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An interactive effect of Diosmin and High-intensity interval training on gene expression and activity of antioxidant enzymes in patients with type 2 diabetes

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Abstract

Background: There is strong evidence that the development of type 2 diabetes (T2DM) is significantly influenced by elevated oxidative stress. Despite the fact that earlier research has demonstrated the benefits of high-intensity interval training (HIIT) for T2DM, But no human study has yet looked into how Diosmin affects T2DM. Given that the redox status of patients with type-2 diabetes is significantly impacted by exercise and the ingestion of medicinal plants with antioxidant qualities, This study's objective was to determine how 12 weeks of high-intensity interval training (HIIT) and ingestion of Diosmin affected the gene expression and activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in patients with type 2 diabetes.
Methods: Based on the inclusion criteria, 93 overweight (BMI > 25) T2DM patients were chosen to participate in this randomized, double-blind study. They were divided into four groups at random and placed in each for 12 weeks: Diosmin (DIS, N = 23), HIIT + Diosmin (Tr + DIS, N = 24), HIIT + placebo (Tr + PL, N = 23), and placebo (PL, N = 23). Before and after participation in all groups, blood samples were taken from every person to measure biochemical markers like lipid profile analysis, fasting blood glucose (FBG), insulin, glycated hemoglobin (HbA1c), and the homeostasis model of assessment-insulin resistance (HOMA-IR). Blood tests using real-time PCR are used to detect the expression of RCT elements.
Results: The interaction between HIIT and Diosmin ingestion significantly boosted CAT, SOD, and GPx gene expression and activity. Additionally, the effect of HIIT alone dramatically elevated SOD and CAT gene expression. However, Diosmin alone had no discernible effects on the expression of the GPx gene. All intervention groups experienced a substantial decrease in serum levels of glucose, HbA1c, HOMA-IR index, TC, and TG compared to the corresponding levels in the control group.
Conclusions: Based on the findings of this study, HIIT with a Diosmin supplement can enhance antioxidant and metabolic conditions. However, using a Diosmin supplement with HIIT at the same time can have beneficial synergistic benefits.

Keywords: Diosmin, High-intensity interval training, Type 2 diabetes, antioxidant enzymes, gene expression.

Introduction

One of the most prevalent metabolic illnesses, type 2 diabetes (T2DM), is currently regarded as a serious global public health issue (1, 2). According to numerous publications (1, 2), oxidative stress may play a role in the etiology of type 2 diabetes. Increased oxidative stress has a significant role in the onset, progression, and occurrence of several problems associated with diabetes (3). In order to create appropriate treatment options, it is crucial to investigate the connection between free radicals, diabetes, and its complications, as well as the mechanisms through which elevated oxidative stress hastens the onset of diabetic problems(4-7). Increased antioxidant availability can neutralize oxidants, which may lessen the effects of oxidative stress in T2DM. Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) are enzymes that work with vitamins A, C, and E, glutathione, lipoic acid, carotenoids, trace minerals like copper, zinc, and selenium, and coenzyme Q10 to combat free radical-mediated damage. The use of antioxidants offers an appealing treatment strategy for the delay or reversal of diabetic problems, given the involvement of oxidative stress in the development of diabetes. In this regard, natural products are recognized as antioxidant therapies since they have been shown to lower oxidative stress (8, 9).

The primary goal of T2DM treatment is to treat patients' metabolic problems, improve their clinical symptoms, and avoid the development of long-term consequences because T2DM has a complex pathophysiology. The first-line therapies for T2DM include dietary and activity modifications (10). Numerous clinical investigations have offered convincing proof of the strategic value of dietary management in the care of T2DM patients. Diosmin is one of the auxiliary antioxidant agents that has received a lot of attention recently.

A flavone glycoside known as diosmin was discovered in the dried pericarps of various citrus fruits. Diosmin has shown anti-inflammatory, antioxidative, and anticancer properties. Diosmin has recently been shown to have

metabolic ramifications in addition to its role in vascular protection and inflammation since it improves bile duct ligation-induced liver abnormalities and promotes glucose homeostasis in type I diabetes model organisms (11-13).

