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Grape seed extract attenuates lung parenchyma pathology in ovalbumin-induced mouse asthma model: An ultrastructural study

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ABSTRACT

Due to the growing incidence of asthma and because of the non-specificity and side effects of the conventional drugs, the development of novel agents for the treatment of asthma has become considerably important. Natural plant products offer promising alternatives for the development of effective and safe treatments. Grape seed extract (GSE) is one such phytochemical supplement that has been shown to have potent antioxidant and anti-inflammatory effects. Thus, the present study aimed to investigate the effect of GSE to suppress lung parenchyma pathology and inflammation in ovalbumin-induced murine asthma model. Ovalbumin exposure was associated with many pathological and morphometric alterations in the lungs of asthmatic mice. The alterations involved alveolar size reduction, alveolar wall thickening, cellular infiltration and blood capillary congestion, as well as significant increase in the number of type II pneumocytes and lamellar bodies. However, GSE significantly ameliorated of the pathological changes of ovalbumin-induced asthma. The results support the possibility of GSE as an effective, safe anti-inflammatory dietary supplement to attenuate the pathogenicity of asthma. While these preliminary results appear promising, further studies are required to elucidate the precise mechanism of the modulatory effect of GSE on asthma remodeling.

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1. Introduction

Asthma is one of the most common chronic inflammatory diseases. It affects about 300 million people worldwide, and its prevalence doubles every decade (Masoli et al., 2004). It is characterized by airway infiltration of inflammatory cells, inducing hyperresponsiveness and remodeling (Janson, 2010). Although such remodeling leads to deterioration in respiratory functions, little is known about structural changes in the distal airways and lung parenchyma of asthmatic patients (Xisto et al., 2005). On the contrary, remodeling of large airways has been extensively described, and involves a wide array of pathophysiologic features, including increased epithelial thickness, goblet cell hyperplasia and increased mucous secretion, increased smooth muscle mass, subepithelial fibrosis, and vascular changes (Roh et al., 2005; Tang et al., 2006; Al-Muhsen et al., 2011).

Since inflammation is the chief factor in the initiation and progression of asthma; therefore, suppression of the influx of inflammatory cells and structural changes in asthma is essential for reducing airway hyperresponsiveness and asthmatic symptoms, as well as the improving respiratory functions (Levine, 2003; Kim et al., 2011). Corticosteroids are currently the most effective

anti-inflammatory treatment for reducing the symptoms of asthma; however, they cannot cure the disease or even alter its progression (Childhood Asthma Management Program Research Group, 2000; Hocaoglu et al., 2011). Additionally, corticosteroids are non-specific in their actions and have eminent systemic and local adverse effects (Walsh, 2005; Shahzad et al., 2009). Owing to the chronic nature of asthma and its increasing prevalence, besides the reputed inefficacy of the currently used drugs to definitely cure asthma and the fear of their known side effects, there is an insistent need for new therapies (Li, 2009b). In this respect, recent interest has arisen for the use of natural plant products as a complementary and alternative medicine (Wen et al., 2005; Cho et al., 2008). It is interesting to mention that 4 of the 5 classes of drugs currently used to treat asthma are of herbal origin (Ziment, 2000). *Vitis vinifera*, known as the grapevine, is native to southern Europe and Western Asia. Grape seed and skin contain several active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol (Nassiri-Asl and Hosseinzadeh, 2009). Grape seed extract (GSE) in particular has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as anti-inflammatory (Chao et al., 2011; Gessner et al., 2011; Pan et al., 2011), antioxidative (Vigna et al., 2003; Chis et al., 2009; Dogan and Celik, 2011; Salem and Salem, 2011), antiaging (Balu et al., 2005, 2006), anticarcinogenic (Dinicola et al., 2012), antimicrobial activities (Al-Habib et al., 2010; Su and D'Souza, 2011), as

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well as having hepatoprotective (Sehirli et al., 2008; El-Beshbishy et al., 2010; Khalifa et al., 2011), renoprotective (Mohanasundari et al., 2005; Salem and Salem, 2011), cardioprotective (Yalçin et al., 2010), and neuroprotective effects (Balu et al., 2006). GSE also exhibits minimal toxicity in long-term studies in laboratory animals (Bentivegna and Whitney, 2002; Wren et al., 2002; Yamakoshi et al., 2002). Taken together, these reports propose that GSE may have a potential in ameliorating inflammation and lung parenchyma pathological changes associated with asthma.

Previously, we have shown that GSE resulted in significant reduction in the levels of total immunoglobulin E in serum, and total inflammatory cells, eosinophils and cytokines in bronchoalveolar lavage fluid. It also efficiently attenuated the remodeling in the respiratory airways (Mahmoud and Soliman, 2011). The present study further provides insight into lung parenchyma pathology in ovalbumin-induced asthma, and verifies the protective and therapeutic effects of GSE on these pathological features. The effect of GSE will be compared with that of dexamethasone, a powerful synthetic anti-inflammatory drug (Mager et al., 2003).

2. Materials and methods

2.1. Materials

Grape seed extract (GSE) was obtained from Arab Co. for Pharmaceuticals & Medicinal Plants (MEPACO, Cairo, Egypt) as dried powder commercially known as Gervital. The dose and treatment schedule were selected according to the results of our previous study (Mahmoud and Soliman, 2011). Ovalbumin powder (OVA) was purchased from Sigma–Aldrich Chemical Co. (grade V; Sigma–Aldrich Corp., St. Louis, Missouri, USA) as powdered albumin of chicken egg white. Dexamethasone (DXM) was supplied in the form of 2 mL ampoules, each contained 8 mg of dexamethasone as sodium phosphate, produced by Amriya Pharmaceutical Industries (Alexandria, Egypt). All other reagents were of analytical grade and purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA).

