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The Effect of Eight Weeks of Intense Interval Training Combined with Pomegranate Juice Supplementation on Oxidative Stress and Caspase3 in Mice with Colon Cancer

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Abstract

Introduction: The aim of the present study was to investigate the effect of eight weeks of high-intensity interval training combined with pomegranate juice supplementation on oxidative stress and caspase-3 in experimental mice with colon cancer.

Methods: 35 Balbi mice; colon cancer induced in 28, divided into 4 groups (n=7): DC (disease control), DS (+pomegranate juice), DE (+exercise), DSE (+both). 7 healthy controls (HC). Exercise: 8 weeks HIIT, 5 sessions/week, 30 min/session (warm-up, 5×1-min high-intensity >75% max speed, 4×2-min low-intensity <50%, cool-down). Pomegranate: 5% daily in drinking water. Measured: ROS (ELISA) and caspase-3 gene expression (Real-time PCR). Analysis: SPSS21, Shapiro-Wilk (normality), one-way ANOVA, Bonferroni post-hoc (P≤0.05).

Results: Oxidative stress in the DSE group was significantly lower than in the DC group (P=0.02). Also, the level of oxidative stress in the DC group was significantly higher than in the HC group (P=0.003). In terms of the relative expression of caspase-3, the DSE group was significantly higher than in the DC group (P=0.043). In addition, the relative expression of caspase-3 in the DC group was significantly reduced compared to the HC group (P=0.02).

Conclusions: The combined intervention of high-intensity interval training with pomegranate juice consumption has a positive effect on reducing oxidative stress and increasing caspase-3 levels in colon cancer compared to either exercise training or pomegranate juice supplementation.

Key Words: high-intensity interval training, pomegranate juice supplementation, oxidative stress, caspase 3, colon cancer.

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1. Introduction

Colon cancer is the second leading cause of cancer-related mortality worldwide, arising from heterogeneous tumor growth and progression, and it requires urgent and effective preventive and therapeutic strategies [1]. Multiple factors are involved in the pathogenesis of colon cancer, including genetic predisposition, poor diet, a sedentary lifestyle, and chronic inflammation. Understanding the molecular mechanisms involved in the initiation and progression of this disease, such as oxidative stress and dysregulation of programmed cell death (apoptosis), is critical for the development of new therapeutic interventions [2]. Oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the body's antioxidant defense system, plays a major role in damaging DNA, proteins, and lipids, and can lead to genetic mutations and ultimately cancer. Recent studies have emphasized the prominent role of oxidative stress in various stages of colon cancer development, including tumor resistance to therapy and metastasis [3]. On the other hand, apoptosis, or programmed cell death, is a vital process for maintaining tissue homeostasis and eliminating damaged or cancerous cells. Caspases, a family of cysteine proteases, play a central role in the execution of apoptosis, and caspase-3, as one of the main executioner caspases, is essential for activating apoptotic pathways. Dysfunction of caspase-3 or reduced expression of this protein can lead to the survival of cancer cells and resistance to chemotherapeutic agents. More recent research, including studies conducted on animal and cellular models of colorectal cancer, has shown that modulation of caspase-3 activity may represent a promising therapeutic strategy [4, 5].

