Original Article

The Possible Protective Effect of Magnolol on Triolein-Induced Lung Structural Changes in Rats: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Triolein is a neutral fat derives from an oleic acid. It has been used as a textile lubricant, a plasticizer and present in cocoa butter. Its occupational exposure lead to health hazards especially lung injury. Magnolol is a Chinese herbal complex with anti-inflammatory, antioxidant and antifibrotic actions.

Aim: The present research aimed to evaluate the possible protective effect of magnolol on triolein-induced lung structural changes in rats using histological and immunohistochemical study.

Materials and Methods: 45 male Wistar rats were used. Their weights were from 120 to 150 gm. They were divided into Group 1 (15 rats as control group); Group 2: 15 rats that received 0.2 ml triolein at the caudal vein then were sacrificed after 2, 4 and 21 days; Group 3: 15 rats that received 50 mg/kg oral dose of magnolol daily 60 minutes before giving triolein then were sacrificed after 2, 4 and 21 days.

Results: Group 2 showed significant thickened inter-alveolar septa, inflammatory cellular infiltrations, congested blood vessels, luminal inflammatory exudates, disorganized sloughed epithelium and lost cilia at H&E stained sections. Group 2 also showed significantly increased deposition of collagen fibers, significantly increased expression of alpha –Smooth muscle actin (α-SMA) in the interalveolar septa, wall of the blood vessels and bronchioles and significant increase in the number of macrophages with positive CD68 (Cluster of Differentiation 68) immunohistochemical reaction. Group 3 showed improvements of the previous pathological findings.

Conclusion: Magnolol could protect the lung from the injurious effect of triolein possibly due to its anti-inflammatory, antioxidant and antifibrotic actions. This may favor the use of magnolol for the treatment of lung injuries.

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Key Words: Histology; immunohistochemistry; lung; magnolol; triolein.

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INTRODUCTION

Triolein is a triglyceride derived from oleic acid. Triolein also is a neutral fat, constitutes the major component of bone marrow[1]. It can be found at cocoa butter as it is one of the principal ingredients of the non-drying oils and fats such as olive oil. It constitutes about 70-80% of the olive oil[2]. It is also can be used as a textile lubricant as well as a plasticizer which may lead to its release to the environment by different waste streams[3]. Different data indicates that the occupational exposure to triolein at the workplaces where it is produced or used might be due to its dermal contact as well as the ingestion of food products contains triolein[4].

Different experimental studies were used to evaluate the effect of triolein on the pathophysiology of different tissues as a model of fat embolism that takes place in patients after traumatic injury as well as inflammation[5]. Fat embolism like manifestation induced by triolein can lead to hypoxia, petechial rash, neurological manifestations, petechial hemorrhages in the brain, skin and retina besides pulmonary manifestations[6].

The possible effect of triolein on the pathophysiology of different tissues is due to its effect on the renin angiotensin system (RAS) which affects the production as well as release of angiotensin (Ang) II besides, fat embolization of the blood vessels[7].

Magnolol is the hydroxylated biphenyl bioactive complex present in Houpu magnolia (Magnolia officinalis) or in M. grandiflora. It has been used in the traditional Chinese as well as Japanese medicine for the treatment of different diseases such as gastrointestinal disorders and allergic diseases[8,9]. It possesses an anti-inflammatory action through the inhibition of cytokine production and leucocyte activation[10]. It also has an antioxidant, anti-fibrotic, anti-platelet, anti-thrombotic, anticancer, anti-atherosclerosis, anti-fungal and anti-allergic actions[9,11].

According to the previous mentioned data; the present research was designed to evaluate the possible protective effect of magnolol on triolein-induced lung structural changes in rats by means of histological as well as immunohistochemical study.

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MATERIALS AND METHODS

For the present research; 45 male Wistar rats were used. Their weights ranged from "120 to 150 grams" and were obtained from the animal house, Tanta University, Egypt. Animals were situated at hygienic environment with a normal room temperature. Rats then, were allowed to have a free access to diet and water ad libitum.

The safety measures of the experiment were fulfilled to be consistent with the guideline and care of the Committee of Tanta University, Institution of Research Ethics with the Approval code NO. 34476/2/21.

Animals were randomly divided into the following groups:

1. Group 1 (Control): included 15 rats which were further subdivided into subgroup 1a) 5 rats left without any treatments; subgroup 1b) 5 rats were given 0.2 ml saline at the caudal vein (vehicle for triolein) and subgroup 1c) 5 rats were given an oral dose of DMSO (vehicle for magnolol) corresponding to their experimental group for the same duration.
2. Group 2: included 15 rats which received triolein in a dose of 0.2 ml in the caudal vein then rats were sacrificed after 2, 4 and 21 days (5 rats sacrificed at each date)[12,13]
3. Group 3: included 15 rats that received a daily oral dose of magnolol (50 mg/kg) through an intra-gastric tube 60 minutes before giving triolein. Then, rats were sacrificed after 2, 4 and 21 days (5 rats sacrificed at each date)[14].

At the last day of the experiment; rats were anesthetized with 50 mg/kg pentobarbital sodium (injected intraperitoneal). The lung was then dissected and immediately fixed in 10% formol buffered saline. Then, lung specimens were dehydrated, cleared and then were embedded in paraffin. Finally, a rotatory microtome (Leica, US) was used to obtain 5 µm sections.

Hematoxylin & Eosin (H&E) stain procedure

The obtained lung sections were deparaffinized, hydrated and were stained with hematoxylin followed by counterstaining by 1% Eosin. Then, sections were dehydrated, cleared and mounted in Canada balsam. The staining tissue showed pink cytoplasm, and connective tissue fibers, while the nuclei appeared blue[15].

Mallory's trichrome stain procedure

Lung sections were deparaffinized, then rehydrated and re-fixed in Bouin's solution. Sections then were stained with Wiegert's iron hematoxylin and Biebrich scarlet-acid fuchsine. Followed by differentiation in phosphotungstic acid solution and aniline blue stain. Eventually, sections were dehydrated, cleared and mounted. This method stained collagen fiber with bluish coloration while nuclei stained red.

Alpha Smooth Muscle Actin (α-SMA) & Cluster of Differentiation 68 (CD68) immunohistochemical evaluation

Lung specimens were deparaffinized and rehydrated. Then, sections were microwaved for 15 minutes for antigen retrieval. After that, sections were treated with a blocking solution for 20 min then incubation with 1:100 α-SMA (Catalog No. sc-53142, Santa Cruz Biotechnology, Dallas, Texas) and 1:25 CD68 (Catalog No. sc-17832, Santa Cruz Biotechnology, Dallas, TX, USA) primary antibody at room temperature for half an hour[16,17]. Later, secondary antibody was added for 10 minutes. Finally, one to two drops of DAB (diaminobenzidine) was added for 10 minutes. At last, lung sections were counterstained with Mayer's hematoxylin, then dehydrated and cleared.

A light microscope (Olympus, Japan) was used for section examination. By which α-SMA expressed brownish coloration of the wall of the lung blood vessels, bronchioles and myofibroblasts. While, CD68 stained the interstitial lung macrophages with brownish positive.

As regards negative control; they were obtained by replacing the step of adding primary antibody with PBS. On the other hand, skin blood vessels were the positive control for α-SMA (www.ihcworld.com; cat. No. IW-MA1106). While, tonsil is the positive control for CD68 (https://biocare.net/product/cd68-antibody).

