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Effects of Short-Term Ziziphus jujuba Supplementation on Neutrophil Apoptotic Markers Following Circuit Resistance Exercise

Seyed Morteza Tayebi *
Associate Professor, Department of Exercise Physiology, Faculty of Sport Sciences, Allameh Tabataba'i University, Tehran, Iran.

Mitra Khademosharie
Department of Sport Sciences, Faculty of Humanities, Kosar University of Bojnord, Bojnord, Iran.

Hajar Nasiri
Department of Exercise Physiology, Faculty of Humanities, Kerman Branch, Islamic Azad University, Kerman, Iran.

Fatemeh Chireh
Department of Exercise Physiology, Faculty of Humanities, Kerman Branch, Islamic Azad University, Kerman, Iran.

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* Corresponding Author: tayebism@gmail.com.
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Abstract

Purpose: Intense exercise, such as circuit resistance training, induces physiological stress and inflammation, potentially accelerating neutrophil apoptosis. This study investigated whether seven days of jujube supplementation could modify key apoptotic proteins in neutrophils following strenuous exercise.

Method: Fourteen healthy young men (mean age ~25 years) were randomly assigned to either a jujube supplementation group (0.5 g/kg/day) or a placebo group for seven days. Participants had not engaged in regular exercise during the two months preceding the study. After the supplementation period, all participants completed a circuit resistance protocol consisting of three rounds of nine exercises performed at 75% of one-repetition maximum. Blood samples were collected before exercise, immediately after, and two hours into recovery. Neutrophils were isolated, and the expression levels of Bcl-2, Mcl-1, Bid, and Caspase-9 proteins were measured by ELISA. ($p = 0.005$).

Results: Significant time × group interactions were observed for Bcl-2 ($p = 0.004$) and Bid. In the jujube group, Bcl-2 levels increased, and Bid levels decreased during recovery compared with placebo. No significant changes were found for Mcl-1 ($p = 0.112$). Caspase-9 also showed no statistically significant differences ($p = 0.091$).

Conclusion: Short-term jujube supplementation was associated with changes in Bcl-2 and Bid during recovery following circuit resistance exercise. However, these findings reflect changes in selected apoptosis-related proteins and do not establish an improvement in overall immune function or recovery.

Keywords: Ziziphus jujuba, circuit resistance exercise, neutrophil, apoptosis, oxidative stress.

Introduction

The most common type of leukocyte is the neutrophil, which works as the main line of defense for the body as it is the first form of protection against infections and damage (Amulic et al., 2012). However, there are certain mechanisms put in place in order to avoid uncontrolled damage, one of these being the induction of apoptosis (Amulic et al., 2012). Performing high-intensity exercises such as resistance training puts a lot of physiological stress on the body through inflammation and oxidation processes, which reduce neutrophil survival (Tayebi et al., 2014; Tayebi et al., 2017) and efficiency (Peake et al., 2017; Powers et al., 2016; Suzuki, 2019). Recent research studies show how ROS produced during exercise have been found to affect neutrophil apoptosis by changing the Bax/Bcl-2 ratio, which regulates intrinsic apoptosis, thus affecting the process (Campbell & Turner, 2018; Luo & Loison, 2008). Bid and Caspase-9, which aid in apoptosis, and Bcl-2 and Mcl-1, which support cell survival, are important proteins in this response (Youle & Strasser, 2008). Therefore, changes in these proteins may provide information about apoptosis-related responses of neutrophils following strenuous exercise, although they do not by themselves establish changes in overall immune function or recovery (Walsh et al., 2011).

Previous human studies indicate that acute exercise can alter neutrophil number, activation, oxidative status, and apoptotic responses (Tayebi et al., 2016; Tayebi et al., 2020; Tayebi et al., 2021). However, the magnitude and direction of these responses depend on exercise intensity, duration, and training status. Studies of strenuous exercise have reported alterations in neutrophil apoptosis and redox status, whereas repeated or moderate exercise may induce different adaptive responses (Syu et al., 2011). In addition, exercise-related delays in neutrophil apoptosis have been linked to regulatory mechanisms such as granulocyte colony-stimulating factor (G-CSF), suggesting that the response of neutrophils to exercise is not uniform across exercise conditions (Mooren et al., 2012). More broadly, recovery of the immune system after exercise appears to depend on the intensity and

