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Formulation and Evaluation of the Anti-inflammatory, Anti-oxidative, and Anti-remodelling Effects of the Niosomal Myrtenol on the Lungs of Asthmatic Rats

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ABSTRACT

Asthma is a common chronic allergic disease that affects a significant percentage of the world's population. Niosomes are nanoparticles consisting of non-ionic surfactants that can be used for drug delivery. This research was designed to investigate the impacts of inhalation of simple and niosomal forms of myrtenol against adverse consequences of asthma in rats.

Asthma induction was performed via injection of ovalbumin, followed by its inhalation. Niosomes were created by a heating protocol, and their physicochemical features were evaluated. Forty-nine male Wistar rats were allotted into 7 groups (n=7 each): Control (CTL), vacant niosome (VN), Asthma, Asthma+VN, Asthma+SM (simple myrtenol), Asthma+NM (niosomal myrtenol), and Asthma+B (budesonide). Lung remodeling, serum immunoglobulin E (IgE), inflammatory and cytokines, and antioxidant factors in the lung tissue and bronchoalveolar fluid (BALF), as well as), were evaluated.

The results showed that myrtenol-loaded niosomes had appropriate encapsulation efficiency, kinetic release, size, and zeta potential. The thickness of the epithelial cell layer in the lungs, as well as cell infiltration, fibrosis, IgE, reactive oxygen species, interleukin (IL)-6, and tumor nuclear factor alpha (TNF-α) levels, decreased significantly. In contrast, superoxide dismutase and glutathione peroxide activity increased significantly in the serum and BALF of the treated groups. The niosomal form of myrtenol revealed a higher efficacy than simple myrtenol and was similar to budesonide in ameliorating asthma indices.

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Inhalation of simple and niosomal forms of myrtenol improved the detrimental changes in the asthmatic lung. The niosomal form induced more prominent anti-asthmatic effects comparable to those of budesonide.

Keywords: Allergic asthma; Inflammation; Histopathological changes; Niosomes Myrtenol; Oxidative stress

INTRODUCTION

Asthma is often accompanied by inflammation in the airways and airway narrowing. The prevalence of asthma is approximately 10% in adults in developed societies, with a higher prevalence in children. The prevalence in developing societies is low but increasing quickly.[1,2] In Iran, the average prevalence is 8.9% in adults and 13.4% in children.[3] Inhaled corticosteroids are the essential therapy for alleviating asthma, and most signs of the disease are relieved by these drugs alone or combined with long-acting β-adrenergic agonists. However, 5% to 10% of asthmatic patients are highly resistant to treatment, which leads to an increase in their healthcare costs and death rate. Therefore, finding medicines with minimal side effects and higher efficacy will greatly benefit the healthcare economy.[4] In this study, we used budesonide as a gold standard drug. This synthetic drug is a strong glucocorticoid that is administered via inhalation for airway inflammation. A high first-pass metabolism and minimal systemic side effects are among the advantages of this drug.[5,6]

In response to external allergens, the production of immunoglobulin (Ig) E, the strongest predictor of bronchial asthma progression, is increased.[7] Interleukin (IL) 6 and tumor necrosis factor-alpha (TNF-α), produced by type 2 helper T and mast cells, are key cytokines in asthma pathogenesis. These cytokines cause airway inflammation by activating eosinophils, macrophages, and dendritic cells.[8] These cytokines can cause airway remodeling by increasing fibroblast proliferation and activation. They also enhance mucus production by activating B cells, producing immunoglobulins, and activating airway epithelial and goblet cells.[9] Inflammatory cells like eosinophils and macrophages increase the production of reactive oxygen species (ROS). Therefore, oxidative stress also rises in patients with asthma.[10] ROS, in turn, can damage lipids, DNA, carbohydrates, and proteins, cause disruption in cellular performance, and enhance inflammation, which is characteristic of allergic asthma.[11]

Herbal medicine is a method for the treatment of diseases.[12-15] *Myrtus communis* is a well-known traditional herb.[16] Investigations have revealed that this plant has antioxidant, anti-inflammatory, analgesic, neuroprotective, anti-aging, and anti-diabetic properties.[17] Myrtenol is one of the active ingredients to which the effects of this plant are often attributed.[18] Our earlier findings have demonstrated that intraperitoneal injection of myrtenol improves pathological alterations in the lungs and the damaged epithelium of the bronchial wall and decreases the inflammatory parameters of lung tissue (e.g., TNF-α and IL-6) and the airways of rats with asthma.[19,20] Additionally, it improves the balance between oxidant and antioxidant agents. Some other studies have shown that myrtenol reduces pulmonary and wound fibrosis.[21,22] However, the inhalation method is usually used for basic therapy, inflammation control, or prevention of bronchoconstriction in asthma, owing to the need for smaller doses of medicines and reduced side effects. Moreover, rapid clinical response and fewer negative impacts are seen as a result of the targeted delivery.[23]

Niosomes are lipid-based vesicular nanoparticles consisting of non-ionic surfactants with cholesterol and are important routes of drug delivery. These nanocarriers of two-layer non-ionic surfactants have been used in chemotherapy since 1980. They have high chemical stability and low price and toxicity.[24] The other benefits of niosomes are their biodegradability and the lack of immunogenicity.[25] Multiple methods have been introduced for niosome preparation. These methods include ether injection, thin-film hydration, microfluidization, and heating.[26] Among the described methods, the heating method uses no toxic solvents or detergents, does not need sterilization, and can be scaled up.[27] Niosomal inhalation administration of some drugs, such as beclomethasone and gemcitabine, has been used for the treatment of lung cancer and chronic obstructive pulmonary disease.[28,29] We have demonstrated the beneficial effects of the systemic administration of a simple form of myrtenol in the treatment of

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experimental asthma.[19,20] In this research, we formulated a niosomal form of the compound and assessed its effectiveness when administered via inhalation in asthma rat models. In this context, the ameliorating effects of niosomal myrtenol were assessed and compared with inhaled simple myrtenol and budesonide, the commonly used standard inhalation drug in treating allergic asthma.

MATERIALS AND METHODS

Niosome Synthesis

The synthesis of niosomes was performed using the heating method.[30,31] Briefly, a mixture containing myrtenol (Sigma Aldrich, USA), glycerol (Sigma Aldrich, USA) (3% v/v), and normal saline was heated and stirred at 60°C and 1000 rpm for 5 minutes.

To incorporate cholesterol (Merck, Germany) into the niosomes, the cholesterol dispersion (30% of total lipid) was added to the mixture and heated to 120°C while stirring at 1000 rpm for 15 minutes. Next, equal amounts of Tween 40 and Span 40 (Merck, Germany) (70% of total lipid) were added to the mixture while heating at 60°C for 1 hour. After sonication and passing through a 0.2-μm filter, the compound was administered to the rats. This intervention reduces their size (around 100 nm) and makes them more uniform.

Size and Zeta Potential Measurement of Niosomes

The vesicle size dispersion of niosomes was assessed by the dynamic light scattering (DLS) method using a particle size analyzer (Cordouan, VASCO II, France). The polydispersity index was used as the size dispersion index. The zeta potential of the vesicles was evaluated by a zeta potential analyzer (Cordouan, WALLIS model, France).

