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The Alleviating Impacts of Quercetin on Inflammation and Oxidant-antioxidant Imbalance in Rats with Allergic Asthma

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ABSTRACT

Asthma is an inflammatory disease of the airways. We assessed the anti-inflammatory and antioxidative impacts of quercetin, a plant derivative, on inflammatory and oxidative indices in lung tissue and serum of rats with asthma.

Asthma was induced by ovalbumin. Rats were divided into 4 groups: control, asthma+vehicle (Receieved normal saline), asthma+dexamethasone, and asthma+quercetin. After asthma induction, quercetin (50 mg/kg) and dexamethasone (2.5 mg/kg) were injected intraperitoneally once daily for 1 week. On day 50, lung histopathology indices; inflammatory factors; tissue gene expression, including *GATA Binding Protein 3* (Gata-3), *Tbx21* (T-bet), *Transforming growth factor-β* (TGF-β), *Il10* (IL-10), *Il1b* (IL-1β), *Il6* (IL-6), *Acta2* (α-SMA), and *Tnf* (TNF-α); and oxidative stress indices (malondialdehyde [MDA], catalase [CAT], glutathione

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peroxidase [GPX], superoxide dismutase [SOD], and total antioxidant capacity [TAC]) in tissue and serum, were evaluated.

The results showed that quercetin reduced *Gata3*, *Tnf*, *Tgfb1*, *Il1b*, and *Acta2* gene expression and increased *Tbx21* gene expression following asthma. Quercetin also improved oxidative stress by decreasing MDA levels and increasing TAC, CAT, SOD, and GPX levels in serum and lung tissue. Furthermore, quercetin decreased *IL6* and *TNFa* levels and increased *IL10* levels in lung tissue after asthma was treated with quercetin.

Quercetin ameliorates oxidative stress and inflammation caused by asthma, especially at the tissue level. Therefore, quercetin can be considered a potent antiasthmatic agent.

Keywords: Asthma; Inflammation; Oxidative stress; Quercetin

INTRODUCTION

Asthma is a disease of the airways with various clinical and pathophysiological features, such as increased mucus secretion, reversible bronchial obstruction, airway hyperresponsiveness and narrowing, goblet cell hyperplasia, and inflammation.[1] About 315 million people worldwide, and 12% to 14% of Iranians, have asthma. This rate increases by 50% every decade and negatively affects public health.[2] Many patients use corticosteroids for treatment; however, 5% to 10% of patients fail to respond to this treatment.[3]

The main feature of asthma is inflammation in the airways. Airway inflammation involves the response of different immune cells and various factors.[4] Inflammatory epithelial cells also produce high levels of cytokines.[5,6] Tumor necrosis factor-alpha (TNF-α) regulates the inflammatory reaction of the airways, whereas interleukin (IL)-1β promotes eosinophil infiltration into the inflamed airways.[7] IL-6 increases type 2 T helper lymphocytes and enhances airway responsiveness. Recent studies report that IL-6 plays a key role in asthma progression.[8]

Oxidative stress can also worsen inflammation in asthma.[9] Asthma is accompanied by increased oxidative stress because active inflammatory cells generate oxidant agents.[10] Antioxidants are divided into 2 groups: enzymatic and nonenzymatic. The enzymatic protective system is the first line of defense against reactive oxygen species.[11] Oxidative stress causes hyperplasia of goblet cells, which worsens inflammation by increasing cytokine release and altering the function of antioxidant enzymes.[12] One of the effective antioxidant agents vital to maintaining protein integrity is total thiol sulfhydryl (T-SH). It also protects against cell and tissue injury induced by oxidative stress.[13]

Herbal medicine use is a common and effective strategy for improving various diseases.[14-16] Quercetin (QS) is one of the most abundant flavonoids in plants. It has high antioxidant properties and is approximately 6 times stronger than vitamin C.[17] QS is found in vegetables, fruits, onions, apples, red grapes, citrus fruits, broccoli, tomatoes, green and black teas, and dark chocolate. QS also preserves serum glutathione levels, decreases malondialdehyde (MDA) levels, inhibits nitric oxide metabolism, limits superoxide generation, and inhibits the release of oxidants and automatic intermediates. This combination has an ameliorative effect on oxidative injury and inflammation.[18] Due to the chronic nature of asthma and the adverse effects of corticosteroids, it is important to find alternative drugs with fewer adverse effects. Therefore, using traditional compounds to supplement standard treatment is useful in chronic diseases such as asthma. Concerning the role of oxidants in asthma progression and airway inflammation, as well as the antioxidant and anti-inflammatory effects of QS, this study aims to determine the effect of this herbal compound on oxidative and inflammatory stress indices in an allergic asthma rat model.