Morphometric evaluation

A- Histopathological scoring

A score from 5 - 30 (minimal to severe damage) was used for the evaluation of each of the following histopathological findings; thickened interalveolar septa, congested blood vessels, bronchiolar affection, and inflammatory cellular infiltrations[12].

B- Collagen and alpha-smooth muscle scoring

Collagen staining as well as alpha-smooth muscle immunostaining were evaluated at the lung blood vessels, interalveolar septa, and peribronchial area through using the same previous scoring for histopathology[12].

C- Number of CD68 positive lung tissue macrophages

An image analysis software (Image J; 1.47v, National Institutes of Health, Bethesda) was used to evaluate the number of CD68 positive lung tissue macrophages that were situated at the interalveolar septa. Five images (at x400 magnification) of the lung tissue stained with CD68 antibody of each group were used. Intersect grid lines of 13 × 13 were made superimposed for each image, then the number CD68-positive macrophages existing in inter-alveolar septa was counted.

Statistical analysis

Data were recorded as means ± SD after using IBM SPSS software (USA). After the evaluation by F-test; the two-sample Student's t-test was used to determine the

significant difference between the experimental groups. Then, the significant values were established when $p < 0.05$.

RESULTS

H& E results

The control group displayed normal histological structure of the lung tissue. It showed alveoli that were separated from each other by a thin interalveolar connective tissue septa containing blood vessels. There were also alveolar sacs, alveolar ducts besides bronchioles lined by simple columnar ciliated epithelium, smooth muscle, and connective tissue (Figure 1A).

Lungs of the rats of group 2 that received triolein and sacrificed after 2, 4, 21 days showed progressive pulmonary histopathological findings. Rats sacrificed after 2 days revealed thickened inter-alveolar septa with inflammatory cellular infiltrations. As for the interalveolar blood vessels; they showed congestion. Moreover, the bronchioles revealed presence of luminal inflammatory exudates with disorganized sloughed epithelium and partially surrounded by inflammatory cellular infiltrations (Figure 1B).

Rats sacrificed after 4 days expressed progressive thickened septa with inflammatory cellular infiltrations. In addition to advanced congestion of the blood vessels. As regards bronchioles; they showed areas of sloughed epithelium, and others with lost cilia besides peri-bronchiolar cellular infiltrations (Figure 1C).

Regarding rats sacrificed after 21 days, they showed marked thickened interalveolar septa with massive inflammatory cellular infiltrations. In addition to markedly congested blood vessels with thickened wall. Regarding bronchioles; there were disorganized lining epithelium with lost cilia of some cells (Figure 1D).

As regards group 3 (rats treated by triolein and magnolol); they showed improvements of the previous H & E findings. A nearly normal histological lung picture was seen except for some vessel congestion for the rats sacrificed after 4 days and some congestion with few areas of thickened interalveolar septa for the rats sacrificed after 21 days (Figures 2 E-G).

As regards the histopathology scoring; it showed significant increase for the groups treated by triolein and sacrificed after 2; 4& 21 days when compared to the control group. While, significant decrease in the groups treated by triolein and magnolol and sacrificed after 2; 4; & 21 days (group 3) as compared to the triolein group (group 2) (Figure 3).

Mallory's trichrome results

Mallory's trichrome stained sections of the control group revealed little amount of collagen fibers in the interalveolar septa, wall of blood vessels as well as around bronchioles (Figure 4A).

Group 2 of triolein that sacrificed after 2; 4 and 21 days showed progressive deposition of collagen fibers in the interalveolar septa, besides the wall of the blood vessels and bronchioles (Figures 4 B-D).

For the rats sacrificed after 2 & 4 days of group 3; they showed few amounts of collagen fibers. However, moderate amounts were seen in rats sacrificed after 21 days (Figures 5 E-G).

Statistically, collagen scoring revealed significant increase in collagen fibers in group2 (triolein group sacrificed after 2; 4& 21 days) as compared to the control group. While, significant decrease in group 3 (triolein and magnolol and sacrificed after 2; 4; & 21 days) when compared to the triolein group (group 2) (Figure 6).

α-SMA immunohistochemical results

Immunohistochemical expression of α-SMA of the negative control showed no α-SMA immunohistochemical reactions (Figure 7A), while the posiitve control group showed positive raction at the wall of the blood vessels , and around broncioles (Figure 7B).

Rats of triolein group (group 2) sacrificed after 2; 4 and 21 days revealed progressive increased expression of α-SMA in the interalveolar septa, as well as the wall of the blood vessels and bronchioles to be more evident for the rats that sacrificed after 21 days (Figures 7C-E).

Rats sacrificed after 2 days of group 3 showed a nearly normal immunohistochemical expression while for that sacrificed after 4 days showed mild α-SMA expression. On the other hand, rats sacrificed after 21 days showed moderate α-SMA immunohistochemical expression of the wall of the blood vessels besides bronchioles while mild expression at the interalveolar septa (Figures 8F-H).

α-SMA scoring for group2 (triolein group sacrificed after 2; 4& 21 days) expressed a significant increase when compared to the control group. On the other hand, a significant decrease was detected in group 3 (triolein and magnolol and sacrificed after 2; 4; & 21 days) as compared to group 2 (triolein group) (Figure 9).

CD68 immunohistochemical results

The negative control showed no any reactions for CD68 (Figure 10 A), while the control group expressed few number of macrophages in the interalveolar septa with brown positively CD68 immunohistochemical reaction. Conversely, in group 2; the number of macrophages with positive CD68 immunohistochemical reaction was progressively increased especially for the rats sacrificed after 21 days (Figure 10 B-E).

For group 3; a nearly normal picture seen for the rats sacrificed after 2 & 4 days while moderate number for the rats sacrificed after 21 days (Figures 11 F-H).

Considering the number of CD68 positive lung macrophages; it showed a significant increase in group2 (triolein group sacrificed after 2; 4& 21 days) in comparison to control. Conversely, a significant decrease was observed in group 3 (triolein and magnolol and sacrificed after 2; 4; & 21 days) when compared to group 2 (triolein group) (Figure 12).

