Original Research

The effect of Morus alba leaves extract and powder on resistin levels and liver transaminase enzymes activities in diabetes

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Abstract: The current study was designed to investigate the changes of the resistin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels of diabetic rats after treatment with Morus alba leaves flavonoid extract (MLE) and Morus alba leaves powder (MLP). Thirty male wistar rats in five groups including control and diabetic groups were included. Diabetic groups consisted of diabetic control, sham and treated group with MLE and MLP. Type 2 diabetes was induced in rats by administration of streptozotocin (STZ) and nicotinamide. The serum concentrations of resistin and insulin in the study groups were identified by ELISA. ALT and AST activities were assayed by spectrophotometer. For the first time, it was shown that the uptake of MLE and MLP by diabetic rats could significantly decrease the serum fasting blood sugar (FBS), resistin levels and enzymes activity of ALT and AST and increases the concentration of serum insulin significantly (P<0.05). in comparison with the sham group and diabetic control. The results showed that there was no significant difference between the anti-diabetic and inflammatory properties of MLE and MLP. In this study, the possible protective effect of MLE and MLP administration was evaluated against destructive effect of STZ on liver and pancreas function in diabetic rats. The results showed that these effects may play an important role in the regulating of adipokines secretion such as resistin and insulin secretion which are involved in the control of diabetes and obesity. MLE and MLP treatment could be useful agents in combination with other therapies in diabetes improvement.

Key words: ALT, AST, Morus alba, resistin, type 2 diabetes.

Introduction

Diabetes is a metabolic disorder with hyperglycemia due to defects in insulin secretion and function. Diabetes mellitus (DM) is a common disease in the world with a metabolic disorder (1). According to the WHO reports, there were 171 million people with diabetes in the world (2.8%) in 2000 and will reach to 366 million (4.4%) in 2030 (2). It is proposed that pathogenesis of type 2 diabetes mellitus (T2DM) is mediated through the simultaneous insulin resistance and subclinical inflammation progression (3). Varieties of adipokines are secreted by adipose tissue such as resistin, adiponectin and leptin (4), which have critical roles in metabolic regulation, insulin resistance and insulin resistance related diseases. Resistin is a pro-inflammatory adipokine and considered as an important pathogenic factor in the etiology of T2DM, human obesity and cardiovascular disease (3). Currently, insulin, different oral hypoglycaemic drugs such as α-glucosidase inhibitors, metformin and sulphonylureas and exercise are used in T2DM patients as common therapy (1). According to available evidences, synthetic drugs despite their usefulness have many side effects. Consumption of herbal compounds and natural medicines is growing in many countries. It has been proved that consumption of different parts of some plants could decrease the glucose and lipid levels, strengthening the body's antioxidant defense system and almost improve the metabolic disorders including diabetes complications.

Morus alba (white mulberry) is one of those plants that have therapeutic application. Morus alba is a tropical and subtropical plant that grows in many parts of Asia (5). It has been shown that Morus alba leaves is rich of different compounds including vitamin C, β-Carotene, protein, iron, zinc, calcium, phosphorous, magnesium, tannic acid, prenylflavore, dihydroxycoumarin, cudrafavone B, Cresverato and oxyreseratrol, bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, alkaloids, amino acids and organic acids, which are found to have anti-inflammatory, anti-diabetic and anti-oxidants properties that serve as free radical scavenger (6, 7). Additionally fruit and different parts of the Morus alba plant are used for high blood pressure, diabetes, bacterial infections, cancer, neuroprotective and atherosclerosis treatment (5, 8). Falvonoids are polyphenolic compounds, which are classified to numerous derivatives including flavonols, flavones, flavanols, flavanones, isoflavones or anthocyanidins according to their chemical structures (9). It has been found that Morus alba leaves are rich of flavonoid components especially quercetin, by which are important for its antioxidant potential property. It

has been identified that *in vitro* and *in vivo* oxidation process could be reduce by mulberry leaves extract or functional components. Root and bark of Morus alba has been used as a component of antidiabetic therapy in Oriental Medicine (8).

In this paper, we assessed the effect of Morus alba leaves extract (MLE) and Morus alba leaves powder (MLP) on resistin and insulin levels, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities in HOMA-IR index of diabetic rats and controls.

