Original Article

Effect of HIIT with almond gum on gene expressions HMGB1, Caspace3, P53 in type2 diabetic rats

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ABSTRACT

Biological responses to exercise training are complex because almost all organs and systems are involved in interactions that lead to many adaptations at the genetic and metabolic levels. The purpose is to investigate the effect of eight weeks of high-intensity interval training with almond gum supplementation on the expression of HMGB1, Caspace3P53 and genes in the heart tissue of type 2 diabetic rats. 40 adult male rats divided into 5 groups of 8, control (CO), diabetes (CD), exercise (DE), supplement (DS), exercise and supplement (DES). Diabetes was induced with a single injection of 55 m/kg STZ. Training group started with 5 intervals of 30 seconds (90% vo2max) and 1 minute of active rest (40% vo2max) in the first week and ended with 12 intervals in the last week. The rats receiving the supplement also received the almond gum supplement by oral gavage for eight weeks. 24 hours after the last training session, the heart tissue of the mice was sampled. Data analysis was done using independent t-tests and one-way ANOVA at a significance level of P≥0.05. The interactive effect of HIIT exercise, along with almond gum supplement, had no significant effect on HMGB1 (p=0.634) and caspase3 (p=0.554) gene expression, but almond gum consumption and intense exercise had a significant effect on HMGB1 (P=0.001) and in Caspase3. Respectively, p=0.031 and p=0.009, each of which was effective on gene expression alone. However, the interactive effect of HIIT exercise and supplement consumption had a significant effect on P53 gene expression (p=0.045).

Keywords: Type 2 diabetes, Caspase3, p53, HMGB1, Almond gum supplement, Streptozotocin.

Introduction

Genes, epigenetics, environment, lifestyle—particularly high-calorie diets—and other variables all play a role in the complex illness known as type 2 diabetes (1). The insulin-secreting β-cells' gene expression and peripheral organs' insulin sensitivity are impacted by these risk factors (2). Empirical data demonstrates the heritability of T2DM phenotypic responses, indicating a potential genetic foundation (3). Hyperglycemia and consistently high blood glucose levels are the hallmarks of diabetes mellitus, sometimes referred to as diabetes mellitus, a complicated metabolic disease. Which is brought about by anomalies in either the action or secretion of insulin, or both (4). As people age, the proportion of adults with diabetes rises and stays elevated. In addition, compared to those without diabetes, those with type 2 diabetes have a higher chance of dying from cardiovascular disease (CVD). Furthermore, even if they are of normal weight, individuals with type 2 diabetes who have low aerobic fitness have a three times higher risk of dying from cardiovascular disease (CVD) and a seven times higher risk of dying from all causes. To increase aerobic fitness, it is advised to engage in regular aerobic activity that targets the main muscular groups in the upper body, arms, and legs. (5). The coexistence of heart failure with type 2 diabetes is widespread (6), and diabetic cardiomyopathy is one of the reasons for mortality in people with type 2 diabetes. (7).

Furthermore, the development and advancement of diabetic vascular problems, including coronary atherosclerosis, are linked to the death of vascular endothelial cells (8). At the moment, all diabetes-related problems are largely caused by inflammatory processes. It is now established that inflammation is linked to issues related to hyperglycemia and that it happens even in the absence of bacterial or viral infections (9).

According to this issue, in response to stress, p53 protein leads to the start of regulation of the transcription of target genes, which can cause various responses such as cell cycle arrest, apoptosis and aging (10). P53 has also been demonstrated to enhance skeletal muscle's ability to do aerobic exercise and to increase the amount of mitochondrial DNA in the muscle. These findings imply that p53 may enhance mitochondrial oxidation in skeletal muscle via controlling mitochondrial biogenesis.

Additionally, one of the most well-known methods for enhancing mitochondrial function and human health in metabolically active tissues is exercise-induced physical activity. In fact, regular exercise completely eliminates the role of p53 in regulating mitochondrial DNA content, respiration, function, oxidative stress, and resistance to skeletal muscle insulin. (11).