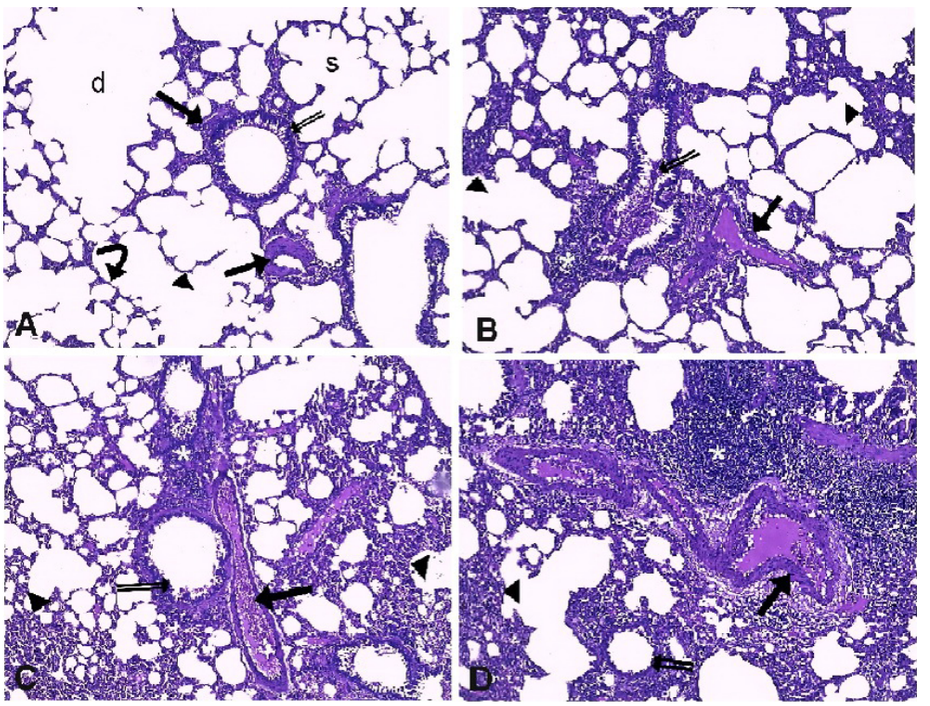

Fig. 1: Effect of magnolol on triolein-induced lung changes stained by H & E (x100). A) Group 1 (Control): alveoli (curved arrow), thin septa (►), blood vessels (→), alveolar sacs (s), alveolar ducts (d), bronchioles lined by simple columnar ciliated epithelium (double arrow). B) Group 2 (rats sacrificed after 2 days): thickened inter-alveolar septa with inflammatory cellular infiltrations (►), Congested blood vessels (→), bronchioles with luminal inflammatory exudates, disorganized sloughed epithelium (double arrow) and partially surrounded by inflammatory cellular infiltrations (*). C) Group 2 (Triolein group) (Rats sacrificed after 4 days): progressive thickened septa with inflammatory cellular infiltrations (►), advanced congestion of blood vessels (→), bronchioles with areas of sloughed epithelium, lost cilia (double arrow) and peri-bronchiolar cellular infiltrations (*). D) Group 2 (Rats sacrificed after 21 days): marked thickened interalveolar septa (►) with massive inflammatory cellular infiltrations (*), markedly congested blood vessels with thickened wall (→), bronchioles with disorganized epithelium and lost cilia of some cells (double arrow).

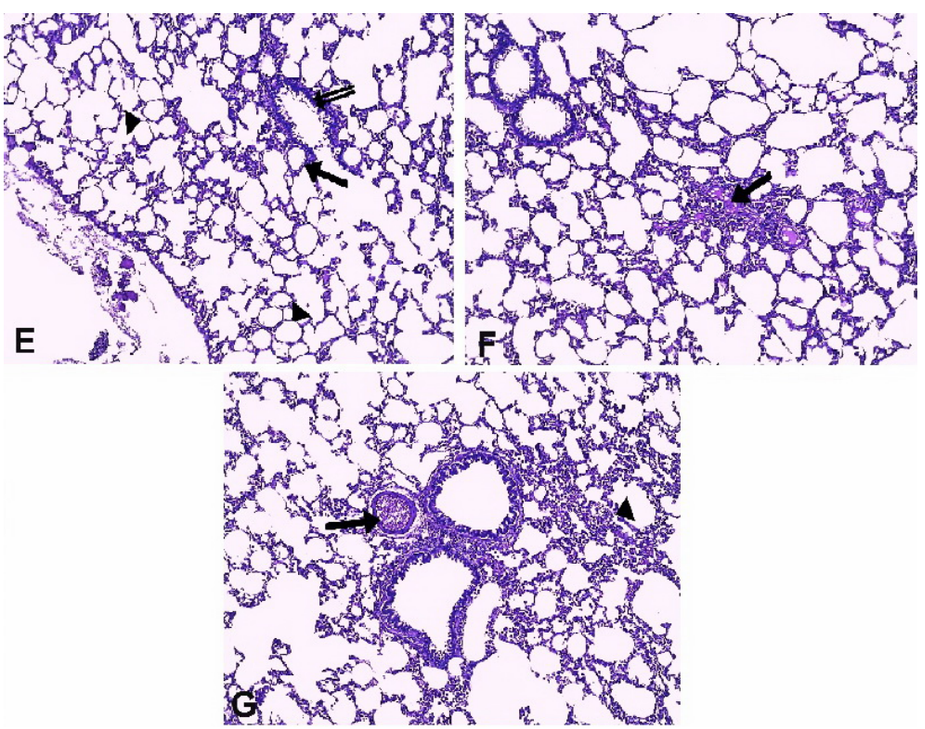

Fig. 2: Effect of magnolol on triolein-induced lung changes stained by H & E (x100). E) Group 3 (Triolein+ magnolol group) (after 2 days): nearly normal alveoli with thin septa (►), blood vessels (→), bronchioles lined by simple columnar ciliated epithelium (double arrow). F) Group 3 (after 4 days): some vessel congestion (→). G- Group 3 (after 21 days): some congestion (→) and few areas of thickened interalveolar septa (►).

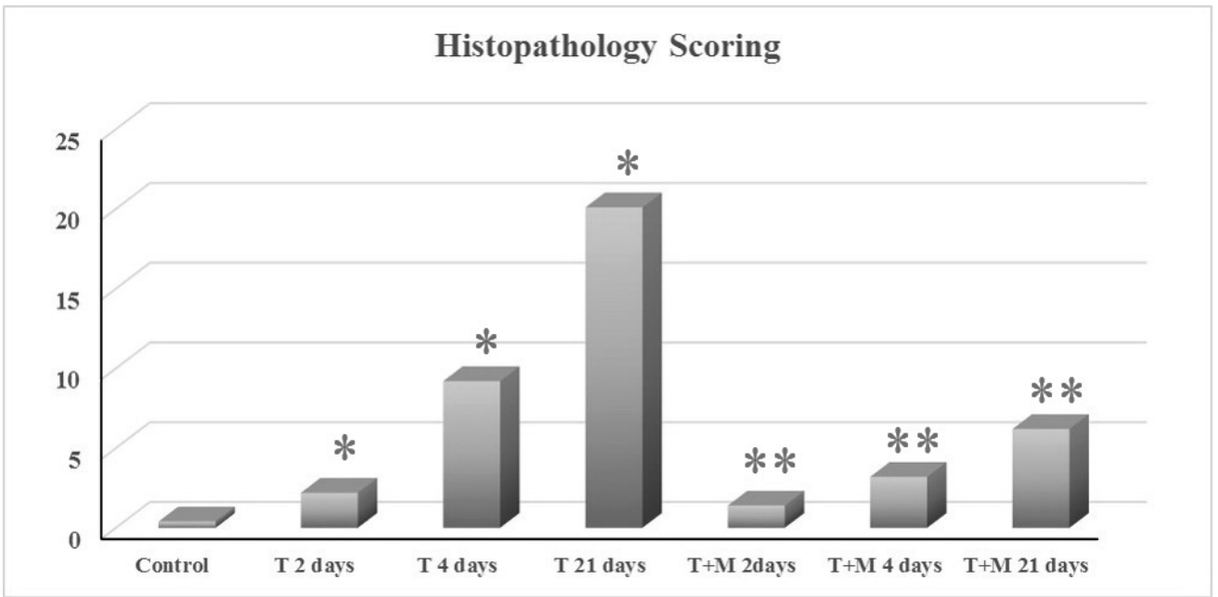

Fig. 3: Histopathology lung score. Data expressed as mean ± SD. T 2; T 4 &T 21 days (Group 2 that received triolein and rats sacrificed after 2; 4 &21 days); * significant increase in comparison to group 1 (control). T+M 2; T+M 4 & T+M 21 days (Group 3 that received magnolol 60 minutes before triolein and rats sacrificed after 2; 4& 21 days); ** significant decrease in comparison to group 2 (triolein group) respectively.