Materials and Methods

Extraction and isolation

The Morus alba leaves were dried under shadow for three weeks and powdered. The powdered materials (2200gr) were extracted three times with ethanol 96% by maceration at room temperature (seven days for each time). The solvent was evaporated under vacuum to give 112 g of extract. The obtained dry extract was suspended in water followed by extraction with water, hexane, ethyl acetate and chloroform for three times consecutively and then were lyophilized (10).

Determination of flavonoid content

The total flavonoid content (TFC) in ethanolic extract and fractions extract of Morus alba leaves (MLE) were determined using AlCl3 reagent. Briefly, 2.5 ml of each sample (and/or quercetin as the standard), previously dissolved in 90% ethanol, was mixed with 2.5 ml of a 2% AlCl3 solution in 90% ethanol. After 40 min, the absorbance of the yellow color product was measured at 415 nm. The TFC [as μg quercetin equivalents/mg of sample] for the sample was calculated on the basis of a linear calibration curve obtained using quercetin ($y = 0.0169x + 0.3526$, $r^2 = 0.995$)(11).

Animals, diet and feeding

All of animal procedures were approved by the Arak University of Medical Sciences ethical committee board. Male Wistar rats weighting (200-250gr) were obtained from the experimental animal unit, faculty of medicine, Arak University of Medical Science, Arak-Iran. They were housed in an air-conditioned room (25°C) and humidity (55±5%) with a 12 hours dark/12 h light cycle which had free access to standard diet and tap water and fed daily (Table 1).

Induction of diabetes

T2DM was induced by injection of a single intra-pe-
ritoneal dose of 55 mg/kg STZ (Sigma-Aldrich, USA) dissolved in 0.1 M citrate buffer (pH 4.5). In order to prevent of complete β cell destruction, 110 mg /Kg body weight of nicotinamide (Sigma-Aldrich) was injected 15 minutes before receiving the STZ. Seven days after STZ injection, blood was collected from the animal's tail vein and fasting level of blood glucose, was measured. Animals were considered as diabetic, if had a fasting blood glucose level over 126 mg/dl and then were recruited in further assessments.

Experimental design

Thirty healthy adult male wistar rats (eight weeks old, 200-250gr weight) were used in this study. The rats were divided into 5 groups (6 rats in each group) as follow: group I: non diabetic control group which received water and normal food; group II: diabetic control group which received water and normal food; group III (sham): diabetic group which received daily 400μl ethanol + 100 μl normal saline via gavage; group IV: diabetic group that were treated with 500 μl of MLE (dissolved 600 mg of MLE in mixture of 400 μl ethanol + 100 μl normal saline solution) via gavage and group V: diabetic rats which treated orally with MLP daily (%25 of daily routine food contained MLP)(12). At the end of the 6th week, all of the rats were anesthetized with ketamine (75mg/kg b.w) and xylazine (10mg/kg b.w), then their blood samples were collected. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels of the study groups were measured by activity assay kit (ZIESTCHEM Company, Tehran, Iran) using spectrophotometer (JENWAY 6505, Europe Union model). The serum concentrations of resistin and insulin in the study groups were identified by ELISA (ELISA plate reader (ELx800TM, Bio Tek, Winooski, VT, U.S.A) according to the manufacturer's protocol (Bioassay technology laboratory Shanghai, China kits producer). HOMA-IR of the study groups was calculated by the formula:

HOMA-IR = [insulin (μU/ml) × glucose (mmol/L)/22.5]

Statistical analysis

All the data were expressed as mean± standard deviation (S.D.) of three replicates for six rats in each group. Stata software, version 13 (Stata Corp, College Station, TX, USA) was used for all statistical analyses. Normality assumption was checked using Shapiro Wilk test. One-way ANOVA was applied for determining differences between mean of the variables in the studied groups. Post hoc Test (Tukey) was used to compare the

Table 1. Ingredients contents of food with or without MLE and MLP.

Ingredients	Non diabetic control, Diabetic control and Sham groups	Diabetic group IV	Diabetic group V
Carbohydrate	60%	60%	45%
Fat	12%	12%	9%
Protein	17.5%	17.5%	13.12%
Fiber	8%	8%	6%
Water	2.5%	2.5%	1.88%
MLE	-	600mg/kg b.w	-
MLP	-	-	25%

data. Values of p<0.05 were considered statistically significant.

