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Propolis as a Protective Agent against Histological Pulmonary Changes Induced by Formaldehyde Inhalation

Yasmin Ramadan[(1)] and Marwa A. Fetouh[(1)]

[1]Department of Anatomy and Embryology, Ain Shams Faculty of Medicine, Cairo, Egypt

Corresponding author: **Marwa A. Fetouh:** *marwa_ali@med.asu.edu.eg*

Abstract

Background: Formaldehyde, a sterilizing and preservative agent, poses detrimental effects to body systems. Propolis, produced by bees, used in the treatment of many diseases by folk medicine has antioxidant, antibacterial, Anti-inflammatory effects.

Aim of the work was to investigate the effects of toxicity of formaldehyde on lungs and protective effects of propolis against these effects.

Material and methods: Thirty-five male Wistar rats were divided into four Groups. Group I comprised the control ones, Group II received propolis alone dissolved in distal water, while Group III were exposed to 10% Formaldehyde inhalation. The rats in Group IV were exposed to 10 % Formaldehyde as in Group III with co-administration of propolis daily for two weeks. After sacrifice, Hx & E and Masson Trichrome stains were applied and immunohistochemical staining for anti NF-Kappa was performed to lung specimens.

Results: FA Group showed fatty cellular infiltration in the pulmonary interstitium and thickening of the inter-alveolar septa. Dilatation and congestion of alveolar septal vessels were prominent with hyperplasia of the bronchiolar epithelium. Propolis-treated Group showed less cellular infiltration, less dilatation of inter-alveolar septal vessels as compared to FA Group. Bronchiolar wall structure almost returned to the normal structure of control Group.

Conclusion: Propolis showed protective and anti-inflammatory activity against FA injurious lung effects.

Keywords: Formaldehyde, Propolis, Lung.

Introduction

Formaldehyde (FA) is a naturally existing, colorless, and inflammable gas that is produced in our bodies in small amounts. It is actually required for cell growth in all living cells **(Burgos-Barragan et al., 2017).** However, increase in its concentration turns it to be dangerous, harmful and in some cases carcinogenic **(Türkoğlu et al., 2008)**. It does not present for a long time in its original form. Most of it is broken down into hydrogen and carbon monoxide molecules once present in the air. On presence in water, it is changed to glycol (**Agency for Toxic Substances and Disease Registry, 2011).**

FA causes irritation of the lining epithelium of the nose, eyes and the throat. This irritation can be caused by low concentrations with people sensitive to odors. Other effects may include nausea, headache, running nose, difficult breathing up to asthmatic attacks (**Abdollahi and Hosseini, 2014).** In addition, studies performed on workers exposed to FA inhalation for long duration showed increased risk of lung cancer (**Marsh et al., 2016).**

Propolis, a natural bee product, varies in its chemical composition according to the geographical origin, the bee species and the feeding trees and flowers (**Kurek-Górecka et al., 2014).** Despite this variation, several studies proved that propolis possesses anti-oxidant (**Mouhoubi-Tafinine et al., 2016),** anti-inflammatory, bactericidal (**Soltani et al., 2017)** and anti-tumor effects (**Benguedouar et al., 2016).**

Aim of the work:

The aim of this study was to evaluate the anti-inflammatory and the antioxidant role of propolis against FA induced lung injury.

Material and Methods

1-Experimental animals

Thirty- five adult male albino rats of 12 weeks age, weighing 200-250 gm, obtained from the Medical Ain Shams Research Institute (MASRI) were used. They were fed standard rat chow, supplied with water ad libitum, and kept under the same conditions throughout the experiment.

2- Experimental Design

The animals were divided randomly into four Groups:

Group I (Control Group): fourteen rats which were subdivided into two subgroups:

Group I-a (negative control): seven rats were left without treatment for 2 weeks.

Group I-b (Positive control): seven rats were exposed to distilled water vapor for 2 hours per day for 2 weeks.

Group II (Propolis Group): seven rats received propolis dissolved in distilled water via an oral gavage-feeding needle in a dose of 50 mg /kg/ day (**Almansour and Jararr, 2017).**

Group III (FA exposure Group): seven rats were exposed to 10% FA inhalation, with a pH 9.2 at room temperature, for 2 hours per day for 2 weeks (5 days per week) **(Leal et al., 2018).** The FA was placed in a small glass container inside a partial-closed wire cages, thus exposing the rats to the vapor. During non-exposure time, all rats were kept away from the source of the vapor. The concentration of FA (ppm) in the atmosphere of the exposed cages was measured (**Hoogenboom et al., 1987).**

Group IV (FA exposure Group treated with propolis): seven rats were exposed to 10 % FA as in Group III with co-administration of propolis as in Group II. Capsules content was dissolved immediately before use in sterile distilled water.

Commercial water soluble propolis crude in the form of capsules (1000 mg) manufactured by Bee Health Limited, East Yorkshire, England, and legally imported was used. Its active ingredients were identified by the quality control of the manufacturer and indicated the following contents: Standardized Purified Propolis Resin, Brewers Yeast Powder, Magnesium Stearate, Silicon Dioxide, Vegetable Source.

3- Histological Study

3.1 Fixation:

After two weeks, all the rats were anesthetized by ether inhalation then euthanized by cervical dislocation. The thoracic cavities of all rats were opened, and lungs were excised and gently rinsed with running tape water. Specimens from the upper lobes were fixed in 10 % formaldehyde solution for 48 hours, then were processed as paraffin blocks, followed by cutting of serial paraffin sections of 5 µm thickness

3.2 Staining:

Tissue sections were stained by Hematoxylin-Eosin and Masson's trichrome stains.

3.3 Immunohistochemistry:

Some sections from all specimens were picked upon positive slides for immunohistochemical staining using Anti- Nuclear Factor- Kappa (NF-Kb).

The tissue sections were deparaffined and antigen retrieval was done. Endogenous peroxidase was blocked by immersion of the sections in 3% hydrogen peroxide solution for 10 minutes and washed by phosphate buffer, followed by incubation of sections with rabbit polyclonal anti-NF-Kb/ p65 (Rel A) Ab-1 antibody that was followed also by washing. Sections were then covered by 4-5 drops of Ultra Vision biotinylated goat anti- polyvalent secondary antibody; incubated at room temperature for 10 minutes. The technique used in staining was a standard avidin- biotin complex staining procedure. Then slides were counter-stained with hematoxylin (**Li et al., 2010).**

3.4 Microscopic Examination:

Histological sections of all rats included in the study were examined using Olympus light microscope. The digital photography was carried out by using Olympus optical microscope with digital camera.

4 - Morphometric Study

4.1 Image Analysis:

Seven fields from seven stained sections of seven different rats were examined in each Group to measure number of goblet cells (of bronchiolar epithelium) per high power field. Measurements were taken using the image analyzer Leica (Q 500 MC program, Wetzlar, Germany).

4.2 Statistical Analysis:

Data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one-way analysis of variance (ANOVA). Bonferroni Post Hoc test were used to detect significance between every two individual Groups. Probability (P-value) of ≥ 0.05 was considered non- significant, ≤ 0.05 was considered significant and ≤ 0.01 was considered highly significant. All the data were expressed as the Mean ± Standard Error of Mean (SEM).

