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Effect of 8 weeks of Intense Intermittent Exercise with Thyme Extract on the Expression of Apoptosis Indicators Bax and p53 in Liver Tissue and Insulin Resistance in Type 2 Diabetic Rats

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A B S T R A C T

Objective: The present study aimed to investigate the effect of eight weeks of exhaustive interval training combined with thyme extract supplementation on the expression of apoptosis markers Bax and P53 in liver tissue and insulin resistance in male rats with type 2 diabetes.

Methods and Materials: The study population consisted of 36 male Wistar rats. After 20 weeks of a high-fat diet and intraperitoneal injection of 25 mg/kg STZ, the rats developed diabetes. A fasting blood glucose level between 150 to 400 mg/dL indicated type 2 diabetes. The diabetic rats were then divided into four groups: control, interval training, thyme extract, and interval training-thyme extract groups. The exercise protocol consisted of eight weeks of interval training, five sessions per week, with 2-minute high-intensity intervals (2 to 8 intervals) at 80-90% VO2max and 1-minute rest intervals at 50-56% VO2max. Thyme extract was administered at a dose of 200 mg/kg dissolved in distilled water, given orally via gavage 5 days per week before exercise.

Findings: Two-way ANOVA analysis showed that the gene expression of apoptosis markers Bax and P53 in liver tissue significantly increased in the interval training and thyme extract groups compared to the diabetic control group. Moreover, the expression of these markers was significantly higher in the thyme extract group compared to the control group. However, the expression of Bax and P53 in liver tissue in the interval training and combined interval training-thyme extract groups showed a non-significant increase compared to the control group. Additionally, findings indicated an improvement in insulin resistance and glucose levels in the interval training and combined interval training-thyme extract groups, with significant results observed in the interval training group.

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Conclusion: It appears that high-intensity interval training and thyme extract consumption may reduce the expression of apoptotic markers Bax and P53 in liver tissue and improve liver health in Wistar rats. Consequently, researchers may utilize these findings to assess improvements in the condition of individuals with type 2 diabetes.

Keywords: *Interval training, Thyme, Apoptosis markers, Insulin resistance index, Liver tissue*

1. Introduction

Diabetes mellitus is the most common metabolic disorder, characterized by hyperglycemia due to absolute or relative insulin deficiency (1). Despite standard methods and the use of chemical drugs to reduce blood sugar in these patients, these approaches are still insufficient to prevent complications such as liver disorders, cardiovascular diseases, eye conditions, neuropathy, and kidney failure. It seems necessary to explore alternative treatments for this disease, which is now considered a latent epidemic. Three major lesions are considered in diabetic hepatopathy: 1) Cellular lesions, 2) Hepatosclerosis, and 3) Hyaline necrosis (2). The most significant liver lesion is hepatosclerosis, which involves the diffuse increase of connective tissue matrix accompanied by the proliferation of fibroblasts, often associated with the thickening of the connective tissue in the periportal space and the space of Disse (1). Hepatocytes in diabetic patients may undergo degenerative changes due to excessive glycogen accumulation. In cases of severe hyperglycemia, the osmolality of liver cells increases, leading to degenerative changes. Regular physical activity is one of the therapeutic and preventive strategies for mitigating the complications of this disease. For this reason, exercise is recommended as a special regimen in the treatment of diabetes (3).

The primary therapeutic goals in diabetes include reducing insulin resistance and stimulating insulin secretion through dietary modification, exercise, and pharmacotherapy (3, 4). Diabetes can also cause tissue damage and cell death or apoptosis. Apoptosis is a programmed and highly regulated form of cell death that plays a critical role in organ development, homeostasis, and the removal of worn-out cells (4). Research indicates that defects in this pathway can lead to the accumulation of mutated cells and, ultimately, to patient mortality (5).

Recent evidence suggests that high-intensity interval training (HIIT) is among the most effective exercise modalities. In addition to saving time for individuals who are unable to engage in regular exercise due to time constraints and busy schedules, HIIT produces remarkable physiological effects and serves as a powerful and practical method for combating obesity. HIIT typically involves exercising at intensities greater than 90% of the maximum heart rate, with short rest periods and total exercise durations of less than 20 minutes. This approach effectively engages more muscle fibers and strongly recruits metabolic and fuel-utilization systems, thereby positively influencing overall body metabolism (6).

