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Niosome nanocarrier enhances the ameliorating effects of myrtenol in the lungs of rats with experimental asthma

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ABSTRACT

We assessed the anti-inflammatory, anti-oxidative and anti-remodeling impacts of a synthesized myrtenol-loaded niosome in rats with allergic asthma. Forty-nine rats were divided into seven groups of control, vacant niosome (VN), Asthma, Asthma+VN, Asthma+SM (simple myrtenol), Asthma+NM (niosomal myrtenol, 8 mg/kg), and Asthma+*B* (budesonide, 41 μg). Ovalbumin-induced asthmatic animals were exposed to daily inhalation of drug/vehicle for one week. Histopathology and inflammatory and oxidative stress indices in the lungs were assessed. Myrtenol-loaded niosomes showed appropriate physicochemical properties. Airway smooth muscle thickness, inflammatory cell infiltration, goblet cell hyperplasia, NO, IL-17, and MDA level decreased, and IL-10 and TAC levels increased in tissue and/or BALF of treatment groups. Niosomal myrtenol showed high potency comparable to budesonide in alleviating disease parameters. In conclusion, inhalation of niosomal myrtenol ameliorated inflammation, oxidative stress and tissue remodeling in asthmatic animals more potently than simple myrtenol and could be a target for production of an anti-asthmatic medicine.

1. Background

Asthma is a chronic and inflammatory illness of the pulmonary system, which is related to elevated immune cell infiltration, particularly of eosinophils, in the lung, elevated mucosal secretion, and impairment of pulmonary function [1]. This disorder is accompanied by multiple signs, including inflammation of airways, mucus hyper-secretion, and airway hyper-responsiveness (AHR) [2]. The involvement of IL-17, a T helper17 (Th17)-secreted cytokine, has been demonstrated in the progression of asthma [3]. Th17 lymphocytes play a main role in lung inflammation. These cells produce the IL-17 cytokine, which triggers the smooth muscle of the lung airways [4]. As an anti-inflammatory cytokine, IL-10 is lower in patients with asthma [5]. It has been well demonstrated now that oxidant-antioxidant imbalance also performs an essential role in asthma. Various oxidant parameters are crucial to the inflammatory

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response through augmentation of pro-inflammatory signaling pathways [6]. Asthmatic patients have shown elevated oxidative stress, such as raised MDA (malonildyaldeide) levels [7]. Corticosteroids are utilized as the major medication for the treatment and amelioration of asthma signs, but owing to the chronic nature of asthma and the adverse effects of long-term administration of corticosteroids, it is crucial to find alternative drugs with lower adverse effects [8]. Budesonide, which has anti-inflammatory and anti-oxidant effects, is one of the main drugs used for asthma treatment [9,10].

Many investigations have demonstrated the curative impact of traditional medicines [11–14]. Myrtle (*Myrtus communis*) is a herb that is often utilized globally in traditional herbal medicine [15,16]. The active chemical of Myrtle is a two-ring monoterpene alcohol named myrtenol, which is responsible for the most significant curative impacts of the plant [17].The inhalation route is often utilized to prescribe agents for alleviating pulmonary disorders. By this route the drug can directly access the site of action, resulting in high pulmonary (and low systemic) concentrations [18]. Niosomes, as a new system for drug delivery, can ameliorate the stability and solubility of pharmaceuticals. They are designed for targeting specific cells and releasing pharmaceutical compounds in a controlled manner. Niosomes stay in the bloodstream for a suitable length of time, which is applicable for targeted drug delivery [19,20]. To improve vesicle stability, a biodegradable polymer chain made of polyethylene glycol (PEG) is attached to the surface of the niosomes. PEGylation hides niosomes from the immune system and enables them to circulate longer in the bloodstream. Span-40 is a sorbitan that is often used to solubilize essential oils into water-based products. Polysorbates such as Tween-40 are oily liquids derived from PEGylated Span-40. Due to the structural difference between niosomes and liposomes (absence of Tween in the structure of liposomes), PEGylation is one of the advantages of niosomes compared to liposomes [21,22]. Moreover, compared with other nanocarriers, niosomes have better stability and lower cost and are more easily formulated and scaled-up. Their stability is because of their non-ionic surfactants, which are more physically and chemically stable than those of lipids [23]. Our previous studies showed that systemic administration of myrtenol significantly reduced oxidative stress, inflammation, and tissue remodeling in the lungs of asthmatic rats [24,25]. However, the need for smaller doses of medicine, and reduction in the adverse effects of the systemic route, and the direct release of the drug at the location of the disorder are superior strategies. Considering to the specifications of niosomal drug delivery, in this study, we aimed to produce a niosomal form of myrtenol and investigate its effects compared to the simple form by the inhalation route on the inflammatory, oxidative, and histopathological indices of the lungs of rats with experimental asthma for the first time. The current gold standard drug for the treatment of asthma, budesonide, was also used in a group of animals to evaluate the potency of the niosomal form of myrtenol comparatively.

2. Methods

2.1. Niosome preparation

Myrtenol (purchased from Sigma Aldrich, USA) was dissolved in DMSO 0.5%. For niosome preparation, the heating method was used [26]. Niosomes were prepared at a concentration of 1 mM. Briefly, a mixture including myrtenol (equivalent to 11 mg) and glycerol (ultimate concentration of 3% v/v) was dissolved in 21 ml normal saline while heating (60 °C) and stirred (1000 rpm) on a stirrer (IKA® 104 C. MAG HS7, Malaysia) for 5 min. In order to incorporate cholesterol (Chol) in the vesicles, the Chol (Merck, Germany) (30% in 1 mM total lipid concentration) dispersion was heated (120 °C) while being stirred (1000 rpm) for 15 min. The reminder 70% (equally made by Tween-40 and Span-40, both purchased from Sigma Aldrich, US), was added to the mixture and heated (60 °C) for 1 h. The niosomal samples were left at room temperature overnight to stabilize. A light microscope was used to ensure vesicle formation. Before administration to the animals, the niosomes were sonicated and passed through a 0.2 μm filter.

2.2. Size analysis and assessing zeta potential

The niosome particle size and size distribution were determined by dynamic light scattering (DLS) technique employing a Nano Zetasizer (VASCO particle size analyzer, Cordouan, France). Polydispersity index (PdI) was utilized as an index of size distribution. The zeta potential of vesicles was assessed by a Nano Zetasizer (WALLIS Zeta potential analyzer, Cordouan, France).