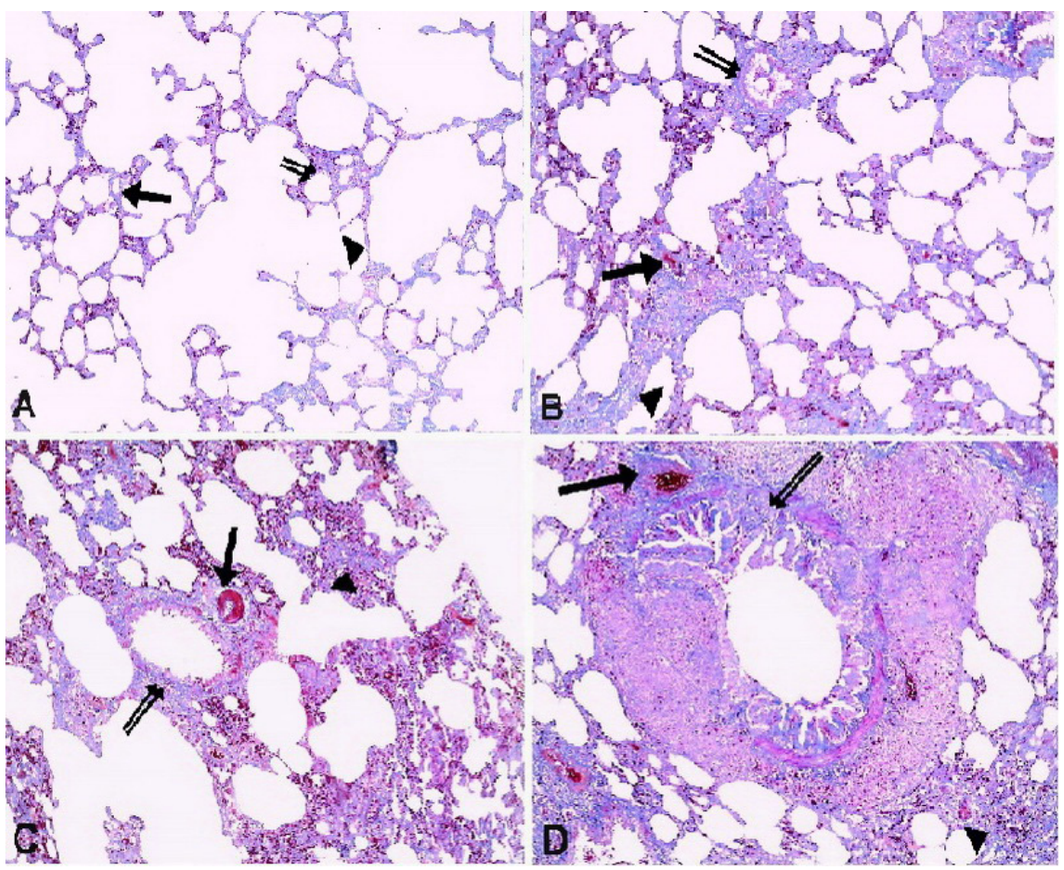

Fig. 4: Effect of magnolol on triolein-induced lung changes stained by Mallory's trichrome (x100). Group 1 (Control): little amount of collagen fibers in the interalveolar septa (►), wall of blood vessels (→) and around bronchioles (double arrow). B) Group 2 (rats sacrificed after 2 days): mild deposition of collagen fibers in the interalveolar septa (►), wall of the blood vessels (→) and around bronchioles (double arrow). C) Group 2 (rats sacrificed after 4 days): moderate deposition of collagen fibers in the interalveolar septa (►), wall of the blood vessels (→) and around bronchioles (double arrow). D) Group 2 (rats sacrificed after 21 days): progressive amounts of collagen fibers in the interalveolar septa (►), wall of the blood vessels (→) and around bronchioles (double arrow).

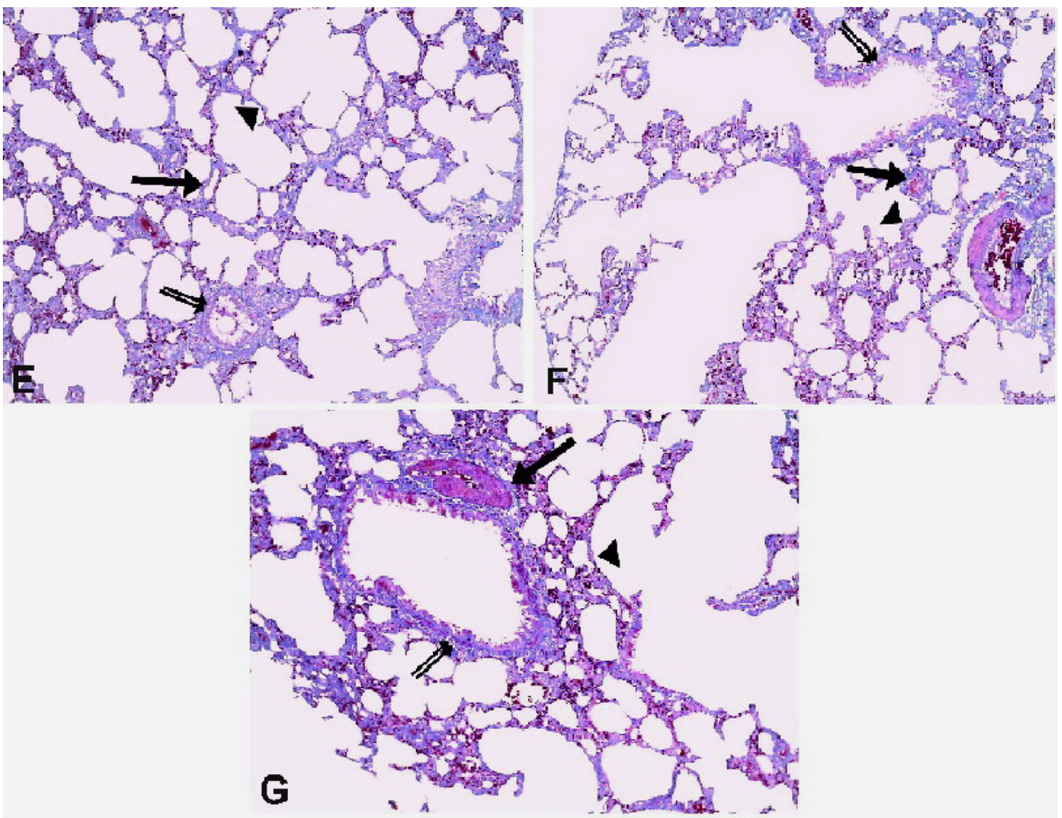

Fig. 5: Effect of magnolol on triolein-induced lung changes stained by Mallory's trichrome (x100). E) Group 3 (after 2 days): little amount of collagen fibers in the interalveolar septa (►), wall of blood vessels (→) and around bronchioles (double arrow) like control. F) Group 3 (after 4 days): little amount of collagen fibers in the interalveolar septa (►), wall of blood vessels (→) and around bronchioles (double arrow). G) Group 3 (after 21 days): few amount of collagen fibers in the interalveolar septa (►), wall of blood vessels (→) and around bronchioles (double arrow).

