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Effects of Capsaicin Supplementation on Eccentric Resistance Training-induced inflammation

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Research Article

Keywords: Capsaicin, Resistance Training, Inflammation, NF-kappa B, Rats, Wistar

Posted Date: November 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11344176/v1

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Additional Declarations: No competing interests reported.

Abstract

Inflammation is a protective response to injury and infection, yet uncontrolled inflammation contributes to chronic diseases. Eccentric resistance training induces muscle damage and acute inflammation. Capsaicin, a chili pepper-derived compound, exhibits anti-inflammatory properties. This study evaluated capsaicin supplementation on exercise-induced inflammation. Thirty-two male Wistar rats were randomly assigned to four groups (n = 8/group; final n = 5 after 30% attrition), control, eccentric resistance training (6 weeks), training + capsaicin (4 mg/kg/day oral), and capsaicin alone. Biceps muscle histopathology (H&E staining) assessed inflammation, degeneration, necrosis, and atrophy. Gene expression of IL-1β, TNF-α, p50, and NF-κB was quantified via real-time PCR. One-way ANOVA with $p < 0.05$ determined significance. The training group exhibited significantly increased histopathological inflammation (inflammatory cell infiltration, degeneration, necrosis, atrophy) and elevated IL-1β (3.06 ± 0.45-fold), p50 (2.21 ± 0.18), NF-κB (1.86 ± 0.25), and TNF-α (2.35 ± 0.35) compared to controls (1.00 ± 0, $p < 0.05$). Capsaicin supplementation significantly reduced these markers relative to control levels, training + capsaicin lowered IL-1β by 72% (0.27 ± 0.22), p50 by 64% (0.35 ± 0.19), NF-κB by 84% (0.15 ± 0.10), and TNF-α by 48% (0.52 ± 0.13) compared to the control group ($p < 0.05$). The capsaicin-only group showed the greatest reductions (IL-1β: 0.08 ± 0.007; p50: 0.14 ± 0.07; NF-κB: 0.09 ± 0.25; TNF-α: 0.27 ± 0.10). Histopathological findings corroborated these molecular results. Capsaicin supplementation attenuates eccentric resistance training-induced inflammation by downregulating the NF-κB/p50 pathway, suggesting potential as a complementary non-pharmacological intervention for exercise-related inflammation.

1. Introduction

Inflammation is a biological and immunological response to infection, injury, and toxic substances. While it serves as a protective mechanism that helps to limit damage or fight infection during acute episodes, it can also lead to sickness or tissue damage in various organs due to either acute or chronic responses (1). Inflammation plays a crucial role in restoring tissue balance. However, when it becomes uncontrolled, it can lead to chronic conditions (2, 3). Notably, inflammation is a primary cause of age-related disorders and a significant trigger for several illnesses, including infections, cancer, autoimmune diseases, and arteriosclerosis. Acute inflammation plays a crucial role in immune responses and is also important in metabolic disorders. It is vital for tissue regeneration and controlling pathogens, but it must be carefully regulated to minimize harm to the host (4, 5).

The inflammatory response is initiated by cell surface pattern recognition receptors that detect pathogenic stimuli. These receptors trigger inflammatory pathways, leading to the release of inflammatory markers and the attraction of inflammatory cells. This process activates intracellular signaling pathways, including the mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways (6, 7). A wide variety of genes involved in immune responses and inflammation are regulated by the NF-κB family of inducible transcription factors. This family consists of five members: NF-κB 1 (p50), NF-κB 2 (p52),

RelA (p65), RelB, and c-Rel. These factors control the transcription of target genes by binding to a specific DNA element known as the κB enhancer, either as homo- or heterodimers. NF-κB plays a crucial role in regulating inflammasomes and stimulates the expression of certain pro-inflammatory genes, including those that code for cytokines and chemokines (8–10). Pro-inflammatory and anti-inflammatory cytokines are released by the immune system to regulate immune responses. Cytokines are proteins that play a crucial role in influencing the survival, proliferation, differentiation, and function of immune cells, as well as other organ systems. Various types of cells, including neutrophils, activated macrophages, fibroblasts, endothelial cells, and damaged muscle cells, can secrete cytokines. In fact, muscles themselves can release cytokines in response to contractions of motor units (11, 12). Exercise has a significant impact on how the immune system functions, promoting an anti-inflammatory state that is vital for improving health, especially in individuals with chronic illnesses. Regular exercise can reduce the risk of chronic metabolic and cardiorespiratory disorders, as well as certain types of cancer (13).