2.3. Encapsulation efficiency

Samples (vacant and myrtenol-loaded niosomes) were centrifuged at 20,000 g for 45 min at 4 °C. The concentrations of free and total myrtenol in the supernatant and pellet mixtures were measured by spectrophotometry at 247 nm. The entrapment efficiency (EE) percentage was calculated as follows [27]:

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

2.4. In vitro release

After preparation, vacant and myrtenol-loaded niosomes were centrifuged at 25,000 g at 4 °C for 45 min. After discarding the supernatant, an equivalent volume of normal saline was added to the pellet. Following resuspension, the vesicles were poured into the dialysis bag (12–14 KDa) and maintained for 24 h. The normal saline in the beaker around the dialysis bag was sampled at different times to check the release of myrtenol from the vesicles. The released myrtenol concentration was assayed by spectrophotometry at

247 nm.

2.5. Animals

The research protocols were reviewed and verified by the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (ethics code IR.KMU.REC.1398.295). Forty-nine male Wistar rats (eight weeks old, 200–250 g) purchased from the animal farm of the University were kept at 23 ± 2 °C and 12 h/12 h light/dark cycle. The animals were divided into seven groups ($n = 7$ in each group): 1- CTL, normal rats with no interventions, 2- VN CTL rats, which only inhaled vacant niosome, 3- Asthma, OVA-sensitized rats that had been challenged with OVA and received no treatment, 4- Asthma+VN, asthmatic animals that inhaled vacant niosomes, 5- Asthma+SM, asthmatic animals that inhaled 8 mg/kg simple form of myrtenol, 6- Asthma+NM, asthmatic animals that inhaled 8 mg/kg of myrtenol in noisome-loaded form, and 7- Asthma+*B*, asthmatic rats that inhaled budesonide (AstraZeneca Co) (41 μg in each rat) as the gold standard drug.

2.6. Asthma induction and treatment protocols

Fig. 1 illustrates the experimental protocol and timeline. On days 1 and 7, in asthmatic rats, ovalbumin (OVA, 1 mg) and aluminum hydroxide (200 μg) (both purchased from Sigma Aldrich) dissolved in 0.5 ml PBS were injected to rats (via intraperitoneal injection). The OVA-injected rats inhaled 1% aerosolized OVA for 30 min, every other day from day 14 to 42 for 30 min, in a closed chamber (30 × 50 × 60 cm) utilizing a nebulizer (Omron CX3, Japan) [24,28,29]. Rats in the treatment groups inhaled (via nebulization) vacant niosomes, simple myrtenol, or niosomal myrtenol once a day for 30 min for one week (from day 43 to day 49) [25,30]. According to the calculation by the following formula, each animal receives approximately 2 mg of myrtenol (8 mg/kg) and 40 μg of budesonide (160 μg/kg) in 30 min:

$$D = \frac{C \times MV \times T}{BW}$$

where D is the dose of drug (mg/kg body weight), C is the drug concentration in the chamber (mg/l), T is the duration of exposure (min), BW is the average of group body weights (kg) and MV is the animal minute ventilation (l/min) [31].

2.7. BALF collection

After the rats were sacrificed with anesthesia overdose (ketamine 80 mg/kg and xylazine 50 mg/kg), BALF (bronchoalveolar lavage fluid) collection was done from the left lungs [32]. Briefly, at the end of the experiment, median sternotomy was performed, the trachea was isolated, and the right main bronchus was clamped. A catheter was inserted to the left main bronchus of the animal and 2.5 mL of normal saline was instilled into the bronchoalveolar space of the left lung. The instilled fluid was then harvested by aspiration into the syringe. The right lung was not washed to keep it intact for histopathology evaluation. The BALF was instantly centrifuged (4 °C, 10 min, 1000 g), and the supernatant was used for measuring the levels of cytokines and oxidative stress factors. 50 μl of PBS (phosphate buffer saline) was added to the pellet, from which 10 μl was smeared on a glass slide. The slides were dried in the air, fixed in methanol for 15 min, and then stained with Giemsa to evaluate their white blood cell content [24,32].

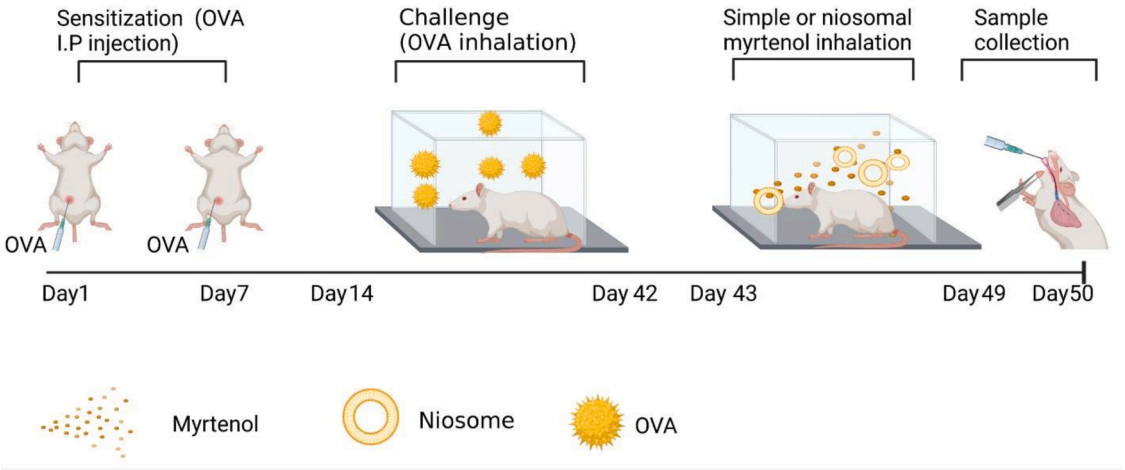

Fig. 1. Time-line diagram showing the experimental protocol used for asthma induction and treatment.