With a different physiological stimulus than regular exercise, high-intensity interval training (HIIT) has recently attracted interest as a time-effective exercise technique for enhancing cardiometabolic health (14). A recent meta-analysis found that HIIT was better than conventional continuous exercise for enhancing insulin sensitivity and glucose control. Numerous studies have demonstrated significant glucose-lowering and cardiovascular health advantages from HIIT(15, 16), These findings point to the potential value of HIIT as a treatment plan for T2D (17).

Many studies have discovered the effects of HIIT or Diosmin supplementation alone on anthropometric indices, lipid profiles and insulin resistance.25,26 . Nevertheless, rare studies have been done on the simultaneous effects of Diosmin and HIIT on these factors. Thus, the purpose of the current study was to examine the effect of 12-week HIIT and Diosmin supplementation on metabolic parameters, antioxidant enzymes activity and gene expression in T2DM patients.

Martial and Methods

Adult participants between the ages of 18 and 65 were chosen from those receiving referrals to the Baqiyatallah Hospital's Diabetes Clinic in Tehran, Iran. Fasting plasma glucose (FPG) of 126 mg/dL, glycated hemoglobin (HbA1C) of 6.5%, or the usage of anti-diabetic medications were required for admission. Pregnancy or breastfeeding, inability to provide informed consent, participation in a concurrent trial, the presence of cancer, chronic liver disease (alanine aminotransferase levels three times above the limit of the normal value range), renal failure (serum creatinine 2.0 mg/dL or being on dialysis), acute infections, chronic inflammatory diseases like rheumatoid arthritis, endocrine disorders other than T2DM (such as hypothyroidism), and malignancies

Study design

Fig. 1 displays a flowchart of the study protocol. Using a list generated by a random number generator, the subjects (n = 93) were divided into four groups: diosmin supplementation (DIS, N = 23), HIIT + diosmin supplementation (Tr + DIS, N = 24), HIIT + placebo (Tr + PL, N = 23), and placebo (PL, N = 23). The DIS and Tr+ DIS groups received two Diosmin capsules each day (450 mg/day) for a period of 12 weeks, while the Tr+PL and PL groups received a placebo daily. Before and 12 weeks after the intervention, questionnaires about the menstrual cycle characteristics were completed (Fig. 1).

The recommended dosage of the product was one coated tablet of smin® Plus (Giellepi S.p.A., Lissone, Italy), which is comparable to 450 mg of diosmin, once per day. Smin Plus® is a well-known oral flavonoid with venoprotective effects that contains 90% total flavonoids and 80% micronized diosmin.

Fig 01. A flowchart of the study protocol

Anthropometric measurements

One observer (SD) measured the anthropometric indexes. With subjects wearing minimal clothing and no shoes, body weight was measured using electronic digital scales, which were accurate to 0.1 kg. A flexible stadiometer was used to measure height in standing posture to the nearest 0.1 cm. By dividing body weight (kg) by the square of height (m), the BMI was computed. According to established procedures, measurements of the waist and hip circumferences (HC; to the nearest 0.5 cm) were taken while the subjects were standing. Using a regular measuring tape, the WC was measured from the front at the narrowest point between the rib cage and iliac crest following complete expiration, and the HC was measured from the side at the maximum extension of the buttocks. The formula used to compute the waist-to-hip ratio (WHR) was WC (cm) divided by HC (cm) (18).

High-Intensity Interval Training

Each HIIT session lasted 23 minutes and included the following components: (1) a 2-minute warm-up at 50% of peak power output; (2) two 8-minute blocks of interval training with 30-second work periods at 80% to 100% of peak power output interspersed with 30-second active recovery (16-minute conditioning phase); (3) a 1-minute cooldown at 25% of peak power output on an upright cycle ergometer. The effort was gradually increased by 10% after every other session, starting at 50% of the participants' peak power output, until patients were able to exercise at 80% to 100% of their peak power output throughout an exercise training session. Patients were able to reach 150% of their maximal power output to account for changes in exercise tolerance over the course of the

12 weeks. The number of exercise classes participants attended was used to gauge their level of exercise adherence.

Blood sampling

10 ml of blood samples that had been fasted overnight were taken at the beginning and end of the trial. The samples were allowed to coagulate for approximately 30 min, after which the serum was obtained by centrifuging at 750g for 10 min. Before measurements, serum samples were aliquoted and frozen at 80 °C.