Ethical Consideration

All the experiments were conducted in accordance with the guidelines approved by the Committee of Animal Research Ethics, Ain Shams University Faculty of Medicine.

Results

After 2 weeks of FA inhalational exposure, there was no mortality in both Groups I, II and IV (control, propolis and propolis- treated Groups respectively), however, there was one mortality recorded in Group III (FA Group).

1- Anatomical results:

The gross appearance of the lungs of Groups I, II and IV (control, propolis and propolis-treated Groups respectively) revealed normal rosy pink color of the examined lobes. There was no changes in size and consistency. On the other hand, lungs of Group III (FA Group) revealed congestion in most of the lobes with no significant apparent changes in the size.

2- Histological results:

Microscopic examination of the lungs of Group I (control) and Group II (propolis) rats stained with Hematoxylin and Eosin (H & E) revealed multiple alveoli and alveolar sacs with clear alveolar cavities and bronchiolar passages. Alveoli were separated by thin alveolar walls and surrounded by capillary networks. Pulmonary vessels were normally distributed within the pulmonary parenchyma. Normal architecture of bronchiolar

epithelium and normal parabronchiolar lymphoid aggregation were also observed. (Figs.1, 2).Masson Trichrome stain revealed alveolar septa with normal thickness of collagen fibers and no abnormality in alveolar septal blood capillaries (Fig.3).

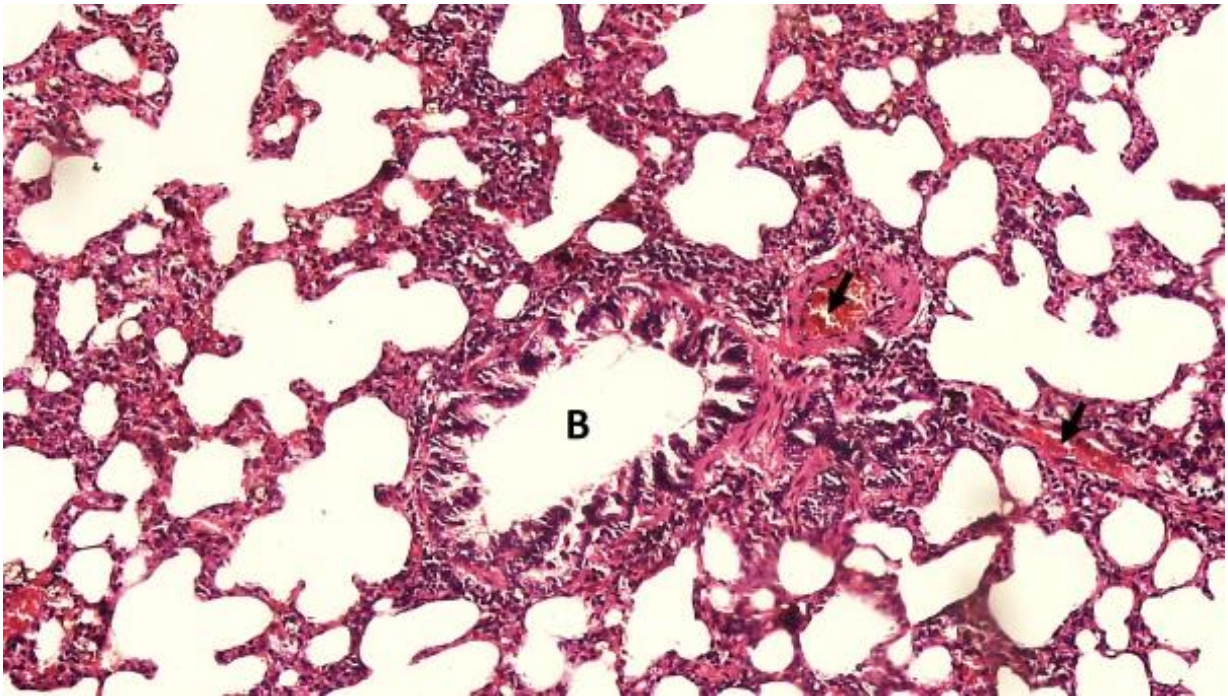


Fig. (1): A photomicrograph of a section of a rat's lung of Group I (control Group) showing clear bronchiolar passage (B) with normal mucosal lining. Pulmonary vessels (arrow) were normally distributed.

H&E, x100

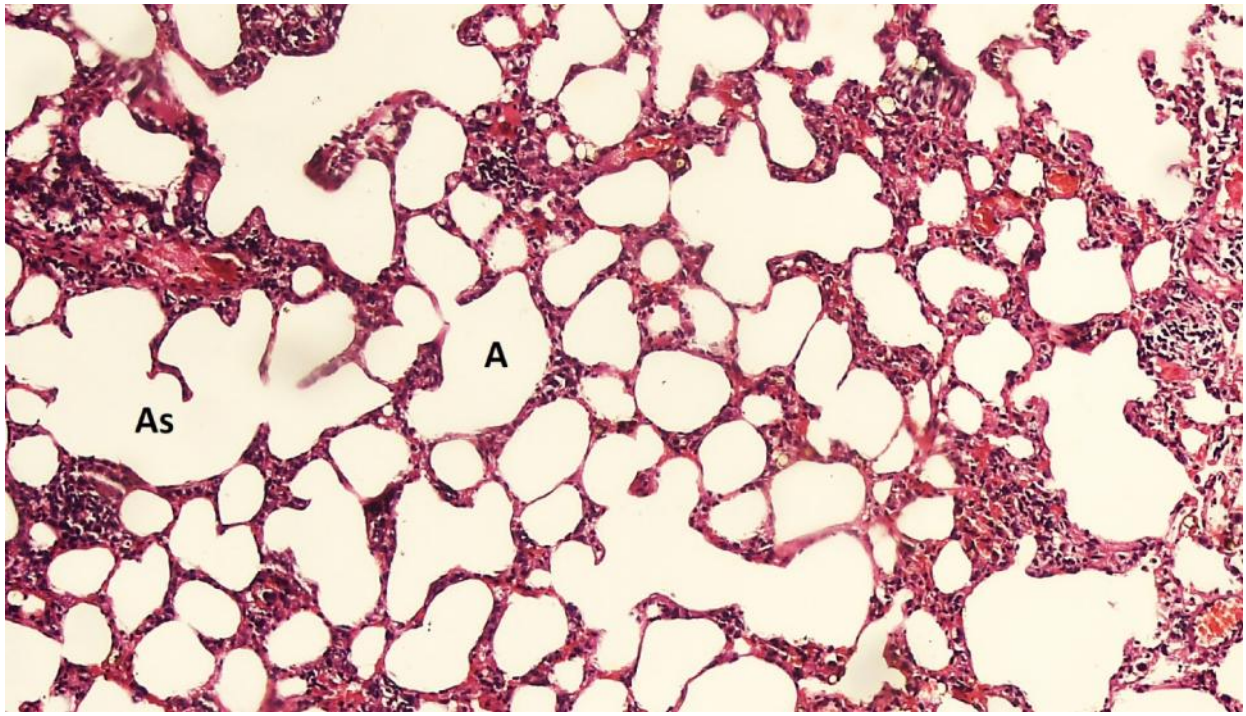


Fig. (2): A photomicrograph of a section of a rat's lung of Group I (control Group) showing multiple clear alveoli (A) and alveolar sacs (As) separated by thin alveolar walls.