Short-term, high-intensity exercise not only reduces fat mass more effectively but also positively impacts lean body mass, increasing its quantity (2). Numerous reputable sources confirm that HIIT increases the type and number of muscles involved and the mechanisms of energy production and consumption within the body. Consequently, the utilization of fats and sugars is elevated (7). The physiological effects of HIIT persist for up to 48 hours after each training session, whereas the effects of long-duration, low-intensity aerobic exercise dissipate shortly after the session ends (8). Many biochemistry experts have reported that HIIT improves insulin sensitivity, reduces insulin resistance, increases secondary glucose transporters such as GLUT-4 in muscle cells, lowers triglycerides and low-density lipoproteins, and increases plasma HDL. Additionally, HIIT has stronger effects on inflammatory and anti-inflammatory cytokines and myokines, as well as on metabolic regulators (9).

Currently, the use of herbal supplements and extracts to aid in the treatment of diseases and metabolic disorders is gaining popularity. Thyme (Thymus vulgaris), one of the oldest medicinal plants, belongs to the Lamiaceae family and contains compounds such as tannins, flavonoids, saponins, bitter substances, and phenolic compounds like thymol, carvacrol, p-cymene, linalool, cineole, terpenoids, glycosides, caffeic acid, and rosmarinic acid. This plant has various properties, including tonic, digestive, antispasmodic, carminative, antifungal, antibacterial, antiseptic, antiepileptic, anthelmintic, antirheumatic, expectorant, and antioxidant effects (10).

One of the vital organs in the body is the liver, which plays a crucial role in metabolism and maintaining blood glucose levels within the normal range. Elevated blood glucose leads to an imbalance in oxidation-reduction reactions within hepatocytes (11).

This study aims to report the effects of exhaustive interval training combined with thyme extract on the pro- and anti-apoptotic markers in the liver tissue of type 2 diabetic rats. This research seeks to evaluate thyme's antioxidant and anti-inflammatory properties in conjunction with designing an interval training program tailored to the dietary needs of diabetics. After similar human studies, we hope that the findings from this study can be applied in the medical and sports sciences as a potential solution for mitigating complications associated with diabetes, such as diabetic cardiomyopathy and liver damage.

2. Methods and Materials

2.1 Study Design

The present study was experimental in nature, featuring a pre-test and post-test design with four control and training groups.

2.2 Population and Sample

The study population consisted of male rats. A total of 36 male Wistar rats, 4 weeks old and weighing within a range of 110 ± 20 grams, were selected from the population.

2.3 Methodology

Initially, the rats were kept for two weeks to acclimatize to the environment, reaching an approximate weight of 190 ± 20 grams. All rats were fed a high-fat diet (45% to 60% fat) for 5 months or 20 weeks. After the rats became obese, reaching an average weight of around 407 ± 50 grams, type 2 diabetes was induced by intraperitoneally injecting them with 25 mg/kg body weight of low-dose streptozotocin (STZ). One week after diabetes induction, fasting blood glucose was measured, and blood sugar levels ranging from 150 to 400 mg/dL were considered as a criterion for confirming the development of type 2 diabetes. Blood samples were randomly taken from the tails of 7 rats to study the effect of the high-fat diet and STZ on serum glucose, insulin, and insulin resistance indices. The diabetic rats were then divided into four groups: a diabetic control group (6 rats), a high-intensity interval training (HIIT) group (8 rats), a thyme group (4 rats), and a thyme + HIIT group (7 rats). The experimental HIIT and thyme + HIIT groups performed intense aerobic interval training at speeds ranging from 20 to 36 meters per minute (65% to 90% of maximum oxygen consumption) for 16 to 45 minutes per session, 5 sessions per week, for two months (8 weeks). The thyme experimental groups (thyme-only and thyme + HIIT groups) consumed 200 mg/kg of hydroalcoholic extract of Thymus vulgaris dissolved in distilled water, administered orally via gavage 5 days a week before the exercise sessions.

