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The Effect of Combined Exercises and Consumption of Mulberry Leaf Extract on Serum Inflammatory Markers Level in Elderly Type 2 Diabetes Mellitus Men

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Abstract

Objective: This study aimed to investigate the effect of 8 weeks combined exercise and consumption of Mulberry leaf extract on the serum levels of inflammatory and atherosclerosis indicators in elderly men with type 2 diabetes mellitus (T2DM).

Materials and Methods: 40 elderly men T2DM aged between 65 to 70 years old were purposefully selected and randomly divided into five equal groups (training, supplement, training+ supplement, placebo, and control). A daily dose of 1000 mg Mulberry leaf extract, 3 times a day, was used for 2 months by the supplement group. Training groups performed combined exercises for eight weeks, each week three sessions of 90 minutes.

Results: The results showed that the amount of salusin-β and interleukin-6 in the training, training + supplement and supplement groups decreased significantly at the end of the study; Meanwhile, the level of salusin-α increased significantly in the training (P= 0.001), training + supplement (P= 0.001) and supplement (P= 0.01) groups. Also, the results of the covariance analysis showed that the amount of salusin-β and interleukin 6 were lower (P= 0.001), and the level of salusin-α was higher significantly in the training (P= 0.001), training +supplement (P= 0.001), and supplement (P= 0.001) groups compared to the control groups.

Conclusion: It seems that the consumption of Mulberry leaf extract, and combined exercises, is effective in controlling the inflammatory indicators and atherosclerosis related to diabetes in the T2DM elderly.

Keywords: Interleukin 6, Salusin-α, Salusin-β, Diabetes, Elderly

QR Code

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Introduction

Nowadays, diabetes is one of the chronic diseases that human societies are dealing with. Type 2 diabetes mellitus(T2DM) is prevalent in the elderly, and about 50% of patients with diabetes are over 65 years old (1). One of the complications caused by high blood glucose is cardiovascular diseases, caused mainly by changes in inflammatory conditions and the destruction of molecular signaling through inflammatory factors (2)**.** Recent studies have shown that salusin-α and salusin-β peptides play a prominent role in sclerotic, anti-sclerotic processes, and hyperglycemic diseases (3). Salusins are expressed in large amounts in vascular smooth muscle cells and endothelial cells. They are also present in the central nervous system, kidney, plasma, and human urine (4). Salusin-α is an anti-inflammatory peptide. Salusin-α and salusin-β have been shown to reduce and increase inflammatory responses in vascular endothelial, respectively. It has been reported that salusin-β plays a role in creating oxidative stress conditions and inflammation by increasing the levels of inflammatory cytokines (3). Inflammatory cytokines such as interleukin-6 lead to an increase in blood glucose through increasing insulin resistance caused by inhibiting phosphorylation of the insulin receptor and Insulin receptor substrate 1 (IRS-1) (5). Also, epidemiological studies have reported that higher serum interleukin-6 levels are associated with an increased risk of T2DM (6).

In recent years, exercises and supplement consumption have been proposed as a solution for hyperglycemic control. Fuji et al. (2020) reported that eight weeks of aerobic training leads to an increase in salusin**-**α levels in elderly, middle-aged, and young people (7). It has been reported that Mulberry leaf extract has potent of anti-inflammatory and antioxidant effects. Mulberry leaves contain polyphenol compounds, flavonoids, anthocyanins, and carotenoids. Epidemiological studies show that mulberry leaves contain deoxy-nojirimycin (DNJ), flavonoids, and sterols (8). DNJ compounds present in Mulberry leaf extract inhibit alpha-glycosidase and prevent the increase of blood glucose levels (8,9). A review of the background of studies shows that the effect of exercise on salusines is contradictory (7,10,11). Moreover, no study has investigated the simultaneous effect of exercise and consumption of mulberry on salusin level (7,10,12).

Therefore the aim of this study was to investigate the effect of eight weeks combined exercises (aerobic+resistance) along with the consumption of Mulberry leaf extract on serum levels of salusin-α, salusin-β and interleukin-6 in T2DM elderly men.

