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Effects of Aerobic Exercise and Carvacrol Supplementation on NRF2/NF-κB Signaling Pathway in an Ovalbumin-Induced Allergic Asthma Model in Rats

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ABSTRACT

Background: Allergic asthma is a chronic inflammatory airway disease characterized by excessive oxidative stress, persistent inflammation, and structural remodeling of lung tissue. Although pharmacological therapies remain central to asthma control, complementary non-pharmacological approaches are increasingly investigated to improve redox and inflammatory balance. The nuclear factor erythroid 2-related factor 2 (NRF2) and nuclear factor kappa B (NF-κB) signaling pathways play key roles in regulating antioxidant defense and inflammatory responses, respectively. This study aimed to evaluate the effects of aerobic exercise and carvacrol supplementation, alone and in combination, on oxidative stress markers, respiratory function, lung remodeling, and NRF2/NF-κB signaling in an ovalbumin-induced allergic asthma model in rats.

Methods: Male Wistar rats were randomly assigned to control, allergic asthma, asthma + aerobic exercise, asthma + carvacrol, and asthma + aerobic exercise + carvacrol groups (n = 8/group). Allergic asthma was induced by ovalbumin sensitization and challenge. Rats in the exercise groups performed moderate-intensity treadmill running for 4 weeks, while carvacrol was administered intraperitoneally at a dose of 73 mg/kg/day once daily. Lung tissue was analyzed for total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, hydroxyproline content, and protein expression of NRF2 and NF-κB. Respiratory rate was also assessed. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, and effect sizes were estimated using eta-squared and Cohen's d.

Results: Asthmatic rats showed significantly increased oxidative stress, elevated NF-κB expression, higher respiratory rate, and increased lung hydroxyproline content, along with reduced antioxidant enzyme activities and NRF2 expression compared with controls ($P < 0.001$). Aerobic exercise or carvacrol treatment significantly improved antioxidant status, reduced MDA levels, suppressed NF-κB expression, and attenuated lung remodeling ($P < 0.05$–0.01). The combined exercise and carvacrol intervention produced the greatest protective effects, markedly enhancing NRF2 expression while further inhibiting NF-κB signaling and improving respiratory and structural outcomes ($P < 0.001$ vs. asthma).

Conclusion: In this preclinical allergic asthma model, aerobic exercise combined with carvacrol supplementation showed greater protective effects than either intervention alone, probably by improving antioxidant defenses and attenuating NF-κB-mediated inflammatory signaling through modulation of the NRF2/NF-κB pathway. These findings support further investigation of combined lifestyle and nutritional interventions as complementary strategies for asthma management.

Keywords: Allergic asthma, Aerobic exercise, Carvacrol, NRF2, NF-κB.

Introduction

Allergic asthma is one of the most prevalent chronic inflammatory diseases of the respiratory system and is characterized by airway hyperresponsiveness, mucus overproduction, and reversible airflow obstruction (1,2). Increasing evidence indicates that oxidative stress plays a crucial role in the pathogenesis and progression of asthma by amplifying inflammatory responses and promoting airway remodeling (3). Current pharmacological treatment with inhaled corticosteroid (ICS)-containing regimens and

add-on therapies can improve asthma control; however, a subset of patients remain symptomatic, experience treatment burden, poor adherence, steroid-related adverse effects, or require specialized add-on treatment for severe disease. Accordingly, safe and accessible complementary strategies that target oxidative stress and inflammation may be useful as adjuncts to standard care (1,2).

An imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms leads to oxidative damage of lipids, proteins, and nucleic acids in lung tissue (4). The transcription factor NRF2 is the master regulator of cellular antioxidant responses and induces the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (5). In contrast, NF-κB is a key transcription factor that regulates the expression of pro-inflammatory cytokines and mediates chronic airway inflammation (6). Importantly, these pathways are not independent: NRF2 activation can counterbalance NF-κB-driven inflammation by limiting ROS-mediated inflammatory signaling, whereas persistent NF-κB activation may suppress antioxidant gene expression. This reciprocal crosstalk provides a mechanistic basis for interventions that simultaneously enhance NRF2 activity and inhibit NF-κB activation (4-6).

Lung remodeling, a hallmark of chronic asthma, involves extracellular matrix deposition and collagen accumulation, which can be quantified by measuring hydroxyproline content (7). Strategies aimed at enhancing antioxidant defenses and suppressing inflammatory signaling pathways may therefore represent effective complementary non-pharmacological approaches for asthma management.

Carvacrol is a natural phenolic monoterpene predominantly found in essential oils of aromatic plants such as *Origanum vulgare* (oregano) and *Thymus vulgaris* (thyme). It has attracted considerable attention due to its broad range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties. Owing to its phenolic hydroxyl group, carvacrol is capable of scavenging reactive oxygen species and inhibiting lipid peroxidation, thereby contributing to cellular redox homeostasis (8-9).

Accumulating evidence indicates that carvacrol exerts protective effects in various inflammatory and oxidative stress–related disorders (10). Experimental studies have shown that carvacrol can enhance endogenous antioxidant defenses by increasing the activity of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, while simultaneously reducing oxidative damage markers (11). In addition, carvacrol has been reported to modulate key intracellular signaling pathways involved in inflammation, particularly by suppressing NF-κB activation and downstream pro-inflammatory cytokine production (12).