duration of the exercise stimulus and the physiological status of the individual (Peake et al., 2017). These findings highlight the importance of examining neutrophil responses specifically after strenuous resistance exercise, a context that has received less attention than aerobic exercise in human exercise-immunology research. However, previous human studies have generally focused on neutrophil counts, functional responses, apoptosis, or inflammatory and oxidative markers, while evidence regarding specific apoptosis-related proteins following a single session of circuit resistance exercise remains limited. Ziziphus jujube, also known as Jujube, has a variety of bioactive compounds, including polyphenols, flavonoids, and polysaccharides, which exhibit anti-inflammatory, antioxidant, and immunomodulatory properties (Gao et al., 2013). These bioactive compounds, especially the flavonoids and saponins, influence cellular stress responses through the stimulation of the process that ensures survival and inhibition of those that lead to cell death, which are marked by the overexpression of Bcl-2 and downregulation of Bax (Luo & Loison, 2008). Using neuronal tissue experiments, the effect of Jujube in reducing oxidative stress is seen indirectly through its influence in regulating cellular processes such as the strengthening of defensive responses of neurons and blocking of pro-apoptotic signals (Goli, 2016; Tepe et al., 2022). Although Jujube's effects on chronic conditions like diabetes and cardiovascular disease have been studied, little is known about how it might lessen exercise-induced neutrophil apoptosis, especially when resistance training is involved (Plastina et al., 2012).

Elevated ROS levels during intense exercise can activate Caspase-9 and decrease Bcl-2 expression, which may contribute to changes in neutrophil apoptotic signaling (Luo & Loison, 2008). Jujube's antioxidant qualities might mitigate these effects, potentially influencing apoptosis-related signaling (Nieman & Wentz, 2019). Short-term supplementation with Ziziphus jujuba has received little attention compared to the large body of research on chronic supplementation, especially when it comes to resistance exercise, as the

majority of studies have focused on aerobic exercise (Plastina et al., 2012).

The selection of Bcl-2, Mcl-1, Bid, and Caspase-9 was based on their complementary roles in the intrinsic and regulatory pathways of apoptosis. Bcl-2 and Mcl-1 are anti-apoptotic proteins that contribute to mitochondrial integrity and cell survival, whereas Bid is a pro-apoptotic member of the Bcl-2 family that can promote mitochondrial apoptotic signaling. Caspase-9 is an important downstream component of the intrinsic apoptotic pathway and is activated following mitochondrial cytochrome c release (Youle & Strasser, 2008). These markers were therefore selected to provide complementary information on both anti-apoptotic and pro-apoptotic signaling in neutrophils following the acute physiological stress imposed by circuit resistance exercise. Examining these proteins together also allows potential changes at different stages of the mitochondrial apoptotic pathway to be considered rather than relying on a single apoptosis-related marker. Because the present exercise protocol consisted of repeated resistance exercises performed at a relatively high intensity, these markers were considered relevant for evaluating whether short-term jujube supplementation was associated with changes in neutrophil apoptosis-related signaling during the post-exercise recovery period.

The purpose of this study is to examine the acute effects of 0.5 g/kg body weight Ziziphus jujuba supplementation on neutrophil apoptosis-related proteins (Bcl-2, Bid, Mcl-1, and Caspase-9) in healthy young men who had not engaged in regular exercise during the preceding two months. Compared to studies on aerobic exercise or chronic conditions, this research examines short-term supplementation in the context of resistance exercise and selected neutrophil apoptosis-related proteins (Plastina et al., 2012). Jujube's bioactive compounds may influence cellular responses to physiological stress over the course of a one-week supplementation regimen, which may improve antioxidant defenses and affect the balance of apoptotic proteins (Boots et al., 2008). The present study was therefore designed to examine whether short-term jujube

supplementation is associated with changes in selected apoptosis-related proteins following circuit resistance exercise.

Methods

Participants

The present study was approved by the Research Ethics Committee of Bojnord University under the code IR.UB.REC.1404.071 and was conducted in accordance with the policy statement of the Declaration of the Iranian Ministry of Health. To figure out how many people were needed, we ran numbers using GPower 3.1.9.7. Since the analysis focused on repeated measures across two groups, the settings included an alpha of 0.05. The effect size sat at 0.5, which is often called medium, using Cohen's d. Power aimed for 80%, meaning β stayed at 0.2. Twelve individuals turned up as the lowest count required. Six would go into each condition to catch real shifts in the main results. That number came straight from the output when the inputs were locked in. Written informed consent was obtained from 14 healthy young male students. Written informed consent was obtained from 14 healthy young male students. All subjects were asked to complete a medical examination and fill out a medical questionnaire to ensure that during the past month they had not taken any regular medication, smoked, consumed alcohol, or engaged in regular exercise during the previous two months, and were free of cardiovascular or metabolic diseases or recent symptoms of upper respiratory tract infection in the month prior to the start of these tests. Thus, although the participants were young and physically healthy, they were not considered regularly trained athletes at the time of the study. The volunteers were randomly assigned to 2 groups (n=7), including a Circuit Resistance Exercise group with placebo (age: 24.5±2.5 years, height: 171.17±1.7 cm, weight: 67.51±4.92 kg) and a Circuit Resistance Exercise group (n=7) with jujube solution (age: 25.25±1.31 years, height: 179.75±3.63 cm, weight: 74.04±5.78 kg). Although participants were randomly assigned to groups, baseline anthropometric differences were observed between

groups. Therefore, findings should be interpreted cautiously due to the potential influence of body size differences on physiological responses.