Results

Total flavonoid content

The highest amount of flavonoid was found in total extract and the flavonoid content of ethyl acetate fraction was more than other fractions. It is worth to point out that the total extract of flavonoids was used for treatment (Table 2).

Table 2. The flavonoid content of total extract and different fractions.

Extracts and fractions	Total flavonoid content (µg/mgr)
Total extract	114.22±2.78
Ethyl acetate fraction	98.28±3.2
Hexane fraction	40.72±0.5
Water fraction	23.57±0.32
Chloroform fraction	14.88±0.15

Fasting blood glucose and body weight gain

As it was shown in Table 3, there was not a significant difference between the baseline fasting blood sugar (FBS) levels of non-diabetic control (83.5±6.37mg/dl) and the FBS levels (90.83±3.71mg/dl) at day 42nd of study, whereas the FBS levels in diabetic controls (P=0.001) and sham group (P=0.001) was significantly higher than non-diabetic control at day 42nd of study. The baseline FBS levels of treated groups with MLE (255.5±6.12 mg/dl) and MLP (259±6.89 mg/dl) was decreased significantly (P=0.001) to 133.5±6.22mg/dl and 127.16±11.19mg/dl, respectively at the day 42th of treatment. As it was shown in Figure 1, there was no significant difference between the baseline FBS levels of the non-diabetic control and their FBS levels after the study duration (P>0.05), whereas the FBS levels of diabetic controls after treatment period was significantly increased (P=0,0002) in comparison with their baseline FBS levels. On the contrary, the FBS levels of the treated groups with MLE and MLP were significantly decreased (P=0.001) in comparison to their baseline FBS levels. The body weight of studied groups was investigated (Table 3). There was no significant difference (P=0.96) between the body weight of studied groups at the beginning of study. On the contrary, after treatment with MLE and MLP, the weight of all studied groups was changed. A significant increase (P=0.001) was observed in the baseline body weight of the non-diabetic control group and diabetic groups which are treated by
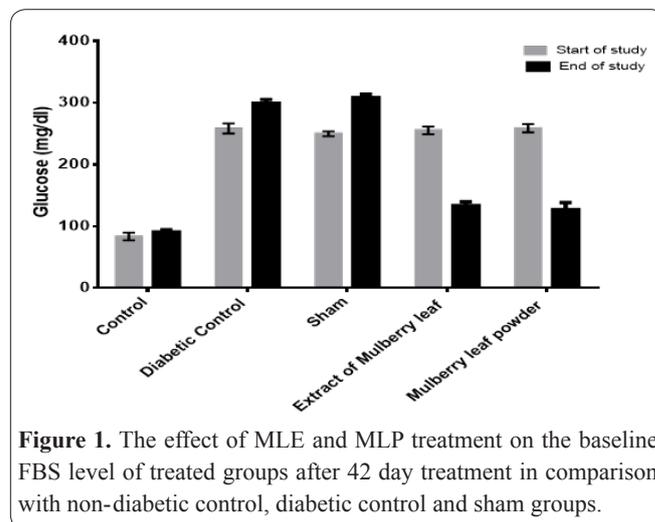
Figure 1. The effect of MLE and MLP treatment on the baseline FBS level of treated groups after 42 day treatment in comparison with non-diabetic control, diabetic control and sham groups.

MLE and MLP at the end of study duration. The body weight of treated diabetic groups with MLE and MLP was also increased significantly (P=0.022 and P=0.01, respectively) when compared with their baseline weight at the beginning of the study.

The effect of the oral consumption of MLE and MLP on body weight (gr) of the studied groups was shown in Table 3. There was a significant difference (P<0.05) between the body weight of group I after 42 days of study, While, the body weight of groups II and III was not changed significantly after 42 days of study. The difference between body weight of groups IV and V was increased significantly (P<0.05) after 42 days treatment in comparison with day 0, but there was no significant difference between body weight (gr) of groups IV and V after 42 days of study. The body weight of group IV was less than controls significantly (P<0.05) after 42 days of treatment. There was a significant decrease (P< 0.05) between body weight of groups II and III in comparison with controls after 42 day of study.

ALT and AST activity enzymes

The ALT and AST enzymes activity (IU/L) of the non-diabetic control and studied groups after study period was also investigated. As it was shown in Figure 2 and Table 4, the enzyme activity change of treated group with MLP was not significant after study period in comparison with non-diabetic control group. The difference between ALT enzyme activity of treated groups with MLE and MLP after treatment period was not significant, while the ALT enzyme activity of other groups increased significantly when compared with each other.