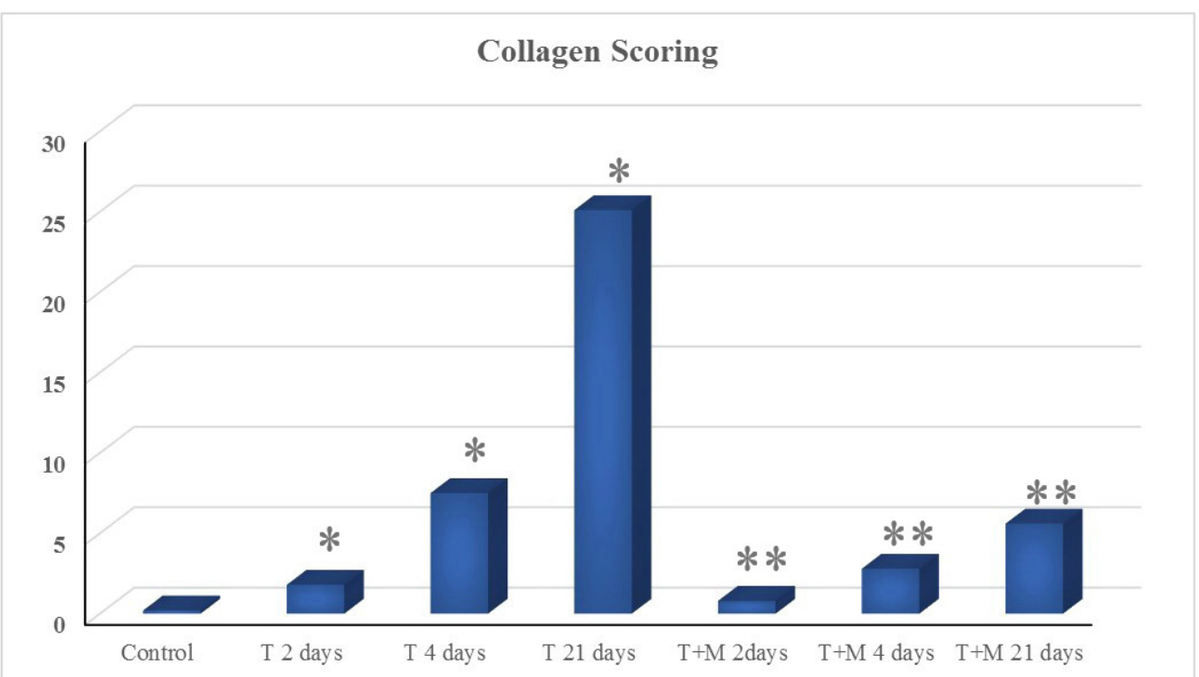

Fig. 6: Collagen score. Data expressed as mean ± SD. T 2; T 4 &T 21 days (Group 2 that received triolein and rats sacrificed after 2; 4 &21 days); * significant increase in comparison to group 1 (control). T+M 2; T+M 4 & T+M 21 days (Group 3 that received magnolol 60 minutes before triolein and rats sacrificed after 2; 4& 21 days); ** significant decrease in comparison to group 2 (triolein group) respectively.

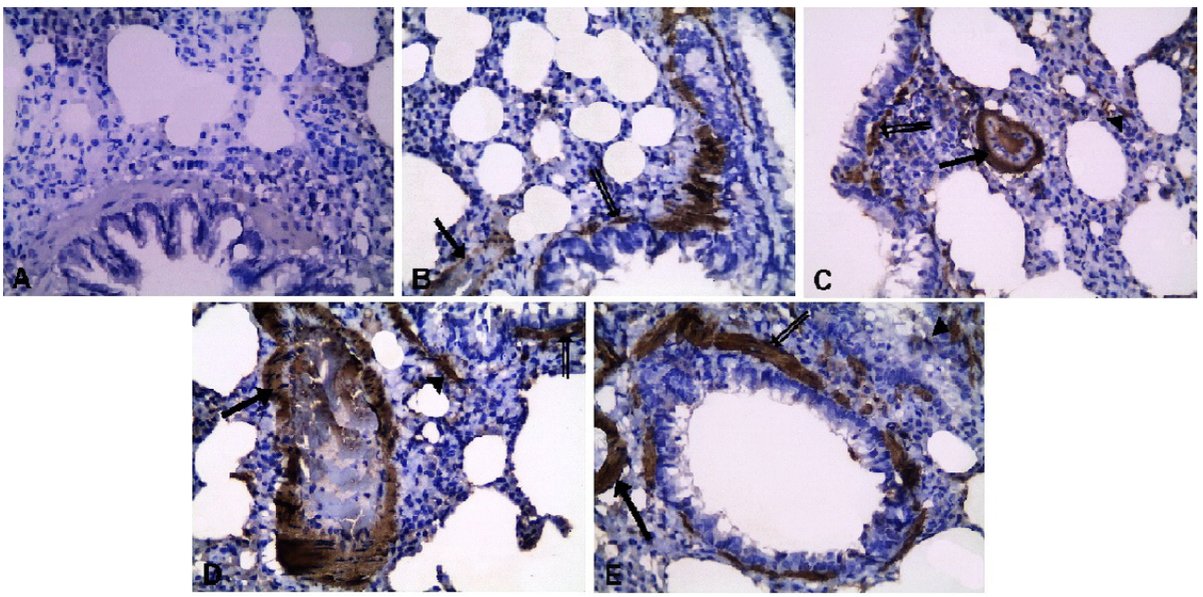

Fig. 7: Effect of magnolol on triolein-induced lung changes stained by α-SMA antibody (x400). A) Negative control: no α-SMA immunohistochemical reactions B) group 1 (control): positive α-SMA raction at the wall of the blood vessels (→) and around broncioles (double arrow). C) Group 2 (rats sacrificed after 2 days): mild increased expression of α-SMA in the interalveolar septa (►), wall of the blood vessels (→) and bronchioles (double arrow). D) Group 2 (rats sacrificed after 4 days): moderate increased expression of α-SMA in the interalveolar septa (►), wall of the blood vessels (→) and bronchioles (double arrow). E) Group 2 (rats sacrificed after 21 days): sever increased expression of α-SMA in the interalveolar septa (►), wall of the blood vessels (→) and bronchioles (double arrow).

