 100 °C for 3 hours in purified water, and the extract was concentrated at 100 °C until the humidity was below 25 %. The extract was dried at 80 °C to

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

achieve < 3 % humidity, pulverized into a fine powder (570 mg from 5 g leaves), and vacuum-sealed for storage at 25 °C and < 75 % humidity. The resulting extract, formulated into DIDALA capsules, was standardized to contain 0.6 mg/g of 1-Deoxynojirimycin (DNJ), as quantified by a certified liquid chromatography-mass spectrometry analysis (LC-MS; ISO/IEC 17025 & GLP). The prepared extract underwent rigorous quality control testing to ensure compliance with the Vietnamese Pharmacopeia, Fifth Edition [14], receiving official certification from MEDIPLANTEX (Registration number: 04P2-004A-20).

2.2. Experimental animals

Male Swiss mice (30 ± 5 g) were procured from the National Institute of Hygiene and Epidemiology, Vietnam. Animals were housed ten per cage under standard laboratory conditions (27 ± 2 °C, 80 ± 10 % humidity, 12-hour light/dark cycle) with free access to a standard diet and water. After random assignment into groups, the mice underwent a 7-day period to acclimate before the initiation of the study. The Scientific Board Committee of Hanoi Medical University approved all animal handling and experimental procedures (IRB: IRB00003121; Ethics Code: 4834/QD-DHYHN, October 27, 2020).

2.3. Experimental arrangement

2.3.1. Induction of T2DM in mice

Type 2 diabetes mellitus (T2DM) was induced using a high-fat diet (HFD) and streptozotocin (STZ; Sigma-Aldrich, Singapore). Mice were divided into Group 1, which received a normal-fat diet (NFD), and Group 2, which received an HFD (43 % saturated fat, 55 % fructose syrup) for 8 weeks [10, 15]. Fasting blood glucose was measured using the tail-vein-sampling technique before the study and after 8 weeks using an on Call EZII Glucometer (ACON Biotech, USA). Subsequently, Group 2 mice received a single injection of STZ (100 mg/kg body weight, i.p.) in citrate buffer (pH 4.5; Thermo Fisher Scientific, Singapore), while Group 1 received citrate buffer alone. After 72 hours, fasting blood glucose was measured, and mice with levels > 10 mmol/L were confirmed diabetic and included in the treatment phase.

2.3.2. Treatment Protocol

Diabetic mice were randomized into four groups (n = 10 each), and NFD mice were the control (Group 1). Treatments were administered orally for 14 days: Group 1 (Normal) and Group 2 (Diabetic model): Distilled water; Group 3 (Gliclazide): Gliclazide (80 mg/kg/day; Diamicron, Servier, France) [16]; Group 4 (Low-dose MAE): MAE (0.8208 g/kg/day); Group 5 (High-dose MAE): MAE (2.4624 g/kg/day).

Establishing appropriate testing doses based on multiple sources: the known safety profile of *Morus alba*, its documented efficacy in managing diabetic complications, official guidelines in the Vietnamese Pharmacopeia (5th ed.), and its long-standing traditional use [14, 17, 18]. Blood glucose levels were then monitored in overnight-fasted animals at three time points: before treatment (T0), and at weeks one (T1) and two (T2). At study completion, mice were euthanized under anesthesia using ketamine (100 mg/kg, i.p.) [19], and blood, liver, and pancreas samples were collected for analysis.

2.3.3. Biochemical analyses

Triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

C) in serum samples were measured using a biochemical analyzer (ERBA Chem., India) with its commercial diagnostic kits.

2.3.4. Malondialdehyde (MDA) assay

From each group, liver and pancreas samples (100 mg per tissue type) were collected from all animals on the last day of the study. Tissue homogenates for MDA analysis were prepared by rinsing samples in ice-cold saline, followed by homogenization in PBS (pH 7.4) and centrifugation (10,000 rpm, 15 min, refrigerated). The supernatant was stored at -20 °C until MDA concentrations were quantified from 100 µl aliquots using an ELISA kit (MBS269473, MyBioSource, USA) as per the manufacturer's protocol, with absorbance read on a BioTek® ELx808 Reader.

2.3.5. Histopathological assessment

Histopathological changes in the liver and pancreas were evaluated in tissue samples collected from three animals per group. After fixation in 10 % neutral buffered formalin, the tissues were subjected to paraffin embedding, sectioned, and stained with hematoxylin and eosin (H&E). Microscopic analysis and image acquisition were performed at 400x magnification using an Olympus BX10 microscope equipped with a DP12 digital camera.

2.3.6. Statistical analysis

All data are expressed as mean ± standard deviation (SD) and were analyzed using SPSS (Version 26.0). Differences between two groups were assessed using Student's t-test, while one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to compare three or more groups. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Effects on weight and fasting blood glucose levels in the diabetic induction period

After 8 weeks, the body weight of all mice increased significantly compared to their baseline weights before the study ($P < 0.001$). In comparison with the NFD group, mice fed the HFD showed a substantially greater increase in body weight, particularly after 8 weeks ($P < 0.05$) (Fig. 1A).

Mice in the NFD group exhibited relatively stable fasting blood glucose levels throughout the induction period. The blood glucose concentration in the HFD group significantly rose compared to the NFD group after 8 weeks of HFD ($P < 0.05$). Following STZ injection (72 hours), blood glucose concentration in the diabetic control group increased remarkably compared to both the NFD group ($P < 0.001$) and to its baseline before STZ injection ($P < 0.001$) (Fig. 1B).