MATERIALS AND METHODS

Animals

Twenty-eight male Wistar rats (weight range, 200–250 g; age, 8 weeks) were kept at standard temperature (22±2°C) and a 12-hour light/dark cycle with free access to water and food.

Animal Groups, Asthma Induction, and Treatment Protocols

Figure 1 shows the timing of the experiment. Quercetin (Sigma-Aldrich) was dissolved in normal saline. On days 0 and 7, the rats received an intraperitoneal injection of 0.5 mL PBS containing 1 mg of ovalbumin (OVA) (Sigma-Aldrich) and 200 μg of aluminum hydroxide (Sigma-Aldrich).. The sensitized rats were exposed to 1% aerosolized OVA (1 g OVA in 100 mL sterile PBS) for 30 minutes every other day from day 14 to day 42 in a closed chamber (30×50×60 cm) using a nebulizer.[7,19-21] The groups included: 1) control (no intervention); 2) Vehicle group (asthmatic rats treated with dimethyl sulfoxide (DMSO); 3) asthmatic rats treated with QS (50 mg/kg),[22] and 4) asthmatic rats treated with dexamethasone (2.5 mg; as the gold standard).[19] The treatments were administered intraperitoneally, daily for 7 consecutive days.[19]

Determination of Oxidative Stress Status and Cytokines Levels

Blood samples were collected from the heart after euthanizing the rats with 80 mg/kg ketamine and 50 mg/kg xylazine. The right lung and airways were harvested for molecular investigations.

Serum nitric oxide was measured using the griess method according to the manufacturer's instructions for the ELISA assay kit.[23] Malondialdehyde (MDA) concentration in lung tissue supernatant was determined by the thiobarbituric acid reaction and absorption at 412 nm.[24] Total antioxidant capacity (TAC) levels in serum and lung tissue were assessed by the FRAP method. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities in lung tissues were evaluated according to the ELISA kit protocols.[25] Serum thiol group (T-SH) levels were also measured using an ELISA kit. Sandwich ELISA was used to quantify TNF-α, IL-6, and IL-10 levels.[7,26]

Real-time PCR

Real-time PCR RNA was extracted from homogenized lung tissue using an RNase-Free Fibrous Tissue Kit (Qiagen) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using a cDNA Synthesis Kit (GeneAll, Korea). Ultimately, the expression of GATA-3, T-bet, TNF-α, IL-1β, Alpha Smooth muscle actin (α-SMA), and TGFβ genes was quantified by SYBR Green-based real-time PCR.[8]

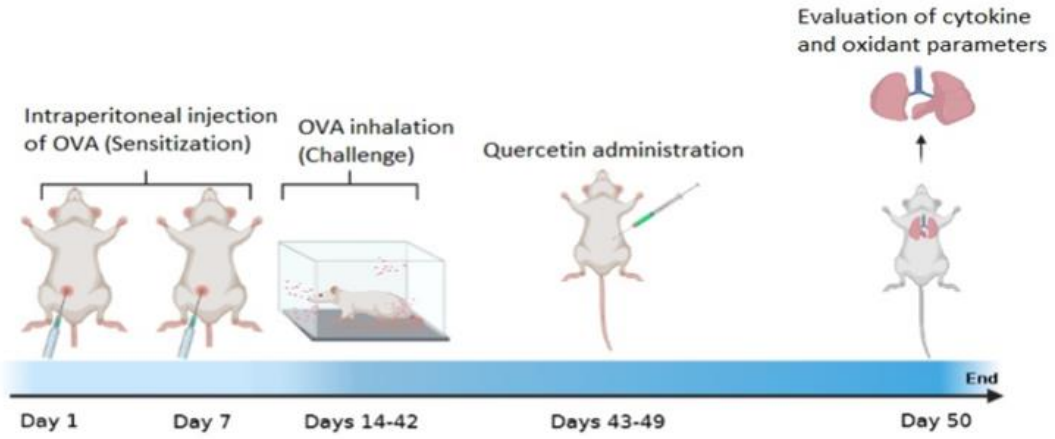

Figure 1. Timeline of the experiment; OVA: ovalbumin.