The two main types of exercise—endurance training and resistance training—produce different yet complementary effects on the body. In recent years, there has been increasing research on how various forms of exercise, including resistance training and high-intensity interval training, influence the composition of immune system cells. This focus has overshadowed studies examining the effects of endurance training (14–16). During endurance training, interleukin 6 (IL-6) levels increase significantly. Muscle-secreted IL-6 has the effect of inhibiting the production of tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) by raising the levels of anti-inflammatory cytokines like IL-10 and IL-1 receptor antagonist (IL-1ra). Except in extreme cases, there is typically no significant increase in TNF-α following exercise. Both IL-1ra and IL-10, which are anti-inflammatory cytokines, increase in response to both resistance and endurance training. In human monocytes stimulated by lipopolysaccharide, IL-10 suppresses the production of IL-1β, TNF-α, and IL-8. Research suggests that chronic resistance training reduces inflammation, as evidenced by an increase in IL-10 following elbow flexor training (11, 17).

Capsaicin is a compound found in chili peppers that causes their burning sensation and irritant effects. It is a phenolic compound (8-methyl-N-vanillyl-6-nonenamide) that gives chili peppers their distinctive taste and pungency (18, 19). Capsaicin acts through its anti-inflammatory properties, contributing to its chemopreventive effects (20, 21). Research has shown a connection between exercise-induced inflammatory injuries and the potential role of capsaicin in reducing these effects (22). Capsaicin possesses anti-inflammatory properties that may help control inflammation caused by exercise. The following sections highlight key findings from relevant studies. Capsaicin activates the TRPV1 channel, which may enhance ATP production and improve vascular function, potentially reducing exercise-induced fatigue and inflammation (23). It has also been shown to inhibit pro-inflammatory cytokines such as TNF-alpha and IL-1beta, thereby reducing inflammatory responses in various models (24, 25). In a study involving exercised rats, capsaicinoids significantly reduced markers of oxidative stress and inflammation, indicating a protective effect against injuries caused by exercise (26). Additionally, the ability of capsaicin to release endogenous somatostatin has been linked to its neuroprotective and anti-inflammatory effects, particularly in ischemic conditions (27, 28). Exercise is well-known for its therapeutic effects on chronic pain, especially in cases of inflammation-induced hyperalgesia (29–31).

Combining exercise with capsaicin may provide an effective strategy for managing both inflammation and pain.

Exercise-induced inflammation requires precise modulation rather than complete suppression to facilitate adaptive muscle remodeling. The necessity of this study stems from a critical gap: although capsaicin exhibits general anti-inflammatory properties, no prior in vivo investigation has systematically examined its supplementation within a progressive eccentric resistance training model. Thus, this study aimed to evaluate the effects of capsaicin supplementation, eccentric resistance training, and their combination on inflammatory cytokine-mediated signaling pathways. Positive findings may support capsaicin and resistance training as non-pharmacological adjuncts or alternatives to conventional anti-inflammatory therapies. Preliminary evidence suggests capsaicin may enhance recovery and reduce soreness, indicating a complementary effect when combined with resistance training, though further research is warranted to fully elucidate this interaction.

2. Materials and Methods

2.1 Animals

In this study, we used 32 male Wistar rats, each weighing 200 ± 20 g and aged between 8 to 10 weeks, sourced from the Pasteur Institute of Iran. The experimental protocols received approval and were conducted in accordance with animal experimentation laws outlined in the "Guide for the Care and Use of Laboratory Animals, Eighth Edition, 2011". The rats were randomly divided into four groups and housed in a controlled environment with a 12-hour light/dark cycle. The groups included: a control group (N = 8), an eccentric resistance training group (N = 8), an eccentric resistance training with capsaicin supplementation group (N = 8), and a capsaicin supplementation group (N = 8). The G*Power program was used to determine the sample size for the study. The parameters included an effect size of 30%, a test power of 80%, and an alpha level of 0.05. We initially included 8 rats in each group. However, we experienced a 30% sample attrition rate during the study. Consequently, the final data analysis and experiments were conducted with 5 rats per group (20).

2.2 Evaluation of the effects of capsaicin supplementation and eccentric resistance training on inflammation.

To determine the rats' one-repetition maximum (1RM) and assess their ability to descend the training ladder, a weight equivalent to 50% of their body weight was attached to their tail. The 1RM refers to the maximum weight that can be lifted once in a specific exercise, such as the bench press or squat. It serves as a clear indication of an individual’s maximum strength capacity in that movement (32). If the rats succeeded, additional weight was added until they could no longer descend the ladder. The ladder was inclined at an angle between 80° and 85°, stood 110 cm tall, and included a chamber at the top. The ladder was constructed using a wire mesh instead of steps (33). Eccentric exercise, which involves lengthening muscles under tension, tends to cause more inflammation in skeletal muscle than other

types of exercise. This increased inflammation can be explained by several mechanisms. Eccentric exercise leads to significant mechanical disruption of muscle fibers, especially within the sarcomeres, the basic contractile units of muscle tissue. This damage triggers a series of inflammatory responses. The initial injury disrupts excitation-contraction coupling and calcium signaling, which further aggravates muscle damage through calcium-sensitive degradation pathways (34). Rats were gavaged with capsaicin (4 mg/kg body weight) for six weeks, and normal saline was added to bring the total volume to 200 µL of capsaicin (Barijessence, Tehran, Iran). In both the capsaicin and training-capsaicin groups, capsaicin was administered orally using a gavage method. The rats in these groups received daily doses of capsaicin five days a week throughout the study. This schedule ensured consistent exposure to capsaicin while allowing adequate recovery time between treatment sessions. The gavage technique was standardized to guarantee accurate and uniform dosing for all subjects.