In recent years, increasing attention has been directed toward the role of a healthy lifestyle, particularly regular exercise, in the prevention and management of cancer. High-intensity interval training (HIIT), characterized by short bouts of vigorous physical activity interspersed with brief recovery periods, has been recognized as an effective strategy for improving metabolic and cardiovascular health. More recent evidence suggests that HIIT may exert anticancer effects through modulation of inflammation, enhancement of immune system function, and reduction of oxidative stress [6]. In addition, researchers have confirmed the positive effects of HIIT on reducing tumor growth and increasing treatment sensitivity in various cancer models, including colon cancer [7]. HIIT may also contribute to the induction of apoptosis in cancer cells by increasing the expression of apoptosis-related proteins such as caspase-3 [8]. In this regard, Parker et al. (2012) demonstrated that following four months of voluntary wheel running, the expression of caspase-3 and caspase-7 increased in aged mice with colorectal cancer[9]. On the other hand, in emerging approaches, a diet rich in plant-derived compounds with antioxidant and anti-inflammatory properties, such as pomegranate juice, has also been proposed as a potential preventive and therapeutic factor in cancer. Pomegranate contains numerous polyphenolic compounds, including ellagitannins, anthocyanins, and flavonoids, which possess strong antioxidant capacity[10]. Furthermore, previous in vitro and animal studies have shown that pomegranate juice can play an effective role in inhibiting cancer cell growth, inducing apoptosis, and reducing oxidative stress in various cancer models, including colon cancer. In particular, evidence suggests that the bioactive compounds present in pomegranate juice are capable of increasing the expression or activity of caspase-3, thereby promoting programmed death of cancer cells[11]. Kaya et al. (2022) conducted a study investigating the modulatory effect of pomegranate extract on caspase-3 expression in a mouse model of colorectal cancer induction. Their findings showed that pomegranate extract significantly increased caspase-3 expression and significantly enhanced antioxidant enzyme levels in intestinal tissue compared with the diseased group[12]. However, most of these studies have focused on the direct effects of pomegranate juice or extract, and the concurrent effect of exercise, particularly HIIT, combined with pomegranate juice consumption on molecular pathways related to apoptosis and oxidative stress has not been thoroughly examined. Therefore, the present study was designed to investigate the combined effect of eight weeks of high-intensity interval training and pomegranate juice supplementation on oxidative stress indices and caspase-3 gene expression in mice with colon cancer, in order to elucidate new cellular and molecular dimensions of the potential synergistic role of exercise and pomegranate juice consumption in modulating oxidative stress and inducing apoptosis.

2. Methods

2.1. Study design

This was an experimental and basic research study. Ethical principles (ethics code: IR.IAU.M.REC. 1402 .068) were observed in accordance with the guidelines for working with laboratory animals based on the Declaration of Helsinki, as approved by the Research Ethics Committee of Islamic Azad University,

Marvdasht Branch .Initially, 35 BALB/c laboratory mice were purchased from the Breeding and Reproduction Center of Laboratory Animals at the Pasteur Institute of Iran and transferred to the Bitaran Laboratory in Shiraz. The mice were housed under standard conditions with respect to light, temperature (22 ± 2°C), relative humidity (55 ± 10%), and a 12:12-hour light-dark cycle, in transparent polycarbonate cages in groups of seven. Food and water were provided ad libitum.

For the induction of colon cancer, CT26 cells were first obtained from the Pasteur Institute of Iran. When the cells formed a complete monolayer covering the bottom of the flask, they were trypsinized, and after three washes, the cell count and percentage of viable cells were determined using the trypan blue exclusion method, a conventional microscope, and a Neubauer hemocytometer. After cell proliferation reached a sufficient level, each mouse was subcutaneously injected in the right flank with 5 × 10^5 viable cells suspended in 150 µL of Hanks' solution. To confirm colon cancer induction, the hematoxylin and eosin (H&E) staining method was used. As shown in Figure 1, differences were observed in the colon adenocarcinoma tissue. In the disease control group, destruction of the normal glandular architecture, alteration of normal tissue structure, excessive cell proliferation, hypertrophied nuclei markedly larger than normal, and an increased nucleus-to-cytoplasm ratio compared with normal tissue were observed (Figure 1). The histological findings and the extent of changes are also presented in Table 1.

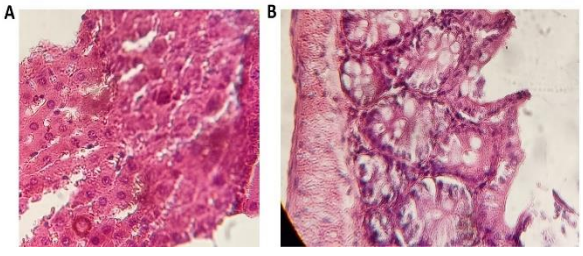

Figure 1. Microscopic appearance of intestinal tissue in the healthy control and disease control groups. (A) H&E-stained colon adenocarcinoma tissue showing loss of normal glandular architecture, marked nuclear atypia with prominent nuclei, and a high nucleus-to-cytoplasm ratio (magnification: 100×). (B) H&E-stained normal colon tissue showing pseudostratified ciliated columnar epithelium without cellular atypia (magnification: 100×).