2.8. Lung histopathology

The animals' right lungs and airways were harvested and immersed in 10% formalin. Then, slices were sectioned (using a microtome, Slee Co., Germany) and stained with H&E (Hematoxylin & Eosin), Periodic acid-Schiff (PAS), and Giemsa [24,32]. The scoring system evaluation of the goblet cell hyperplasia (by PAS staining) [33], pathologic changes [24], grading system of peri-bronchial cells [34], and damage to the airway epithelium [24] were assessed by H&E staining as listed in Table 1. The number of blood vessels within the airway wall and smooth muscle thickness were also evaluated by H&E staining. The sub epithelial smooth muscle thickness of the bronchial wall was evaluated by a micrometer [35]. H&E and Giemsa staining were also utilized to evaluate inflammatory cell infiltration in the lung tissue and BALF, respectively.

2.9. Biochemical measurements in BALF and lung tissue supernatant

Total proteins were assessed by Bradford method. Malondialdehyde (MDA), as an index of lipid peroxidation, was estimated using the concentration of thiobarbituric acid reactive substances (TBARS) at 550 nm according to the kit′s instructions (Navand Salamat Co., Iran). Total antioxidant capacity (TAC) was determined by the ferric reducing ability of plasma (FRAP) assay at 593 nm via the related kit (Navand Salamat Co., Iran). Also, nitric oxide metabolites (NOx) and total protein levels in BALF were evaluated using their assay kits (Navand Salamat Co., Iran), based on the manufacturers' protocols [35,36]. The levels of tissue and BALF cytokines (IL-17 and IL-10) were assessed by ELISA kits (Karmania pars gene Co., Iran). More detailed information about the biochemical measurements are provided in previous publications [37].

2.10. Dose-response study

Based on the dose-response assessment, the lowest dose of myrtenol that showed maximum efficacy in reducing pathological changes (mentioned in Section 2.8) at histopathologic level and at molecular level (tissue IL-10 level) was selected as the optimum dose (8 mg/kg) (Fig 2), and this dose was then used (in both simple and niosomal forms) for the rest of the study. The optimum dose was selected among tested doses of 2, 4, 8, and 16 mg/kg.

2.11. Statistical analysis

The data in the figures are presented as a mean ± SEM. Shapiro-Wilk test was used for checking the normality of data. One-way ANOVA, followed by Tukey's post hoc test, was utilized for data analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Niosome characterization

The vesicle size of niosomes was 420 ± 87 nm for vacant niosomes and 412 ± 73 nm for myrtenol-loaded niosomes. DLS evaluations showed a roughly narrow dispersion in the vesicle size in most of the formulations, which shows that the niosomes were almost uniform in size and shape (Fig. 3). In this study zeta potential was seen within −27.4 ± 3.1 mv for vacant niosomes and −24.9 ± 4.3 mv for myrtenol-loaded niosomes. High and negative zeta potentials are suggestive of stable preparations. High encapsulation efficiency (EE) ensures the bioavailability of the nutraceuticals. By elevating EE, the concentration of myrtenol in the vesicle is elevated. The EE of the drug was 81.33 ± 0.38%. By *in vitro* testing, a biphasic kinetic release of myrtenol, including the early moderately rapid release (50% in 4 h) and plateau state (slower release phase) was seen in the niosomal form. All (100%) of the simple myrtenol was released during 3 h, while a controlled release of niosomal from was observed for up to several hours.

Table 1
Histopathological indices and their scoring system in the lungs of asthmatic rats.

Score	Index			
	Goblet cell hyperplasia	Pathologic changes	Peri-bronchial cell infiltration	Airway epithelium damage
0	No goblet cells	No lesion	No cells	No damage
1	< 15%	Slight lesion	A few cells	Normal appearance
2	15–30%	Mild lesion	A ring of cells one cell layer deep	Observable loss of cilia, or degeneration or necrosis of < 25% of the ciliated cells, without epithelial sloughing (ES)
3	30–45%	Moderate lesion	A ring of cells 2–4 cells deep	Observable ES and degeneration or necrosis of < 25% of the ciliated cells
4	45–60%	Severe lesion	A ring of cells 4–6 cells deep	ES and degeneration or necrosis between 25% and 50% of the ciliated cells
5	> 60%	Severe lesion	A ring of cells > 6 cells deep	ES and degeneration or necrosis of > 50% of the ciliated cells

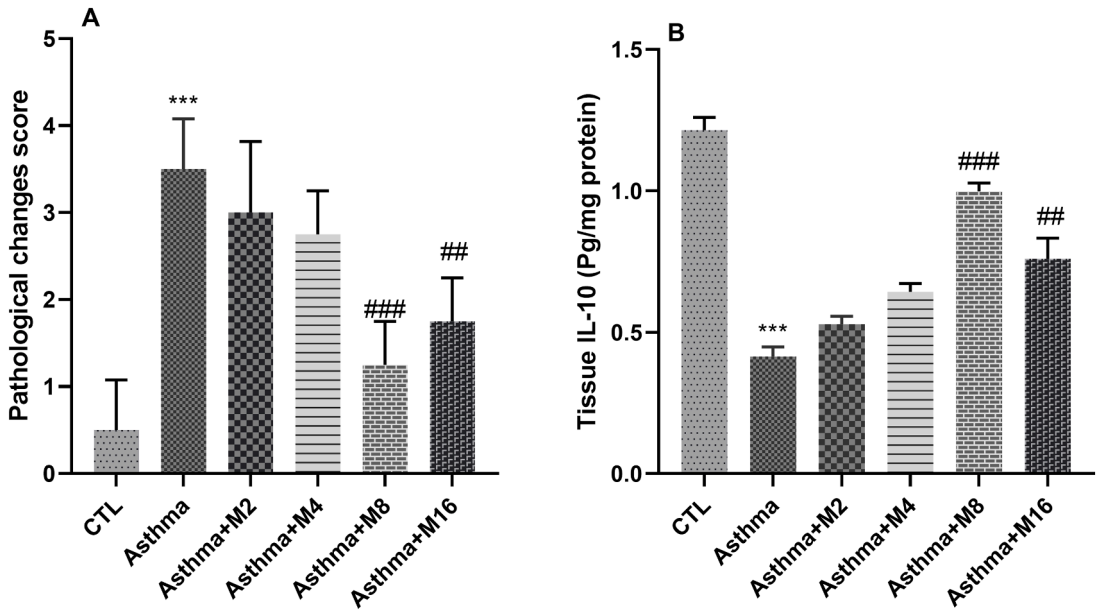

Fig. 2. Dose-response study for determining the optimum dose of niosomal myrtenol according to the effects on pathology and lung tissue IL-10. The lowest dose that exhibited maximum efficacy was 8 mg/kg among 2, 4, 8 and 16 mg/kg administered. CTL: control. Data are Mean ± SEM (n = 7 per group). *** $P < 0.001$ *vs* CTL. ## $P < 0.01$ and ### $P < 0.001$ *vs* Asthma.