The Evaluation of Encapsulation Efficiency

Sample centrifugation (20000*g*, 4°C, 45 min) was performed to evaluate the entrapment efficiency (EE). Spectrophotometry at 247 nm was used to determine free and total myrtenol concentration in the supernatant and pellet. The EE percentage was calculated using the following formula:[32]

$$EE\% = \frac{\text{Total content} - \text{Free content}}{\text{Total content}}$$

In vitro Release

After niosome synthesis, sample centrifugation was performed (25000*g*, 4°C, 45 minutes). The supernatant was discarded, a normal saline volume equivalent to the initial volume of the sample was added to the pellets, and resuspension was performed. A dialysis bag was used for release assessment. Sampling from the normal saline in the beaker and around the dialysis bag was done at various times up to 21 hours. Spectrophotometry at 247 nm was used to check the release of myrtenol from the niosomes.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) (EM208S 100KV, Philips, Netherlands) was performed to study vesicle morphology (magnification ×16700).

Animals

The study protocols were approved by Kerman University of Medical Sciences, Kerman, Iran (ethics code: IR.KMU.REC.1398.295). Forty-nine adult male Wistar rats (weight 200–250 g) were maintained under standard conditions. The rats were allocated to 7 groups (n = 7 in each group). The groups included: CTL (healthy animals), VN (healthy animals that inhaled niosomes without myrtenol, i.e., vacant niosome), Asthma (animals with asthma that received no treatment), Asthma+VN: animals with asthma that received niosomes without myrtenol (vacant niosome), Asthma+SM: animals with asthma that received simple myrtenol (8 mg/kg), Asthma+NM: animals with asthma that received myrtenol-loaded niosomes, and Asthma+B: animals with asthma that received 2.5 mL (41 μg) budesonide (Pulmicort, AstraZeneca Co. UK) as gold standard[33] (positive control group).

Asthma Induction and Treatment Protocols

Myrtenol (obtained from Sigma) was dissolved in dimethyl sulfoxide (DMSO). Figure 1 shows the study timeline. On days 1 and 7, all animals with asthma received 1 mg of ovalbumin (OVA; Sigma Aldrich, USA) for sensitization plus 200 μg of aluminum hydroxide (Sigma Aldrich, USA) dissolved in sterile phosphate-buffered saline (PBS) (0.5 mL) via intraperitoneal injection. On day 14, the animals that received OVA intraperitoneally started inhaling 1% OVA aerosolized with a nebulizer (Omron CX3, Japan) every other day for 4 weeks in a chamber (30 × 50 × 60 cm) for 30 minutes each time.[20,34,35] This is a model for

allergic asthma, as OVA acts as an allergen. The animals in the treatment groups inhaled niosomes without myrtenol (vacant niosomes), simple myrtenol, or myrtenol-loaded niosomes for 1 week (30 minutes per day from day 43 to 49) (19). Approximately 2 mg of myrtenol (8 mg/kg) and 40 µg of budesonide (160 µg/kg) were administered to each rat in 30 minutes, based on the determination by the formula below:

$$D=(C\times MV\times T)/BW$$

where D is the dose of compound (mg/kg), C is the concentration of the compound in the exposure chamber (mg/L), T is the time of exposure (min), BW is the body weight (kg), and MV is the animal minute volume (L/min).[36]

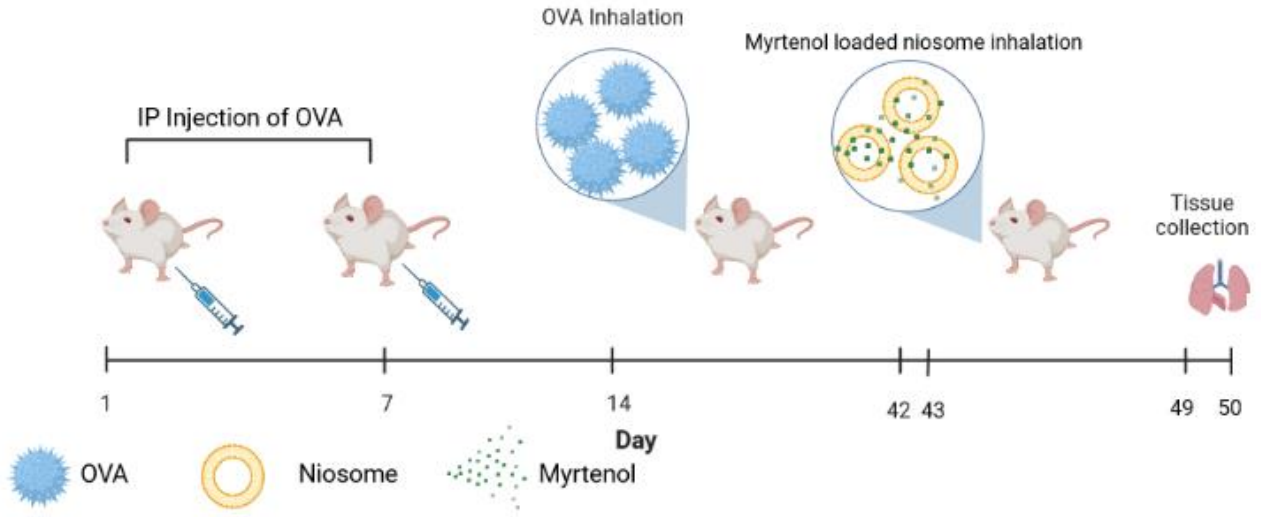

Figure 1. Timeline diagram demonstrating the intervention protocol used in the study groups.

Broncho-alveolar fluid (BALF) Collection

Euthanasia was performed under deep anesthesia with high doses of ketamine (80 mg/kg) and xylazine (50 mg/kg). To collect BALF, a catheter was inserted into the left main bronchus of the animal while the right main bronchus was clamped, and 2.5 mL of normal saline was injected into the left lung. The injected normal saline was then aspirated into the syringe after 2 minutes. The collected BALF (75% to 80% of the injected saline) was centrifuged at 1000*g* for 10 minutes at 4°C, and the supernatant was used to evaluate the oxidative stress and inflammation parameters. PBS (50 µL) was added to the sedimentation and shaken. Then 10 µL of the suspension was smeared on a glass slide. Then Giemsa staining was performed to assess the BALF cells.[20,37]

Lung Histopathology

After euthanasia, the right lungs were removed and sectioned into slices, and hematoxylin/eosin (H&E), Masson trichrome, and Giemsa staining were performed. The sections were assessed by a blinded pathologist[20,37] to evaluate the pathological properties in the lungs. In H&E stained specimens, alveolar macrophage count was scored as 0 = absence, 1 = 1–4 cells, 2 = 5–9 cells, 3 =10 cells, and 4 = abundant cells [38]. Giemsa staining was used to assess the precise number of mast cells in lung tissue and macrophages in the BALF.[19] Masson trichrome staining was used to measure tissue fibrosis according to the scoring system described by Hübner et al.[39]

Biochemical Evaluations in BALF and Lung Tissue

The Bradford protocol was used for the assessment of total protein. The superoxide dismutase (SOD) and Glutathione peroxidase (GPx) activities were determined by kits from Navand Salamat Co., Iran. ROS levels were assessed based on the kit′s instructions (Kushanzist Co., Iran). Evaluation of IL-6 and TNF-α was performed using enzyme-linked immunosorbent assay (ELISA; Karmania Pars Gene Co., Iran). Serum

IgE was determined using the related kit (Abcam Co, UK) according to the manufacturer's protocol. An ELISA plate reader (Model ELX-80 MS, Biotech, USA) was used for sample analysis[19,20,40].