In the training-capsaicin group, capsaicin was given several hours after the exercise session. This approach was taken to avoid potential adverse effects that could arise from administering it immediately after exercise, such as reflux or gastrointestinal discomfort. By timing the administration in this way, recovery was enhanced, and the risk of complications associated with combining physical exertion and capsaicin intake was minimized.

The six-week timeframe was chosen based on effective training periodization and physiological adaptation principles. A duration of six weeks is widely recognized as optimal for observing significant changes in muscle strength, inflammation, and other physiological responses to resistance training. This period allows for gradual increases in training intensity while providing the rats with sufficient time to recover and adapt to progressively increasing loads. Additionally, it strikes a balance between training intensity and recovery, which is essential for preventing overtraining and injuries. In summary, adopting a six-week training protocol is based on the necessity for proper adaptation to exercise and established frameworks in sports training methodologies (35).

The eccentric resistance training involved a training ladder with an 80–85% incline and 26 steps, which required the rats to control their descent on each step, as illustrated in the graphical abstract. The training involved a closed training ladder with a wire running between the two sides, designed to guide the rats' movements and help them maintain their balance. The steps of the ladder were spaced 2.5 cm apart to ensure a consistent and controlled descent. The weights were gradually increased to 50%, 75%, 90%, 100%, and 110% of the maximum load throughout the six-week training period. Since the 1RM for each rat was determined individually, the exercise intensity was tailored to each rat's 1RM. As a result, the maximum loads varied among the rats based on their individual strength capacities.

The weights were progressively increased to 50%, 75%, 90%, 100%, and 110% of each rat's maximum load. The 110% load refers to a weight that is 10% higher than a rat's individual 1RM. For example, if a rat's 1RM was 100 grams, the 110% load would be 110 grams. This progressive increase in load was implemented during the eccentric resistance training protocol as the rats adapted to the rising intensity. The resistance training method consisted of three sets, each comprising six trials. There was a one-

minute rest between each trial and a two-minute break between sets. If the rats were unable to complete the workout or remained motionless, an electric shock of 0.2–0.3 mA was applied to encourage them to move. If a rat received three consecutive shocks without any movement, the training was halted, and the rat did not move again (36). All four groups were housed under the same conditions during gavage administration and resistance training. The only difference between the groups was the type of intervention applied—exercise, capsaicin, or a combination of both. This controlled environment ensured that any observed effects were a result of the interventions rather than differences in housing or handling. All rats experienced the same training conditions and duration.

2.3 Isolation of biceps muscles and RNA

After the trial, rats were anesthetized using ketamine hydrochloride (60 mg/kg) and diazepam hydrochloride (4 mg/kg). Biceps muscle tissue samples were collected because the biceps brachii experience significant eccentric contractions during the lowering phase of resistance training, particularly when descending a ladder. This type of muscle contraction causes mechanical strain, leading to microdamage and inflammation. Samples were taken from both the left and right biceps to evaluate potential differences in damage or inflammation between the two limbs. Although both arms participate in the eccentric movement, they may endure varying forces. The tissue samples were extracted manually and then lysed using a TRIzol™ RNA extraction kit from Thermo Fisher Scientific (Waltham, MA, USA). They were utilized to investigate the effects of eccentric resistance training and capsaicin supplementation on inflammation by assessing inflammatory markers and cytokines, including IL-1β, TNF-α, p50, and NF-κβ, through real-time polymerase chain reaction (Real-Time PCR).

2.4 Histopathological assessment

The biceps muscles of the rats were excised after they were anesthetized to assess inflammation. The muscle tissue was then fixed in paraffin. Once the paraffin blocks were sectioned to a thickness of 6 µm, hematoxylin and eosin (H&E) stains were applied to detect inflammation. An Olympus light microscope (Olympus, Tokyo, Japan) was utilized to examine the stained sections and evaluate inflammation in muscle tissue following eccentric resistance training and capsaicin supplementation. After deparaffinizing and hydrating the tissue slices in ethanol, they were incubated in a solution of 10% acetic acid and 95% ethanol for 8 to 16 hours at 60°C. The slides were dehydrated in 70% ethanol for 20 to 30 seconds after rinsing with distilled water and 95% ethanol. Differentiation was stopped by washing the tissue with distilled water until it turned white. Neutral red staining was applied for dedifferentiation and counterstaining. After a quick rinse with 95% ethanol, the slides were mounted on glass coverslips, clarified, and dried. Light microscopy was performed in a blinded manner to evaluate the sections. The following criteria were used to identify inflammation: the presence of inflammatory cells, the presence of macrophages, the rate of degeneration, necrosis, and atrophy.

