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Contributed Paper

Protective Effect of Propolis Against Pulmonary Histological Alterations Induced by 10 nm Naked Gold Nanoparticles

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ABSTRACT

Little is known about propolis protective effect against the toxicity induced by gold nanoparticles (GNPs). The present investigation was carried out to investigate the protective role of propolis against the histological alterations induced in the lung tissues by naked 10 nm GNPs. Male albino Wistar rats were exposed to 10 nm GNPs at a dose of 2 mg/kg together with or without propolis (50 mg/kg) for15 days. Lung biopsies from all rats under study were subjected to histological examinations. Exposure to 10 nm GNPs has induced thickened alveolar wall, inflammatory cells infiltration, interstitial macrophages invasion, emphysema, pulmonary edema, dilatation and congestion of the interalveolar capillaries, atelectasis and fibrocytes proliferation. Propolis combination with GNPs demonstrated full protection from pulmonary edema and alveolar hypersensitivity while the lung tissues were partially protected from interstitial thickening, inflammatory cells infiltration, emphysema, dilatation and congestion of the interalveolar capillaries. On the other hand, propolis failed to protect the lung tissues from fibrosis, macrophages invasion and atelectasis induced by GNPs. The findings indicated protective role for propolis against some histological damage in the pulmonary tissues induced by GNPs toxicity.

Keywords: gold nanoparticles, lung, nanotoxicity, propolis, pulmonary tissues

1. INTRODUCTION

Gold nanoparticles are currently used in many diagnostic and therapeutic purposes including drug delivery, cancer therapy and in vivo imaging for different biomedical applications [1-3]. Gold NPs hold a promise for many health disorders specially autoimmune diseases such as arthritis, cardiovascular complication and lung cancer [4]. These fine particles can be novel therapies in lung cancer as can be endocytosed by lung cancer cells and facilitate cell invasion [5]. Gold NPs conjugate with methotrexate (MTX) showed cytotoxic and antitumor effect in suppressing tumor growth of Lewis lung carcinoma and in treatment of primary and metastatic tumors [6]. Recently, GNPs

are being used successfully to distinguish between normal and cancerous lung cells [7]. Furthermore, GNPs biosensor is being used to detect lung cancer by analyzing individual's exhaled breath [8].

Gold NPs are biologically active with long blood circulating time and can accumulate in the vital body organs including the lungs [9]. On the other hand, some studies reported toxic effects of GNPs in relation to their surface area, shape, size and charge [10-13]. Additionally, gold nanoparticles are able to induce oxidative stress by interacting with cell components that could result damage to tissues, cells and macromolecules [14-16]. Previous reports demonstrated that small, rod-shaped and positively charged naked GNPs were more toxic than larger, spherical and ionic ones respectively [12].

Lung tissues receive high blood flow and have high exposure to GNPs with long circulating residue [12]. Gold NPs were reported to cause significant oxidative stress and cytotoxicity that could reveal a high risk potential on vital organs [15-17]. Some studies reported toxic effects of GNPs in the pulmonary tissue with relation to the size and time of exposure [18].

Propolis is a natural resinous substances collected from plants by honeybees [19]. It has been used for centuries in folk medicine as antimicrobial, anti-oxidative, anti-ulcer, hypotensive agent, immune system stimulant and is being invested highly in cosmetic applications [20-21]. This natural crude is characterized by its antioxidant properties due to its flavinoids, phenolics and essential oils contents. Propels demonstrated partial hepatoprotectivity against GNPs toxicity and was found to have protective effects against toxicity induced by certain chemicals and drugs [22].

Limited studies have been carried out on propolis role in augmentation the pulmonary histological alterations induced by GNPs exposure. With this objective, the present work was conducted to investigate the protective role of propolis against the histological alterations induced in the lung tissues by 10 nm GNPs.

2. MATERIALS AND METHODS

2.1 Animals

Forty-eight male Wistar albino rats of 12 weeks age and weighing 210-230 gm were obtained from the animal house of King Saud University. The rats were randomly assigned and separately caged to three test groups and a control one (12 rats each) with access to food and water *ad libitum*. All experiments were carried out at an ambient temperature of 24±2 °C.

2.2 Gold Nanoparticles

Spherical naked colloidal monodisperse GNPs (10 nm) stabilized in 0.1 mM PBS, were purchased from Sigma-Aldrich, USA with the following physicochemical characterization: 5.98×10^{12} nanoparticles/ml, concentration of 1.01×10^{8} molar Ext M^{-1} Cm^{-1}, reactant free with absorption at ~520 nm.