2.4 Diabetes Induction Method via STZ Injection

Diabetes was induced using a high-fat diet for 20 weeks, followed by the intraperitoneal injection of a freshly prepared STZ solution in physiological saline (25 mg/kg). This study aimed to use a high-fat diet and a minimal STZ dose to minimize pancreatic cell destruction and induce type 2 diabetes. One week after the injection, fasting blood glucose levels were measured by placing a drop of blood from a small tail wound on a glucometer strip, then read by a glucometer. Blood glucose levels between 150 and 400 mg/dL indicated diabetes. To ensure accuracy and confirm the diabetes induction in rats, random blood samples were taken from 10 rats, and glucose, insulin, and insulin resistance indices were measured. The results of these measurements are provided in the findings tables (12).

2.5 Thyme Preparation and Administration Method

The leaves and young shoots of the plant under study were collected from the heights of Arsanjan in Fars Province, and the University of Shiraz confirmed a herbarium sample. After drying the collected samples in the shade, they were ground into powder using a mill. The powder was then placed in closed 1-liter glass containers, to which 70% medical alcohol and 30% distilled water were added. The mixture was allowed to soak for 72 hours. After this period, the extract was obtained using the percolation method (extraction under pressure). The collected extract was concentrated using a rotary evaporator and then further dehydrated using an incubator as much as possible. The solid extract was reconstituted with distilled water to form a solution. During the experimental period, the thyme and the thyme + HIIT groups were administered 200 mg/kg of the thyme extract, diluted in distilled water, orally via gavage (13).

2.6 Interval Training Protocol

The 8-week aerobic training program consisted of five sessions per week with a gradual increase in the intensity of intervals, from speeds of 22 to 38 meters per minute (80% to 90% of VO2max) and rest intervals at speeds of 16 to 22 meters per minute (50% to 56% of VO2max), with durations

ranging from 15 to 34 minutes, performed as treadmill running. The running duration increased from 16 minutes in the first week to 34 minutes in the eighth week. The rats were familiarized with the treadmill one week before starting the protocol by walking on the treadmill for 10, 12, and 15 minutes at a speed of 5 meters per minute with a 0% incline three days a week. The control group also walked on the treadmill following the same protocol. The maximum speed was determined using the protocol described by Rodríguez et al. (2007). Due to the unavailability of direct tools (such as respiratory gas analysis devices) for measuring maximum oxygen consumption (VO2max), an indirect protocol was used with high accuracy based on previous research. Every two weeks, the rats were subjected to an exercise test, starting with a 5-minute warm-up at 10 meters per minute, followed by running at 15 meters per minute for 2 minutes. Every 3 minutes, the speed was increased by 3 meters per minute until the rats could no longer continue and reached exhaustion, as indicated by remaining on the shock grid (14).

Table 1. Interval Training Protocol

Week	Warm-Up Intensity	Number of Intense Intervals	Intense Interval Duration	Intense Interval Speed	Rest Interval Duration	Rest Interval Intensity	Cool-Down Intensity	Total Time (min)
1st & 2nd	5 minutes	2 intervals	2 minutes	80% of maximum speed (30 m/min)	1 minute	50% (16 m/min)	10 m/min	16
3rd & 4th	5 minutes	4 intervals	2 minutes	85% (32 m/min)	1 minute	52% (18 m/min)	10 m/min	22
5th & 6th	5 minutes	6 intervals	2 minutes	90% (34 m/min)	1 minute	54% (20 m/min)	10 m/min	28
7th & 8th	5 minutes	8 intervals	2 minutes	95% (36 m/min)	1 minute	56% (22 m/min)	10 m/min	34

2.7 Sampling

At the end of the training period and 48 hours after the last training session, the experimental groups of rats were anesthetized with ether and euthanized after a 12-hour fast. Blood samples were collected via cardiac puncture and stored at -20°C. Glucose levels were measured using an auto-analyzer, and insulin was measured using a specific kit from Pars Azmoon. The Insulin Resistance Index (HOMA-IR) was calculated using the following formula (11):

Insulin Resistance (HOMA-IR) = Glucose (mg/dl)×Insulin (µUI/ml) / 405

2.8 Statistical Analysis

Descriptive statistics (mean and standard deviation) were used to describe the data. The Kolmogorov-Smirnov test was used to determine the normality of data distribution, and Levene's test was used for variance homogeneity. Inferential statistics were used to compare group differences, including one-way ANOVA and Bonferroni post hoc tests. Two-way ANOVA and effect size measures were used to compare the impact of each independent variable. Statistical analysis of glucose, insulin, and HOMA-IR was performed using SPSS version 22. Gene analysis for Bax and p53 was conducted similarly. The significance level for all data was set at $p \leq 0.05$

3. Results

Table 2 shows the weight and descriptive information for glucose, insulin, and insulin resistance index, as well as the lipid profiles of the rats in the research groups used to diagnose type 2 diabetes.