2.5 CDNA synthesis and Real-Time PCR

Total RNA, including mRNA, was isolated from muscle tissue using a TRIzol™ RNA extraction kit (Thermo Fisher Scientific), following the manufacturer's instructions. The quality and integrity of the RNA were

assessed using 1% agarose gel electrophoresis. To do this, a 1% agarose gel was prepared, and the RNA samples were separated at 100 V for 30 minutes. The gel was then visualized under UV light with a gel documentation system. Afterward, the RNA concentration and purity were determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). The absorbance at 260 nm was measured to determine RNA concentration, while the A260/A280 ratio was used to assess RNA purity. Total RNA isolated using TRIzol was converted into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), following the manufacturer's protocol. Specific primers for GAPDH, IL-1β, p50, NF-κB, and TNF-α were designed using Allele ID version 6 (Primer Design). The primer sequences are provided in Table 1. Real-time PCR amplification was conducted using the Qiagen Rotor-Gene Q Real-Time PCR System, with a total reaction volume of 10 µl for 40 cycles. The results were visualized using SYBR Green I dye (Applied Biosystems, ThermoFisher Scientific). Each reaction was performed in duplicate, and relative expression levels were calculated using the 2-ΔΔCT method. GAPDH was used as the internal control gene for normalization, and the relative expression values were presented as the final results.

Table 1
The primers sequences of the GAPDH, IL-1β, p50, NF-kβ, and TNF-α genes

Gene	Sequence (5'-3')	Product length (nt)	Accession number
IL-1β	**F**: GGATGATGACGACCTGCTA	192	NM_031512.2
	R: GTCGTTGCTTGTCTCTCCT		
TNF-α	**F**: ATGGGCTCCCTCTCATCAGT	106	NM_012675.3
	R: GCTTGGTGGTTTGCTACGAC		
NF-κβ	**F**: CGTGAGGCTGTTTGGTTTGA	89	NM_199267.2
	R: TCTGCCCTCCTGACTCTACT		
p50	**F**: CATCCACCTTCATGCTCAGC	144	NM_001276711.2
	R: TCCACCACATCTTCCTGCTT		
GAPDH	**F**: CAAGTTCAACGGCACAGTCA	102	NM_017008.4
	R: CCCCATTTGATGTTAGCGGG		

2.6 Statistical Analysis

The data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for comparisons. Statistical analyses were performed using GraphPad Prism version 9, with a significance level set at $p < 0.05$.

3. Results

3.1 Histopathological Findings

Histopathological examination of biceps muscle tissue using Hematoxylin and Eosin (H&E) staining was conducted to investigate the effects of capsaicin supplementation and eccentric resistance training on muscle inflammation. The presence of multinucleated inflammatory cells, macrophages, edema, degenerative changes, necrosis, and muscle atrophy were evaluated (Fig. 1). Our results showed the Training group showed higher presence of inflammatory cells compared to the control groups, indicating the resistance training induced inflammation in the rats. Moreover, we observed significant degenerative changes, necrosis, and muscle atrophy in the training groups compared to the control group (Fig. 1, B). The Training-Capsaicin group displayed lower levels of inflammatory cells, degenerative changes, edema, necrosis, and muscle atrophy compared to the Training group, while still exhibiting elevated levels compared to the control (Fig. 1, C). This group exhibited the lowest levels of all evaluated parameters, including inflammatory cells. Notably, no degenerative changes, necrosis, or muscle atrophy were observed in the Capsaicin group (Fig. 1, D). The histopathological findings suggest that capsaicin supplementation may have a potential to attenuate some of the exercise-induced inflammatory effects on muscle tissue. Altogether, the Capsaicin group demonstrated reduced levels of inflammatory cells and a diminished incidence of severe muscle degeneration, necrosis, and atrophy compared to the Training group (Fig. 1, E).

3.2 Expression Levels of the Inflammation Markers

The expression levels of GAPDH, IL-1β, p50, NF-κB, and TNF-α were assessed using RT-PCR. The statistical analysis revealed that the levels of IL-1β increased approximately 3.06-fold in the training group compared to the control group ($P < 0.05$). Specifically, the mean ± SD for the training group was 3.06 ± 0.45, while for the control group it was 1 ± 0 (Fig. 2). In comparison, the levels of IL-1β decreased by 91% in the capsaicin group and by approximately 72% in the training-capsaicin group relative to control levels. In contrast, the training group saw an increase in IL-1β levels by 206% ($P < 0.05$). As shown in Fig. 2, the mean ± SD of IL-1β in the training-capsaicin group was 0.27 ± 0.22, while in the capsaicin group it was 0.08 ± 0.007. The genes p50, NF-κB, and TNF-α showed expression patterns similar to that of IL-1β. In the training group, the levels of p50, NF-κB, and TNF-α increased by 2.2-fold, 1.8-fold, and 2.3-fold, respectively, compared to the control group ($P < 0.05$). The mean ± SD for p50 in the training group was 2.21 ± 0.18, for NF-κB it was 1.86 ± 0.25, and for TNF-α it was 2.35 ± 0.35. In contrast, the mean ± SD for the control group was 1 ± 0. In the training-capsaicin group, the p50 values decreased by approximately 64% relative to control, and in the capsaicin group, the reduction was about 85%. Meanwhile, in the training group, the p50 values had increased by 122% ($P < 0.05$). The mean ± SD for p50 in the training-capsaicin group was 0.352 ± 0.19, while in the capsaicin group, it was 0.14 ± 0.07.