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

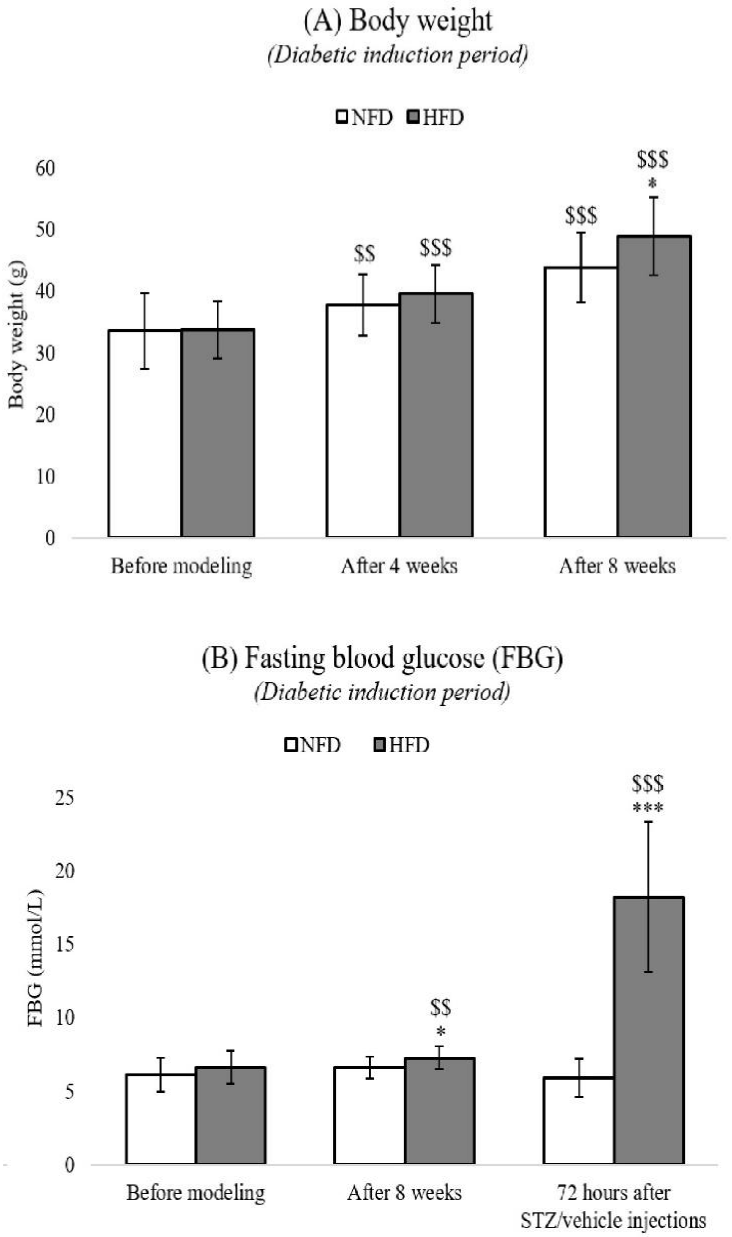


Fig. 1. Body weight and fasting blood glucose during the diabetic induction period. (A) Body weight changes in the NFD group (n = 10) or the HFD group (n = 40); (B) Fasting blood glucose (FBG) changes in NFD and HFD groups. $^{\$\$,\$\$\$}$: $P < 0.01$, $P < 0.001$, respectively, compared to baseline; *,***: $P < 0.05$, $P < 0.001$, respectively, compared to NFD group.

3.2. Effects of MAE on weight and fasting blood glucose in the treatment period

Mice in the normal control group exhibited normal weight gain, with a statistically significant increase after 2 weeks of treatment ($P < 0.05$ compared to T0). Mice in the diabetic control, gliclazide, and high-dose MAE groups showed a sustained decrease in body weight over the 2 weeks of treatment. The low-dose MAE group maintained its body weight, with a tendency to increase, but no marked changes were observed compared to T0 ($P > 0.05$) (Fig. 2A).

The gliclazide-treated group showed a reduced fasting blood glucose effect, which is

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

significant when compared to the diabetic control group at 2 weeks ($P < 0.05$). In comparison with the diabetic control group, mice receiving low-dose MAE (0.8208 g/kg/day) for two consecutive weeks also had significantly decreased blood glucose levels at both 1 week and 2 weeks ($P < 0.05$). Treatment with high-dose MAE (2.4624 g/kg/day) tended to reduce blood glucose levels after 1 week compared to the diabetic control group. Still, this effect was not statistically significant and was not observed after two weeks ($P > 0.05$) (Fig. 2B).

