Original Article

Impact of Mulberry Leaf Extract and Mulberry Leaf Powder on Serum Nesfatin-1 Level in Nicotinamide/Streptozotocin-Induced Type 2 Diabetic Rats

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Abstract

Background & Objective: In traditional medicine, leaves of white mulberry (Morus alba L.) are used as antidiabetic agents. Considering the presence of flavonoids, this plant exhibits insulin mimetic activities. Nesfatin-1 is described as an anorexigenic regulatory peptide, which can influence glucose metabolism via insulin sensitivity enhancement. This study examined the effects of both mulberry leaf powder and extract (MLP and MLE) on fasting blood glucose (FBG), nesfatin-1, and insulin in diabetic Wistar rats.

Materials & Methods: Five groups of rats (n, 40) were included and examined in this study. A group was selected as the healthy control (I), while the other groups received streptozotocin and nicotinamide (55 and 110 mg/kg bw, respectively) for diabetes induction. Diabetic rats were then grouped as follows: control group (II); sham group (receiving ethanol) (III); treatment group receiving 600 mg/kg/day of MLE (IV); and treatment group receiving 25% MLP (V). After 6 weeks, we measured insulin, nesfatin-1, and FBG in the groups.

Results: The FBG level decreased in the treatment groups, while serum insulin increased in comparison with the diabetic controls. In addition, serum level of nesfatin-1 improved significantly (to an almost normal level) in MLP rats in comparison with the diabetic controls. (pvalue<0.05) The effects of MLE on serum nesfatin-1 was similar to MLP but not significant. (pvalue>0.05)

Conclusion: Considering the reduction in insulin level and the rise in blood glucose, the diabetic control group showed an increase in serum nesfatin-1 with a compensatory mechanism, while hyperglycemia improved in the MLE and MLP groups, and the level of nesfatin-1 reduced. MLP showed greater efficacy than MLE in the improvement of nesfatin-1, which might be related to the presence of ethanol in MLE.

Keywords: Nesfatin-1, Type 2 diabetes, Mulberry Leaf, Rat

Introduction

Type II diabetes mellitus (T2DM), which is recognized as an important endocrine disorder (1), has different effects on global economy and healthcare (2). T2DM is a metabolic disorder characterized by insulin resistance and pancreatic beta cell dysfunction as a consequence of unsettled hyperglycemia (3). Adipose tissues are not only involved in triacylglycerol accumulation, but also secrete adipocytokines, which contribute to insulin resistance and pathogenesis of T2DM (4).

Nesfatin-1 is identified as an adipokine from a nucleobindin-2 (NUCB2) gene precursor molecule (5), containing 82 amino acids with a half-life of 6 hours. Nesfatin-1/NUCB2 expression in different hypothalamus sites (6), tissues (adipose and stomach tissues), pancreatic β cells, and gastric oxyntic mucosa has been

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documented (7-10). The intracerebroventricular (ICV) nesfatin-1 injection decreased body weight and food intake; therefore, nesfatin-1 can be described as a satiety molecule (11). Studies have confirmed the involvement of this molecule in controlling glucose homeostasis (12). In mice, nesfatin-1 improves insulin secretion of islet β-cells by integrating Ca^{2+} influx in calcium channels (L-type) (13). According to recent studies, nesfatin-1 improves the secretion and sensitivity of insulin by changing AKT phosphorylation and translocation of GLUT4 to the membrane in adipose tissues, as well as skeletal muscles (14).

Despite the common prescription of advanced synthetic drugs for diabetes, more attention has been directed towards medicinal plants, given the disadvantages of synthetic drugs. Some herbal medicines are known to improve diabetes. *Morus alba* (mulberry tree), which belongs to the *Moraceae* family, is endemic to temperate and tropical regions. Some parts of this plant are used to reduce serum glucose, cholesterol, and lipids (15, 16). Extract of mulberry leaf has potential effects on inflammation, oxidative stress, and cardiovascular defense (15, 17, 18).

With this background in mind, the present study evaluated the anti-diabetic activity of mulberry leaf extract (MLE) and mulberry leaf powder (MLP) and examined its effects on nesfatin-1 and other biochemical parameters in type II diabetic rats.

Materials & Methods

In this experimental study, streptozotocin (STZ) and nicotinamide adenine dinucleotide (NAD) were supplied by Sigma-Aldrich. Also, ethanol Merck provided other chemicals of an analytical grade. Commercial kits (Pars Azmun, Tehran, Iran) were used to measure the serum level of fasting blood glucose (FBG) with a spectrophotometer (JENWAY 6505, Europe Union). Moreover, to determine insulin level, the ELISA kit (Bioassay Technology Laboratory, China) was used. Finally, the level of nesfatin-1 was measured with an ELISA kit (Cusabio, China), using an ELISA plate reader (BioTek ELX800TM, USA).