Also, caspase 3 is known as the most effective caspase in apoptosis. In humans, this gene is a cytoplasmic protein that is highly expressed in the lungs, spleen, heart, liver, kidneys, and cells of the immune system (12), (13). Researchers have found that stress markers, such as caspase-3, have a specific predictive value in heart problems and can be used as serum biomarkers for the diagnosis of HF (14). (15), also discovered that pyroptosis, a pro-inflammatory kind of controlled cell death, is linked to an increased risk of diabetes, and that during exercise, apoptosis is reduced because of a drop in components of the Fas-dependent apoptotic pathway, such as Fas protein. Death domain associated with (FADD), -ligand, Fas death receptor, TNFR-1, caspase-8, and caspase-3. (14).

Pyroptosis differs from apoptosis and necrosis, two other types of cell death, in terms of morphology, mechanism, and pathophysiology. It is typified by a fast rupture of the plasma membrane, releasing pro-inflammatory mediators including IL-1β, IL-18, and HMGB1 as well as intracellular contents. Pyroptosis may be connected to atherosclerosis and play a crucial part in the instability of atherosclerosis lesions, according to recent research (16). Furthermore, HMGB1 belongs to a higher family within the HMG protein group. It functions to establish chromosomal structure and regulate transcription inside the cell; following acetylation, it is transferred from the nucleus to the cytoplasm, where it eventually finds extracellular location. (17). In order to discover the role of autophagy in the pathophysiology of heart function in diabetes, researchers use pharmaceutical agents and even lifestyle modification (diet and exercise) and types of exercise that include short periods of intensive exercise and high-intensity interval (HIIT) followed by periods of They used low-intensity rest that can provide maximum health in a short period of time (18). Exercise positively influences the P53 gene, which encourages injured cells to repair the damage, and it has been demonstrated that the levels of P53 and cytochrome C proteins rise following exercise. If this is not the case, self-destruction may be encouraged if repair is possible (19). Furthermore, exercise—both short-term (3 days) and long-term (3 weeks)—may decrease TNF-α, caspase-8, and caspase-3, which in turn can decrease myocardial apoptosis in rats. On the other hand, sustained activity outperforms transient exercise (20). Researchers discovered in their investigations that HMGB1 contributes to the aetiology of diabetes and that both diabetic humans and animal models had high amounts of HMGB1 (8). A six-month exercise-based cardiac rehabilitation program decreased plasma HMGB1 levels, which significantly improved maximal oxygen consumption and heart rate recovery, an important autonomic functional marker. This suggests that the availability of HMBG1 may be a key factor in microglia activation (17, 21). Meanwhile, in the blood and tissue of diabetic rats, aerobic exercise lowers the amount of HMGB1 (17). It was postulated that a bitter almond gum supplement might enhance cardio-metabolic indicators in the study of the impact of bitter almond gum as a functional food on immunological, oxidative stress, inflammatory, and mental health biomarkers in women with T2 diabetes. Enhance mental well-being, oxidative stress, and inflammation in women with type 2 diabetes by modifying their gut flora (22). Rahbar Ghazi and colleagues found in their review studies on melatonin, exercise, and heart tissue function in type 1 and type 2 diabetes that by controlling lipid profile, antioxidant capacity, apoptosis, and inflammation, exercise and supplements can lessen the detrimental effects of diabetes on the heart (23). By introducing brief bursts of moderate-to-intense physical activity (e.g., up to three minutes), high-intensity interval training proves effective in reducing blood glucose levels. Oddly enough, it may have somewhat greater glycemic advantages to exercise in the afternoon as opposed to the morning and after a meal. The best exercise recommendations when taking food, medicine, and/or other habits into account remain uncertain, despite the fact that exercise is an excellent way to manage type 2 diabetes (24).

So far, few studies have been conducted on the effect of sports activities, especially HIIT exercises and the consumption of almond gum supplements, and most of the researches conducted in this field have examined the effects of endurance, strength and aerobic activities without almond gum supplements. Therefore, according to the researches that have been done and the lack of sufficient researches regarding the effect of sports activities, especially HIIT exercises and the consumption of almond gum supplements on the expression of HMGB1, Caspase3, P53 genes, also according to the principle of exercise characteristics that each exercise has characteristics. It creates its own adaptations and adaptations on different organs and also due to the different and contradictory results regarding the mechanism and role of the above genes in the prevention and treatment of type 2 diabetes, the purpose of this research is to investigate the effect of exercise. The severity of almond gum supplementation on the expression of HMGB1, Caspace3, P53 genes in the heart tissue of type 2 diabetic rats was investigated.