H&E, x100

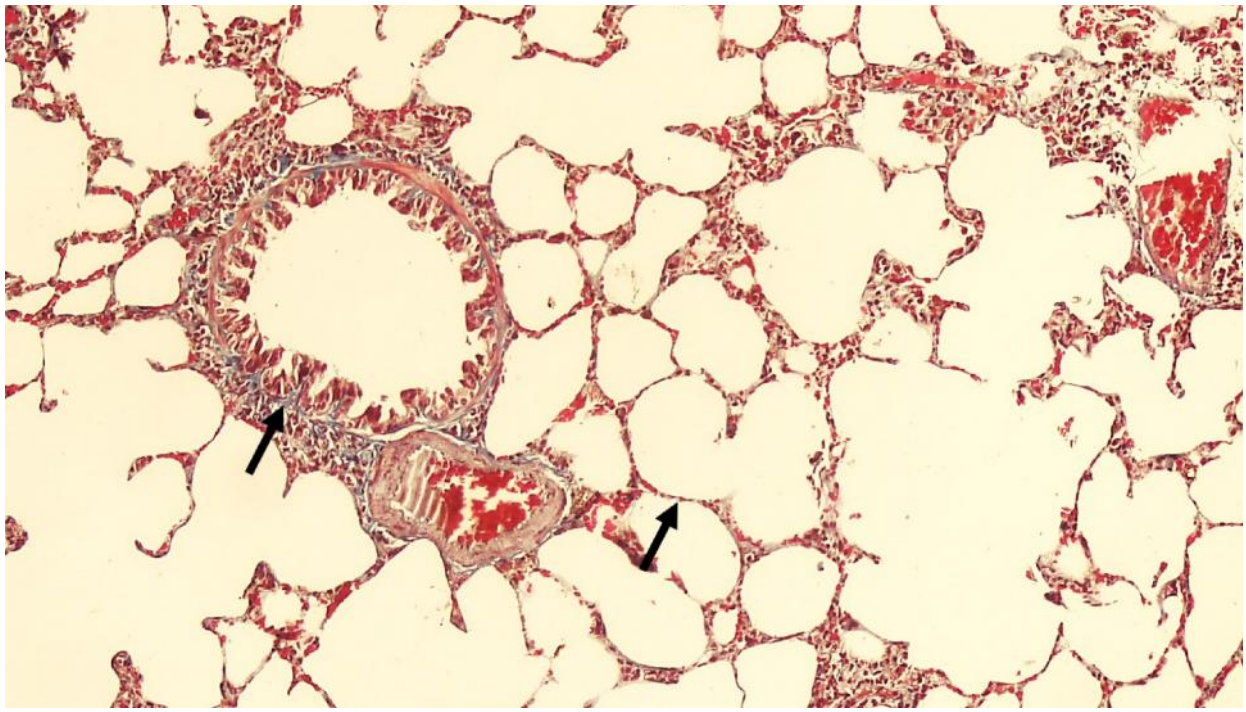


Fig. (3): A photomicrograph of a section of a rat's lung of Group I (control Group) showing normal thickness of alveolar septal and bronchiolar collagen fibers (arrow). **Masson trichrome, x100**

Regarding immunohistochemical reaction to NF-Kappa antigen, sections of control Group showed negative reaction in the form of faint brownish cytoplasmic and nuclear discoloration (Fig.4).

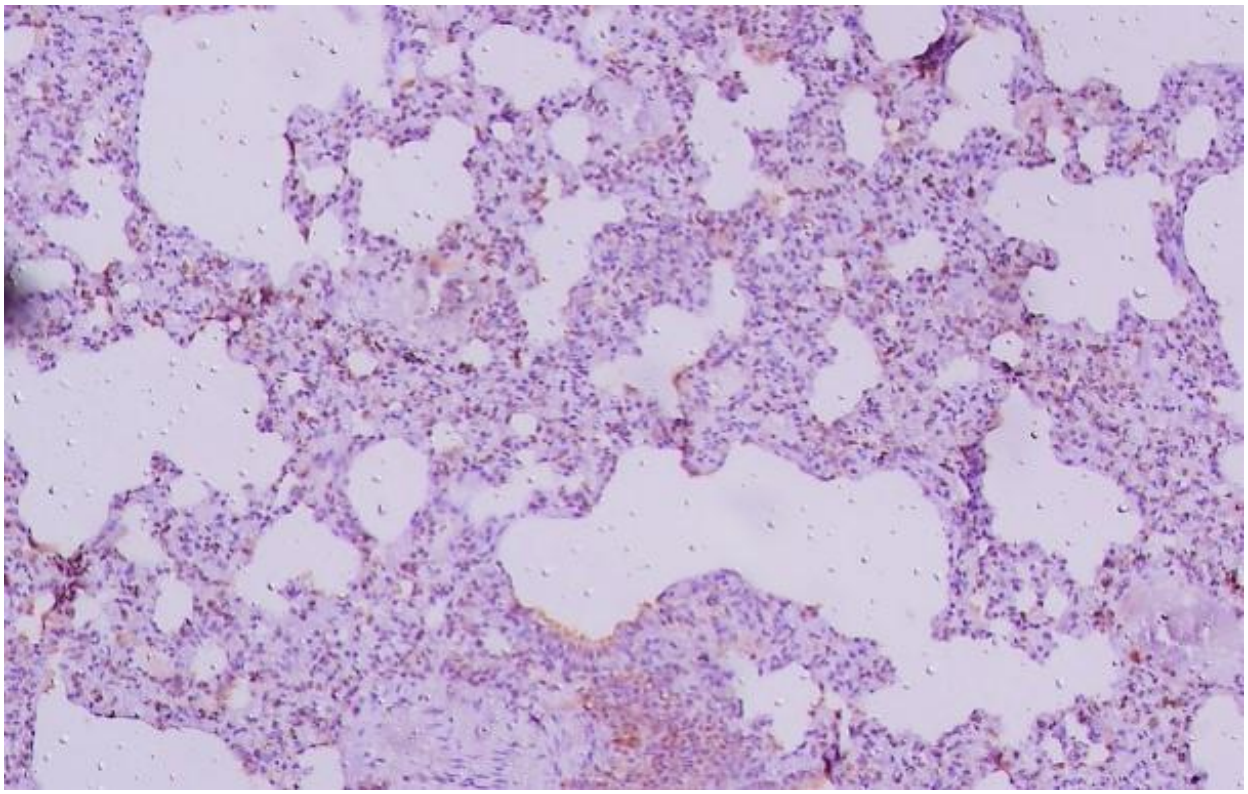


Fig. (4): A photomicrograph of a section of a rat's lung of Group I (control Group) showing negative reaction. **Anti-NF-Kappa antibody, x400**

Sections of the lungs obtained from Group III rats (FA Group) stained with H & E showed irregular interalveolar septal thickening characterized by intense, diffuse interstitial and peribronchiolar inflammatory cell infiltration. Some areas showed sloughed mucosal bronchiolar lining (Fig.5). Thin-walled large airspaces with destructed septa were also present. Inflammatory cell infiltration and extensive interstitial hemorrhage were also noticed (Fig.6). Congestion of blood vessels in the thickened alveolar walls was also seen with leakage of blood cells in the interstitium associated with intra-alveolar hemorrhage in some areas (Fig.7).