Biochemical Assessment

On the Biolis 24i Premium (DiaSystem, Husqvarna, Sweden), the serum concentrations of FBS and triglycerides, total cholesterol, HDL-c, and LDL-c were measured using commercially available kits (Pars Azmoon, Tehran, Iran). Electrochemiluminescence (ECL) was used to measure the insulin levels in serum (Roche, Switzerland; Elecsys 2010 Immunoassay Analyzer). The G8 HPLC Analyzer (Tosoh Bioscience, San Francisco, USA) was used to measure HbA1c. The activity of the enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) was measured using the SOD assay kit from ZellBio GmbH in Germany. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) approach was used to determine insulin resistance: HOMA-IR=fasting insulin (U/mL) and fasting blood glucose (mmol/L)/22.5 [24].

Real-Time PCR (RT-PCR)

HiSep LSM 1077 (Himedia, Mumbai, India) was used to separate PBMCs, which were then preserved in 1 ml of RNAiso Plus reagent (Takara Bio Inc., Shiga, Japan) and kept at 80°. With the help of the RNX-plus kit (Cinnacolon, Tehran, Iran), RNA was extracted from blood samples. Until cDNA was produced, the RNA suspension was frozen at 20°C. Each sample's total RNA was extracted, and then the amount of RNA in each sample was calculated using a UV spectrophotometer. Each sample's OD 260/280 ratio of 1.7–2.1 indicated that there was no protein- or DNA-related contamination. Isolated RNA was reverse transcribed to a cDNA library using Moloney murine leukemia virus reverse transcriptase (RT). Mononuclear cells (PBMCs) from peripheral blood were used to conduct gene expression studies for GPx, SOD, and CAT utilizing quantitative RT-PCR and Light Cycler technology (Roche Diagnostics, Rotkreuz, Switzerland). As a housekeeping gene, primers for the gene actin were employed (Table 1). Relative transcription levels were calculated using Pffafi or $2^{-\Delta\Delta CT}$ methods

Table 1. Specific primers used for real-time quantitative PCR (19, 20).

Gene Name	Sequences 5----3	
SOD	F	GGGCAATGTGACTGCTGACAAAGATGG
	R	CTTTCTTCATTTCCACCTTTGCCCAAGTC
CAT	F	CTCCACTGTTGCTGGAGAAT
	R	CGAGATCCCAGTTACCATCTTC
GPx	F	GCTGTGGAAGTGGATGAAGAT
	R	TCGATGAGGAACTGTGGAGA
β-actin	F	GCAAGCAGGAGTATGACGCTAG
	R	GTCACCTTCACCGTTCCAGTGTC

Data analysis

All data were expressed as mean ± SD. The data's normality and variance equality were assessed using the Shapiro-Wilk and Levene's tests, respectively. A dependent *t* test compared within-group differences, and the analysis of covariance (ANCOVA) and Bonferroni post hoc tests determined between-group differences and inspected research hypotheses. The significance level was set at $P \leq 0.05$, and SPSS version 22 software (IBM, Armonk, NY, USA) analyzed the data.

Results

Anthropometric measurements

The dependent sample t-test results revealed that all intervention groups' posttest weight and BMI values were considerably lower than pretest values. In the Tr groups, the posttest levels of WC and WHR were considerably lower than the pretest. The findings demonstrated that all intervention groups had considerably lower weight and BMI values than the PL group. Additionally, the Tr+ DIS group had a much lower weight and BMI than the DIS group did. When compared to the Tr+PL group, the WC and WHR levels in the Tr+DIS group did not change substantially (Table 2).

Variables		PL	DIS	Tr+PL	Tr+ DIS	P-value
Weight (kg)	Before	82.32±11.03	81.93±12.21	82.29±13.01	83.01±9.23	0.647
	After	83.11±12.10	79.64±6.08[a]	77.18±9.58[ab]	75.33±7.02[abc]	-
	P-value	0.592	0.025	0.016	0.006	
BMI ($kg.m^{-2}$)	Before	30.02±1.43	30.32±1.01	29.77±2.01	30.13±1.78	0.861
	After	29.88±0.91	29.11±0.81[a]	28.72±1.83[ab]	27.19±1.33[abc]	-
	P-value	0.621	0.036	0.028	0.018	
WC (cm)	Before	98.32±7.93	99.45±5.39	100.33±6.72	99.37±7.84	0.755
	After	97.88±6.94	98.66±6.82	97.29±5.12[a]	95.37±5.69[ab]	-
	P-value	0.453	0.711	0.031	0.016	
WHR (cm)	Before	0.96±0.05	0.94±0.03	0.95±0.06	0.96±0.08	0.641
	After	0.97±0.03	0.93±0.06	0.87±0.03[ab]	0.87±0.03[ab]	-
	P-value	0.757	0.688	0.022	0.008	