Eligibility Criteria

Participants were eligible for inclusion if they were healthy young men, were free of cardiovascular and metabolic diseases, had no symptoms of upper respiratory tract infection during the month preceding the study, had not taken regular medication during the preceding month, had not smoked or consumed alcohol, and had not engaged in regular exercise during the preceding two months. Participants were also required to complete the medical examination and health questionnaire and to provide written informed consent before participation.

Exclusion Criteria

Participants were excluded if they developed symptoms of an upper respiratory tract infection, used regular medication, smoked or consumed alcohol during the study period, engaged in additional regular physical exercise, or did not comply with the supplementation and study procedures. Participants were also excluded if they were unable to complete the circuit resistance exercise protocol or failed to provide the required blood samples.

Exercise Protocol and Blood Collection

Participants were taken to the weight room and weighed three times before the main trial. A strength test was performed at first and second visits to determine one repetition maximum (1-RM) of all participants for each of the 9-resistance exercises employed in the study. 1-RM value was determined by trial, by adding or removing weights after each attempt, as required. Subjects were allowed to take as much time as they felt necessary to recover from each attempt. Subjects completed a practice session to ensure that each participant was able to complete the entire exercise session on the third visit and to confirm that weight lifting was inducing fatigue by the end of the session. This was

confirmed by visual and verbal feedback from participants. Records of 1-RM are presented in Table 1.

Table 1. Exercise's 1-RM Records of participants

Number	Variables (kg)	groups	Mean ± SE
1	crunch	placebo	127.12 ± 8.66
		Jujube solution	129.49 ± 10.21
2	back extension	placebo	199.76 ± 24.46
		Jujube solution	270.77 ± 82.12
3	biceps curl	placebo	54.01 ± 5.38
		Jujube solution	62.55 ± 4.49
4	triceps press	placebo	57.39 ± 4.29
		Jujube solution	60.97 ± 4.67
5	knee extension	placebo	156.15 ± 13.07
		Jujube solution	173.69 ± 11.27
6	knee curl	placebo	100.43 ± 9.67
		Jujube solution	110.35 ± 18.12
7	standing calf raise	placebo	152.63 ± 15.20
		Jujube solution	166.08 ± 10.25
8	chest press	placebo	73.29 ± 3.38
		Jujube solution	77.56 ± 9.83
9	seated row	placebo	126.46 ± 7.73
		Jujube solution	149.15 ± 11.45

Jujube Preparation

The semi-dried fruits of Ziziphus Jujube were washed, and the seeds were separated, and the soft red parts were removed. The samples were dried at 50°C and ground to a powder using a mortar (Kim et al., 2012).

Combination Assessment of jujube extraction by Gas Chromatography-Mass Spectrometry (GC-MS). Compounds of jujube extraction were detected by GC-MS using a semi-quantitative method. The contents of jujube extraction compounds were quantified using an internal standard (3-octanol, 99%, Sigma-Aldrich). Wine volatile compounds were analyzed using an Agilent 5975 Mass Spectrometer coupled to an Agilent 7890A Gas Chromatograph (Agilent, Santa Clara, USA). A DB-WAX column (60 m×0.25 mm ID and 0.25 µm film thickness) was used for separation. The working parameters were as follows: injector temperature of 210°C, EI source of 230°C, MS Quad of 150°C, and transfer line of 210°C. The initial temperature was 30°C for 8 min, which was increased to 150°C at a rate of 3°C/min. Injector port temperature was 290 oC and helium was used as carrier gas at a flow rate of 1.5 ml/min. A total of 15 compounds were positively or tentatively identified by GC-MS, which contain 92.27% the area under the peak (Table 2). All Ziziphus jujuba samples were obtained from the same source and prepared under identical laboratory conditions to minimize batch-to-batch variability. However, quantitative analysis of total polyphenol and flavonoid contents was not performed, which represents a limitation of the present study.

Table 2. Combination Assessment of jujube extraction by Gas Chromatography-Mass Spectrometry (GC-MS).

Combination	The area under the peak (%)	Retention time (min)
furfural	51.33	20.21
4-Pyrone	9.51	17.07
Oleic acid	6.31	39.00
palmic acid	4.15	35.70
Imidazole	3.03	23.59
Cyclononasiloxane	2.03	42.05
Cyclodecasiloxane	1.75	35.63
Oxantin	1.61	10.65
Guanine	1.58	27.20
gamma.-Sitosterol	1.17	44.56
Niphimycin	1.16	26.89
Iron	1.10	45.65
Butanediol	1.07	27.00
Phthalic acid	1.02	45.48
Pentasiloxane	1.01	48.34
Dodecanoic acid	0.97	27.616
octadecamethyl	0.95	32.604
Methyl 2-furoate	0.92	14.28
1,4-dicarbonic acid	0.86	51.997
Tetradecanoic acid	0.74	31.840
Total	92.27	

Exercise Protocol

Recent studies have shown that appetite hormones such as ghrelin increase during starvation and before feeding (breakfast, lunch, and dinner), and this serves to increase absorption levels (Muller et al.).