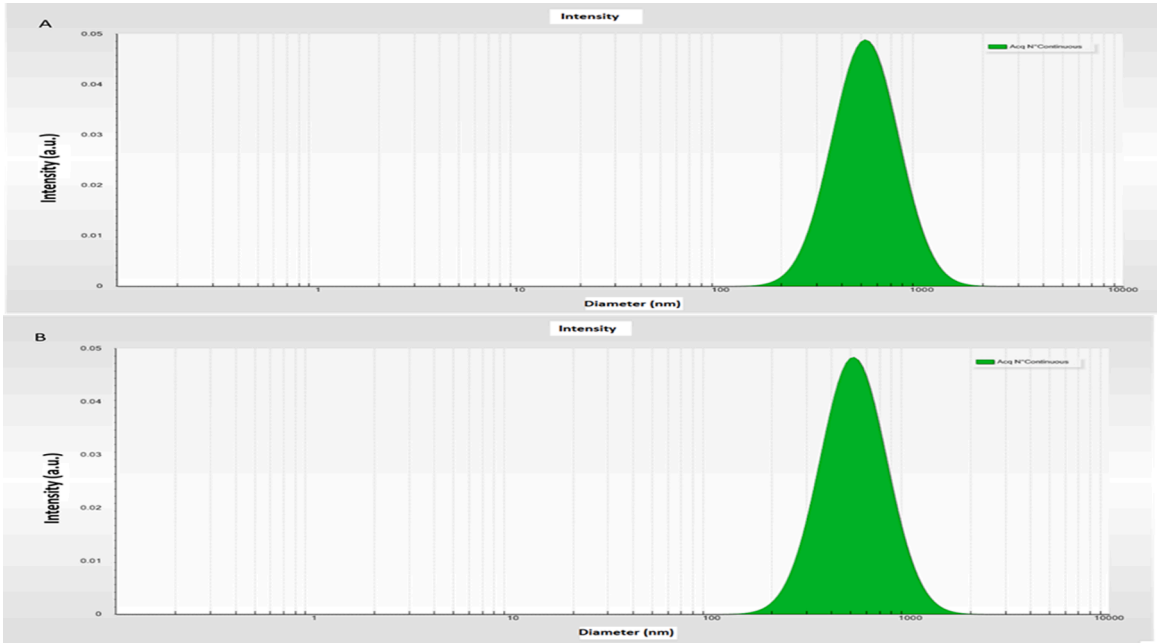

Fig. 3. Particle size distribution of vacant (A) and myrtenol-loaded (B) niosomes, measured by DLS at 25 °C. Before administration to the animals, the niosomes were sonicated and passed through a 0.2 micrometer filter. This makes the size of niosomes smaller and uniform in the form of unilamellar vesicles in the range of 25–100 nm.

3.2. Histopathologic observations in the lung tissue and airways

The pathological and airway epithelium damage score incremented in the asthmatic rats in comparison with the CTL group and treatment with niosomal and simple forms of myrtenol significantly reduced these pathological scores compared to the asthmatic rats. Inflammatory cell infiltration and airway vascularity were remarkably elevated in the asthma group compared to the CTL group. Inhalation of niosomal and simple forms of myrtenol markedly decreased inflammatory cell infiltration and airway vascularity

compared to the asthma group. Infiltration of inflammatory cells increased in the peribronchial and perivascular area of the asthmatic group compared to the CTL group. These parameters were also remarkably ameliorated by inhalation of niosomal and simple forms of the compound. Fig 4 illustrated that the sub-epithelial smooth muscle thickness in the asthma group remarkably increased compared to the CTL group. This increase was inhibited by niosomal and simple forms of myrtenol compared to the asthma group. The number of goblet cells increased in the lungs of rats with asthma compared to the healthy rats (Fig 5H). This parameter significantly decreased in groups treated with niosomal and simple forms of myrtenol in comparison with the asthma group. Also, our results revealed that budesonide inhalation improved histopathological indices in comparison with the asthma group. Moreover, niosomal myrtenol had similar or better effect than budesonide.

3.3. Cytokines in lung tissue and BALF

In the asthma group, the level of IL-17 was higher and IL-10 was lower in the lung tissue and BALF in comparison with the CTL group. One week of inhalation therapy with niosomal and simple myrtenol (8 mg/kg) caused an improvement in disease induced-disturbances of IL-17 and IL-10 levels (Figs. 6 and 7). Niosomal myrtenol showed stronger improving effect than simple myrtenol, comparable to the effect of budesonide.

3.4. Oxidant and antioxidant indices in the lung and BALF

In the OVA exposure group, the level of MDA in tissue and BALF and NO in BALF increased compared to the CTL group (Fig. 7). Niosomal and simple forms of myrtenol diminished the level of MDA and NO in comparison with the asthma and vehicle groups. In this regard, niosomal myrtenol showed a significantly more potent effect compared to the simple form. Niosomal and simple forms of myrtenol increased TAC levels compared to the asthma group. Again, the niosomal form of myrtenol was significantly more potent in recovering the TAC level. Furthermore, OVA in the asthma group leads to a remarkable elevation in BALF total protein in comparison with the control group. Compared to the asthma group, animals that has inhaled simple and niosomal forms of myrtenol revealed significant reduction in total protein in their BALF. Also, there was a significant difference between simple and niosomal forms of myrtenol in the total protein in BALF (Fig. 7).