Histopathological Examination

The left lung was harvested from the rats on day 50 and fixed in 10% formalin. The tissues were then stained with hematoxylin and eosin (H&E) staining. The sections were examined by a blinded pathologist under a light microscope. Bronchus and bronchiole destruction, alveolar damage, and inflammation were evaluated by a pathologist.[27]

Statistical Analysis

Data were analyzed using GraphPad Prism version 8. One-way analysis of variance and Tukey post hoc test

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were utilized to compare the groups. A *p* value of less than 0.05 was considered statistically significant.[28,29]

RESULTS

Oxidative Stress Indices

We found a remarkable difference in serum TAC between asthma and control groups, and QS treatment significantly increased serum TAC compared to the asthma group. However, we did not observe significant differences in serum T-SH and NO among the groups (Figure 2). In contrast, tissue TAC in the asthma group was significantly lower than in the control group. QS treatment increased tissue TAC compared to the asthma group, and the MDA level was higher in the asthma group than in the control group. QS treatment also reduced tissue MDA in asthmatic rats. SOD, GPX, and CAT activities were lower in the asthma group than in the control group. QS treatment enhanced the activities of SOD, GPX, and CAT compared to the asthma group (Figure 3).

Cytokines Levels in Lung Tissue

We found that the TNFα and IL-6 levels were significantly higher in the asthmatic group than in the control group. QS treatment significantly reduced TNFα and IL-6 levels. IL-10 levels were lower in the asthma group than in the control group, and QS treatment increased the level of IL-10 in the asthma group. (Figure 4).

Histopathological and Molecular Findings

The histopathological examination of lung tissue showed alveolar injury and terminal bronchial damage in asthmatic rats. In contrast, we did not observe any alveolar injury or terminal bronchial devastation in the control group. These pathogenic changes seem to be caused by the infiltration of inflammatory cells into the bronchial tissues. However, our findings indicate that quercetin, like dexamethasone, can significantly reduce inflammation and improve morphological features .

Real-time PCR results showed that the expression of the GATA-3, α-SMA, IL-1β, TNFα, and TGF-β genes was higher in the lung tissue of the asthma group than in the control rats. QS and dexamethasone decreased the expression of the GATA-3, α-SMA, IL-1β, TNFα, and TGF-β genes compared to the asthma group. T-bet expression increased after QS treatment in asthmatic rats (Figures 5, 6 and 7 and Table 1).