Accordingly, participants were taken to the test location after a 12 h overnight fast. All participants performed a session of circuit resistance exercise in two cycles, simultaneously. Each cycle contained 9 exercises (crunch, back extension, biceps curl, triceps press, knee extension, knee curl, standing calf raise, chest press, seated row, machines were used in all exercises). The test included three non-stop circuits with a 3-minute active rest period between circuits. Each exercise was performed for 30 s (about 10-14 repeats) with one repeat maximum (1RM) of 75%. The exercise protocol is shown in Figure 1.

Supplement Protocol

The groups received oral jujube solution (0.5 g/kg body weight in 2.5cc distilled water) and placebo (2.5cc/kg of body weight in distilled water sweetened by sugar with no calories, and colored by food dye); this supplement was taken daily for one week in a double blind manner without any physical training during this period. To minimize inter-individual variation in nutrient intake, all participants followed a strictly standardized dietary protocol during the seven-day supplementation period. Participants were provided with a detailed, individualized meal plan based on their body weight: breakfast (10 kcal·kg−1 BW), lunch (10 kcal·kg−1 BW), and dinner (18 kcal·kg−1 BW), maintaining a consistent macronutrient distribution (70% carbohydrate, 15-18% protein, 12-15% fat). To ensure strict adherence, we implemented a three-layered monitoring system: 1) Daily Dietary Logs: Participants were required to maintain a daily log recording all food consumption and the timing of the jujube/placebo supplementation. 2) Mandatory Reporting: Research staff conducted daily check-ins (either in-person or via direct communication) to confirm adherence to the prescribed menu and supplement protocol. 3) Verification: Any deviation from the prescribed diet or missed supplement dose was immediately flagged and discussed. Participants who failed to demonstrate >95% adherence to the protocol were subject to exclusion criteria. This stringent protocol ensured that dietary intake

was controlled and consistent across both groups, mitigating the influence of nutritional variance on the measured apoptotic markers. Subjects in both groups arrived at the test location at 08:00 and rested for about 30 minutes. All subjects performed circuit resistance exercise in two cycles at 08:30, simultaneously. The first peripheral venous blood samples were taken on the 8th day and after 12 hours of overnight fasting at 08:30. The second set of blood samples was taken immediately after exercise at 09:00; then subjects remained seated for 120 minutes. The third set of blood samples was taken at 11:00. The research design and blood collection of the second supplementation protocol are shown in Figure 2.