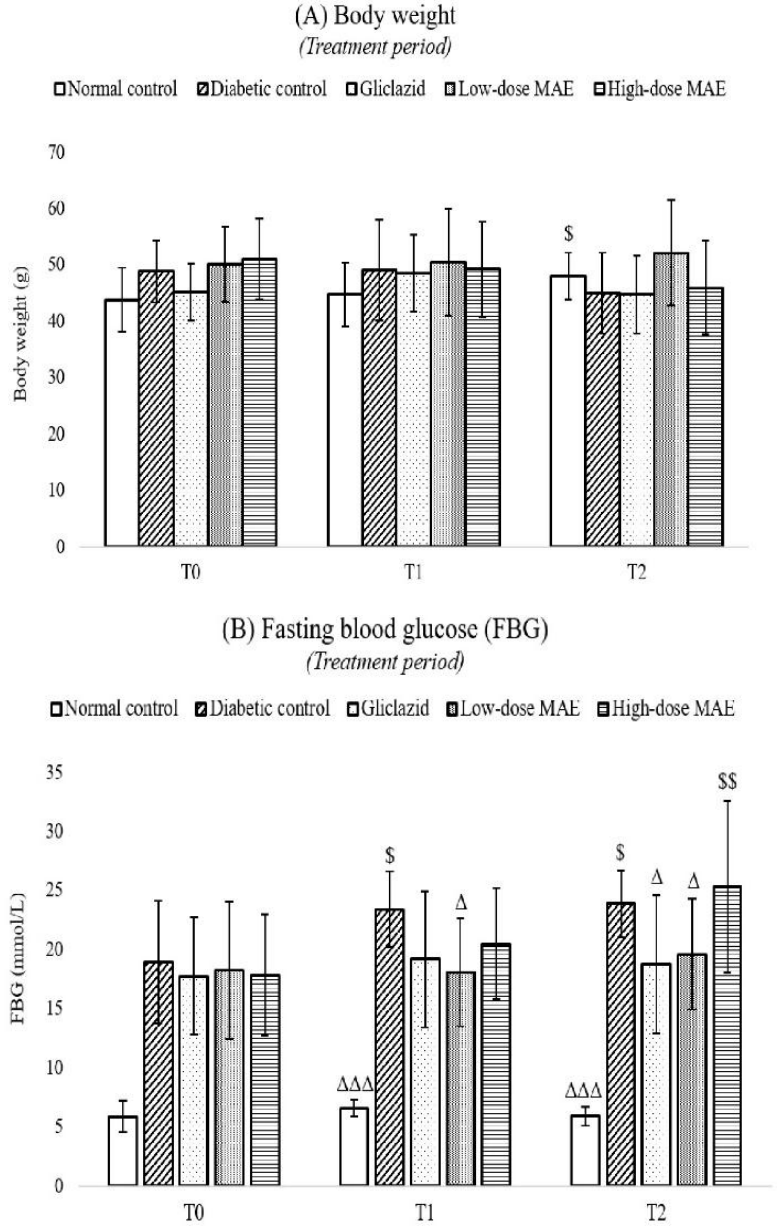


Fig. 2. Body weight and fasting blood glucose during the treatment period. (A) Body weight changes in normal control, diabetic control, gliclazide, low-dose MAE, and high-dose MAE groups; (B) Fasting blood glucose (FBG) changes in the same groups (n = 10). T0, T1, T2: before treatment, after 1 week of treatment, and after 2 weeks of treatment. $^{\$, \$\$}$: $P < 0.05$, $P < 0.01$, respectively, compared to T0 for that group; $^{\Delta, \Delta\Delta\Delta}$: $P < 0.05$, $P < 0.001$, respectively, compared to the diabetic control group at the same time point.

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

3.3. Effect of MAE on blood lipid profiles

In comparison with the normal control group, concentrations of TC, TG, and LDL-C in the HFD-STZ-induced diabetic control group were significantly increased, while HDL-C was also elevated ($P < 0.01$ or $P < 0.001$). Gliclazide (80 mg/kg/day) for 2 weeks tended to reduce TC, TG, and LDL-C compared to the diabetic control group, but such changes were not statistically significant ($P > 0.05$ for TC, TG, LDL-C; HDL-C remained significantly elevated compared to normal control). Both low and high doses of MAE significantly reduced LDL-C compared to the diabetic control group ($P < 0.001$ and $P < 0.05$, respectively). The low-dose MAE (0.8208 g/kg/day) substantially reduced TC levels compared to the diabetic control group ($P < 0.001$), whereas the high-dose MAE did not produce a statistically significant effect. Low-dose MAE significantly reduced HDL-C compared to the diabetic control group ($P < 0.01$) (Table 1).

3.4. Effect of MAE on MDA concentration

In comparison with the normal control group, liver and pancreatic MDA levels in the diabetic control group were significantly increased ($P < 0.01$ for liver MDA; $P < 0.05$ for pancreatic MDA). Treatment with MAE at both low and high doses significantly decreased MDA levels in both liver ($P < 0.01$) and pancreas ($P < 0.05$) compared to the diabetic control group. While gliclazide treatment did not significantly alter pancreatic MDA levels ($P > 0.05$), it effectively reduced them in the liver ($P < 0.05$) compared to the diabetic control group (Fig. 3).

3.5. Effect of MAE on liver and pancreas histopathology

Histopathological examination showed that the diabetic control group experienced degenerative changes in the liver, such as hepatocyte disarray and vacuolar degeneration, and in the pancreas, characterized by deformed islets of Langerhans and degenerated beta cells, when compared to the normal control group, which showed normal liver and pancreas architecture. Treatment with gliclazide and MAE (both low and high doses) ameliorated these histopathological changes in both the liver and pancreas compared to the diabetic control group, indicating a protective effect (Figures 4 and 5).

Table 1. Blood lipid index after 2 weeks of MAE treatment

Group	Lipid markers (mmol/L)			
	TG	**TC**	**LDL-C**	**HDL-C**
Normal control	1.09 ± 0.10	1.93 ± 0.25	0.91 ± 0.30	0.53 ± 0.09
Diabetic control	1.62 ± 0.53	3.11 ± 0.52***	1.44 ± 0.37**	0.94 ± 0.23***
Gliclazide	1.48 ± 0.49*	2.99 ± 0.45***	1.36 ± 0.41*	0.98 ± 0.20***
Low-dose MAE	1.96 ± 0.38***	2.19 ± 0.33$^{\Delta\Delta\Delta}$	0.62 ± 0.21$^{\Delta\Delta\Delta}$	0.68 ± 0.09**$^{\Delta\Delta}$
High-dose MAE	2.01 ± 0.46***	2.74 ± 0.57**	1.00 ± 0.24$^{\Delta}$	0.82 ± 0.25*

*,**,***: $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the normal control (Group 1);

$^{\Delta}$, $^{\Delta\Delta}$, $^{\Delta\Delta\Delta}$: $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the diabetic control (Group 2).