Regular aerobic exercise is a non-pharmacological intervention that can improve cardiopulmonary function, modulate immune responses, and, when prescribed at moderate intensity, induce adaptive antioxidant signaling (13). Carvacrol, a phenolic monoterpene derived from aromatic plants, exhibits potent antioxidant and anti-inflammatory properties in respiratory and systemic inflammatory models (8-12, 14). Combining exercise-induced hormetic activation of antioxidant pathways with the direct antioxidant and anti-inflammatory properties of carvacrol may produce additive or synergistic effects by decreasing ROS availability, facilitating NRF2 signaling, and attenuating NF-κB activation (15-18).

However, limited data are available regarding the combined effects of aerobic exercise and carvacrol on lung remodeling in allergic asthma. Therefore, the present study investigated whether combined aerobic exercise and carvacrol supplementation would exert greater protective effects than either intervention alone by modulating NRF2/NF-κB signaling and thereby mitigating oxidative stress, inflammation, respiratory dysfunction, and airway remodeling in ovalbumin-induced allergic asthma.

Materials and Methods

Animals and Experimental Design

Male Wistar rats (8–10 weeks old, weighing 220–250 g) were obtained from laboratory animal breeding center of Ahvaz Jundishapur University of Medical Sciences and housed under standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 50–60%, and a 12 h light/dark cycle). Animals had free access to standard laboratory chow and water throughout the experimental period. All experimental procedures

were conducted in accordance with the ethical committee of Iran Islamic Azad University, Ahvaz Branch (Ethics Code: IR.IAU.AHVAZ.REC.1404.048). After a one-week acclimatization period, rats were randomly assigned into five experimental groups (n = 8 per group): (1) Control, receiving saline and no intervention; (2) Allergic Asthma, subjected to ovalbumin (OVA) sensitization and challenge; (3) Asthma + Aerobic Exercise (Asthma+EX); (4) Asthma + Carvacrol (Asthma +CR); and (5) Asthma + Aerobic Exercise + Carvacrol (Asthma +EX+CR).

Allergic asthma was induced using an established ovalbumin sensitization and challenge protocol. Briefly, rats in the asthmatic groups were sensitized by intraperitoneal injection of ovalbumin (10 mg OVA emulsified in 1 mg aluminum hydroxide) on days 0 and 14. From day 21, animals were exposed to aerosolized 1% ovalbumin for 30 minutes per day, three times per week, for three consecutive weeks. Control animals received equivalent volumes of saline following the same schedule.

Following completion of the OVA sensitization/challenge phase, aerobic exercise and/or carvacrol administration were initiated and continued for 4 weeks. The aerobic exercise protocol in the present study was performed continuously at moderate intensity on a treadmill. The main exercise program was performed for four weeks with a frequency of five sessions per week. Before starting the main exercise, the animals underwent a two-week familiarization phase (five sessions per week) by running at a speed of 5 to 15 m/min. To determine maximal running speed, an incremental treadmill test to exhaustion was used. The main exercise intensity was determined as a percentage of the maximal speed achieved in the exhaustion test. The percentages of maximal speed are equivalent to intensities of 45 to 70% of maximal oxygen uptake (VO_2max). In the first week, the exercise intensity was 50 to 55% of maximal speed and gradually increased each week until it reached 65 to 70% in the fourth week. The duration of each session varied between 20 and 24 minutes. The structure of the sessions was designed as a combination of two running periods with different intensities (Table 1).

Carvacrol (≥98% purity; Sigma-Aldrich, St. Louis, MO, USA; CAS No. 499-75-2) was freshly prepared in sterile normal saline containing 0.5% dimethyl sulfoxide as vehicle and administered intraperitoneally at 73 mg/kg/day in a final volume of 1 mL/kg, once daily between 09:00 and 10:00 a.m. for six weeks. On training days, carvacrol was administered approximately 30 min after completion of the exercise session. Non-carvacrol groups received an equivalent volume of vehicle according to the same schedule.

At the end of the experimental period, animals were anesthetized and sacrificed 48 hours after the final intervention to avoid acute effects. Lung tissues were harvested for biochemical, molecular, and histological analyses.

Assessment of Antioxidant Status (TAC and SOD)

Lung tissue samples were rapidly excised, rinsed with ice-cold phosphate-buffered saline (PBS), and homogenized in cold buffer. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the supernatants were collected for biochemical analyses.

Table 1. Exercise protocol.

	Week 1		Week 2		Week 3		Week 4	
	Time (min)	intensity	Time (min)	intensity	Time (min)	intensity	Time (min)	intensity
Session 1	20	50%	20	55%	20	60%	20	65%
Session 2	16	50%	16	55%	16	60%	16	65%
	4	55%	4	60%	4	65%	4	70%
Session 3	12	50%	12	55%	12	60%	12	65%
	8	55%	8	60%	8	65%	8	70%
Session 4	8	50%	8	55%	8	60%	8	65%
	12	55%	12	60%	12	65%	12	70%
Session 5	4	50%	4	55%	16	60%	4	65%
	16	55%	16	60%	4	65%	16	70%

Total antioxidant capacity (TAC) was determined using a commercially available FRAP-based colorimetric assay kit (ZellBio GmbH, Ulm, Germany; Cat. No. ZB-TAC-96A) according to the manufacturer's instructions and expressed as mmol Trolox equivalents per gram of tissue. Superoxide dismutase (SOD) activity was measured using a commercial colorimetric SOD assay kit (ZellBio GmbH; Cat. No. ZB-SOD-96A) based on inhibition of superoxide radical autoxidation and expressed as U/mg protein. Protein concentration was measured using the Bradford method.