Analysis of biochemical parameters

According to the dependent sample t-test results, there was no discernible difference between pre- and post-test levels of glycemic indices and lipid profiles in any group. However, all intervention groups had post-test levels of glucose, insulin, TC, and TG that were significantly lower than pre-test values. However, the post-test HDL levels in the intervention groups were noticeably greater than the pre-test levels. In comparison to the PL group, the DIS, Tr+PL, and Tr+DIS groups had significantly lower levels of blood sugar, insulin, the HOMA-IR index, TC, and TG, according to the data. Compared to the DIS group, blood glucose levels were considerably lower in the Tr+ DIS group. In the Tr+ DIS group compared to the DIS group, the levels of insulin, TC, and TG did not differ significantly (Table 03).

Table 03. Biochemical parameters before and after 12-week of intervention.

Variables		PL	DIS	Tr+PL	Tr+ DIS	P-value
FBS (mg/dL)	Before	153.92±26.13	151.29±25.44	154.02±27.37	153.12±27.83	0.427
	After	151.22±27.39	146.30±21.82[a]	147.49±25.75[a]	143.33±25.91[abc]	-
	P-value	0.829	0.042	0.024	0.001	
Insulin (µU/mL)	Before	14.57±3.52	14.38±3.26	15.11±4.33	14.93±5.20	0.835
	After	14.42±3.18	12.01±3.12[a]	12.56±3.52[a]	10.02±5.59[abc]	-
	P-value	0.192	0.34	0.015	0.003	
HOMA-IR	Before	4.31±1.87	4.15±1.55	4.38±1.28	4.03±1.85	0.633
	After	4.66±1.43	3.23±1.29[a]	3.17±1.61[a]	2.35±1.47[abc]	-
	P-value	0.696	0.022	0.019	0.001	
HbA1c (%)	Before	7.49±1.03	7.26±0.62	7.44±1.05	6.88±1.37	0.247
	After	7.66±1.22	6.77±0.82[a]	6.62±0.99[a]	6.01±0.64[abc]	
	P-value	0.490	0.018	0.015	0.001	
TC (mg/dL)	Before	183.15±28.43	187.99±27.74	191.44±27.93	190.66±26.44	0.554
	After	185.96±23.11	178.11±25.94[a]	180.87±24.18[ab]	171.35±29.74[abc]	-
	P-value	0.662	0.012	0.009	0.0001	
TG (mg/dL)	Before	161.77±25.54	163.84±29.33	166.91±28.44	159.22±19.88	0.386
	After	163.33±28.11	151.05±25.02[a]	153.55±29.05[a]	141.44±21.18[abc]	-
	P-value	0.388	0.030	0.022	0.0001	
HDL-c (mg/dL)	Before	41.22±4.44	41.77±4.31	42.82±5.01	42.37±4.44	0.533
	After	41.11±3.78	44.82±3.29[a]	45.33±4.46[a]	51.63±6.27[abc]	-
	P-value	0.706	0.032	0.024	0.001	
LDL-c (mg/dL)	Before	116.32±18.44	113.57±17.44	115.87±19.36	117.05±21.39	0.289
	After	112.10±17.99	110.33±16.03	109.44±18.91	113.36±20.27	-
	P-value	0.677	0.086	0.102	0.091	

FBS: Fasting blood glucose; HOMA-IR, homeostatic model assessment for insulin resistance;; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol. *Values are showed as mean ± SD. a: Significantly different compared to PL. b: Significantly different compared to CUR. c: Significantly different compared to Tr+PL.. †Statistical analysis was done by paired sample t-test. *Statistical analysis were determined using One-way ANOVA.*

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Activity assessment and gene expression of antioxidant enzymes