Table 2. Initial Descriptive Data for Weight, Glucose, and Insulin Resistance in Rats Following HFD and STZ-Induced Diabetes for Diagnosing Type 2 Diabetes.

Initial Protocol Weight (g)	Weight After Obesity (g)	Glucose (mg/dl)	Insulin (µUI/ml)	HOMA-IR
197.7 ± 19.46	402.75 ± 51.69	363 ± 124.5	3.92 ± 0.49	3.56 ± 1.43

Table 3. Description of Weight, Glucose, Insulin, HOMA-IR, and Hepatic Gene Expression of Bax and Bcl2 in Different Rat Groups

Variable/Group	Control (6 rats)	Interval Exercise (8 rats)	Thyme Extract (4 rats)	Exercise + Thyme Extract (7 rats)
Weight After High-Fat Diet	386.66 ± 48.42 g	407.37 ± 64.64 g	414.75 ± 43.49 g	421.85 ± 65.53 g
Weight After 8 Weeks	317.00 ± 71.3 g	373.12 ± 54.28 g	332.25 ± 67.73 g	352.71 ± 39.31 g
Glucose (mg/dl)	333.83 ± 39.39	40.04 ± 138.25	117.00 ± 1.41	121.28 ± 6.39
Insulin (µUI/ml)	3.89 ± 0.53	6.22 ± 1.35	11.22 ± 1.25	9.64 ± 1.51
HOMA-IR	3.18 ± 0.33	2.04 ± 0.35	3.24 ± 0.38	1.69 ± 0.36
Bax Gen. Fold Change	1.11 ± 0.54	0.29 ± 0.16	1.06 ± 0.57	0.37 ± 0.21
P53 Gen. Fold Change	1.02 ± 0.25	0.48 ± 0.27	0.72 ± 0.37	0.55 ± 0.31

The data in Table 4 show that interval training significantly affects Bax gene expression in the studied groups. The mean Bax gene expression ratio in the interval training group (0.29) and the interval training + thyme group (0.37) was significantly lower compared to the control group. However, the expression in the thyme group (1.06) did not differ significantly from the control group (1.11) (P = 0.996).

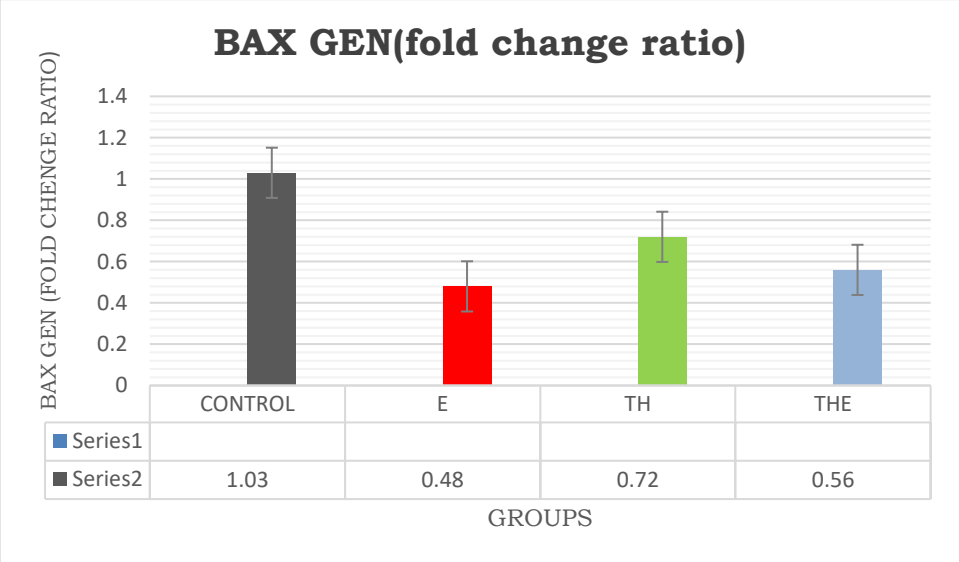

Figure 1. Bax Gene Expression in Different Groups

Table 4. Results of Two-Way ANOVA Test for Determining the Effects of Interval Training and Thyme Extract on Bax Gene in Diabetic Male Rats.