In the study, NF-κB levels were significantly affected by different treatments. In the training-capsaicin group, NF-κB levels decreased by approximately 84% relative to control, while in the capsaicin-only group, they decreased by about 90%. In contrast, NF-κB levels increased by 86% in the training-only group ($P < 0.05$). The mean ± standard deviation (SD) of NF-κB levels was 0.15 ± 0.1 for the training-capsaicin

group and 0.09 ± 0.25 for the capsaicin group. Similarly, TNF-α levels showed a decrease of approximately 48% in the training-capsaicin group relative to control and a 72% decrease in the capsaicin group. However, in the training group, TNF-α levels increased by 135% ($P < 0.05$). The mean ± SD of TNF-α levels was 0.52 ± 0.13 for the training-capsaicin group and 0.27 ± 0.10 for the capsaicin group. Altogether, the data suggests that resistance exercise significantly upregulated the expression of pro-inflammatory cytokines (IL-1β, p50, NF-κB, and TNF-α). Capsaicin supplementation effectively attenuates the exercise-induced upregulation of these pro-inflammatory cytokines. However, the combination of capsaicin supplementation and resistance exercise did not completely abolish the exercise-induced increase in cytokine expression, but it did significantly reduce it compared to the exercise-only group. This suggests that capsaicin may have a mitigating effect on exercise-induced inflammation.

4. Discussion

Eccentric resistance training has shown to elicit a more pronounced inflammatory response and greater muscle injury compared to concentric contractions performed at the same intensity and duration (37). This heightened response is attributed to the nature of eccentric contractions, which involve muscle lengthening under tension. This mechanism results in increased mechanical strain and greater structural damage at the sarcomere level compared to concentric contractions, where the muscle shortens. Consequently, eccentric exercise is associated with a higher incidence of injury and a more robust inflammatory response, characterized by elevated levels of inflammatory markers such as creatine kinase and C-reactive protein (38–40). The existing literature on exercise-induced inflammation following eccentric resistance training reveals consistent patterns in inflammatory responses, muscle damage, and recovery mechanisms. Interestingly, Capsaicin has demonstrated anti-inflammatory properties in numerous studies, suggesting its potential therapeutic applications. For instance, capsaicin has been shown to reduce inflammation in sepsis by inhibiting PKM2 and LDHA, key enzymes involved in the Warburg effect and COX-2 expression (41–43). Furthermore, capsaicin reduces the activation of NF-κB, a crucial transcription factor responsible for the expression of pro-inflammatory cytokines, in models of H. pylori infection and intestinal inflammation (44). Additionally, capsaicin has been shown to influence macrophage activity by inhibiting the MAPK signaling pathway, a key pathway involved in the inflammatory response (45). Given these anti-inflammatory effects, this study aimed to investigate the impact of capsaicin supplementation on exercise-induced inflammation *in vivo*.

Eccentric exercise has been shown to significantly increase the levels of TNF and IL-6 mRNA in skeletal muscle, highlighting the tissue-specific nature of the inflammatory response (46, 47). Studies on both young and master resistance-trained athletes have demonstrated similar increases in creatine kinase and interleukin-6 levels after exercise, indicating comparable muscle damage and inflammatory responses (48). In the present study, we found that training group exhibited increased inflammatory cell infiltration, indicative of exercise-induced inflammation, along with significant degenerative changes, necrosis, and muscle atrophy. The training-capsaicin group displayed lower levels of inflammatory cells, edema, and degenerative changes compared to the training group, indicating that capsaicin might somehow attenuate the damages caused by exercise. Teixeira et al. found that eccentric exercise has

been associated with elevated levels of C-reactive protein and plasminogen activator inhibitor-1, both of which are linked to inflammation and reduced fibrinolysis (49). Systemic anti-inflammatory responses have also been observed, with notable increases in IL-10 and TNF-α after exercise, suggesting a balanced regulation of pro- and anti-inflammatory cytokines (50).