Table 3. Longitudinal study of the MLE and MLP Oral consumption effect on Fasting Blood Glucose Levels and Body Weight in Studied groups.

Body weight(gr)		FBG(mg/dl)		Study groups
Day 0	Day 42	Day 0	Day 42	
279.16±9.82	214±16.63	90.83±3.71	83.5±6.37	I
238.5±8.04 [b]	214.33±27.12	299.16±6.3 [b]	258.5±8.11 [b]	II
229.5±10.25 [b]	217.5±26.86	308.66±5.6 [b]	249.8±3.97 [b]	III
246±24.38 [b]	211.33±8.18	133.5±6.22 [bcd]	255.5±6.12 [b]	IV
254.33±17.87	210±15.67	127.16±11.19 [bc]	259±6.89 [b]	V

[a] Each value is mean ± SD .

[b] P< 0.05 in comparison with normal control rats.

[c] P< 0.05 in comparison with diabetic rats.

[d] P< 0.05 in comparison with sham rats.
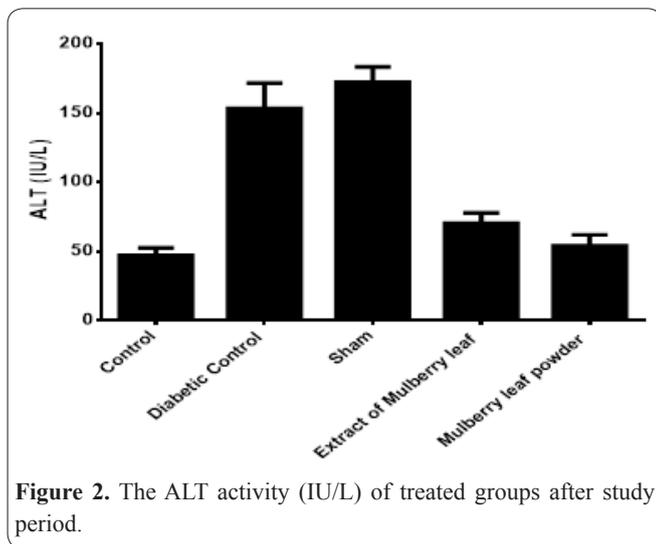
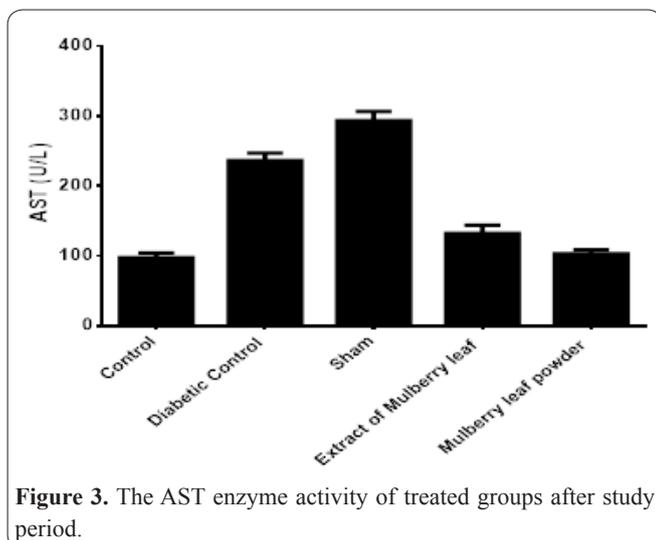**Figure 2.** The ALT activity (IU/L) of treated groups after study period.

According to Figure 3 and Table 4, AST enzyme activity (IU/L) of the diabetic control and sham groups increased significantly in comparison with non-diabetic control group, while the enzyme activity of the treated groups with MLE and MLP decreased significantly in comparison with diabetic control and sham groups. Enzyme activity of the treated group with MLE was significantly less than the treated group with MLP. The difference between enzyme activity of the treated group with MLP and non-diabetic control was not significant (P=0.827).

Resistin levels

The resistin levels of the non-diabetic control and treated groups after study period were shown in Figure 4 and Table 5. The resistin levels of all studied groups were increased significantly in comparison with non-diabetic control, but this increase in treated group with MLE and MLP was less than diabetic controls and shams. It is worth to note that difference between resistin levels of the two treated groups with MLE and MLP was not significant.