Table 1. Results of the histopathological evaluation of colon tissue changes in the experimental rat groups

Group	Sample 1	Sample 2	Sample 3
Healthy Control	Normal Tissue	Normal Tissue	Normal Tissue
Disease Control	Severe Change	Severe change	Moderate change

Note: The interpretation of the table is as follows:
Normal tissue: Tissue with no pathological alterations, identical to healthy tissue.
Mild change: Minor alterations during tissue status assessment.
Moderate change: Moderate alterations during tissue status assessment.
Severe change: Major/substantial alterations in tissue architecture and status.

After confirming the induction of colon cancer, 28 tumor-bearing mice were allocated into four groups: (1) Disease Control (DC) (n=7), (2) Disease+ pomegranate juice Supplement(DS) (n=7), (3) Disease Exercise (DE) (n=7), and (4) Disease +pomegranate juice Supplement + Exercise(DSE) (n=7). In addition, to evaluate the effects of cancer induction on the study variables, seven mice were considered as the Healthy Control (HC) group (Figure 2).

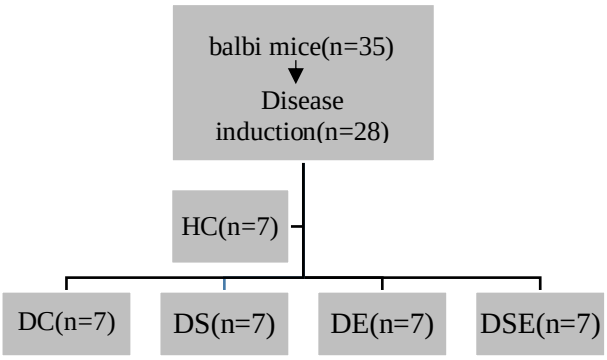

Figure 2: Experimental design and group allocation during the study. HC: Healthy Control, DC: Disease control, DS: Disease+pomegranate juice supplement, DE: Disease exercise, DSE: Disease +pomegranate juice supplement + Exercise.

2.2. Exercise protocol

Mice in the high-intensity interval training (HIIT) and HIIT + pomegranate juice (HIIT+PJ) groups underwent an 8-week training program (5 sessions/week), which began with a 1-week treadmill familiarization period. Following familiarization, maximal running speed was determined using an

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incremental exercise test to exhaustion. The animals first warmed up for 3 min at 10 m/min on a 5° incline. Treadmill speed was then increased by 3.3 m/min every minute on a 10° incline until exhaustion. Exhaustion was defined as the inability of the mice to maintain the running speed, indicated by three consecutive contacts with the shock grid (or back of the treadmill) within a 1-minute period. Following the exhaustion test, the mice performed an active cool-down for 3 min at a speed below 10 m/min before being returned to their home cages. The HIIT protocol was structured in two distinct phases: **Phase 1 (Weeks 1–2):** The animals trained for 30 min per session (5 sessions/week). Each session consisted of a 10-min warm-up at <50% of maximal running speed, followed by five 1-min high-intensity bouts (>75% of maximal running speed) interspersed with four 2-min low-intensity recovery intervals (<50% of maximal running speed). The session concluded with a 10-min cool-down. **Phase 2 (Weeks 2–8):** Training sessions began with a 3-min warm-up at <50% of maximal running speed. The interval training consisted of 1-min high-intensity bouts (85–90% of maximal running speed) interspersed with 1-min active recovery intervals at <50% of maximal running speed. To satisfy the progressive overload principle, the treadmill incline was increased to 15° during weeks 6–8, and the number of high-intensity intervals was progressively increased from 8 bouts in week 2 to 16 bouts by week 8 [13]. The detailed timeline and parameters of the training protocol are summarized in Table 2.

Table 2. High-intensity interval training (HIIT) protocol in experimental mice.

Week	Warm-Up/Cool-Down		Main Training		
	Speed (% of V_{max})	Duration (min)	High-Intensity Intervals (sets)	Running Intensity (% of V max)	Incline (°)
Week1	<50	10	5	>75	0
Week2	<50	10	5	>75	0
Week3	<50	3	8	>85	10
Week4	<50	3	10	>85	10
Week5	<50	3	12	>85	12
Week6	<50	3	14	>90	12
Week7	<50	3	16	>90	15
Week8	<50	3	16	>90	15

2.3. Supplementation protocol

Pomegranate supplementation was prepared from the edible portions of the fruit (arils only) using a blender to extract the juice. The obtained juice was first filtered through paper filter, then centrifuged, and kept undisturbed until the clear supernatant separated. This transparent supernatant was considered pomegranate juice and was stored at −20∘C. Due to the sensitivity and limited shelf life of pomegranate juice, it was prepared every 3 days, and the extraction procedure was repeated for the subsequent 3-day period. A 5% pomegranate juice solution was prepared daily by mixing the juice with the animals' drinking water, which was then provided ad libitum to the experimental mice [14].