Material and methods

Participants

This study was a semi-experimental, single-blind, with a pre-test and post-test trial that was conducted among T2DM patients, referred to diabetes center in Ardabil city, Iran and had medical records. The estimation of the sample size was done using the software (G Power 3.1). The statistical power of 0.95 in the effect size equal to 0.80 with the alpha level of 0.05 was determined equal to 8 subjects in each group. 44 people were included in the study. 3 people withdrew and 1 person was excluded from the study due to illness.

Then, 40 people were selected among this statistical community in a purposeful way and according to the inclusion criteria. The purpose of the study, possible benefits, and risks were explained to the volunteers; then informed consent was obtained from the participants **.**The men were eligible to enter the study if they were aged 65-70, T2DM between 1 and 10 years, not taking more than one type of oral anti-diabetic pill daily (all subjects were taking the same amount of metformin), no insulin injection, having the basic level of glycosylated hemoglobin

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between 6.6 and 9.9%, fasting blood glucose 160 to 250 mg/dL, and being able to exercise. Individuals with covid-19, participating in a regular exercise program in the last six months, history of cardiovascular, renal and eye diseases, history of any complications of diabetes (neuropathy, nephropathy, and retinopathy) and smoking were excluded. Due to the fact that elderly people with type 2 diabetes were at risk of falling and prone to cardiovascular diseases, permission was issued for the presence of cardiologists and orthopedists to participate in the exercises.

The subjects were randomly divided into five groups of eight subjects in each group, including combined exercises (aerobic+ resistance) group, combined exercises (aerobic + resistance) + mulberry leaf extract, mulberry leaf extract group, placebo group, and control group. In this way, using the lottery method, the names of the subjects were written on separate papers and placed in a container.

Then the names of the subjects were taken out randomly and they were placed in the intervention, placebo and control groups, respectively. Study participants were asked to continue their medication and diet plan. Due to the spread of the covid-19 pandemic, all preventive measures such as disinfection of tools, proper ventilation of the training place, daily fever measurement, and social distancing were carried out. People in the control group were asked to continue their previous lifestyle. In addition, the subjects and doctors were also asked to inform us if there was a change in their treatment plan.

Training protocol

One repetition maximum (1RM) was determined by sub-maximum repetitions until the point of exhaustion at the beginning of the first and fifth weeks of training (13). The combined training program (aerobic + resistance) was performed for eight weeks, three sessions per week, and each session for 90 minutes with at least one day of rest between sessions. Each training session included a 10-minute warm-up period (including muscle stretching, and walking) and aerobic exercises for 10 to 30 minutes with an intensity between 50 and 70% of the maximum heart rate (HR_{max}) (14,15); HR_{max} was obtained using the following formula.

HR_{max}=220-age

After performing aerobic exercises, there was a rest between 3 to 5 minutes, and then resistance exercises were performed for 30 to 40 minutes with intensity between 40 and 70% of a maximum repetition. Resistance exercises for each subject included the large muscles of the upper body and lower body, so that he could repeat each movement 8-12 times at each station. Some of the performed exercises include Triceps extension, Biceps curl, Bench press, Latissimus pull down, Calf raises, Seated leg press, Hamstring flexion, Abdominal crunches, and Low back extensions.

In the end, to recover the body to its original state and cool down, subjects did 10 minutes of walking and muscle stretching. There was 1- minute rest between sets and 2 minutes between stations (Table 1) (15-17).

Extract and supplement consumption

Training+ supplement and supplement groups used 1000 mg capsules of Mulberry leaf extract manufactured by Nanjing NutriHerb BioTech Co., China. They took the extract supplement three times daily with

Table 1. Combined training program (aerobic + resistance)

Group	Training type		Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Combined exercise	Aerobic (10 to 30 minutes)	Time(min)	10	10	15	20	20	25	30	30
		Intensity= % of HR_{max}	%50	%50-%60	%50-%60	%60-%70	%60-%70	%60-%70	%60-%70	%60-%70
	Resistance Intensity= % of one repetition maximum)		%40-%60 (one repetition maximum) intensity				%60-%70 (one repetition maximum) intensity			
	Time= (30 to 40 minutes)		Time= 30 min					Time= 40		

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meals for eight weeks. Mulberry leaf extract supplement contains 10 mg of deoxynogirimycin (DNG), prescribed to patients with the advice and supervision of a diabetes specialist. The method used to prescribe the dosage was taken from the research of Rich et al. (2017) (18). Participants in the placebo group received capsules (pills containing wheat flour), which were similar in appearance to the Mulberry leaf extract supplement, three times a day (19). In addition, in order to monitor the consumption of the capsules, the subjects were monitored in daily phone interviews.