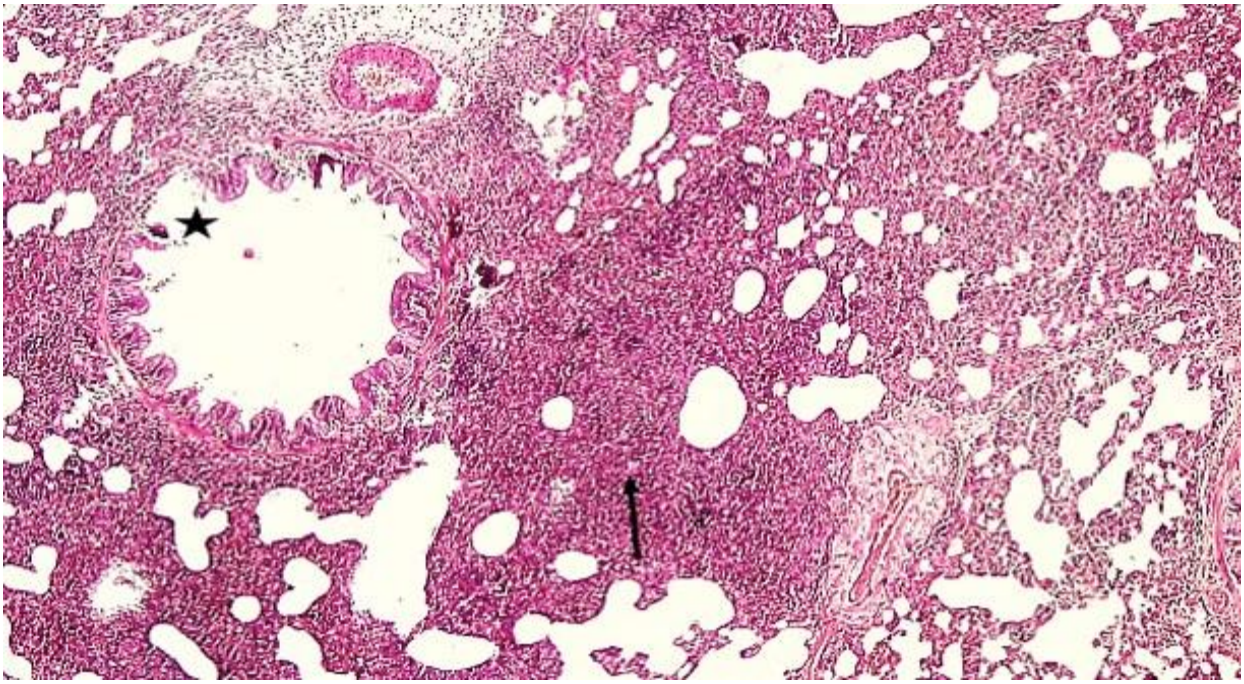


Fig. (5): A photomicrograph of a section of a rat's lung of Group III (FA Group) showing thickening of interalveolar septa by intense, diffuse interstitial and peribronchiolar inflammatory cell infiltration (arrow). Some areas showed sloughed mucosal bronchiolar lining (star). **H&E, x100**

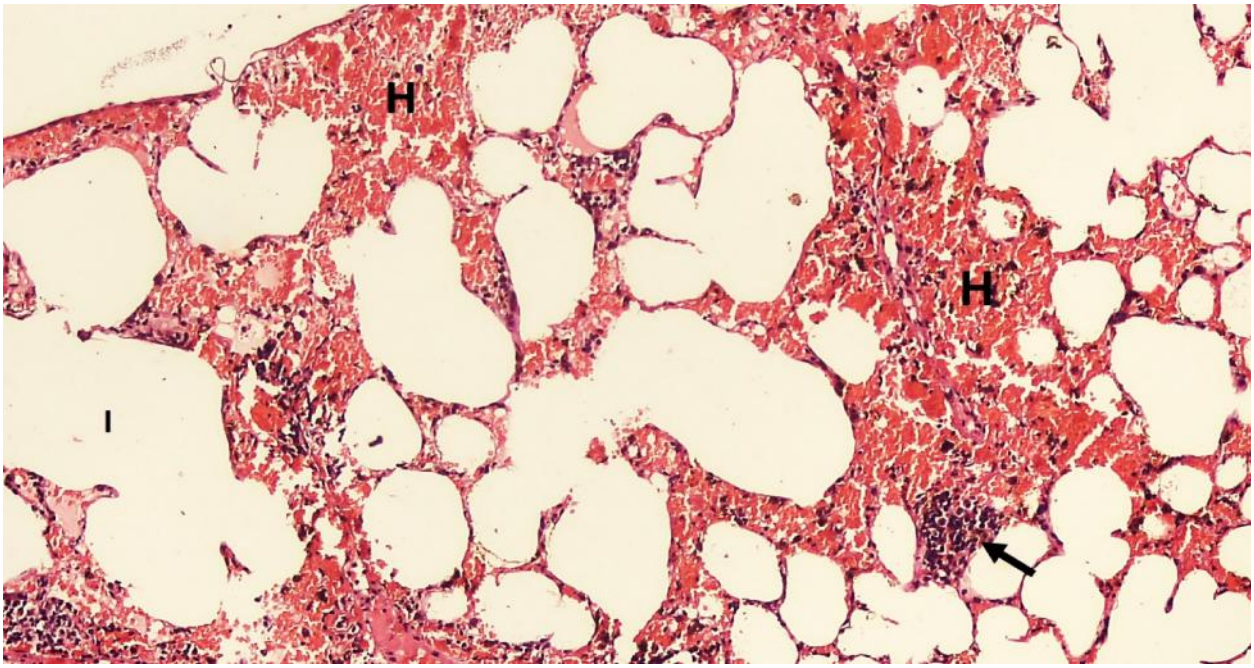


Fig. (6): A photomicrograph of a section of a rat's lung of Group III (FA Group) showing destructed interalveolar septa (I), inflammatory cell infiltration (arrow) and extensive interstitial hemorrhage (H) **H&E, x100**

