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Original Article

High-intensity interval training and *Crataegus pentagyna* extract enhance metabolic and oxidant profiles in ovariectomized Wistar rats

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Abstract

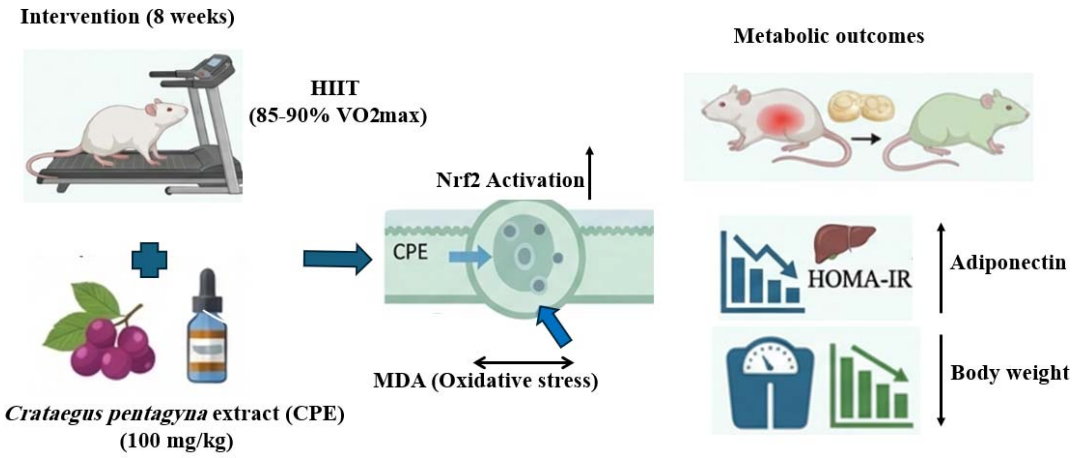
Background and purpose: High-intensity interval training (HIIT) improves body composition in obese individuals but increases fat oxidation and oxidative stress. This study aimed to evaluate the effects of chronic supplementation with *Crataegus pentagyna* fruit extract (CPE), combined with HIIT, on metabolic syndrome indices and oxidative stress.

Experimental approach: Female Wistar rats (200-220 g) were randomized into seven groups (8 rats each) including: ovariectomized control (Ovx + saline), exercise (Ovx + Exe), CPE alone (100, 200, 300 mg/kg), Ovx + CPE + Exe: HIIT plus CPE (100 mg/kg, i.p); and sham. . At the end of the training, body weight, body mass index, waist circumference, visceral fat, fasting blood glucose, serum insulin, lipid profiles, MDA, TAC, Nrf2, and adiponectin were measured. The phytochemical contents were evaluated.

Findings/Results: Co-treatment with HIIT and CPE (100 mg/kg) produced greater reductions in visceral fat (-72.8%) than HIIT alone (-33.7%) and comparable reductions to CPE alone (-65.5% visceral fat, -20.3% waist circumference), with no dose-dependent effects across 100-300 mg/kg ($P > 0.05$).

Conclusion and implications: Combined HIIT and 100 mg/kg CPE extract supplementation for 8 weeks significantly ameliorated metabolic syndrome and oxidative stress in ovariectomized rats, likely *via* adiponectin and Nrf2 upregulation, suggesting a potential adjunctive therapy for postmenopausal women.

Keywords: Adiponectin; *Crataegus pentagyna*; Insulin resistance; Nuclear factor erythroid 2-related factor 2; Malondialdehyde; Ovariectomy.



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INTRODUCTION

Metabolic syndrome (MS) is a complex condition characterized by central adiposity, insulin resistance (IR), dyslipidemia, hyperglycemia, and hypertension, with a high prevalence among postmenopausal women, increasing their risk of cardiovascular diseases (1). This study addresses the challenge of menopause-induced MS by exploring non-pharmacological interventions, specifically high-intensity interval training (HIIT) and supplementation with ethanolic extract of *Crataegus pentagyna* fruit (CPE), a native species in northern Iran known for its antioxidant properties (2).

Many studies have reported that visceral adipose tissue accumulation and chronic inflammation play a key role in the pathophysiology of MS (3). The adipose tissue produces and secretes various proinflammatory and anti-inflammatory mediators, including the adipokines (4). Adipokines, known as cell-signaling molecules (cytokines), play important roles in body energy/metabolic process, obesity, and inflammation (5). They are considered a core component of MS. Adiponectin is one of the most important adipokines (5), is capable of decreasing inflammatory cytokines and oxidative stress, and alleviates IR. It has been assumed that a low level of adiponectin in obese individuals significantly affects the pathogenesis of atherosclerosis and cardiovascular diseases associated with obesity and MS (6).

On the other hand, oxidative stress has a potential role in MS. It is associated with chronic diseases, including diabetes, atherosclerosis, hypertension, and obesity (7). Nuclear factor E2-related factor 2 (Nrf2) is an essential transcription factor responsible for protecting the cell against oxidative stress. It controls the expression of many antioxidant genes involved in metabolic homeostasis, inflammatory reactions, and anti-apoptosis in cells. It remarkably affects energy metabolism, improves insulin receptor sensitivity, and increases glucose uptake (8-10).