Control of diet

A 3-days dietary record (over two weekdays and one weekend day) was completed by participants. All subjects were asked to list all the foods and beverages they had consumed in the past 24 hours. Household scales were used to help the participants remember more accurately the amount of food consumed. This 24-hour food reminder was completed for each of the subjects in 20 non-consecutive times (3 times a week during the research period).

The mentioned amounts of food were converted into grams using the guide of household scales. Then, each food was coded according to the instructions of the food processing software program FP2 (Food Processor 2) and was analyzed by a nutrition expert in order to evaluate their energy and nutrients (20). Assessment of variables Body weight and height of each participant were measured at the baseline of trial while wearing minimal clothing by a trained researcher. Body mass index (BMI) was calculated as weight (in kilograms) divided by height in meters squared (21). Fasting blood samples were acquired to measure salusin β, salusin-α, and Interleukin 6. Participants were fasted for 10-12 h and samples were obtained between 8.00 am to 9.00 am at baseline and post- treatment (week 8) for biochemical analysis. Finally, after blood collection, the blood samples were placed at room temperature for 20 minutes to clot. Then Blood was centrifuged for 10 minutes at a speed of 3000-3500 rpm, and obtained serum was separated into four separate microtubes, and was stored at -20 ˚C until further analysis. To measure salusin alpha and salusin beta, a human salusin-α, kit with a sensitivity of 0.51 pg/ml (Stibopharm Company) and a human salusin-β kit with a sensitivity of 5.22 pg/ml (Stibopharm Company) were used respectively. Interleukin 6 were measured by ELISA sandwich method (International GmbH LBL company and with a sensitivity of 0.92 pg/ml).

Statistical method

All data are presented as mean ±standard deviation. The Shapiro-Wilk test was used to check the normality of the data distribution, and the Levene's test was used for homogeneity of variances. The paired t-test was applied to compare significant differences between pre and post-tests in each group. Analysis of covariance and Bonferroni's post hoc test were used to check the difference of post-tests between groups. The percentage of changes was calculated as the difference the between pre-test and post-test; divided by the post-test, multiplied by 100. All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 23. A value of $P< 0.05$ was regarded as statistically significant**.**

Ethical considerations

The present study protocol was confirmed by the Ethics Committee of Honorable Vice-Chancellor of Research of Mohaghegh Ardabili University (IR.UMA.REC.1401.002) and was registered in the Iranian registry of clinical trials (http://www.irct.ir: IRCT 20201128049510N1).

Results

No significant difference was found between groups in term of age, weight, height, body fat percentage, BMI, HbA1C, fasting blood glucose, and the duration of diabetes at baseline of the study (Table 2). Also, the results of consumption food processing

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Table 2. Descriptive characteristics of participants in the studied groups