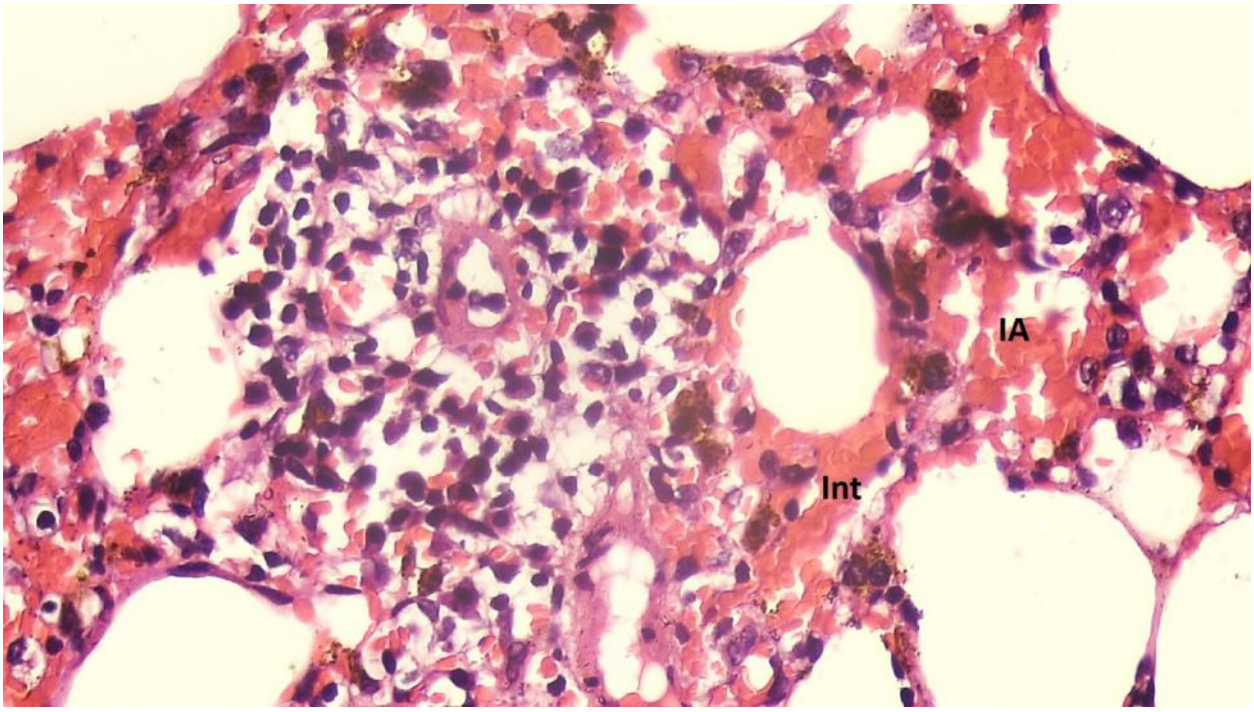


Fig. (7): A photomicrograph of a section of a rat's lung of Group III (FA Group) showing congestion of blood vessels with leakage of blood cells in the interstitium (Int) and intra-alveolar hemorrhage (IA)**.**
H&E, x400

Some bronchi showed abnormal morphology of the lining bronchiolar epithelium with increased nuclear cytoplasmic ratio and increased number of epithelial layers (Fig.8).

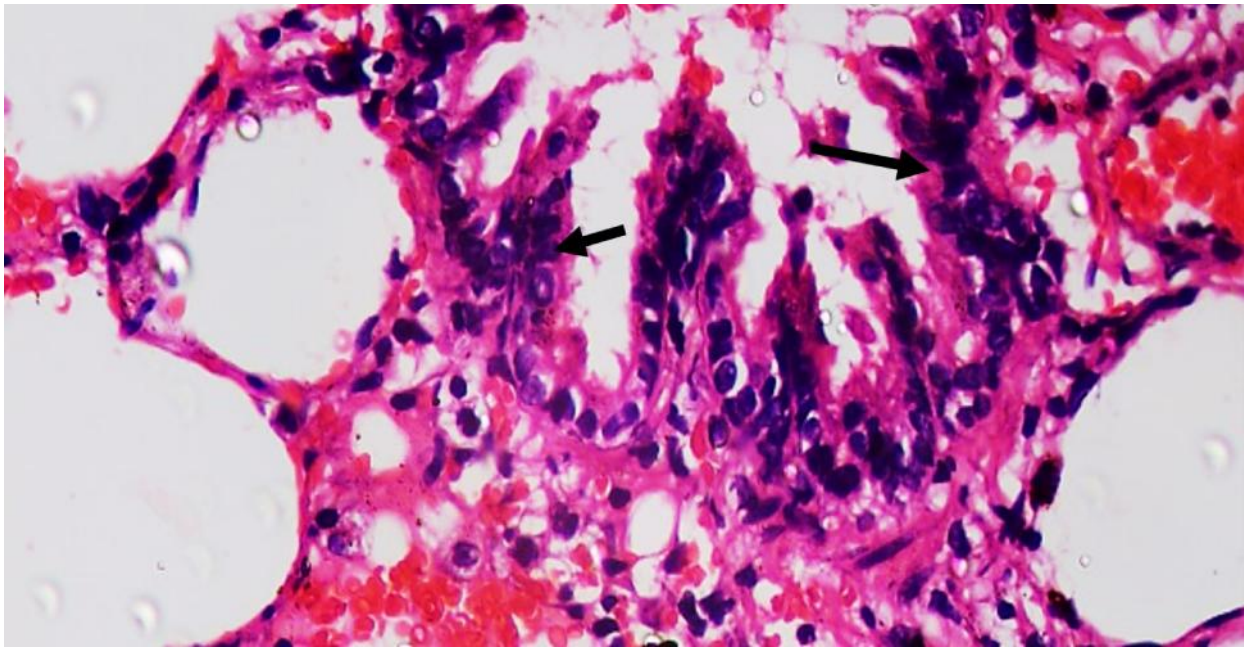


Fig. (8): A photomicrograph of a section of a rat's lung of GroupIII (FA Group) showing increased thickness of bronchiolar epithelial lining with increased nuclear cytoplasmic ratio of the lining cells (arrow)**.**
H&E, x400

Masson Trichrome stain revealed thickening of the bronchiolar walls, associated with increased perivascular and interstitial connective tissue (Fig.9).