2.2. Animals

Six- to 8-week-old male CD-1 strain mice, weighing 18–20 g, were purchased from the Medical Research Centre at the Faculty of Medicine, Ain Shams University (Cairo, Egypt). Mice were transported to the animal care facility of the Zoology Department at the Faculty of Science, Ain Shams University, two weeks prior to the initiation of the experiments for acclimatization to the laboratory conditions. Mice were reared in hygienic polypropylene cages (290 mm × 220 mm × 140 mm) and bedded on autoclaved wood shavings. A temperature of $25 \pm 2^\circ\text{C}$ and 12 h light/dark cycle were maintained. Mice were given free access to water and standard pelleted diet, containing 24% protein, 4% fat and about 4–5% fiber; and obtained from the Schistosoma Biological Supply Program at Theodor Bilharz Research Institute (Giza, Egypt). All aspects of the present study agreed strictly with the regulations and guidelines set by the University of Ain Shams Ethics Committee for care and use of experimental animals.

2.3. Ovalbumin-induced asthma sensitization model

The experimental protocol for inducing asthma was performed as previously described by Hrvacic et al. (2009). Briefly, mice were sensitized with 2 intraperitoneal injections of $10\ \mu\text{g}/0.1\ \text{mL}$ of sterile ovalbumin, emulsified in alum as an adjuvant, on days 0 and 14 of the experiment. Under light anesthesia, mice were intranasally challenged with $100\ \mu\text{g}$ of ovalbumin, dissolved in phosphate buffered saline, in a total volume of $50\ \mu\text{L}$ on day 20. Mice in normal control and GSE groups received saline, instead of

OVA, with alum intraperitoneally on days 0 and 14 of the experiment and were challenged with saline on day 20 of the study.

2.4. Experimental protocol and treatments

Mice were randomly divided into six groups (8 mice each): normal, GSE control, OVA control, OVA + DXM, GSE + OVA, and OVA + GSE. GSE control mice were administered GSE orally at a dose of $150\ \text{mg}/\text{kg}$ body weight every day for 7 days. All OVA groups were sensitized and challenged with OVA as described above. Normal and OVA control mice were orally administered saline instead of GSE for 7 days. OVA + DXM mice were intraperitoneally administered DXM on experimental days 21–23, at the dose of $1\ \text{mg}/\text{kg}$ body weight. GSE + OVA (GSE prophylactic group) was orally treated with GSE for 7 days, and then sensitized and challenged with OVA. OVA + GSE (GSE therapeutic group) was orally administered GSE for 7 days after the sensitization and challenge with OVA. Twenty-four hours after the last ovalbumin and drug exposure, mice were anaesthetized with pentobarbital ($60\ \text{mg}/\text{kg}$), and the lungs were inflation-fixed and processed for light and electron microscopical assessments as well as morphometric analysis.

2.5. Histology

Left lungs were removed from the thoracic cavity, inflated with air to a pressure of $25\ \text{cm H}_2\text{O}$, and immediately fixed at this pressure in aqueous Bouin's fluid for an hour. After that, a slice from the middle zone of each lung was fixed for 24 h, dehydrated, cleared, and then embedded in paraffin wax. At least five representative sections of each sample were randomly chosen at $250\text{-}\mu\text{m}$ intervals and stained with hematoxylin and eosin. Slides were examined and photographed with an Olympus microscope equipped with a high-resolution Olympus camera at the Medical Military Academy (Cairo, Egypt). Ten images from non-overlapping parenchymal fields in each lung section were captured at a $40\times$ objective magnification.

2.6. Electron microscopy

Right lungs from 5 mice in each group were processed for ultrastructural assessment. Inflated lungs were perfusion-fixed *in situ* via $0.1\ \text{M}$ phosphate buffered 2.5% glutaraldehyde at pH 7.2, then were sagittally cut into 3-mm-thick slices with sharp razor blades. Lungs were fixed in glutaraldehyde for 90 min, and then samples were again cut into 1-mm-thick, and rinsed three times in the buffer and left overnight. Later, the tissues were post-fixed for 2 h in 1% buffered OsO_4 , dehydrated through ascending graded series of acetone, embedded in epoxy resin (Araldite) and were left to polymerize overnight at 70°C . Five tissue blocks were prepared from each group. Semithin sections ($\sim 0.5\ \mu\text{m}$) were cut with glass knives, stained with 1% toluidine blue O and examined with a light microscope at $400\times$ magnification. Areas of interest were selected and blocks were trimmed accordingly. Ultrathin sections ($80\text{--}90\ \text{nm}$) were cut with a Diatom diamond knife, collected on 200-mesh nickel grids and stained in uranyl acetate and lead citrate. Ultrathin sections were examined and photographed using Jeol 100S transmission electron microscope in the Regional Mycology and Biotechnology Centre at Al-Azhar University. Ten electron micrographs were taken from each block, giving a total of 50 micrographs per group.