Notably, the capsaicin group exhibited the lowest levels of all evaluated parameters, including inflammatory cells, with no observable degenerative changes, necrosis, or muscle atrophy. These findings suggest that capsaicin supplementation may have a potential to attenuate some of the exercise-induced inflammatory effects on muscle tissue, as evidenced by the reduced levels of inflammatory cells and the diminished incidence of severe muscle degeneration and atrophy in the capsaicin group compared to the training group. A previous study found that capsaicin inhibited the production of pro-inflammatory mediators in macrophages stimulated by LPS by deactivating NF-κB. The study also examined whether capsaicin influences PPARγ activity, which regulates TNFα production. It was observed that capsaicin significantly suppressed TNFα production in a dose-dependent manner (51).

Our gene expression analysis supported the histopathological findings. The training group exhibited a significant upregulation of IL-1β, p50, NF-κB, and TNF-α expression compared to the control group, which showed the induction of inflammation following receiving training in the rats. While the combination of training and capsaicin supplementation did not fully suppress the exercise-induced increase in inflammatory marker expression, it significantly attenuated it compared to the exercise-only group. Notably, in rats receiving capsaicin supplementation, the upregulation of IL-1β, p50, NF-κB, and TNF-α induced by exercise was significantly attenuated. The significant reduction in inflammation markers expression observed in the capsaicin group suggests that capsaicin can exert an anti-inflammatory effect on the exercise-induced inflammation. This is supported by previous studies demonstrating the anti-inflammatory effects of capsaicin. Capsaicin has been shown to mitigate muscle wasting induced by agents such as dexamethasone and cisplatin (52, 53). Its potential mechanisms of action include the activation of TRPV1 receptors and the release of calcitonin gene-related peptide, both of which play a role in regulating muscle cell signaling and apoptosis (54). Li et al. investigated the effect of capsaicin on macrophage inflammatory factors in a lipopolysaccharide-induced inflammation model. They demonstrated capsaicin inhibits NF-κB and MAPK signaling pathways, leading to a decreased release of pro-inflammatory cytokines, including interleukins, TNF-α, and nitric oxide (NO), ultimately attenuating the inflammatory response triggered by lipopolysaccharide in macrophage (45).

This study makes three novel contributions. First, it provides the first in vivo demonstration that oral capsaicin supplementation attenuates eccentric resistance training-induced inflammation in a progressive overload model. Second, it identifies NF-κB/p50 downregulation as the primary mechanistic pathway. Third, it shows that capsaicin produces partial (not complete) suppression of inflammatory markers, a pattern that may preserve adaptive signaling while mitigating excessive tissue damage. In our assessment, these findings are clinically meaningful because capsaicin could serve as a nutritional adjunct for individuals at risk of excessive exercise-induced inflammation, including untrained

populations beginning eccentric training or athletes in heavy training phases. The partial reduction observed (72% decrease in IL-1β, 64% in p50, 84% in NF-κB, and 48% in TNF-α relative to control) suggests modulation rather than suppression, which is likely preferable for maintaining beneficial adaptation. Key strengths of this study include the randomized four-group design, progressive overload from 50% to 110% of 1RM, and concurrent histopathological and molecular outcome measures.

5. Limitations

This study has several limitations. First, reliance on RT-PCR for gene expression analysis may not reflect actual protein levels or functional activity, future studies should incorporate Western blot or ELISA validation. Second, histopathological assessment of muscle damage was limited, additional measures such as muscle force production, contractile function, and biochemical injury markers are warranted. Third, findings are specific to capsaicin in resistance exercise-induced inflammation and may not generalize to endurance training or different exercise intensities. Finally, the relatively small sample size per group may limit statistical power and generalizability.

6. Conclusion

The single most important outcome of this study is that capsaicin supplementation (4 mg/kg/day for six weeks) attenuates eccentric resistance training-induced muscle inflammation via downregulation of the NF-κB/p50 pathway and its downstream effectors IL-1β and TNF-α. Interpreting this finding at a higher level of abstraction, capsaicin acts as a modulator rather than a suppressor of exercise-induced inflammation, a distinction that is mechanistically important because partial preservation of inflammatory signaling may be required for adaptive muscle remodeling. We have successfully addressed the need stated in the Introduction; this is the first demonstration that a natural, safe compound can mitigate excessive eccentric training-induced inflammation in vivo while maintaining a level of inflammatory response potentially compatible with beneficial adaptation. These data provide proof-of-concept evidence supporting capsaicin as a potential nutritional adjunct for individuals undergoing eccentric resistance training protocols. Further investigations are warranted to elucidate the long-term effects on muscle function and to translate these findings to human populations.

Declarations

Ethics approval and consent to participate

The experimental protocols received approval from the Ethics Committee of the Sport Sciences Research Institute of Iran (ethics code: IR/SSRI.REC.2023.15209.2320). The study was conducted in full compliance with all ethical principles concerning the use of laboratory animals, following the ARRIVE guidelines. All methods were performed in accordance with the relevant guidelines and regulations.