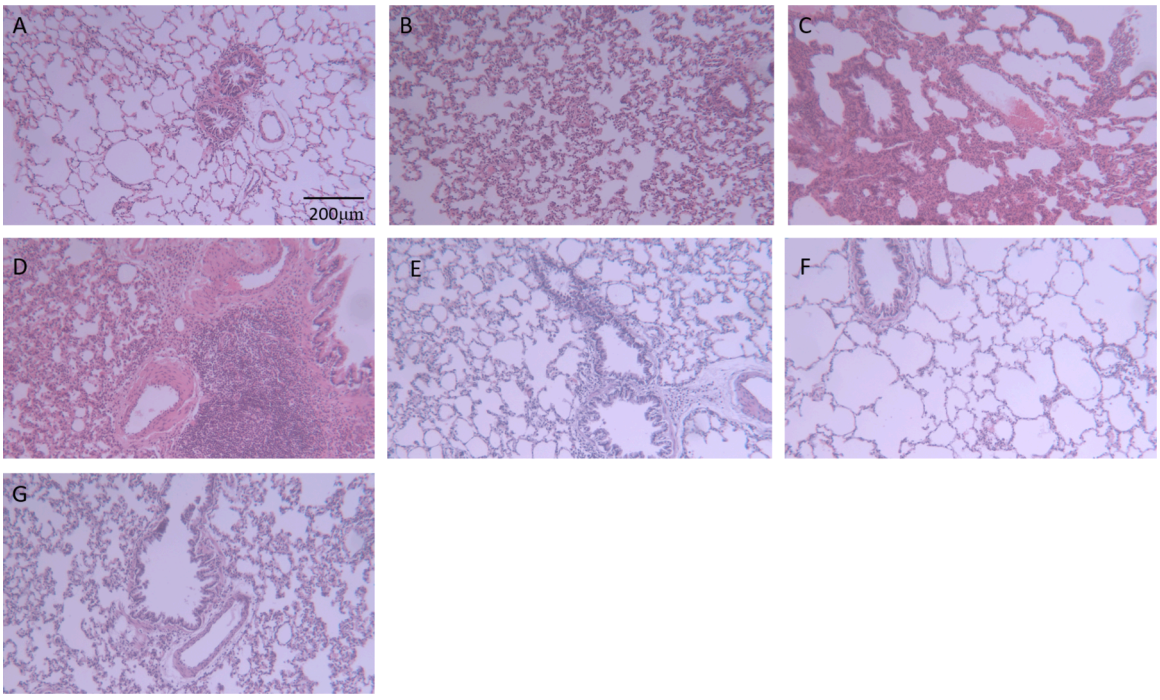

Fig. 4. Tissue micrographs stained with H&E (A-G). A: CTL: B: VN: control+vacant niosome; C: Asthma; D: Asthma+VN; E: Asthma+SM (simple myrtenol); F: Asthma+NM (niosomal myrtenol); G: Asthma+*B* (budesonide). The quantitative data showed as Pathological scores (H), inflammatory cell infiltration score (I), airway damage score (J), number of vessels per bronchus (K), smooth muscle thickness (L), and inflammatory cell infiltration in tissue and BALF (M) in asthmatic rats in different groups ($n = 7$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs* CTL, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ *vs* VN, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ *vs* Asthma, $ $P < 0.05$, $$ $P < 0.01$, $$$ $P < 0.001$ *vs* Asthma+VN, ^ $P < 0.05$ Asthma+*B* *vs* Asthma+SM..

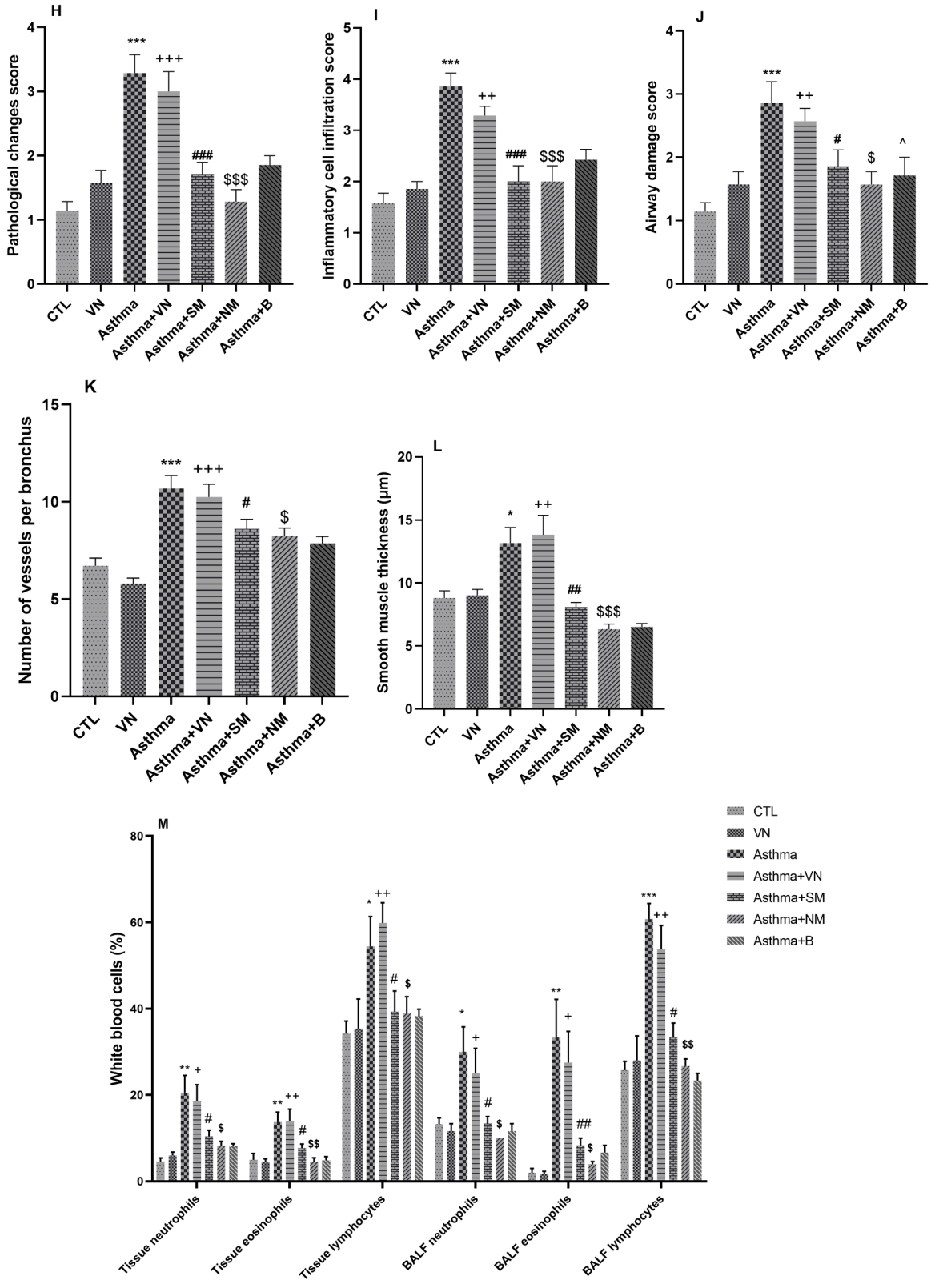

Fig. 4. (*continued*).