2.3 Propolis

Commercial water soluble propolis crude in the form of capsules (1000 mg) manufactured by Marnys Spanish Company (Spain) and legally imported by Saudi Arabian Dug Store Ltd (Saudi Arabia) was used. Its active ingredients were identified by the quality control of the manufacturer and indicated the following contents: Phenolic acids (caffeic acid, tocopherol, sinapic acid, cinamic acid, coumaric acid and ferulic acid) and flavonoids (quercetin, kaempferol, rutin and apigenin) together with amino acids and vitamins.

For the use of propolis in the present

work, capsules content, was dissolved immediately before use in sterile distilled water. The rats were subjected to propolis in a daily single dose of GNPs with or without propolis for 15 consecutive days as follows:

Group 1: Each member of this group received no GNPs nor propolis but a single intraperitoneal (ip) injection of 100 µl of the sterile distilled water for consecutive 15 days.

Group II: Each member of this group received a daily ip injection of 100 µl GNPs of size 10 nm at a dose of 2 mg/kg for consecutive 15 days.

Group III: Each member of this group received a daily ip injection of 100 µl GNPs of size 10 nm at a dose of 2 mg/kg, and subjected to oral dose of propolis (50 mg/kg) for consecutive 15 days.

Group IV: Each member of this group received a daily oral dose of propolis (50 mg/kg) for consecutive 15 days.

2.4 Physical Observation

Daily observation throughout the study was made for mortality, general well being and behavior patterns in the three test groups and the control one.

Food consumption: Weekly ratio of food consumption (g) to rat body weight (g) after treatment for each group was calculated.

Water intake: Weekly ratio of water intake to rat body weight (ml/g) after treatment was measured.

Body weight monitoring: The rats body weight was monitored at the beginning of treatment, then after 7 days of treatment and on the day of dissection.

2.5 Sample Preparation

All members of all groups were euthanized by cervical dislocation after 15 days of treatment. Fresh lung biopsy from each rat of all groups were cut rapidly, fixed in neutral buffered formalin, dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%), cleared in xylene, impregnated then embedded and blocked out in paraffin wax. Paraffin sections (4-5 µm) of the control and GNPs treated rats were stained according to Jarrar and Taib [23] with hematoxylin and eosin stain, trichrome stain, Periodic Acid-Schiff (PAS) method and Prussian blue reaction.

2.6 Microscopic Examination

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

2.7 Experimental Protocol

The animals were handled and the experiments were conducted in accordance with the protocols approved by King Saud University ethical committee. The doses and route of administration were carried out according to previous studies protocols and confirmed data from the literature (19-22).

3. RESULTS AND DISCUSSION

After 15 days of treatment, no mortalities or signs of toxicity were detected in any of the experimental groups of the present study. Moreover, no macroscopic anomalies were seen in the appearance and behavior of rats subjected to GNPs with or without propolis.

3.1 Morphometric Alterations

Effect on the average weight: After 15 days of GNPs exposure, a non significant decline (p-value > 0.05) of the average weight of treated rats was seen (Table 1). On the other hand, the decline of the

average weight of rats exposed to GNPs plus propolis was also non significant (p-value > 0.05) but lower than rats received propolis. Control rats had normal weight gain during the treatment period.

The average body weight gain in the GNPs treated rats with or without propolis was slightly lower, but failed to reach the statistic significant (p-value = 0.23, t-test), in comparison with the control ones.

Table 1. Rat average weight (g) ± standard deviation for each group (n=12) during the period of treatment.

Group	Starting weight	Weight after 7 days of treatment	Weight after 15 days of treatment
Group I (Control)	218.7±12.2	223.5±33.3	233.8±22.4
Group II (Received GNPs only)	222.3±16.3	226.5±29.7	226±21.5
Group III (Received GNPs plus propolis)	219.2±19.2	223.8±21.3	225.8±22.6
Group IV (Received propolis only)	217.8±13.1	219±35.5	230.5±25.7

Effect on food consumption: As seen in Table 2, the amount of food consumed (gram) per gram of body weight gain of the GNPs treated group was slightly less than that of the control rats and those received GNPs plus propolis. However, rats treated with propolis only showed significant (p-value < 0.05) increase in food consumption (17 %) than GNPs treated rats.

Table 2. Food consumption (g) to rat body weight (g) for each group (n=12) during the period of treatment.

Group	Starting food consumption	Consumption after 7 days of treatment	Consumption after 15 days of treatment
Group I (Control)	9.14±1.2	12.15±1.7	19.44±2.7
Group II (Received GNPs only)	8.96±1.3	13.36±1.5	16.19±1.3*
Group III (Received GNPs plus propolis)	9.6±1.3	12.16±1.6	18.35±1.9
Group IV (Received propolis only)	9.4±1.3	11.89±1.7	19.25±2.1

"*" represents significant p value <0.05 in comparison with control group.