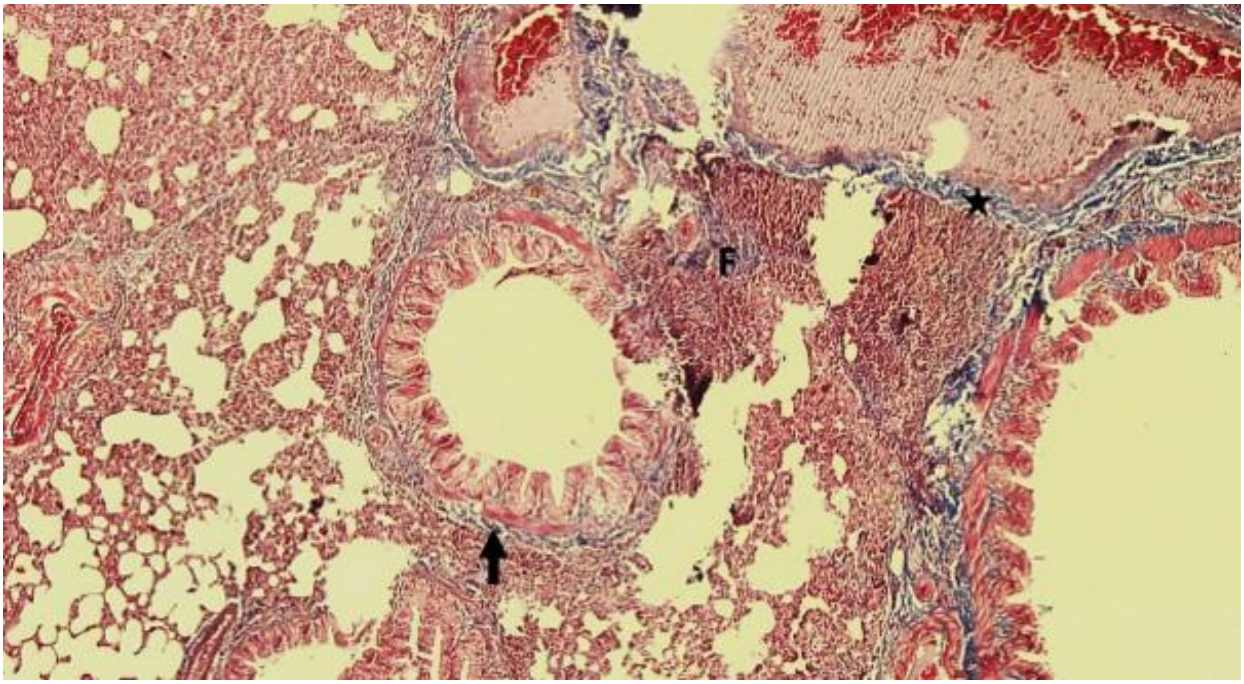


Fig. (9): A photomicrograph of a section of a rat's lung of GroupIII (FA Group) showing thickening of the bronchiolar walls (arrow), associated with increased perivascular (star) and interstitial connective tissue (F). **Masson trichrome, x100**

Regarding immune-histochemical reaction to NF-Kappa antigen, sections of Group III (FA Group) showed strong positive nuclear and cytoplasmic reaction (Fig.10).

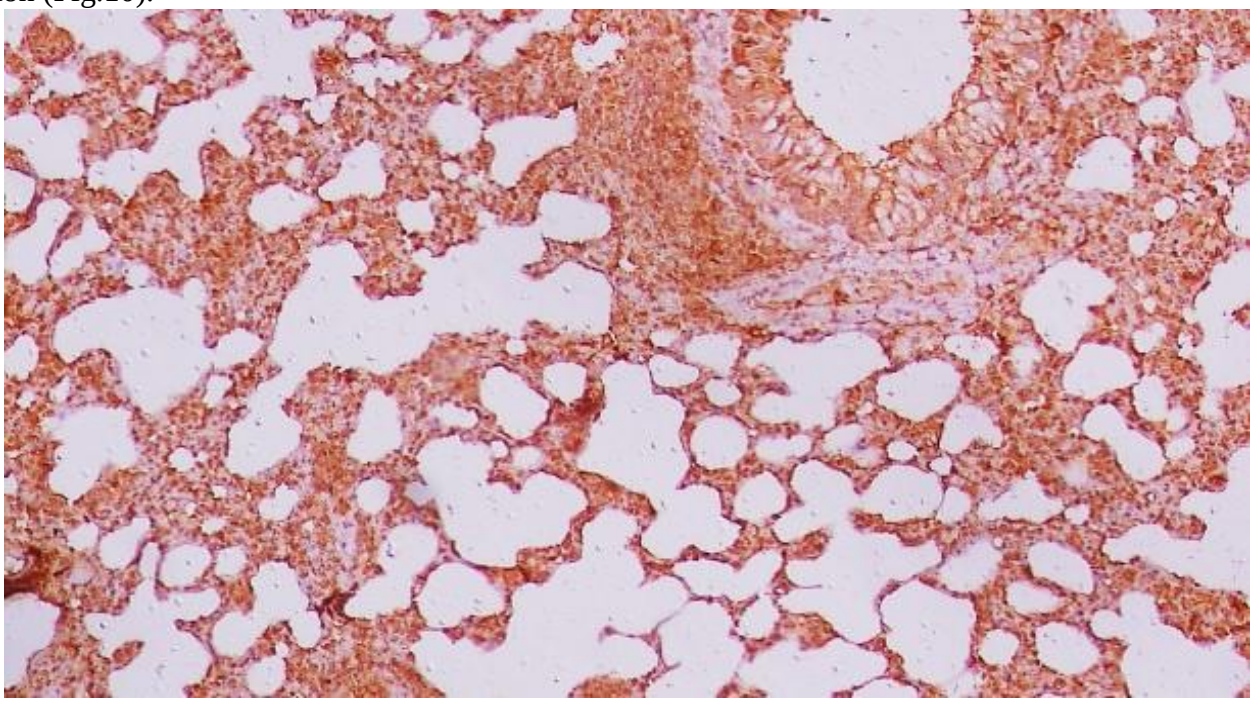


Fig. (10): A photomicrograph of a section of a rat's lung of Group III (FA Group) showing strong positive reaction. **Anti-NF-Kappa antibody, x100**

In Group IV (FA treated with propolis Group), sections stained with H & E showed partial improvement in the form of decreased thickness of alveolar walls and decreased degree of vascular congestion of inter-alveolar capillaries. No extravascular or intra-alveolar hemorrhage were noticed (Fig.11). Inflammatory cell infiltration was less prominent in the peri-alveolar interstitium (Fig.11). Sections stained with Masson Trichrome revealed decreased amount of connective tissue around the bronchi and the alveolar walls compared to FA Group (Fig.12).