One successful strategy to combat MS is physical activity (11,12). Our recent work showed that HIIT successfully alleviates visceral adiposity and dyslipidemia (13). HIIT consists of a series of high-intensity workouts interspersed with relief periods (14). Despite its beneficial effects, HIIT produces reactive oxygen species (ROS), increasing lipid peroxidation, inflammation, malondialdehyde (MDA) levels, and cellular toxicity (15-20). Based on the theory of hormesis, constant exposure to low levels of stress factors improves the cell's ability to respond to higher levels of stress (21). Many investigations have suggested that moderate-intensity exercise is essential for producing a beneficial level of ROS, thereby increasing the expression of antioxidant enzymes. This, in turn, leads to a reduction in the ageing process and improved health condition (21).

Some medicinal plants and dietary supplements could improve insulin sensitivity, reduce blood glucose, cholesterol, triglycerides (TG), low-density lipoprotein-cholesterol (LDL) levels, body fat percentage, and blood pressure (22).

The genus name *Crataegus*, known as hawthorn, belongs to the Rosaceae family. This genus includes small-sized trees with ovoid, deep red fruits (23). It comprises over 1000 species that are widely distributed in Asia, Europe, and North America (24). Several hawthorn species have been mainly used for the treatment of cardiovascular diseases (25). Studies showed that hydro-alcoholic extracts from leaves and fruits of *Crataegus* are rich sources of bioactive compounds such as polyphenols and flavonoids with antioxidant capacity. Several pharmacological activities, such as anti-hypertension, anti-atherosclerosis, anti-inflammatory, and antilipidemic effects, are also reported (24,26,27). Among them, *Crataegus pentagyna* Waldst. et Kit. ex Willd is a native species in the north of Iran (25). Limited studies have explored the phytochemical composition and potential benefits of *C. pentagyna* for MS indices. No prior studies have investigated *C. pentagyna*'s potential to mitigate HIIT-induced oxidative stress, a critical gap given exercise's dual role in improving metabolic health while increasing ROS. We hypothesized that *C. pentagyna* would synergize with HIIT by enhancing adiponectin and Nrf2 levels, thereby improving MS indices while counteracting oxidative stress. Therefore, this study evaluated the effects of eight weeks of *C. pentagyna* ethanolic fruit extract supplementation, combined with HIIT, on MS indices, IR, serum adiponectin, total antioxidant capacity (TAC), and Nrf2 in ovariectomized rats, a model of menopause-induced MS. The phytochemical content of the extract was also characterized.

MATERIALS AND METHODS

Preparation of CPE

C. pentagyna fruits were collected from Rudbar, Guilan Province, Iran, in October 2021, and a voucher specimen (Voucher No. 424HGUM) was deposited at the herbarium of the Faculty of Pharmacy, Guilan University of Medical Sciences. Fruits (2.5 kg) were washed, macerated in 5 L of 96% ethanol for 24, 48, and 72 h at ambient temperature, filtered, and concentrated using a rotary vacuum evaporator (Heidolph, Germany) at 45 °C. The crude extract was stored at -4 °C for administration *via* intraperitoneal (i.p.) injection. The extraction yield was approximately 15.4 µg/mL, calculated as the ratio of the weight of the dried ethanolic extract to the initial dry fruit weight. Intraperitoneal injection was chosen to ensure consistent dosing, although oral administration may be more clinically relevant for human translation.

Determination of total phenolic and flavonoid content

The Folin-Ciocalteu method was used to measure the total phenolic content. Gallic acid was used as the reference for plotting the calibration curve. In this assay, 1 mL of extract solution (1 mg/mL) was added to 5 mL of Folin-Ciocalteu reagent (diluted tenfold with distilled water) and incubated for 10 min. Then, 4 mL sodium bicarbonate solution (75 g/L) was added, and the mixture was incubated for 30 min in the dark. Finally, the absorbance was measured at 765 nm using a UV/Vis spectrophotometer (PerkinElmer, USA). All the experiments were repeated three times. The total phenolic contents were expressed as mg of gallic acid equivalents (GAE)/g extract.

The total flavonoid content was measured by the Dowd method (28). The quercetin was used as the standard compound. In this test, 2.5 mL of aluminum trichloride solution (2% in methanol) was mixed with 2.5 mL of the sample (1 mg/mL). The mixtures were incubated for 10 min at room temperature. Lastly, the absorbance was obtained at 415 nm using a UV/Vis spectrophotometer (29). All the experiments were repeated three times. The total flavonoid content was expressed as mg of quercetin equivalents / g of extract.

Determination of DPPH radical scavenging activity

The radical scavenging activity of the extract was investigated by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (30). First, 21 mL of DPPH solution (40 µg/mL in methanol) was added to 0.5 mL of the sample. After 30 min, the absorbance was measured at 517 nm. Butylated hydroxyanisole (BHA) was used as a standard antioxidant. All the experiments were repeated three times. The control contained the sample (1 mL) and distilled water (2 mL). The blank included DPPH solution (2 mL) and distilled water (1 mL). The percentage of inhibition was calculated using the following equation (31):

$$\text{Inhibition (\%)} = 100 - \left[\frac{(\text{Sample absorption} - \text{control absorption})}{\text{Blank absorption}}\right] \times 100 \quad (1)$$

The IC_{50} values (indicating the concentration of the extract (µg/mL) providing 50% inhibition) were calculated from the graph-plotted scavenging percentage against sample concentration.