The expression profiles of genes involved in antioxidant function (CAT, SOD, GPx,) in all groups were measured using the qRT-PCR technique. As the results indicate in, Diosmin supplement and HIIT significant increased the expression of CAT compared to the PL group. Also, Tr+DIS significantly increased the expression of CAT compare to DIS group. But, the expression of CAT gene expression wasn't significantly different between Tr+PL and Tr+DIS groups (Figure 02.A1). The results of CAT activity showed Diosmin supplement, HIIT and their combination significantly increased the CAT activity compared to the PL group. There wasn't significant different between experimental groups for CAT activity (Figure 02.A2). Diosmin supplement, HIIT, and their combination increased the expression of SOD, compared to the PL group. These differences were significant only in the Tr+PL and Tr+DIS groups (Figure 02.B1). Also SOD activity in all intervention groups significant increased compared to PL group, Also SOD activity in the Tr+ DIS was significant higher than Tr+PL and DIS group (Figure 02.B2). The Diosmin supplement and HIIT groups increased the expression of GPx compared to the placebo group, but the increases were not statistically significant. On the other hand, their combination significantly increased the expression of GPx (Figure 02.C1). Also GPx activity in DIS and Tr+DIS groups significant increased compared to PL group. But, there wasn't significant different between experimental groups for GPx activity (Figure 02.C2).

3.3. SOD mRNA expression and enzyme activity

In all participants, following the interventions, negative correlation were noted between changes in CAT, and SOD genes expression and activities with changes in glycemic indices. But the changes in GPx gene expression and activity wasn't a negative significant correlation with the changes in HbA1c and HOMA-IR.

Table 04. Variables' correlations.

Variables	antioxidant enzymes gene expression			antioxidant enzymes activities		
	CAT	SOD	GPx	CAT	SOD	GPx
Glucose	-0.275*	-0.098*	-0.109*	-0.077*	-0.146	-0.294*
Insulin	-0.168*	-0.135*	-0.135*	-0.130*	-0.293*	-0.372*
HOMA-IR	-0.402*	-0.211*	-0.591	-0.395*	-0.115*	-0.057
HbA1c	-0.311*	-0.184*	-0.292	-0.190*	-0.084*	-0.719

. * *Significant correlation*

In all participants, following the interventions, negative correlation were noted between changes in CAT, and SOD genes expression and activities with changes in glycemic indices. But the changes in GPx gene expression and activity wasn't a negative significant correlation with the changes in HbA1c and HOMA-IR.

Discussion

According to the findings of the current study, patients with T2DM who received Tr and DIS for 12 weeks saw a significant reduction in their anthropometric indices (weight and BMI). Despite being significant in the Tr and DIS groups alone, this drop was more obvious in the Tr+DIS group. In a similar vein, Osama et al. evaluated the anti-obesity effects of diosmin in obese diabetic patients with metabolic syndrome and found that weight and BMI were significantly decreased (21). An enhanced basal metabolic rate, enhanced free fatty acid oxidation, and lower levels of inflammatory cytokines may all play major roles in how turmeric improves anthropometric measures (11-13). In line with the findings of the current investigation, Banitalebi et al. (22) and Jiang et al. (23) reported that Tr increased maximum fat oxidation in T2DM patients, which decreased body weight (BW) and body fat percentage (BFP). The intake and release of fat from visceral fat stores may be the fundamental mechanism of HIIT-induced body weight loss.

Glycemic indices

The current study's findings showed that following Tr + DIS, the glycemic index underwent considerable alterations. As a result, during the 12-week intervention, there was a significant drop in the levels of insulin, FBS, HOMA-IR, and HbA1C. The interaction effect of Tr and DIS was noticeably stronger, even though this drop was seen following Tr and DIS alone.

Liu et al., developed A systematic review and meta-analysis of 13 studies examining the impact of HIIT on glucose management and cardio-pulmonary fitness (24). According to Jelleyman et al., HIIT significantly decreased IR, HbA1c, and fasting blood glucose in T2DM patients compared to the sedentary group (17). Boff et al. did not appreciate any changes in HbA1c after 8 weeks in either HIIT, in contrast to our trial (25).