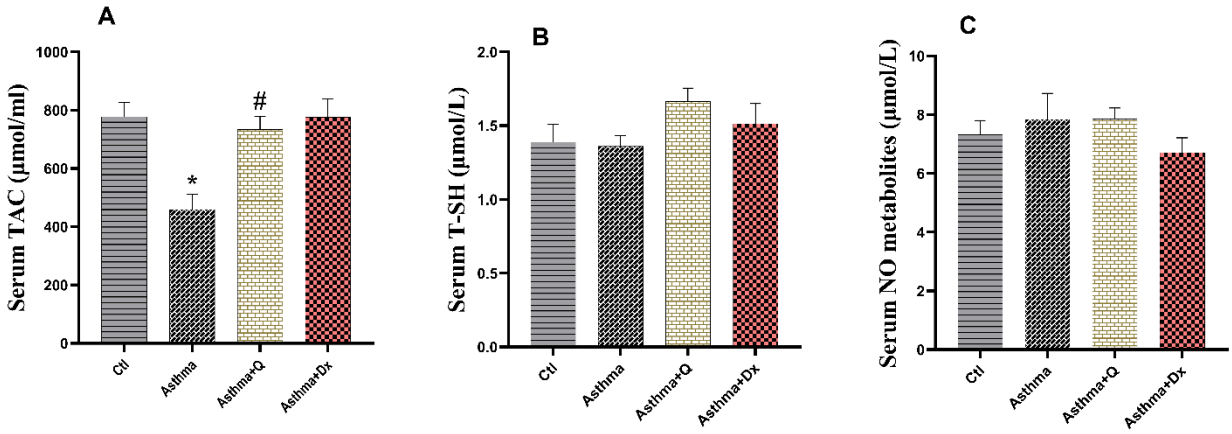

Figure 2. Effects of quercetin and dexamethasone on serum total antioxidant capacity (TAC), thiol (T-SH), and nitric oxide (NO) levels in asthmatic rats. TAC (A), T-SH (B), and NO (C). Data are expressed as mean ± standard error of the mean (SEM) (n=7 rats per group).There was no significant difference among all groups in T-SH and NO metabolites. Ctl: control; Asthma+Q: asthmatic rats treated with quercetin; Asthma+DX: asthmatic rats treated with dexamethasone. * *p*<0.05 vs Ctl, # *p*<0.05 vs Asthma.

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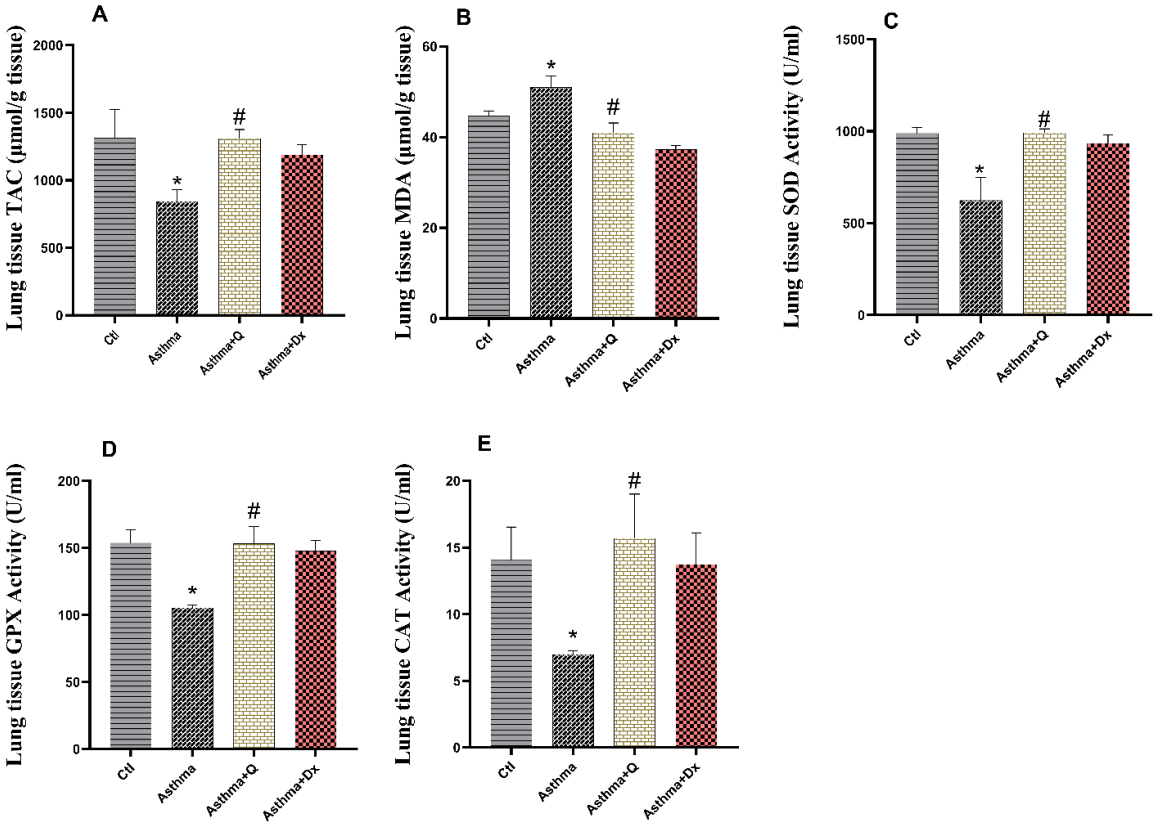

Figure 3. Effects of quercetin and dexamethasone on total antioxidant capacity (TAC), malondialdehyde (MDA), and antioxidant enzyme activities in lung tissue of asthmatic rats. TAC (A), MDA (B), superoxide dismutase (SOD) activity (C), glutathione peroxidase (GPX) activity (D), and catalase (CAT) activity (E). Data are expressed as mean ± standard error of the mean (SEM) (n = 7 rats per group). Ctl: control; Asthma+Q: asthmatic rats treated with quercetin; Asthma+DX: asthmatic rats treated with dexamethasone. * p<0.05 vs Ctl, # p<0.05 vs Asthma.