Dose-Response Study

Inhalation of simple myrtenol by asthmatic rats (2, 4, 8, and 16 mg/kg) was performed for the dose-response study. The effective dose was the lowest, with high potency in reducing inflammation at both biochemical and histological levels (8 mg/kg) (Figure 2).

Statistical Analysis

The data are shown as a mean ± SEM. The Shapiro-Wilk test was performed to check for normal distribution. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for data analysis. The significance level was considered $p<0.05$.

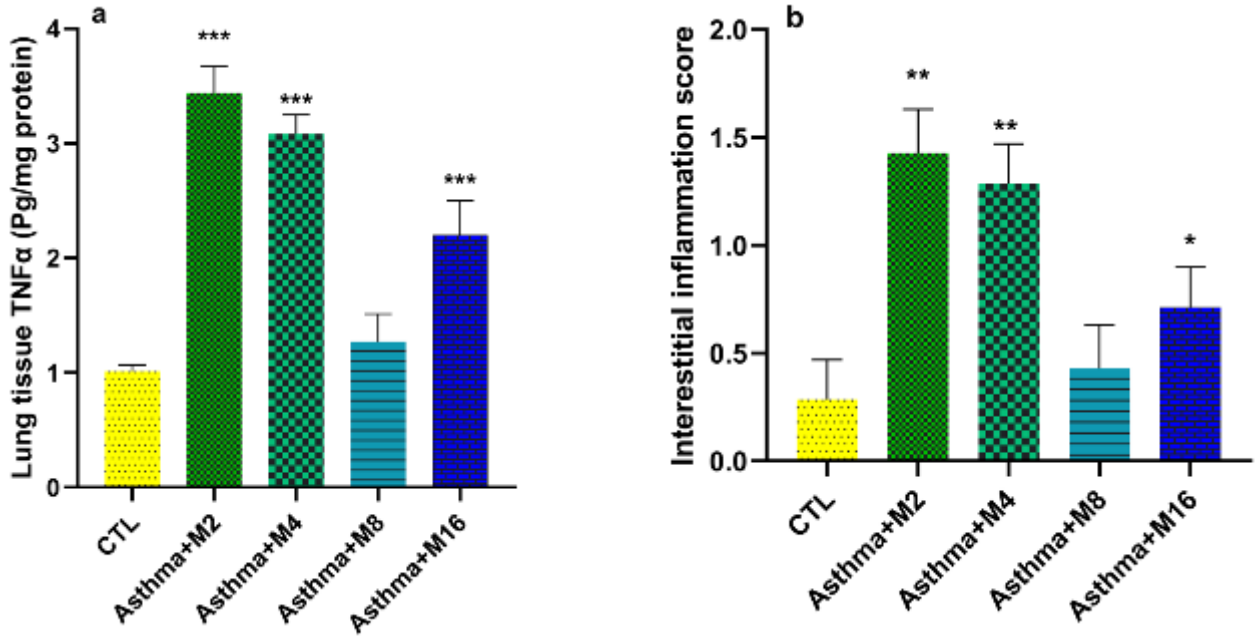

Figure 2. Dose-response results for determining the optimum dose of niosomal myrtenol inhalation according to the effects on the lung tissue TNF-α((Tumor necrosis factor α) (a) and interstitial inflammation (b). The lowest dose of myrtenol that exhibited maximum efficacy was 8 mg/kg among 2 (M2), 4 (M4), 8 (M8), and 16 (M16) mg/kg doses administered. CTL: control group. Data are reported as mean ± SEM (n=7 per group). *** $p < 0.001$, ** $p < 0.01$ and, * $p < 0.05$ vs. CTL.

RESULTS

The Results Related to Niosomes

The mean size of non-ionic surfactant nanocarriers is an important index influencing their biological and physical features as well as entrapment efficiency. The range of niosome particle size was 420±87 nm for niosomes without myrtenol and 412±73 nm for niosomes with myrtenol. DLS estimations revealed that the niosomes were approximately uniform in size and shape.

The zeta potential is an important parameter of the relations between colloidal vesicles. The zeta potential indicates that particles are inclined to disperse each other, meaning that there will be little inclination for the vesicles to aggregate. This demonstrates the niosomes suspension in water, which is vital for their storage. In this research, the zeta potential was found to be −27.4±3.1 mv for niosomes without myrtenol and −24.9±4.3 mv for niosomes with myrtenol. Negative and high zeta potential values are indices of stable formulations. After loading myrtenol, the zeta potential of the niosomes with myrtenol showed a nonsignificant

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increase. The TEM images of the chosen samples are illustrated in Figure 3. The morphology of the prepared niosomes revealed an almost spherical shape and a unilamellar membrane.

One of the remarkable physicochemical indices in niosomes is EE. A high EE guarantees the bioavailability of nutraceuticals. With an increase in EE, the amount of myrtenol in the niosomes increases. The EE of the formulation was 81.33%±0.38%.

The results of the evaluation of the release pattern of myrtenol from the niosomal formulae are depicted in Figure 3. For drug delivery systems, the drug release rate is an essential index. Figure 4 shows the in vitro release of simple myrtenol and the myrtenol in the niosome formulation. The biphasic kinetic release of myrtenol was comprised of an early moderately rapid release and a plateau state (slower release phase). Almost 100% of the free (i.e., simple form) myrtenol was released during 3 hours; however, a controlled release of the niosomal form was seen for several hours.

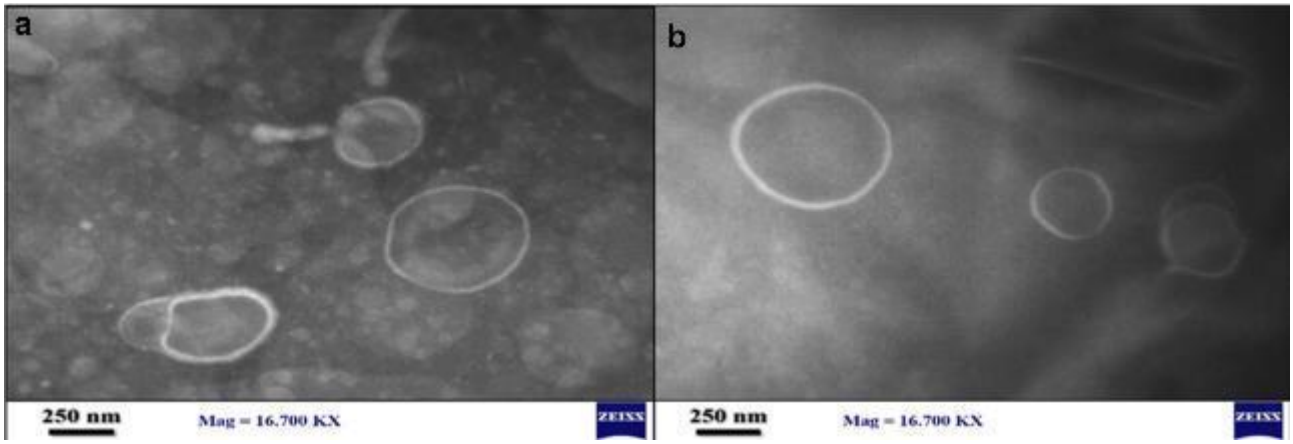

Figure 3. Transmission electron microscopy (TEM) image of vacant niosome (a) and myrtenol-loaded niosome (b) formulations. Original magnification: ×6700.