2.7. Morphometric quantification

Using the image analysis software Leica Qwin 500, digital images were analyzed and the following parameters were estimated: mean alveolar diameter (μm), interalveolar septum thickness (μm), type II pneumocytes count/microscopic field at $400\times$ magnification,

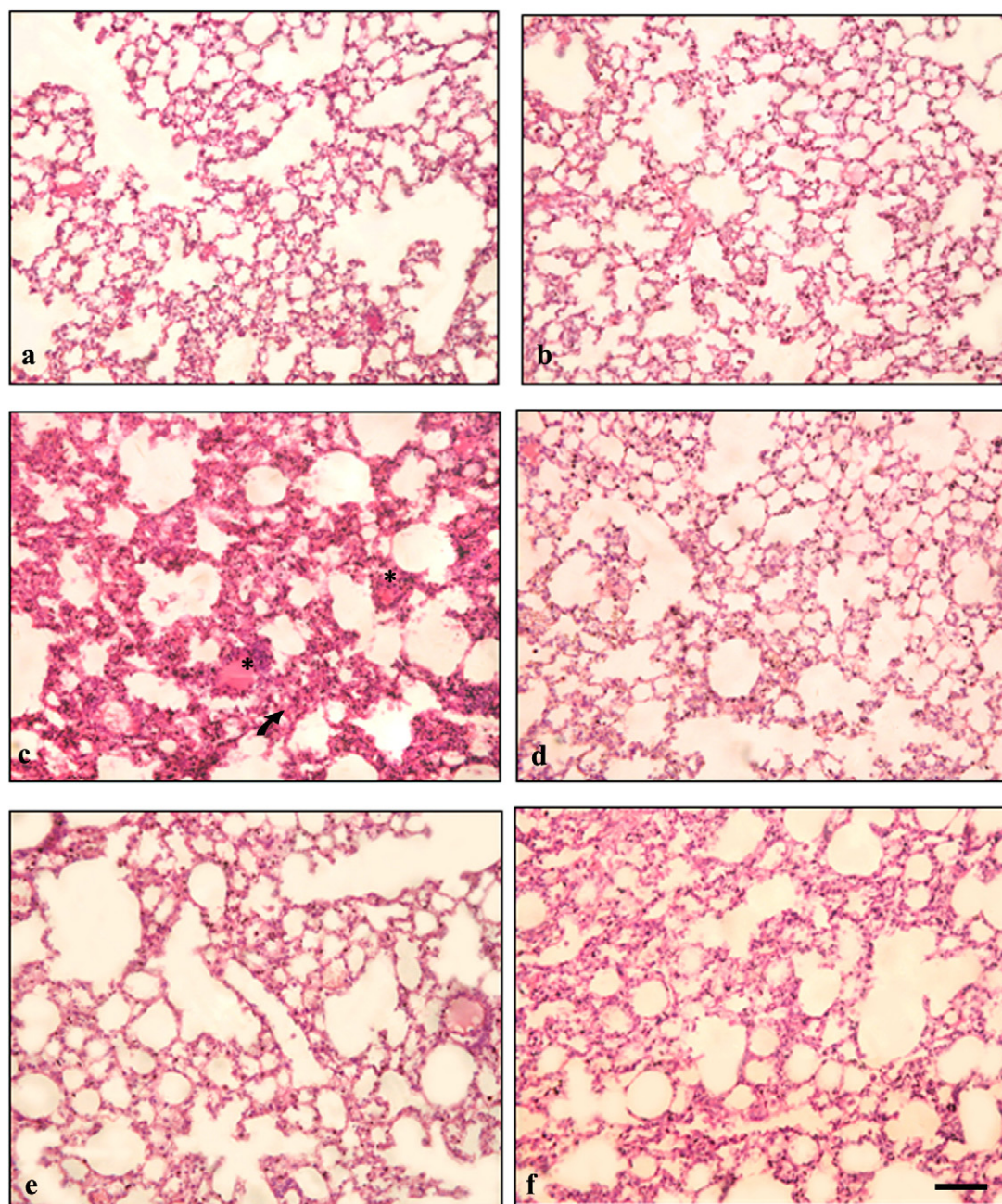


Fig. 1. Representative photomicrographs showing general morphology of lung parenchyma. (a) Normal control mice. Note the spongy appearance of the alveoli; (b) GSE control; (c) OVA control. Note alveolar collapse (arrow), eosinophilia, vascular congestion and inflammatory response (asterisks); (d) OVA + DXM; (e) GSE + OVA (prophylactic group); and (f) OVA + GSE (therapeutic group). Hematoxylin and eosin staining; scale bar = 50 μ m; original magnification = 400 $\times$.

and lamellar body count/type II cells. Data were expressed as mean $\pm$ standard error of mean (SEM). The significance of the interrelation of the treated groups to the control was tested using One-Way Analysis of Variance, ANOVA and Tukey's multiple comparison tests using GraphPad PrismTM (version 4.03, GraphPad software, San Diego, CA, USA). Difference values were considered significant when $P \leq 0.05$.

3. Results

Lung sections of the normal group showed the characteristic spongy histological appearance of normal pulmonary parenchyma, having uncountable number of polygonal alveoli separated by thin interalveolar septa (Fig. 1a). The alveolar lining epithelium was

made up of two cell types, type I and type II pneumocytes. Type I pneumocytes are flattened cells that occupy most of the lining of the alveoli (Figs. 2a and 3a). Type II cells were identified as large cuboidal cells with dome-shaped free surfaces, occupying very little of the alveolar lining and are commonly located at the angles between neighboring alveolar septa. These cells have large nuclei and few scattered microvilli on their free surfaces. The most characteristic feature is the presence of lamellar bodies, which contain the pulmonary surfactant (Figs. 3a and 4a). The air–blood barrier consists of the cytoplasm of type I pneumocytes, fused basal lamina of type I pneumocytes and capillary endothelial cells, and the cytoplasm of endothelial cells lining the interstitial capillaries (Fig. 5a). GSE treatment did not affect the familiar lung architecture, as the lung sections of this group appeared similar to those of the normal