Figure 1. Exercise protocol

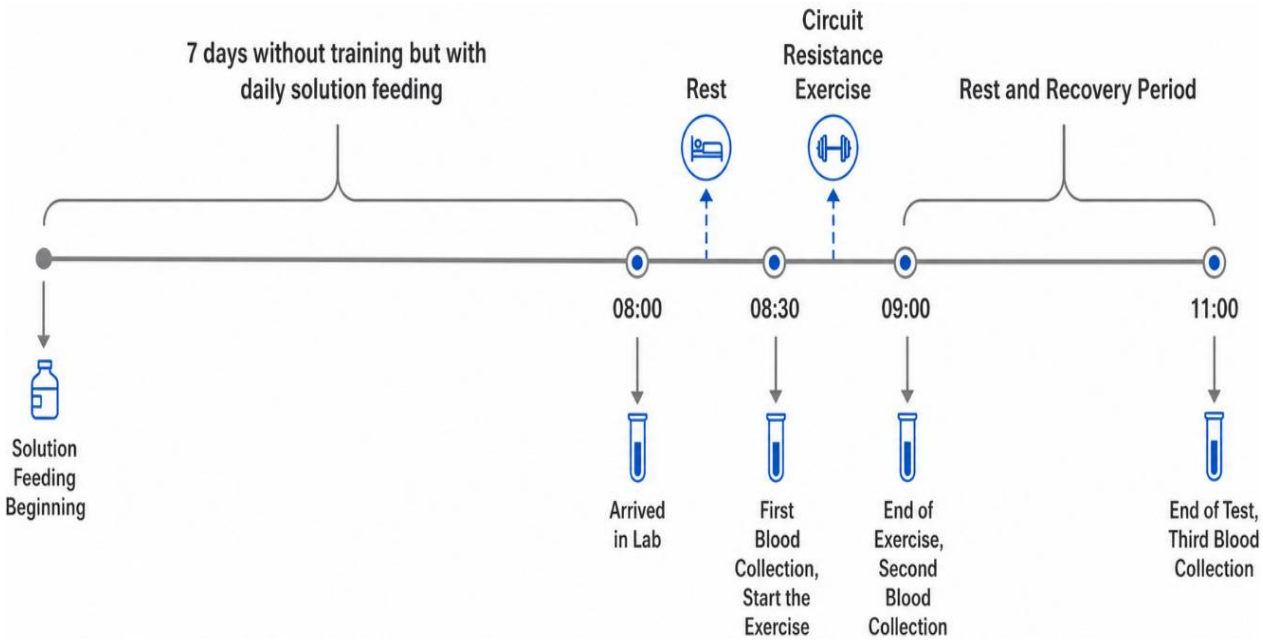


Figure 2. Research Design and Blood Collection of the Second Supplement Protocol

Reagents

Anticoagulant ACD [mixture of citric acid (Sigma Aldrich, Germany), sodium citrate (Sigma Aldrich, Germany), dextrose (Sigma Aldrich, Germany), and Deionized Sterile Distilled Water/dsdH_2O (Baharafshan, B.I.R.D, Iran)], Dextran 6% [combination of dextran with at least 100000 MW (grade B, BDH lab, GPR^{TM}, England), 0.9% NaCl (Sigma Aldrich, Germany), and dsdH_2O], 0.6 M KCl [mixture of KCl (Sigma Aldrich, Germany), and dsdH_2O], Phosphate buffered saline/PBS solution [combination of NaCl, KCl, Na_2HPO_4 (Sigma Aldrich, Germany), KH_2PO_4 (Sigma Aldrich, Germany), and dsdH_2O] with pH 7.4 and Autoclaved, Hank's Balanced Salt Solution/HBSS without Ca & Mg [NaCl, KCl, Na_2HPO_4, KH_2PO_4, $NaHCO_3$ (Sigma Aldrich, Germany), glucose (Sigma Aldrich, Germany), and dsdH2O]

with pH 7.4 and Autoclaved, Ficoll-Hypaque 10.77 (Baharafshan, B.I.R.D, Iran).

Neutrophil isolation. Neutrophils were purified from venous blood treated with ACD from healthy volunteers by 3 steps: Dextran sedimentation, Hypotonic lysis, and Ficoll sedimentation (BOYTUM, 1968). Briefly, a solution of 6% Dextran & 0.9% NaCl was added to the mixture of ACD & blood, and after adequate mixing, it stood down at room temperature until separation was complete. Then, the yellowish supernatant was separated and centrifuged using a low brake. After breaking the pellet by discarding the supernatant and resuspending in dsdH_2O at 20 s, it was mixed with 0.6M KCl, and the solution was diluted with PBS. Then, it was centrifuged using a high brake. The supernatant was discarded, and the pellet was resuspended in PBS. The cell suspension was layered over Ficoll-Hypaque and centrifuged using a low brake. Finally, the supernatant was discarded, and the neutrophil pellet was resuspended in HBSS. Trypan Blue Viability Test using a hemocytometer chamber was performed to assay cell viability, and it was ≥ 96%. Although neutrophil viability was confirmed, neutrophil purity was not directly quantified, which should be considered when interpreting protein expression results.

Neutrophil Proteins Assessments

Determination of caspase-9 (E20120710032, 16-1000 IU/L), Bcl-2 (E20120713065, 0.5-100 ng/ml), Mcl-1 (E20120713064, 0.1-40 ng/ml), and Bid (E20120713063, 0.01-30 ng/ml) is done by Human ELISA Kits (Glory Science Co., Ltd, China).

Statistical Analysis

Prior to analysis, normality of data distribution was assessed using the Shapiro–Wilk test. Homogeneity of variance was evaluated using Levene's test, and Mauchly's test was applied to assess the assumption of sphericity. Repeated measures (two-way) ANOVA was used to determine the effects of TIME × SOLUTION by SPSS software at a significance level of p =

0.05. The Bonferroni post-hoc test was used to examine differences between groups. All data were presented as means with the standard error of the mean.