Determination of total antioxidant capacity by phosphomolybdenum reduction assay

The antioxidant activity of the sample was also determined by the phosphomolybdenum reduction assay (PRA) (30). The α-tocopherol was used as the standard substance for plotting the calibration curve. In this test, 0.3 mL of the extract sample was mixed with 3 mL of a reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Next, it was incubated in an oil bath (90 °C, 90 min). After cooling to room temperature, the absorbance was measured at 695 nm. All the experiments were repeated three times. The total antioxidant capacity of each sample was expressed as mg of α-tocopherol equivalent / g extract.

Animals

Fifty-six female Wistar rats, aged 3 months and weighing 200-220 g, were used in this study. All rats were kept in standard conditions (temperature of 22 to 24 °C), relative humidity of 55%, 12/12-h dark/light cycle, and free access to water and food (32). Experimental protocols were

approved by the Ethics Committee of Ardebil University (IR.ARUMS.REC.1400.098), which is in accordance with NIH principles of laboratory animals' care.

Ovariectomy surgery

All animals (except the sham group) were ovariectomized under general anesthesia with an intraperitoneal injection (i.p) of 60 mg/Kg ketamine (Alfasan, Holland) and 5 mg/Kg xylazine (Alfasan, Holland). After deep anesthesia, the ovaries were accurately removed through a midline incision (33), then the abdominal muscles and skin were sutured accurately, and the animals were left in warm cages to recover.

Animal grouping

One-month post-ovariectomy, rats were randomized into seven groups (n = 8): including Ovx + saline: sedentary control receiving saline (i.p.); Ovx + Exe: HIIT only; Ovx + CPE 100: CPE (100 mg/kg, i.p., 5 days/week for 8 weeks); Ovx + CPE 200: CPE (200 mg/kg, i.p, 5 days/week for 8 weeks); Ovx + CPE 300: CPE (300 mg/kg, i.p, 5 days/week for 8 weeks); Ovx + CPE + Exe: HIIT plus CPE (100 mg/kg, i.p, 5 days/week for 8 weeks); and sham: anesthesia and incision without ovariectomy (Fig. 1).

The dose of 100 mg/kg was selected based on dose-response data showing no significant differences between doses, prioritizing the lowest effective dose to minimize potential toxicity, although no formal toxicity assessment was conducted. This lack of dose-dependent effects suggests a plateau effect at 100 mg/kg, where further dose increases (200 and 300 mg/kg) provided no additional benefits.

HIIT

Rats underwent a one-week treadmill familiarization (10 m/min, 10 min, 0° gradient) using a rodent treadmill (Model XYZ, Manufacturer, Country). The HIIT protocol was performed on a treadmill (5 days/week, 8 weeks, 10-12 bouts of 2 min at 85-90% VO_2max, 1 min rest (Fig. 2) (34). VO_2max was measured by incrementally increasing treadmill speed from 3 m/min every 3 min until exhaustion, defined as three collisions with the treadmill's end within 1 min. CPE was administered 30 min post-HIIT to align with peak exercise-induced oxidative stress, maximizing the antioxidant effects of *C. pentagyna* flavonoids, based on prior pharmacokinetic studies of *Crataegus* extracts (34,35).

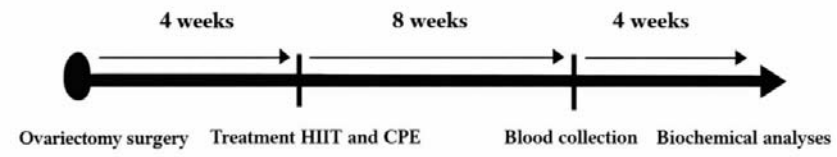

Fig. 1. Design of the study. HIIT, High-intensity interval training; CPE, ethanolic extract *of Crataegus pentagyna* fruit.

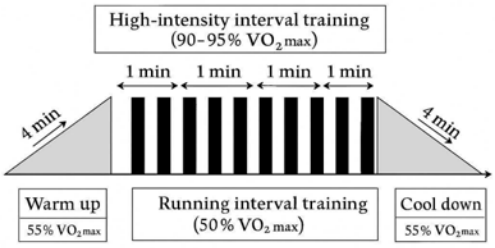

Fig. 2. High-intensity interval training protocol.

Anthropometrical assessments

Twenty-four hours after the last training session and 12 h fasting, rats were sacrificed under deep anesthesia to measure height, weight, and waist circumference. Then, blood samples were drawn from the inferior vena cava, and sera were separated by centrifugation at 2000 rpm/ 15 min and stored at -80 °C. Immediately after blood sampling, all intra-abdominal fat depots, including mesenteric, urogenital, and retroperitoneal, were dissected out and weighed using a digital scale.

Determination of serum insulin, glucose, cholesterol, HDL, and TG levels

Insulin was measured using a Rat Insulin ELISA kit (Cat. No. DEIA1897, Pkg. USA; sensitivity 0.2 mIU/L). Glucose was determined using a GOD-PAP kit (Pars Azmon, Cat. No. 1500017). Cholesterol, HDL, and TG levels were measured colorimetrically using kits (CHOLESTEROL(CHOD) Cat. No. 110500; HDL Precipitant Cat. No. 111150, GPO-PAP Cat. No. 132500). LDL was estimated using the Friedewald equation (36).

$$LDL - C = Total\ cholesterol - HDL - C - (TG\ /\ 5) \quad (2)$$

Homeostasis model assessment of insulin resistance index

Homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as follows:

$$HOMA - IR = \left[\frac{Fasting\ insulin\left(\frac{mU}{mL}\right) \times fasting\ glucose\left(\frac{mmol}{L}\right)}{\frac{22}{5}}\right] \quad (3)$$

Determination of serum adiponectin and Nrf2 levels

Serum adiponectin and Nrf2 levels were measured using commercial ELISA kits according to the manufacturer's instructions. Adiponectin was quantified with the Aat Adiponectin/Acrp30 ELISA kit (Cat. No. DY3100-05, R&D Systems, USA & Canada), while Nrf2 was measured using the at NRF2 ELISA kit (Cat. No. LS-F8159, LifeSpan BioSciences, USA).