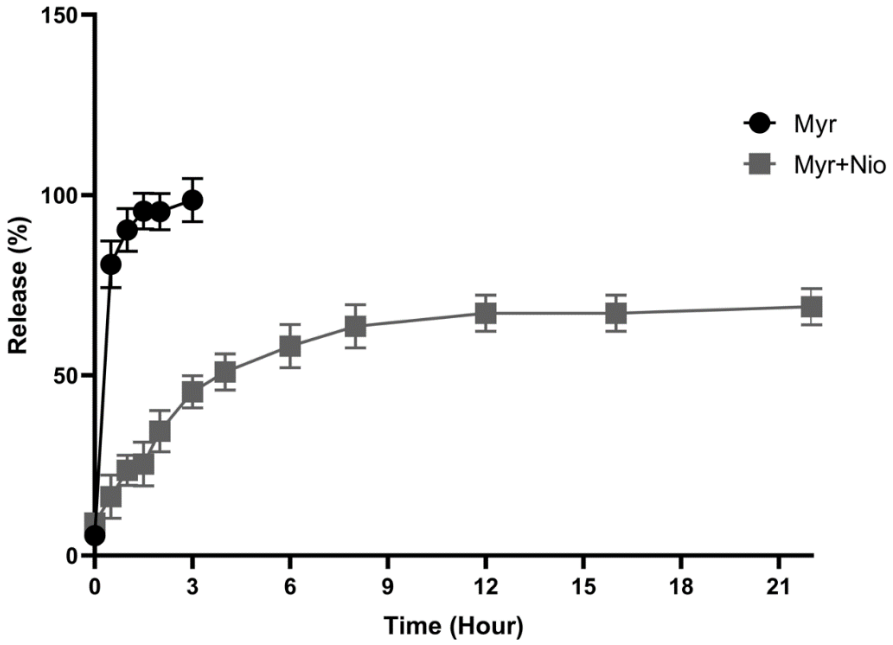

Figure 4. Myrtenol release pattern from niosomes. Cumulative release (%) of the drug from vesicles (Myr+Nio) is gradual, but simple myrtenol (Myr) is released quickly. Myr+Nio = niosomal myrtenol.

Assessment of BALF and Lung Tissue Cytokines and Serum IgE

In asthmatic rats, the levels of TNF-α and IL-6 were remarkably higher compared to the CTL groups (p<0.001 for all groups). Treatment with simple and niosomal myrtenol resulted in a noticeable reduction in the levels of TNF-α (p<0.001) and IL-6 in tissue and BALF (p<0.05 and p<0.001) (Figure 5a-d). In this section, the results showed that niosomal myrtenol reduced inflammatory cytokines in both tissue and BALF more effectively than simple myrtenol. Our results demonstrated that serum IgE levels increased in asthmatic rats (p<0.001), while treatment with simple and niosomal myrtenol significantly reduced serum IgE levels towards the control level (p<0.001). Furthermore, niosomal myrtenol was more effective than the simple form in suppressing IgE levels (Figure 5e).

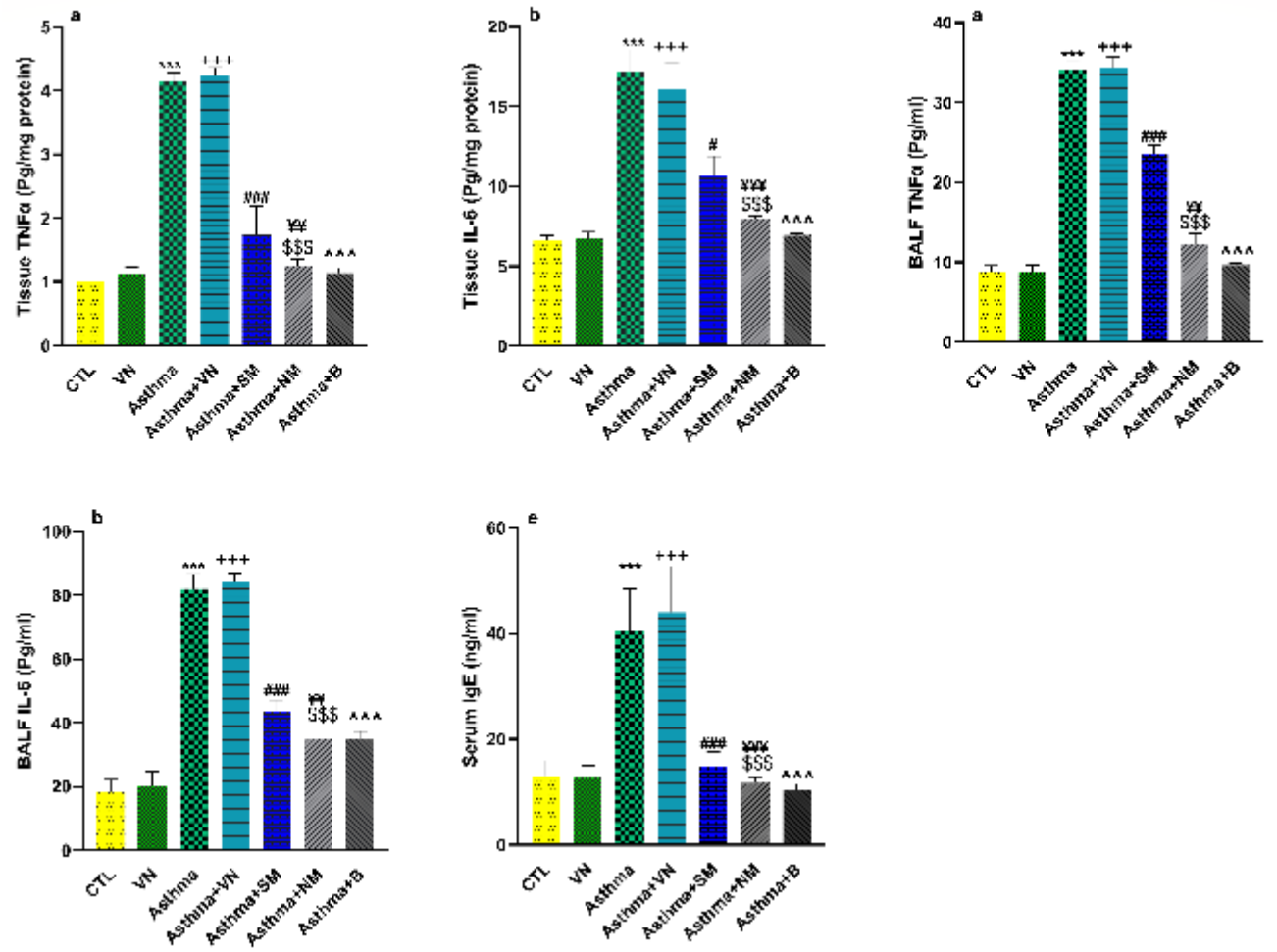

Figure 5. ELISA (Enzyme-linked immunosorbent assay) analyses of inflammatory cytokines and IgE (Immunoglobulin E) in the lung tissue, BALF (Broncho alveolar lavage fluid), and serum of different groups. Asthma significantly increased the levels of TNF-α (Tumor necrosis factor α) (a and c) and IL-6 (Interleukin 6) (b and d) in the lung tissue and BALF. Data are presented as mean±SEM (n=7 rat per group). CTL: control; VN: control+vacant noisome; Asthma+VN: asthma+vacant niosome; Asthma+SM: asthma+simple myrtenol (8 mg/kg); Asthma+NM: asthma+niosomal myrtenol (equivalent to 8 mg/kg myrtenol); Asthma+B: asthma+budesonide (41 µg). *** p<0.001 *vs.* CTL, +++ p<0.001 *vs.* VN, # p<0.05 and ### p<0.001 *vs.* Asthma, $$$ p<0.001 *vs.* Asthma+VN, ¥¥ p<0.01 & ¥¥¥ p<0.001 Asthma+NM *vs.* Asthma+SM, ^^^ p<0.001 Asthma+B *vs.* Asthma+SM.

