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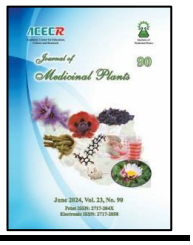

Research Article

Silymarin supplementation could improve the effect of exercise training on high-fat diet-induced metabolic disorders

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ABSTRACT

Background: The use of dietary herbal products as potential anti-obesity agents has gained considerable traction in recent years. **Objective:** The purpose of the current study was to assess the effects of *silymarin* supplementation with and without exercise training on a high-fat diet-induced metabolic disorder in male Wistar rats. **Methods:** 36 male Wistar rats were divided into six groups; 1) control group with a regular diet; 2) high-fat diet (HFD); 3) HFD + *Silymarin* 25 (S25); 4) HFD + *Silymarin* 50 (S50); 5) HFD +S25 + ART (aerobic resistance training); 6) HFD + S50+ART (n = 6 for each group). HFD + S25 + ART and HFD + S50 + ART groups performed aerobic exercise three days/week and resistance exercise two days/week. Blood specimens were obtained for biochemical assessments and gene expression at the end of the 12-week intervention. The data were analyzed using one-way ANOVAs and Tukey's post hoc test with SPSS21 at a significance level of $P < 0.05$. **Results:** Final body weight, the levels of glucose, insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in all of the treatment groups were significantly less compared with the HFD group ($P < 0.05$). Lipid profile was significantly improved in treatment groups in comparison with the HFD group ($P < 0.05$). Leptin and Neuropeptide Y (NPY) levels were significantly lower in HFD + S50, HFD + S25 + ART, and HFD + S50 + ART groups compared to HFD group ($P < 0.05$). Exercise plus *silymarin* consumption (HFD + S25 + ART and HFD + S50 + ART) increased Peptide YY levels (PYY) ($P= 0.013$ and $P < 0.001$, respectively). **Conclusion:** Our findings suggest that combined *silymarin* consumption and exercise training is a promising non-pharmacological treatment for multiple simultaneous HFD-induced risk factors.

Abbreviations: ART, Aerobic Resistance training; FEC, Functional Exercise Capacity; HDL, High-Density Lipoprotein; HFD, High-Fat Diet; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LDL, Low-Density Lipoprotein; MLT, Maximal Load Test; NPY, Neuropeptide Y; PYY, Peptide YY; S, Silymarin; TC, Total Cholesterol; TG, Triglyceride

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1. Introduction

Tackling rising levels of obesity is a global challenge [1]. There is a growing interest in how exercise, diet, and non-pharmacological supplementation can interact to help manage obesity and the associated risk factors. Some studies show that well-structured exercise programs and calorie restrictions can help prevent or reduce obesity levels; however, these strategies are not effective for everyone and don't always lead to long-term changes. Thus, there remains a need for further research into additional strategies that can help tackle obesity and other metabolic disorders [2].

In recent years, dietary herbal products have garnered significant attention because of their anti-obesity properties [2]. *Silybum marianum*, is a flower that has been utilized for an extensive period of time for medicinal purposes. *Silymarin* has been shown to have anti-inflammatory, antioxidant, hepatoprotective, anticancer, and cardioprotective activities [3]. As such, several studies have examined whether *silymarin* could improve lipoprotein profile in rats [4-6]. In one study, Sayin et al. reported that *silymarin* supplementation improved insulin resistance, hyperlipidemia, and hepatopathy in response to a high-fat diet (HFD) [7]. Thus, adding *silymarin* supplementation to an exercise program could support metabolic health.

Neuropeptide Y (NPY) and peptide YY (PYY) are expressed by cell systems at various points along the gut-brain axis, contributing to the regulation of appetite. In this regard, researchers suggest that PYY affects not only food intake and energy expenditure, but systemic administration of PYY may also diminish the drive to pursue high-fat food after exposure to pellet priming or pellet cues [8]. This indicates that PYY may help to regulate the desire to consume a high-fat diet [9]. As *silymarin* could improve insulin resistance and hyperlipidemia following a high-fat diet [7], it may also positively influence serum PYY or NPY. To date, little is known about the association between serum PYY levels, NPY expression, and *silymarin* supplementation or the potential synergistic effect of *silymarin* consumption and exercise training on serum PYY levels and NPY expression.

Therefore, this study aimed to determine whether different doses of *silymarin* supplementation could ameliorate metabolic disorders in HFD-fed rats. We also sought to determine whether various doses of this supplementation would augment the advantageous effects of exercise training on metabolic outcomes in HFD-fed rats.