Determination of TAC and MDA concentrations

Adiponectin and Nrf2 were measured using rat-specific ELISA kits per manufacturer protocols: Rat Adiponectin/Acrp30 Kit (Cat. No. DY3100-05, R&D Systems, USA & Canada) and Rat NRF2 ELISA Kit (Cat. No. LS-F8159, LifeSpan BioSciences, USA), respectively.

Statistical analysis and food intake monitoring

Data normality was confirmed using the Shapiro-Wilk test. Data are expressed as mean ± SD, and One-way ANOVA with Tukey's post hoc test was used for group comparisons (GraphPad Prism 8.0.2, San Diego, CA, USA). A priori power analysis indicated eight animals per group provided 80% power to detect a 20% difference in body weight ($\alpha = 0.05$). Food intake was monitored weekly, showing no significant differences, supporting peripheral mechanisms.

RESULTS

Phytochemical analysis

Phytochemical investigation of *C. pentagyna* fruit extract was carried out by the determination of total phenolic and flavonoid contents. The total phenolic content was measured regarding the gallic acid standard curve ($y = 0.0189x - 0.0276$, $R^2 = 0.999$). Moreover, the total flavonoid content was determined using the quercetin standard curve ($y = 0.0197x - 0.0312$, $R^2 = 0.998$). All the results are represented in Table 1. In this study, the antioxidant activity of the extract was tested by two methods, including the DPPH radical scavenging test and phosphomolybdenum reduction assay. In the presence of antioxidant phytochemicals, $DPPH^{\bullet}$ accepts a hydrogen (H) atom and reduces to $DPPH_2$, and its purple color changes to yellow, which is measured spectrophotometrically. Also, in the total antioxidant capacity test, the reduction of Mo (VI) to Mo (V) by antioxidants leads to the production of green phosphate Mo (V) complex at acidic pH, which is used for quantitative detection of antioxidant capacity by the spectrophotometric method. The results are shown in Table 1.

Table 1. Antioxidant Activity and phytochemical analysis of the CPE. Values are mean ± SD, n = 3.

Sample	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	DPPH (µg/mL)	PRA (mg αTE/g extract)
CPE	44.83 ± 4.9	8.38 ± 1.03	151.81 ± 1.12	378.14 ± 13.11

TPC, Total phenolic content; TFC, total flavonoid content; DPPH, 2,2'-diphenyl-1-picrylhydrazyl; PRA, phosphomolybdenum reduction assay; GAE, gallic acid equivalents; QE, quercetin equivalents.

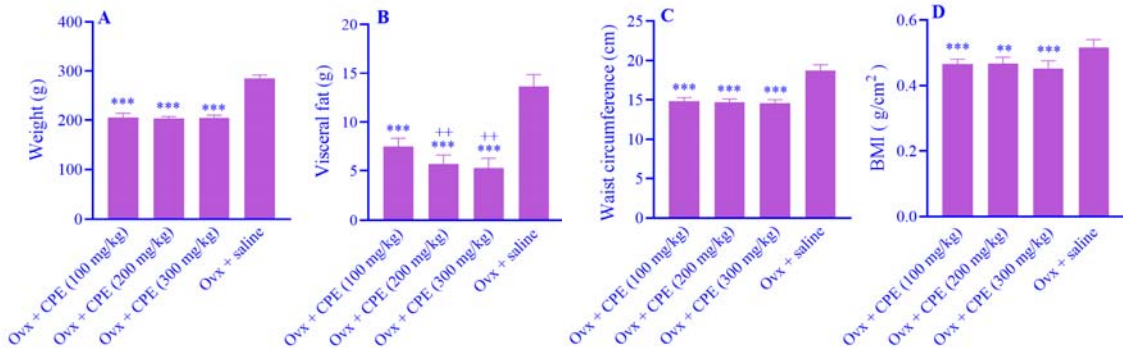

Fig. 3. Dose-dependent effects of CP Extract. Effects of different doses of CPE (100, 200, 300 mg/kg) on (A) body weight (g), (B) visceral fat (g), (C) waist circumference (cm), and (D) body mass index (BMI, g/cm²). Values are mean ± SD, n = 8. No significant differences were observed among different doses ($P > 0.05$). $^{**}P < 0.01$ AND $^{***}P < 0.001$ indicates the significant differences in comparison with the Ovs + saline group. Ovx, Rats underwent ovariectomy; CPE, ethanolic extract of *Crataegus pentagyna* fruit; BMI, body mass index.