Effect on water intake: As shown on Table 3, during 15 days of treatment, water intake (ml) per gram of body weight was increased significantly (p-value < 0.05) in GNPs treated rats with or without propolis than the control rats. However, water intake by rats subjected to propolis only was almost similar to that of the control rats.

Table 3. Ratio of water intake (ml) to rat body weight (g) for each group (n=12) during the period of treatment.

Group	Starting water ratio intake of treatment	Water intake ratio after 7 days of treatment	Water intake ratio after 15 days of treatment
Group I (Control)	3.96±0.5	4.34±0.5	5.18±0.7
Group II (Received GNPs only)	3.82±0.6	4.94±0.9	5.68±0.5*
Group III (Received GNPs plus propolis)	3.74±0.6	4.54±0.9	6.06±0.7*
Group IV (Received propolis only)	3.68±0.7	4.47±0.9	5.25±0.8

"*" represents significant p value <0.05 in comparison with control group.

3.2 Lung of The Control Rats

Microscopic examination of the control rat lungs revealed normal alveolar architecture. The thin-walled alveoli consisted of simple epithelium surrounded by blood capillaries with normal distribution of pulmonary parenchyma vessels (Figure 1). The interalveolar septa of this group of rats were free from fibrosis, edema, inflammatory cell infiltration or any other abnormalities.

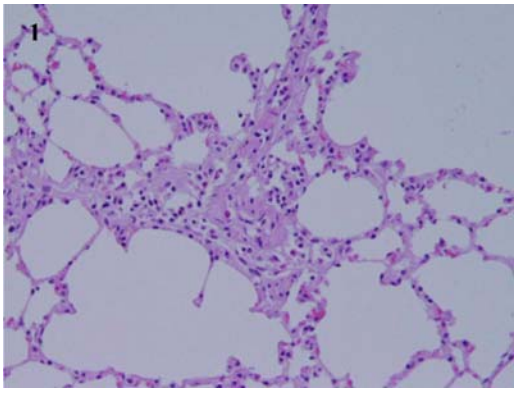


Figure 1. Light micrograph of section in the lung of control rat demonstrating normal lung tissue. H&E stain, x160.

3.3 Lung of Rats Treated with GNPs

Gross morphological examination showed mild congestion in the lung of rats subjected to GNPs with noticeable increase in the lung size compared with the control lungs. Microscopic examination of lung tissue of rats exposed to 10 nm GNPs showed the following abnormalities:

3.3.1 Thickened alveolar wall

In comparison with control rats, irregular interalveolar septa thickening characterized by infiltration with inflammatory cells and to lesser extent with fibroblasts was observed in the pulmonary tissue of rats exposed to GNPs (Figures 2a&b). This change might be due to increased cellularity and fibrosis of the alveolar walls induced by GNPs. Moderate congestion in the thickened alveolar walls was also seen.

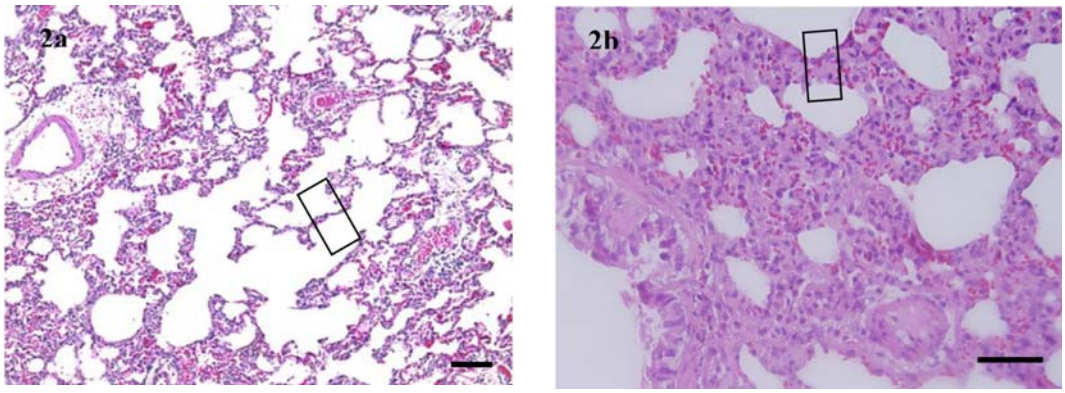
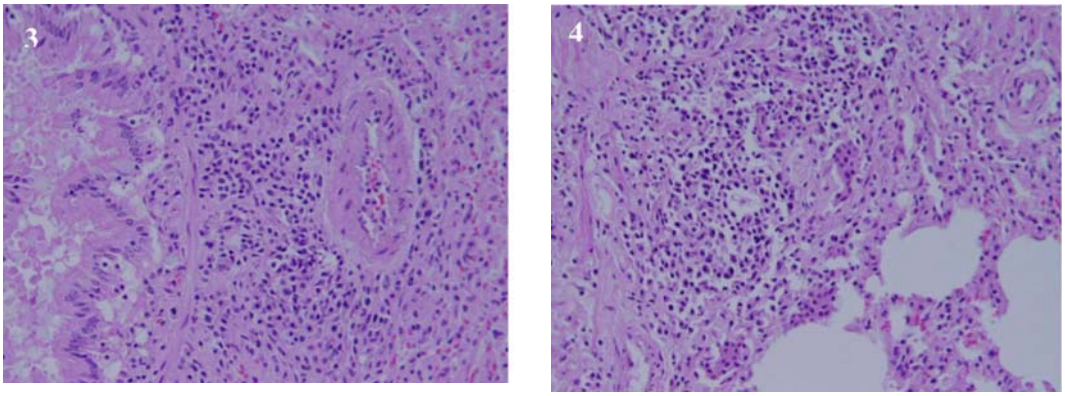