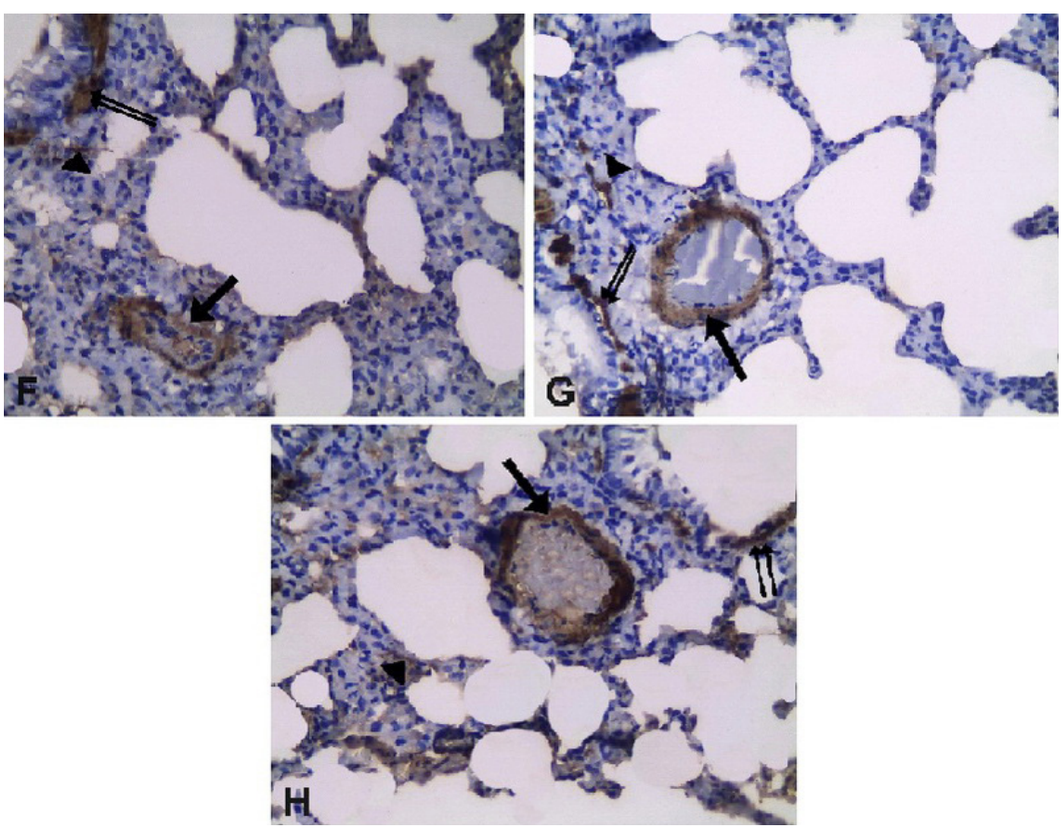

Fig. 8: Effect of magnolol on triolein-induced lung changes stained by α-SMA antibody (x400). F) Group 3 (rats sacrificed after 2 days): nearly normal with mild increased expression of α-SMA in the interalveolar septa (►), wall of the blood vessels (→) and bronchioles (double arrow). G) Group 3 (rats sacrificed after 4 days): mild increased expression of α-SMA in the interalveolar septa (►), wall of the blood vessels (→) and bronchioles (double arrow). H) Group 3 (rats sacrificed after 21 days): moderate increased expression of α-SMA at the wall of the blood vessels (→), bronchioles (double arrow) and mild expression at the interalveolar septa (►).

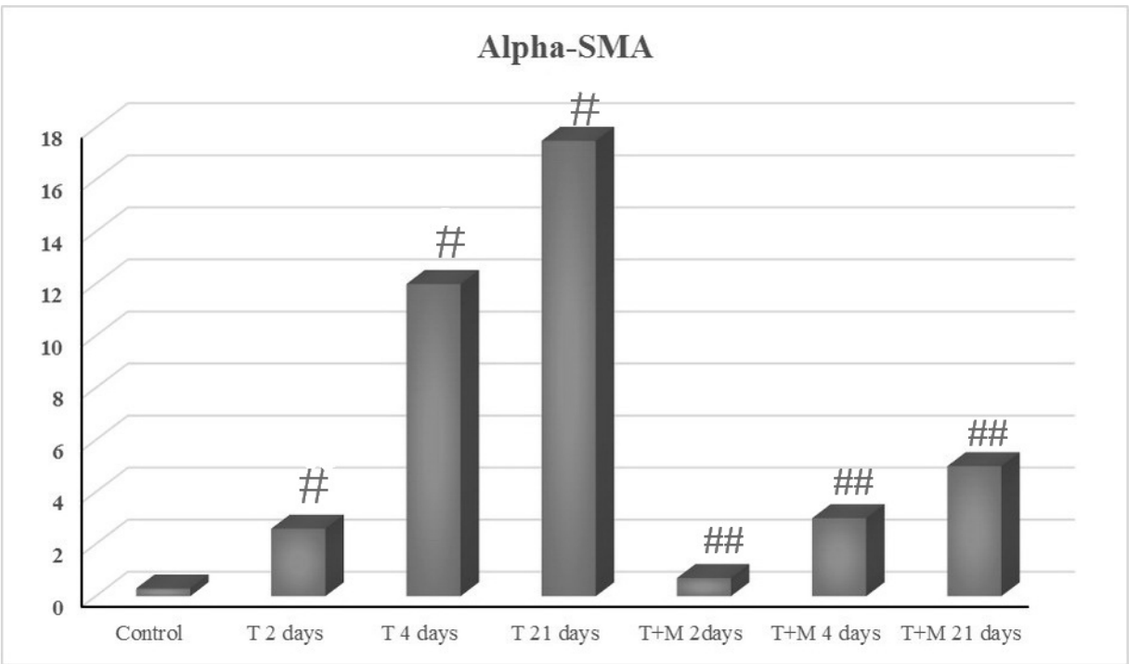

Fig. 9: α- SMA score. Data expressed as mean ± SD. T 2; T 4 &T 21 days (Group 2 that received triolein and rats sacrificed after 2; 4 &21 days); # significant increase in comparison to group 1 (control). T+M 2; T+M 4 & T+M 21 days (Group 3 that received magnolol 60 minutes before triolein and rats sacrificed after 2; 4& 21 days); ## significant decrease in comparison to group 2 (triolein group) respectively.

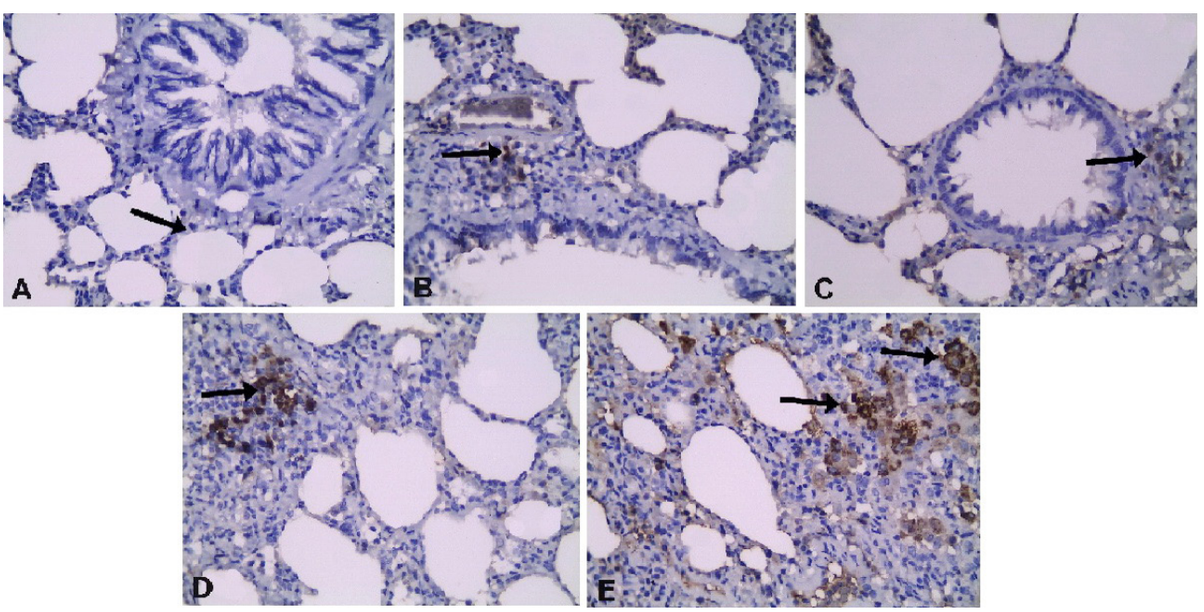

Fig. 10: Effect of magnolol on triolein-induced lung changes stained by CD68 antibody (x400). A) Negative control: with no primary antibody showed no any reactions (→). B) group 1 (control): few number of macrophages in the interalveolar septa with brown positively CD68 immunohistochemical reaction (→). C) Group 2 (rats sacrificed after 2 days): mild increase of the number of macrophages with positive CD68 immunohistochemical reaction (→). D) Group 2 (rats sacrificed after 4 days): moderate increase of the number of macrophages with positive CD68 immunohistochemical reaction (→). E) Group 2 (rats sacrificed after 21 days): sever increase of the number of macrophages with positive CD68 immunohistochemical reaction (→).