Measurement of Oxidative Stress Marker (Malondialdehyde, MDA)

Lipid peroxidation in lung tissue was assessed by measuring malondialdehyde (MDA) levels using a commercial thiobarbituric acid reactive substances (TBARS) assay kit (ZellBio GmbH; Cat. No. ZB-MDA-96A) according to the manufacturer's instructions. Briefly, lung tissue homogenates were mixed with thiobarbituric acid reagent and heated in a boiling water bath. After cooling and centrifugation, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. MDA concentrations were calculated using a standard curve and expressed as nmol/g tissue.

Evaluation of Respiratory Function (Respiratory Rate)

Respiratory rate was assessed in conscious animals under resting conditions. Each rat was placed individually in a transparent observation chamber and allowed to acclimatize for 10 min before measurement. Thoracoabdominal respiratory movements were then counted visually by two observers blinded to group allocation for three separate 60-s intervals, with 2-min rest periods between counts. One complete inspiration-expiration cycle was recorded as one breath. The average of the three counts was used for statistical analysis and expressed as breaths per minute (breaths/min). Measurements were performed at the same time of day to minimize circadian variations.

Assessment of Lung Remodeling (Hydroxyproline Content)

Lung collagen content was evaluated by measuring hydroxyproline levels as an index of lung remodeling and fibrosis. Lung tissue samples were hydrolyzed in 6 N hydrochloric acid at 110 °C for 18 hours. The hydrolysates were neutralized and reacted with chloramine-T followed by Ehrlich's reagent. Absorbance was measured at 560 nm, and hydroxyproline concentration was calculated using a standard curve. Results were expressed as µg hydroxyproline per gram of lung tissue.

Analysis of NRF2 and NF-κB Protein Expression (Western Blot)

Protein expression levels of NRF2 and NF-κB in lung tissue were analyzed by Western blotting. Lung tissues were homogenized in RIPA lysis buffer containing protease and phosphatase inhibitors. After centrifugation, total protein concentration was determined using the Bradford assay.

Equal amounts of protein (30–50 µg) were separated by SDS–PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1 h at room temperature and incubated overnight at 4 °C with rabbit anti-NRF2 antibody (NRF2 (D1Z9C) XP® Rabbit mAb (#12721); dilution 1:1000), rabbit anti-NF-κB p65 antibody (p-NFκB p65 (27.Ser 536): sc-136548; dilution 1:1000), and mouse anti-GAPDH antibody (GAPDH (D16H11) XP® Rabbit mAb; dilution 1:5000). After washing, membranes were incubated with HRP-conjugated goat anti-rabbit IgG (Abcam; Cat. No. ab6721; dilution 1:5000) or HRP-conjugated goat anti-mouse IgG (mouse anti-rabbit IgG-HRP: sc-2357 Santa; dilution 1:5000) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system and quantified by densitometric analysis. GAPDH was used as the internal loading control for both NRF2 and NF-κB. Western blot analysis was performed using biological samples from all animals in each group (n = 8/group), and densitometric quantification was confirmed across three independent experimental repetitions.

Statistical Analysis

Data were analyzed using SPSS software. The normality of data distribution was assessed using the Shapiro–Wilk test. For normally distributed data, differences among multiple groups were analyzed using one-way analysis of variance (one-way ANOVA), followed by Tukey's post hoc test for pairwise comparisons. In addition to