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

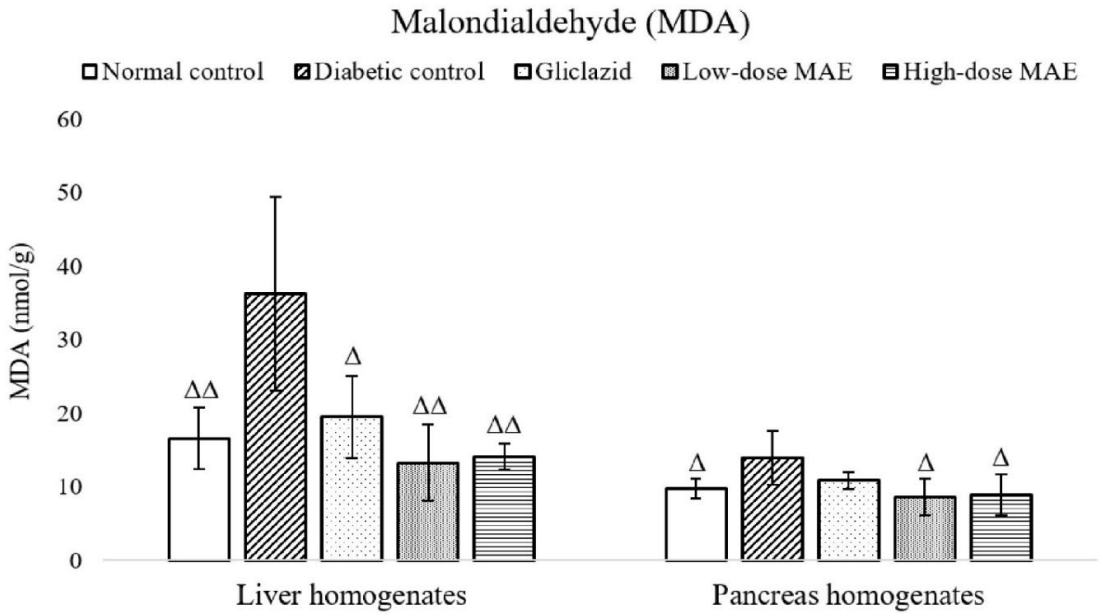


Fig. 3. Liver and pancreatic MDA concentrations after 2 weeks of MAE treatment. Bar graphs showing MDA levels in liver and pancreas homogenates for normal control, diabetic control, gliclazide, low-dose MAE, and high-dose MAE groups (n = 10). $^{\Delta}$, $^{\Delta\Delta}$: $p < 0.05$ and $P < 0.01$ compared to the diabetic control group.

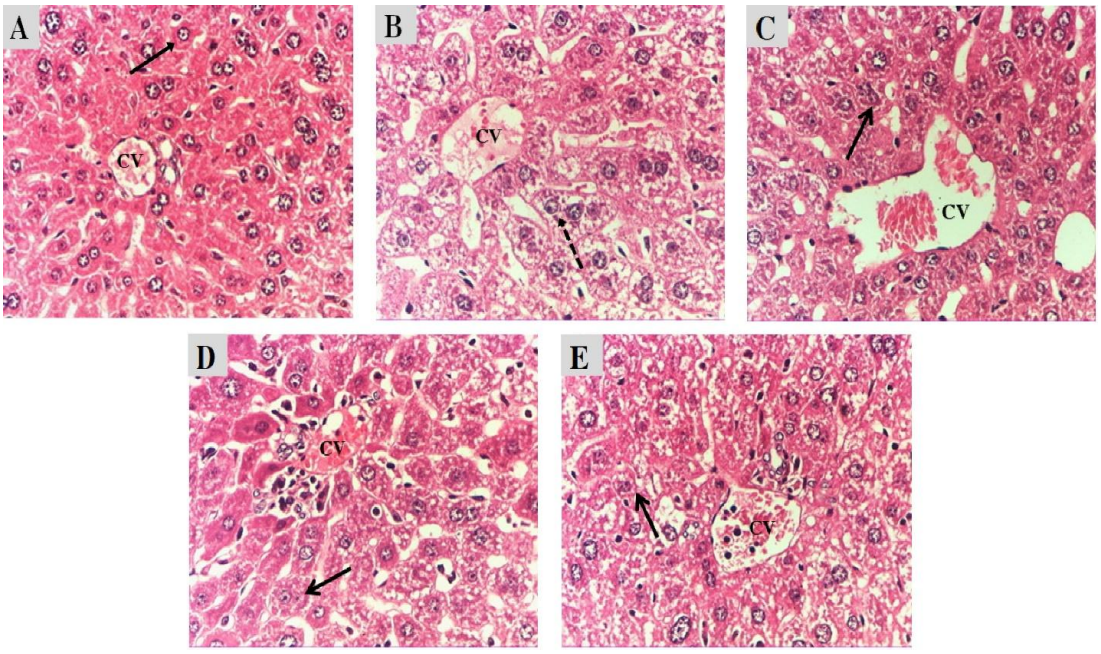


Fig. 4. Microhistopathological images of the liver (H&E staining, x400 magnification). (A) Normal control group shows normal hepatocyte architecture; (B) Diabetic control group shows hepatocyte disarray, sinusoidal congestion, and moderate vacuolar degeneration of hepatocytes (dashed arrow); (C) Gliclazide group shows reduced degeneration compared to the diabetic control group, with some mild vacuolar degeneration (open arrow); (D) Low-dose MAE group shows significant improvement, with mostly normal hepatocytes and some areas of mild vacuolar degeneration (open arrow); (E) High-dose MAE group shows improvement compared to the diabetic control, with evidence of mild to moderate vacuolar degeneration of hepatocytes. (Solid arrow: normal hepatocyte; Dashed arrow: moderate vacuolar degeneration of hepatocytes; Open arrow: mild vacuolar degeneration of hepatocytes; CV: central venule.)

