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Original Article

High-Intensity Interval Training and Crocin Effects on Doxorubicin-Induced Heart Apoptosis

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ABSTRACT

Background: Doxorubicin (Dox) has been shown to negatively impact the function and structure of the heart in several studies. This experimental study examined the effects of 8 weeks of high-intensity interval training (HIIT) and crocin (Cr) on Dox-induced apoptosis in the heart tissue of male Wistar rats.

Methods: Fifty healthy male Wistar rats (8 weeks, 200–220 g) were utilized in this study. The groups under investigation included Dox (2 mg/kg), Dox + HIIT (5 days/week for 8 weeks), Dox + Cr (10 mg/kg), and Dox + Cr + HIIT. HIIT involved 1 hour of running at 40%–60% of maximum speed, 5 days per week. Apoptosis and histological changes were assessed in the heart tissue of the rats.

Results: Dox significantly increased the expression of Bax while decreasing Bcl-2 expression levels in the cardiac tissue of animals ($P < 0.01$). The combination of HIIT and Cr reduced the heart injury score, TUNEL-positive cells, and the Bax/Bcl-2 ratio in Dox-treated rats compared with HIIT or Cr alone.

Conclusions: Cr and HIIT, either individually or combined, demonstrated potential in suppressing Dox-induced cardiotoxicity through the attenuation of apoptosis and histological injury. ***(Iranian Heart Journal 2025; 26(1): 81-92)***

KEYWORDS: Cardiotoxicity, High-intensity interval training, Crocin, Apoptosis

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The rate of cancer is on the rise due to factors such as population growth and aging. [1] Alongside the increasing incidence of cancer, advancements in treatment options have improved patient survival rates. Nonetheless, cancer treatments can also lead to side effects like cardiotoxicity. [2] Doxorubicin (Dox), a chemotherapeutic agent, is widely employed for treating various cancer types, including solid tumors, leukemia, lymphoma, and breast cancer. Despite its efficacy, the clinical

application of Dox is limited due to its potential to inflict irreversible damage on heart tissue. [3] Dox triggers cardiotoxicity by activating apoptosis signaling pathways. [4] Apoptosis is a physiological event that eliminates damaged or aged cells during development, embryogenesis, and tissue repair, maintaining tissue homeostasis. [5] In healthy heart muscle, apoptosis is a rare occurrence but is observed in chronic and acute heart diseases. [6] Apoptosis is initiated through 2 pathways: extrinsic and intrinsic. The intrinsic pathway commences inside the cell via signals such as free radicals and involves Bcl-2 family proteins found in the outer mitochondrial membrane. [7] Bax and Bcl-2 proteins play crucial roles in forming mitochondrial channels, regulating mitochondrial permeability, and transmitting apoptosis-related signals. [8] Physical activities have been shown to support heart health by enhancing antioxidant levels and reducing cell death. [9-11] Engaging in physical activities can aid in the prevention or management of cardiovascular diseases. For individuals with heart damage, exercise tolerance is influenced by the duration and intensity of the physical activity. [12] Previous research has demonstrated the beneficial effects of exercise in mitigating Dox-induced cardiac dysfunction. [9-11] High-intensity interval training (HIIT) is an effective, safe, and cost-efficient intervention for individuals with stroke or heart failure. [13,14] Moreover, HIIT is a well-tolerated and safe exercise modality for patients with cancer undergoing chemotherapy, as it enhances maximum oxygen consumption, heart rate, and physical performance. [15] Combining natural products with chemotherapy to minimize side effects has emerged as a novel approach to cancer management. [16] Crocin (Cr), a water-soluble carotenoid derived from saffron stigmas and gardenia fruits, exhibits numerous medicinal properties, including protection of the nervous system, cognitive function improvement, reduction of serum lipids, and promotion of kidney health. [17-21] Studies have demonstrated the potential of saffron and Cr in preventing oxidative damage resulting from ischemia-reperfusion blood flow in rats. [22] Cr has been shown to alleviate Dox-induced cardiotoxicity by inhibiting inflammatory and apoptotic pathways. [23] As a result, this research serves as the pioneering investigation into the combined effects of HIIT and Cr consumption during Dox therapy on apoptosis and cardiac tissue damage in rats.