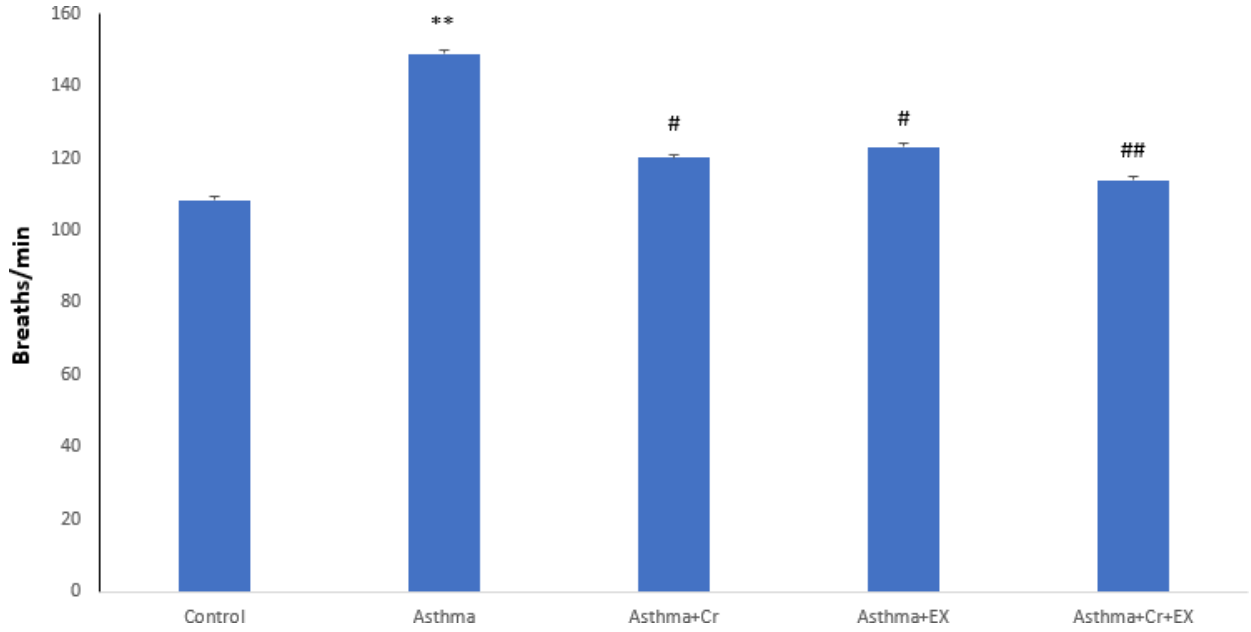

Figure 1. Effects of aerobic exercise and carvacrol on respiratory rate in ovalbumin-induced asthmatic rats. Respiratory rate (breaths/min) was assessed under resting conditions. Data are presented as mean ± SD (n = 8 rats/group). ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. Asthma group.

P values, effect sizes were calculated to improve interpretability. Eta-squared (η^2) was used to estimate the magnitude of the overall ANOVA effect, and Cohen's d was used for pairwise contrasts. Effect sizes were interpreted according to conventional thresholds: η^2 values of 0.01, 0.06, and 0.14 and Cohen's d values of 0.20, 0.50, and 0.80 were considered small, medium, and large effects, respectively. Statistical significance was set at $P < 0.05$.

Results

Effects of aerobic exercise and carvacrol on respiratory rate in asthmatic rats

Respiratory rate was significantly elevated in the Asthma group compared with the Control group ($P < 0.001$), reflecting impaired respiratory function (Figure 1). Both aerobic exercise and carvacrol treatment significantly reduced respiratory rate compared with the Asthma group ($P < 0.05$–0.01). The Asthma+CR+EX group showed the most pronounced reduction in respiratory rate, with values approaching those of the Control group ($P < 0.001$ vs. Asthma). Effect-size analysis indicated a large overall group effect based on eta-squared interpretation, supporting the biological relevance of the observed changes.

Effects of aerobic exercise and carvacrol on Lung Remodeling Assessed by Hydroxyproline Content

Lung hydroxyproline content, an index of collagen deposition and airway remodeling, was significantly increased in asthmatic rats compared with controls ($P < 0.001$), as shown in Figure 2. Aerobic exercise and carvacrol supplementation each significantly reduced hydroxyproline levels relative to the Asthma group ($P < 0.05$–0.01). The combined treatment group exhibited the greatest reduction in lung hydroxyproline content ($P < 0.001$ vs. Asthma), suggesting an additive effect of exercise and carvacrol on attenuating asthma-induced lung remodeling. The overall group effect was large according to eta-squared interpretation.

Effects of Aerobic Exercise and Carvacrol on Oxidative Stress Markers in Lung Tissue

As shown in Figure 3, induction of allergic asthma resulted in a significant reduction in total antioxidant capacity (TAC) and antioxidant enzyme activity, including superoxide dismutase (SOD), compared with the Control group ($P < 0.001$). In parallel, malondialdehyde (MDA) levels were markedly increased in the Asthma group, indicating enhanced lipid peroxidation and oxidative stress ($P < 0.001$). Aerobic exercise (Asthma+EX) and carvacrol supplementation (Asthma+CR) significantly increased TAC and SOD activities compared with the Asthma group ($P < 0.05$–0.01), while MDA levels were significantly reduced. Notably, the combined intervention (Asthma+CR+EX) produced a greater improvement in antioxidant status than either treatment alone, restoring antioxidant parameters closer to control values and resulting in the lowest MDA levels among the asthma-treated groups ($P < 0.001$ vs. Asthma). Effect-size estimates for the oxidative stress markers were in the large range, indicating robust treatment-related differences.