Source	F	Significance Level	Result	Effect Size	Power
Interval Training	23.65	0.0001	**	0.530	0.996
Thyme Extract	0.008	0.928		0.000	0.051
Interval Training & Thyme Extract	0.199	0.660		0.009	0.071

Table 5. Results of the Bonferroni Post Hoc Test for Comparing Bax Gene in rat.

Group	Diabetic Control	Interval Training	Thyme Extract	Training and Thyme Extract
Diabetic Control	M = 1	P = 0.003**	P = 0.996	P = 0.009**
Interval Training		M = 0.29	P = 0.015**	P = 0.937
Thyme Extract			M = 1.06	P = 0.038**
Training and Thyme Extract				M = 0.37

In this regard, the Bonferroni post hoc test indicated that the mean ratio of Bax gene expression in the interval training group (0.29) and the training-thyme group (0.37) was significantly lower compared to the control, while the expression in the thyme group (1.06) did not differ significantly from the control group (1.11).

As shown in Table 6, interval training did not significantly affect the expression of the P53 gene among the study groups. The mean ratio of P53 gene expression was significantly higher in the interval training group (1.73) and the training-thyme group (1.27) compared to the control group (1.07), while the expression in the thyme group (0.81) did not differ significantly from the control group (1.07) (P = 0.947).

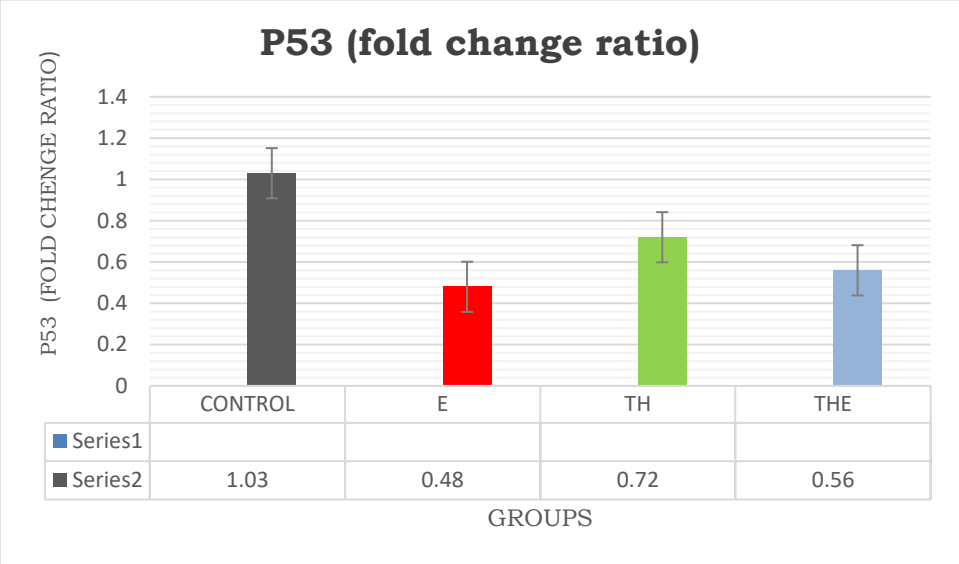

Figure 2. Expression of the P53 Gene in Different Groups

Table 6. Results of the Two-Way ANOVA Test to Determine the Effects of Interval Training and Thyme Extract on Gene P53 in Diabetic Male Rats.

Source	F	Significance Level	Result	Effect Size	Power
Interval Training	3.37	0.08	**	0.138	0.418
Thyme Extract	1.38	0.25		0.062	0.202
Interval Training and Thyme Extract	0.10	0.74		0.005	0.061

*p<0,05, **p<0,01

Table 7. Results of the Bonferroni Post Hoc Test for Comparing Gene P53 in Rats

group	Diabetic Control	Interval Training	Thyme Extract	Training + Thyme Extract
Diabetic Control	M=1	P=0.014**	P=0.401	P=0.045*
Interval Training		M=0.48	P=0.575	P=0.962
Thyme Extract			M=0.72	P=0.821
Training + Thyme Extract				M=0.56

*Significant at the 0.05 level.