The ability of HIIT to engage more muscle fibers and quickly deplete muscle glycogen stores may be the mechanism by which it improves glucose management, as this would lead to a higher improvement in post-exercise muscle insulin sensitivity(26). Due to the fact that a single bout of exercise increases muscle insulin sensitivity over 48 hours afterward, HIIT may be a useful technique for enhancing glucose management both immediately and over the long term. The additional benefits of decreasing abdominal adipose tissue and boosting lower-body muscle mass may result from performing HIIT over a longer length of time (e.g., 12–16 weeks) (15, 16). Exercise would also increase the translocation of GLUT4 receptors at the muscle level, resulting in an increase in peripheral glucose absorption (27, 28). However, HIIT increases VO2max absorption in T2DM patients, a measure of the maximum oxygen uptake and use by our cells that also serves as a predictor of glucose clearance via plasma insulin (29).

On the other hand, previous studies have shown that Diosmin treatment resulted in a significant restoration of the plasma glucose, insulin, glycosylated hemoglobin, and the activities of carbohydrate metabolic enzymes. However, prior research has demonstrated that therapy with diosmin led to a considerable recovery of plasma glucose, insulin, glycosylated hemoglobin, and the activity of carbohydrate metabolic enzymes. According to Pari et al., Diosmin (in various doses) administered orally for 45 days can enhance glycemic control (30). Cheng et al., showed that diosmin encourages the synthesis of insulin from the remaining cells in the pancreas, which increases the activity of glycolytic enzymes and modifies glucose metabolism [Cheng, 2002 #65]. Diosmin is a potential therapeutic agent for the treatment of metabolic illnesses because Yu et al. showed that it protected mice from diet-induced insulin resistance, obesity, and fatty liver by blocking PPAR phosphorylation without causing any obvious side effects (31). Additionally, Zhang et al. demonstrated that in rats with gestational diabetes mellitus (GDM), diosmin significantly decreased serum glucose level, insulin resistance, insulin, C peptide, and HbA1c via the AGEs-RAGE signaling pathway (32).

Lipid profile

Symptoms of metabolic dyslipidemia in persons with T2DM include hypercholesterolemia, lipid deposition in hepatic organs, and changes in plasma lipid and lipoprotein profiles (33, 34). There has also been conflicting evidence in the literature on the effects of HIIT-induced benefits on lipid profiles in T2DM patients. Studies have shown benefits for LDL cholesterol alone, LDL and HDL cholesterol together, and not at all (35-37). In T2DM patients, the same trials did not find any HIIT-induced reductions in triglycerides (38, 39). In the current study, we found that 12-week HIIT improved triglyceride and HDL cholesterol levels but not LDL cholesterol levels. Disparities between studies on training-induced improvements in lipid profiles may be explained by variations in exercise volume and intensity, as well as in the length of the intervention.

Numerous strategies through which HIIT may alter the lipid profile have been reported in studies. Lipid oxidation has been demonstrated to increase during HIIT (40). Furthermore, lipolysis-related hormones and enzyme levels could rise (40, 41). HIIT has been associated with increases in hormones like catecholamine and growth hormone. Catecholamine enhances lipid oxidation and accelerates lipolysis by activating adrenergic receptors in target tissues, primarily adipose tissue. After HIIT, beta-hydroxyacyl coenzyme-A dehydrogenase levels rise; this enzyme controls lipid oxidation (41, 42).

Along with its contribution to intermittent exercise, diosmin has also been connected to better lipid metabolism in diabetics. With diosmin therapy, plasma lipids, tissue lipids, and plasma lipoproteins can all be effectively normalized (11-13). Osama et al showed hesperidin and diosmin significantly lowered blood glucose, TG, and LDL in diabetic patients with metabolic syndrome (21). Diosmin was found to increase HDL levels while decreasing TG, LDL, and TC levels in GDM, according to a number of investigations (32). According to Srinivasan and Pari, administering Diosmin to diabetic rats may have potential antihyperlipidemic effects, as seen by the further decline in LDL and VLDL levels and the increase in HDL levels (43). The positive effects of diosmin and serum lipid have been attributed to a variety of pathways, including HMG-CoA enzyme inhibition, LPL activation, decreased hepatic fatty acid synthesis, increased cholesterol excretion through bile acids, and increased LCAT activity (11-13) .