Insulin levels and alteration of HOMA-IR

The insulin levels of non-diabetic control and diabetic groups after study period were shown in Table 6. Serum insulin level of the treated rats significantly increased in comparison with diabetic control and sham groups (P=0.001). However, there was no significant

Figure 3. The AST enzyme activity of treated groups after study period.

Table 4. Longitudinal study of the MLE and MLP oral consumption effect on ALT and AST in studied groups[a,b].

| Groups	ALT(U/L)	AST(U/L)
I	47.00±5.44	97.5±6.65
II	153.33±18.34[c]	236.66±10.11[c]
III	172.33±11.07[cde]	293.66±13.04[cde]
IV	70.16±7.52[d]	132.00±12.01[df]
V	54.16±7.7	103.00±5.86

[a] Each value is mean ± SD .

[b] Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[c] P< 0.05 in comparison with normal control rats.

[d] P=0.001 in comparison with diabetic rats.

[e] P= 0.001 in comparison with sham rats

[f] P= 0.001 in comparison with treatment with Extract of Mulberry leaf.
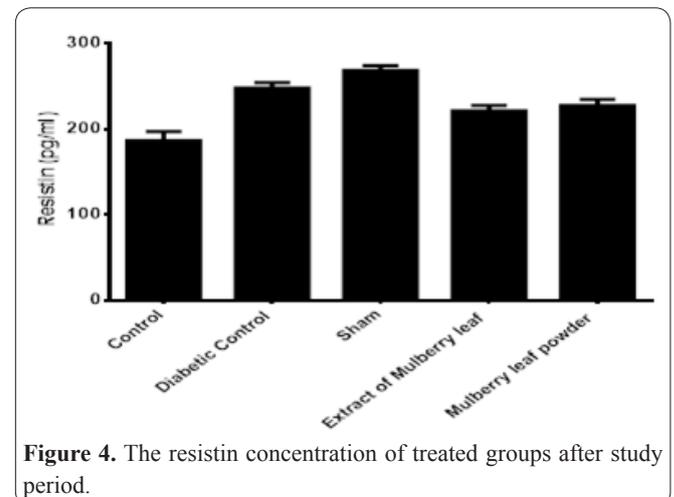
Figure 4. The resistin concentration of treated groups after study period.

Table 5. Longitudinal study of the MLE and MLP oral consumption effect on resistin levels in studied groups [a].

Groups	Resistin(pg/ml)
I	186.66±10.78
II	248.16±6.43[b]
III	268.66±5.75[b]
IV	221.5±6.12[bcd]
V	227.5±7.28[bc]

[a] Each value is mean ± SD .

[b] P=0.001in comparison with normal control rats.

[c] P=0.001 in comparison with diabetic rats.

[d] P= 0.001 in comparison with sham rats.

difference between the two treated groups (P=0.978). The difference between insulin levels of treated groups and non-diabetic control was not significant. According to Table 6, the HOMA-IR index of the diabetic control and sham groups increased significantly in comparison with the non-diabetic control group. HOMA-IR index of treated groups with MLE and MLP decreased significantly when compared with the diabetic control and sham groups (P=0.001). Difference between HOMA-IR index of treated groups and non-diabetic control was not significant. There was no significant difference between HOMA-IR index of treated group with MLP and non-diabetic control (P=0.059).

Table 6. Longitudinal study of the MLE and MLP oral consumption effect on insulin and HOMA-IR in studied groups [a].

Groups	Insulin(mIU/L)	HOMA-IR(%)
I	4.4±0.2	2.95±0.06
II	2.83±0.31 [b]	6.26±0.56 [b]
III	2.91±0.29 [b]	6.65±0.55 [b]
IV	3.96±0.45 [cd]	3.9±0.29 [bcd]
V	3.85±0.42 [c]	3.59±0.16 [c]

[a] Each value is mean ± SD .

[b] P=0.001 in comparison with normal control rats.

[c] P=0.001 in comparison with diabetic rats.

[d] P= 0.001 in comparison with sham rats.