Materials and Methods

Examples of research:

The current study is an experimental one that was conducted in a lab setting. The study employed forty adult male Wistar rats, weighing 260 ± 30 and aged 12 weeks, that were acquired from the Pasteur Institute in Tehran and brought to the Animal House of Medical Sciences in the province of Qom. Mice housed in polycarbonate cages for 12 hours were kept in a dark environment with a temperature of 22 ± 1 °C. Water for the animals was always freely provided in a 500-ml bottle for laboratory animals for the whole study process (10). Every stage of the current study was conducted in accordance with the National Research Council's guidelines for the use and care of

laboratory animals, and every attempt was made to minimize the animals' discomfort. The Qom Faculty of Medical Sciences ethics and research committee examined and approved the study's protocol, which was given the ethics identification number IR.MUQ.AEC.1402.021. Every attempt was made to limit animal suffering and the number of animals used in the study.

Induction of diabetes:

Mice were given a single dosage of streptozocin (STZ) to induce diabetes. A single intraperitoneal injection of 55 ml/kg Streptozotocin was used to produce diabetes; blood sugar levels more than 250 mg/dl seventy-two hours following the injection were deemed indicative of caused diabetes (25).

Research implementation method

Training protocol:

The mice were trained to run on a treadmill for five days, for ten minutes each day, at a pace of ten meters per minute. The training program was then implemented using the HIIT protocol, one week following the transfer and acclimatization to the new environment. At the beginning of the training period, each training session started with five 30-second intervals at 90% VO2max speed with one minute of active rest between two intervals at 40% VO2max speed and one additional interval every week based on the maximum speed test on the tape speed. Battalion was added. The number of repetitions in the last week of 12 repetitions and running speed was calculated based on the maximum speed protocol of each week and based on about 90% of the maximum oxygen consumption. The training started with 5 intervals of 30 seconds and 1 minute of active rest (17:30 minutes) at a speed of 25 m/min and ended at the end of the eighth week with 12 intervals and a speed of 33 m/min (28 minutes). Warming up and cooling down were also done at a speed of 10 m/min at the beginning and end of each training session (26), (12).

Table 1. the weekly protocol

weeks	Warm up (10 m/min)	Reps 30"	Maximal speed in min	Rep speed in min	Rest (10 m/min)	Cool down (10 m/min)	Total time
1	5	5	25	4	5	5	17:30
2	5	6	27	5	6	5	19:00
3	5	7	28	6	7	5	20:30
4	5	8	29	7	8	5	22:00
5	5	9	30	8	9	5	23:30
6	5	10	31	9	10	5	25:00
7	5	11	32	10	11	5	26:00
8	5	12	33	11	12	5	28:00

Maximum speed measurement test:

The conveyor belt's maximum speed was measured at 5 m/min at the beginning of the test, and it was raised by 5 m/min every 3 minutes until the rats became weary (the mice adhered to the end of the conveyor belt). The maximum speed of the rats is defined as the pace at which they become fatigued. According to the conditions of adaptation to the practice test, the maximum speed test was performed every week (12).

Supplement:

Persian gum (Amygdalus scoparia) is an anionic gum that is naturally secreted from the mountain almond tree. Persian gum was collected from almond trees grown in Shiraz (Kazron) province, Iran, and then ground (27).

Consuming almond gum supplement:

Mice receiving the supplement were treated with almond gum in distilled water at a concentration of 15% by weight, equivalent to 1 CC, by oral gavage for eight weeks, five days a week and once a day (28).

Laboratory methods

Tissue sampling method:

Following the conclusion of the training session, the rats were eliminated in accordance with ethical principles and intraperitoneally injected with a combination of ketamine (30 to 50 mg/kg body weight) and xylazine (3 to 5 mg body weight) to induce anesthesia. The rats were then promptly frozen using liquid nitrogen at -80 for additional measurements in order to eradicate the sudden impacts of training and unexpected stress factors of the subjects during the implementation of the training program. The samples in the laboratory were kept in a freezer at -80 degrees until conducting tests to evaluate the amount of gene expression changes (29).

Preparation of histopathology sample:

After dissection, to take tissue sample, the tissue will be placed in containers containing buffered formalin (10%) for stabilization. After a week has passed and the tissue samples have been fixed, the rest of the steps will be carried out in the autotechnicon machine and paraffin casts will be prepared from them. Using a microtome, 5 micrometer slices are prepared from the paraffin molds and one section is selected from every 20 slices and a slide is prepared from it. Then the slides will be stained with hematoxylin-eosin method (H&E). In each slide, the parts observed in the entire tissue section will be examined morphometrically and descriptively with a light microscope (30).