2. Materials and methods

2.1. Animals and treatment protocol

Thirty-six male Wistar rats (150-170 g) of 8 - 10 weeks were used in this study. All the animals were housed in a standard animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 AM) and the temperature maintained at 20 ± 4 ^{o}C. Rats in the control group were fed a standard food, including crude carbohydrate (48.5 %), crude protein (23 %), crude fat (3.5 %), crude fiber (4-5 %), ash (8 %), calcium (0.95-1 %), phosphorus (0.65 %), moisture (10 %) and rats in other groups were fed a high-fat food including crude carbohydrate (43 %), crude Protein (17 %) and crude Fat (40%) contained powdered rat feed (68 %), maize oil (6 %), ghee (6 %), milk powder (20 %) [10]. All studies and experimental protocols were approved by the Ethics committee of the Sport Sciences Research Institute of Iran (IR.SSRI.REC.1403.053) and adhered to the EU animal experiments ethical guidelines (2010/63/EU). The overall characteristics of the experimental groups are depicted in Table 1.

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2.2. Experimental groups

At first, 36 male Wistar rats were divided into six groups comprising the control group with a regular diet, high-fat diet (HFD), HFD + S25, HFD + S50, HFD + S25 + ART, and HFD + S50 + ART (n = 6 for each group) (Fig. 1).

Table 1. Aerobic exercise training protocol

Weeks	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	12
Speed (m/min)	6	8	10	12	15	18	21	24	25	25	25	25
Slope (°)	0	0	0	0	0	5	5	5	10	10	10	10
Duration (min)	10	14	18	22	26	30	34	38	40	40	40	40

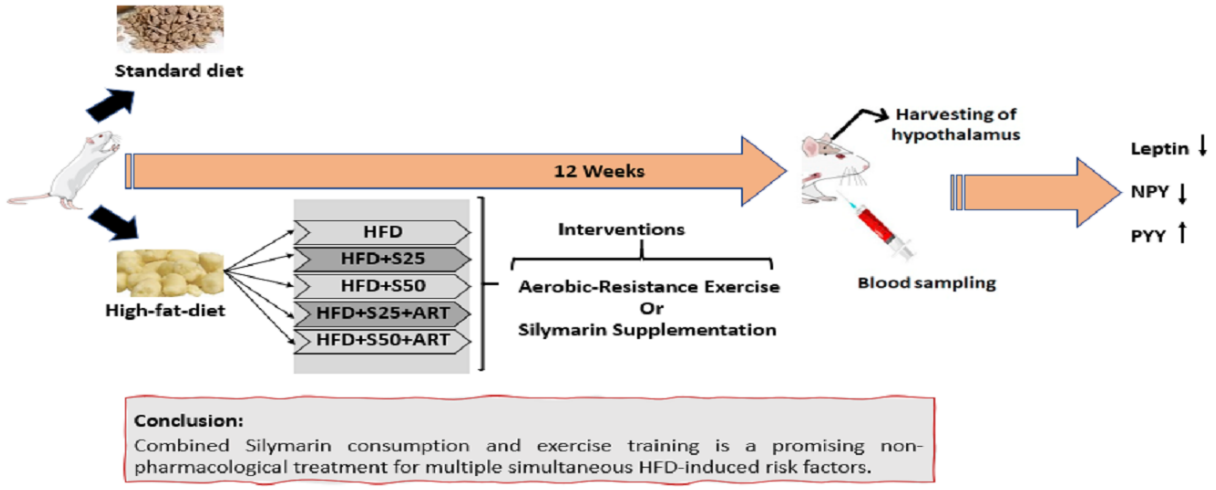

Fig. 1. Experiment design. After a ten-day acclimation period to the new environment, HFD groups (n = 30) were divided into five groups, HFD, HFD + S25, HFD + S50, HFD + S25 + ART, and HFD + S50 + ART (n = 6 for each group). These groups were fed a high-fat food. The control group (n = 6) was fed the standard food. Following this, aerobic-resistance training or *Silymarin* supplementation was administered for 12 weeks. Finally, biochemical assessments and molecular studies were conducted by drawing blood samples and removing the hypothalamus, respectively. Exercise plus *silymarin* consumption can decrease some multiple simultaneous HFD-induced risk factors by affecting leptin, peptide YY (PYY), and neuropeptide Y (NPY) levels.

2.3. Silymarin supplementation

Silymarin (25 and 50 mg/kg) [11] was purchased from Sigma Aldrich (St. Louis, MO, USA). *Silymarin* was mixed with olive oil based on the manufacturer's guidelines (Barij Essence Pharmaceutical Company) and administered by gavage for 12 weeks.