Variable	Training Mean (±SD)	Training + supplement Mean (±SD)	Supplement Mean (±SD)	Placebo Mean (±SD)	Control Mean (±SD)	*P*
Age(year)	66.50 (±1.41)	66.75(±1.66)	67.2 5(±1.66)	67.00 (±1.30)	67.87 (±1.45)	0.43
Weight(kg)	73.62 (±2.87)	73.37 (±3.20)	72.87 (±3.13)	72.00 (±2.56)	71.75 (±3.05)	0.21
Height (cm)	174.87 (±3.31)	175.12 (±2.47)	174.00 (±4.69)	174.87 (±3.52)	174.12 (±3.18)	0.95
Body fat (percentage)	24.12 (±1.55)	24.87 (±1.64)	25.00 (±2.72)	24.50 (±2.44)	25.50 (±2.97)	0.55
BMI* (kg/m^2)	24.10 (±0.89)	24.00 (±0.86)	24.01 (±0.74)	23.52 (±0.88)	23.66 (±0.671)	0.74
HbA1C **(percentage)	7.15 (±0.32)	7.18 (±0.54)	7.16 (±0.38)	7.20 (±0.46)	7.20 (±0.5)	0.99
Fasting glucose	185.75 (±6.31)	185.79 (±6.93)	186.96 (±7.82)	185.20 (±7.09)	185.93 (±7.41)	0.98
Duration of diabetes (years)	7.50 (±1.60)	7.32 (±1.43)	7.87 (±1.55)	7.66 (±1.31)	7.62 (±1.59)	0.95

-Values reported as mean ± SD. P-value was obtained from ANOVA.
*BMI: Body Mass Index. **HbA1C: Hemoglobin A1C.

analysis showed that there was no significant difference in any of the macronutrients, minerals and vitamins consumed between subjects of different groups during the implementation of the research. No side effects were reported during the study.

The results of this study show that a significant increase was detected in salusin-α (pg/ml) and a significant reduction was detected in salusin-β (pg/ml) and interleukin-6 (pg/ml) after intervention in compare with pre-test in the training, supplement, and training + supplement groups. Whilst, we failed to find any significant changes in salusin-α, salusin-β and interleukin-6 in the placebo and control groups (Table 3).

The result of covariance analysis for comparing post-tests of variables between study groups was significant. Therefore Bonferroni's post hoc test was used to examine differences between paired groups.

There were significant differences for salusin-α mean values in the post-test phase, between training and supplement groups (*P*= 0.001), training and placebo groups (*P*= 0.001), training and control groups (*P*= 0.001), training + supplement and supplement groups (*P*= 0.001), training + supplement and placebo groups (*P*= 0.001), and training + supplement and control groups (*P*= 0.001).

However, there was no significant difference between training + supplement and training groups (*P*= 0.12), supplement and placebo groups (*P*= 1.000), supplement and control groups (*P*= 1.000), and placebo and control groups (*P*= 1.000).

Comparison of salusin-β in post-test demonstrated significant differences between training and placebo groups (*P*= 0.03), training and control groups (*P*= 0.02), supplement and training + supplement groups (*P*= 0.003), training + supplement and placebo groups (*P*= 0.001), training + supplement and control groups (*P*= 0.001).

However, there was no significant difference between training + supplement and training groups (*P*= 1.000), supplement and placebo groups (*P*= 1.000), supplement and control groups (*P*= 1.000), training+supplements (*P*= 0.18) and placebo and control groups (*P*= 1.000).

There was a significant difference in interleukin 6 values in the post-test between training+ supplement and placebo groups (*P*= 0.04), training+ supplement, and control groups (*P*= 0.006). Nevertheless, differences between training + supplement and training groups (*P*= 1.000), training and supplement groups (*P*= 1.000), training and placebo groups (*P*= 1.000), training and control groups (*P*= 1.000), supplement and training + supplement groups (*P*= 0.35), supplement and placebo groups (*P*= 1.000), supplement and control groups (*P*= 1.000) and placebo and control groups (*P*= 1.000) were not significant.

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Table 3. Average changes and standard deviation of the research variables in the studied groups