Preparation and isolation of mulberry leaf extract (MLE)

After collecting the leaves from a farm situated in Tehran, Iran, they were completely washed with tap water and dried in shade; then, an electric blender was used to grind the leaves into powder. Via maceration at room temperature, extraction of dried leaf powder (2200 g) was performed twice with ethanol 96%. (19)

Mulberry leaf powder (MLP) preparation

The dried MLP was incorporated in the diet. For its preparation, the powdered leaves were mixed with a standard feed (25%) (20).

Animals

The rats (male,200-250 g) were supplied by the animal house of Tehran University of Medical Sciences. During the study, the animals were kept at humidity of 55±5% and temperature of 23±2ºC with access to a pellet diet, containing fat 12%(w/w), fiber 8% (w/w), carbohydrate 60% (w/w), and protein 17.5% (w/w), as well as water; they were in a 12:12 h light/dark cycle.

T2DM Induction

To induce T2DM, a single dose of STZ (55 mg/kg bw) was intraperitoneally injected in male Wistar rats 15 minutes after intraperitoneally injection of a single dose of NAD(110 mg/kg bw). NAD was dissolved in normal saline and STZ in citrate buffer (pH, 4.5). On the other hand, the controls were administered normal saline and vehicle citrate buffer (21) For confirming T2DM induction, a glucometer was used to measure blood glucose, 72 h and then on day 7 after injection. (single puncture of lateral tail vein using 30G needle was sufficient for taking 1–2 drops of blood for glucometer) (22). Blood glucose level exceeding 126 mg/dL confirmed diabetes, these rats were considered as T2DM rats. (23).

Experiments

Five groups of rats (n:40) were divided via simple randomization (8 rats per group). One group (group I) was designated as the control group. T2DM rats formed groups II to V as follows:

Group I: non-diabetic control (water and normal diet)

Group II: diabetic control (water and normal diet)

Group III: diabetic sham (water, 0.1 mL; ethanol, 0.4 mL: gavaged)

Group VI: diabetic + MLE (ethanol,0.4 mL; MLE,600 mg/kg; water,0.1 mL: gavaged)

Group V: diabetic + MLP (a standard feed mixture and 25%MLP).

Treatment was carried out for 6 weeks. The baseline and final FBG level and body weight were measured under fasting conditions. An Accu-Chek Advantage II glucometer (Roche Diagnostics, Germany) was used to measure the

level of blood glucose. For anesthesia induction, xylazine and ketamine (10 and 75 mg/kg bw, respectively) were injected intraperitoneally. After collecting blood samples via cardiac puncture, serum was immediately removed.

Data analysis

Values are presented as mean ± SD of 3 replicates for 8 rats per group. For statistical analysis, Stata 13 (Stata Corp., TX, USA) was used. To examine the normality assumption, Shapiro-Wilk test was performed. Moreover, to determine the differences in the mean values of variables in the groups, one-way ANOVA was applied. Finally, for the comparison of data, Tukey's test was applied, and the significance level was 0.05.

Results

The effects of MLE and MLP treatment on FBG level are presented in Chart 1. The serum glucose level was determined at baseline and 42 days after oral administration of MLP and MLE. Based on the findings, FGB significantly increased in diabetic groups versus the controls 72 hours after STZ injection. Based on the findings, 42 days of MLE and MLP treatment caused a decline in FBG level in the treatment groups versus diabetic rats without treatment (P=0.001); nonetheless, treatments were not significantly different.

Table 1 demonstrates the effects of oral MLP and MLE on serum insulin. Based on the findings, insulin significantly reduced in diabetic rats, while an increase was detected in insulin secretion in all rats following 42 days of MLP and MLE treatment. Although MLE was more effective in insulin secretion compared to MLP, no significant difference