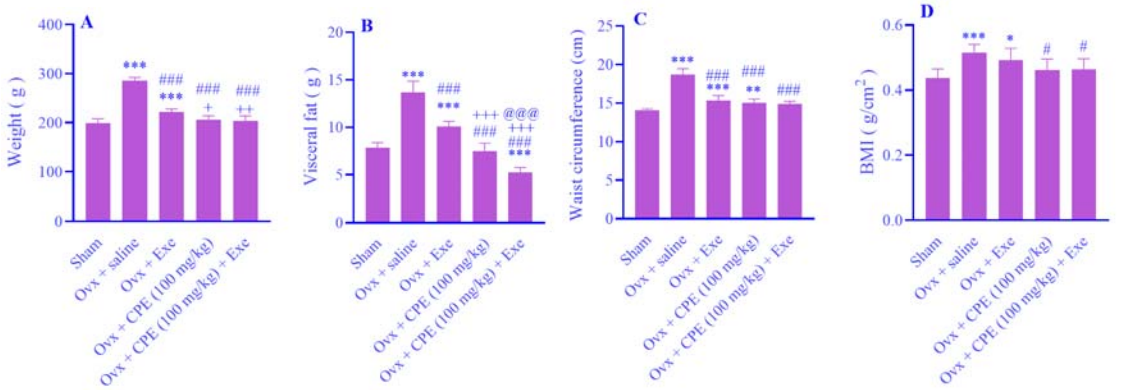

Fig. 4. Effects of HIIT and CPE on metabolic syndrome indices. Effects of HIIT and CPE (100 mg/kg) on (A) body weight, (B) visceral fat, (C) waist circumference, and (D) BMI after 8 weeks. Values are mean ± SD, n = 8. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicate the significant differences in comparison with the sham group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs* Ovx + saline; $^{+++}P < 0.001$ against Ovx + Exe; $^{@@@}P < 0.05$ vs Ovx + CPE (100 mg/kg). HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise; BMI, body mass index.

Dose-response effect of CPE on MS indices

No significant differences were observed in body weight, waist circumference, or visceral fat across 100, 200, and 300 mg/kg CPE doses compared to Ovx + saline (Fig. 3), suggesting a plateau effect. The 100 mg/kg dose was selected for co-treatment due to its efficacy and lower potential for toxicity, consistent with prior studies on *Crataegus*.

Co-treatment of HIIT and CPE (100 mg/kg) on MS indices

Co-treatment (Ovx + CPE + Exe) reduced body weight by 31.2%, visceral fat by 72.8%, waist circumference by 29.9%, and BMI by 20% compared to Ovx + saline, outperforming Ovx + Exe (22%, 33.7%, 16%, 4.8%, respectively) and Ovx + CPE (29.8%, 65.5%, 20.3%, 20%, respectively) (Fig. 4). Notably, the combined treatment (Ovx + CPE + Exe) reduced visceral fat significantly more than exercise alone and showed comparable effects to CPE alone, indicating a synergistic effect of the combined treatment.

Co-treatment of HIIT and CPE (100 mg/kg) on glucose, insulin, and HOMA-IR

Serum glucose levels decreased by 71.4% in the Ovx + Exe group, 66.5% in the Ovx + CPE + Exe group, and 60.0% in the Ovx + CPE group compared to the Ovx + saline group (Fig. 5A). Regarding insulin levels, the Ovx + Exe group exhibited the most significant reduction of 75.0%, whereas the Ovx + CPE and Ovx + CPE + Exe groups showed decreases of 48.5% and 52.0%, respectively, compared to the saline group (Fig. 5B). Consequently, HOMA-IR improved markedly in all intervention groups, with the most profound effect observed in the exercise-trained groups (Fig. 5C).

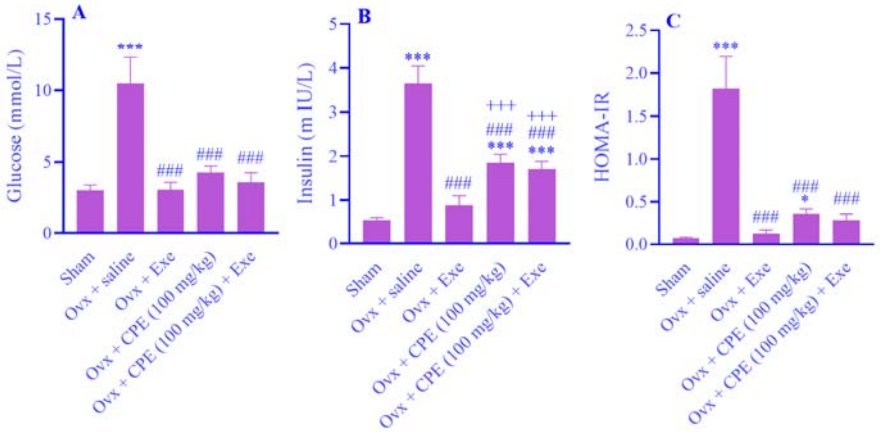

Fig. 5. Effects of HIIT and CPE (100 mg/kg) on (A) serum glucose, (B) serum insulin, and (C) HOMA-IR. Values are mean ± SD, n = 8. Values are mean ± SD, n = 8. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate the significant differences in comparison with the sham group; $^{\#\#\#}P < 0.001$ *vs* Ovx + saline; $^{+++}P < 0.001$ against Ovx + Exe. HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise; HOMA-IR, Homeostasis model assessment of insulin resistance.