Results

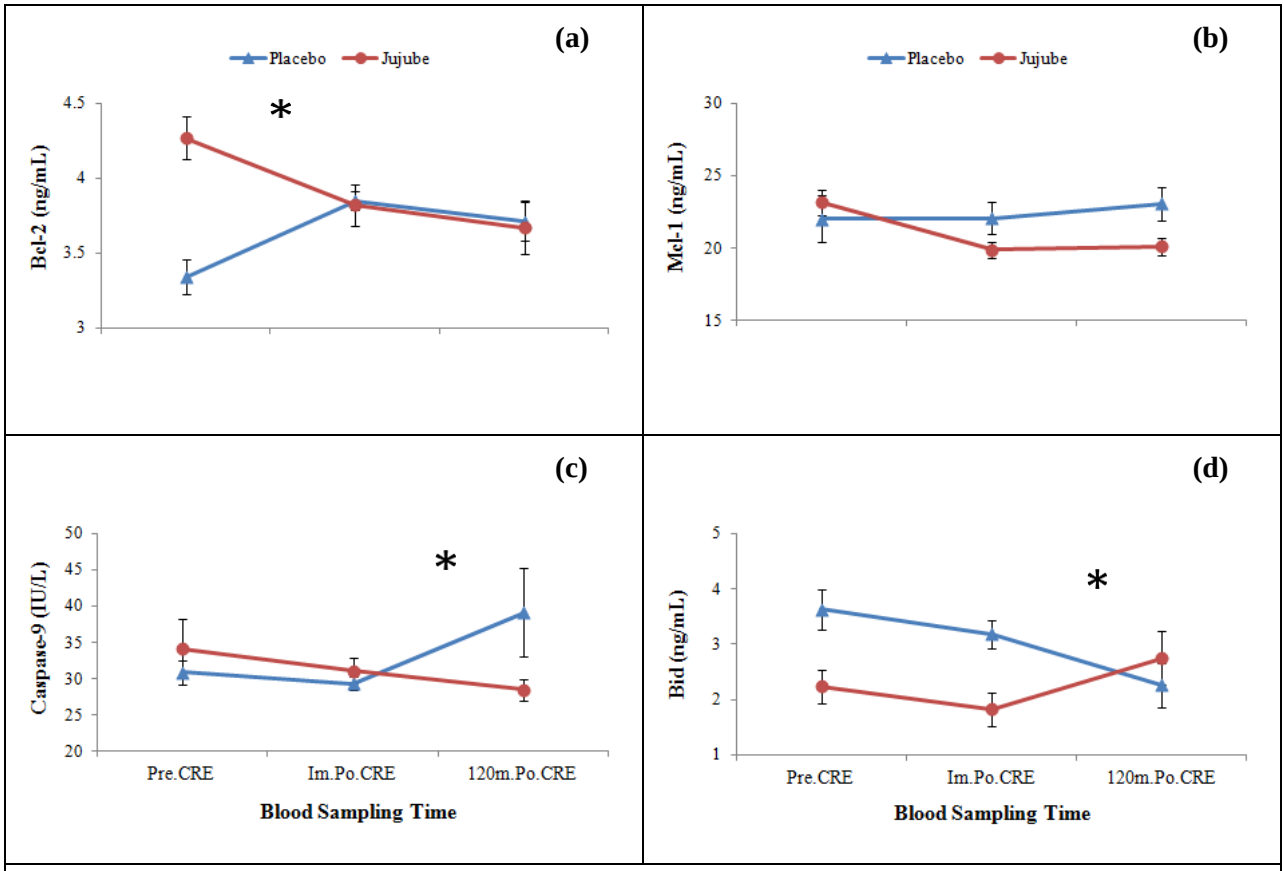
Bcl-2. Mauchly's test of sphericity was non-significant (W = 0.943, p = .726). The TIME × GROUP interaction was significant, $F_{(2, 24)}$ = 6.933, p = 0.004, partial η^2 = 0.366, 95% CI [0.081, 0.547], indicating a very large effect size (36.6% of variance explained). The interaction was linear, $F_{(1, 12)}$ = 10.954, p = 0.006, partial η^2 = 0.477, 95% CI [0.104, 0.678], indicating a very large effect size (47.7% of variance explained). [Figure 1a]. When Bcl-2 was increased in placebo group from Pre.CRE to Im.Po.CRE, it was decrased in Jujube group ($p<0.05$). The changes to recovery period was insignificant ($p>0.5$).

Mcl-1. Mauchly's test of sphericity was non-significant (W = 0.909, p = 0.59). The TIME × GROUP interaction was not significant, $F_{(2, 24)}$ = 2.401, p = 0.112, partial η^2 = 0.167, 95% CI [0.000, 0.364]. (medium-to-large effect size suggests that 16.7% of the variance in Mcl-1 levels is accounted for by the interaction); [Figure 1b].

Caspase-9. Mauchly's test of sphericity was non-significant (W = 0.771, p = 0.239). The TIME × GROUP interaction was not significant, $F_{(2, 24)}$ = 2.659, p = 0.091, partial η^2 = 0.181, 95% CI [0.000, 0.381], a medium-to-large effect size suggests that 18.1% of the variance in Caspase-9 levels is explained by the interaction. The linear component was also not significant, $F_{(1, 12)}$ = 3.054, p = 0.106, partial η^2 = 0.203, 95% CI [0.000, 0.456], indicating a medium effect size. [Figure 1c]. The changes of Caspase-9 from Pre.CRE to Im.Po.CRE was insignificant ($p>0.5$); but during recovery, when it was increased in placebo group , it was decrased in Jujube group ($p<0.05$).