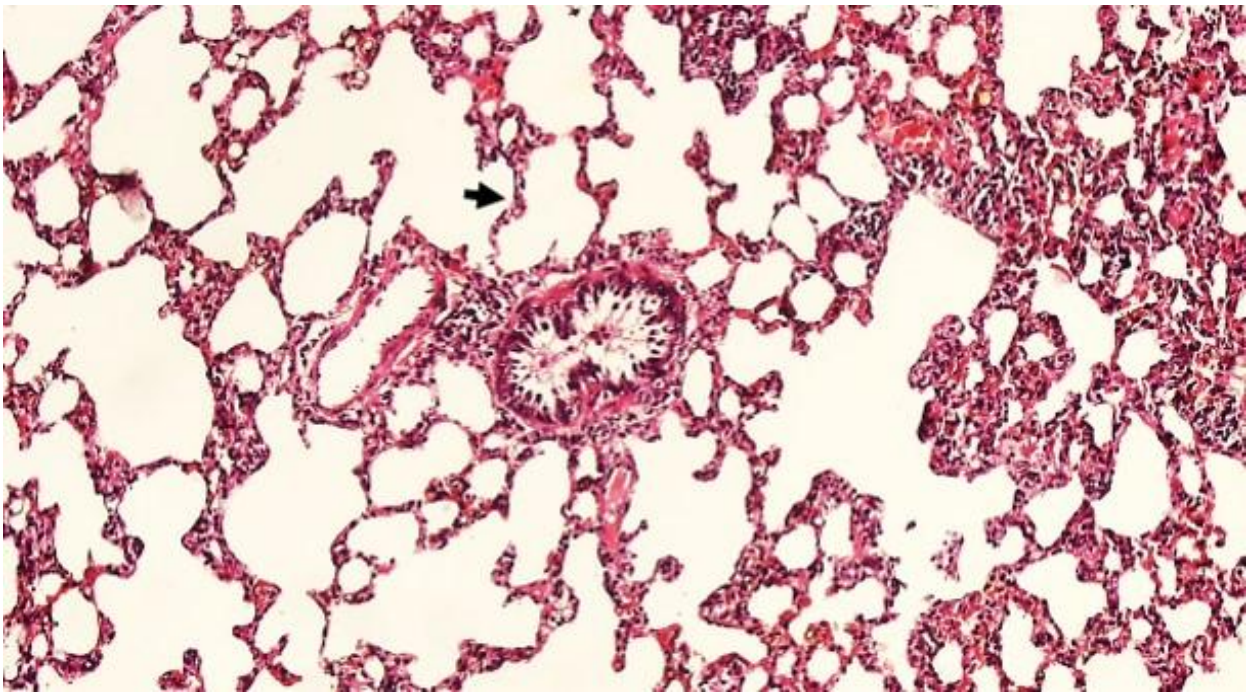


Fig. (11): A photomicrograph of a section of a rat's lung of Group IV (FA treated with propolis) showing decreased alveolar wall thickness with less inflammatory cell infiltration (arrow).No extravascular or intra-alveolar hemorrhage was noticed. **H&E, x100**

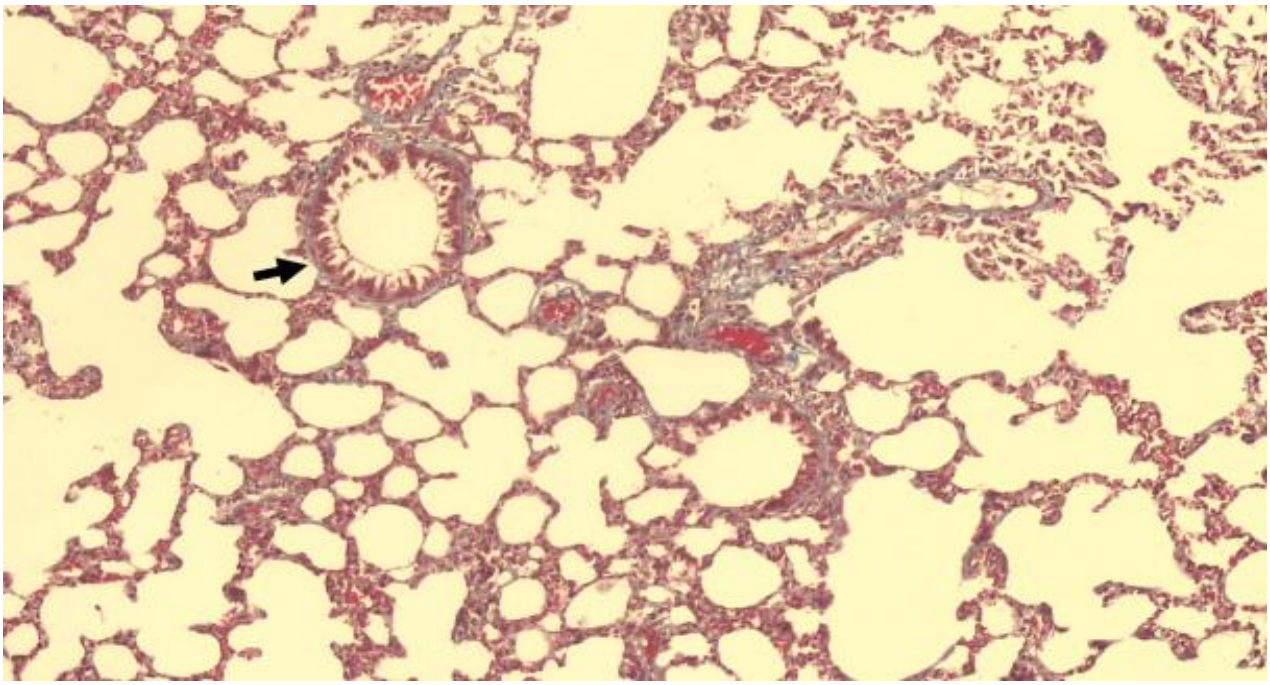


Fig. (12): A photomicrograph of a section of a rat's lung of Group IV (FA treated with propolis) revealing decreased amount of connective tissue around the bronchi and the alveolar walls (arrow) compared to Group III (FA Group)**. Masson trichrome, x100**

Regarding immune-histochemical reaction to NF-Kappa antigen, sections Group IV showed weak positive reaction (Fig.13).