METHODS

Study Design

Fifty healthy male Wistar rats (8 weeks, 200–220 g) were utilized. The rats were housed in clean and transparent polycarbonate cages, maintained at 22 ± 3 °C with an air humidity of 35 ± 5%, and exposed to a 12–12 hour light-dark cycle. To acclimate the rats to their new environment and familiarize them with treadmill activity, a light training program consisting of 10 sessions (each session involving 5 to 10 walks at a speed of 8–10 meters/minute) was implemented over 2 weeks. The rats were allocated into the following 5 groups (10 rats per group):

1. **Control**: The animals were treated with normal saline for 8 weeks.
2. **Dox**: The animals were administered 2 mg/kg Dox for 8 weeks.
3. **Dox + HIIT**: The animals underwent Dox treatment combined with HIIT (5 days per week for 8 weeks).
4. **Dox + Cr**: The animals received Dox and 10 mg/kg Cr for 8 weeks.
5. **Dox + Cr + HIIT**: The animals were administered combined Dox, Cr, and HIIT interventions for 8 weeks.

Dox) was administered via intraperitoneal injection on Fridays at 10 AM, while Cr was given concurrently through gavage at a dosage of 10 mg/kg. HIIT was also performed simultaneously with Cr administration.

Two days after the last training session, the rats were anesthetized with a ketamine (90 mg/kg) and xylazine (10 mg/kg) mixture while in a resting condition. The heart tissues were then meticulously dissected and promptly preserved at −70 °C for subsequent examinations.

Sports training program

The primary training program was carried out over 8 weeks (5 days per week), commencing from the third week. This program consisted of 2-minute intervals of treadmill running without incline, specifically designed for rodents. The main stage included the following:

1. **First week**: Two-minute intervals at 80% of maximum speed (32 meters per minute)
2. **Second week**: Four 2-minute intervals at 85% of maximum speed (34 meters per minute)
3. **Third to eighth weeks**: Six 2-minute intervals at 90% of maximum speed (36 meters per minute)

From the start of the fourth week until the end of the course, the rats completed 8 HIIT sessions. Low-intensity intervals, each lasting 2 minutes, were performed with the following specifications:

1. **First to third weeks**: 40% of maximum speed (15 meters per minute)
2. **Fourth to eighth weeks**: 30% of maximum speed (12 meters per minute)

The total training time was as follows:

- **First week**: 16 minutes
- **Second week**: 24 minutes
- **Third week**: 32 minutes
- **Fourth to eighth weeks**: 40 minutes

Histology

Under deep anesthesia, the cardiac tissues were dissected and fixed in 10% formalin. The samples were then embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin and eosin (H&E). For quantitative analysis, histological photomicrographs were assessed using a scoring system. Based on inflammatory cell infiltration, congested vessels, and myofibrillar loss, scores of 0 (typical), 1 (mild), 2 (moderate), or 3 (severe) were assigned. Photomicrographs were captured using an Olympus BX43 microscope equipped with a DP26 camera.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was extracted from cardiac tissue using the RNeasy kit by Qiagen Company. Complementary DNA (cDNA) was synthesized through reverse transcription of RNA utilizing a kit from Qiagen Company. The PCR reaction mixture contained cDNA, DEPC water, forward and reverse primers (Table-s1), and SYBR Green Master Mix (Qiagen). Next, qRT-PCR was performed with 45 cycles: 95 ºC for 50 seconds, 95 ºC for 30 seconds, and 60 ºC for 35 seconds. Relative gene expression was normalized against GAPDH. Data analysis was carried out using REST software (2009).