Discussion

T2DM is a metabolic disorder that growing constantly around the world. This disorder is characterized by defect of insulin secretion and insulin resistance in peripheral tissues (13). One of the critical side effects of hyperglycemia is an imbalance in reduction and oxidation reactions in hepatocytes (14). The tissue damage due to free radicals in streptozotocin-induced diabetic has been investigated (15). Oxidative stress in diabetes is increased via glycosylation, non-enzymatic glycation and changes in energy metabolism, inflammatory mediators and defense of antioxidant systems (5). Although the result of a study showed that injection of STZ and high fat diet uptake in rats does not affect inflammation and does not cause significant liver damage. The protective effects of quercetin (QE) as an antioxidant flavonoid against β-cell damage in diabetic rats was identified by Omer et al (17). Diabetic liver injury is induced by a several factors and cannot be controlled only with inhibiting hyperglycemia. Thus, controlling of blood glucose alone is not sufficient to delay or prevent diabetes complications. Therefore, other relevant factors must be considered. Several studies investigated the effects of traditional herbal medicine in diabetes treatment. It has been established that some herbs can decrease glucose, lipids levels and improve the body's antioxidant defense system, which improve diabetes complications (5). It has been also identified that some components of Morus alba leaves such as 1-deoxynojirimycin (DNJ), inhibits intestinal α-glucosidases and have good effects on the postprandial suppression of digestion and absorption rate of carbohydrates from intestine (18).

In an experimental approach, we adapted, diabetes was induced by a single intraperitoneal STZ injection. In order to prevent complete β cell destruction, the amount of 110 mg /Kg body weight of nicotinamide was injected 15 minutes before receiving the STZ. Metabolic profile of the animals including hyperglycemia and insulin resistance, resembling metabolic characteristics of humans with type 2 diabetes were detected. Thus, this model could be useful in studying of anti-diabetic properties of natural compounds of plant origin. In the present study Morus alba leaves and its flavonoid extract were added directly to the diets of diabetic rats to provide constant supply of bioactive components as dietary supplement during food ingestion, allowing us to investigate the effects of this bioactive component from food intake on several biochemical markers levels.

The results showed that FBS of treated diabetic rats with both MLE and MLP after 42 consecutive days decreased significantly in comparison with diabetic control (II) and sham (III) groups (Table 3). The administration of MLE and MLP in treated groups IV and V significantly increased insulin levels (Table 6) in comparison with the groups II and III. However, difference between insulin levels of both treated groups (IV and V) and non-diabetic control group (I) was not significant. The HOMA-IR (%) of treated groups IV and V also decreased to control range (Table 6) after treatment. These changes elucidate the effectiveness of this herb intake in diabetic rat as comparison with diabetic control and sham groups. Our results showed that hyperglycemia and insulin resistance condition, which was induced due to STZ intake in study rats, were improved significantly after MLE and MLP consumption. The presence of some flavonoids components in MLE and MLP likely regulate the glucose uptake in the cells and peripheral tissues utilization could counter this effect.

Blood glucose decrease by Morus alba leaves in the adipose tissue in diabetic rats could be attributed to transfer of GLUT4 to plasma membrane and increased uptake of cellular glucose (18).

Consistent with our results, Magdalena et al.(16) showed that Morus alba extracts significantly improve disturbed metabolism of carbohydrate in STZ-induced diabetic rats. The antidiabetic properties of these compounds were attributed to likely involving the intracellular pathways in insulin signaling or glucose homeostasis. Our results are supported by the results of a study conducted by Singab et al (19). They showed that dose of 600 mg kg(-1)day(-1) of 70% alcohol extract of the Morus alba root bark administration to diabetic rats for 10 consecutive days reduced the amount of the glucose by 59% as compared to STZ-diabetic rats and increased insulin production by 44%. In a study Osama et al. (20) showed that Rutin, a member of bioflavonoids, could protect the β cells of rats against STZ-induced stress oxidative, resulting in increased insulin secretion and decreased blood glucose levels.

It has been suggested that anti-diabetic property of polyphenol compounds could be modulated via various mechanisms including reduction of glucose absorption from mucosa of intestinal and proximal renal tubules and inhibition of a-glucosidase and sodium–glucose symporter in the peripheral tissues. Decrease in hepatic gluconeogenesis, adrenergic stimulation of muscle glucose uptake and insulin release from pancreatic β cells are also induced by polyphenol compounds (5).