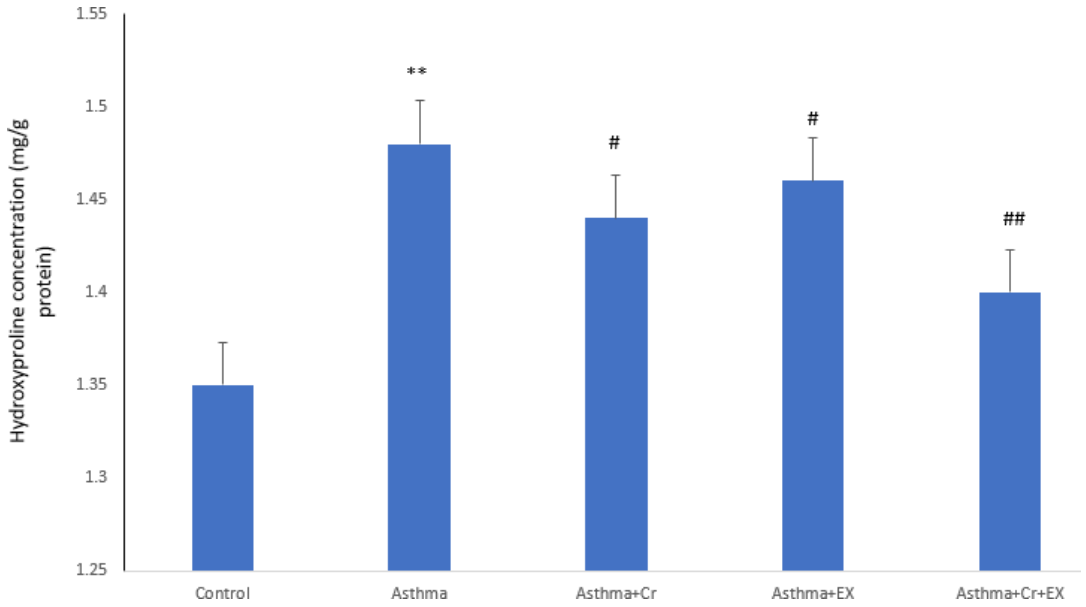

Figure 2. Effects of aerobic exercise and carvacrol on lung hydroxyproline content in ovalbumin-induced asthmatic rats. Hydroxyproline levels were measured as an index of collagen deposition and lung remodeling. Values are expressed as mean ± SD (n = 8 rats/group). ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. Asthma group.

Effects of aerobic exercise and carvacrol on NRF2 protein expression in lung tissue of asthmatic rats

Western blot analysis demonstrated a significant decrease in NRF2 protein expression in the Asthma group compared with the Control group ($P < 0.001$) (Figure 4). Treatment with aerobic exercise or carvacrol significantly increased NRF2 expression compared with the Asthma group ($P < 0.05$–0.01). Importantly, the Asthma+CR+EX group showed the highest NRF2 protein expression among all asthmatic groups ($P < 0.001$ vs. Asthma), indicating a synergistic-like activation of the NRF2 antioxidant pathway. The magnitude of the overall group effect was large based on eta-squared interpretation.

Effects of aerobic exercise and carvacrol on NF-κB protein expression in lung tissue of asthmatic rats

NF-κB protein expression was significantly elevated in the Asthma group compared with the Control group ($P < 0.001$) (Figure 5). Both aerobic exercise (Asthma+EX) and carvacrol supplementation (Asthma+CR) significantly reduced NF-κB expression relative to the Asthma group ($P < 0.05$ and $P < 0.01$, respectively). The combined intervention (Asthma+CR+EX) resulted in a further and more pronounced decrease in NF-κB levels ($P < 0.001$ vs. Asthma). GAPDH was used as the loading control. The overall group effect was large according to eta-squared interpretation.

Taken together, the molecular results showed an inverse pattern between NRF2 and NF-κB expression: interventions that enhanced NRF2 protein expression also reduced NF-κB protein expression, with the most prominent reciprocal shift observed in the Asthma+CR+EX group. This pattern supports a coordinated modulation of antioxidant and inflammatory signaling in response to the combined intervention.

Discussion

The present study investigated the effects of aerobic exercise and carvacrol supplementation, alone and in combination, on oxidative stress, airway inflammation, lung remodeling, and NRF2/NF-κB signaling pathways in an ovalbumin-induced allergic asthma model in rats. The main findings demonstrate that allergic asthma is associated with increased oxidative stress, elevated inflammatory signaling, impaired respiratory function, and enhanced lung collagen deposition, whereas aerobic exercise and carvacrol attenuate these pathological changes. Because the study was performed in a rat model, the findings should be interpreted as preclinical evidence.