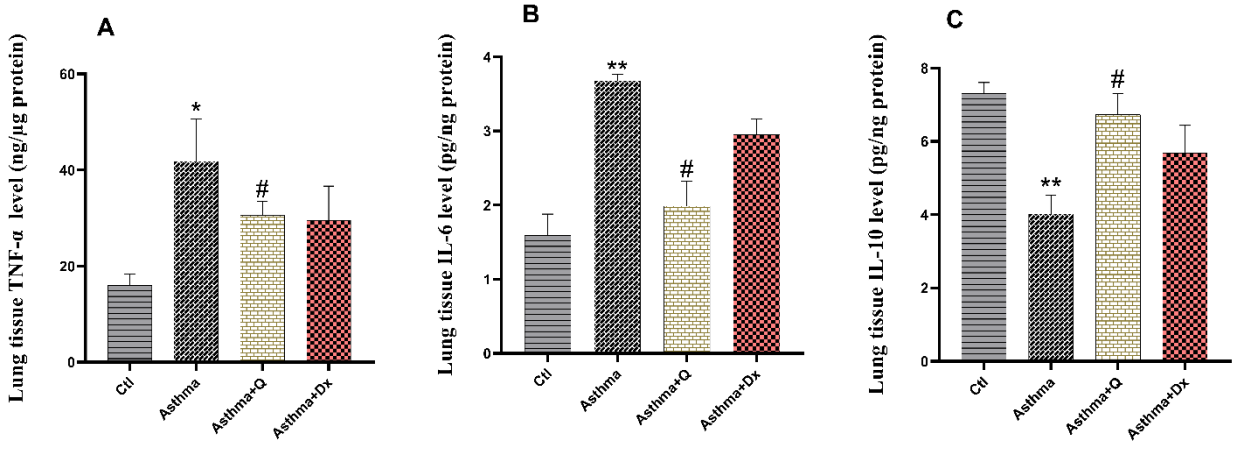

Figure 4. Effects of quercetin and dexamethasone on inflammatory cytokine levels in lung tissue of asthmatic rats measured by enzyme-linked immunosorbent assay (ELISA). Asthma significantly increased tumor necrosis factor α (TNF-α) (A) and interleukin 6 (IL-6) (B) levels and decreased interleukin 10 (IL-10) levels in lung tissue. Data are expressed as mean ± standard error of the mean (SEM) (n = 7 rats per group). Ctl: control; Asthma+Q: asthmatic rats treated with quercetin; Asthma+DX: asthmatic rats treated with dexamethasone. * p<0.05 and ** p<0.01 vs Ctl, # p<0.05 vs Asthma.