2.4. Exercise protocol

The exercise training protocol was carried out in the afternoon (2.00-4.00 p.m.). We initially performed a pilot study on five rats to estimate maximal load test (MLT) and functional exercise capacity (FEC). Afterward, animals belonging to the groups of HFD + S25 + ART (aerobic-resistance training) and HFD + S50 + ART performed combined aerobic and resistance training three days/per week.

To estimate the FEC, the rats were performed running on a graded treadmill for 3 min, while the speed and the incline progressively increased. Exhaustion was characterized as a condition in which a rat was avoiding running for about 90 seconds (50 % of the target time) or staying on the shock grid for 10 consecutive seconds.

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The training session included running at the speed of 6 m/min for 10 minutes, then the training period was gradually increased to 26 min/day at 15 m/min, 0 % slope (fifth week), up to 40 min/day at 25 m/min, 10 % slope for the last four weeks. The exercise sessions were equivalent to 70-85 % VO_2max (Table 1) [12, 13].

To estimate MLT, all rats carried a load equal to 75 % of their body weight and this load was progressively increased by 15 % each subsequent climb until they were unable to climb to the next step of the ladder after three attempts. The resistance training protocol was performed 2 days a week, 15-20 climbs per session, with a 1-min rest between each interval per session, with a 1-min rest between each climb, from a moderate to a high intensity (70-85 % of the MLT) for 12 weeks (Table 2) [14].

Table 2. Resistance exercise training protocol

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Load (MLT)	70	70	75	80	85	85	70	70	75	80	85	**85**
Times	15	16	17	18	19	20	15	16	17	18	19	**20**

2.5. Biochemical assessments

Blood specimens were gathered under anesthesia with ketamine 75 mg/ kg and xylazine 10 mg/kg from the vena cava, and then the samples were centrifuged [14]. The collected serums were stored at -20$^{C^\circ}$ for additional biochemical assays. Serum total cholesterol (TC) and triglyceride (TG) were measured using commercial ELISA Kits (Cat. No, MBS161594; MyBioSource, USA and Cat. No, MBS726298; MyBioSource, USA, respectively), and serum levels of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using an HDL and LDL Assay Kit (Cat. No, KA1671; Abnova, Taiwan). Fasting plasma glucose was determined using a commercial kit (Cat. No, MBS7233226; MyBioSource, USA). Serum insulin levels were determined using a commercial radioimmunoassay kit (Cat. No, CSB-E05070R; CUSABIO, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by the formula: [glucose (nmol/L) × insulin (μU/mL)/22.5] [15]. According to the manufacturer's instructions, plasma leptin and PYY levels were determined using a Rat Leptin ELISA Kit (Cat. No, RAB0335; Sigma-Aldrich; Merck) and Rat PYY ELISA Kit (Cat. No, MBS702436; MyBioSource, USA), respectively. Finally, under deep anesthesia, visceral adipose tissue in rats (VAT) was dissected and weighed (epididymal, perirenal, and omental fat) [16].

2.6. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Immediately after blood sampling, the hypothalamus was removed. Then, tissues stored in liquid nitrogen were homogenized. Total RNA was isolated from the hypothalamus using RNAzol (Sigma-Aldrich; Merck KGaA) following the manufacturer's instructions. Then, cDNA synthesis was performed with the Enhanced Avian RT First-Strand Synthesis Kit (Sigma, St. Louis, MO, USA). qPCR for Neuropeptide Y (NPY) was performed using SYBR-Green PCR master mix (Sigma-Aldrich; Merck KGaA) and primer sequences: Forward, 5'-GTGGATCTCTTCTCTCACAGAGG-3' and reverse, 5'-GCCCAAACACACGAGCAGAG-3'. The PCR cycling conditions for the gene expression were as follows: Pre-denaturation temperature was first set up at 5 min at 95°C, 30

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cycles of 60 secs at 95°C, 40 secs at 55°C, and 60 secs at 72°C. GAPDH was run in parallel and served as a loading control. The qPCR reactions were performed in triplicate, and the raw data were analyzed [17].

2.7. Statistical Analyses

Normality for all variables was estimated by Shapiro-Wilk tests. After confirming data distribution ($P < 0.05$ considered normally distributed), multiple one-way ANOVAs with Tukey's *post hoc* test were used to test for between-group differences for all outcomes. The level of significance was $P < 0.05$ in all statistical evaluations. Data were analyzed in Graph Pad Prism 8 version 8.4.3. (GraphPad Software, San Diego, CA, USA) and expressed as mean ± SD.