In this context, the Bonferroni post hoc test indicated that the mean ratio of P53 gene expression in the interval training group (0.48) was significantly reduced compared to the control group (1.02) (P=0.009). However, the P53 expression in the exercise–thyme group (0.55) and the thyme group (0.72) did not show a significant reduction compared to the control.

4. Discussion and Conclusion

The present study's findings showed that high-intensity interval training and thyme extract led to a significant

reduction in glucose levels, an increase in insulin levels, and a significant decrease in insulin resistance in type 2 diabetic rats fed a high-fat diet. Additionally, these interventions reduced the expression of the pro-apoptotic gene Bax and decreased the expression of the anti-apoptotic gene P53 in the liver of type 2 diabetic rats fed a high-fat diet. Ahmadi Asl and colleagues (2007) showed that 12 weeks of moderate-intensity aerobic exercise did not affect myocardial apoptosis in Wistar rats; however, 24 and 36 weeks of endurance exercise significantly reduced apoptosis (15). Finally, Marsh et al. (2001) showed that long-term aerobic exercise (14 weeks) did not affect the levels of BAX, Bcl-2, and BAX/Bcl-2 ratios in endothelial cells of Wistar rats. They reported a reduction in Bax expression and an increase in Bcl2 following six weeks of aerobic exercise and L-carnitine supplementation, suggesting that the increase in anti-apoptotic Bcl2 with exercise and L-carnitine supplementation might be due to the combined antioxidant and fat-reducing effects of L-carnitine and exercise. However, L-carnitine alone had no significant effect on Bax levels in the liver (10).

Cell sensitivity to apoptosis depends on the balance and ratio of pro-apoptotic factors (Bid and Bax) and anti-apoptotic factors (Bcl-xl and Bcl2). The average ratio of these proteins determines the cell's fat (15). Previous studies have observed that exercise can prevent apoptosis by reducing pro-apoptotic Bax protein and increasing anti-apoptotic Bcl2 protein, thereby inhibiting cytochrome c release and preventing caspase 9 activation, which can lead to positive regulation of the apoptosis process (16). Other research has shown that exercise can inhibit apoptosis and DNA fragmentation through both intrinsic and extrinsic pathways by reducing the activity of caspase-9 and caspase-3 (16).

Based on the results of this study, it can be concluded that high-intensity interval training combined with thyme consumption can lead to a reduction in the expression of pro-apoptotic genes such as Bax and improve glucose levels through the effects of genetic components involved in hepatic glucose release in type 2 diabetic patients. Due to its diverse vitamin and protein compounds, phenolic compounds, and its roles as an antioxidant and anti-inflammatory agent, Thyme can help regulate carbohydrate metabolism, particularly glucose and lipid metabolism, and reduce hyperglycemia, dyslipidemia, and insulin resistance. However, thyme alone may not be effective in these areas and changes in apoptotic and anti-apoptotic genes, while the combination of thyme supplementation with interval training can enhance its efficacy (17).

Recent studies have shown that exercise significantly reduces Bax levels. In this regard, Cai et al. (2016) showed that high-intensity interval training and moderate-intensity continuous exercise both reduced BAX expression compared to the control group (18). Lee et al. (2014) also reported that levels of Fas ligand and receptor, caspase-3 and caspase-8 activity, Bax protein levels, and the Bax to Bcl-2 ratio were significantly lower in exercised obese rats compared to sedentary obese rats (19). Research has shown that aerobic exercise significantly reduces visceral fat and improves insulin resistance. Additionally, aerobic exercise may reduce liver fat (19). As demonstrated in the present study, after the exercise intervention, liver Bax levels in diabetic rats were reduced. Regular aerobic exercise also strengthens the body's antioxidant capacity, which may reduce cellular damage at the level of liver cells. The results also showed that the combined intervention of aerobic exercise and supplementation reduced Bax factors in the liver of diabetic rats. Although the precise mechanism of Bax changes is not clearly defined, it is known that Bcl-2 prevents the increase of Bax The findings of the present study demonstrated that high-intensity interval training and thyme extract led to significant reductions in glucose levels, increased insulin levels, and significant decreases in insulin resistance in type 2 diabetic rats fed a high-fat diet, and reduced the expression of the tumor suppressor gene and obesity-related gene P53 in liver tissue compared to the control (20).