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

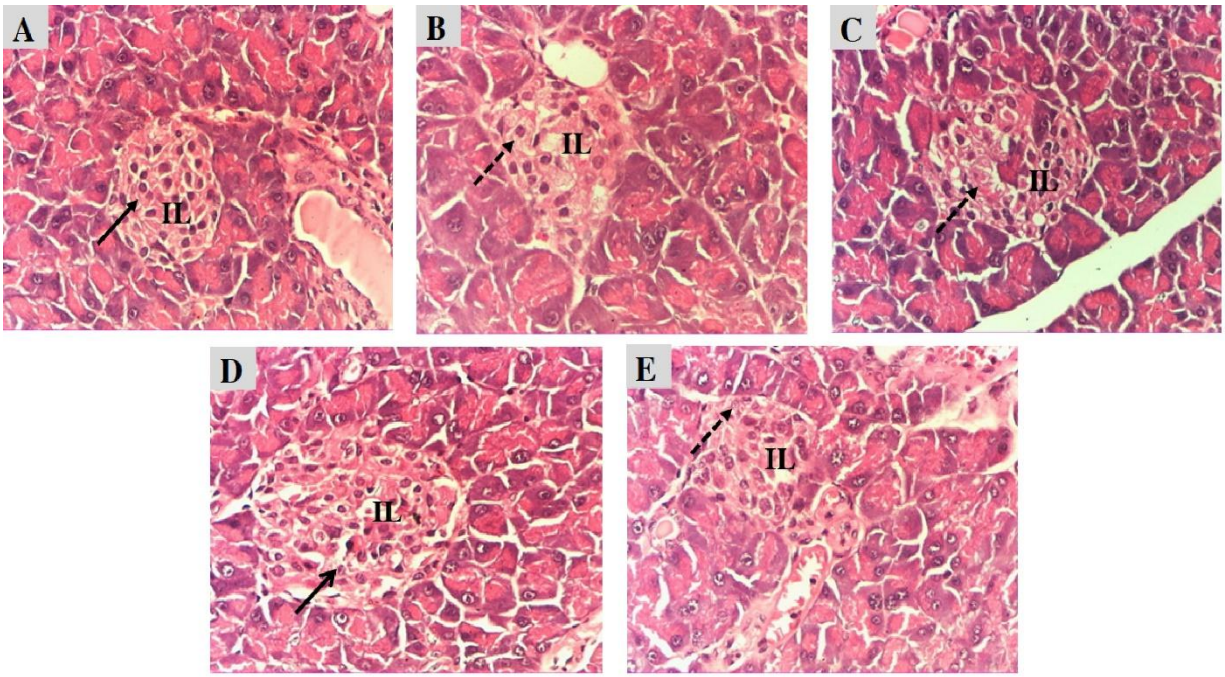


Fig. 5. Microhistopathological images of the pancreas (H&E staining, x400 magnification). (A) Normal control group shows normal pancreatic acini and well-defined islets of Langerhans with intact beta cells (solid arrow); (B) Diabetic control group shows degenerated beta cells, shrunken and deformed islets, and reduced islet size (dashed arrow indicates degraded cells); (C) Gliclazide group shows some recovery in islet structure, though some deformation and degradation of pancreas cells may persist (dashed arrow); (D) Low-dose MAE group shows noticeable improvement with better-preserved islet morphology, slight increase in islet size, and reduced beta cell degeneration; some mild degradation of pancreas cells (open arrow) may be present; (E) High-dose MAE group shows some pancreatic islets with deformation and degradation of cells (dashed arrow), indicating less protection compared to low-dose MAE. (Solid arrow: normal pancreas cell; Dashed arrow: degraded pancreas cells; Open arrow: mild degradation of pancreas cell; IL: islet of Langerhans.)

4. Discussion

Diabetes mellitus (DM) continues to be a major public health concern worldwide. At its core, the disease is defined by a profound dysregulation of metabolism. The pathophysiology of T2DM typically involves adiposity-induced insulin resistance coupled with progressive pancreatic β-cell dysfunction [20]. To evaluate potential therapeutic interventions effectively, animal models that closely approximate human T2DM pathophysiology are essential. The present study utilized a combined HFD and STZ approach to establish a T2DM model in mice. Streptozotocin (STZ), characterized by its diabetogenic properties, selectively targets pancreatic β-cells while preserving α and δ cells [7, 9]. Reed et al. first proposed the combination of a single STZ injection with HFD to induce β-cell dysfunction in 2000 [8], a protocol subsequently validated by numerous investigations demonstrating its capacity to recapitulate key features of human T2DM [10, 15]. This model offers both implementation simplicity and pathophysiological relevance. Our study utilized male mice exclusively, given the documented resistance of female mice to STZ-induced diabetogenesis [21] and the potential confounding effects of hormonal fluctuations on experimental outcomes [11]. The findings that HFD-conditioned mice treated with STZ exhibited remarkable weight gain and

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

hyperglycemia confirm the successful establishment of a T2DM model characterized by the hallmark features of adiposity and hyperglycemia.

During the treatment period, the body weights of mice exhibited notable differences across groups. The normal control group exhibited steady weight gain, consistent with healthy growth. In contrast, the diabetic control, gliclazide, and high-dose MAE groups experienced a sustained weight decrease over the 14-day treatment period, likely due to the catabolic state induced by persistent hyperglycemia and insulin deficiency in the diabetic control group, or potential side effects of gliclazide, such as gastrointestinal disturbances [22]. However, the low-dose MAE group maintained body weight with a non-significant tendency toward weight gain. This stabilization suggests that low-dose MAE may mitigate the catabolic effects of diabetes, potentially by improving insulin sensitivity or glucose utilization [23, 24]. Our findings align with those of Zhao et al. (2022), who demonstrated that polysaccharides derived from MAE could ameliorate metabolic disorders [25], as well as 1-deoxynojirimycin (DNJ) in *M. alba*, which enhances glucose metabolism, both of which may contribute to weight stabilization by reducing muscle wasting [23]. These results suggest that low-dose MAE may confer metabolic benefits without the adverse weight gain associated with conventional antidiabetic interventions [26].

Administration of MAE at 0.8208 g/kg/day for 2 weeks significantly reduced fasting blood glucose levels compared to untreated diabetic controls, with efficacy comparable to Gliclazide (80 mg/kg/day). Paradoxically, high-dose MAE (2.4624 g/kg/day) demonstrated diminished glycemic control, particularly after two weeks of administration, where its efficacy appeared to worsen compared to its effect at 1 week. The primary hypoglycemic mechanisms of MAE likely involve DNJ-mediated α-glucosidase inhibition since DNJ enhances glucose uptake in peripheral tissues [23]. A key mechanism of 1-deoxynojirimycin (DNJ) is the attenuation of postprandial glycemic excursions. It achieves this by acting as a potent inhibitor of intestinal sucrase and isomaltase, thereby retarding the breakdown of carbohydrates into absorbable monosaccharides [23]. Additionally, MAE contains fagomine, which not only inhibits α-glucosidase but also lowers the risk of developing insulin resistance and facilitates gut microbiota modulation [27]. Our results corroborate findings by Wang et al. that mulberry extract (100-200 mg/kg) effectively ameliorated hyperglycemia through multiple mechanisms, including strong α-glucosidase inhibitory and radical-scavenging effects [28]. The same results are observed in T2DM rats thanks to the important role of chlorogenic acid and rutin that comes from MAE [29]. However, this inverse dose-response relationship represents an intriguing pharmacological phenomenon that warrants mechanistic exploration. High-dose MAE may induce saturation of enzymes, which are responsible for carbohydrate digestion and glucose absorption, because inhibitory activities for sucrase, maltase, and isomaltase are competitive [30]. In addition, the increased concentration of DNJ and other compounds may lead to reduced absorption efficiency in the gastrointestinal tract due to saturation of transporters or enzymatic metabolism [31]. Finally, the higher dose may have exerted pro-oxidant effects since excessive concentrations of certain polyphenols transition from antioxidant to pro-oxidant activity [32], leading to off-target effects, such as mild