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) test

The TUNEL assay kit (Invitrogen, Germany) was used to identify apoptotic cells in the heart tissue. Initially, deparaffinized slides were permeabilized and incubated with the TUNEL reagent solution for 1 hour. Following a washing step, the slides were incubated with 50 µL of converter solution for 40 minutes and then stained with diaminobenzidine tetrachloride. For a quantitative assessment of apoptotic cells, the apoptosis index was calculated as the percentage of TUNEL-positive cardiomyocytes.

Data Analysis

The Shapiro-Wilk test was employed to confirm the normal distribution of data. A one-way analysis of variance test (SPSS,

version 21), followed by the least significant difference (LSD) or Kruskal-Wallis variance test, was utilized for data analysis. The significance level was set at $P \leq 0.05$.

Table s1: Primer Sequences

Genes	Forward	Reverse
Bax	GGATGCCTTTGTGGAACTGT	TCACTTGTGGCCCAGATAGG
Bcl-2	ACCCAGAAGACTGTGGATGG	TTCTAGACGGCAGGTCAGGT
GAPDH	GCTGGACATTGGACTTCCTC	ACCACTGTGACCTGCTCCA

RESULTS

Relative heart weight

No significant differences in body weight were found between the experimental and control groups. Nonetheless, a significant reduction in heart weight relative to body weight was observed in Dox-injected rats compared with the control group ($P < 0.01$). Both HIIT and Cr interventions led to increased relative heart weight compared with the Dox group ($P < 0.05$). Furthermore, the combination of HIIT and Cr resulted in a greater relative heart weight increase compared with HIIT or Cr alone (Fig. 1).

Histology

The control groups displayed a typical heart tissue structure. A significant increase in heart injury score was observed in the Dox group compared with the control ($P < 0.001$). Heart injury scores were significantly reduced in Dox-treated animals that received HIIT or Cr treatment. The combination of HIIT and Cr led to a further decrease in heart injury scores compared with HIIT or Cr alone (Figs. 2 & 3).

qRT-PCR

The Bax/Bcl-2 ratio was significantly elevated in Dox-treated animals compared with the control group ($P < 0.001$). Both HIIT and Cr interventions led to a significant reduction in the Bax/Bcl-2 ratio compared with the Dox-injected group. The combination of HIIT and Cr resulted in a more significant decrease in the Bax/Bcl-2 ratio compared with either treatment alone (Fig. 4).

TUNEL assay

The apoptosis index showed a significant increase in the Dox group compared with the control ($P < 0.01$). Simultaneous treatment with Cr and HIIT led to a reduction in the apoptosis index of the Dox group. HIIT demonstrated a more significant effect on TUNEL-positive cardiomyocytes in Dox-treated rats compared with the Cr + Dox group (Figs. 5 & 6).