3. Results

Table 3 illustrates the attributes of the animals. There was no significant difference in their initial body mass or food intake, but after the 12-week intervention body mass was significantly higher in the HFD group compared with the control group (22.1 %, $P < 0.001$). Compared with HFD, final body mass was significantly lower in the exercise and supplementation groups ($P < 0.05$). The greatest reduction in mass was observed in the HFD+S50+ART group (12.5 %, $P < 0.001$) compared with the HFD group (Table 3).

Table 3. Characteristics and measured indices after eight weeks of the treatment protocol

Groups	Control	HFD	HFD+S25	HFD+S50	HFD+S25+ART	HFD+S50+ART
Initial body weight (g)	166 ± 4.3	159 ± 7.3	163 ± 5.7	160 ± 6.4	160 ± 6.3	163 ± 6.7
Final body weight (g)	276 ± 8.3	337 ± 8.7***	320 ± 10	318 ± 7.4	305 ± 17 ^{c}	295 ± 10 $^{c,\&\&,\dagger}$
Food intake (g/day)	117.6 ± 5	128 ± 7.8	122 ± 8.1	119 ± 7.9	126 ± 5.8	121 ± 6.2
visceral fat (g)	5.8 ± 1.2	15.02 ± 1.88***	12.75 ± 1.12	12.23 ± 1.37	9.68 ± 1.29$^{c,\&}$	9.06 ± 1.47$^{c,\&\&,\dagger}$
Glucose (mg/dl)	220 ± 42	321 ± 21***	273 ± 18^{a}	265 ± 18^{b}	264 ± 19^{b}	256 ± 18^{c}
Insulin (ng/ml)	1.96 ± 0.7	3.66 ± 0.32***	3.02 ± 0.19^{a}	2.90 ± 0.35^{b}	2.89 ± 0.26^{b}	2.30 ± 0.27$^{c,\&\&}$
HOMA-IR	11.50 ± 2.2	30.6 ± 3***	23.8 ± 3.3^{a}	21.8 ± 4.1^{b}	20.45 ± 2^{c}	15.91 ± 4.7$^{c,\&\&}$
TG (mg/dl)	86.8 ± 7	138.6 ± 9***	118 ± 8^{a}	116 ± 7^{b}	110 ± 12^{c}	94 ± 6$^{c,\&\&,\dagger\dagger}$
TC (mg/dl)	169 ± 6	216 ± 7***	187 ±12	182 ± 12^{a}	156 ± 22^{c}	140 ± 15$^{c,\&\&\&,\dagger\dagger}$
HDL (mg/dl)	38 ± 4	43 ± 7.8	51 ± 7	52 ± 5.6	57 ± 6.5^{a}	60 ± 7.7^{b}
LDL (mg/dl)	43 ± 4.7	56 ± 5.4*	51 ± 8.4	48 ± 4.7	44 ± 4^{a}	40 ± 5.9$^{c,\&}$

Data are presented as mean ± SD.

TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HFD, high-fat diet; $S_{25,50,}$ *Silymarin* 25 and 50 mg/kg; ART, aerobic-resistance training.

$^{*, ***}$ (significant increase vs Control group; $P < 0.05$, and $P < 0.001$, respectively)

$^{a, b, c}$ (significant increase vs HFD group; $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively)

$^{\&, \&\&, \&\&}$ (significant increase vs HFD + S25 group; $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively)

†, †† (significant increase vs HFD + S50 group; $P < 0.05$ and $P < 0.01$, respectively)

3.1. Glucose and insulin levels

Whereas the levels of glucose (51 %, $P < 0.001$), insulin (87 %, $P < 0.001$), and HOMA-IR (166 %, $P < 0.001$) in the HFD group were significantly greater than those of the control group, in the treatment groups these values were significantly lower than the HFD group ($P < 0.05$). Compared with HFD, the most significant reduction in glucose, insulin, and HOMA-IR belonged to the HFD + S50 + ART group (20 %, 37 %, and 48 %, respectively; $P < 0.001$ for all) (Table 1).

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3.2. Lipid profile

Serum TG, TC, and LDL concentrations were significantly higher in the HFD group compared with the control group (change, 28 %, P < 0.001; 30 %, P = 0.011; 61 %, P < 0.001, respectively). However, TG levels were significantly lowered in all treatment groups in comparison with the HFD group (P < 0.05); the most significant reduction was found in the HFD + S50 + ART group (32 %, P < 0.001). Serum TC levels decreased significantly in all treatment groups except the HFD + S25 group compared to the HFD group, with the greatest reduction in the HFD + S50 + ART group (35 %, P < 0.001). LDL concentrations decreased only in the HFD + S25 + ART and HFD + S50 + ART groups (Table 1). By contrast, HDL levels did not significantly differ between HFD and the control group (P = 0.913). Nevertheless, HDL concentrations increased in the HFD + S25 + ART and HFD + S50 + ART groups (Table 1).