Bid. Mauchly's test of sphericity was non-significant (W = 0.721, p = 0.166). The TIME × GROUP interaction was significant, $F_{(2, 24)}$ = 6.634,

p = 0.005, partial η^2 = 0.356, 95% CI [.075, .539], a large effect size indicates that 35.6% of the variance in Bid levels is explained by the interaction. The interaction was linear, $F_{(1, 12)}$ = 11.332, p = 0.006, partial η^2 = .486, 95% CI [.110, .684], reflecting a very large effect size (48.6% of variance explained). [Figure 1d]. The changes of Bid from Pre.CRE to Im.Po.CRE was insignificant (p>0.5); but during recovery, when it was decrased in placebo group, it was increased in Jujube group (p<0.05).



Graph 1. The Effect of One Week Supplementation with Ziziphus jujuba on some Pro- and Anti-Apoptotic Protein Levels of Human Neutrophils in Response to a Session of Circuit Resistance Exercise. Pre: previous. CRE: circuit resistance exercise. Im.po: Immediately Post. 120m: 120 minutes.

*: interaction effect of GROUP×TIME is significant at p<0.05.

Discussion

The present study investigated the effects of short-term Ziziphus jujuba supplementation on neutrophil apoptosis-related proteins following circuit resistance exercise. The principal findings were increased Bcl-2 levels and reduced Bid levels during recovery in the supplementation group compared with the placebo. However, no statistically significant changes were observed for Mcl-1 or Caspase-9.

The present findings should be considered in the context of previous research examining exercise-related changes in neutrophil apoptosis. Previous experimental work has demonstrated that acute strenuous exercise can modify neutrophil apoptotic responses and redox status. For example, Syu et al. reported that acute severe exercise increased oxidative stress and was associated with subsequent changes in neutrophil apoptotic responses, whereas regular moderate exercise produced a different adaptive pattern. These observations are relevant to the present study because they indicate that the direction and magnitude of neutrophil apoptotic responses may depend on exercise intensity, training status, and the physiological context in which the exercise is performed.] Similarly, Mooren et al. reported that both intensive resistance exercise and other forms of acute exercise could alter the timing of neutrophil apoptosis, suggesting that acute exercise does not necessarily produce a uniform pro-apoptotic response. In comparison with these studies, the present investigation did not directly quantify neutrophil apoptosis by flow cytometry or assess redox and inflammatory markers; instead, it examined selected apoptosis-related proteins. Therefore, the observed increases in Bcl-2 and decreases in Bid should be interpreted as changes in selected apoptotic signaling markers rather than direct evidence of altered neutrophil apoptotic rates.

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apoptotic signaling markers rather than direct evidence of altered neutrophil apoptotic rates.

The increased Bcl-2 activity in jujube consumption is congruent with the antioxidant and anti-inflammatory properties of the plant. Indeed, Bcl-2 serves as a mitochondria integrity facilitator that prevents cytochrome c release and thus avoids caspase stimulation, which is why the longevity of leukocytes can be attributed to the activity of Bcl-2 (Youle & Strasser, 2008). The observed increase in Bcl-2 may be consistent with modulation of cellular stress-related signaling; however, the mechanism responsible for this change cannot be established from the present data. (Nieman & Wentz, 2019). Quercetin demonstrates the same behavior pattern since under electrophilic stress induced by reactive molecules, Bcl-2 engagement increases in the immune effector cells (Boots et al., 2008). Taking Jujube daily for seven days may have influenced apoptosis-related signaling during the recovery period. Later on, lower Bid amounts suggest Jujube may have been associated with reduced pro-apoptotic signaling. [REVISED: This interpretation is consistent with the role of Bid as a pro-apoptotic member of the Bcl-2 family, although the present study cannot determine whether these protein changes resulted from altered oxidative stress or other upstream mechanisms (McFarlin et al., 2016). Because oxidative stress biomarkers and inflammatory cytokines were not directly measured in the present study, the proposed mechanisms regarding antioxidant-mediated modulation of apoptotic signaling remain speculative.

The insignificance of Mcl-1 (partial η^2=.167) and Caspase-9 (partial η^2=.181) requires further study. While Mcl-1 performs similarly to Bcl-2, Mcl-1 degrades quickly and is further enhanced when cells are stressed to degrade through ubiquitination (Youle & Strasser, 2008). Since changes are quickly realized regarding Mcl-1, recent studies show that results may only be seen with longer durations of supplementation (Perciavalle et al., 2012). Samples were taken right after CRE and then 120 minutes later; yet these times may be too soon to capture any changes since Mcl-1 changes quickly and thus may

account for these null results. Little change in Caspase-9 activity suggests that Jujube might be having an effect on the earlier steps in the intrinsic apoptotic pathway, which may involve the regulation of Bcl-2 or Bid. This trend is also observed with curcumin, where its effects on caspases can differ based on exercise and supplementation duration (McFarlin et al., 2016).