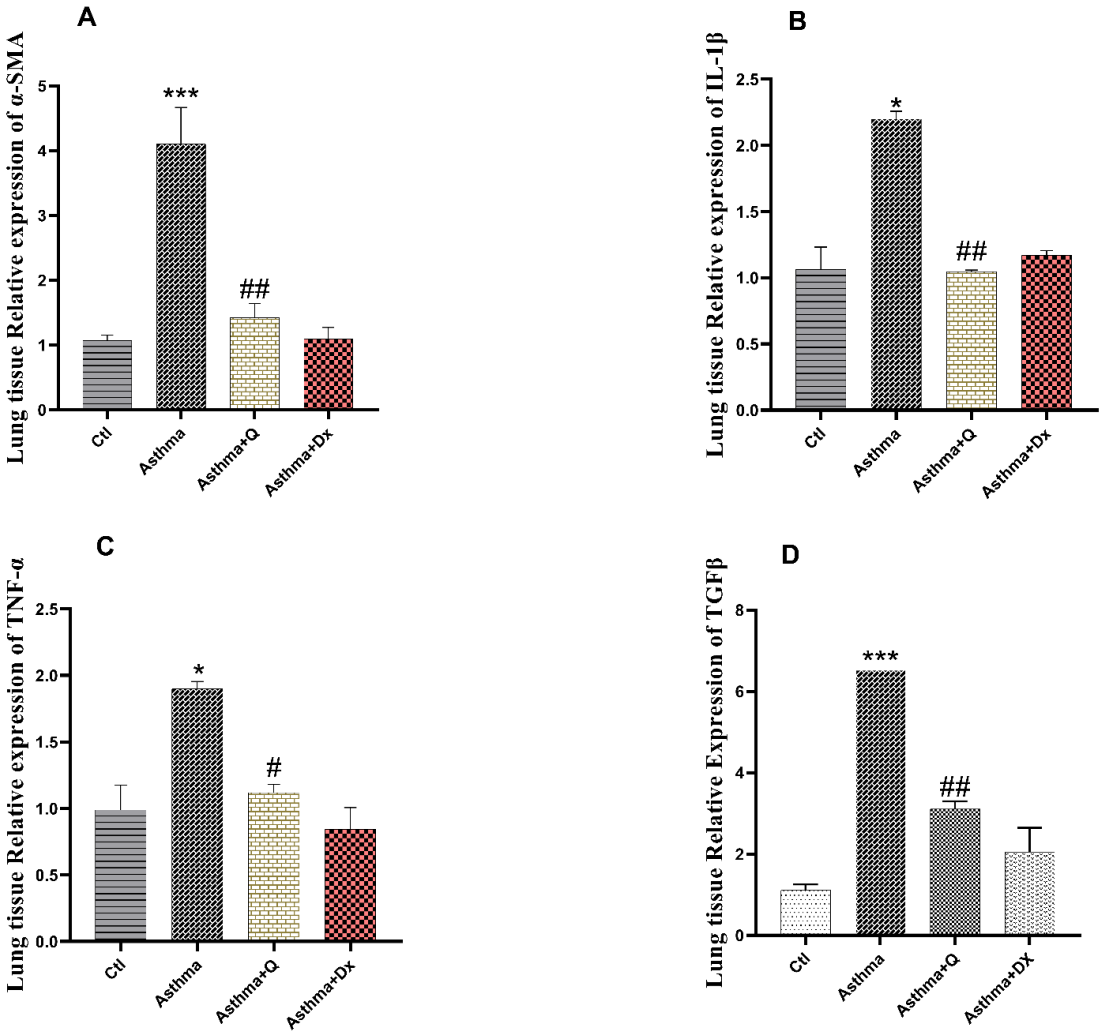

Figure 5. Effects of quercetin and dexamethasone on inflammatory cytokine gene expression in lung tissue of asthmatic rats measured by real-time polymerase chain reaction (PCR). Asthma significantly increased gene expression of α-smooth muscle actin (α-SMA) (A), interleukin 1β (IL-1β) (B), tumor necrosis factor α (TNF-α) (C), and transforming growth factor β (TGFβ) (D) in lung tissue. Data are expressed as mean ± standard error of the mean (SEM) (n = 7 rats per group). CTL: control; Asthma+Q: asthmatic rats treated with quercetin; Asthma+DX: asthmatic rats treated with dexamethasone. * p<0.05 ***p<0.001 vs CTL, # p<0.05 and ## p<0.01 vs Asthma.