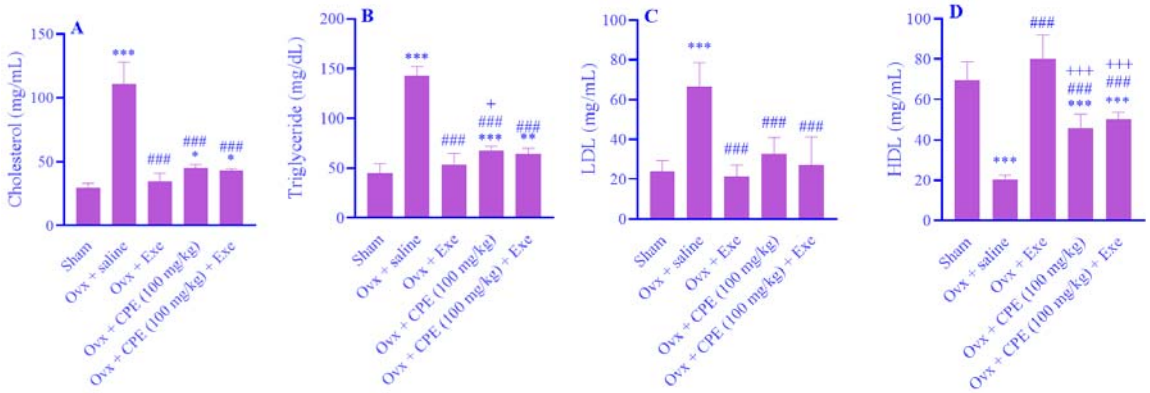

Fig. 6. Effects of HIIT and CPE on lipid profiles. Effects of HIIT and CPE (100 mg/kg) on (A) cholesterol, (B) triglyceride, (C) LDL, and (D) HDL. Values are mean ± SD, n = 8. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate the significant differences in comparison with the sham group; $^{\#\#\#}P < 0.001$ *vs* Ovx + saline; $^{+}P < 0.05$ and $^{+++}P < 0.001$ against Ovx + Exe. HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Co-Treatment of HIIT and CPE (100 mg/kg) on lipid profiles

Ovx + CPE + Exe reduced total cholesterol (-64%), TG (-58%), and LDL-C (-66.4%) vs. Ovx + saline, outperforming Ovx + Exe (-43.2% LDL-C) and Ovx + CPE (21.8%; P > 0.05), while significantly increasing HDL-C (Fig. 6).

Co-Treatment on TAC, MDA, and Nrf2

TAC increased by 473% (Ovx + Exe), 453% (Ovx + CPE), and 420% (Ovx + CPE + Exe) versus Ovx + saline (Fig. 7A). MDA decreased by 57%, 41%, and 92%, respectively (Fig. 7B). Nrf-2 levels were reduced by 50% (Ovx + Exe), 33% (Ovx + CPE), and increased 67% (Ovx + CPE + Exe) relative to Ovx + saline (Fig. 7C), with Ovx + Exe showing significantly greater reduction than Ovx + CPE (Fig. 7C and Table 2).

The effect of co-treatment on adiponectin

Adiponectin levels were significantly higher in all treatment groups compared to Ovx + saline (Fig. 8), with the greatest increase in Ovx + Exe (73%), followed by Ovx + CPE (40%) and Ovx + CPE + Exe (35%). However, no significant differences were observed between the treatment groups ($P = 0.51$).

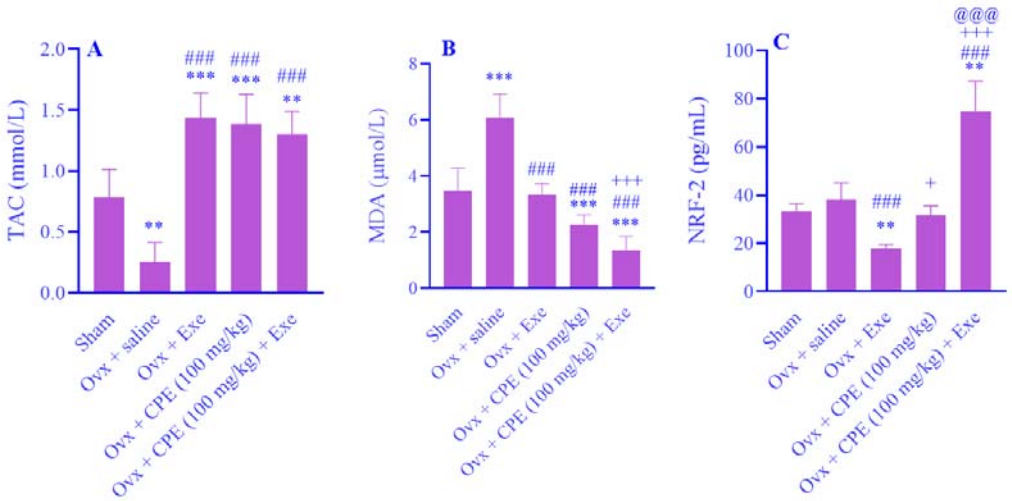

Fig. 7. Effects of HIIT and CPE (100 mg/kg) on (A) TAC, (B) MDA, and (C) Nrf2. Values are mean ± SD, n = 8. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate the significant differences in comparison with the sham group; $^{\#\#\#}P < 0.001$ *vs* Ovx + saline; $^{+}P < 0.05$ and $^{+++}P < 0.001$ against Ovx + Exe; $^{@@@}P < 0.05$ vs Ovx + CPE (100 mg/kg). HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise; TAC, total antioxidant capacity; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2.