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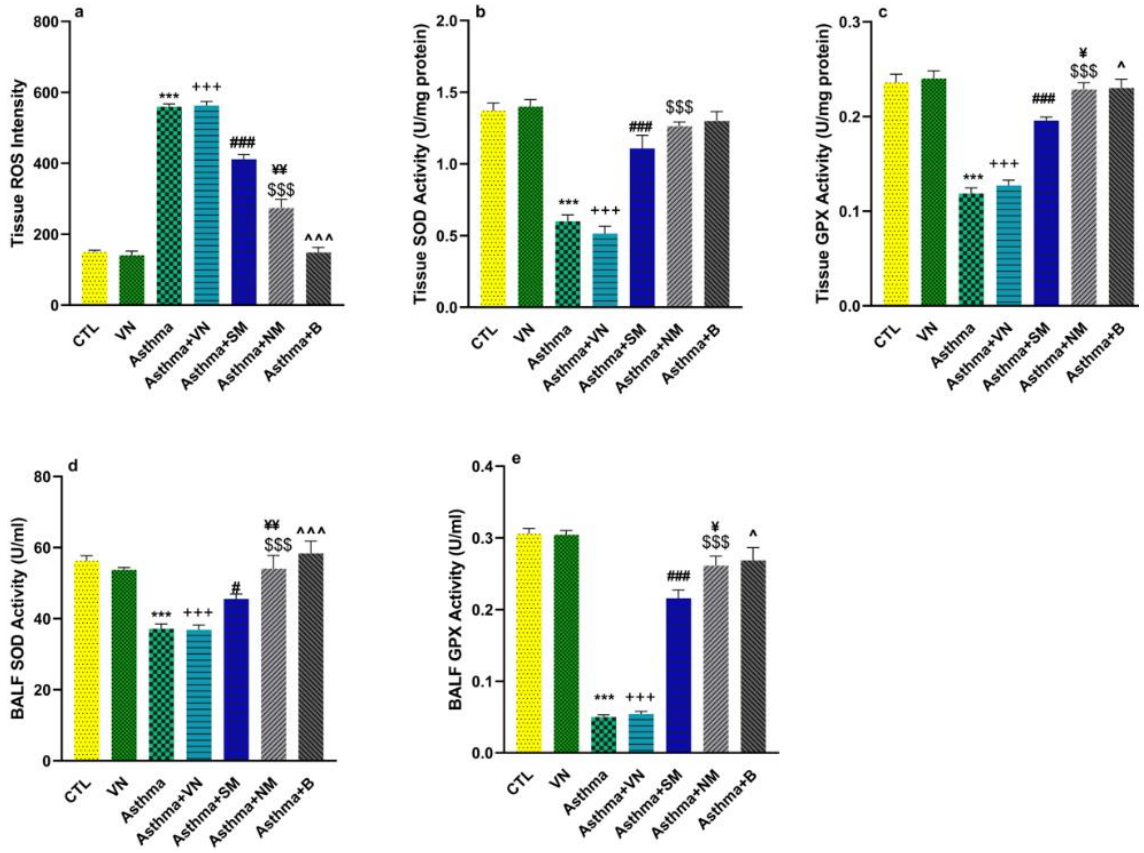

Figure 6. Alterations of the reactive oxygen species (ROS) and antioxidant enzymes in the lung tissue and BALF (Broncho alveolar lavage fluid) in different study groups. ROS (a), Superoxide dismutase (SOD) activity (b and d), Glutathione peroxidase (GPx) activity (c and e). Data are presented as mean±SEM (n=7 rats per group). CTL: control; VN: control+vacant niosome; Asthma+VN: asthma+vacant niosome; Asthma+SM: asthma+simple myrtenol (8 mg/kg); Asthma+NM: asthma+niosomal myrtenol (equivalent to 8 mg/kg myrtenol); Asthma+B: asthma+budesonide (41 µg). *** $p < 0.001$ *vs.* CTL, +++ $p < 0.001$ *vs.* VN, # $p < 0.05$ and ### $p < 0.001$ *vs.* Asthma, $$$ $p < 0.001$ *vs.* Asthma+VN, ¥ $p < 0.05$ and ¥¥ $p < 0.01$ Asthma+NM *vs.* Asthma+SM, ^ $p < 0.05$ and ^^^ $p < 0.001$ Asthma+B *vs.* Asthma+SM.

The Impacts of Treatments on Oxidative Stress

In asthmatic rats, the SOD and GPx activities in lung tissue and BALF decreased significantly compared to the CTL and VN groups (p<0.001), while the amount of ROS in the lung tissue increased (p<0.001). Treatment by simple and niosomal myrtenol inhalation for 1 week significantly restored these alterations toward normal (Figures 6 a-e). In this part, the findings revealed that the niosomal myrtenol in all parameters, except tissue SOD activity, had a more prominent effect than the simple myrtenol.

Histopathology Findings

Tissue remodeling in animals with asthma included elevated interstitial inflammation (p<0.05), bleeding (p<0.05), emphysematous changes (p<0.01), epithelial damage (p<0.001) (Figure 7h), thickening of the bronchiole epithelium (P<0.01) (Figure 7k), and increased number of macrophages in lung tissue (p<0.05) and alveolar macrophage score (p<0.05) (Figures 7i and 7j) compared with the control groups. One week of remedy with simple and niosomal forms of myrtenol recovered these impairments to a great extent (p<0.05 for all indices). In addition, Masson's trichrome staining revealed fibrotic changes owing to collagen sedimentation in the lung tissue of asthmatic rats (p<0.001) (Figure 8h), and Giemsa staining revealed increased lung tissue mast cells (p<0.05) (Figure 9h) and BALF macrophage infiltration (p<0.001) (Figure 7i) compared to the control groups. All these unfavorable alterations were dramatically ameliorated by inhaling simple and niosomal myrtenol.