2.4. Sample Collection and RNA Extraction

Forty-eight hours after the final training session, the mice were anesthetized using a combination of ketamine (55 mg/kg) and xylazine (15 mg/kg). The colon tissue was then carefully excised and stored at −80∘C for subsequent analyses. To evaluate the gene expression of Caspase-3, total RNA was extracted using the FavorPrep™ Tissue Total RNA Mini Kit (Favorgen, Taiwan; Cat. No. FATRK001). For the intestinal tissue, a minimum of 5 mg of fresh tissue or 10 mg of frozen tissue was utilized as the starting material, yielding at least 2 µg of total RNA with an optical density (OD) 260/280 ratio ranging from 1.8 to 2.0. The average extraction yield for the colon tissue within this weight range was 15±5µg of RNA per 20 mg of tissue.The relative expression level of the target gene was analyzed via Real-Time PCR using the AMPLIQON RealQ Plus 2x Master Mix Green (Denmark; Cat. No. A325402). All primers were designed using Primer3 software and verified using the NCBI BLAST tool. TATA-binding protein (TBP) was used as the reference gene to normalize the raw Real-Time PCR data (Table 3).

Table 3. Forward and reverse primer sequences of the target genes utilized for Real-Time PCR analysis.

Genes	Primer Sequences	Sizes (bp)
TBP	*Forward:5'-* GAATTGTACCGCAGCTTCAAAAT *-3'*	*161*
	Reverse: 5'- *AATCAACGCAGTTGTCCGTG -3'*	
Caspase3	Forward: 5'- GCTTGGAACGGTACGCTAAG -3'	*112*
	Reverse: 5'- CCACTGACTTGCTCCCATGT -3'	

2.5. Statistical analysis

Statistical analyses were performed using SPSS software (version 21). After confirming the normal distribution of the data using the Shapiro-Wilk test, a one-way analysis of variance (ANOVA) was conducted to evaluate differences between the groups. To determine the exact location of pairwise differences between groups, the Bonferroni post-hoc test was applied. The level of significance for all statistical analyses was set at P≤0.05.

3. Results

Table 4 presents the descriptive data for the study variables in the groups under investigation.

Table 4. Descriptive statistics of the study variables

Variable	Group	Mean±SD	Mini	Max	Number
Oxidative Stress *(Relative Expression)*	HC	64.93±11.68	36.96	99.83	6
	DC	112.11±4.90	101.2	127.13	6
	DS	86.48±9.69	56.96	108.42	6
	DE	83.74±4.33	76.34	97.44	6
	DSE	74.53±6.78	53.42	88.42	6
Caspase-3 *(Relative Expression)*	HC	1±0.75	0.25	2.08	6
	DC	0.36±0.09	0.27	0.51	6
	DS	0.48±0.16	0.32	0.72	6
	DE	0.86±0.42	0.44	1.48	6
	DSE	1.20±0.53	0.67	1.95	6

The results of the one-way analysis of variance showed a significant difference in the relative expression of oxidative stress among the studied groups (P = 0.005). Furthermore, the Bonferroni post hoc test revealed that oxidative stress was significantly increased in the DC group compared with the HC group (P = 0.003). In contrast, a significant decrease was observed in the DSE group compared with the DC group (P = 0.02) (Figure 1).