Table 2. Summary of changes in metabolic syndrome indices, adiponectin, Nrf2, TAC, and MDA across treatment groups. Values represent percentage changes relative to Ovx + saline.

Parameter	Ovx + saline	Ovx + Exe	Ovx + CPE (100 mg/kg)	Ovx + CPE (100 mg/kg) + Exe	Sham
Body weight (g)	High	-22%	-29.8%	-31.2%	Low
Visceral fat (g)	High	-33.7%	-65.5%	-72.8%	Low
Waist circumference (cm)	High	-16%	-20.3%	-29.9%	Low
Glucose (mmol/L)	High	-70%	-70%	-70%	Low
Insulin (mU/mL)	High	-70%	-70%	-70%	Low
HOMA-IR	High	Improved	Improved	-94%	Low
Cholesterol (mg/dL)	High	Reduced	Reduced	-64%	Low
Adiponectin (µg/mL)	Low	+63%	+47%	+78%	High
Nrf2 (ng/mL)	Low	-45%	-53%	+188.94%	High
TAC (µmol/L)	Low	+473%	+453%	+420%	High
MDA (µmol/L)	High	-57%	-41%	-92%	Low

CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise; TAC, total antioxidant capacity; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; HOMA-IR, Homeostasis model assessment of insulin resistance.

The effect of co-treatment on metabolic Z score

The metabolic syndrome Z-score, calculated according to ATP III criteria (37), was significantly reduced in the Ovx + CPE + Exe, Ovx + Exe, and Ovx + CPE groups compared to the Ovx + saline group (Fig. 9).

Metabolic syndrome severity was assessed using a continuous Z-score adapted from the Adult Treatment Panel III (ATP III) criteria (37). The Z-score was calculated using the following equation:

$$\text{Z Score} = \frac{40-HDL}{5.49} + \frac{TG-150}{149.53} + \frac{fasting\ blood\ glucose-110}{22.69} + \frac{waist\ circumference-102}{11.13} \quad (4)$$

where HDL, triglycerides (TG), and fasting glucose are in mg/dL, and waist circumference in cm.

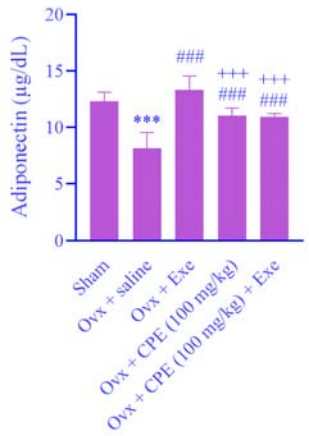

Fig. 8. Effects of HIIT and CPE (100 mg/kg) on serum adiponectin after 8 weeks. Values are mean ± SD, n = 8. ***P < 0.001 indicates the significant differences in comparison with the sham group; $^{###}P$ < 0.001 *vs* Ovx + saline; and ^{+++}P < 0.001 against Ovx + Exe. HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise.

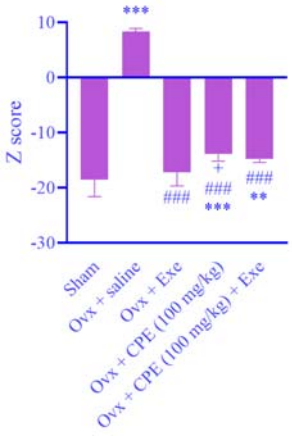

Fig. 9. Changes in metabolic Z score after 8 weeks of treatment. Values are mean ± SD, n = 8. **P < 0.01 and ***P < 0.001 indicate the significant differences in comparison with the sham group; $^{###}P$ < 0.001 *vs* Ovx + saline; and ^{+}P < 0.05 against Ovx + Exe. HIIT, High-intensity interval training; CPE, ethanolic extract of *Crataegus pentagyna* fruit; Ovx, rats underwent ovariectomy; Exe, exercise.

Food intake

Food intake was monitored weekly and showed no significant differences across groups (Ovx + saline: 18.5 ± 1.2 g/day, Ovx + Exe: 18.2 ± 1.0 g/day, Ovx + CPE: 18.7 ± 1.3 g/day, Ovx + CPE + Exe: 18.4 ± 1.1 g/day; P > 0.05), indicating that observed metabolic improvements were not due to changes in food consumption.

DISCUSSION

Given the finding of the present study, ovariectomy caused a significant increase in body weight, visceral fat, insulin, serum LDL, TG and cholesterol, but reduction in adiponectin and NRF2 level; both monotherapy and combined treatments with HIIT and CPE (100 mg/kg) alleviated MS indices; cotreatment with exercise and CPE significantly elevated serum level of adiponectin and BMP-9, but reduced lipid peroxidation and oxidative stress markers. These findings declare that improvement in MS indices by simultaneous administration of CPE and HIIT protocol might be achieved *via* elevation in adiponectin. Food intake was monitored and showed no significant differences (data not shown), supporting peripheral mechanisms, but further studies are needed to confirm this.

Previously, it was reported that HIIT stimulates fatty acid oxidation and suppresses liver fatty acid synthesis (38). On the other hand, adiponectin increased by HIIT is capable of reducing serum insulin and glucose (39,40), by inserting glucose transporters into the cell membranes and activating AMP-activated protein kinase (AMPK) (41,42). Also, adiponectin binds to its receptors, AdipoR1 and AdipoR2, in the skeletal muscles and liver, and enhances fatty acid oxidation and uptake, triglyceride catabolism, and removes cholesterol from the liver ducts (43).