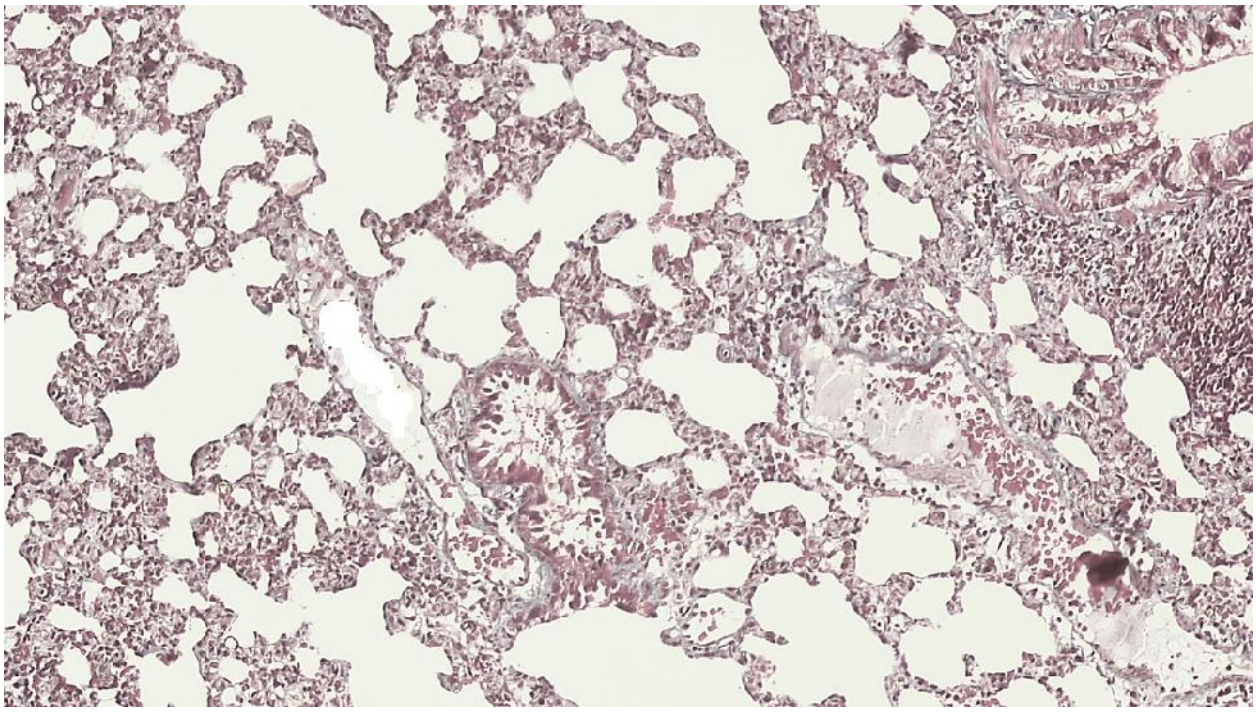


Fig. (13): A photomicrograph of a section of a rat's lung of Group IV (FA treated with propolis) showing decreased positive reaction compared to Group III (FA Group)**. Anti-NF-Kappa antibody, x100**

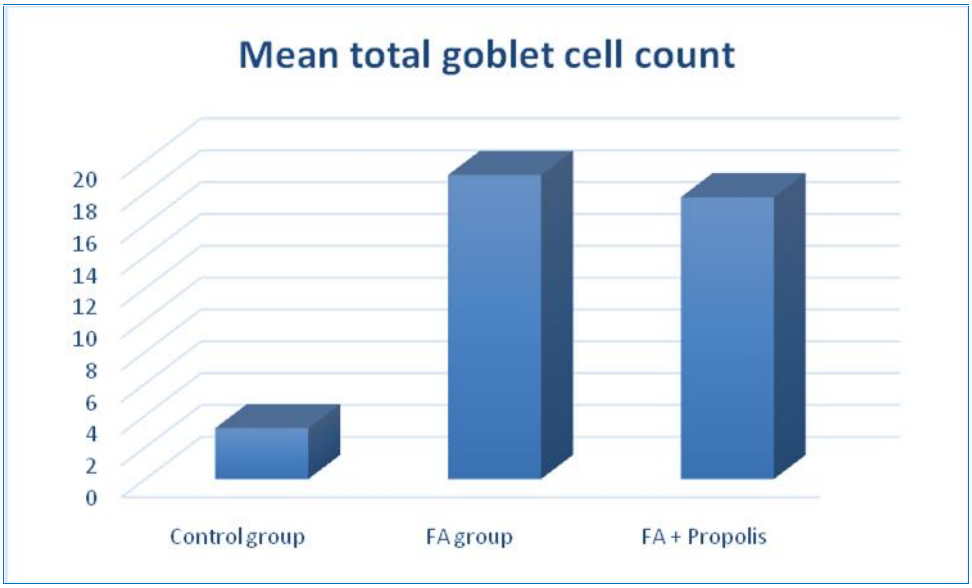
3- Morphometric results and statistics

The mean total goblet cell count (of bronchiolar epithelium) per high power field in FA Group was greater than in the control Group showing highly significant statistical difference *(P ≤ 0.000001)*. The propolis –treated Group had a lower mean total goblet cell count compared to FA Group with non-significant difference *(P ≤ 0.3)* but greater than that of the control Group with significant difference *(P ≤ 0.00007) (Table1 and Histogram 1)*

Table 1: Comparison between Mean Total Goblet cell counts (of bronchiolar epithelium) /high power field among the experimental Groups

	Control Group	**FA Group**	**FA + Propolis**
Mean total goblet cell count	**3.2 ± 0.44**	**19.1 ± 0.31**	**17.7 ± 1.14**
		*P ≤ 0.000001**	*P ≤ 0.253***
			*P ≤ 0.00007****
* Highly significant greater than control Group			
** No significant difference compared to FA Group			
*** Highly significant greater than control Group			

Histogram 1: Mean Total Goblet cell count (of bronchiolar epithelium) /high power field among the experimental Groups



Discussion

Inhalation is the main exposure pathway of atmospheric pollutants, which makes the respiratory tract the first target organ of airborne pollutants **(Bernstein et al., 2008).** Formaldehyde is a potent respiratory irritant that reacts directly with tissue constituents, and its cytotoxicity is presumably a function of this reactivity **(Kimbell et al., 2001).** It was observed that FA caused histopathological and morphometric changes in the lungs of the rats. Histological examination showed thickened alveolar septum, bronchiolar epithelial hyperplasia, congested capillaries, hyperplasticparabronchiolar lymphocytic aggregations, increased peri- bronchiolar connective tissue multiple areas of hemorrhages, which was consistent with the findings in the rabbit lungs after FA exposure **(Neelam et al., 2011),** also detected in rats by (**Turkoglu et al., 2008),** mice by (**YU et al, 2004)** and in children by **(Casset et al., 2006).**

Formaldehyde leads to oxidative damage in the lungs (**Zararsiz et al., 2004).** Different types of propolis have a variety of biological activities, including antioxidant power. It has been suggested that the therapeutic activities of propolis depend mainly on the presence of polyphenols, specifically flavonoids and caffeic acid which are essential for the anti-inflammatory activity of propolis (**Mouhoubi-Tafinine et al., 2016).**

In this study propolis treated Group showed less inflammatory cells and fewer signs of inflammation. Propolis was reported to be a potent inhibitor of lipoxygenase, and through this activity, leukocyte chemotaxis and inflammatory activity are abolished **(Koksel et al 2005). Calikoglu et al**., **2003** indicated that propolis might be effective in protecting the injury of remote organs caused by oxidative stress and neutrophil accumulation.

In addition, propolis treated Group showed less vascular congestion. Studies investigating propolis in an *in vivo* model of chronic inflammation demonstrated that it suppresses the cell migration. However, the deposition ofcollagen was not affected, suggesting that propolis can be used to control the inflammatory response without compromising the tissue repair process **(Valente et al., 2011).**

Nuclear factor kappa B (NF-κB) is a nuclear transcription factor. It plays a pivotal role in immune and inflammatory responses through the regulation of the expression of several proteins, including proinflammatory cytokines, chemokines, and adhesion molecules **(Oršolić et al., 2013).**

In the current work, Group III rats (FA Group) showed strong positive nuclear and cytoplasmic reaction, while Group IV rats (FA treated with propolis) showed decrease in the positive reaction compared to Group III, but not to the degree of the Group I rats (control Group),showing that propolis has anti-inflammatory action.

In addition, number of goblet cells showed significant increase in Group III and IV compared to Group I. This agreed with **Mayara Peres Leala et al**., (**2018)** who demonstrated elevated mucous production associated with Formaldehyde exposure.

In conclusion, it was detected that FA- exposure leads to inflammation and injury of the lung tissue components. Propolis showed protective and Anti-inflammatory activity against these harmful effects.

Conflict of interest:

We declare that we have no conflict of interest, intent of financial gain, or commercial associations regarding this research.

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