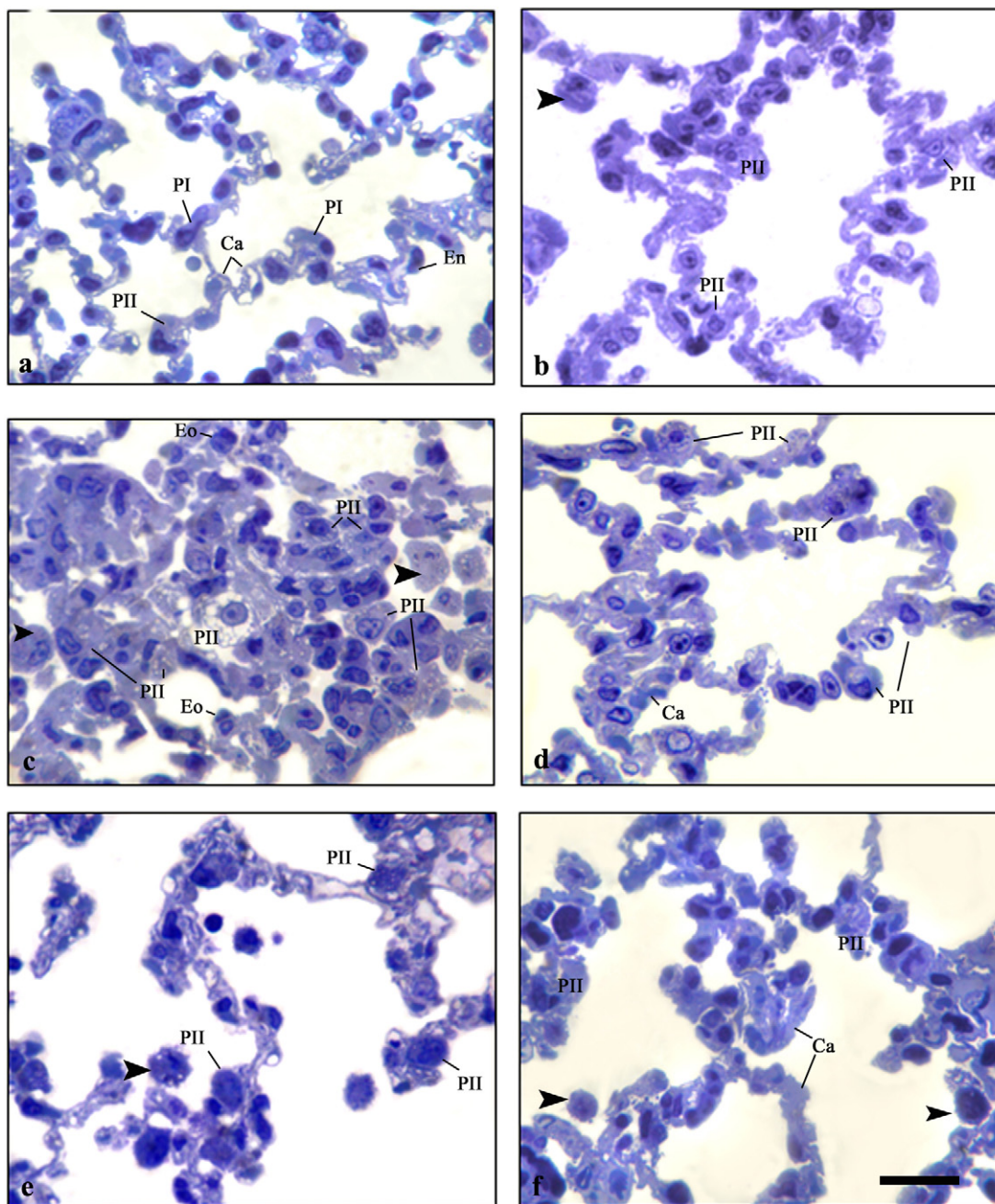


Fig. 2. Photomicrographs of semithin sections of alveoli showing the abundance of type II pneumocytes. (a) Normal mice; (b) GSE control; (c) OVA control. Note hyperplasia of type II cells; (d) OVA + DXM; (e) GSE + OVA; and (f) OVA + GSE. Arrowhead: macrophage; Ca: capillary; En: endothelial cell; Eo: eosinophil; PI: type I pneumocyte; PII: type II pneumocyte. Toluidine Blue staining; scale bar = 20 μ m; original magnification = 1500 $\times$.

mice (Figs. 1b, 2b, 3b, 4b, and 5b). Moreover, the results presented in Table 1 revealed no statistically significant differences in the assessed morphometric parameters, relative to the normal group.

Histological examination of the pulmonary parenchyma of ovalbumin-induced asthmatic mice showed increased eosinophilia (asterisks, Fig. 1c) and evident alterations when compared with control samples. Ovalbumin sensitization also resulted in the emergence of thicker-walled alveoli with small diameters (arrow, Fig. 1c), which were significantly different relative to the normal group (Table 1). The thickened septa showed inflammatory reaction in the form of edema, inflammatory cell infiltration and vascular congestion (asterisks, Figs. 1c and 2c). Electron microscopy revealed that the thickening of the alveolar walls was due to vasodilatation, cellular infiltration, and increased amounts of extracellular

matrix elements, such as collagen fibers (Fig. 3c). Type II pneumocytes suffered from hyperplasia (Table 1), and appeared vacuolated with swollen mitochondria and empty hypertrophied lamellar bodies (Fig. 4c). The air–blood barrier was commonly thickened and disrupted by the deposition of collagen fibers (Fig. 5c).

On the other hand, dexamethasone alleviated most of the morphological changes induced by ovalbumin challenge in asthmatic animals. Other than the incomplete recovery of the thickness of the air–blood barrier (Fig. 5d, Table 1), lung tissues of this group showed considerable improvement, and most alveolar cells regained nearly their normal morphology (Figs. 1d and 4d), and morphometry (Table 1).