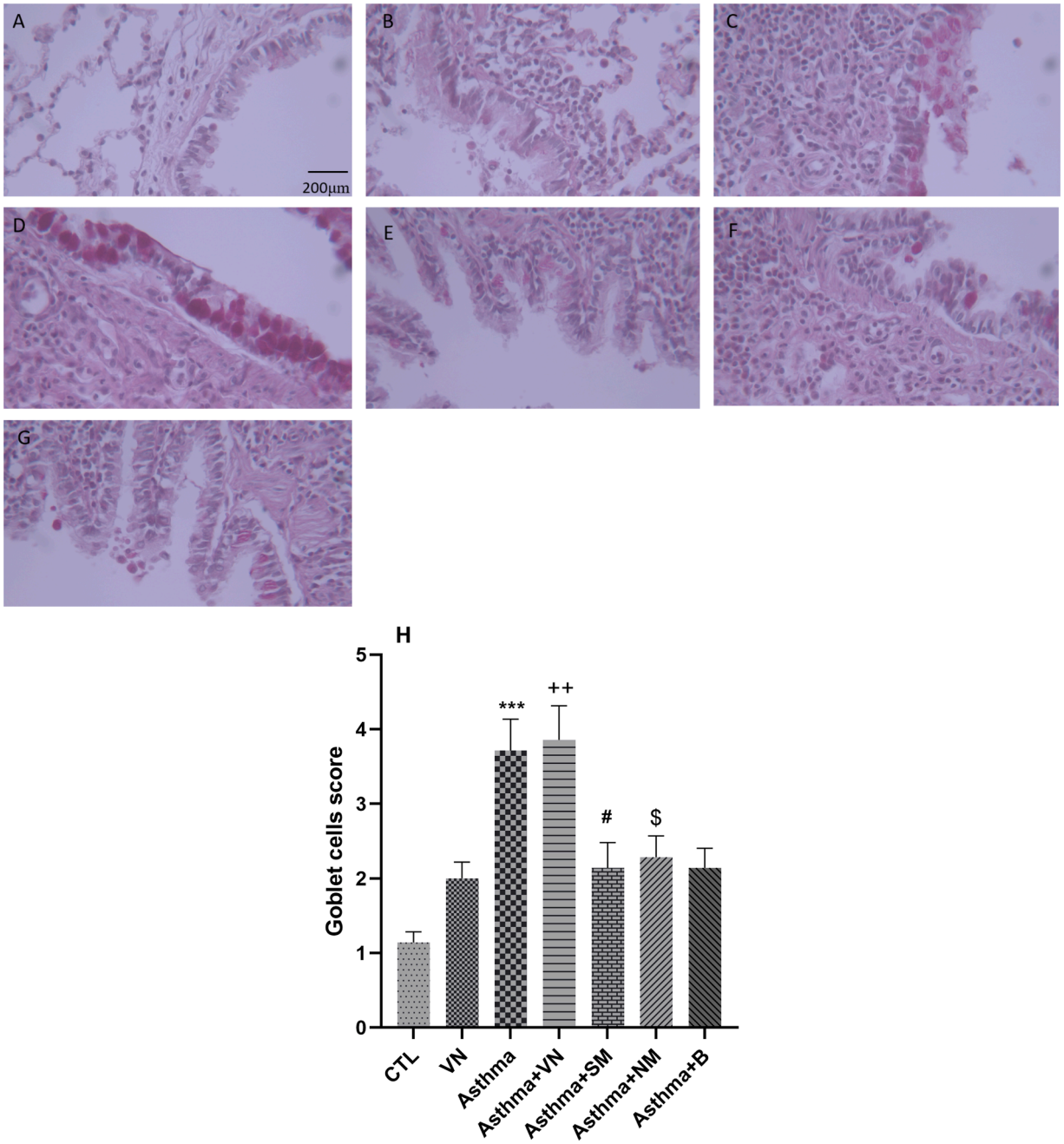

Fig. 5. Lung micrographs stained with PAS (A-G). A: CTL; B: VN: control+vacant niosome; C: Asthma; D: Asthma+VN; E: Asthma+SM (simple myrtenol), F: Asthma+NM (niosomal myrtenol); G: Asthma+*B* (budesonide) and H: the quantitative data ($n = 7$ in each group). *** $P < 0.001$ *vs* CTL, ++ $P < 0.01$ *vs* VN, # $P < 0.05$ *vs* Asthma, $ $P < 0.05$ *vs* Asthma+VN.

4. Discussion

This study investigated the impact of the inhalation of niosomal and simple forms of myrtenol on inflammatory markers and oxidative and histopathological indices of rat lungs with allergic asthma.

Our results showed that myrtenol in both niosomal and simple forms was able to decrease the level of IL-17 and elevate the level of IL-10 in lung tissue and BALF. Our findings also demonstrated that the increased level of MDA, NO, and total protein was normalized, and the reduced TAC levels were compensated following inhalation of niosomal and simple forms of myrtenol in asthmatic rats. In general, the niosomal form of myrtenol had a more potent effect than the simple form, which was comparable to the effects of the currently used golden standard drug, budesonide.