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

hepatotoxicity, pancreatic stress, or other gastrointestinal adverse reactions, which could impair glucose regulation. This might explain the less pronounced histopathological improvements in the high-dose group compared to the low-dose group, despite reductions in liver and pancreatic MDA levels.

MAE administration significantly improved lipid profiles, primarily by reducing total cholesterol and LDL-C concentrations. These findings align with observations by Jia-Shang Li et al., who attributed the lipid-lowering effects of mulberry leaf to its alkaloid, flavonoid, and polysaccharide constituents [24]. This effect may be mediated by AMP-activated protein kinase (AMPK), which is a key regulator of energy and lipid metabolism, directly phosphorylating SREBP-1c's analogous target, lowering the mRNA production for SREBP-1c and inhibiting the synthesis of fatty acids. After the mulberry leaf extract intervention, activation of AMPK not only promotes the oxidation but also inhibits the synthesis of fatty acids [33]. Although this study did not directly assess AMPK activation, the observed reductions in total cholesterol and LDL-C by MAE are consistent with the known downstream effects of AMPK activation. AMPK acts as a cellular energy sensor; its activation typically leads to inhibition of cholesterol synthesis and fatty acid synthesis [34], and further studies would be conducted to compare MAE with metformin, a well-known AMPK activator, to strengthen the scientific evidence [35]. Compounds found in Morus alba, such as flavonoids (e.g., quercetin, rutin mentioned in the introduction) and potentially DNJ, have been reported in other studies to modulate AMPK activity [24, 33]. The lack of direct AMPK measurement in our study limits mechanistic conclusions, but the observed lipid profile improvements support a potential AMPK-mediated pathway, warranting further investigation with enzymatic assays or gene expression analyses.

The antioxidant properties of MAE were evident through an improvement in MDA levels of liver and pancreas samples, indicating a reduction in oxidative stress. Malondialdehyde (MDA) is a well-established marker of lipid peroxidation, reflecting damage caused by reactive oxygen species (ROS) to cellular membranes. Chronic hyperglycemia in diabetes promotes ROS generation, leading to increased oxidative stress, which plays a crucial role in the progression of diabetic complications and organ damage [36]. This study demonstrated that both low and high doses of MAE significantly decreased MDA levels in these organs. This can be explained by the fact that it contains antioxidant compounds such as quercetin and rutin known to be present in Morus alba leaves [5,37]. These compounds can scavenge free radicals, chelate pro-oxidant metal ions, or upregulate endogenous antioxidant enzyme systems [5]. The reduction in oxidative stress, as indicated by lower MDA levels, likely contributed to the amelioration of hepatic and pancreatic histopathological changes observed in MAE-treated groups. By mitigating oxidative damage, MAE (formulated as DIDALA hard capsules) may help preserve organ function and protect against the detrimental effects of diabetes. The findings are in line with numerous studies highlighting the antioxidant potential of MAE and their protective effects in various models of oxidative damage [28, 38]. This is one of the drug's antioxidant mechanisms, which supports studies on the qualities of cell protection to prevent problems from diabetes. However, more in-depth experimental studies, with different oxidative markers examinations, are still necessary.

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

In summary, MAE, particularly at 0.8208 g/kg/day, demonstrates robust hypoglycemic, lipid-lowering, and antioxidant effects in an HFD-STZ-induced T2DM mouse model, with potential AMPK-mediated mechanisms and significant MDA reduction contributing to its efficacy. These findings support using MAE as a promising natural therapeutic agent for T2DM management. Further studies are needed to elucidate AMPK activation, quantify antioxidant enzyme activities, compare MAE to other established agents in the management of diabetes mellitus with its complications, and validate these results in clinical trials.

5. Conclusion

After 14 days of treatment, both low (0.8208 g/kg/day) and high (2.4624 g/kg/day) doses of MAE significantly lowered fasting blood glucose, total cholesterol, and LDL-cholesterol in the HFD/STZ-induced diabetic mice. The treatment also markedly reduced MDA levels in both the liver and pancreas, indicating a reduction in systemic oxidative stress. The low-dose MAE (0.8208 g/kg/day) showed effects comparable to the reference drug gliclazide, with additional improvements in histopathological changes in the pancreas. These findings suggest that MAE formulated in products like DIDALA hard capsules, particularly at the lower dose, holds promise as a natural therapeutic agent for managing type 2 diabetes mellitus, warranting further mechanistic and clinical studies.

Author contribution

HTTN: Supervision, Conceptualization, Resources; NKN: Conceptualization, Project administration, Methodology, Formal analysis, Original writing; HTTD: Data curation, Formal analysis, Writing – review & revision; QVT: Data curation, Formal analysis, Software, Writing – review & revision; QTNN: Investigation, Data curation, Original writing; HTTN: Project administration, Methodology, Supervision, Original writing; NV: Resources, Software, Investigation; NTD: Resources, Software, Writing – review & revision; PXP: Methodology, Supervision, Visualization; VATP: Conceptualization, Supervision, Project administration, Investigation, Visualization, Validation, Writing – review & revision.

Acknowledgement

The authors acknowledge the Department of Physiology at Hanoi Medical University (Hanoi, Vietnam) for its support. We also extend our gratitude to the HMU staff for their invaluable technical assistance and advice.