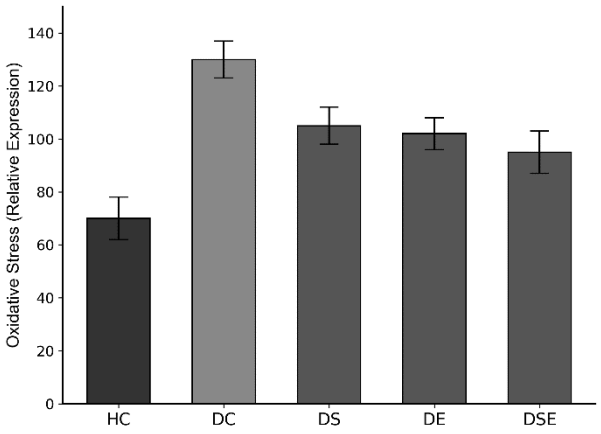

Figure 1. Oxidative stress levels across the experimental groups. Significant decrease in oxidative stress in the Disease + Supplement + Exercise (DSE) group compared to the Disease Control (DC) group (P = 0.02). #Significant increase in oxidative stress levels in the Disease Control (DC) group compared to the Healthy Control (HC) group (P = 0.003).

In addition, regarding caspase-3, the results of the one-wayANOVA showed a significant difference among the studied groups (P = 0.02). Post hoc Bonferroni comparisons indicated that caspase-3 expression was significantly increased in the DSE group compared with the DC group (P = 0.04). Furthermore, the relative expression of caspase-3 in the DC group was significantly lower than that in the HC group (P = 0.02) (Figure 2).

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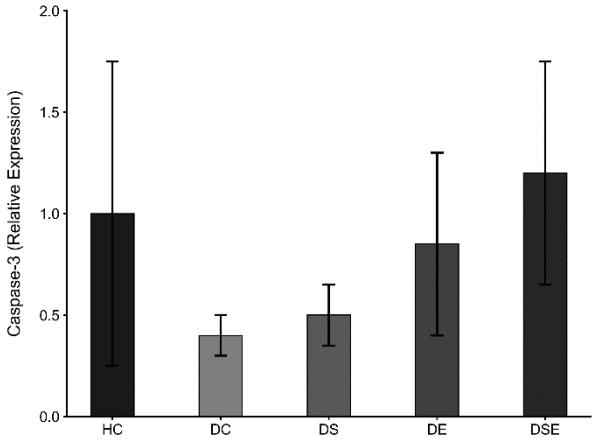

Figure 2. Caspase-3 gene expression levels across the experimental groups. Significant increase in the DSE group compared with the DC group (P = 0.04).

4. Discussion

The present study was conducted to investigate the effects of eight weeks of high-intensity interval training (HIIT) combined with pomegranate juice supplementation on oxidative stress and caspase-3 levels in mice with colon cancer. The findings demonstrated that oxidative stress in the 'Disease - exercise-supplement' group was significantly lower than that in the 'Disease-control' group. Additionally, the level of oxidative stress in the 'Disease-control' group was significantly higher compared to the 'healthy-control' group. In explaining this finding, it can be argued that oxidative stress, a state arising from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system, is increasingly recognized as a critical factor in the pathogenesis of colon cancer [15]. Although ROS are natural byproducts of cellular metabolism, their elevated levels can lead to damage to vital macromolecules, including DNA, lipids, and proteins. If not effectively repaired, this damage can result in genetic mutations, impaired cellular signaling, and ultimately, malignant transformations in colon cells [16]. Given that the findings of the present study showed that oxidative stress levels were significantly higher in the Disease-control group than in the healthy-control group, it can be suggested that colon cancer cells often exhibit higher levels of ROS and oxidative damage compared with healthy cells. This increase may be attributed to mitochondrial dysfunction, chronic inflammation within the tumor microenvironment, and/or mutations in genes involved in the regulation of redox homeostasis. In this regard, recent studies have demonstrated that even subtle changes in ROS levels can affect signaling pathways associated with cell growth, survival, and metastasis[17]. Moreover, the results of the present study indicated that oxidative stress was significantly lower in the Disease -exercise + supplementation group compared with the Disease control group. In this regard, the synergistic effects of exercise and pomegranate juice supplementation can be considered as a combined strategy for attenuating oxidative stress. The present findings are consistent with those of Souda et al. (2021), who reported that pomegranate extract in animal models of colon cancer markedly reduced ROS levels and inflammation, thereby inhibiting tumor growth [18]. In this context, elevated oxidative stress has been proposed as an additional biomarker for the detection of colorectal cancer and other toxicity-related manifestations [12]. Furthermore, glutathione (GSH) concentration, as a molecular marker in colorectal cancer, varies significantly depending on the type of treatment and the site of metastasis[19]. The increase in malondialdehyde (MDA) levels as a marker of oxidative stress, and the decrease in glutathione (GSH) levels as a natural antioxidant marker in the body during colorectal cancer, can be directly associated with cellular damage, membrane channel dysfunctions, and lipid peroxidation. Due to its rich phenolic and flavonoid content, pomegranate extract can exhibit significant antigenotoxic effects when appropriately administered, protecting DNA which determines the course of cancer. It has been noted that evaluating these effects with broader parameters in an appropriate animal model is essential.[18]. In the present study, the findings showed that pomegranate extract administration alone could not exert a potential significant improvement in reducing colorectal cancer-induced oxidative stress, which diverges from the findings of previous studies. Potential reasons for this discrepancy include differences in the intervention duration, administered doses of pomegranate extract, disease severity, and the type of cancer. Despite previous research findings in this field, it is plausible that the dosage of pomegranate extract used for treatment in the current study was insufficient to independently exert its suppressive effect on apoptosis and oxidative stress. However, the findings of the present study demonstrated that when this same dose of pomegranate extract is combined with HIIT, it can cause a significant reduction in oxidative stress compared to the untreated cancer group. This result suggests that the combination of HIIT with pomegranate extract consumption likely leads to a reduction in oxidative stress and consequently protects cellular integrity by regulating the expression