Figure 2. (a&b). Light micrographs of sections in the lungs of demonstrating the thickness alveolar septa In: (a) Control rat(rectangle). H&E stain, Scale bar = 40 µm (b) GNPs-treated rat (rectangle). H&E stain. Scale bar 40 µm.

3.3.2 Inflammatory cells infiltration

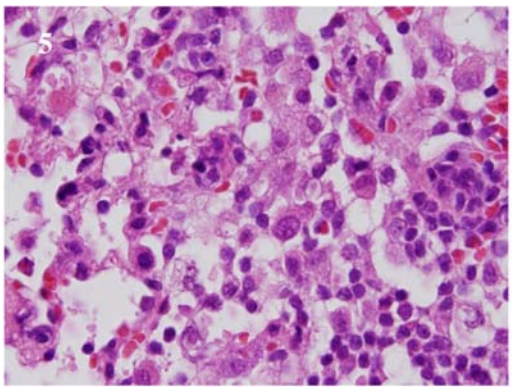
Intense, diffuse and evenly diffused interstitial and peribronchial mononuclear inflammatory cell infiltration mainly lymphocytes was seen in the lungs of all members exposed to 10 nm GNPs (Figures 3&4). Plasma cells and eosinophils were also seen.

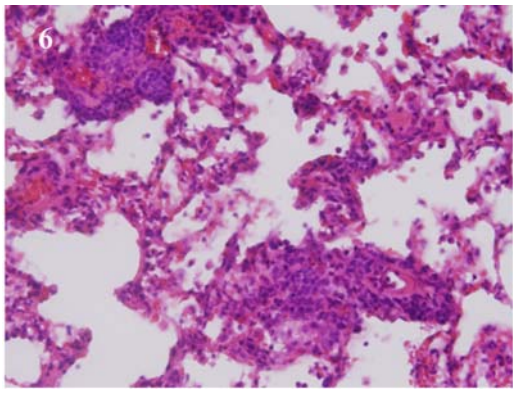


Figures (3-4). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: 3) Interstitial mononuclear inflammatory cell infiltration. H&E stain, ×198. (4) Peribronchial inflammatory cell infiltration. H&E stain, ×198.

3.3.3 Macrophages invasion and sloughing

Foamy interstitial alveolar macrophages were predominant in the ineralveolar interstitial tissue (Figure 5). Some macrophages loaded with brown pigments were seen sloughed in the lumen of some alveoli (Figure 6). The predominance of macrophages due to GNPs exposure might indicate a compensatory response to cellular debris clearance accumulated due to the toxicity of these nanoparticles. Macrophages stimulate lymphocytes and other immune cells to respond to foreign substances and to participate in regeneration function [24].

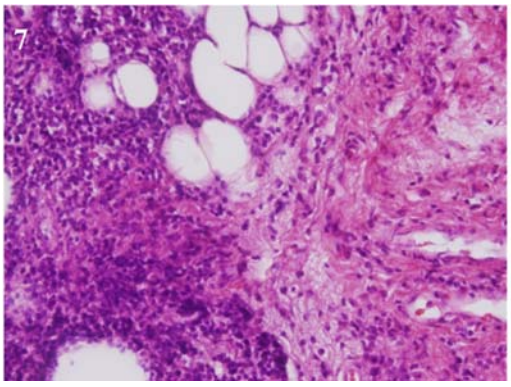


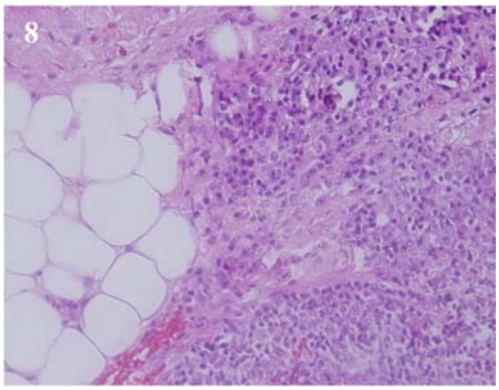


Figures (5-6). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: (5) Interstitial macrophages invasion. H&E stain, ×480. (6) Macrophages sloughing in the alveolar sacs. H&E stain, ×160.