Likewise, treating asthmatic mice with GSE, before or after ovalbumin-challenge, provoked notable recovery of the

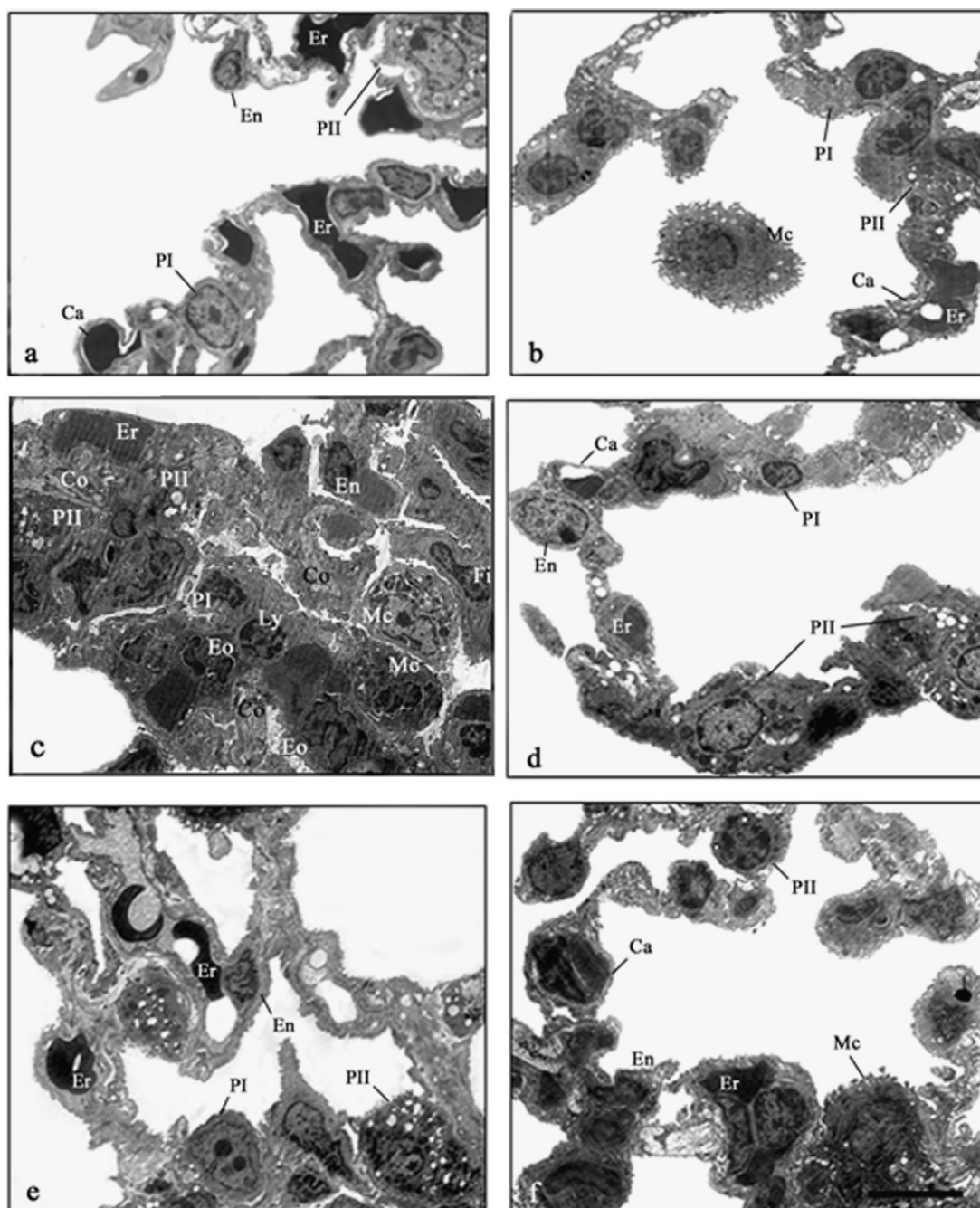


Fig. 3. Electron micrographs of alveoli showing ultrastructure of alveolar cells. (a) Normal control mice; (b) GSE control; (c) OVA control mice. Note the alveolar wall thickening, and the emergence of inflammatory cells and collagen fibers; (d) OVA + DXM; (e) GSE + OVA; and (f) OVA + GSE. Ca: capillary; Co: collagen fibers; En: endothelial cell; Eo: eosinophil; Er: erythrocyte; Fi: fibroblast; Ly: lymphocyte; Mc: macrophage; PI: type I pneumocyte; PII: type II pneumocyte. Scale bar = 10 μ m; original magnification = 5000 $\times$.

Table 1

Effect of dexamethasone and grape seed extract on lung morphometric parameters in ovalbumin-induced asthmatic mice.

	Alveolar diameter (μ m)	Alveolar septum thickness (μ m)	Pneumocytes II/high power field	Lamellar bodies/type II pneumocyte
Normal	28.06 $\pm$ 0.86	4.24 $\pm$ 0.13	8.10 $\pm$ 0.28	6.79 $\pm$ 0.44
GSE control	27.50 $\pm$ 0.72	4.71 $\pm$ 0.23	8.65 $\pm$ 0.09	7.50 $\pm$ 0.27
OVA control	5.48 $\pm$ 0.36**	18.62 $\pm$ 1.20**	20.16 $\pm$ 0.62**	18.61 $\pm$ 0.42**
OVA + DXM	25.41 $\pm$ 0.61	6.20 $\pm$ 0.23	9.42 $\pm$ 0.26	10.78 $\pm$ 0.21*
GSE + OVA	28.83 $\pm$ 0.88	7.25 $\pm$ 0.31*	8.00 $\pm$ 0.35	20.08 $\pm$ 0.50**
OVA + GSE	20.51 $\pm$ 0.71	9.12 $\pm$ 0.46*	7.10 $\pm$ 0.23	19.18 $\pm$ 0.63**

Data are expressed as mean $\pm$ SEM (n = 5/group).