of TRP channels in the cell membrane. The transient receptor potential melastatin 2 ($TRPM_2$) channel is modulated by oxidative stress and plays a key role in stress-induced cell death responses[20]. TRP channels directly regulate the influx of cations, such as Ca^{2+}, Mg^{2+}, and Na^{+}, into living cells. Among these, TRPM channels are considered crucial therapeutic targets in the management of pro-inflammatory diseases and cancers[21]. The $TRPM_2$ channel, which is modulated by organic, inorganic, or physical stimuli, such as temperature variations and voltage fluctuations, is highly sensitive to oxidative stress. It has been documented that $TRPM_2$ expression triggers cell death responses during oxidative stress. $TRPM_2$ functions as a host defense responder against infections, and blocking these channels may represent a primary therapeutic strategy to mitigate reactive oxygen species (ROS) burden and oxidative stress in various pathologies where oxidative stress is central to pathogenesis [22]. Indeed, $TRPM_2$ activation can be induced by H_2O_2, which is a major source of ROS, mimicking the microenvironment observed in cancer. It has been suggested that corilagin, a tannin found in pomegranate, may act as a TRP channel antagonist against capsaicin stimulation[23]. In the current study, although the pomegranate extract (rich in phenolic and flavonoid compounds) combined with HIIT led to a non-significant reduction in oxidative stress compared to the cancer group, the authors hypothesize that this intervention may have also exerted a direct suppressive effect on $TRPM_2$ expression. Improving the regulation of this channel could, in turn, attenuate inflammatory mechanisms and upregulate key apoptotic markers, including caspase-3 [21]. Consequently, it is plausible that the significant upregulation of caspase-3 observed in the 'supplement + exercise' group is biochemically linked to the improved modulation of $TRPM_2$ channels. Nevertheless, this remains a hypothesis and is acknowledged as one of the limitations of the present study. Consistent with these mechanisms, Darband et al. (2020) investigated the modulating effects of exercise on inflammatory biomarkers and apoptosis in colorectal cancer rat models induced by 1,2-dimethylhydrazine (DMH). Their findings revealed that exercise training significantly increased the expression levels of activated caspase-3 and promoted apoptosis compared to the DMH-induced control group. Ultimately, they concluded that exercise training can effectively attenuate DMH-induced elevation of inflammatory markers, and that exercise-induced apoptosis occurs downstream of this modulated inflammatory response[24]. Therefore, exercise may act as an anti-inflammatory modulator to exert protective effects against colon cancer[23]. In this context, the findings of the present study revealed a significant increase in caspase-3 expression following HIIT combined with pomegranate extract supplementation compared to the cancer group; this result is consistent with the findings of Ghazizadeh Darband et al. (2020). In other words, the upregulation of caspase-3 promotes apoptosis in malignant cells, thereby enhancing cancer cell death and reducing the resulting inflammation. A body of recent studies has reported an inverse relationship between physical activity and the risk of colorectal cancer, proposing various underlying mechanisms such as the suppression of inflammatory responses and the induction of apoptosis. However, limited data exist regarding the precise benefits of exercise on inflammation-induced apoptosis in the context of colorectal cancer[25]. Consequently, we investigated the impact of physical activity on downstream apoptotic markers in a murine model of colorectal cancer. Overall, the combination of exercise and pomegranate juice supplementation led to a significant increase in the expression levels of key apoptosis-related markers, specifically caspase-3, in mice with colorectal cancer. Furthermore, exercise training ameliorated cancer-induced structural damage in the colon. Murine models of colorectal cancer represent one of the most effective tools for evaluating the effects of physical activity on tumorigenesis, as they facilitate the control of exercise modality, intensity, and duration, as well as the precise assessment of specific disease stages. Such models provide greater feasibility for examining physiological responses to exercise. Previous research has consistently reported the beneficial preventive effects of physical activity in murine models of colorectal cancer [26]. Collectively, these findings from our study demonstrate that exercise training exerts beneficial effects in colorectal cancer models. We evaluated the effects of exercise on oxidative and apoptotic markers in colon tissue. Given the downregulation of the apoptotic marker (caspase-3) and the elevation of oxidative stress in the colorectal cancer model, it is widely suggested that oxidative stress plays a pivotal role in multiple aspects of oncogenesis, particularly by suppressing apoptosis in cancer cells. The present study demonstrates that the combination of training and pomegranate juice supplementation can reverse this trend; specifically, it reduces oxidative stress and upregulates caspase-3 expression, thereby promoting cancer cell apoptosis in this pathology. Furthermore, a growing body of recent evidence indicates that the interplay and integration of inflammatory mechanisms, invasion, angiogenesis, and metastasis—along with their respective biomarkers, including pro-inflammatory