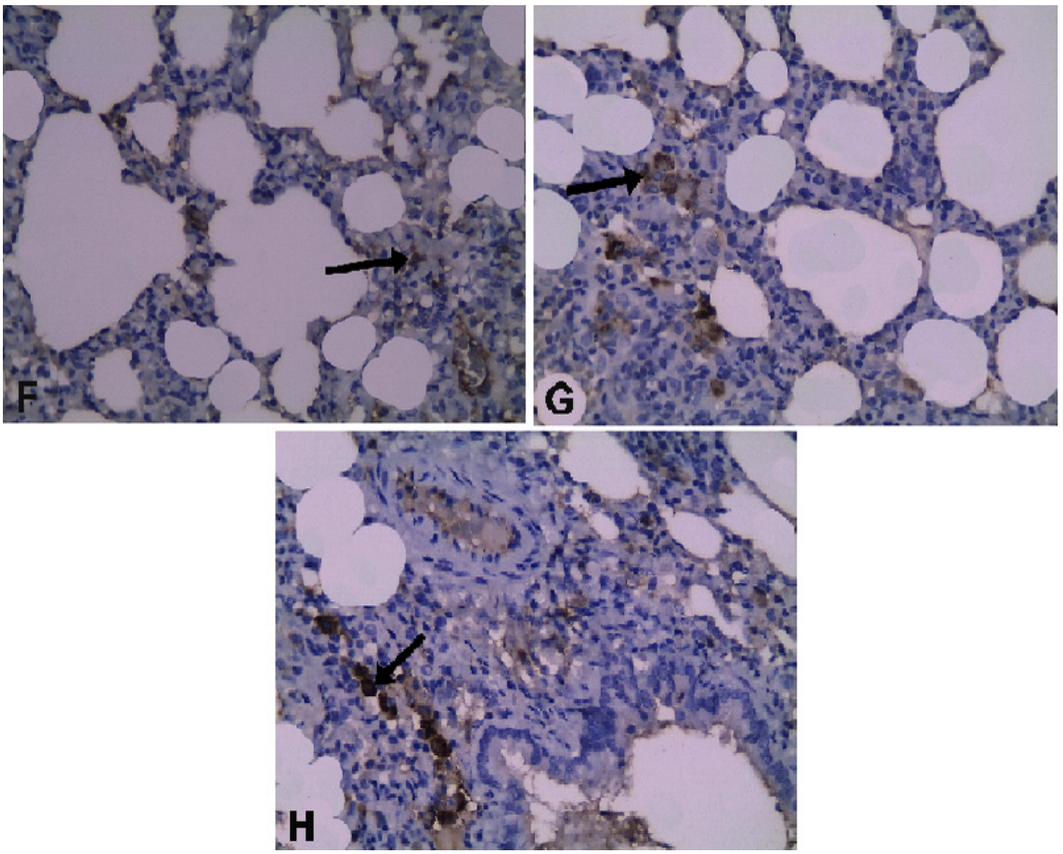

Fig. 11: Effect of magnolol on triolein-induced lung changes stained by CD68 antibody (x400). F) Group 3 (rats sacrificed after 2 days): nearly normal with mild number of CD68 positive macrophages (→). G) Group 3 (rats sacrificed after 4 days): mild number of CD68 positive macrophages (→). H) Group 3 (rats sacrificed after 21 days): moderate number of CD68 positive macrophages (→).

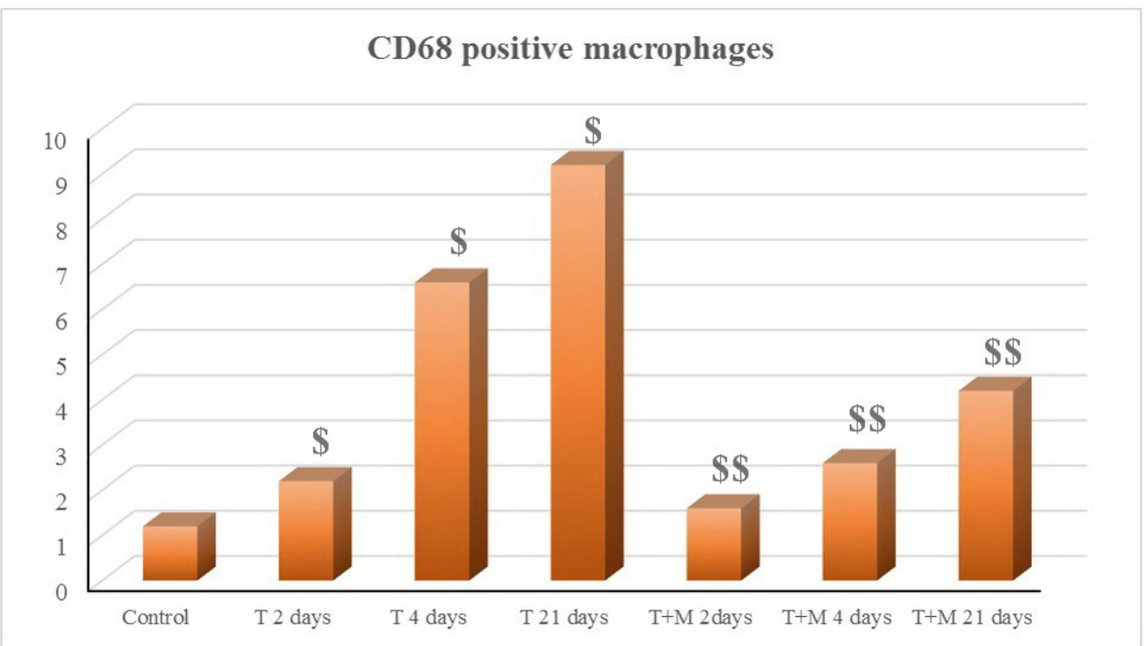

Fig. 12: Number of CD68 positive lung macrophages. Data expressed as mean ± SD. T 2; T 4 &T 21 days (Group 2 that received triolein and rats sacrificed after 2; 4 &21 days); $ significant increase in comparison to group 1 (control). T+M 2; T+M 4 & T+M 21 days (Group 3 that received magnolol 60 minutes before triolein and rats sacrificed after 2; 4& 21 days) $$ significant decrease in comparison to group 2 (triolein group) respectively.

DISCUSSION

The present research revealed that triolein has induced several structural lung changes in a time course manner. These were in the form of thickened inter-alveolar septa, inflammatory cellular infiltrations, congested blood vessels, luminal inflammatory exudates, disorganized sloughed epithelium and lost cilia which were seen at H&E stained sections. Also, there was significant progressive deposition of collagen fibers, and significantly increased expression of α-SMA in the interalveolar septa, wall of the blood vessels and bronchioles.

Besides, a significant increase in the number of macrophages with positive CD68 immunohistochemical reaction. These were in agreement with Mclff *et al*. 2010[12] who showed a progressive histopathological changes of triolein at the lung tissue in a time- course manner. Moreover, Poisner *et al*. (2018)[18] has proved that, the peak histopathological findings were for the rats were given i.v triolein then were sacrificed after 21 days.

The histopathological changes found in H&E stained sections of triolein group (group 2) might be due to its action on lung angiotensin II (Ang II) that will further increase the expression of lipase in the vascular tissues consequently, increased the breakdown of triolein to oleic acid, a more toxic material than triolein[19]. Moreover, Ang II leads to increased permeability from the blood vessels with subsequent pro-inflammatory as well as pro-fibrotic effects manifested by the increased tissue exudates and cellular infiltrations[19]. In addition, the Ang II-dependent pathological pathways are considered to be the chief source of generating reactive oxygen species (ROS) with subsequent cellular damage[20].