Real-time PCR:

First, do triplicate PCRs for each gene in each cDNA sample. Then, create the following master composition, which comprises one to two extra reactions per gene. Materials in each reaction (ll) for reactions x (ll) 2) SYBR green reagent 12.5 × 12.5 Forward/reverse primer mixture (50 mM each) 0.125 x 0.125 molecular biology-grade water. 7.375 x 7.375 then, first place, molecular biology-grade water. In a sterile disposable well, dilute the cDNA at 1:50 or 1:100. Using a multichannel pipette, add 99 mL of water into the PCR strip tube. Use a multichannel pipette to dispense 1 mL of cDNA. (For example, Rainin L8-10) into the designated strip tubes. Mix the solution 20 times with a multichannel pipette, such as the Rainin L8-200. Please adjust the pipette to 75% capacity for effective mixing. To prevent cross-contamination, add solution (in this case, cover 75 ml of undiluted cDNA with fresh strip caps). Mark distinct places on the plate with a marker with a fine tip. And then add 20 cc of the master mix to each sample using a repeat pipette (Rainin, E12-20). One row at a time, add. Apply 5 milliliters of diluted cDNA using a multichannel pipette to every well. Using sealing tools, apply optical adhesive coating and seal. Run a quick spin on a centrifuge that has a 96-well plate adaptor, up to 1500 rpm. Utilizing a real-time device, carry out PCR in accordance with the manufacturer's instructions. For SYBR green detection, 40 cycles of 15 s at 95 °C and 60 s at 60 °C are usually carried out, after which the thermal separation process is used (31).

Statistical analysis:

After collecting the data for statistical analysis, Shapro-Wilk test was used to ensure the normal distribution of the collected data, Levine's test was used for the homogeneity of variances, and analysis of variance test (one-way and two-way) was used to compare the outgroups.) ANOVA and analysis was done using SPSS 26 software at a significance level of <0.05. The results were presented in the form of mean and standard deviation, and the data was analyzed.

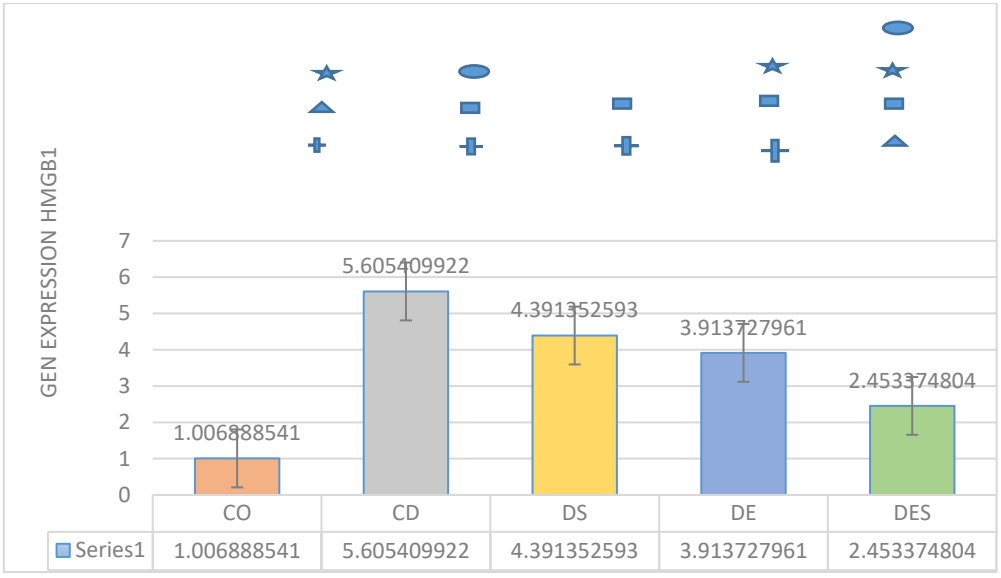
Results and Discussion

The results of this research showed that the healthy control group gained weight during these 8 weeks, while the other groups that had become diabetic, their weight decreased slightly or did not change. And according to the initial and final sugar, no change was observed in the healthy group, but in other diabetic groups, we saw a sharp increase in blood sugar, which is the result of streptozotocin injection. It should be noted that the supplement and exercise group had lower final sugar than the diabetic control group, which showed the effect of the supplement and intermittent high-intensity exercise on type 2 diabetic rats.