* Significantly different from normal (P < 0.05)

** Highly significant (P < 0.001).

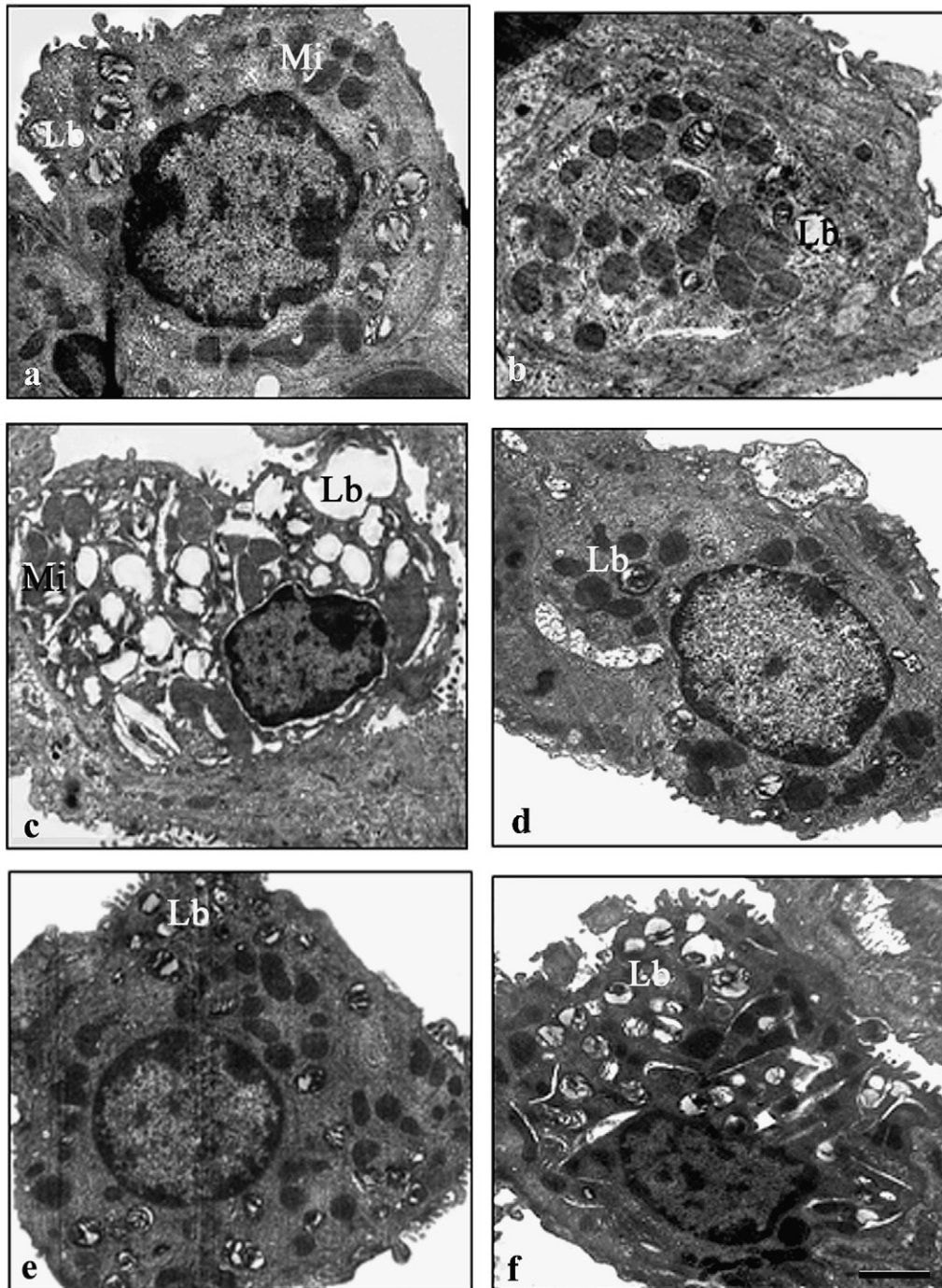


Fig. 4. Electron micrographs of type II pneumocytes. (a) Normal control mice; (b) GSE control; (c) OVA control mice. Note mitochondrial swelling, cytoplasmic vacuolation and loss of lamellar pattern of lamellar bodies; (d) OVA + DXM; (e) GSE + OVA; and (f) OVA + GSE. Lb: lamellar body; Mi: mitochondria. Scale bar = 2 μ m; original magnification = 12,000 $\times$.

pathological alterations induced by ovalbumin. In the GSE prophylactic group, light microscopic examination showed that the ovalbumin-induced alveolar collapse, septal thickening, inflammation and vascular congestion were significantly alleviated (Figs. 1e and 2e, Table 1). At the ultrastructural level, the alveoli showed nearly normal structure and the severely affected cells that were seen in asthmatic mice were hardly seen in GSE protected animals (Fig. 3e). An improvement of type II cells, represented by the increase in their dense lamellar contents and the absence of cytoplasmic vacuoles, was also observed (Fig. 4e, Table 1). Moreover, the

air–blood barrier possessed moderately thin walls except for few foci where there was slight fibrous deposition as the one shown in Fig. 5e. Treating asthmatic mice with GSE after ovalbumin challenge also suppressed lung parenchyma pathology. However, this ameliorating effect was less than that of the prophylactic group. Mild thickening of some alveolar septa (Table 1), in addition to mild cell infiltration were still noticed (Figs. 1f and 3f). Type II cells of this group regained nearly their normal appearance (Figs. 3f and 4f), except for the hyperplasia of the lamellar bodies. The air–blood barrier was found thin but irregular (Fig. 5f).