In one study, rodents fed a high-fat, high-sucrose diet or with obesity showed increased p53 protein levels in the liver (21). Another study reported that p53 regulates a set of gluconeogenic genes and gluconeogenesis regulatory genes in primary cultured liver cells and p53-deficient liver cells observed with disrupted glucose production (22).

Exercise can improve glycemic control by reducing PGC-1α and subsequently decreasing gluconeogenesis through reduced expression of PEPCK, and can also enhance insulin effects through short-term effects and primarily through insulin-independent glucose uptake. Previous research indicates moderate-intensity aerobic exercise enhances peripheral glucose uptake beyond liver glucose production, reducing blood glucose levels. Aerobic exercise may affect glucose metabolism in diabetic individuals through two separate mechanisms: increasing mitochondrial oxidative capacity in peripheral and liver tissues and suppressing hepatic glucose production. Therefore, the results of the

present study show that high-intensity interval training significantly reduces P53 expression in the liver, whereas thyme alone did not significantly reduce this gene expression. This suggests that combining thyme extract with its medicinal and antioxidant properties with interval training can improve its efficacy by further reducing P53 expression. Suppressing hepatic glucose production along with reduced P53 expression can effectively improve diabetes treatment, indicating that targeting components in the gluconeogenic pathway can alleviate hyperglycemia (23, 24).

The results of the present study showed that eight weeks of interval training and thyme supplementation in type 2 diabetic rats led to significant reductions in fasting glucose and significant increases in serum insulin levels. However, regarding insulin changes with interval training, Fakhr Fatemi et al. (2023) reported increased serum insulin with decreased blood glucose in response to long-term HIIT in type 2 diabetic rats (25); Kharghani et al. (2023) found that an aerobic exercise program led to significant reductions in fasting glucose levels in the diabetic aerobic group compared to the control diabetic group, and serum insulin levels were higher in the aerobic diabetic group compared to the control diabetic group, although this difference was not statistically significant (26).

Overall, high-intensity interval training, especially combined with thyme, can reduce P53 gene expression and improve glucose levels through effects on genetic components involved in hepatic glucose release in type 2 diabetic patients. Thyme, due to its diverse vitamin, protein, and phenolic compounds, serves as a good substitute for glucose and has multiple antioxidant, anti-inflammatory, and other roles, contributing to the regulation of carbohydrate metabolism, particularly glucose, lipid metabolism, and reduction of hyperglycemia, dyslipidemia, and insulin resistance. However, thyme alone may not be effective in these areas and changes in P53 expression can be enhanced by combining it with interval training. Further and complementary studies in this area are necessary (27).

Overall, the results of the present study indicate that thyme extract, combined with high-intensity interval training, leads to an improvement in hepatic apoptosis in diabetic subjects. Based on the findings, it appears that thyme extract in conjunction with interval training may provide protective effects by reducing apoptotic factors such as Bax and P53 in liver tissue and improving liver condition in diabetic rats. Therefore, for individuals with diabetes, incorporating thyme extract along with regular physical activity is recommended.

This study has several limitations that should be considered. First, there was no precise control over the amount of food each rat received, which could have influenced the results. Additionally, the daily activities of the rats were not monitored in detail, potentially affecting their overall behavior and metabolism. The rats' biological characteristics and circadian rhythms were also not tightly controlled, which might have introduced variability in the findings. Another significant limitation was the lack of control over physical injuries resulting from interactions among the rats outside the exercise periods and potential injuries sustained during exercise sessions. Finally, potential stressors affecting the rats during and outside exercise sessions were not monitored, which could have impacted their physiological responses. These factors should be taken into account when interpreting the results and considering future research directions.

Authors' Contributions

N.A. conceived the study, designed the experimental framework, and supervised the project. A.S. carried out the primary laboratory experiments and data collection. H.N. contributed to the interpretation of the results and assisted with the statistical analyses. H.H. provided critical input on the experimental protocols and helped draft and revise the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

Ethical approval was granted by Islamic Azad University (ethical code: IR.IAU.SRB.REC.1399.004), and all procedures followed relevant animal care guidelines.

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