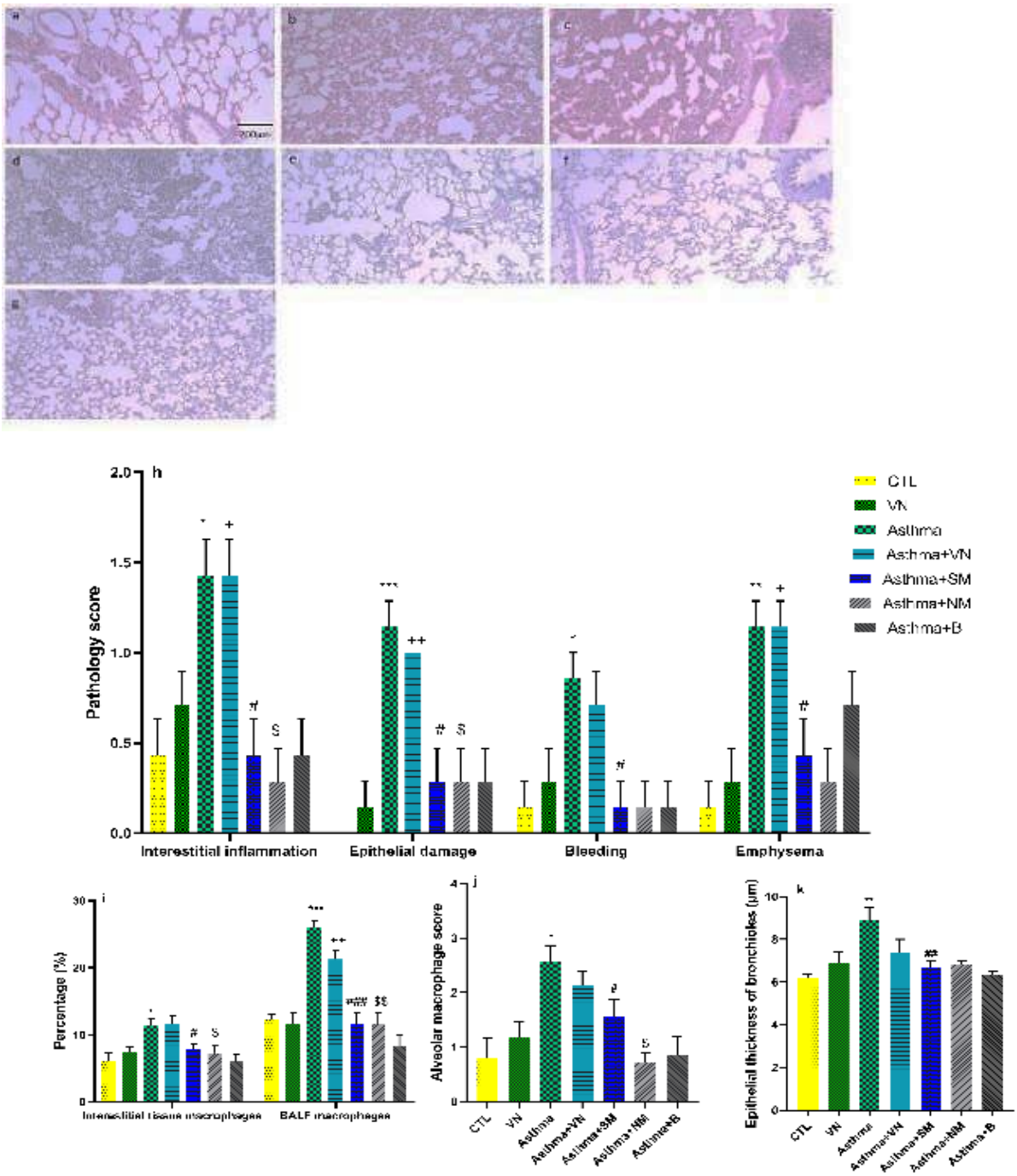

Figure 7. Micrographs of the lung stained with H&E (Hematoxylin & Eosin) showing the effect of inhaled simple and niosomal forms of myrtenol on interstitial inflammation, bleeding, emphysema, and epithelial damage (h), lung interstitial, BALF, and alveolar macrophages (i and j), and epithelial thickness of bronchioles (k) in asthmatic rats in different groups (n=7 in each group) (magnification: ×100, Scale bar: 200 µm). Data are presented as mean±SEM. a: CTL (control); b: VN (control+vacant noisome); c: Asthma; d: Asthma+VN; asthma+vacant niosome; e: Asthma+SM; asthma+simple myrtenol (8 mg/kg); f: Asthma+NM: asthma+niosomal myrtenol (equivalent to 8 mg/kg myrtenol); g: Asthma+B: asthma+budesonide (41 µg). * p<0.05, ** p<0.01, *** p<0.001 *vs.* CTL, + p<0.05, ++ p<0.01 *vs.* VN, # p<0.05, ### p<0.001 *vs.* Asthma, $ p<0.05, $$$ p<0.001 *vs.* Asthma+VN.

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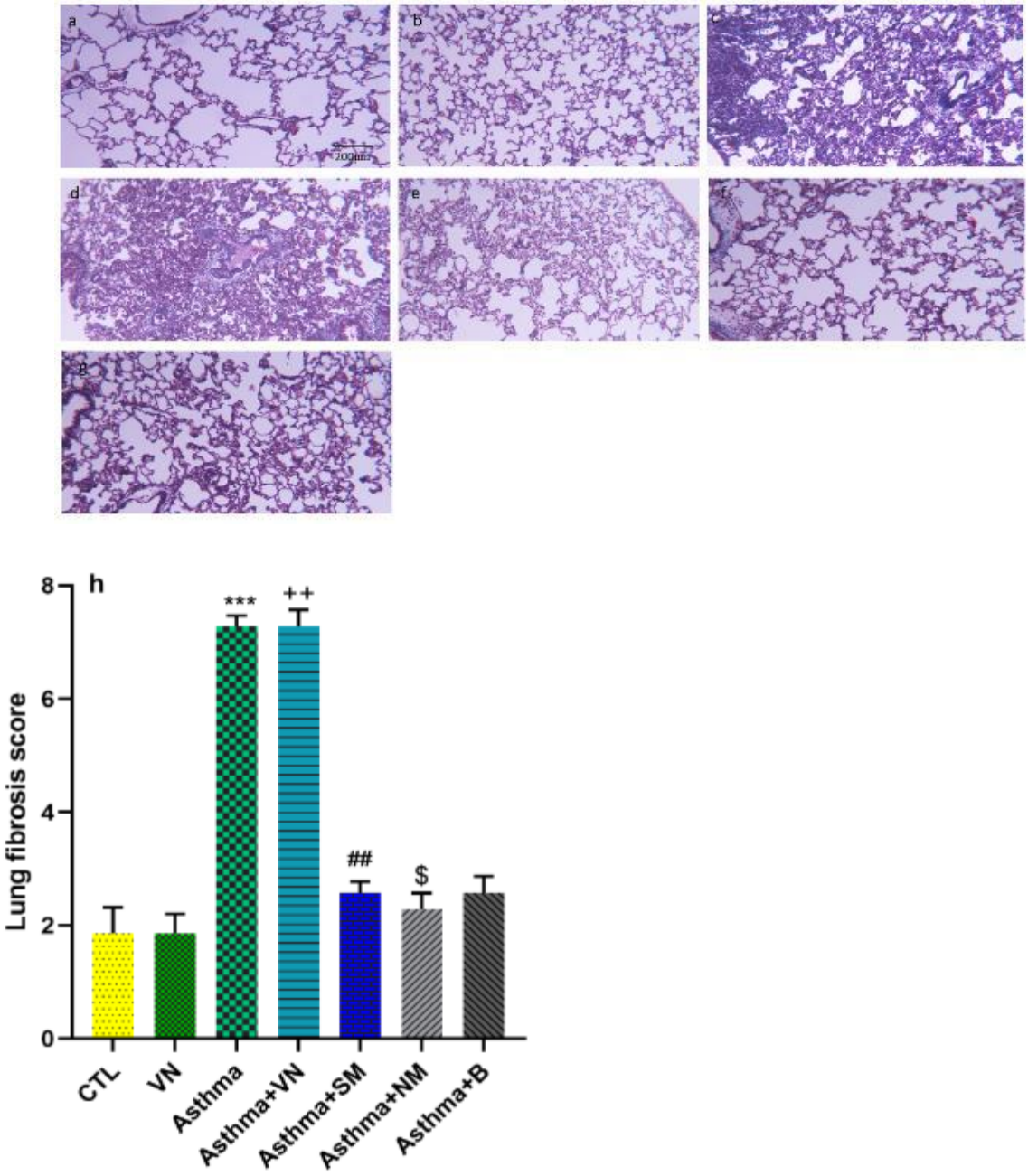