3.3.4 Emphysema

Thinned wall large airspaces with compressed septa and bloodless capillaries were seen in the lung tissues exposed to 10 nm GNPs (Figure 7). This alteration might indicate a pathophysiological disturbances in the lung as a result of GNPs toxicity. The emphymatous holes were seen together with inflammatory cells infiltration which might indicate pulmonary parenchymal attenuation due to GNPs toxicity (Figure 8). Emphysema is a sort of irreversible alveolar walls destruction leads to loss of lung tissue elasticity [25].

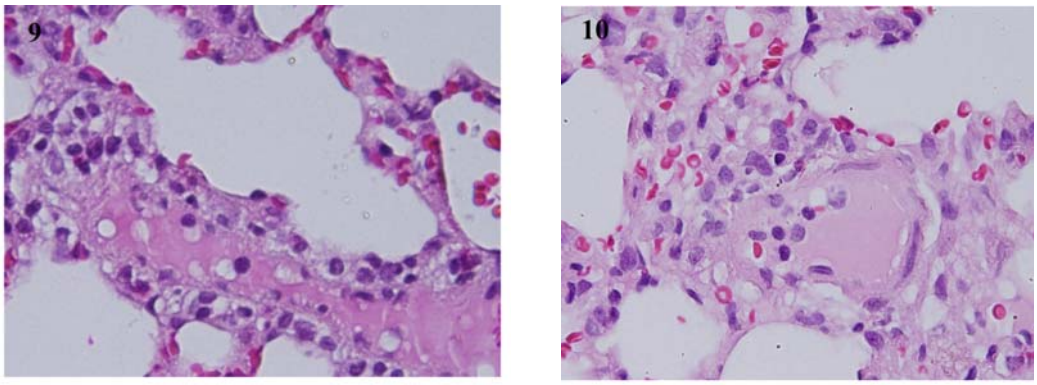




Figures (7-8). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: (7) Emphymatous holes seen together with inflammatory cells infiltration. H&E stain, ×160**.** (8) Alveolar walls destruction with emphysema. H&E stain, ×198.

3.3.5 Pulmonary edema

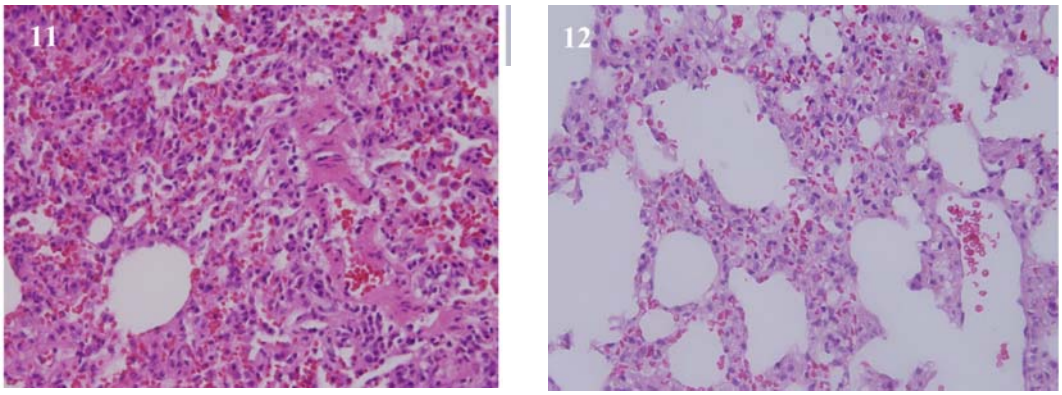
Pulmonary interstitial edema in the lung tissue of all GNPs treated rats was observed (Figure 9). Lung tissue edema is an air spaces obstruction complication related to lung inflammation and pulmonary tissue fluid flooding. This finding might indicate that GNPs toxicity could induce hydrostatic forces on the alveolar capillary by increasing their permeability. Perivascular pulmonary edema was also detected in the lung tissue of rats exposed to 10 nm GNPs (Figure 10).



Figures (9-10). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: (9) Pulmonary interstitial edema. Note the accumulated pink materials in the interstitium suggesting high protein content. H&E stain, ×480. (10) Perivascular edema. H&E stain, ×480.