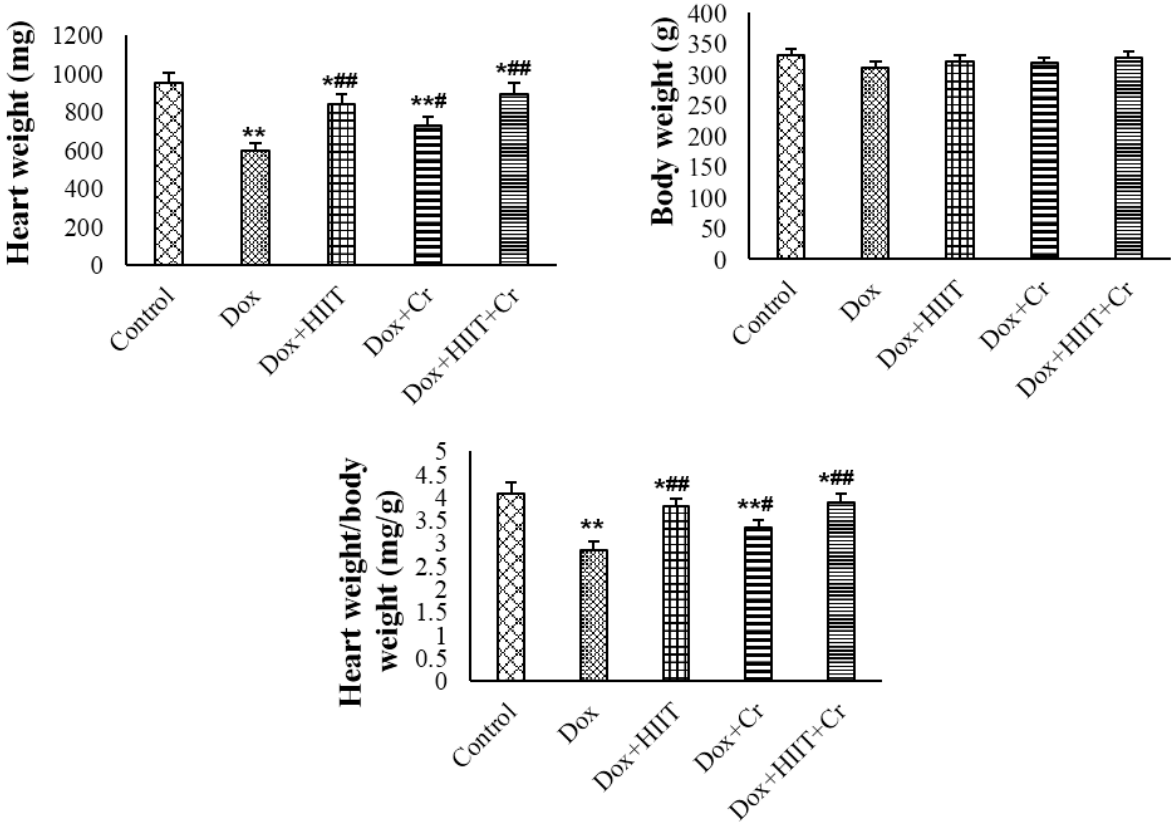

Figure 1: The images show heart weight, body weight, and relative heart weight in the different studied groups (mean ± SD; n=10).
* $P < 0.05$, ** $P < 0.01$, # $P < 0.05$, ## $P < 0.01$
The * and # symbols indicate a comparison between the control and Dox groups.
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