Antioxidant

The first line of defense against free radical toxicity is comprised of antioxidants. Diabetes causes oxidative stress and a decline in antioxidant status, which might exacerbate the harmful effects of free radicals (3). The enzymes SOD, CAT, GPx, and GST, which are vital in scavenging the hazardous intermediate of incomplete oxidation, represent the first line of defense against ROS in the body. Diabetes patients were found to have reduced SOD, CAT, and GPx activities. The inactivation of enzymatic antioxidants by free radicals or enzymatic glycation may be the cause of their decreased activity (4-6).

The results of this study demonstrated that HIIT for 12 weeks increased CAT, GPX, and SOD activity and gene expression levels in T2DM significantly. The CAT, GPX, and SOD antioxidant enzymes have been shown to improve after 12 weeks of moderate-to-high intensity training and eight weeks of HIIT (8,9). Increases in GPx and decreases in MDA were noted following HIIT training in the study by Mitranun et al. (44). Bafghi et al., showed that the exercise-induced increases in SOD and CAT concentrations show either a decrease in the formation of advanced glycation end products (AGEs) or a reduction in the glycation of antioxidant enzymes (8). Regarding the impact of sports, the level of exertion can cause the release of free radicals, which in turn stimulate the antioxidants' metabolic pathways. The SOD is the main antioxidant defense line that is activated at the start of any action that starts at a low intensity, meaning that the amount of free radical formation is significantly smaller. However, as exercise intensity increases, GPXs become engaged and deactivate H_2O_2 (45). The intensity and duration of sports activities, as well as the quick rise in oxygenation levels and free radical production, can all be noted to support the increase in antioxidant defense enzymes after exercise. The results of the current investigation agree with those of Hamakawa et al. High levels of oxidative stress in the skeletal muscle appear to be necessary for altering the amount of antioxidant enzymes produced as a result of exercise (46). Regarding the effects of exercise on the rise in GPX levels, it should be noted that HIIT increases the gene expression of antioxidant enzymes and reduces the oxidative stress brought on by high-intensity exercise through phosphate and calcium-dependent pathways, the activity of AMPK-dependent and calmodulin-bound kinase enzymes, as well as the activation of the FOXO3 pathway (47, 48).

On the other hand, the expression and activity levels of the SOD and CAT enzymes would significantly increase after 12 weeks of diosmin administration in diabetic patients. By scavenging free radicals, flavonoids help reduce or eliminate oxidative damage caused by illnesses. By reducing the formation of free radicals and boosting antioxidant levels, the dietary flavonoid diosmin may shield cells from oxidative damage (43). Zhang et al., exhibited Diosmin also decreased the level of TBARS and enhanced the levels of GSH, CAT, and SOD, according to an experimental investigation (32). In another investigation, Yasm et al. found that adding diosmin-hesperidin combo to the diet significantly boosted serum levels of GPX and SOD, demonstrating this medication's antioxidative capabilities (49).

Reelatship Antioxidant with hyperglycemia

Table 4 shows a negative correlation between changes in glycemic indices, antioxidant enzyme activity, and gene expression. In T2DM patients, Monnier et al. found a significant positive connection between glycemic status and a marker of oxidative stress(50). One of the main sources of the hyperglycemia-induced triggers of diabetic complications has been identified as increased oxidative stress (51). However, by causing insulin resistance, dyslipidemia, cell dysfunction, and reduced glucose tolerance, oxidative stress has been linked to the development of diabetes in general (52). Reactive oxygen species are overproduced as a result of various mechanisms connected to hyperglycemia, including the production of AGEs, the activation of protein kinase C (PKC), the accumulation of sorbitol, and the hyperactivity of the hexosamine pathway(53).

Conclusion

According to the results, HIIT with DIS administration during a 12-week period considerably improved glycemic, lipid, and antioxidant status. This is known as the interaction effect. Additionally, HIIT with DIS exhibited greater effects than HIIT on the improvement of lipid profiles and antioxidant enzymes. It appears that HIIT combined with DIS consumption can enhance the lipid and insulin-lowering effects of DIS, even though there was no study to determine the interactive effects of HIIT and DIS consumption on the lipid profile, antioxidant enzymes, and glycemic indices of diabetic individuals to compare to the present study. It is advised that additional studies examine the impact of HIIT with various protocols of volume and DIS consumption at various doses on the lipid profile and glycemic indices of patients with T2DM. Volume is one of the principles of training, and different doses of herbal extracts can have different effects on lipid profiles and glycemic indices.

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