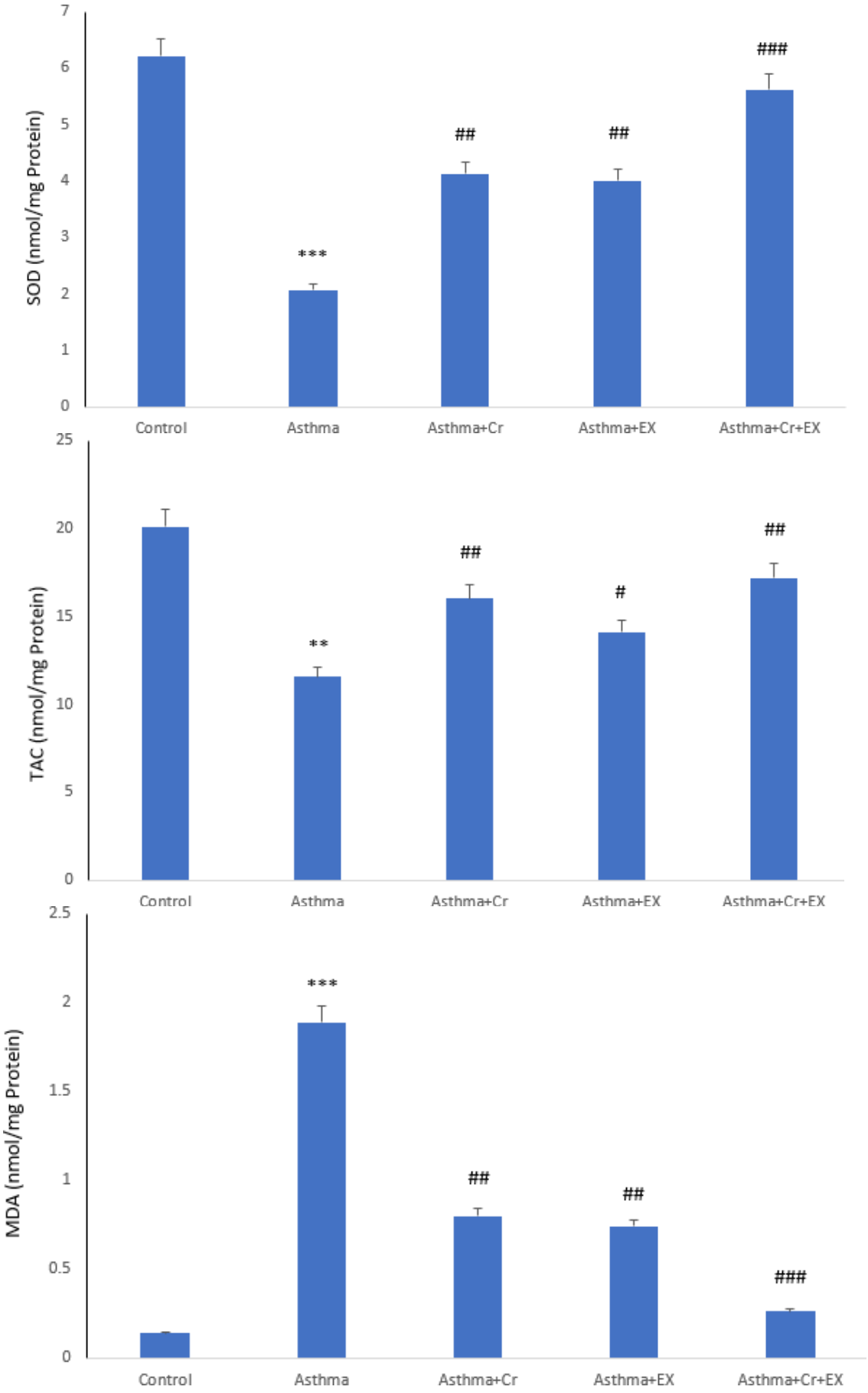

Figure 3. Effects of aerobic exercise and carvacrol on oxidative stress markers in lung tissue of ovalbumin-induced asthmatic rats. Total antioxidant capacity (TAC), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured in lung tissue. Data are expressed as mean ± SD (n = 8 rats/group). ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. Asthma group.