3.3. Leptin, PYY, and NPY levels

Serum leptin levels were significantly higher in the HFD group than those in the control group (P < 0.001). Compared to HFD, exercise and *silymarin* supplementation significantly decreased leptin in HFD + S50, HFD + S25 + ART, and HFD + S50 + ART groups (P < 0.05). Moreover, serum leptin concentrations were lower in the HFD + S50 + ART than in the HFD + S25 group (P < 0.05). There was no change in serum leptin levels in the HFD+S25 group (Fig. 2A).

HFD significantly decreased PYY concentrations compared with the control rats (24 %, P = 0.004). However, exercise accompanied by silymarin consumption (HFD + S25 + ART and HFD + S50 + ART) was beneficial in increasing PYY. Moreover, PYY concentrations were significantly different in the HFD + S25 + ART and HFD + S50 + ART groups compared to the HFD + S25 group (Fig. 2B).

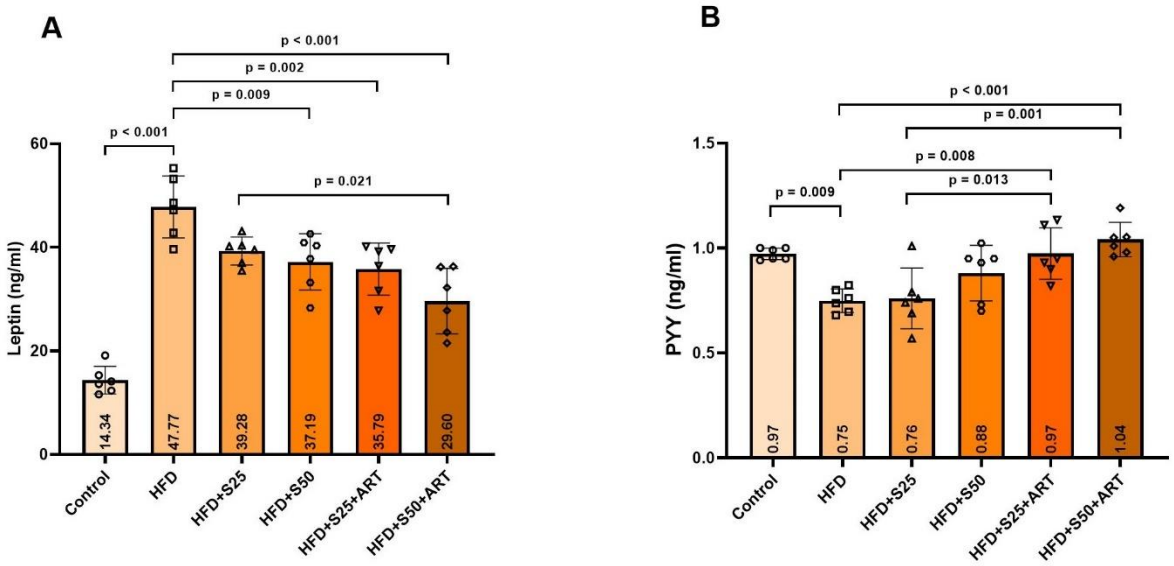

Fig. 2. Serum levels of leptin (A) and PYY (B). Data are expressed as mean ± SD (N = 6 for each group). HFD, high-fat diet; $S_{25,50}$, *silymarin* 25 and 50 mg/kg; ART, aerobic-resistance training; PYY, peptide tyrosine.

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The serum NPY levels were significantly higher in the HFD group than those in the control group (P = 0.020). However, NPY levels decreased substantially in HFD+S50, HFD + S25 + ART, and HFD + S50 + ART groups compared to the HFD group (P = 0.009, 0.007, and 0.001, respectively) (Fig. 3).