The present findings can also be considered alongside studies of other plant-derived compounds that have been investigated in relation to exercise, oxidative stress, and inflammatory responses. Quercetin has been reported to influence oxidative protection and apoptosis-related pathways (Boots et al., 2008), while curcumin supplementation has been associated with changes in inflammatory and muscle-damage biomarkers following exercise (McFarlin et al., 2016). Unlike these studies, however, the present investigation specifically examined selected apoptosis-related proteins in human neutrophils following circuit resistance exercise. Therefore, comparisons with quercetin or curcumin should be considered contextual rather than evidence that Jujube produces greater or equivalent effects. Seven days after the start of treatment with jujube extract, the Bcl-2 and Bid levels were altered during recovery. These findings provide preliminary evidence that short-term jujube supplementation may be associated with changes in selected neutrophil apoptosis-related proteins following resistance exercise.

Consumption of Jujube over one week means that the organism interacts with bioactive components contained in it for a much longer period than in shorter studies, where only direct effects are evaluated. Such interaction may allow cells to respond differently to physiological stress, and changes in Bcl-2 and Bid were observed in the present study. However, because broader immune function, oxidative stress, inflammatory cytokines, and functional recovery were not assessed, the present findings cannot establish that Jujube improves immune function or recovery after exercise.

However, some limitations exist despite these achievements. The number of subjects who underwent examination was very limited since

it included only fourteen participants. This limitation may affect the discovery of true alterations in Mcl-1 or Caspase-9 amounts, and more participants will have to be involved in future studies to prove it. In addition, the present study assessed only four apoptosis-related proteins and did not directly measure neutrophil apoptotic rate, oxidative stress biomarkers, inflammatory cytokines, or broader indicators of immune function or recovery. Therefore, the observed changes in Bcl-2 and Bid should not be interpreted as evidence of improved overall immune function or exercise recovery.

What is more, only healthy, young males participated in the experiment. Thus, there might be doubts about the generalization of these results to females and elderly people because their physiological reactions after intense exercise may differ significantly (Peake et al., 2017). In addition, long-term experiments will be required in order to determine how jujubes influence the organism of a person when used on a regular basis. It might provide more information about alterations, which are undetectable within a short time. Another direction for future research could be an analysis of various dosages and the comparison of Jujube with other antioxidants.

Some limitations need to be mentioned here. For one thing, the sample size was small, possibly reducing the power of statistics. For another, only young and healthy males participated in the study, thus confining the applicability of the results. In addition, no assessment was made as to the purity of neutrophils. Last but not least, no measurements of oxidative stress markers and inflammation-related cytokines were carried out.

Conclusion

After seven days of taking Ziziphus jujuba, changes were observed in Bcl-2 and Bid within human neutrophils following intense circuit resistance exercise, indicating an association between short-term jujube supplementation and selected apoptosis-related protein responses during recovery. Still, because no significant changes occurred in Mcl-1 or Caspase-9, the full effect on the measured apoptosis-related

signaling pathways remains unclear. These findings should be interpreted cautiously because only selected apoptosis-related proteins were assessed, while direct measures of neutrophil apoptosis, oxidative stress, inflammatory responses, immune function, and exercise recovery were not included. Looking ahead, further studies should explore these responses over longer supplementation periods, in larger and more diverse populations, and using broader measures of neutrophil apoptosis and immune-related responses.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

Mitra Khademosharie: Conceptualization, Data collection, Writing- Reviewing and Editing, Methodology, Hajar Nasiri: Conceptualization, Formal analysis, Writing- Original draft preparation and Editing, Methodology, Fatemeh Chireh: Writing- Reviewing and Editing, Visualization, Seyed Morteza Tayebi: Supervision, Writing- Reviewing and Editing, Data curation and Methodology.

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Informed consent

Informed consent has been obtained from all individuals included in this study.

Ethical approval

The research related to human use has complied with all the relevant national regulations and institutional policies, has followed the tenets of the Declaration of Helsinki, and has been approved by the Research Ethics Committee of Bojnord University under the code IR.UB.REC.1404.071.

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ORCID

Seyed Morteza Tayebi	**https://orcid.org/0000-0003-0459-4443**
Mitra Khademosharie	**https://orcid.org/0000-0003-4422-8093**
Hajar Nasiri	**https://orcid.org/0009-0000-1237-2328**
Fatemeh Chireh	**https://orcid.org/0009-0005-1292-6243**

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* Corresponding Author: tayebism@gmail.com.
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