Conflict of interest

The authors declare no conflicts of interest in relation to the content of this article.

Funding

The authors received no external funding for the study, authorship, and/or publication of this work that could have influenced its outcome.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; 33(Suppl 1): S62-S69. doi: 10.2337/dc10-S062.

2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ and Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045.

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

Diabetes Res. Clin. Pract. 2022; 183: 109119. doi: 10.1016/j.diabres.2021.109119.

3. Lada P and Idrees U. Toxicity of oral agents used to treat diabetes. *J. Pharm. Pract.* 2005; 18(3): 145-156. doi: 10.1177/0897190005277239.

4. Salehi B, Ata A, V. Anil Kumar N, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, Abdulmajid Ayatollahi S, Valere Tsouh Fokou P, Kobarfard F, Amiruddin Zakaria Z, Iriti M, Taheri Y, Martorell M, Sureda A, N. Setzer W, Durazzo A, Lucarini M, Santini A, Capasso R, Adrian Ostrander E, -ur-Rahman A, Iqbal Choudhary M, C. Cho W and Sharifi-Rad J. Antidiabetic potential of medicinal plants and their active components. *Biomolecules* 2019; 9(10): 551. doi: 10.3390/biom9100551.

5. Putra IMWA, Fakhrudin N, Nurrochmad A and Wahyuono S. A review of medicinal plants with renoprotective activity in diabetic Nephropathy animal models. *Life* 2023; 13(2): 560. doi: 10.3390/life13020560.

6. Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J. Diabet. Investig*. 2014; 5(4): 349-358. doi: 10.1111/jdi.12235.

7. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. *Curr. Protoc. Pharmacol.* 2015; 70: 5.47.1-5.47.20. doi: 10.1002/0471141755.ph0547s70.

8. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM and Reaven GM. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000; 49(11): 1390-1394. doi: 10.1053/meta.2000.17721.

9. Srinivasan K and Ramarao P. Animal models in type 2 diabetes research: an overview. *Indian. J. Med. Res.* 2007; 125(3): 451-472.

10. Barrière DA, Noll C, Roussy G, Lizotte F, Kessai A, Kirby K, Belleville K, Beaudet N, Longpré J-M, Carpentier AC, Geraldes P and Sarret P. Combination of high-fat/high-fructose diet and low-dose streptozotocin to model long-term type-2 diabetes complications. *Sci. Rep.* 2018; 8: 424. doi: 10.1038/s41598-017-18896-5.

11. Saadane A, Lessieur EM, Du Y, Liu H and Kern TS. Successful induction of diabetes in mice demonstrates no gender difference in development of early diabetic retinopathy. *PLoS One* 2020; 15(9): e0238727. doi: 10.1371/journal.pone.0238727.

12. Jan B, Zahiruddin S, Basist P, Irfan M, Abass S and Ahmad S. Metabolomic profiling and identification of antioxidant and antidiabetic compounds from leaves of different varieties of *Morus alba* Linn grown in Kashmir. *ACS Omega* 2022; 7: 24317-24328. doi: 10.1021/acsomega.2c01623.

13. Jeong HI, Jang S and Kim KH. *Morus alba* L. for Blood Sugar Management: A Systematic Review and Meta-Analysis. *Evid. Based. Complement. Alternat. Med.* 2022; 2022(1): 9282154. doi: 10.1155/2022/9282154.

14. Vietnamese Ministry of Health. Vietnamese Pharmacopeia Fifth edition. Ha Noi: Medical Publishing House Co., Ltd.; 2017, 1136-1137.

15. Racine KC, Iglesias-Carres L, Herring JA, Wieland KL, Ellsworth PN, Tessem JS, Ferruzzi MG, Kay CD and Neilson AP. The high-fat diet and low-dose streptozotocin type-2 diabetes model induces hyperinsulinemia and insulin resistance in male but not female C57BL/6J mice. *Nutr. Res.* 2024; 131: 135-146. doi: 10.1016/j.nutres.2024.09.008.

16. Binh PQ, Thuan ND, Hang VV, Phuong PT, Su PQ, Phu DV, Thuong PT, Thu Hang DT and Van Anh PT. Antidiabetic activity of *Gomphogyne bonii* Gagnep. extract against high-fat diet and Alloxan-Induced type 2 diabetes in mice. *Evid. Based. Complement.*

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

Alternat. Med. 2021; 2021: 8648209. doi: 10.1155/2021/8648209.

17. Fauzi A, Kifli N, Noor MHM, Hamzah H and Azlan A. Bioactivity, phytochemistry studies and subacute *in vivo* toxicity of ethanolic leaf extract of white mulberry (*Morus alba* Linn.) in female mice. *J. Ethnopharmacol.* 2024; 325: 117914. doi: 10.1016/ j.jep.2024.117914.

18. Li Y, Zhang X, Liang C, Hu J and Yu Z. Safety evaluation of mulberry leaf extract: Acute, subacute toxicity and genotoxicity studies. *Regul. Toxicol. Pharmacol.* 2018; 95: 220-226. doi: 10.1016/j.yrtph.2018.03.007.

19. Institutional Animal Care and Use Committee. Anesthesia (Guideline). Iowa, United States of America: University of Iowa; 2023.

20. Martínez-Sánchez F, Diaz-Jarquin A, Vargas-Abonce VP, Torres-Cuevas JL, Guerrero-Castillo AP, Medina-Julio D, Meza-Arana C, Gulías-Herrero A and Gómez-Sámano M. Comparison of adiposity indices and their association with Insulin resistance and *β*-Cell dysfunction in primary prevention for diabetes in Mexican population. *Diabet. Epidemiol. Manag.* 2022; 8(10053): 100091. doi: 10.1016/j.deman.2022.100091.

21. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, Eberhardt NL and Kudva YC. Single dose streptozotocin-induced diabetes: Considerations for study design in islet transplantation models. *Lab. Anim.* 2011; 45(3): 131-140. doi: 10.1258/la.2010.010090.