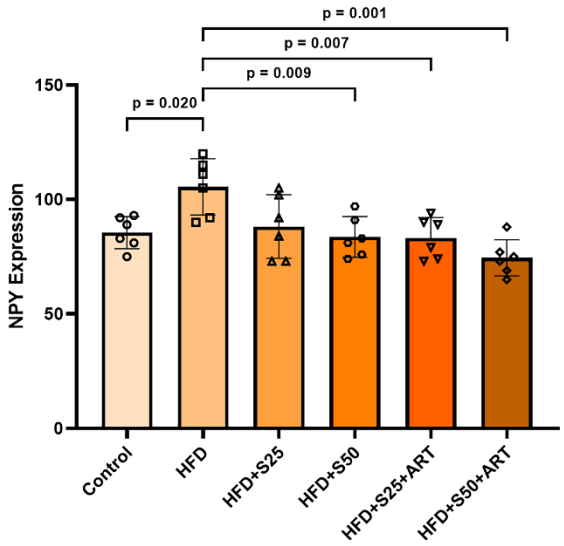

Fig. 3. Serum levels of NPY. Data are expressed as mean ± SD (N = 6 for each group). HFD, high-fat diet; $S_{25,50}$, *silymarin* 25 and 50 mg/kg; ART, aerobic-resistance training; PYY, Neuropeptide Y.

4. Discussion

In experimental studies, HFDs are a strategy used to induce obesity and dyslipidemia in animals [18]. In the present study, the body weight and visceral fat of rats fed an HFD for 12 Weeks significantly increased compared to the control group and dyslipidemia was evident. The main finding was that a combined exercise and *silymarin* intervention (HFD+S25+ART and HFD + S50 + ART) significantly attenuated the negative effects of an HFD, diminishing gains in body mass and improving lipid and glucose profiles. While *silymarin*-only interventions (HFD + S25 and HFD + S50) did not affect body weight, suggesting the positive effects in the combined groups stemmed from the exercise, they did improve insulin sensitivity and lipid profile. In addition, the negative impacts of an HFD on serum leptin, NPY, and PYY levels were significantly decreased following the administration of *silymarin* alone (HFD + S50) or in conjunction with exercise (HFD + S25 + ART and HFD + S50 + ART), reducing increases in leptin and NPY levels and enhancing serum levels of PYY.

On the contrary, Sayin et al. reported that 11 weeks of *Silymarin* supplementation in HFD-fed rats decreased body weight and body mass index. This disparity might be due to the dosage of *Silymarin* consumed in this study (200 mg/kg/day). *Silymarin* administration may reverse HFD's effects on body weight and BMI, and this effect may be attributed to decremented fat absorption. Other potential mechanisms such as fatty acid synthesis reduction and increment of fatty acid oxidation need further investigation [7].

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Consistent with Hamza et al [19], our results confirm that HFD-induced metabolic defects are associated with of type 2 diabetes, such as higher glucose levels, insulin concentrations, and HOMA-IR. Recent studies have begun to examine whether these symptoms can be modulated by *Silymarin* supplementation in HFD-induced obese animal models [20-22]. When *Silymarin* was ingested by mice alongside a HFD, it significantly improved glucose tolerance, ameliorated insulin resistance, and prevented excessive weight gain [20]. In another study, *Silymarin* has been reported to increase lipolysis favorably, decrease visceral fat, ameliorate insulin resistance, and suppress gluconeogenesis through the down-regulation of related genes [21]. In the current study, the most striking result from the data is that glucose level, insulin concentration, and HOMA-IR ameliorated after 12 weeks of administration of *Silymarin*, either 25 or 50 mg/kg doses. This amelioration was increased when a high amount of *Silymarin* was supplemented with exercise training. Our results are consistent with previous studies [23-25]. To be mentioned specifically, glucose level was significantly lower in all groups than in the HFD group, which can be concluded that low and high doses of *Silymarin* supplementation effectively decreased the glucose level.

Moreover, in the present study insulin concentration and HOMA-IR were improved by *Silymarin* supplementation. These improvements were greatest when a high dose was consumed in conjunction with exercise training. Several previous studies show that that different kind of exercise training elicits improvements in insulin concentrations and HOMA-IR [26, 27]. A novel finding of the current study is that adding *Silymarin* supplementation to exercise training led to further improvements in these markers of metabolic health. Several studies reported that *Silymarin* not only could improve endocrine function and pancreatic morphology but also the pancreatic activity of antioxidant enzymes in diabetic models [20, 22, 28, 29].

In line with previous studies, Dyslipidemia is characterized by an increase in TG, TC, and LDL-C levels; changes we observed in the HFD-fed rats in our study. Interestingly, we found that their lipid profiles significantly improved following combined *Sylimarin* and exercise training, especially in HFD + S25 + ART and HFD + S50 + ART groups. Other recent studies have also shown that *Silymarin* improves lipid profile in rats and humans [4, 30]. However, these findings are not consistent; Hadad et al. (2011), reported that 12 weeks of *Silymarin* administration in HFD-fed rats had no significant influence on TC concentrations, despite reducing LDL levels [4]. However, Skottova and Ramakrishnan showed that *Silymarin* supplementation decreased TC levels in hepatocellular carcinoma and high cholesterol-fed rats. Additionally, Sobolova et al. found that *Silymarin* attenuates TC absorption in rats fed on high cholesterol diet, attenuating changes in TC, TG, and VLDL in the liver [5, 6].