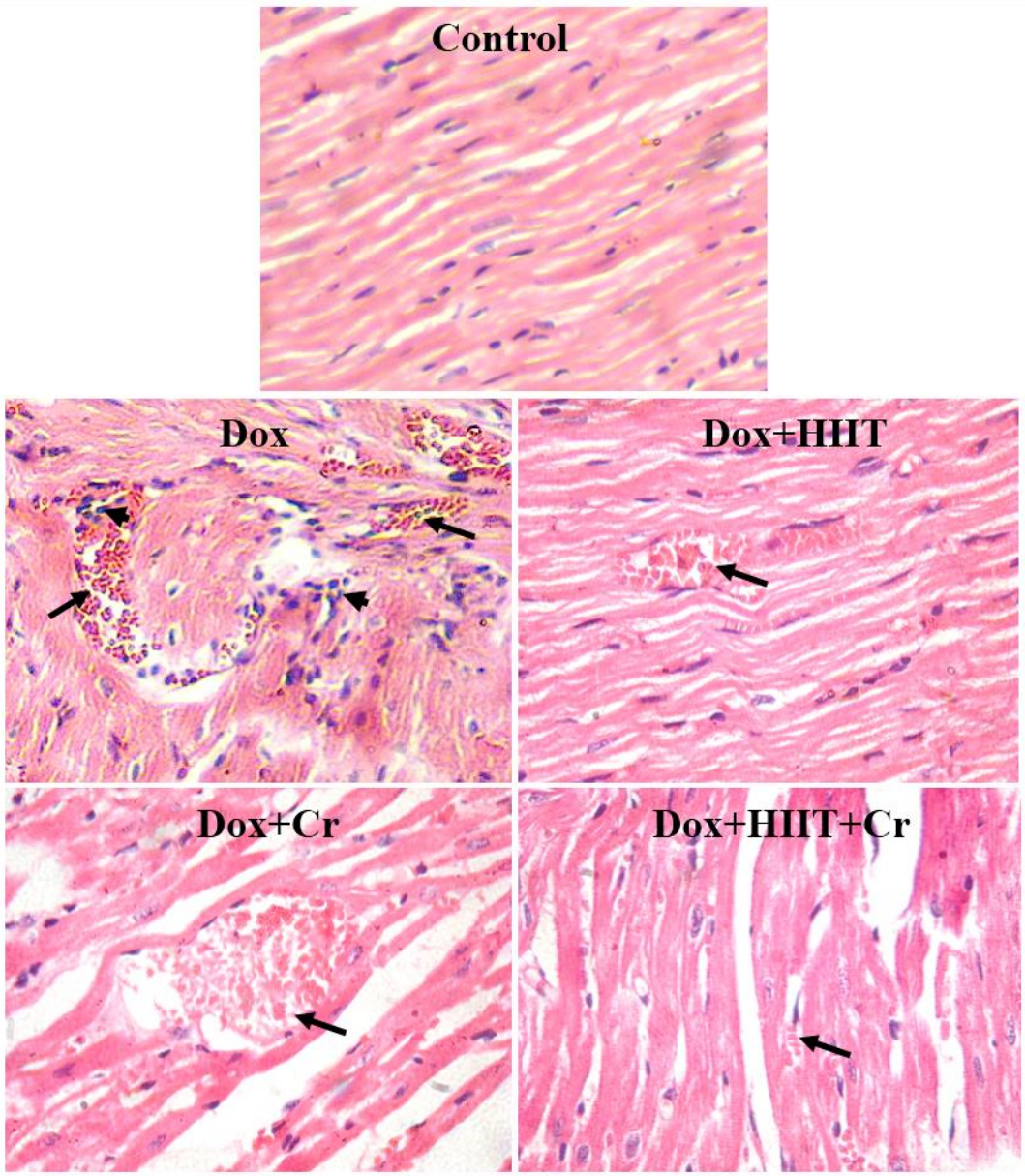

Figure 2: The images present light microscopy of cardiac tissue from the different studied groups. The arrows show congested vessels, and the arrowheads indicate inflammation.
H&E staining; magnification: X250
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

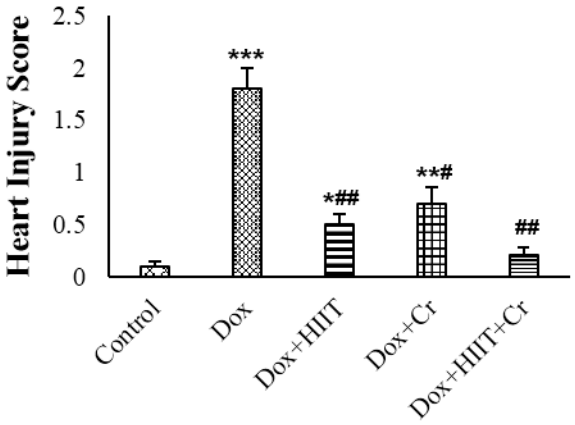

Figure 3: The image presents heart injury scores in the different studied groups (mean ± SD; n=10).
* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, # *P* < 0.01, ## *P* < 0.001.
The * and # symbols indicate a comparison between the control and Dox groups.
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