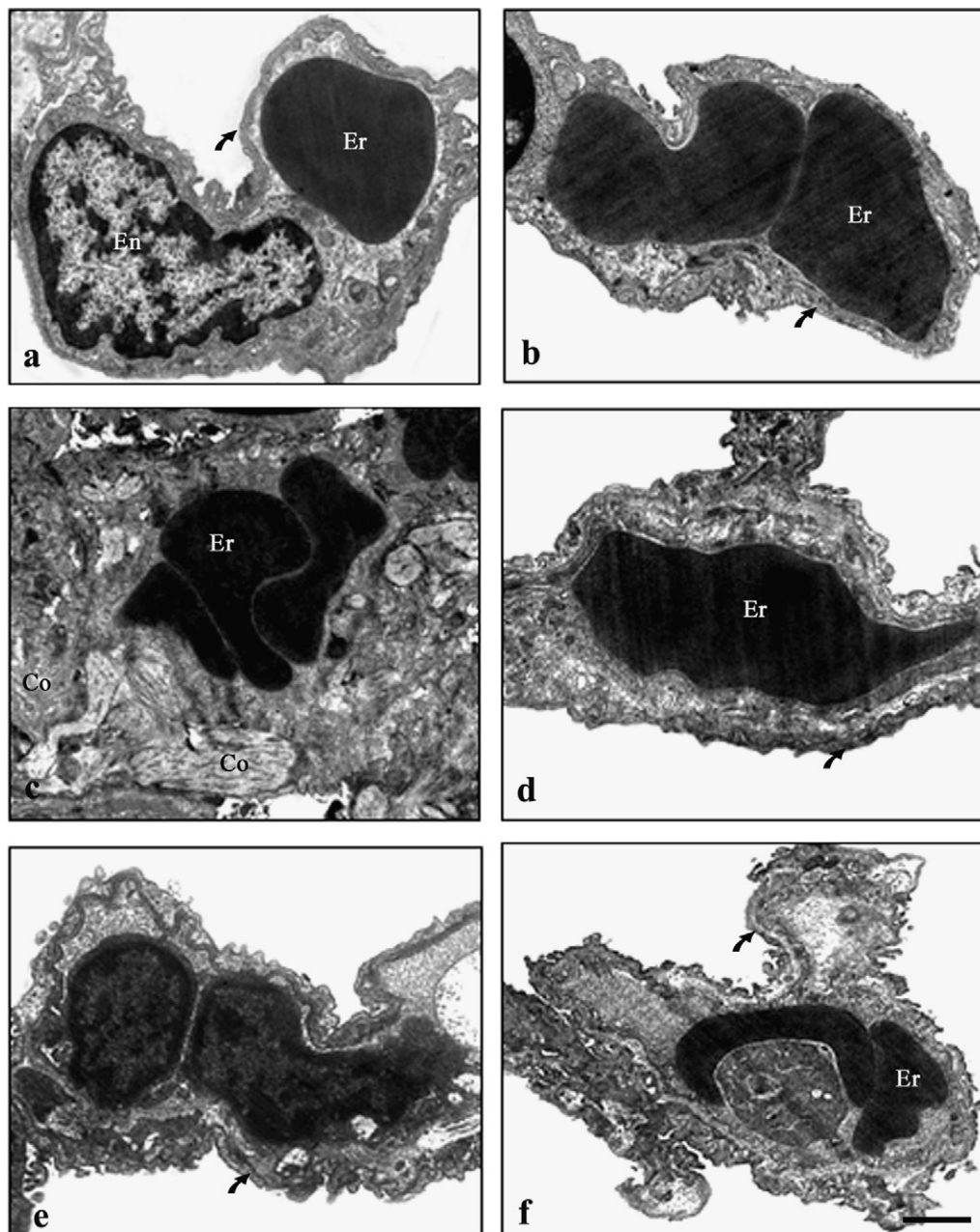


Fig. 5. Electron micrographs of pulmonary blood capillaries. (a) Normal mice; (b) GSE control; (c) OVA control. Note the congested blood capillary, with thick basement membranes containing collagen deposits. (d) OVA + DXM; (e) GSE + OVA; and (f) OVA + GSE. Arrows: air–blood barrier; Co: collagen fibers; En: endothelial cell; Er: erythrocyte. Scale bar = 2 μ m; original magnification = 15,000 $\times$.

4. Discussion

In this study, we examined the effect of grape seed extract on lung alterations in ovalbumin-induced asthma. Ovalbumin exposure interfered with the morphological and morphometric characteristics of the pulmonary parenchyma, leading to alveolar septa thickening, inflammatory cell infiltration, vascular congestion and eosinophilia. Ultrastructurally, evident accumulation of the extracellular matrix and fibroblasts, besides, hyperplasia of type II pneumocytes and empty lamellar bodies were depicted. These findings are in continece with the observations of Zeng et al. (2001) who studied the pathological changes in the peripheral airways and lung tissues in guinea pig asthma model. They reported widespread inflammation with eosinophilic infiltration in the alveolar walls, which were significantly thickened. They also

noticed cell swelling, evacuation of lamellar bodies and partial cell desquamation in type II alveolar cells. Using different asthma protocols, Xisto et al. (2005) and Oberholzer and Pretorius (2009) also reported the presence of marked eosinophilia and vascular congestion in the lung parenchyma together with the influx of inflammatory cells in the alveolar walls of asthmatic mice. They also observed increase in the thickness of the alveolar interstitium due to the deposition of extracellular matrix elements.