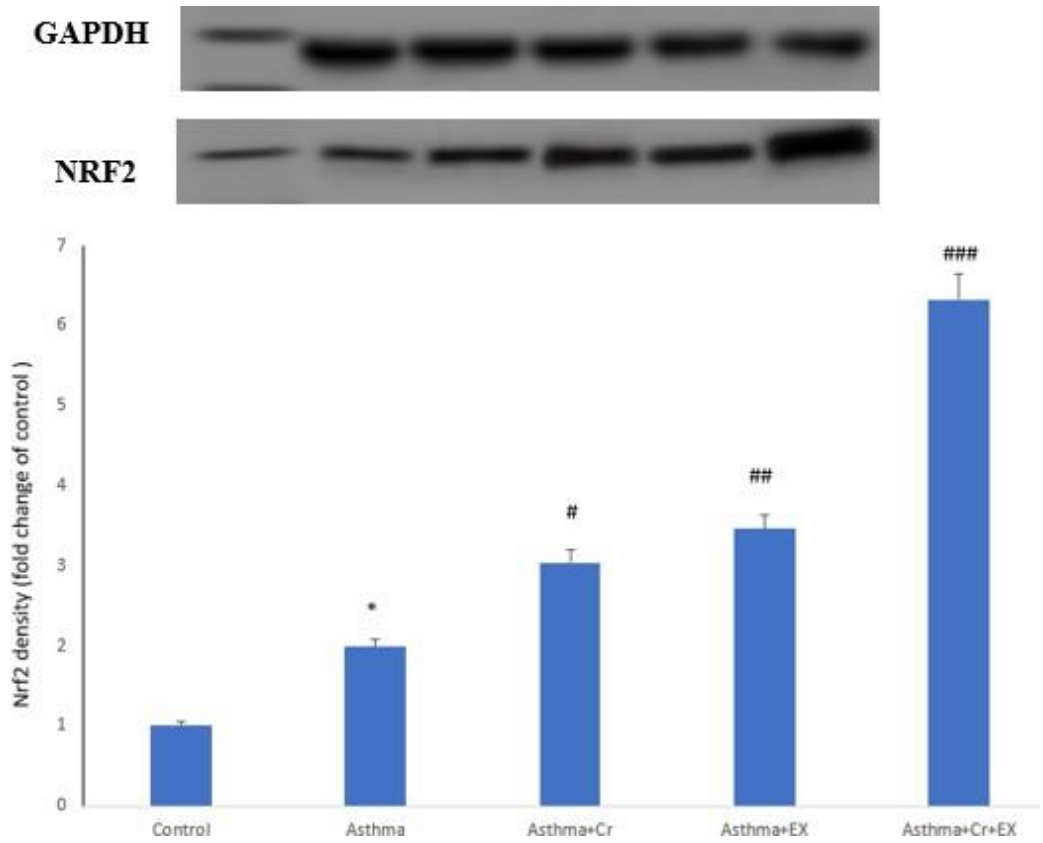

Figure 4. Effects of aerobic exercise and carvacrol on NRF2 protein expression in lung tissue of ovalbumin-induced asthmatic rats. Representative Western blot images and quantitative densitometric analysis of NRF2 protein expression normalized to GAPDH are shown. Data are expressed as mean ± SD (n = 8 rats/group; three independent Western blot repetitions). ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. Asthma group.

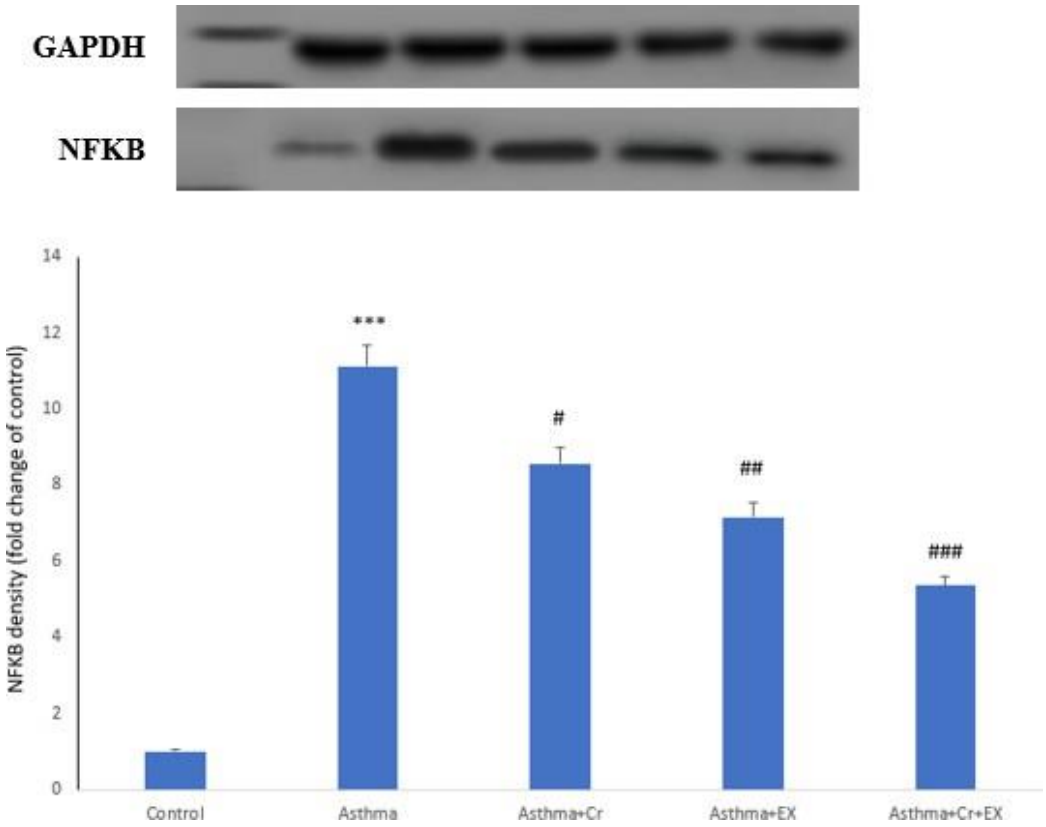

Figure 5. Effects of aerobic exercise and carvacrol on NF-κB protein expression in lung tissue of ovalbumin-induced asthmatic rats. Representative Western blot images and quantitative analysis of NF-κB protein expression normalized to GAPDH are presented. Data are expressed as mean ± SD (n = 8 rats/group; three independent Western blot repetitions). ***P < 0.001 vs. Control group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. Asthma group.

Asthmatic rats exhibited a marked reduction in antioxidant capacity and antioxidant enzyme activities accompanied by increased lipid peroxidation, confirming the critical role of oxidative stress in asthma pathophysiology. These findings are consistent with previous studies reporting excessive reactive oxygen species production and weakened antioxidant defenses in asthmatic airways (19-21). The observed decrease in NRF2 expression in asthmatic animals further supports the notion that suppression of endogenous antioxidant signaling contributes to disease progression.

Aerobic exercise significantly improved antioxidant status and reduced oxidative damage in lung tissue. Exercise-induced activation of redox-sensitive signaling pathways may enhance NRF2 nuclear translocation and transcription of antioxidant enzymes, thereby strengthening cellular defense mechanisms. In this context Maira et al, showed that physical exercise prevents, at least partially, the oxidative damage caused by lipopolysaccharide induced lung injury, suggesting that exercise may have an important role as protector in this condition (22).