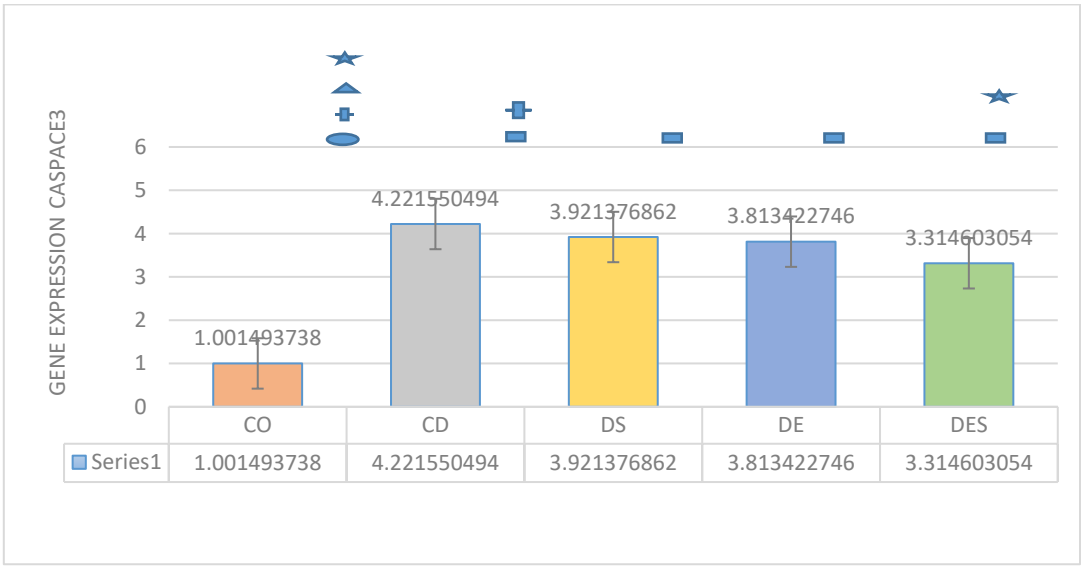
Table 2. The results of primarily and final weight and sugar

groups	NO	primarily WEIGHT	FINAL WEIGHT	primarily sugar	FINALY sugar
CO	8	236	293.38	92.38	112.88
CD	6	251.6	194.8	80	587
DS	7	218.57	209.86	104.43	474.43
DE	7	244.86	238.57	157.43	472.57
DES	7	228.57	231.43	76.43	355.71

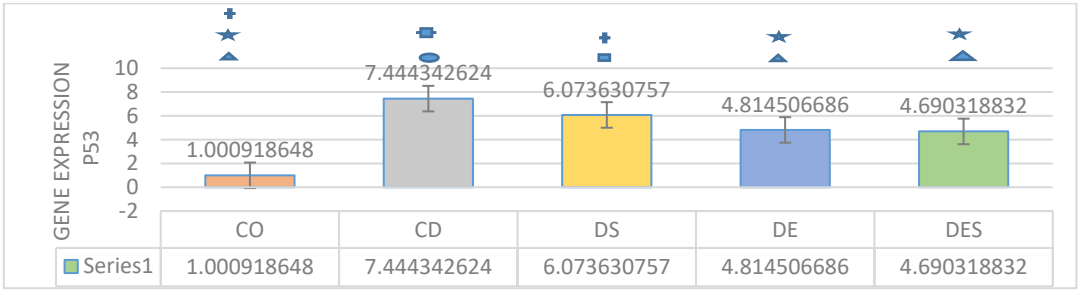
Normality results show that HMGB1, P53 and Caspase3 test groups follow a normal distribution. In all these stages, the significance of the Shapiro-Wilk test is greater than 0.05. The results of Levin's test also show that the significance of this test for the variables in the groups and stages of the experiment is greater than the 5% error, therefore the variance of the groups is the same. And the assumptions of the two-way analysis of variance test are performed for the above variables.



(A)



(B)



(C)

Figure 1. The relative expression of HMGB1, CASPASE3, P53 gene expression values in the research groups after a period of exercise and supplement consumption (mean, standard deviation): noteworthy distinction from the diabetes control group, noteworthy distinction from the exercise group, noteworthy distinction from the supplement group, noteworthy distinction from the healthy group, and noteworthy distinction with activity supplement group (P≤0.05).