Figure 8. Micrographs of the lung stained with Masson trichrome showing the effect of inhalation of myrtenol on lung fibrosis. Data are presented as mean ± SEM (*n* = 7 in each group). (Magnification: ×100, Scale bar: 200 µm) a: CTL = control; b:VN = control+vacant noisome; c: Asthma; d: Asthma+VN = asthma+vacant noisome; e: Asthma+SM = asthma+simple myrtenol (8 mg/kg); f: Asthma+NM = asthma+niosomal myrtenol (equivalent to 8 mg/kg myrtenol); g: Asthma+B = Asthma+budesonide (41 µg); h: Quantitative data of lung fibrosis score in study groups. * *p*<0.001 *vs.* CTL, ++ *p*<0.01 *vs.* VN, ## *p*<0.01, *vs.* Asthma, $ *p*<0.05, *vs.* Asthma+VN.**

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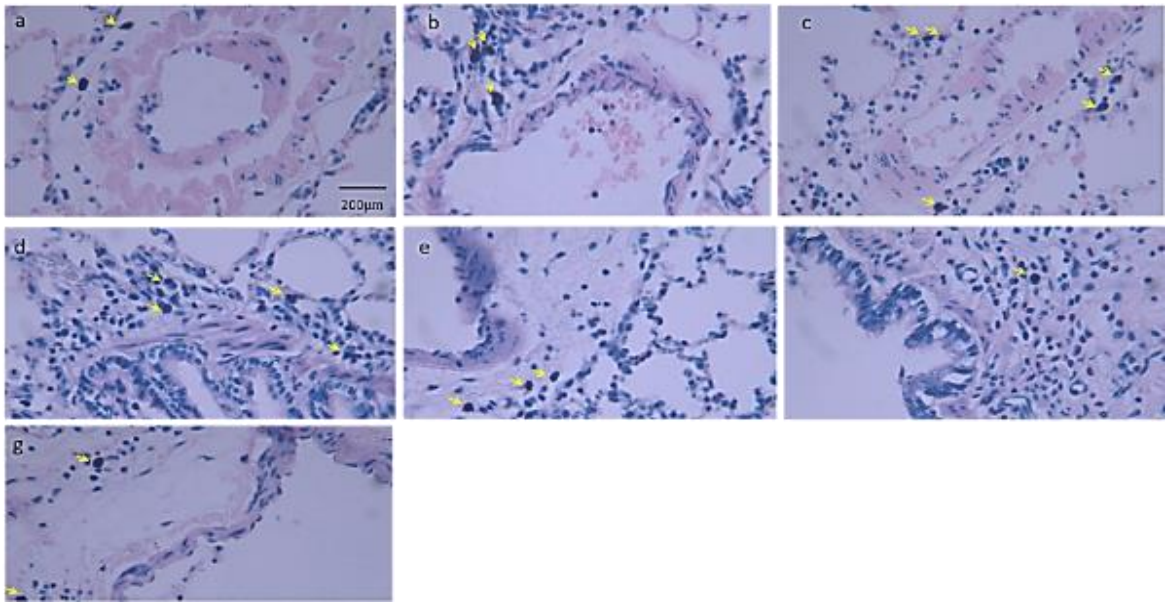

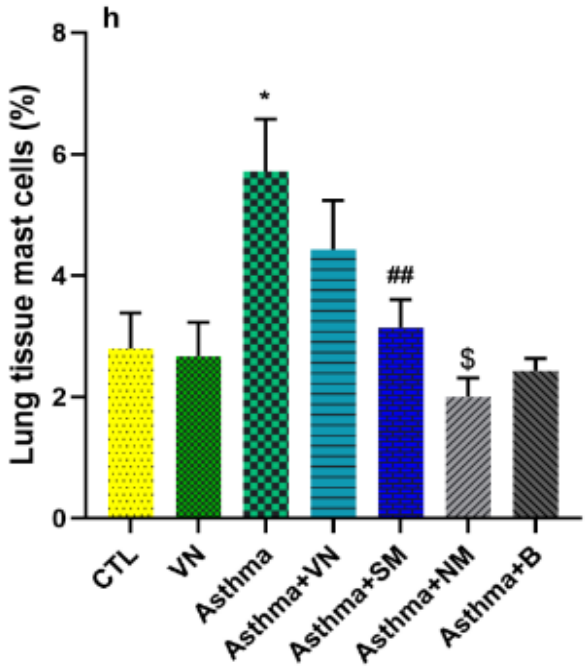

Figure 9. Micrographs of the lung stained by Giemsa showing the effect of inhalation of myrtenol on lung tissue mast cells. Data are presented as mean±SEM ($n = 7$ in each group). (Magnification: ×100, Scale bar: 200µm). a: CTL (control); b: VN=(control+vacant noisome); c: Asthma; d: Asthma+VN=Asthma+vacant noisome; e: Asthma+SM=asthma+simple myrtenol (8 mg/kg); f: Asthma+NM=asthma+niosomal myrtenol (equivalent to 8 mg/kg myrtenol); g: Asthma+B=asthma+budesonide (41 µg); h: Quantitative data of lung tissue mast cells in the study groups. * p<0.05 *v.s* CTL, ## p<0.01, *vs.* Asthma, $ p <0.05, *vs.* Asthma+VN.

DISCUSSION

The purpose of this research was to synthesize a niosomal form of myrtenol and to evaluate and compare the effects of inhaling this form of myrtenol with its simple form on oxidative and inflammatory mediators and histological indices in asthmatic rats. The results demonstrated that treatment through inhalation of niosomal myrtenol induced more prominent ameliorative impacts in the lungs of asthmatic animals.

Niosomes are nanoparticles with great stability, ease, and low cost of production, and the potential to be scaled up. They are an appropriate carrier for the delivery of drugs in both hydrophobic (associated with the lipid bilayer) and hydrophilic (encapsulated in the aqueous interior compartment) forms.[41,42]

Previous studies have reported successful inhaled administration of the niosomal form of clarithromycin, salbutamol, retinoic acid, ciprofloxacin, and rifampicin.[28,43] Niosomes can stay inside the respiration system for a longer time owing to their inclination to gel formation.[44] Similarly, Wong et al. showed that niosomes loaded with curcumin downregulate mRNA expression of some cytokines (IL-6, IL-8, IL-1β, and TNF-α) in asthma.[45] Consistent with the mentioned investigations, our findings showed that myrtenol-loaded niosomes had a significant effect, comparable to budesonide, in reducing inflammation and oxidative stress in allergic asthma.