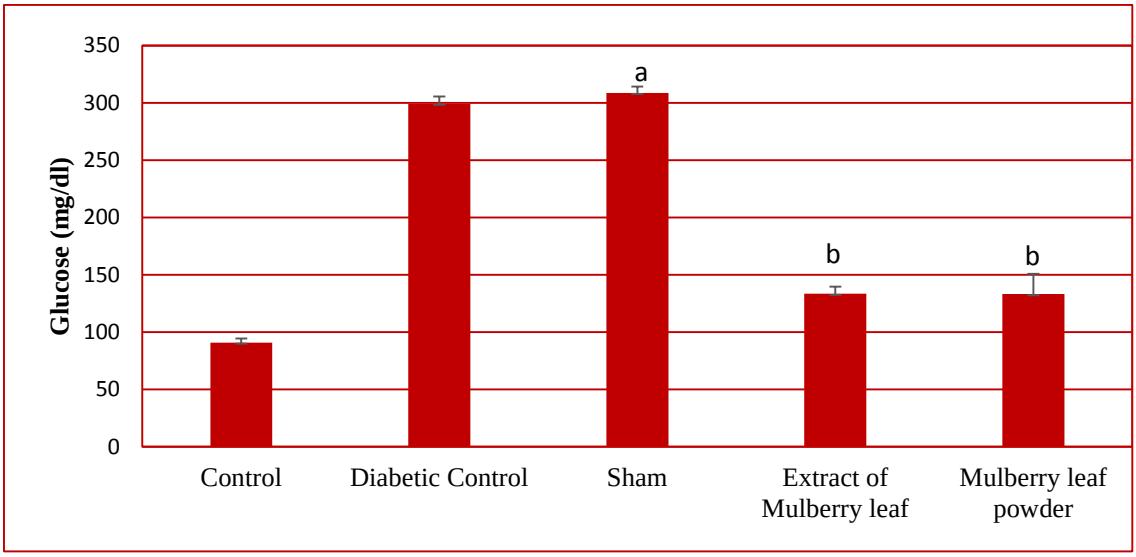

Chart 1. The level of FBG in the diabetic MLE, diabetic MLP, and control groups; a, P< 0.05 versus controls; b, P< 0.05 versus diabetic rats.

Table 1. Serum insulin in the control and diabetic MLE and MLP groups

Groups	Insulin level (mlU/L)
Control	**4.40**
Diabetic control	$\mathbf{2.83}^{a}$
Sham	$\mathbf{2.91}^{a}$
MLE	$\mathbf{3.96}^{b,c}$
MLP	$\mathbf{3.85}^{b,c}$

was found between MLE and MLP treatment.

a: P<0.05 in comparison with normal control rats, *b*: P <0.05 in comparison with diabetic control. *C*: P< 0.05 in comparison with sham rats.

The effects of MLE and MLP on nesfatin-1 are presented in Chart 2. An increase in nesfatin-1 level was reported in diabetic rats in comparison with the controls, although the difference was insignificant. The serum level of nesfatin-1 significantly improved towards the normal range in MLP-treated rats versus the diabetic controls(b).

A significant increase (P<0.05) was observed in the baseline body weight of the non-diabetic control group and diabetic groups which are treated by MLE and MLP at the end of study duration. (Data not shown)

changes. T2DM develops, since NAD can protect the cytotoxic potential of STZ through free radical scavenging, thereby causing trivial damage to pancreatic β-cells (25). The FBG level increased in this model, while serum insulin significantly reduced.

High fiber content and/or trigonelline bases of mulberry leaves may account for hypoglycemic activity; in addition, moran A and/or moranoline present in the leaves may be involved (26). There are also other compounds in MLE, including deoxynojirimycin (DNJ), which has major hypoglycemic activities. DNJ, as an inhibitor of intestinal α-glucosidases, influences carbohydrate absorption and digestion and results in postprandial hyperglycemia suppression (27). Structurally, DNJ is similar to glucose, and a glucose transporter regulates the intestinal absorption at the