The disorganized sloughed epithelium and lost cilia presented in the present work can be attributed to the fact that triolein is the precursor of free fatty acids by the action of endothelial lipases and its histopathological effects are due to the activation of phospholipases A2. It hydrolyzes membrane phospholipids with the synthesis of arachidonic acid. Moreover, by the effect of cyclooxygenase; arachidonic acid is converted into prostaglandins, thromboxanes, and oxygen-free radicals. Consequently, structural changes of the bronchial epithelial cells[21].

The significantly increased collagen in the present work of triolein group (group 2) can be explained by the release of pro-fibrotic mediators from macrophages by triolein so, initiating an inflammatory response. As a result, increased deposition of collagen fibers with thickened interalveolar septa together with inflammatory cellular infiltrations[13]. Additionally, previous studies reported the effect of renin angiotensin system activation in local mast cells with the induction of lung injury and fibrosis especially with the stimulation of macrophages and other inflammatory cells[22].

α-SMA is a marker for the myofibroblasts in fibrotic lung. These cells are smooth muscle-like cells originated from differentiated fibroblast that in-turn acquired a contractile property like smooth muscle cells. These can be identified in the pulmonary interstitial spaces[23]. By increased α-SMA expression; an increased deposition of the extracellular matrix occurs leading to increased collagen deposition consequently, fibrosis[24]. Sun *et al*. (2016)[25] added that the increased expression of α-SMA as a marker of activated fibroblasts is strongly indicates the presence of fibrogenesis.

CD68 is an integral membrane glycoprotein expressed by tissue macrophages. It is expressed on its intracellular lysosomes. It has an important role in the different phagocytic activities of tissue macrophages. Especially the intracellular lysosomal metabolism besides, the extracellular cell to cell as well as cell to pathogen interactions[26].

The significantly increased number of macrophages stained by CD68 in the immunohistochemical results of group 2 can be explained by So, by the lung injury and fibrosis induced by triolein with the stimulation of lung macrophages and other inflammatory cells. Consequently, stimulation of the renin angiotensin system in the locally situated mast cells leading significant positive CD68 expression[22].

The naturally occurred products are good sources of a new therapeutics. Magnolol, a plant derived complex. It is isolated from Magnolia officinalis. It exerts different pharmacological actions like antioxidant and anti-inflammatory ones. It was proved to have effective improvements of the lung injury through attenuation of inflammatory cells migration beside the reduction of inflammation[14].

In the present work, magnolol induced improvements of the time-course triolein-induced lung structural changes seen in group 3. These were in the form of significantly decreased histopathological findings in H&E stained sections, significantly decreased collagen fibers, and significantly decreased α-SMA and Cd68 immunohistochemical expression.

Magnolol inhibited the release of signal transducer and activator of transcription 6 (STAT6) which regulates the release of different inflammatory cellular infiltrations. So, its inhibition leads to decreased lung inflammation, exudates and the deposition of the inflammatory cellular infiltrations[14].

Moreover, the improved epithelial as well as endothelial cell functions in group 3 could be due to the inhibition of cytokine production and nuclear factor-kB activation by magnolol. Furthermore, it suppresses leukocytes through the inhibition of myeloperoxidase activity, leukotriene formation and histamine release improving the blood vessel functions[27]. Besides, inhibition of tumor Necrosis Factor-α, matrix metalloproteinase-1 and nitric oxide (NO) (which causes dysfunction and damage of the epithelial and endothelial cells). The inhibition of nitric oxide release

is through the inhibited iNOS expression and activation. So, inhibits the release of the oxygen free radicals and ROS production as well as improvements of the endothelial as well as epithelial functions[20].

Significant decrease in collagen production in group 3 was due to the possible inhibition of the increase of TNF-α as well as TGF-β by magnolol. These cytokines were proved to have a role in lung fibrosis[27].

The significantly decreased α-SMA immunohistochemical expression could be due to the inhibited proliferation of vascular smooth muscle cells as well as the proliferation of fibroblast through the inhibition of ROS generation by magnolol[20].

The significantly decreased CD68 immunohistochemical expression in group 3 can be explained by the fact that magnolol suppresses IL-6-induced ICAM-1 that is released by the endothelial cells. Also, suppresses NF-κB-regulated inflammatory gene products with the inhibition of leucocyte adhesion. Consequently, attenuating the inflammatory processes with the inhibited stimulation of macrophages[27].

CONCLUSION

Form the previously mentioned data we can conclude that magnolol can protect the lung from the injurious effect of triolein through its anti-inflammatory, antioxidant and antifibrotic actions. These findings may support the potential application of magnolol, introduce a new insight for using magnolol in the treatment of different lung injuries.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائي المحتمل للمجنولول على التغيرات التركيبية الرئوية المستحثة بالتريولين في الجرذان: دراسة هستولوجية وهستوكيميائية مناعية

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المقدمة: تريولين هو مادة دهنية متعادلة مشتقة من حمض الأوليك. يتم إستخدامه كمادة لتشحيم الأنسجة وملدن وموجود في زبدة الكاكاو. التعرض المهني له يؤدي إلى مخاطر صحية وخاصة إصابة الرئة. مجنولول هو مركب عشبي صيني ذو نشاط مضاد للإلتهابات ومضاد للأكسدة ومضاد للتليف.

الهدف: البحث الحالي يهدف لتقييم التأثير الوقائي المحتمل للمجنولول على التغيرات التركيبية الرئوية المستحثة بالتريولين في الجرذان بإستخدام دراسة هستولوجية وهستوكيميائية مناعية.

المواد والطرق: تم إستخدام ٤٥ من ذكور جرذان ويستار (١٢٠ - ١٥٠ جم). تم تقسيمهم إلى المجموعة ١ (١٥ جرذًا كمجموعة تحكم)؛ المجموعة الثانية: ١٥ جرذًا تلقت ٠,٢ مل من التريولين في الوريد ثم تم التضحية بها بعد ٢ و ٤ و ٢١ يومًا ؛ المجموعة ٣: ١٥ جرذًا تناولت ٥٠ مجم / كجم جرعة عن طريق الفم من المجنولول يوميًا ٦٠ دقيقة قبل التريولين ثم تم التضحية بها بعد ٢ و ٤ و ٢١ يومًا.

النتائج: أظهرت المجموعة الثانية وجود زيادة كبيرة في الحواجز بين الحويصلات ، إرتشاح خلوي إلتهابي ، وإحتقان في الأوعية الدموية ، وإفرازات التهابية تجويفية ، ونسيج طلائي غير منظم ومنفصل ، وأهداب مفقودة ، مع زيادة كبيرة في ترسب ألياف الكولاجين ، وزيادة كبيرة في التعبير عن alpha -SMA في الحاجز بين الحويصلات وجدار الأوعية الدموية والقصيبات وزيادة كبيرة في عدد البلاعم مع تفاعل CD68 المناعي الإيجابي. ، وقد أظهرت المجموعة ٣ تحسينات في هذه النتائج المرضية السابقة.

الخلاصة: مجنولول يحمي الرئتين من التأثير الضار للتريولين من خلال نشاطه المضاد للإلتهابات ومضاد الأكسدة ومضاد للتليف. هذا قد يوفر نظرة ثاقبة جديدة للمجنولول لعلاج إصابات الرئة.