TOXICOLOGICAL AND HISTOLOGICAL EFFECTS OF SILVER NANOPARTICLES ON THE LUNG OF ADULT MALE ALBINO RAT AND PROTECTIVE ROLE OF GREEN TEA EXTRACT

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ABSTRACT

Silver nanoparticles (AgNPs) are incorporated into a large number of consumer and medical products. AgNPs has been reported as the materials with high toxicity especially after the systemic uses. The present work aimed to evaluate the toxic effects of two different doses 0.5, 10 mg/kg of AgNPs (35±8.5nm) on the lung of adult male albino rat following 30 days oral administration and also to assess the protective role of green tea extract (GT). Sixty rats were classified into six groups (ten rats/ group); Group1:(control group) allowed distillate water, Group2: Rats were given GT at a concentration of 1.5%, Group3: AgNPs treated rats (0.5mg/kg/day), Group4: AgNPs treated rats (0.5mg/kg/day)–co administered GT, Group5: AgNPs treated rats (10mg/kg/day), and Group6: AgNPs (10mg/kg/ day)–co administered GT. This aim was undertaken through adopting certain parameters including, animal observation, changes in body weight, and biochemical studies for antioxidant enzymes {super oxide dismutase (SOD) and catalase (CAT)}. Absolute and relative lung weights have been carried as well. Histological examination of lung tissue using different stains; H&E, Mallory trichrome, and immunohistochemical study for surfactant protein B was done followed by morphometric and statistical studies. **Results:** Normal daily activity was observed in all groups. A statistically significant increase in the mean body weight in groups treated with AgNPs; whereas a nonsignificant increase in GT group and groups treated with GT+ AgNPs at the end of experiment as compared to the initial value. AgNPs significantly decreased (CAT) while, increased SOD level. GT significantly increased relative lung weight while, a nonsignificant increase in AgNPs groups as compared to control. Histological examination of lung tissue revealed histological alterations in groups treated with AgNPs which were more pronounced in high dose (10mg) including; thickening of the alveolar wall, destruction of the alveoli, dilated alveoli, mononuclear cellular infiltration associated with marked collagen deposition and weak immunoexpression for surfactant protein B**.** Coadministration of GT with AgNPs caused significant amelioration of biochemical, histological and immunohistochemical changes induced by AgNPs**. Conclusion:** Silver nanoparticles caused oxidative damage, biochemical changes and histological alterations in the lung of male rat. This study demonstrates the benefits of green tea as it reduces oxidative damage by virtue of its antioxidant properties thus improving the structural integrity of lung tissue and eventually alleviates the histological changes as well as the biochemical perturbations.

Keywords: Silver nanoparticles (AgNPs), Antioxidant enzymes, Lung tissue, Histology, Immunohistochemistry, Green tea extract, Rats.

INTRODUCTION

Nanotechnology is an important modern and rapidly growing field dealing with design, synthesis and manipulation of particles structure ranging approximately from 1100 nm. These small dimensions result in a high surface area to volume ratio determining unique chemical, physical and biological properties different from those of bulk material with the same composition **(Barnes et al., 2008).**

Silver nanoparticles (AgNPs) are presently one of the most frequently used nanomaterials in consumer products and have attracted the attention in diverse areas such as medicine, catalysis, nanobiotechnology, electronics, magnetics, optics and water treatment because of its specific biological properties and proven applicability. Moreover, Silver nanoparticles have significant inhibitory effects against microbial pathogens, and are widely used as antimicrobial agents **(Marin et al., 2015).**

However, extensive use of AgNPs may lead to environmental contamination and human exposure by inhalation, dermal and oral routes, raising concerns about their potential environmental impact and toxicity **(Shahare and Yashpal,2013).** Silver nanoparticles has been reported as the materials with high toxicity as compared to other materials especially after the systemic uses, the nanoparticles is small enough to pass from smallest capillary vessel of body and biological membranes and be effective on physiology of any cells in body **(Sharma et al.,2006).** Nanoparticles can damage organs and different tissues through producing free radical and stress oxidation mechanism by attacking free radical to tissues **(Akradi et al., 2012).**

Herbal medicines derived from plant extracts are being utilized as adjunct treatment options for a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities**(Mandel et al., 2006).**Today green tea is the most widely used beverage next to water. It has many beneficial effects. It is made from unfermented leaves and contains the highest concentration of powerful antioxidants called polyphenols **(Thasleema, 2013).**

The increasing interest in the health properties of green tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapy in several diseases **(Mandel et al., 2006**).

The present study was designed to investigate the histological and toxicological effects of silver nanoparticles and the protective role of green tea extract on the lung of adult male albino rat.

MATERIALS AND METHODS

- **Preparation of Silver nanoparticles:**

Silver nitrate ($AgNO_3$) (99%) and sodium citrate ($Na_3C_6H_5O_7$) were obtained from SigmaAldrich Chem. Co. All chemicals were used as received without further purification. Silver colloidal nanoparticles were prepared according to **Monteiro et al., (2012).**

Silver nanoparticles were prepared at Egyptian petroleum research institute.