According to *Silymarin's* impact on HFD-fed animals in this study and others suggests this herb influences lipoproteins [31]. It has been suggested that *Silymarin* affects lipoprotein concentration not only by decreasing cholesterol absorption from the intestine [32] but also via up-regulation of the ABC transporters associated with lipid metabolism [31]. Accordingly, suppression of cholesterol absorption by *Silymarin* may be the major mechanism driving improvements in lipid metabolism [32]. In the present study, we found

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that HDL levels were significantly raised when *Silymarin* was consumed in conjunction with exercise training but not *Silymarin* alone; thus, changes in HDL and LDL levels in the present study were likely a result of the exercise training, as shown before [33].

A recent study reported that *Silymarin* reduces circulating leptin levels [7]. Our result demonstrates that a high dose of *Silymarin* could attenuate a rise in leptin levels in HFD rats. This improvement was augmented when low and high doses of *Silymarin* were consumed with exercise training. Leptin reduction owing to *Silymarin* supplementation may be attributable to its effects on adipose tissue, as it has been reported that *Silymarin* remarkably reduced pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β levels at both the mRNA and protein level [22, 34]. As leptin may reduce insulin sensitivity and alter insulin signaling in hepatocytes, increases in this hormone could contribute to hepatic steatosis [35]. Combined with our results on insulin concentrations, the decrease in leptin levels by *Silymarin* supplementation suggests this herb may play an adjunctive role in managing the potentially deleterious effects of chronically consuming a HFD [7].

We found that HFD significantly reduced PYY compared with the control group, indicative of an increased appetite. However, neither low nor high doses of *Silymarin* supplementation altered PYY levels when consumed alone, but when combined with exercise training, both doses markedly increased PYY levels. It has recently been documented that exercise training has a favorable impact on PYY. Martins et al. [36] reported that postprandial PYY concentrations were significantly increased in overweight/obese men and women after a 12-week exercise intervention. In addition, Jones et al. [8] found that fasting plasma PYY concentrations following 32 weeks of exercise training, which resulted in significant body fat loss, were raised in overweight males and females. Collectedly, these findings suggest that exercise can positively influence appetite and appetite-regulating hormones such as PPY facilitating a negative energy balance and weight loss [37]. Since it has been shown that chronic PYY administration shifts substrate mobilization in favor of fat oxidation in rodents [38-40], reduced body weight and visceral fat in the present study following consumption of *Silymarin* in conjunction with exercise training could be related to the increase in PYY concentrations. Postprandial PYY levels are also correlated with postprandial energy expenditure and the thermic effect of food [41]. Collectively, these findings suggest that PYY increases after exercise training, and this may help to regulate body weight by increasing energy expenditure and reducing food intake.

HFD-induced NPY values were significantly higher compared with the control condition. Recent studies have also demonstrated that NPY increases in response to HFD. The combined *Silymarin* and exercise training groups had significantly reduced NPY concentrations in comparison with the HFD-induced NPY values. Additionally, HFD + S50-induced NPY levels were also significantly lower than the HFD-induced NPY levels. That exercise decreased NPY levels is consistent with the results of Wu et el. (2019) but differs from Khajehnasiri et al.'s study [43]. Wu and colleagues identified that the expression of NPY and its receptors in the vasculature was decreased by physical activity, and this expression was significantly correlated with the progress of atherosclerosis [42]. They also stated that exercise might

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decrease macrophage activity by down-regulating the expression of NPY Y1 receptors, thereby decreasing the release of inflammatory cytokines. On the other hand, Khajehnasiri and colleagues found that NPY levels remained unchanged after one month of regular intensive exercise in male Wistar rats [43]. In another study, it was revealed that HFD-induced metabolic syndrome was inverted by exercise training, illustrating that these beneficial exercise impacts may be mediated by shear stress-induced Akt/eNOS pathway activation. Hence, the authors stated that exercise training may be a valuable approach to invert almost a wide range of risk factors related to obesity [44]. The evidence confirmed that the reduced NPY levels after exercise are likely because of the rising negative energy balance in the organism as previously mentioned [45] and appears to validate earlier discoveries indicating that energy balance is the primary factor influencing changes in leptin levels during exercise [46, 47].