3.3.6 Interalveolar capillaries dilatation and congestion

Pulmonary congestion with dilated interalveolar septal capillaries and leakage of blood cells were seen (Figures 11&12). The appearance of extravasated erythrocytes in the alveolar sacs may indicate compression due to edema and/or thickening of the alveolar walls.



Figures (11-12). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: (11) Dilated interalveolar septal capillaries. H&E stain, ×160. (12) Extravasated erythrocytes in the alveolar sacs. H&E stain, ×160.

3.3.7 Atelectasis

Focal narrowing and deflation of some alveolar sacs in the lung tissue of some GNPs treated rats were detected (Figure 13). These abnormalities might be resulted from partial blockage of alveoli in the affected area of the pulmonary tissue with interstitial exudates accumulation by GNPs toxicity.

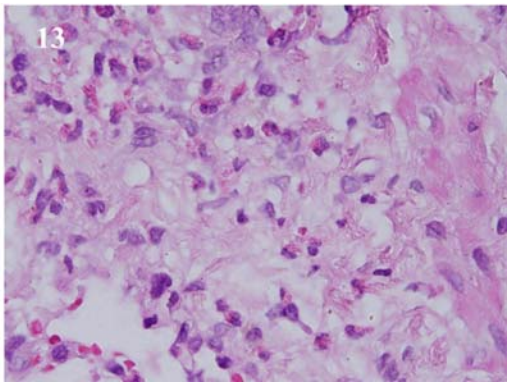
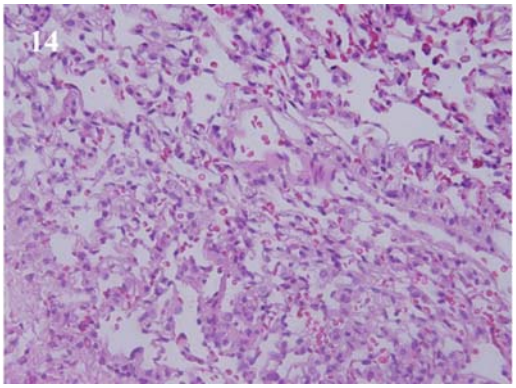
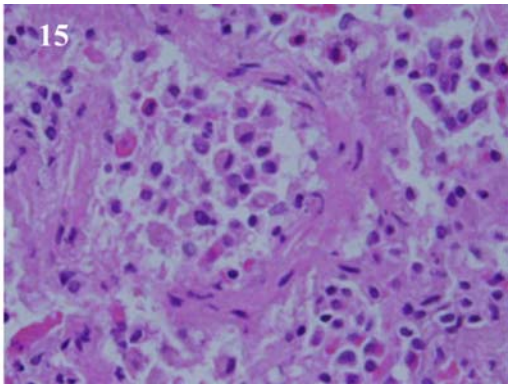


Figure 13. Light micrograph of section in the lung of GNPs-treated rat demonstrating atelectasis. H&E stain, ×160.

3.3.8 Alveolar hypersensitivity

Considerable number of eosinophils and plasma cells were seen in the thickened flamed interstitium and pulmonary blood vessels (Figures 14&15). This finding might indicate allergic alveolitis induced by GNPs.

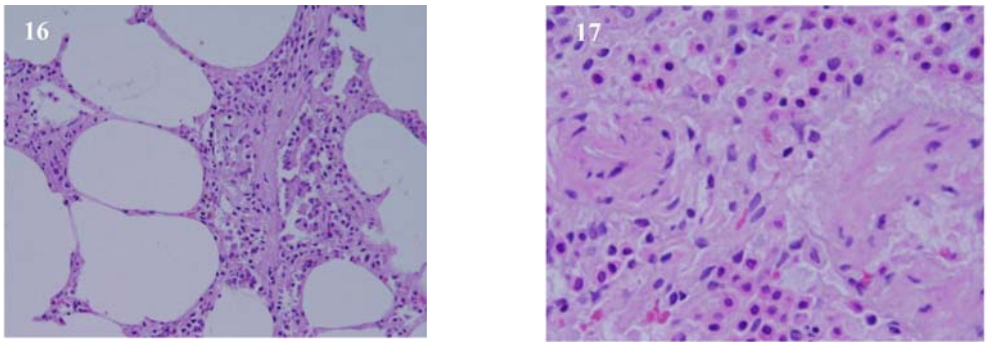




Figures (14-15). Light micrographs of sections in the lung of GNPs-treated rat demonstrating: (14) Large number of eosinophils in thickened flamed interstitium. H&E stain, ×480. (15) Crowded plasma cells in a pulmonary blood vessels. H&E stain, ×480.