Despite all the beneficial effects of HIIT on MS indices mentioned above, HIIT increased the lipid peroxidation as it was evident by elevation in MDA, and reduction in TAC and Nrf2. The reduction in Nrf2 levels with HIIT alone may be attributed to the increased production of ROS during high-intensity exercise, which can overwhelm the antioxidant defense system and lead to a temporary suppression of Nrf2 activation. Elevation in oxidative stress marker of MDA reflects shifting the redox balance in favor of oxidative stress after HIIT (44), which could drive the pathogenesis of cell toxicity (9). Surprisingly, supplementation with CPE (100 mg/kg) for eight weeks not only alleviated MS indices efficiently and synergistically but also elevated TAC and Nrf2 and reduced MDA.

Our findings indicated that CPE is a rich source of polyphenols and flavonoids. So far, various phytochemicals, such as organic acids, phenolic acids, flavonoid O- and C-glycosides, flavonoid aglycones, and proanthocyanidins have been determined in other investigations. For example, Bujor *et al.* reported the presence of epicatechin, caffeic acid, rutin, quercetin, and apigenin glycosides in fruits of *C. pentagyna* growing in Romania (45). Moreover, quercetin, chlorogenic acid, spiraeoside, isoquercetin, epicatechin, and procyanidin (46-48) were reported as well. Mateos *et al*. reported that the main flavonoid of this species was quercetin (49). Quercetin potently exerts antioxidant, anti-inflammatory, and hypoglycemic effects (50) by inhibiting alpha-amylase and upregulating glucose transporter 4 (51).

This study has several limitations. The use of intraperitoneal administration limits translatability to humans, as oral administration is more clinically relevant. The standardization of the CPE relied on spectrophotometric assays (total phenolic and flavonoid contents), and the absence of advanced profiling techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) may limit the robustness and reproducibility of the extract's chemical fingerprint. The absence of a formal toxicity assessment (*e.g.,* liver or kidney function tests) represents a study limitation. Tissue-level data on Nrf2 expression and blood pressure measurements were not included due to resource constraints, limiting a comprehensive evaluation of metabolic syndrome. The animal model may not fully replicate human pathophysiology. Variability in CPE composition due to environmental factors was not assessed. The lack of a non-ovariectomized exercise group limits insights into menopause-specific effects. The interaction effects between HIIT and CPE supplementation were not assessed using two-way ANOVA, which could have provided deeper insight into their synergistic effects.

Furthermore, our findings showed that supplementation with CPE improved TAC as an indicator of serum antioxidants (52,53). Quercetin, as a main antioxidant in the CPE, activates Nrf2 signaling (54), and then Nrf2 protects cells from oxidative stress (9). Like other antioxidants such as resveratrol and curcumin, the quercetin in CPE upregulates Nrf2 to bolster antioxidant defenses (55). However, the diverse phytochemical profile of *C. pentagyna* may provide additional metabolic advantages over single-compound antioxidants. Additionally, the VO_2max protocol used to determine exercise intensity was not specifically validated for ovariectomized rats, which may influence exercise performance due to hormonal changes, representing a limitation of the study. When Nrf2 is released from Kelch-like ECH-associated protein (56), it translocates to the nucleus and binds to antioxidant responsive elements and activates the transcription of several antioxidative genes (57), to prevent oxidative stress, inflammation, and lipid peroxidation (58,59). Nfr2 also negatively regulates the NF-κB signaling pathway (56), an important mediator of both inflammation and IR (60). Future research should involve randomized controlled trials in postmenopausal women, integrating HIIT with oral CPE supplementation, to confirm these findings and evaluate long-term efficacy and safety. Comparing *C. pentagyna* with other antioxidants or *Crataegus* species could clarify its unique benefits. Long-term studies should assess the sustainability of these effects and the potential side effects of chronic CPE supplementation. Taken together, we showed that 8 weeks of HIIT combined with intraperitoneal administration of ethanolic CPE synergistically alleviated the MS Z score. Also, CPE successfully targets and nullifies oxidative stress induced by HIIT *via* Nrf2 signaling. From a clinical importance view, the combination of HIIT and CPE may serve as a successful strategy to combat MS progression and prevent atherosclerosis development.

CONCLUSION

The combination of HIIT and CPE (100 mg/kg) supplementation for 8 weeks significantly ameliorates metabolic syndrome and oxidative stress in ovariectomized rats, associated with increased adiponectin and Nrf2 levels. This combination enhances HIIT's metabolic benefits while mitigating its oxidative risks, suggesting a promising adjunctive therapy for postmenopausal metabolic dysfunction.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

F. Sadeghi and S. Falahati contributed to data curation, formal analysis, investigation, writing, review, and editing the article; P. Babaei, M. Nabilpour contributed to conceptualization, data curation, formal analysis, methodology, project supervision, resources, validation, visualization, writing, review, and editing the article; A. Damirchi and F. Seify contributed to conceptualization, technical and experimental facilities, exercise protocol providing; F. Seify and F. Yousefbeyk contributed to investigation, providing plant extract, validation, writing, review, and editing the article.

All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

AI declaration

During the preparation of this work, the authors used Grok to improve readability and language. After using this tool, the authors reviewed and edited the content and take full responsibility for the content of the publication.

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