TNF-α has been demonstrated to be a crucial mediator in asthma.[46] IL-6 is also regarded as a byproduct of continued inflammation in the lungs.[47] In the present study, we found that myrtenol in the form of simple and niosomal inhalation reduced TNF-α and IL-6, with the niosomal form showing higher potency. Also, when inhaled, both forms were found to be more effective than when administered via the systemic route, which we had used previously against the same model of asthma.[19,20] Furthermore, it has been shown that niosomal formulations of some medications, including sodium diclofenac, nimesulide, flurbiprofen, and ammonium glycyrrhizinate, demonstrate more anti-inflammatory activity than their simple formulations.[48,49]

ROS has deleterious effects on the airways. SOD and GPx are antioxidants that neutralize these effects of ROS.[50] Our previous research showed that myrtenol's systemic administration ameliorated SOD and GPx abnormalities in allergic asthma.[19,20] Here, inhalation of niosomal myrtenol also showed its antioxidative effect.

The binding of IgE to its receptor on target cells, like mast cells, triggers the cascade of inflammatory responses leading to the release of inflammatory factors and the occurrence of chronic and acute symptoms of allergic airway diseases.[51] Elevated IgE levels in serum and BALF have been shown in two recent studies.[52,53] Our results demonstrated that serum IgE increased following asthma and that niosomal myrtenol recovered serum IgE levels.

Fibrosis is an important remodeling component of the lungs and airways in asthma. In asthma, the balance between the production and degradation of collagen and extracellular matrix components is shifted in favor of production, resulting in the remodeling of airways.[54] In this research, myrtenol in both simple and niosomal forms ameliorated fibrosis. Whether myrtenol exerts its antifibrotic effects by reducing collagen in the extracellular matrix or acts on the metalloproteinase enzymes or TGF-β expression requires further study. Our results also showed that asthma induces epithelial thickening, and myrtenol also alleviates this abnormality.

In asthma, mast cell activation by allergens generally occurs through IgE binding to its receptor (FcεRI).[55] This triggers a cascade of inflammatory responses, leading to cytokine release and chronic and acute symptoms of allergic airway diseases.[56] Histamine and bioactive lipids are mast cell mediators and account for some of the physiological consequences seen in asthma.[57] Myrtenol decreased mast cells in lung tissue, and niosomal and simple types of myrtenol reduced serum IgE levels. This property can potentiate the alleviating impact of myrtenol.

Macrophages participate in asthmatic inflammation by reducing cytokines.[58] Our findings disclosed an elevation in the number of macrophages in the alveoli and BALF of rats with asthma, while niosomal and simple myrtenol inhalations reduced the number of macrophages.

The results revealed that inhalation of myrtenol has a significant protective effect against asthma (Figure 10). When myrtenol is formulated in niosomal form, it exerts stronger ameliorative effects. This is related to the gradual release of the drug, which increases the duration of exposure of the cells to the drug. This would also decrease the dose or frequency of drug administration and minimize its probable adverse effects.

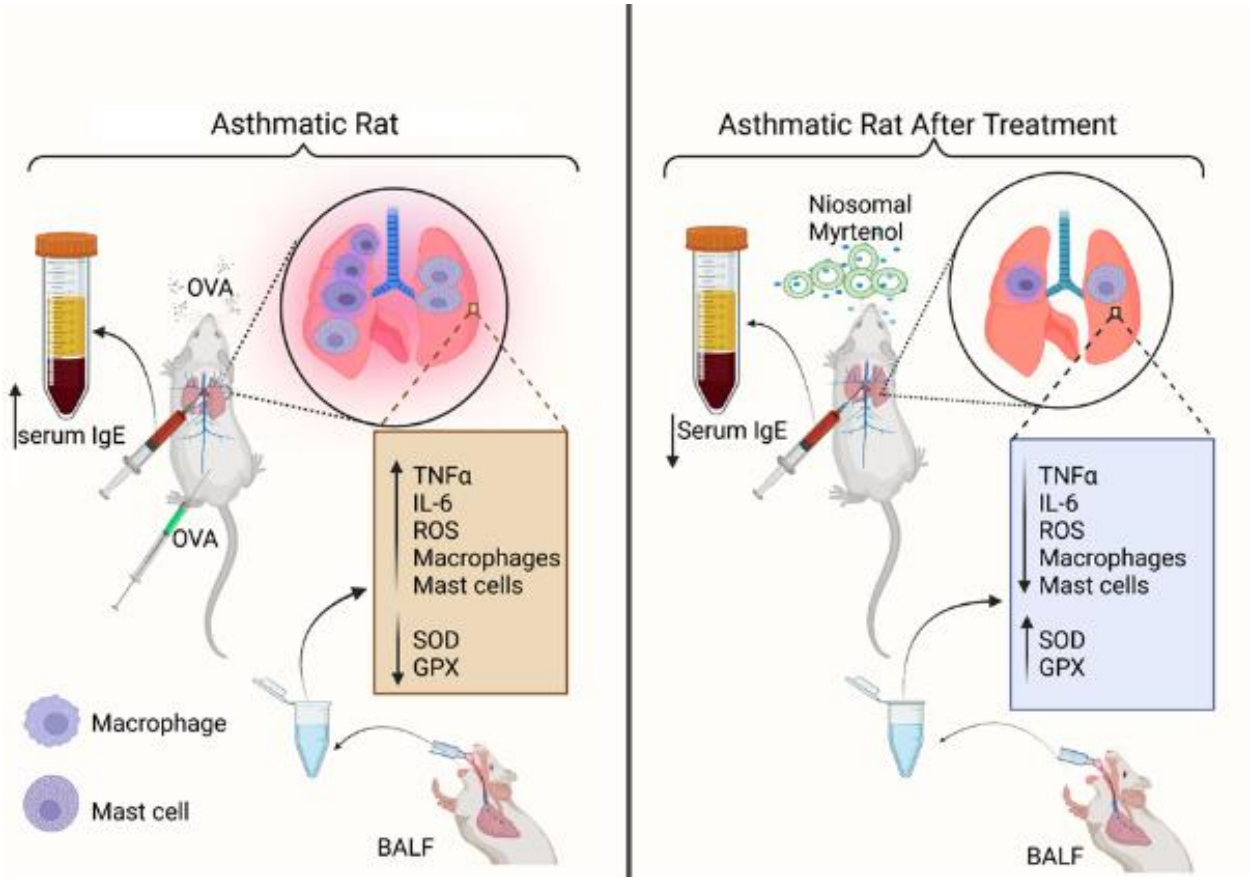

Figure 10. Inhalation of niosomal form of myrtenol could improve the inflammatory and oxidative consequences of asthma in the lung tissue and BALF of male rats.
BALF: bronchoalveolar lavage fluid; OVA: Ovalbumine; IgE: Immunoglobulin E; TNF: tumor necrosis factor; IL6: Interleukine 6; ROS: Reactive oxygen species; SOD: Superoxide dismutase; GPX: Glutathione peroxidase

STATEMENT OF ETHICS

All experimental protocols have been verified by the Ethics Committee of Kerman University of Medical Sciences for animal studies (ethics code IR.KMU.REC.1398.295).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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