Similarly, carvacrol supplementation effectively increased antioxidant enzyme activities and reduced MDA levels, which may be attributed to its phenolic structure and free radical–scavenging properties. A study by Carvalho et al, reported that carvacrol alleviates oxidative stress and lung histological damages induced by smoke inhalation in rodents (23).

Importantly, the combined intervention of aerobic exercise and carvacrol produced greater improvements than either treatment alone, suggesting an additive or synergistic-like effect on antioxidant defense. This enhanced effect was accompanied by a pronounced upregulation of NRF2 protein expression, indicating that simultaneous lifestyle and nutritional interventions may more effectively restore redox balance in asthmatic lungs. The rationale for this combined approach is that moderate aerobic exercise may induce adaptive, hormetic antioxidant signaling, whereas carvacrol may provide direct radical-scavenging and anti-inflammatory effects; convergence of these mechanisms may reduce ROS burden and facilitate suppression of downstream inflammatory signaling.

Inflammation is a hallmark feature of allergic asthma, and NF-κB plays a central role in regulating pro-inflammatory gene expression (24). In the present study, NF-κB expression was markedly increased in asthmatic rats, whereas both exercise and carvacrol significantly suppressed NF-κB activation. The combined intervention resulted in the greatest inhibition of NF-κB signaling, highlighting the close interplay between oxidative stress and inflammatory pathways. The simultaneous increase in NRF2 and decrease in NF-κB suggests that restoration of antioxidant signaling may contribute to inhibition of inflammatory transcriptional activity.

Respiratory rate and lung hydroxyproline content were also significantly improved following exercise and carvacrol treatment. Reduced respiratory rate indicates improved airway function, while decreased hydroxyproline levels suggest attenuation of collagen deposition and airway remodeling. These findings are clinically relevant, as airway remodeling is often resistant to conventional asthma therapies.

The inverse regulation of NRF2 and NF-κB observed in this study supports the concept of crosstalk between antioxidant and inflammatory signaling pathways. Activation of NRF2 may inhibit NF-κB signaling directly or indirectly by reducing ROS-dependent IκB kinase activation, inducing cytoprotective enzymes such as heme oxygenase-1 and NAD(P)H quinone oxidoreductase-1, and limiting cytokine-driven oxidative amplification (15-17). Conversely, sustained NF-κB activation can perpetuate pro-oxidant and pro-inflammatory networks, thereby weakening endogenous antioxidant capacity. Therefore, the concurrent increase in NRF2 expression and reduction in NF-κB expression in the combined treatment group provides a plausible molecular explanation for the improvements observed in MDA, TAC, SOD, respiratory rate, and hydroxyproline content.

Some findings in the broader literature appear inconsistent with the present results. For example, exhaustive or high-intensity exercise may increase ROS formation and aggravate inflammatory responses, and excessive antioxidant supplementation can blunt redox-dependent adaptive responses to exercise. Differences in exercise intensity and duration, timing relative to allergen challenge, species and disease model, baseline severity of airway

inflammation, carvacrol dose or formulation, and the time point at which outcomes are measured may explain divergent findings. In the present study, the progressive moderate-intensity exercise protocol was designed to avoid excessive exercise-induced airway stress while still promoting adaptive antioxidant responses, which may account for the beneficial effects observed when exercise was combined with carvacrol (25).

Despite the promising findings, certain limitations should be acknowledged. The study was conducted in an animal model, and extrapolation to human asthma requires caution. In addition, inflammatory cytokines and histopathological assessments were not evaluated, which could further clarify the mechanisms involved. Future studies should include dose-response designs, additional molecular endpoints, histological scoring of airway remodeling, and clinical investigations to confirm translational relevance.

In conclusion, the present preclinical findings indicate that aerobic exercise combined with carvacrol supplementation exerts protective effects against allergic asthma by enhancing antioxidant defenses, suppressing inflammatory signaling, and reducing lung remodeling through modulation of the NRF2/NF-κB pathway. These results support further investigation of combined non-pharmacological and nutritional strategies as complementary, rather than replacement, approaches in asthma management.

Conclusion

In conclusion, the present study demonstrates that allergic asthma is associated with increased oxidative stress, enhanced inflammatory signaling, impaired respiratory function, and lung remodeling in an ovalbumin-induced rat model. Aerobic exercise and carvacrol supplementation, administered individually, improved antioxidant capacity, reduced lipid peroxidation, suppressed NF-κB activation, and attenuated pathological changes in lung tissue. The combined intervention produced more pronounced protective effects than either treatment alone, as reflected by increased NRF2 expression, reduced NF-κB signaling, improved respiratory rate, and decreased lung collagen deposition. These preclinical findings suggest that integrating regular aerobic exercise with antioxidant supplementation such as carvacrol may be a promising complementary strategy for allergic asthma management. However, the results do not establish clinical efficacy in humans, and further mechanistic, dose-response, and clinical studies are warranted to confirm these effects and clarify their translational relevance.

Declarations

Ethics Approval and Consent to Participate

All experimental procedures involving animals were conducted in accordance with Iran Islamic Azad University, Ahvaz Branch (Ethics Code: IR.IAU.AHVAZ.REC.1404.048). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by the authors. The first draft of the manuscript was written by the corresponding author, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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