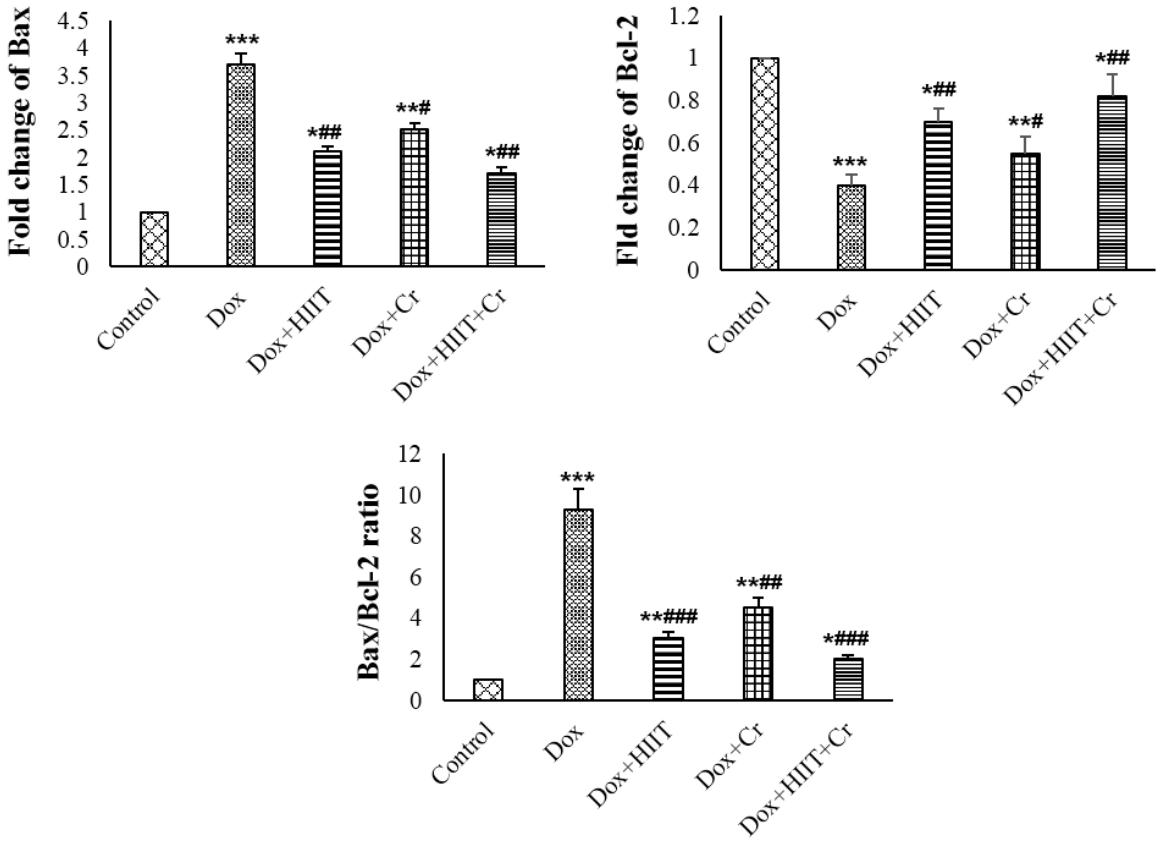

Figure 4: The image illustrates the real-time results of Bcl-2 and Bax expression levels in the different studied groups (mean ± SD; n=5).
* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, # P < 0.05, ## *P* < 0.01, ### *P* < 0.001.
The * and # symbols indicate a comparison between the control and Dox groups.
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