Using the two-way ANOVA test, the expression levels of Hmgb1, Caspase3 and P53 genes in the heart tissue of type 2 diabetic rats in different research groups were investigated. (A), the simultaneous use of supplements and exercise is greater than 0.05, and as a result, the interactive effect of supplements and exercise on HMGB1 has no significant effect (p=0.634). However, the calculated effect size value is equal to 0.8, which indicates an excellent effect size. The calculated power is also equal to 1, which indicates the high power of the test. The main effect of supplementation on HMGB1 is also significant because the level of significance obtained is smaller than 0.05 (p < 0.001). The calculated effect size is equal to 0.701, which indicates a high effect size. The calculated power is equal to 0.99, which indicates the high power of the test. According to the above interpretation, supplements alone and exercise alone can have a significant effect on HMGB1. And the use of supplements with exercise does not have a significant effect.

(b), the significance level in the sports supplement group is greater than 0.05, and as a result, the interactive effect of the supplement and exercise on caspase 3 does not have a significant effect (p=0.554), but the calculated effect size is equal to 0.44, which indicates a medium effect size. The calculated power is also equal to 0.81, which shows the high power of the test. The main effect of the supplement on Caspase3 is also significant because the significance level obtained is smaller than 0.05 (p<0.031). The calculated effect size is equal to 0.33, which indicates a medium effect size. The calculated power is equal to 0.615, which indicates the high power of the test. According to the above interpretation, supplements alone and exercise alone can have a significant effect on Caspase3. And the use of supplements with exercise does not have a significant effect.

(c) The simultaneous interaction effect of supplementation and exercise on P53 had a significant effect (p=0.045). It should be noted that the effect of the test is 0.2, which means that it is not very high, and the power of the test is 0.05, which indicates the low power of the test. The main effect of training on P53 is significant and training alone can be effective on P53 (p < 0.001). The main effect of the supplement on P53 is also significant because the significance level obtained is smaller than 0.05 (p<0.020). The main effect of exercise supplement was also significant (p<0.045). According to the above interpretation, supplement alone and exercise alone can also have a significant effect on P53.

nd can be used as serum biomarkers for the diagnosis of HF. Exercise causes components of the mitochondrial-dependent apoptotic system, including cytochrome c, caspase-9, caspase-3, Bak, Bad, p-Bad, and t-Bid, to decrease (Pahlavani, 2022). Furthermore, eight weeks of high-intensity training (HIIT) combined with almond gum intake had a substantial impact on

P53 gene expression, per the results of this study. Research by Rahbar Ghazi et al. (2023), Akbarian et al. (2023), Qane et al. (2022), and Hay et al. is consistent with the findings of the current study. P53 has been demonstrated to enhance skeletal muscle's ability to do aerobic exercise and to increase the amount of mitochondrial DNA in the muscle. This suggests that, via controlling mitochondrial biogenesis, p53 may also enhance skeletal muscle's mitochondrial oxidation. Additionally, one of the most well-known methods for enhancing human health and mitochondrial function in metabolically active tissues is exercise-induced physical activity. It has been thoroughly demonstrated that p53 has a role in controlling the amount of mitochondrial DNA, respiration, oxidative stress, and insulin resistance in skeletal muscles after consistent exercise (Elraf et al., 2023). In the male rat cardiac muscle, the expression of the P53 and cytochrome C genes was not significantly affected by either rose or endurance exercise alone. Furthermore, in male rats, body weight, myocardial weight, and the ratio of myocardial weight to body weight were significantly impacted by both endurance exercise and the rose supplement alone. They found that the expression of the cytochrome C and P53 genes was not significantly affected by 12 weeks of endurance training using rose extract (Abdollahi-Diba et al., 2022).

In diabetic rats, Anwar et al. looked into the anti-atherogenic properties of almond oil. The findings revealed that the diabetic group receiving almond oil had much greater HDL cholesterol than the diabetic group and significantly lower average levels of cholesterol, triglycerides, and LDL cholesterol. The diabetic group receiving almond oil had considerably lower mean levels of ICAM-1, H2O2, and percentage of DNA damage, and significantly greater mean concentrations of insulin, NOx, and GPX activity than the diabetic group. In STZ-induced diabetic mice, the findings validated almond oil's ability to operate as an antioxidant to alleviate oxidative stress and demonstrated that it efficiently enhances endothelial function and prevents the development of atherosclerosis (33). An eight-week study of yoga exercises also showed a significant effect of almond consumption and diet on the sexual performance of diabetic women. But after the intervention in the control group, no significant effect on sexual performance was observed. Therefore, yoga exercises, almond consumption and diet improved the sexual performance of diabetic women (34).