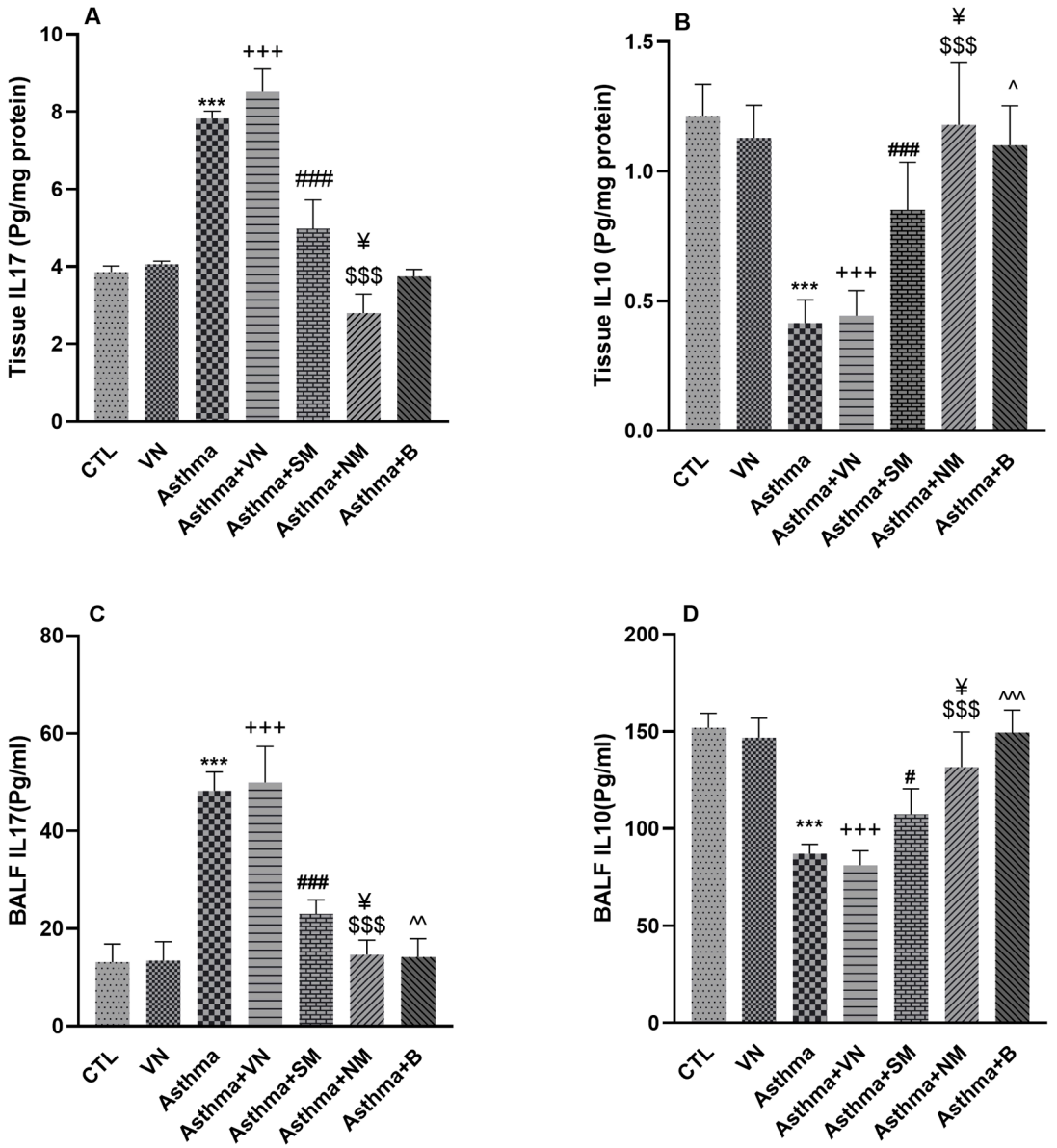

Fig. 6. ELISA analyses of cytokines in the lung tissue and BALF of different groups. Asthma significantly increased the levels of IL-17 (A and C) and decreased IL-10 (B and D). Data are means ± SEM ($n = 7$). A: CTL: B: VN: control+vacant niosome; C: Asthma; D: Asthma+VN; E: Asthma+SM (simple myrtenol); F: Asthma+NM (niosomal myrtenol); G: Asthma+*B*: (budesonide). *** $P < 0.001$ *vs* CTL, +++ $P < 0.001$ *vs* VN, # $P < 0.05$ and ### $P < 0.001$ *vs* Asthma, $$$ $P < 0.001$ *vs* Asthma+VN, ¥ $P < 0.05$ Asthma+NM *vs* Asthma+SM, ^ $P < 0.05$, ^^ $P < 0.01$ and ^^^ $P < 0.001$ for Asthma+*B* *vs* Asthma+SM.

Previous studies have confirmed the impact of IL-17 elevation in coordinating the airway response against infection by different pathogens [4]. However, the inflammatory function of IL-17 influences the airway mucosa [3]. Th17 cytokines, including IL-17, play a role in neutrophilic inflammation in severe asthma and may be involved in airway remodeling [38]. Our results showed that the amounts of this cytokine increased in the tissue and BALF. IL-10 is a strong anti-inflammatory cytokine and its generation by a wide range of cell types has been described [39]. Our results also showed diminished IL-10 levels in the lung tissue and BALF of asthmatic animals. It has been shown that IL-10 can suppress AHR and negatively regulate eosinophilic and neutrophilic migration into the tissues [40]. Niosomal myrtenol normalized the disturbances in the production of these two interleukins, which occurred in the process of disease, as potently as budesonide. These observations show that myrtenol in niosomal form has a more potent anti-inflammatory effect. Certain anti-inflammatory drugs are frequently administered in niosomal form to potentiate the curative and anti-inflammatory efficiency of the drug. Niosomes are one of the best choices for localized drug action with reduced systemic adverse effects [41,42].

Oxidant-antioxidant imbalance leads to pathophysiological consequences associated with asthma, such as vascular permeability,