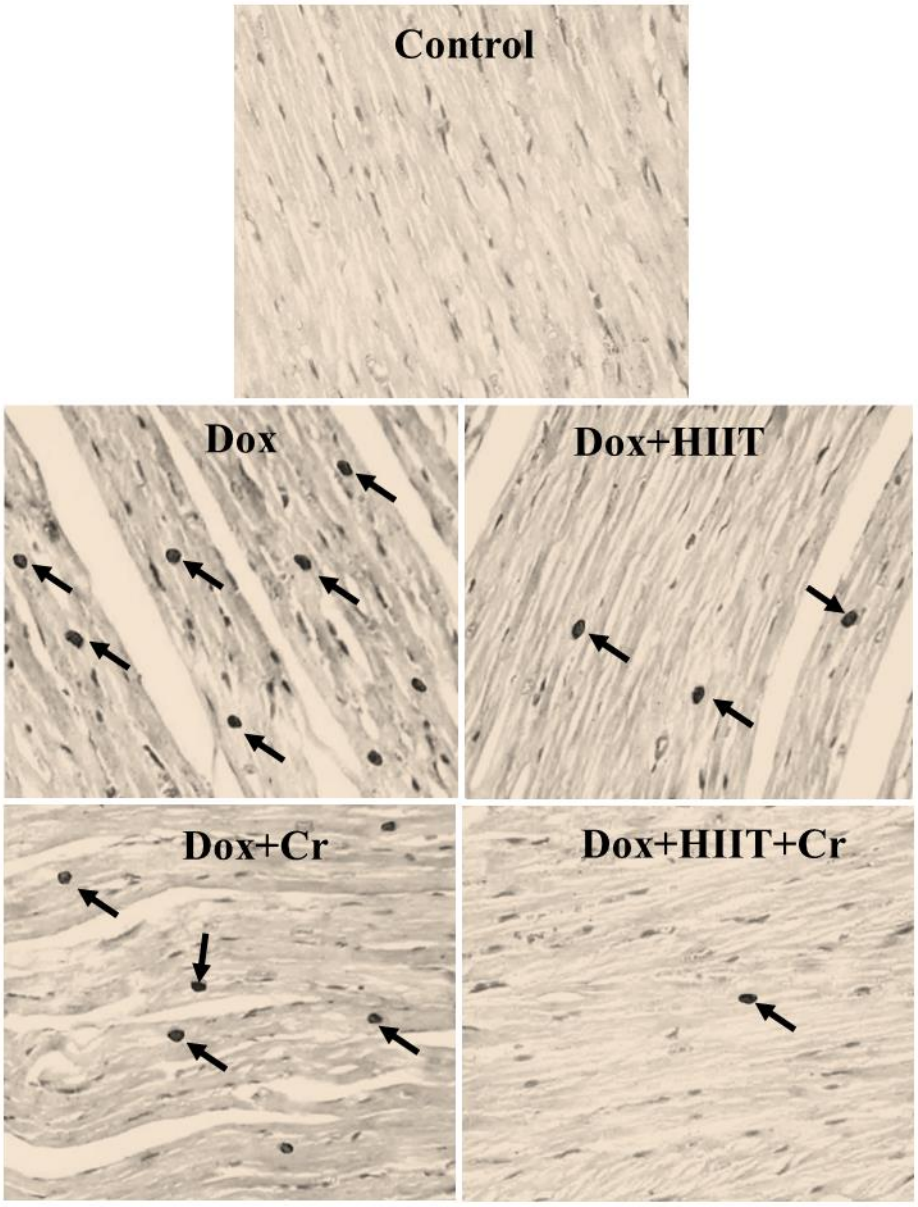

Figure 5: Light microscopy of TUNEL staining from the different studied groups. The arrows show TUNEL-positive reactions (apoptotic cells).
magnification: X250
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

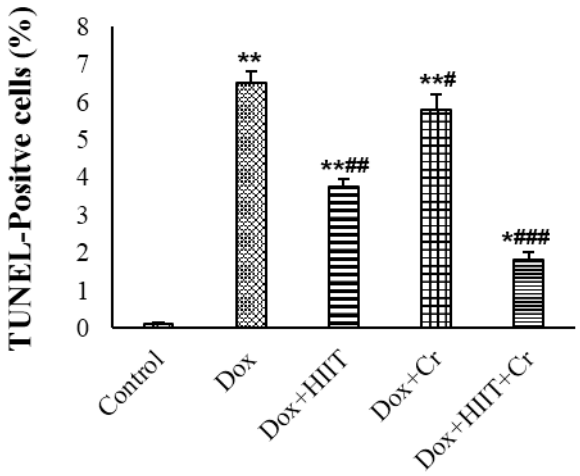

Figure 6: The image provides the percentage of TUNEL-positive cells (the apoptotic index) in the different studied groups (mean ± SD; n=3).
* *P* < 0.05, ** *P* < 0.01, # *P* < 0.05, ## *P* < 0.01, ### *P* < 0.001
The * and # symbols indicate a comparison between the control and Dox groups.
Dox: doxorubicin, Cr: crocin, HIIT: high-intensity interval training