Author Contribution

MD and AB designed the study. MD, EK, and AB wrote the original manuscript. MD, EK, and AB analyzed the data. NE supervised the writing, analyses, and revision of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

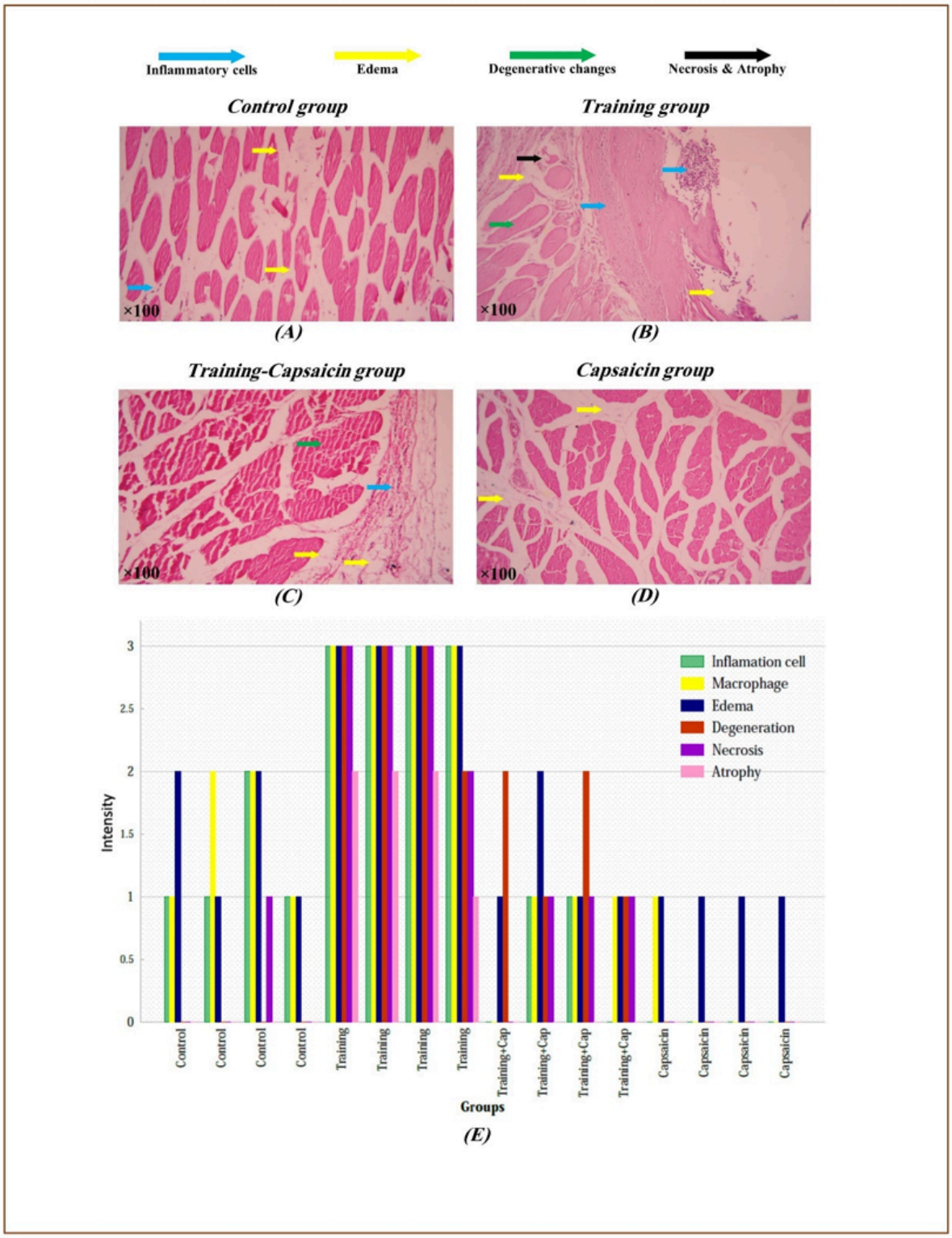


Figure 1

Hematoxylin and eosin staining was performed on tissue slices from various experimental groups. Panel A displays images from the control group, while Panel B presents images from the training group, which exhibited more pronounced edema, macrophage infiltration, and a higher presence of inflammatory cells compared to the control. Panel C depicts the training-capsaicin group, which showed a reduced number of inflammatory cells compared to the training group. Panel D depicts the capsaicin

supplementation group, which showed a reduced number of inflammatory cells, lower levels of necrosis, muscle atrophy, and degenerative changes compared to the training group and training-capsaicin group. Panel E depicts assessment of inflammation, edema, degeneration, necrosis, and atrophy in muscle sections from each group. Y-axis represents the mean score for each parameter. The intensity of each parameter was scored on a scale of 0 to 3 (0: Absent, 1: Mild, 2: Moderate, 3: Severe). The Capsaicin supplementation group showed a significant decrease in inflammation, lower levels of necrosis, muscle atrophy, and degenerative changes compared to the other groups. The analysis of pathological inflammation was conducted by evaluating inflammatory indices in each tissue slice. All images were captured at a magnification of ×100. Each group consisted of four tissue slices (n=4), and representative images are shown.