5. Conclusion

Our results showed that *silymarin* consumption, especially a higher dose, accompanied by exercise training, can reverse some of the negative effects of a chronic HFD on metabolic health. Overall, the combination of exercise and *silymarin* was more effective than *silymarin* alone. Since it seems likely that taking *silymarin* enhances the beneficial effects of exercise training, it is suggested that *silymarin* consumption with exercise training may be a potential nonpharmacological treatment for multiple simultaneous HFD-induced risk factors.

Author contribution

MM, BR, MF designed the study. MM, BR, and GMR performed the experiments, MM analyzed the data. MM, BR, GMR, and TC prepared this manuscript. All authors reviewed and endorsed the final manuscript.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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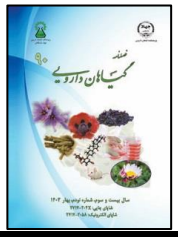

مقاله تحقیقاتی

مکمل‌یاری سیلی‌مارین، اثر تمرین ورزشی بر اختلالات متابولیکی ناشی از رژیم غذایی پرچرب را بهبود می‌دهد

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اطلاعات مقاله

گل واژگان:

رژیم غذایی پرچرب

تمرین هوازی مقاومتی

شاخص‌های متابولیکی

سیلی‌مارین

اشتها

چکیده

مقدمه: در سال‌های اخیر، استفاده از محصولات گیاهی رژیمی به عنوان عوامل بالقوه ضد چاقی مورد توجه قرار گرفته است. **هدف:** هدف مطالعه حاضر ارزیابی اثر مکمل‌یاری سیلی‌مارین با و بدون تمرین ورزشی بر اختلالات متابولیکی ناشی از رژیم غذایی پرچرب در رت‌های ویستار نر بود. **روش بررسی:** ۳۶ رت ویستار نر به شش گروه تقسیم شدند؛ ۱) گروه کنترل با یک رژیم غذایی منظم؛ ۲) رژیم غذایی پرچرب (HFD)؛ ۳) HFD+ سیلی-مارین ۲۵ (S25)؛ ۴) HFD+ سیلی‌مارین ۵۰ (S50)؛ ۵) ART + S25 + HFD (تمرین هوازی مقاومتی)؛ ۶) ART + S50 +HFD (۶ = n برای هرگروه). گروه‌های ART + S25 + HFD و ART + S50 + HFD، سه روز/هفته تمرین هوازی و دو روز/هفته تمرین مقاومتی را انجام دادند. نمونه‌های خونی به منظور ارزیابی بیوشیمیایی و بیان ژن در پایان مداخله ۱۲ هفته‌ای جمع‌آوری شدند. داده‌ها با استفاده ازآزمون تجزیه و تحلیل واریانس یک‌طرفه و آزمون تعقیبی توکی با نرم‌افزار SPSS 21 و سطح معنی‌داری P < ۰/۰۵ مورد تجزیه و تحلیل قرار گرفت. **نتایج:** وزن بدن، سطوح گلوکز، انسولین و ارزیابی مدل هومئواستاتیک برای مقاومت انسولین (HOMA-IR) نهایی در همه گروه‌های درمانی در مقایسه با گروه HFD به طور معنی‌داری کمتر بود (P <۰/۰۵). نیمرخ چربی در گروه‌های درمانی در مقایسه با گروه HFD به طور معنی‌داری بهبود یافت (P <۰/۰۵). سطوح لپتین و نوروپپتید Y (NPY) در گروه‌های HFD + S50، HFD + S25 + ART و HFD + S50 + ART در مقایسه با گروه HFD به طور معنی‌داری کمتر بود (P <۰/۰۵). تمرین ورزشی بعلاوه مصرف سیلی‌مارین (HFD + S25 + ART و HFD + S50 + ART)، سطوح پپتید YY (PYY) را افزایش داد (P <۰/۰۵). **نتیجه‌گیری:** یافته‌های ما پیشنهاد می‌کند که ترکیب مصرف سیلی‌مارین و تمرین ورزشی یک درمان غیردارویی امیدوارکننده برای چندین عامل خطرزای ناشی از رژیم غذایی پرچرب است.

مخففها: ART، تمرین هوازی مقاومتی؛ FEC، ظرفیت ورزشی عملکردی؛ HDL، لیپوپروتئین با چگالی بالا؛ HFD، رژیم غذایی پرچرب؛ HOMA-IR، ارزیابی مدل هومئواستاتیک برای مقاومت انسولین؛ LDL، لیپوپروتئین با چگالی پایین؛ MLT، آزمون بار حداکثر؛ NPY، نوروپپتید Y؛ PYY، پپتید YY؛ S، سیلی‌مارین؛ TC، کلسترول تام؛ TG، تری‌گلیسرید

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