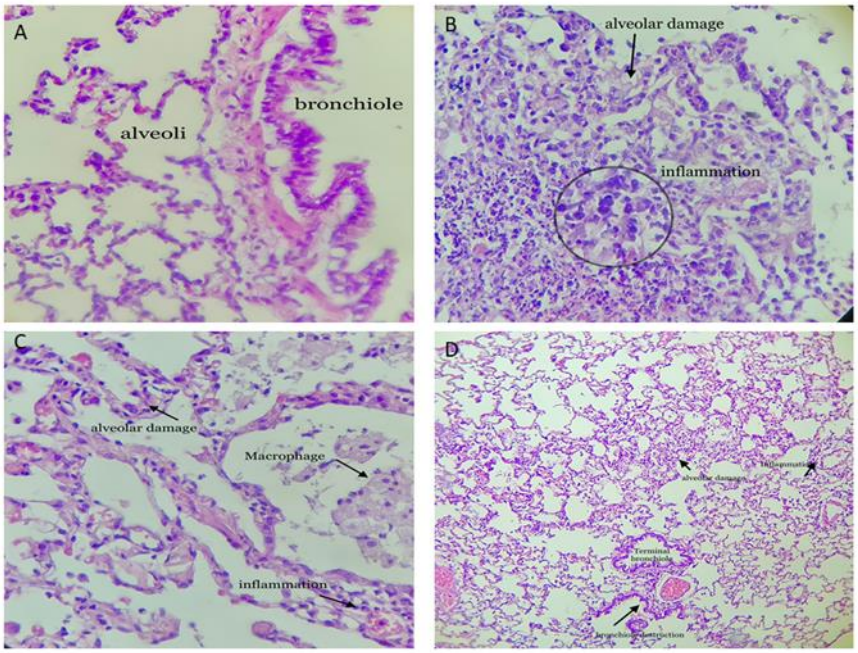

Figure 6. Effects of quercetin and dexamethasone on histopathologic changes in lung tissue of asthmatic rats stained with hematoxylin and eosin (10×). (A) Control; (B) asthma; (C) asthmatic rats treated with 50 mg/kg of quercetin; (D) asthmatic rats treated with 2.5 mg/kg of dexamethasone.

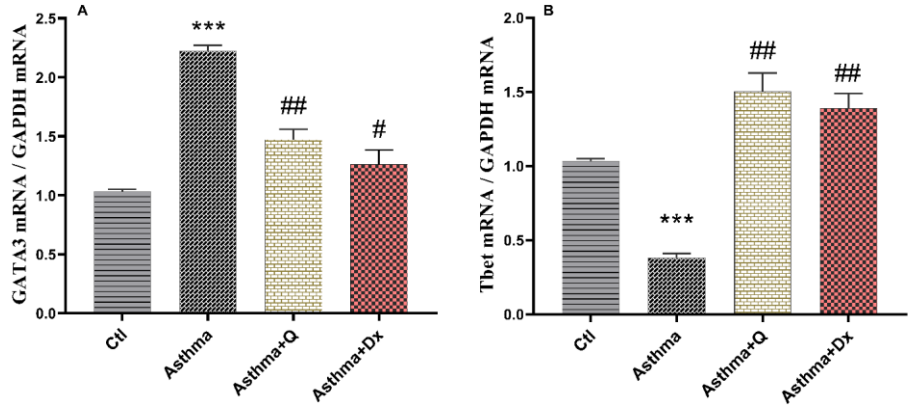

Figure 7. Effects of quercetin and dexamethasone on GATA-binding protein 3 (GATA3) and T-box transcription factor TBX21 (T-bet) gene expression in lung tissue of asthmatic rats measured by real-time polymerase chain reaction (PCR). Asthma significantly increased GATA3 gene expression (A) and decreased T-bet gene expression (B) in lung tissue. Data are expressed as mean ± standard error of the mean (SEM) (n = 7 rats per group). Ctl: control; Asthma+Q: asthmatic rats treated with 50 mg/kg of quercetin; Asthma+DX: asthmatic rats treated with 2.5 mg/kg of dexamethasone. *** p<0.001 vs Ctl, # p<0.05 and ## p<0.01 vs Asthma.

Table 1. Histopathological changes among study groups

Groups	Main Bronchus Destruction	Terminal Bronchiole Destruction	Alveolar Damage	Inflammation	Vessels
Control	Normal	Normal	Normal	Normal	Normal
Asthma	5%	20%	50%	20%	Normal
Asthma+Quercetin	5%	15%	15%	10%	Normal
Asthma+Dexamethasone	5%	10%	15%	10%	Normal

DISCUSSION

This study aimed to examine the effects of QS and dexamethasone on various lung indicators, including those that measure inflammation, oxidative stress, and histopathology, in rats with asthma. The results revealed that QS reduced histological alterations in the lungs of asthmatic rats. Furthermore, it decreased TNF-α and IL-6 levels and increased IL-10 levels. Moreover, QS decreased the expression of TNFα, IL1β, TGFβ, and α-SMA and increased antioxidants in lung tissue.