3.3.9 Interstitial fibrosis

Connective tissue fibrosis with spindle shaped fibrocytes was demonstrated in the lung interstitium of some rats exposed to GNPs (Figure 16). Scared fibrous tissue was seen in the damaged inflamed thickened walls and accompanied by blood vessel thickening (Figure 17). Pulmonary fibrosis cause the lung to lose its elasticity [26].



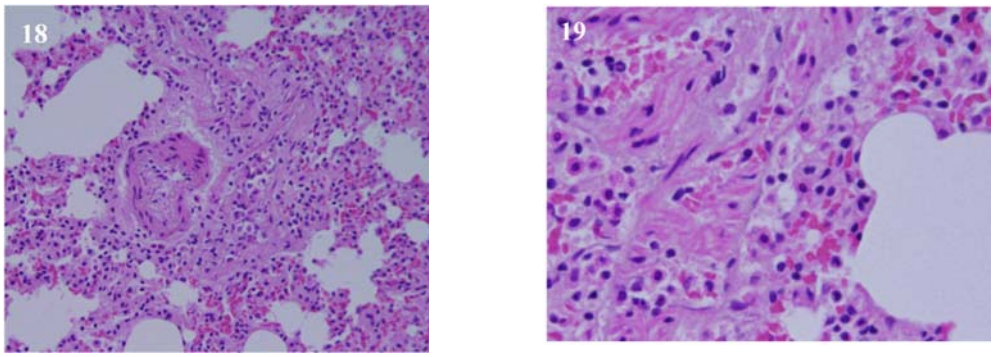
Figures (16-17). Light micrographs of sections in the lung of GNPs-treated rats demonstrating: (16) Interstitial fibrosis together with emphysema and inflammatory cells infiltration. H&E stain, ×160. (17) Scared fibrous tissue accompanied by blood vessel thickening. H&E stain, ×480.

Some of the above pulmonary described alterations induced by 10 nm GNPs were reported by some previous studies [18]. Other abnormalities such as atelectasis, pulmonary edema, macrophages invasion and alveolar hypersensitivity were not described before and are reported for the first time by the present work.

3.4 Lung of Rats Treated with GNPs Plus Propolis

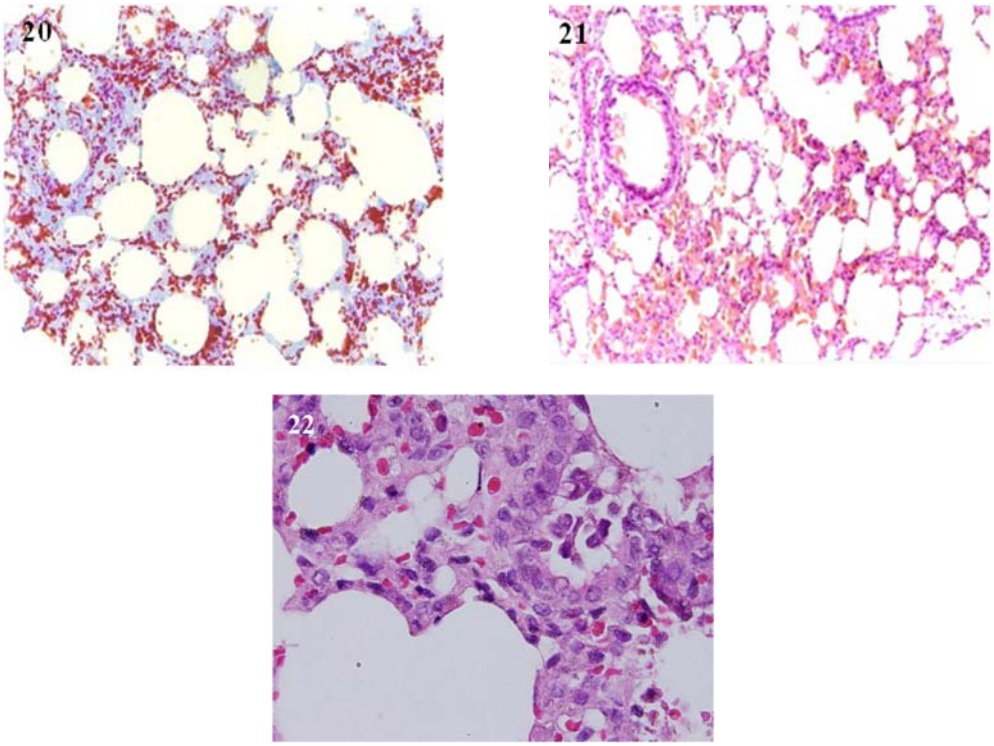
The lung tissues of rats subjected to GNPs plus propolis demonstrated full protection from pulmonary edema and allergic alveolitis. This protection may indicate that propolis can reverse the change in the ion balance and fluid hemostasis that affect the ion transport through cell membrane due to GNPs toxicity. Some investigators reported that propolis could repair cellular structures by inhibiting membrane free radical formation and lipid peroxidation by activation of some antioxidant enzymes [27].