22. Belcher G, Lambert C, Edwards G, Urquhart R and Matthews DR. Safety and tolerability of pioglitazone, metformin, and gliclazide in the treatment of type 2 diabetes. *Diabet. Res. Clin. Pract.* 2005; 70(1): 53-62. doi: 10.1016/J.DIABRES.2005.02.011.

23. Li Y-G, Ji D-F, Zhong S, Lin T-B, Lv Z-Q, Hu G-Y and Wang X. 1-deoxynojirimycin inhibits glucose absorption and accelerates glucose metabolism in streptozotocin-induced diabetic mice. *Sci. Rep.* 2013; 3: 1377. doi: 10.1038/srep01377.

24. Li J, Ji T, Su S, Zhu Y, Chen X, Shang E, Guo S, Qian D and Duan J. Mulberry leaves ameliorate diabetes via regulating metabolic profiling and AGEs/RAGE and p38 MAPK/NF-κB pathway. *J. Ethnopharmacol.* 2022; 283: 114713. doi: 10.1016/j.jep.2021.114713.

25. Zhao X, Fu Z, Yao M, Cao Y, Zhu T, Mao R, Huang M, Pang Y, Meng X, Li L, Zhang B, Li Y and Zhang H. Mulberry (*Morus alba* L.) leaf polysaccharide ameliorates insulin resistance- and adipose deposition-associated gut microbiota and lipid metabolites in high-fat diet-induced obese mice. *Food Sci. Nutr.* 2022; 10(2): 617-630. doi: 10.1002/fsn3.2689.

26. Cheng V and Kashyap SR. Weight considerations in pharmacotherapy for type 2 diabetes. *J. Obes.* 2011; 2011: 984245. doi: 10.1155/2011/984245.

27. Gómez L, Molinar-Toribio E, Calvo-Torras MA, Adelantado C, Juan M, Planas J, Cañas X, Lozano C, Pumarola S, Clapés P and Torres JL. D-Fagomine lowers postprandial blood glucose and modulates bacterial adhesion. *Br. J. Nutr.* 2012; 107(12): 1739-46. doi: 10.1017/ S0007114511005009.

28. Wang Y, Xiang L, Wang C, Tang C and He X. Antidiabetic and antioxidant effects and phytochemicals of Mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. *PLoS One* 2013; 8(7): e71144. doi: 10.1371/ journal.pone.0071144.

29. Hunyadi A, Martins A, Hsieh T-J, Seres A and Zupkó I. Chlorogenic acid and rutin play a major role in the in vivo anti-diabetic activity of *Morus alba* leaf extract on type II diabetic rats.

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]

PLoS One 2012; 7(11): e50619. doi: 10.1371/journal.pone.0050619.

30. Oku T, Yamada M, Nakamura M, Sadamori N and Nakamura S. Inhibitory effects of extractives from leaves of *Morus alba* on human and rat small intestinal disaccharidase activity. *Br. J. Nutr*. 2006; 95(5): 933-938. doi: 10.1079/bjn20061746.

31. Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A and Rémésy C. The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. *Eur. J. Nutr*. 2006; 45: 88-96. doi: 10.1007/S00394-005-0568-5.

32. Nowak M, Tryniszewski W, Sarniak A, Wlodarczyk A, Nowak PJ and Nowak D. Concentration dependence of anti- and pro-oxidant activity of polyphenols as evaluated with a light-emitting Fe2+-Egta-H2O2 system. *Molecules* 2022; 27(11): 3453. doi: 10.3390/molecules27113453.

33. He L, Xing Y, Ren X, Zheng M, Yu S, Wang Y, Xiu Z and Dong Y. Mulberry leaf extract improves metabolic syndrome by alleviating Lipid accumulation *in vitro* and *in vivo*. *Molecules* 2022; 27(16): 5111. doi: 10.3390/molecules27165111.

34. Foretz M, Even P and Viollet B. AMPK activation reduces hepatic Lipid content by increasing fat oxidation *in vivo*. *Int. J. Mol. Sci.* 2018; 19(19): doi: 10.3390/ijms19092826.

35. Owaki R, Aoki H, Toriuchi K, Inoue Y, Hayashi H, Takeshita S, Kakita H, Yamada Y and Aoyama M. AMPK activators suppress cholesterol accumulation in macrophages via suppression of the mTOR pathway. *Exp. Cell. Res*. 2023; 432(1): 113784. doi: 10.1016/j.yexcr.2023.113784.

36. Zhang Z, Huang Q, Zhao D, Lian F, Li X and Qi W. The impact of oxidative stress-induced mitochondrial dysfunction on diabetic microvascular complications. *Front. Endocrinol.* 2023; 14: 1112363. doi: 10.3389/fendo.2023.1112363.

37. Morales Ramos JG, Esteves Pairazamán AT, Mocarro Willis MES, Collantes Santisteban S and Caldas Herrera E. Medicinal properties of *Morus alba* for the control of type 2 diabetes mellitus: A systematic review. *F1000Res* 2021; 10: 1022. doi: 10.12688/f1000research.55573.1.

38. Soni R, Gupta D, Gupte SS, Rathour A, Shrivastava S and Shukla S. Phytochemical screening and evaluation of antioxidant and antiproliferative potential of *Morus alba* (L.) leaves extracts against breast cancer cell lines. *Toxicol. Int.* 2023; 30(3). doi: 10.18311/ti/2023/v30i3/33811.

How to cite this article: Thi Thu Nguyen H, Kim Nguyen N, Thi Thu Dinh H, Vinh Trinh Q, Thi Nhu Nguyen Q, Thi Thanh Nguyen H, Vu N, Trong Duong N, Xuan Pham P, Thi Pham VA. Antidiabetic effects of *Morus alba* Linn leaf extract in a high-fat diet and streptozotocin-induced type 2 diabetic mouse model. ***Journal of Medicinal Plants*** 2025; 24(95): 45-59.
doi: 10.61882/jmp.24.95.45

[Downloaded from jmp.ir on 2026-09-15]

[DOI: 10.61882/jmp.24.95.45]