We have also showed that MLE and MLP consumption did not affect body weight of treated group significantly in comparison with the controls and other groups (Table 3). Normal body weight that was observed in the treated group suggests that MLE is safe for consumption at the experimental dose. This finding was in agreement with studies of Song-Tao et al.(21), Davoud et al. (22) and Singab et al. (19) who observed a body weight gain upon betterment of the diabetes status.

We showed that MLE and MLP consumption by diabetic rats after a 42 days' period could decrease the serum ALT and AST enzymes activity significantly when compared with non-diabetic control and other groups. We have also showed that the effect of MLP consumption on reduction of enzymes AST activity

was more significant when compared with the effect of MLE consumption (Figure 3). Whereas, the effect of the MLP consumption on ALT enzyme activity decrease was not different significantly in comparison with MLE consumption effect. These results could be attributed to hepatoprotective potential effect of MLE and MLP, which is demonstrated by significantly lower levels of ALT and AST of treated groups when compared to their levels in the untreated groups (controls and shams). In line with our results, in a study, Nazari et al.(5), have shown that, Morus alba leaf extracts (MAE) significantly decreases the liver enzymes ALT, AST levels in T2DM rats in comparison with the diabetic control groups.

These findings strongly suggest the potential of MLE and MLP in controlling the liver damage that caused by STZ. However, the underlying mechanism of function for these effects is still unclear. The presence of antioxidants like flavonoids and polyphenol compounds in ML may help to reduce inflammation in the body due to their cytoprotective and anti-inflammatory properties.

As it was shown in Table 5, the resistin levels of treated groups IV and V significantly decreased in comparison to sham and diabetic controls, although these amount was still a little bit more than non-diabetic control group. For the first time, we showed that Morus alba leaves consumption in both extract and powder forms could significantly decrease the resistin levels of diabetic rats when compared with untreated groups. It is suggested that anti-inflammatory and anti-oxidants properties of MLE and MLP components such as flavonoids could neutralize the inconvenient potential properties of resistin and modulate the insulin action, glucose uptake and hepatic function. It is also suggested that insulin-sensitizing effects of MLE and MLP may be partly due to the regulation of the biosynthesis and secretion of adipose-derived proteins, including resistin and probably other adipokines. Resistin influences insulin sensitivity, metabolism of lipid and a variety of inflammatory pathways. Resistin is a cysteine-rich and unique signalling adipokine molecule that is released by adipocytes and macrophages (23). In addition to adipose tissue, resistin expression and its co-localization with insulin and glucagon in pancreatic cells was also identified via immunofluorescence technique by Suhail et al (24). They showed that many of pancreatic cells expressing resistin increased significantly after the onset of T2DM. They also showed that degree of co-localization was higher in the pancreases of diabetic patients compared to normal. It was suggested that resistin may play a regulatory role in pancreatic β cells function. Resistin levels are reduced by anti-diabetic drug rosiglitazone uptake and increased in genetic and diet induced forms of obesity. Claire et al. (23) showed that administration of anti-resistin antibody improved blood sugar and insulin action in mice with diet-induced obesity. They showed that treatment of normal mice with recombinant resistin impaired glucose tolerance and insulin action. Insulin-stimulated glucose uptake by adipocytes was enhanced by neutralization of resistin and was reduced by resistin treatment. They introduced resistin as a hormone that potentially links obesity to diabetes.

The relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic type 1 and high fat diet-fed rats (HFD) was also investi-gated by Mohammad et al (25). They showed a significant increase in resistin levels (p<0.001) of diabetic and high fat diet-fed rats (HFD) group in comparison with controls, which was correlated positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index (p<0.001), atherogenic lipid profile and hyper-coagulability markers (except for platelet count). Hyperresistinemia was speculated to represent a probably link between metabolic signals, atherosclerosis, and hypercoagulability in T2DM rats. Whereas no role was found for resistin in metabolic and haemostatic changes in type 1 diabetic rats (25).

Taking together our studies showed that the MLE and MLP consumption in rat caused: (i) a significant decrease in HOMA-IR index, FBS and resistin levels, ALT and AST activities (ii) a significant increase in insulin levels. It is worth to point that no significant difference was found between two kinds of treatment methods. These results indicate that a Morus alba based therapeutic pattern might serve as a basis for the development of novel potential therapies in T2DM. Further studies and development in this direction of diabetic drug development are required.

Acknowledgments

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