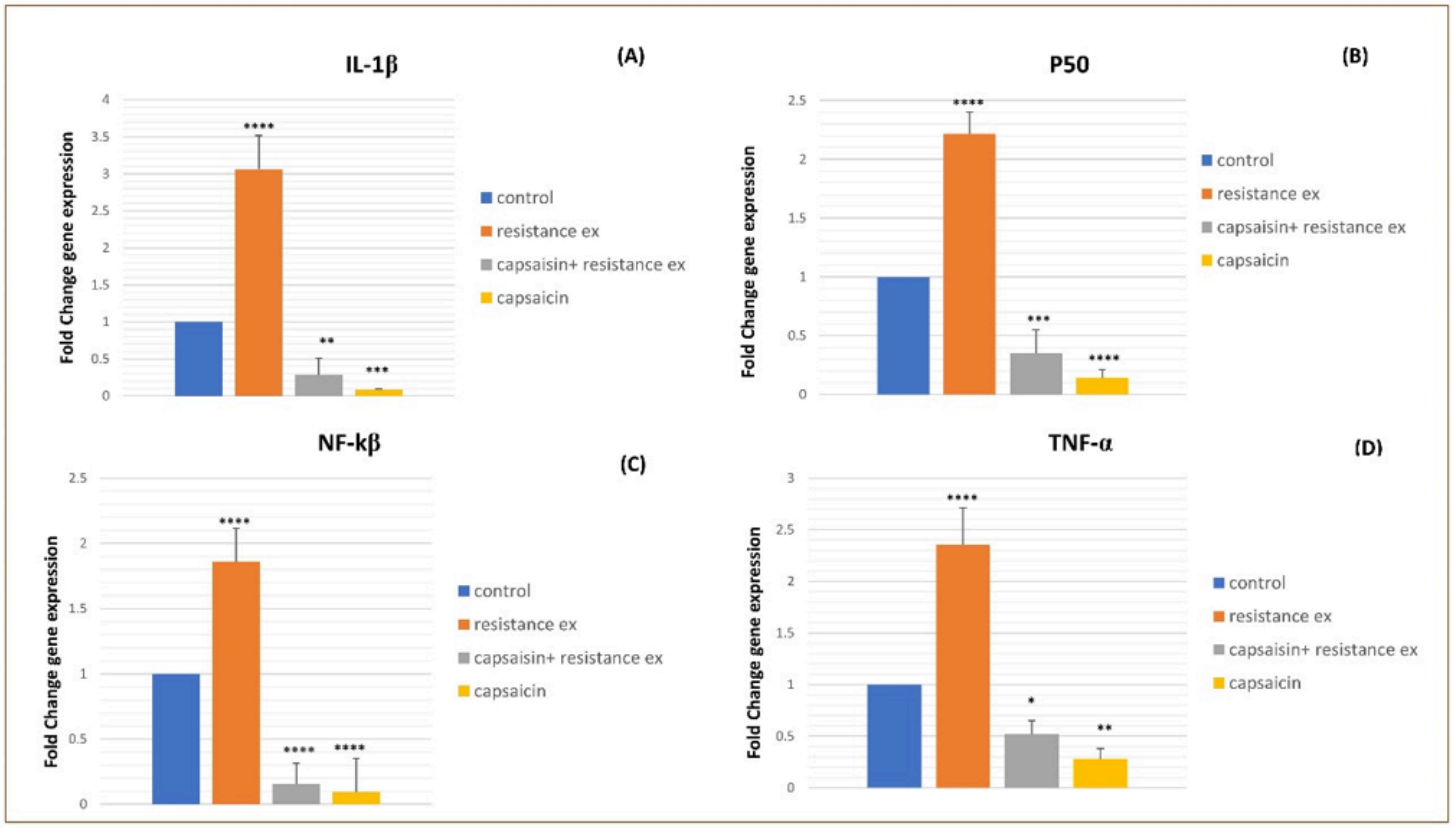


Figure 2

Comparison of IL-1β, p50, NF-κB, and TNF-α expression in muscle tissue among the training, capsaicin, and training + capsaicin groups, relative to the control group. Muscle tissue samples were collected from each group, and the expression levels of IL-1β, p50, NF-κB, and TNF-α were quantified using RT-PCR. Results from the One-Way ANOVA analysis showed that the expression of all four genes was significantly higher in the training group compared to the control (Panels A, B, C, and D). In contrast, the expression levels in the training + capsaicin and capsaicin groups were significantly lower than those in the training group (Panels A, B, C, and D). Each bar represents the mean ± SD, with *** indicating significance at the 0.0001 level.