TNF-α has been involved in several aspects of asthma pathology.[30] Our results showed that TNFα levels increased due to asthma, and many studies approved our results and reported the elevation of TNFα in asthma.[6,7,31] Additionally, in this study, TNFα gene expression increased in lung tissue after asthma, and these data were consistent with other investigations.[32,33]

IL-6 is increased in various lung disorders but was regarded as a byproduct of inflammation in the lung.[34] Many studies, like our findings, revealed elevated levels of IL-6 in asthma.[35-37]

IL-10 is a critical modulating cytokine needed to control asthma.[38] Consistent with previous research, we found that IL-10 levels were lower in rats with asthma.[7,39]

According to the findings of several studies, asthma is accompanied by an elevated expression of IL1β in lung tissue.[7,40] Our results were also were in agreement with these investigations.

Min et al, disclosed that QS inhibits IL-1β mRNA expression by reducing NF-κB in the human mast cell line.[41] Furthermore, our results showed that QS alleviated the impact of IL-1 on lung tissue from asthmatic rats. Specifically, we found that QS improved IL-10 while decreasing IL-6 and TNF-α levels in asthmatic rats. Zhu et al, found that QS significantly reduced mRNA expression of TNF-α and IL-6 in newborn rats with asthma.[42] Sozmen et al, reported the impacts of QS on histological aspects as well as inflammation. Compared to asthmatic mice, QS treatment resulted in fewer pathogenic alterations. These findings propose that QS reduces chronic histopathological alterations and that its alleviating impacts on inflammation may be owing to cytokine modifiers.[43]

Many investigations showed reduced expression of T-bet and increased expression of GATA-3 following asthma.[44,45] Our results were also consistent with these investigations. Our findings suggest that QS's anti-inflammatory benefits in asthma may be mediated, at least in part, by its ability to modulate GATA-3 and T-bet expression. TGF-β is an essential parameter that acts on tissue remodeling in asthma-affected lungs.[46] Many studies, like our research, reported elevated levels of TGFβ in asthmatic rats.[47,48] Some investigations revealed the inhibiting role of QS on TGFβ expression in some tissues such as the liver, lung, kidney, and heart.[49,50] Our results also revealed the beneficial role of QS on TGFβ mRNA in lung tissue.

αSMA is a parameter for the active fibroblast populations known as the myofibroblast. Wu et al, showed that mRNA expression of αSMA in the lung increased in rats with asthma.[51] Ren et al, revealed that high αSMA expression in the lung tissue of asthmatic rats is accompanied by the initiation of asthma attacks.[52] Also, we reported in the current study that the expression of αSMA increased in lung tissue following asthma. Some studies revealed that QS could diminish the αSMA in tissues like the liver.[53] Our data also revealed the alleviating impacts of QS against αSMA in the lung of rats with asthma.

We found that TAC levels were greatly reduced in asthmatic rats and dramatically elevated in QS-treated rats. However, we found no statistically significant variations in TAC levels between groups in the serum.

Numerous studies showed the elevation of TAC in lung tissue in asthmatic animals,[54,55] and some investigations revealed the increasing effects of QS on TAC levels in lung tissue.[56,57] Consistent with our findings, other studies found that asthmatic rats have an increased MDA level,[7,26] and QS improved this factor.[58] Many studies showed a decrease in SOD,[59,60] GPX,[61,62] and CAT,[63,64] in asthma. Our results also were consistent with other investigations. Consistent with our findings, QS has been shown to increase the activity of these antioxidant enzymes in lung tissue.[65,66] Our findings did not reveal significant differences between all groups in serum NO, T-SH. Serum-measured variables can typically be influenced by a wide range of variables, whereas tissue-measured variables are more specific and less influenced by other variables. As a result, it appears that the impacts of other components in serum are one of the potential causes of the lack of significant changes in the factors mentioned above between different groups.

The impacts of QS on asthma are comparable to the effects of many flavonoids, such as hesperetin,[67] naringenin,[68] and resveratrol.[69]

Our findings showed that QS exerts its protective effects against asthma by reducing inflammation, oxidative stress, and tissue remodeling. It seems that modulation of the NF-κB pathway and regulation of Th1/Th2 balance are the other main mechanisms of QS action in asthma.[70]

In general, our data demonstrated that QS has strong asthma-protective effects. Our results demonstrated the antioxidative and anti-inflammatory impacts of QS on lung tissue. Finally, additional molecular and physiological research is required to evaluate the precise mechanisms driving QS's effects.

STATEMENT OF ETHICS

This animal study has been approved by the ethics committee of Kerman University of Medical Sciences, Kerman, Iran (IR.KMU.REC.1400.244).

FUNDING

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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