Variable	Group	levels	Mean (±SD)	P within group	Percentage of changes	*P*
Salusin-α (pg/ml)	Training	Pre-test Post-test	678.80 (±19.89) 706.69 (±19.98)	0.001*	3.94 (±1.89)	0.001†
	Training + Supplement	Pre-test Post-test	685.03 (±15.52) 725.22 (±16.06)	0.001*	5.54 (±1.89)	
	Supplement	Pre-test Post-test	678.72 (±11.03) 683.34 (±11.97)	0.01*	0.67 (±6.50)	
	Placebo	Pre-test Post-test	671.44 (±22.89) 669.62 (±14.25)	0.82	-.027 (±7.85)	
	Control	Pre-test Post-test	689.88 (±15.21) 683.67 (±14.77)	0.052	-0.90 (±2.97)	
Salusin-β (pg/ml)	Training	Pre-test Post-test	816.06 (±32.0) 780.65 (±24.13)	0.001*	-4.53 (±32.61)	0.001†
	Training + Supplement	Pre-test Post-test	819.63 (±44.89) 764.44 (±28.06)	0.001*	-6.73 (±56.95)	
	Supplement	Pre-test Post-test	817.89 (±62.93) 811.41 (±43.86)	0.001*	-0.79 (±43.47)	
	Placebo	Pre-test Post-test	818.63 (±45.28) 819.76 (±46.58)	0.82	0.13 (±2.79)	
	Control	Pre-test Post-test	816.33 (±39.48) 819.53 (±37.25)	0.47	0.39 (±5.98)	
Interleukin 6 (pg/ml)	Training	Pre-test Post-test	7.66 (±0.90) 6.89 (±1.00)	0.0001*	-11.17 (±10.00)	0.001†
	Training + Supplement	Pre-test Post-test	7.72 (±1.12) 6.28 (±0.85)	0.02*	-22.92 (±31.76)	
	Supplement	Pre-test Post-test	7.50 (±1.13) 7.12 (±1.12)	0.02*	-5.33 (±88.39)	
	Placebo	Pre-test Post-test	7.72 (±1.10) 7.64 (±1.06)	0.66	1.04 (±3.77)	
	Control	Pre-test Post-test	7.70 (±1.01) 7.61 (±1.20)	0.76	1.18 (±15.83)	

-Values reported as mean ± SD. **P* was obtained from paired T-test. †*P* was obtained from ANCONA
*Intragroup statistical significance
†between groups statistical significance

Discussion

This study aimed to investigate the effect of 8 weeks of combined exercise (aerobic+ resistance) along with the consumption of Mulberry leaf extract on the serum levels of salusin-α, salusin-β and interleukin 6 in elderly men with type 2 diabetes. The results of the present study showed that there were significant increase in serum levels of salusin-α in the training +supplement group (5.54 percent), training group (3.94 percent) and supplement group (0.67 percent). salusin-β was significantly reduced in the supplement+ training group (6.73%), training group (4.53%), and supplement group (0.79%).

The results of the study on increase of Salusin-α and decrease of salusin-β in the exercise group are in line with the findings of Paaho et al. (10) and Nik Sarasht et al (22). In a study, Nik Sarasht et al. (2020) showed that 8 weeks of aerobic and resistance training increases salucine alpha and decreases salucine beta in women with type 2 diabetes (23). Some studies have shown that salusin-β levels increase in the blood circulation of people with diabetes. Also, a significant relationship has been reported between the increase of salusin-β and cardiomyopathy in diabetic rats (12,22). It seems that the increase of salusin-α and the decrease of salusin-β are through the decrease of the activation of the levels of inflammatory cytokines and indicators of oxidative stress (23). The improvement of endothelial dysfunction in diabetes may be through the mechanism of peroxisome proliferator-activated receptor gamma (PPARγ) agonists. It has recently been found that salusin-β down regulates PPARγ expression at the protein, mRNA, and gene promoter in Vascular Smooth Muscle Cell (VSMCs) (24). It has been reported that the reduction of β-salocin is done by reducing the formation of macrophage sponge cells by reducing cholesterol ester accumulation in macrophages (down regulation of ACAT1) (24,25). Watanabe et al. (2008) exhibited that

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salusin-α and salusin-β have mutual effects in the formation of macrophage sponge cells so that salusin-α suppresses the formation of foam cells through negative regulation., In contrast, salusin-β increases the formation of sponge cells by positive regulation (3). The other proposed mechanism, the changes in salusines due to exercise can be attributed to the reduction of blood pressure caused by the inhibition of angiotensin II through Janus kinase-2 (Jak-2) activation (7). Salusin-α expression is modulated by angiotensin II through the activation of Jak-2, a well-known regulator of blood pressure (26,27). Apoptosis, proliferation, migration, and irregular angiogenesis of endothelial cells are among the functional disorders in the vessels of people with diabetes, which can be moderated by the beneficial changes of salusins (28,29).