At the moment, all diabetes-related problems are largely caused by inflammatory processes. It is now known that inflammation is linked to problems with hyperglycemia and that it happens not just when host pathogens are under control but even when there are no bacterial or viral infections present (9). P53 deficiency contributes to glycolysis as an energy production pathway. A parallel decrease in cellular oxygen consumption is also associated with a decrease in COX activity. Also, due to a decrease in the synthesis of cytochrome c oxidase subunit (SCO22) in both types of cells (20), caspase-3 is known as the most effective caspase in apoptosis and is known as Yama, known as -1SCA, apopain and CPP32, is a cysteine-aspartate-specific protease. The cytoplasmic protein encoded by this gene is abundantly expressed in human lung, spleen, heart, kidney, and immune system cells (35).According to recent research, proptosis may be a significant factor in atherosclerosis lesions (12). The workout group's protein family is larger (17). The average blood glucose levels of the diabetes exercise group and the control group differed significantly from one another; the control group's average blood glucose levels were higher than those of the exercise group and lower than those of the diabetes and exercise control groups (9). The probiotic compounds identified in bitter almond gum consist of arabinogalactan polysaccharides similar to gum aerobic (22). Further, considering exercise as a health tool for most people with type 2 diabetes (DM2T), exercise is a valuable therapeutic aid to optimize health that is easy to perform. Of course, depending on specific conditions such as the characteristics of exercise, timing in relation to meals, routine blood sugar control in patients and the appropriateness of preventive measures. , the effectiveness of the measures taken is different (36).

In the heart tissue of diabetic rats, the results of this study demonstrated that eight weeks of high-intensity interval training (HIIT) or almond gum supplementation had a significant interactive effect on the expression of HMGB1 and caspase 3 genes. Additionally, the heart tissue of diabetic rats showed significant effects from exercise alone or from almond gum supplementation alone, but the interaction between the two variables did not significantly affect the gene expression of HMGB1 and caspase 3 genes. This suggests that taking the almond gum supplement in conjunction with exercise may produce positive results. Additionally, the results demonstrated that the main effect of the supplement and exercise, as well as their interaction, had a significant impact on the expression of the P53 gene. This suggests that both the supplement and exercise used in combination, as well as either exercise alone or the supplement's consumption alone, can have positive effects. Possess the P53 gene. Exercise is the most appropriate option for improving non-communicable diseases, which type of exercise and its intensity can be prescribed according to the type of disease and its severity as well as the physical condition of the person. Which is suggested for more effectiveness of exercise along with nutrition. In this context, the study's findings demonstrated that the expression of the genes HMGB1 and Caspase3 was impacted in the heart tissue of type 2 diabetic rats after eight weeks of high-intensity training (HIIT) combined with the ingestion of an almond gum supplement. While it didn't have a substantial impact, high-intensity exercise and supplements containing almond gum alone had a notable impact on the expression of these genes. However, it significantly impacted the P53 gene. In HMGB1 and Caspase3 genes, there is no need to consume almond gum together with exercise, and almond gum alone and exercise alone can also affect the expression of these genes. But in the P53 gene, due to the significance of exercise and supplementation, exercise and supplementation will have a greater effect at the same time to optimize conditions and reduce gene expression.

It is suggested that more research be conducted on the effect of various types of sports activities on other unknown gene targets related to type 2 diabetes and related factors, to lead to more accurate results and a complete understanding of their molecular mechanisms. One of the limitations of the present study is the lack of investigation of other effective factors in the recovery process of type 2 diabetes and the lack of investigation in a human model.

Conclusion

The interactive effect of HIIT exercise, along with almond gum supplement, had no significant effect on HMGB1 and caspase3 gene expression, but almond gum consumption and intense exercise had a significant effect on HMGB1 and Caspase3. Each of which was effective on gene expression alone. However, the interactive effect of HIIT exercise and supplement consumption had a significant effect on P53 gene expression.

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Conflict of interest: None

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Ethics statement: None

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