Many hypotheses could explain the mechanism leading to the histopathological events observed herein. Firstly, inflammation is believed to be the driving force behind most of the pathological features of asthma (Al-Muhsen et al., 2011). At the site of inflammation, eosinophils release diverse mediators, which contribute to the pathological alterations of asthma (Wu et al., 2006). Eosinophils also support fibroblast proliferation, collagen

synthesis, and myofibroblast maturation (Kay et al., 2004), which consequently contributes to enhanced collagen deposition and alveolar damage. Moreover, increased inflammatory cytokines, such as interleukin-4 and -5, stimulates the profibrotic transforming growth factor- β , which is expressed in various lung cells such as alveolar macrophage, fibroblasts and endothelial cells, to increase the accumulation of the extracellular matrix and the thickening of the interalveolar septa (Gao et al., 2009; Halwani et al., 2011). The histological findings of the present study are in favor of these propositions to explain lung parenchyma remodeling in ovalbumin-induced asthma. Such mechanism is also supported by the data obtained from phase I of this study, where significant increase in the levels of total immunoglobulin E in serum, and inflammatory cells, eosinophils and cytokines in bronchoalveolar lavage fluid were observed in asthmatic mice.

In the present study, ovalbumin sensitization induced significant increase in the number of type II pneumocytes. Type II cells hyperplasia has been reported after lung injury in many previous studies (Sulkowski et al., 1994; Wirtz and Schmidt, 1996; Baybutt et al., 2000; Aiso et al., 2010). Jeffery et al. (1992) and Soliman (2008) believed that asthma has an irritant effect on pulmonary tissues that might stimulate the proliferation of type II cells as a part of the inflammatory process. Hinata et al. (2003) attributed this increase in the number of type II cells to compensate for the injured pulmonary epithelium; as these cells are known to synthesize essential components that maintain the integrity of the alveolar epithelium and regulate the migration, differentiation and adhesion of fibroblasts and other types of cells (Li, 2009a). Such hyperplasia is also supposed to be part of the inflammatory response of asthma. Alveolar type II cells are known to produce specific molecules, enzymes, and proteins, such as surfactant proteins A, which participate in lung defense against noxious agents (Sano and Kuroki, 2005; Li, 2009a).

Morphometric quantification revealed a significant increase in the count of lamellar bodies. Lamellar bodies are the store of the pulmonary surfactant. Kida et al. (1997) reported that the surfactant forms a covering coat for the particulate matter to facilitate phagocytosis, which explains the hyperplasia of the lamellar bodies after ovalbumin sensitization. It was noted that most lamellar bodies in the asthmatic group appeared as empty sacs. Crim and Simon (1988) postulated that the reactive oxygen species resulting from active macrophages are injurious to type II pneumocytes and may lead to impaired surfactant synthesis.

Phase I of this study showed that GSE reduced total immunoglobulin E levels in serum and total inflammatory cells, eosinophils and cytokines in the bronchoalveolar lavage fluid, and effectively attenuated the pathological alterations in large airways of asthmatic mice. Similar results were obtained by Zhou et al. (2011), who treated asthmatic mice with intraperitoneal injections of proanthocyanidins extracted from grape seeds. They found that treatment with grape seed proanthocyanidins attenuated allergen-induced lung eosinophilia, significantly enhanced gamma interferon level and decreased interleukin-4 and -13 levels in the bronchoalveolar lavage fluid and total immunoglobulin E levels in serum. However, no references could be cited for the ameliorating effect GSE on the lung pathology induced by ovalbumin. In the current study, the significant findings are that asthmatic mice receiving GSE, either before or after inducing asthma, showed alleviation of many of the evaluated pathological changes of lung in the mouse model of asthma, including inflammation, vascular congestion, and thickening of the interalveolar septa and basement membranes of the air–blood barrier. Moreover, GSE restored the normal count and ultrastructure of type II cells, probably as an indication of the attainment of the healing process. Bearing in mind that inflammation is the chief factor in initiation and progression of asthma, the amelioration of the ovalbumin-induced asthma

deleterious effects by the administration of GSE is probably due to its renowned anti-inflammatory and antioxidant potency. GSE is also reported to possess significant multi-organ histological protection against various toxic assaults (Bagchi et al., 2002; El-Ashmawy et al., 2006, 2010; Hemmati et al., 2008; Sehirli et al., 2008; El-Beshbishy et al., 2010; Safa et al., 2010; Yalçın et al., 2010; Khalifa et al., 2011). It is worth noting that the protective effect of GSE was more significant than the curative one. In addition, the ameliorating effect of GSE on asthma-induced remodeling was almost similar to that of dexamethasone.

Dexamethasone and other corticosteroids are powerful suppressors of inflammation (Blyth et al., 2000), and, consequently, as potential airway remodeling modulators. In the present study, dexamethasone suppressed almost all the pathological changes in the lungs of asthmatic mice. However, it could not alleviate the thickness of the air–blood barrier. Data regarding the effect of corticosteroids on remodeling are rather conflicting (Bergeron et al., 2010). Several studies demonstrated a positive effect of corticosteroids on airway structural changes in asthma (Blyth et al., 2000; Trifileff et al., 2000; Zeng et al., 2001; Oberholzer and Pretorius, 2009; Hocaoglu et al., 2011). However, other studies reported no effect of corticosteroids on basement membrane thickness (Trigg et al., 1994; Boulet et al., 2000; Ward et al., 2002). Collectively, corticosteroids have a modulatory effect on some, but not all, components involved in remodeling in asthma (Murray, 2008; Montuschi, 2011).

In summary, the current results show the protective and therapeutic effects of GSE in ameliorating the structural alterations in pulmonary tissues of asthmatic mice. The results support the possibility of GSE as an effective anti-inflammatory dietary supplement to attenuate the pathogenicity of asthma. The novelty of this study lies in providing a detailed insight into lung parenchyma morphological and morphometric alterations in ovalbumin-induced mouse asthma model, as well as the exploration of a promising safe approach for the control of this allergic disease. While these preliminary results appear promising, further studies are required to elucidate the precise mechanism of the modulatory effect of GSE on asthma remodeling.

Conflicts of interest

No potential conflicts of interest were disclosed.

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