DISCUSSION

The present study investigated the effects of HIIT and Cr on the prevention of apoptosis induced by Dox in heart tissue. Dox administration led to a reduction in cardiac weight, indicating its detrimental impact on heart function. The decrease in cardiac weight may be attributed to the increased histological injury score observed in the hearts of Dox-treated rats. The cardiotoxic effects of Dox have been well-documented in previous studies. [25, 3] The reduction in cardiac weight may result from Dox-induced apoptosis in heart tissue. Dox triggers apoptosis through the generation of free radicals and the disruption of the mitochondrial membrane via an intrinsic mechanism. [26, 27]

As observed in the results, HIIT, with or without Cr, could significantly reverse cardiac weight reduction, which may be attributed to the apoptosis-suppressing effects of HIIT. The decreased percentage of TUNEL-positive cells and the Bax to Bcl-2 ratio following HIIT or Cr intervention supports this hypothesis. A previous study demonstrated that 6 weeks of interval exercise training reduced the Bax/Bcl-2 ratio and TUNEL-positive cardiomyocytes in Dox-treated animals. [28] In a study by Marques-Aleixo et al, [29] it was shown that 12 weeks of treadmill training led to a reduction in apoptosis in cardiomyocytes of Dox-treated animals.

Razavi Majd and Ghahramani [30] found that 3 months of aerobic training led to a decrease in the expression of the Bax gene. Furthermore, a separate study demonstrated that regular HIIT reduced the Bax/Bcl-2 ratio in cardiac tissue. [31] These findings align with the observation that Dox induces various detrimental effects on cardiac tissue, including oxidative damage, mitochondrial dysfunction, histological damage, and apoptosis. [32] Cardiomyocytes are particularly susceptible to Dox-induced oxidative stress due to their low antioxidant defense and high mitochondrial volume. As observed in the results, the use of Cr, with or without HIIT, led to reduced apoptosis and histological changes in cardiac tissue following Dox administration. These findings are consistent with several previous studies that have reported the beneficial effects of Cr in preventing Dox-induced cardiotoxicity. For instance, Chu et al [33]

demonstrated that Cr could effectively prevent Dox-induced cardiotoxicity. In a study conducted by Kohpayeh et al, [34] it was shown that continuous exercise combined with Cr supplementation resulted in a reduction in the expression of inflammatory genes in rat cardiac tissue.

In separate studies, it was shown that Cr supplementation reduced the Bax/Bcl2 ratio and apoptosis in rats with cardiotoxicity induced by diazinon and Dox. [35, 36] According to the present results, the combination of HIIT and Cr demonstrated a more pronounced positive effect on heart tissue in Dox-intoxicated rats compared with HIIT or Cr alone. In line with these findings, Shekarriz et al [37] reported that aerobic training and Cr could help prevent apoptosis induced by Dox in cardiac tissue. While this study demonstrates the positive effects of HIIT and Cr on Dox-induced apoptosis, the underlying mechanisms responsible for these observations remain unexplained. One possible explanation is that HIIT and Cr may reduce apoptosis by bolstering the antioxidant defense of cardiomyocytes. This hypothesis is supported by the findings of a previous study, which showed that HIIT, with or without Cr, led to improved antioxidant levels in rat cardiac tissue. [38]

CONCLUSIONS

This study demonstrates that both HIIT and Cr have protective effects against Dox-induced apoptosis in rat hearts. Further research is necessary to elucidate the exact mechanisms involved in these protective effects. Understanding these mechanisms may contribute to the development of strategies for reducing chemotherapy-related toxicity and improving treatment outcomes.

Declarations

Ethics Approval and Consent to Participate: This study adhered to the ARRIVE guidelines for the Care and Use of Laboratory Animals and was approved by the ethics committee of Azad University, Tehran, Iran.

Consent for Publication: Not applicable.

Availability of Data and Materials: Data and materials can be made available upon reasonable request.

Conflict of Interest: The authors declare no conflict of interest.

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Authors' contributions

- Conceptualization: MAA and RK
- Methodology: LK and RK
- Formal analysis and investigation: LK and RK
- Writing and original draft: LK and RK
- Supervision: MAA

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Not applicable.

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