Another finding of the present study was that the levels of salusin-α and-β increased and decreased significantly in training+supplement and supplement groups, respectively. The noteworthy point in the present study was that the supplement + training group caused a more significant response in salusins changes. It seems that the use of antioxidant supplements such as mulberry leaf extract is one of the effective treatment options to reduce inflammatory conditions due to its antioxidant and anti-inflammatory effects (30). According to the effects of combined aerobic and resistance exercises, it has been seen that their use together can exert synergistic effects (17,31). Combined aerobic and resistance exercise seems to improve inflammatory conditions in people with diabetes by reducing oxidative stress and inflammatory factors (32). Phenolic compounds in Mulberry leaf extract inhibit the oxidation reactions of lipid compounds by the following mechanisms: Inhibiting the formation of free radicals, reducing metal ions, deactivating singlet and triplet oxygen, or forming complexes with metal ions (33). Previous studies have demonstrated that Mulberry extract exhibits anti-inflammatory effects by inhibiting the production of prostaglandins and leukotrienes (8). phenolic and quercetin compounds are responsible for their various medicinal effects, including immune regulation, inhibition of tumor formation, reducing inflammation, and being anti-apoptotic (9). Therefore, it seems that the combined exercises and supplement of Mulberry extract have anti-atherosclerotic, anti-inflammatory, and antioxidant effects, and in this way can lead to the improvement of salusins in the intervention groups.

Also, the present study showed that there were significant decrease in interleukin-6 in training + supplement (22.92%), training (11.17%), and supplement groups (5.33) The findings of previous research demonstrated that muscle contractions caused by sports activity lead to a decrease in the expression of inflammatory cytokines such as interleukin-6 (5,6). A number of previous studies have stated that several mechanisms alter the inflammatory index: The amount of adipose tissue is undoubtedly the most related to the concentration of circulating inflammatory markers and it seems that the change in body composition is one of the factors affecting inflammatory conditions (34). Exercise may affect cytokine secretion by T cells through various mechanisms such as changes in circulating factors (lactate, catecholamines, and growth factors), lymph node stimulation, and increased NK cell motility. Also, exercise decreases the release of cytokines from adipose tissue by reducing sympathetic stimulation (35). The primary mediator of the sympathetic nervous system is norepinephrine and epinephrine, which are mainly secreted from the cells of the central part of the adrenal gland. These catecholamines increase the production of pro-inflammatory cytokines such as interleukin 6 and tumor necrosis alpha in the bloodstream through alpha and beta-adrenergic receptors (33). It has been reported that Mulberry leaf extract has anti-diabetic, antioxidant, anti-cancer, and anti-inflammatory properties due to its anthocyanin, quercetin, and polyphenol compounds (36-38). Anthocyanins present in the extract play a role in reducing the

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expression of inflammatory and oxidative factors effective in diabetes such as IL-6, monocyte chemotactic protein (MCP) and Tumor necrosis factor (TNF-α), and by increasing the expression of AMP-activated protein kinase (AMPk) and as a result, increasing the expression of GLUT4 in muscles and fat tissue and reducing Retinol binding protein 4 (RBP4), is effective in reducing blood sugar (39). Anthocyanin reduces the process of hepatic gluconeogenesis by inhibiting the glucose-6-phosphatase enzyme (40). Regarding the inconsistency of some studies with the results of the present study, it seems that the type of exercise protocol, the intensity of the exercise and the gender of the subjects are the reasons for the inconsistency with our study. Accordingly, the response of inflammatory markers depends on age, fitness level, and pathological conditions such as obesity and diabetes (41,42).

Conclusions

According to the results of the present study, it can be suggested that performing combined exercises (aerobic + resistance) for eight weeks and consumption of Mulberry extract have a protective effect against inflammatory and sclerotic indicators in elderly with T2DM. Also, combined exercise along with consumption of this supplement causes a more incredible response in controlling atherosclerosis indicators in T2DM.

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Conflict of Interest

There is no conflict of interest between the authors in this study

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