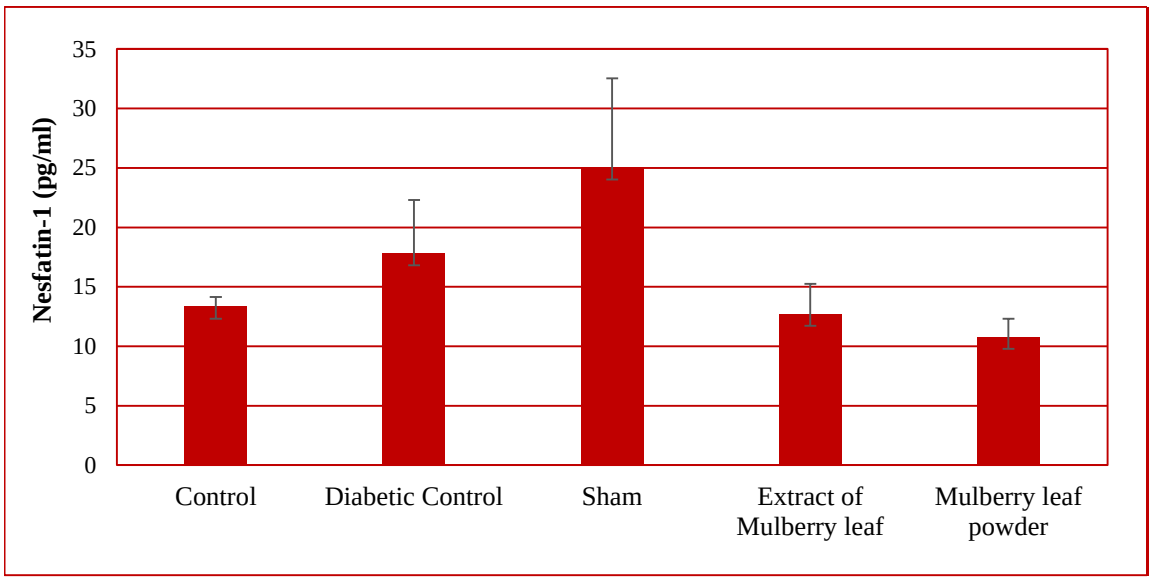

Chart 2. Serum nesfatin-1 in the diabetic MLE, diabetic MLP, and control groups; a, *P*< 0.05 versus the controls: b. *P*< 0.05 versus diabetic rats: c. *P*< 0.05 versus MLE and MLP treated rats.

Discussion

A rat model of T2DM was used in this study, induced by a combination of STZ and NAD. Polyadenosine diphosphate ribosylationis activated and nitric oxide is produced by STZ; in fact, pancreatic cell necrosis is induced by STZ (24). The animals receiving NAD before STZ showed moderate and stable hyperglycemia, while the plasma level of insulin showed no major

small intestine brush border (28).

Glucose uptake improved in fat cells with *Morusalba* in a concentration-dependent manner via GLUT4 translocation, which is mediated by activation of PI3K, possibly due to the presence of gallic acid (29). There are various active compounds, such as rutin in *Morus alba*. Rutin protects β-cells and inhibits STZ-related oxidative stress, leading

to the increased secretion of insulin. According to another study, quercetin aglycone increased insulin secretion and diminished the glucose increase in diabetic rats (30).

In the current study, serum glucose reduced significantly in the treated rats, while the treatment groups had higher FBG levels, compared to the controls. In diabetic rats, oral MLE and MLP administration improved the destruction of pancreas β-islet cells. Therefore, MLE and MLP may be able to increase insulin secretion, resulting in improved glucose uptake of tissues (31). In a study by Mohammad et al., blood glucose, islet diameter, and β-cell count improved in the MLE group in comparison with diabetic rats. Based on biochemical and histological findings, the extract could decrease the level of blood glucose by pancreatic β-cell regeneration (32). (Although we did not do pathologic study).

Generally, nesfatin-1 can control food intake. Its presence in the third brain ventricle of rats can majorly reduce body weight and food intake (11). The present study showed that T2DM rats had increased nesfatin-1 levels. The study by Y. Guoand et al has also presented similar results. In T2DM patients, nesfatin-1 mRNA and proteins of adipose tissues and muscles increased significantly in comparison with the controls (associated with the increased plasma level of NUCB2-1) (33). Furthermore, Ying Zhang et al. analyzed the concentrations of nesfatin-1 in maternal and cord serum, to evaluate the expression of nesfatin-1 in subcutaneous adipose tissue (SAT) from pregnant women with gestational diabetes mellitus (GDM). The GDM group had higher levels of maternal serum and cord blood nesfatin-1, and greater nesfatin-1 expression in SAT. Nesfatin-1 was related to obesity and IR in pregnancy (34). Contrary to the results of these articles and our study, Algul S et al. showed that nesfatin-1 were significantly lower in diabetic patients (Type 2) compared to healthy subjects (35).

Additionally, Zhang Z et al. in their study reported an increase in nesfatin-1 among individuals showing impaired glucose tolerance, along with newly diagnosed T2DM; the increase in nesfatin-1 was related to several clinical parameters, associated with insulin resistance (32). On the other hand, the present findings are inconsistent with a study by Sermin Algulet al., which revealed reduced nesfatin-1 level in T2DM patients (35). Changes in nesfatin-1 level may be a compensatory mechanism or a physiological response to impaired insulin activity (33). Y. Yang et al. reported that In pancreatic beta-cell-specific NUCB2 knockout in vivo and vitro insulin secretion was reduced and blood glucose was elevated (36).

In T2DM patients, the physiological outcomes of increased plasma nesfatin-1 are not determined. In our study, an increase in nesfatin-1 was found in diabetic groups, while MLE and MLP treatment decreased its level significantly; on the other hand, MLP showed greater efficacy than MLE. Considering the increment in blood glucose and reduction in insulin level, serum nesfatin-1 increased in a compensatory manner in the diabetic control group, while MLE and MLP reduced hyperglycemia; therefore, the level of nesfatin-1reduced in the treatment groups. The limitation of this study is the lack of confirmatory test methods for nesfatin-1 assay.

we analyzed nesfatin-1 change at the protein level, also molecular and gene expression analyzing are necessary for confirmation of this result. Since our study was performed for 6 weeks, a longer study is needed to confirm the results. Further studies are necessary to evaluate how MLE and MLP affect nesfatin-1 in diabetes and in glucose metabolism.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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