On the other hand, the lung tissues of rats subjected to GNPs combined with propolis demonstrated no protection from atelectasis, fibrosis and macrophages invasion induced by GNPs (Figures 18&19). This might indicate that propolis could not compensate against these pulmonary alterations.



Figures (18-19). Light micrographs of sections in the lung of rats subjected to GNPs combined with propolis demonstrating:(18) No protection from fibrosis and atelectasis in comparison to rats subjected to GNPs only. H&E stain, x160. (19) Predominance of fibrocytes together with lymphocytes infiltration similar to that seen in the lung tissue of rats exposed to GNPs only. H&E stain, x480.

The lungs of rats exposed to GNPs plus propolis demonstrated partial amelioration of the alveolar walls thickening, inflammatory cells infiltration and alveolar capillaries dilatation with concurrent macrophages invasion (Figures 20-22). The alveolar septa consist of connective tissue and contain lymphatic and pulmonary venules. Once thickening occurred, most likely these components are affected. The observed septal thickening due to GNPs toxicity might be resulted from pulmonary tissues infiltration with inflammatory cells and fibroblasts. These together may indicate protecting role to propolis against cellular infiltration in the alveolar walls. This also might indicate immunostimulant and immunomodulating activity of this bee glue to reduce the activity of pro-inflammatory mediators specially the cytokines. Constant macrophages production concurrent with propolis treatment might indicate regeneration role against GNPs toxicity as a mechanism of apoptotic cells and tissue debris clearance to regain lung tissue elasticity. Macrophages stimulate the production of chemokines and growth factors and can rebuild the injured tissues. Additionally, propolis subjection with GNPs improved emphysema and to lesser extent the interstitial congestion. This might indicate potential capability of propolis to suppress pulmonary capillary expansion.



Figures (20-22). Light micrographs of sections in the lung of rats subjected to GNPs combined with propolis demonstrating: (20) Partial protection against alveolar walls thickening compared with lung tissue of rats exposed to GNPs only. Masson trichrome stain, x160. (21) Partial protection against inflammatory cells infiltration and alveolar capillaries dilatation compared with lung tissue of rats exposed to GNPs only. H&E stain, x160. (22) Predominance of macrophages with foamy cytoplasm similar to that seen in the lung tissue of rats exposed to GNPs only. H&E stain, x480.

3.5 Lung of Rats Treated with Propolis Only

Normal histoarchitectural pattern of the lung tissues was seen in the lung of all rats subjected to propolis only (Figure 23). Propolis therapy has been considered affective in several pulmonary diseases protecting the lungs from damage caused by free radicals and oxidative stress [25].

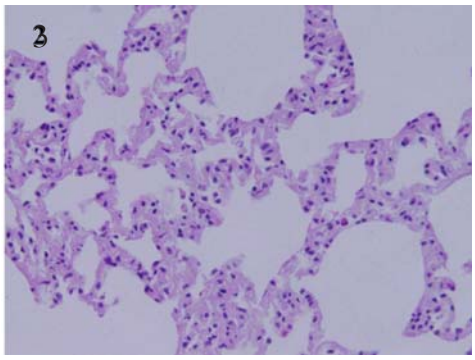


Figure 23. Light micrograph of section in the lung of rat treated with propolis only demonstrating normal histological pattern. H&E stain, ×198.

The findings of the present work might together indicate that propolis could afford potential protection to lung tissues against GNPs toxicity. This protection might be due to the antioxidant activity of propolis against oxidative stress induced in the lung tissues by GNPs. Some studies demonstrated protective role and therapeutic potential for propolis against several chemical and environmental toxicants and [27,29]. One recent study indicated that propolis could be used in preparing electrspun nanofiber used in oral care products [30]. The antioxidative capacity of propolis might be related to its pharmacological and biological contents such as flavonoids and phenolic acids. Furthermore, propolis has the ability to activate antioxidant enzymes to suppress cytochrome p-450 enzymes and to reduce lipid peroxidation [19,31]. In addition, some investigators reported that propolis can inhibit membrane free radical formation and has the capability to protect the mitochondria and cellular macromolecules against oxidative damage [32].

4. Conclusion

It is concluded from the findings of the present study that propolis combined with GNPs can augment the defense against the severity of some alterations in the lung tissues induced by GNPs. In addition, the results may provide evidences for the protective role and therapeutic potential of propolis related to its antioxidant ability to protect pulmonary tissues from oxidative stress induced by GNPs toxicity.

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