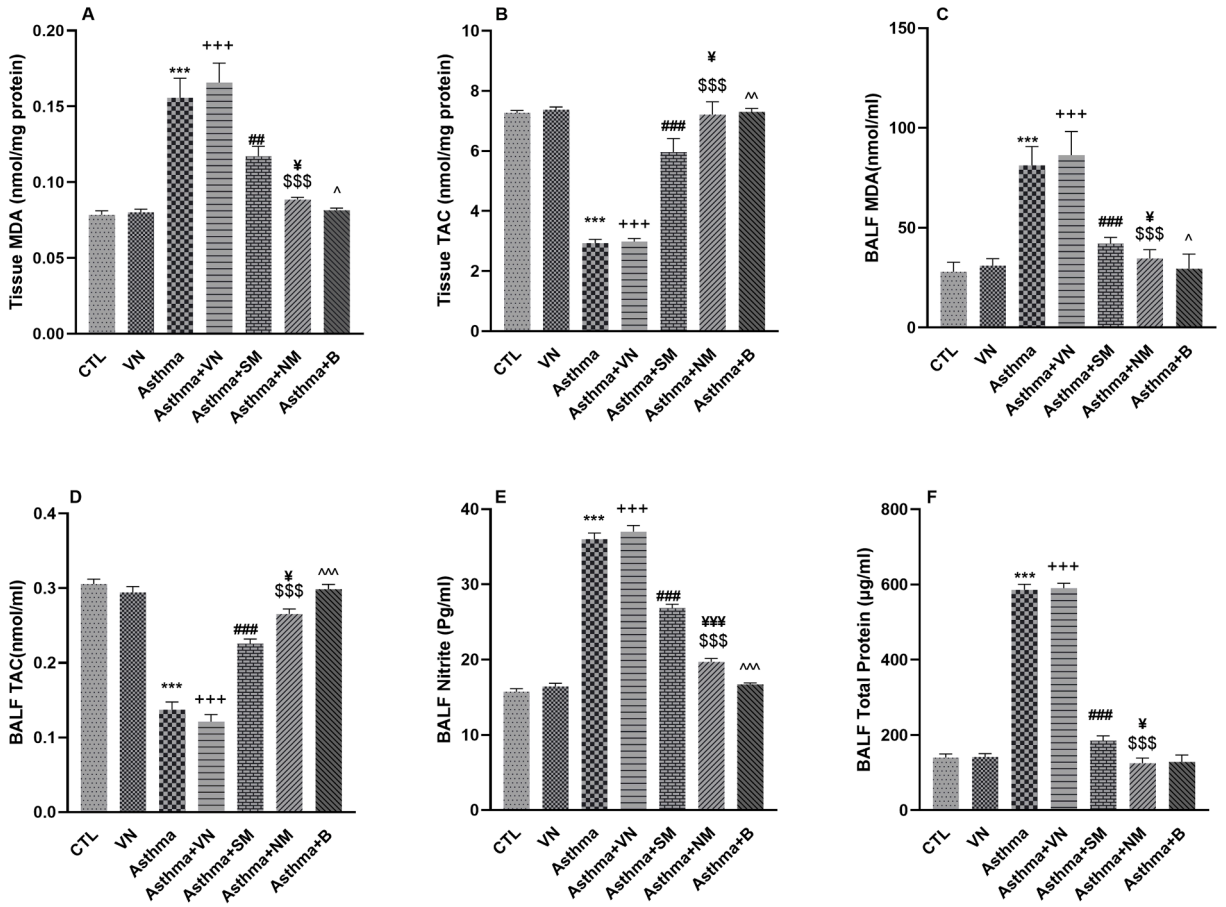

Fig. 7. Alterations in the BALF total protein and nitrite, and MDA and TAC in the lung tissue and BALF in the study groups. Data are means ± SEM ($n = 7$). A: CTL: B: VN: control+vacant niosome; C: Asthma; D: Asthma+VN; E: Asthma+SM (simple myrtenol); F: Asthma+NM (niosomal myrtenol); G: Asthma+*B* (budesonide). *** $P < 0.001$ *vs* CTL, +++ $P < 0.001$ *vs* VN, ## $P < 0.05$ and ### $P < 0.01$ and $P < 0.001$ *vs* Asthma, $$$ $P < 0.001$ *vs* Asthma+VN, ¥ $P < 0.05$ and ¥¥¥ $P < 0.001$ Asthma+NM *vs* Asthma+SM, ^ $P < 0.05$, ^^ $P < 0.01$ and ^^^ $P < 0.001$ Asthma+*B* *vs* Asthma+SM.

mucus hypersecretion, smooth muscle contraction, and epithelial shedding [43]. We have shown that myrtenol decreases MDA levels and increases SOD and GPX activity in asthmatic rats [24,25]. In the present study niosomal myrtenol normalized the elevated level of oxidant factor MDA and the reduced level of antioxidants (TAC) as potently as budesonide did. This observation implies that the ameliorating effect of myrtenol is also due to improving the antioxidant/oxidant imbalance. MDA enhances the vascular permeability and leukocyte chemotaxis and alters prostaglandin synthesis and histamine release, thus perpetuating inflammation [44]. Furthermore, the elevation in iNOS-derived NO observed in asthma, may result in an elevation in peroxynitrite and oxidative stress progression [45]. The decrease in leukocyte infiltration and tissue inflammation (Fig 7) may be due to the reduction in nitrite and MDA levels caused by niosomal (and simple) myrtenol employed in this study.

Sub epithelial hypervascularity and angiogenesis in the airways are part of the structural airway changes in asthma. Increment in iNOS activity may impact extracellular matrix remodeling [46]. Elevated number of goblet cells, increased bronchial vascularity, and hypertrophy of smooth muscle cells also occur in airways in asthma [47]. Our results showed goblet cell hyperplasia and elevation in smooth muscle thickness following induction of allergic asthma, and myrtenol improved many of the features of airway remodeling that had happened as a result of disease progression. Again, niosomal myrtenol was found to have more anti-remodeling effect than the simple form of the compound, almost comparable to that of budesonide. It seems that myrtenol can maintain the integrity of the capillaries and bronchial epithelium and prevent the infiltration of inflammatory cells and secretion of proteins and pro-inflammatory interleukins in the lungs.

Overall, the results showed that myrtenol ameliorates inflammation, oxidative stress and tissue remodeling in asthmatic animals. Inhalation of the niosomal form more potently induces improving effects against allergic asthma than the simple myrtenol. Due to the comparable treatment efficacy with budesonide, we propose niosomal myrtenol as a target for production of anti-asthmatic medications after complementary studies have been conducted in clinical settings.

Human care of the animals

Animal housing conditions and the research protocols were reviewed and verified by the university Ethics Committee for animal studies (ethics code IR.KMU.REC.1398.295).

CRediT authorship contribution statement

Mohammad Amin Rajizadeh: Writing – original draft, Investigation, Methodology. **Mohammad Hadi Nematollahi:** Investigation, Methodology. **Elham Jafari:** Methodology. **Mohammad Abbas Bejeshk:** Writing – original draft, Resources. **Mehrnaz Mehrabani:** Validation, Investigation. **Mohammad Sadegh Razeghinia:** Validation. **Hamid Najafipour:** Conceptualization, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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