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cytokines such as TNF-α\alphaα—can drastically suppress cancer cell apoptosis and its biochemical indicators, including caspase-3. This process also indirectly affects COX-2, the rate-limiting enzyme for prostaglandin synthesis. Notably, COX-2 specifically drives cellular proliferation in colorectal cancer[27]. In other words, in this disease, COX-2 becomes dysfunctional and aberrantly upregulated, which, in conjunction with elevated inflammation, leads to uncontrolled cell proliferation while downregulating apoptotic executioners like caspase-3 [24]. Consistent with our findings, Ao et al. (2013) demonstrated that regular exercise increases caspase-3, caspase-8, and subsequent apoptosis in the colonic mucosal cells of wild-type mice[28]. Similarly, in the current study, a severe downregulation of caspase-3 expression was observed in the cancer-control group. However, the combined intervention of training and pomegranate juice supplementation reversed this pathological trend, resulting in a significant upregulation of caspase-3 expression and a subsequent improvement in apoptosis. These findings suggest that the upregulation of caspase-3 may have potentially led to a concomitant reduction in inflammatory mediators and cellular proliferation markers, such as COX-2. Nevertheless, since these specific factors were not measured in the present study, this remains a limitation of our research.

5. Conclusion

The present study demonstrated that a combined intervention consisting of eight weeks of HIIT together with pomegranate juice supplementation significantly reduced oxidative stress in mice with colon cancer, while simultaneously increasing the level of caspase-3, a key protein involved in the apoptotic pathway. These findings suggest that the combination of HIIT and pomegranate juice may serve as an effective strategy for reducing oxidative stress and inducing cell death in colon cancer cells. This synergistic approach, which appears to act through enhancement of the body's antioxidant defense system and activation of the apoptotic pathway, may be considered a potential adjuvant therapy alongside conventional cancer treatments.

6. Highlights

Future studies are recommended to investigate the long-term effects of the combined intervention of HIIT and pomegranate juice on colorectal cancer progression and patients' quality of life through human clinical trials. Furthermore, exploring the underlying molecular mechanisms and the role of other natural compounds in conjunction with exercise protocols of varying intensities is warranted to facilitate the development of more effective and personalized therapeutic strategies.

Statements

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Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

Authors' Contributions

Homa Sheikhani Shahin: Study design, supervision, data analysis, and corresponding author .

Nazila Dejbakhsh: Study design, data collection, and initial drafting .

Zahra Mosalinejad: Data collection, analysis, and manuscript editing .

Leila Malaei: Literature review, interpretation, and scientific revision.

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