- **Characterization of the prepared NPs:**

UV/VIS/NIR spectrophotometer:

The synthesized NPs were studied through measuring optical absorbance by using near ultraviolet to visible to near infrared instrument (UVVisNIR, V570 model, JASCO, JAPAN) spectrophotometer.

Transmission Electron Microscopy (TEM)

TEM (JEM2100 LaB6, Japan) enables the visualization of internal structure of crystal samples and provides two dimensional images magnified as high as 100,000 times.

Green tea:

Green tea was purchased from SigmaAldrich Chem. Co. It was prepared according to **Maity et al.**, **(1998)** and later adopted by **ElBeshbishy, (2005)** by soaking 15 g of instant green tea leaves in 1 L of distilled water whose temperature did not exceed 90 °C, for 5 min to obtain soluble polyphenols dissolved in the aqueous extract. The solution was filtered to obtain the final 1.5% (w/v) green tea extract. This solution was substituted in the place of water as the sole source of drinking fluid to rats in groups 2,4, and 6.

Animals:

The present study was carried out on sixty adult male albino rats with body weight ranged from 180200 grams. They were housed in clean plastic cages with metal covers, at room temperature. Free access to water and diet were allowed to the animals. They were subjected to 7 days period of passive preliminaries in order to adapt themselves to their new environment and to ascertain their physical wellbeing.

- **Experimental design:**

Experimental groups of animals were administered silver nanoparticles (AgNPs) (35±8.5nm) orally using the oral gavage technique once a day for 30 days. The animals were classified into six groups (ten animals per group) in the following manner:

Group 1: (control group) in which rats were allowed distillate water orally ad libitum.

Group 2: (Green tea group) in which rats were given GT at a concentration of 1.5% orally as the sole drinking fluid substituted in the place of water **(Heikal et al., 2013).**

Group 3: AgNPs (0.5mg/kg/ day) treated rats **(Sardari et al., 2012).**

Group 4: Rats treated with AgNPs(0.5mg/kg/ day)– co administered GT as the sole source of drinking fluid.

Group 5: AgNPs(10mg/kg/ day) treated rats **(Yousef et al., 2012).**

Group 6: Rats treated with AgNPs (10mg/kg/ day)–co administered GT as the sole source of drinking fluid.

In this study the animals were received the treatment of silver nanoparticles by oral route, because AgNPs can already be found in a number of commercial products including food packing materials and kitchen appliances, and is even sold as an alternative "health supplement" **(Loeschner et al., 2011).** Therefore, oral intake of silver nano particles is a relevant route of exposure for the consumer.

During the the treatment period, physical evaluation was performed on each animal and included:

Observation for mortality and general condition: Animals were observed in their cages daily throughout the study for mortality, any deterioration condition and or signs of toxicity or possible illness.

Body weight gains:

Body weights were measured prior to the initiation of treatment, and immediately before sacrificing the animals.

Twentyfour hours after the last dose of treatment, all animals were anaesthetized with diethyl ether inhalation. Blood samples were obtained from the retro orbital sinus puncture into heparinized capillary tubes from each rat before killing. Blood samples were collected in clean dry test tubes and centrifuged at 2000 rpm for 15 minutes. Sera were then separated and kept frozen at 20 °C for subsequent biochemical studies.

After collecting blood samples, both lungs were removed carefully and grossly examined for any abnormalities. The lung weights (absolute) were recorded and lung– to–body weight ratio (relative weights) expressed as [absolute organ weight (g)/body weight (g) ×100] were determined **(Kang et al., 2014).** Then the lungs were fixed in 10% neutral buffered formaldehyde.

The handling of animals followed the rules for the experimental research ethics approved by Research Ethics Committee at faculty of Medicine for Girls AlAzhar University.

- **Biochemical studies:**

The collected sera were used for the estimation of antioxidant enzymes {super oxide dismutase (SOD) and catalase (CAT)}. Commercial Kits were purchased from Bio diagnostic, Giza, Egypt.

- Quantitative estimation of plasma catalase (CAT) was done according to **(Aebi,1984**).
- Quantitative estimation of plasma superoxide dismutase (SOD) was determined by the method described by **(Nishikimi et al., 1972).**

- **Histological studies:**

After proper fixation, the specimens were processed and stained with the following stains:

H&E stain as a routine stain for studying the general histological structure and changes of the lung **(Kieranan, 2001).**

Mallory trichrome stain: to study collagen fibers deposition in the lung **(Drury & Wallington, 1980).**

- **Immunohistochemical study:**

Lung tissue sections were processed according to **(You et al., 2014)** using surfactant protein B (1:200) [US Biological Life Sciences, United States].

- **Morphometric study**

For semiquantitative analysis of lung fibrosis, 10 microscopic fields from each group were randomly selected under a light microscope, and the bluestained area percentage (collagen fibers) (Mallory trichrome stain) / $(\mu m)^2$ surface area in lung tissue was measured using a computerized image system composed of a Leica Qwin 500 image analyser which is connected to a Leica microscope **(Mohamad et al., 2011).**

For the immunohistochemical analyses of surfactant protein B, staining density (optical density) was determined using the same image analysis system under high power magnification for ten fields per group **(You et al., 2014)**.

- **Statistical analysis:**

Data was expressed as mean ± standard deviation (± SD). Comparison of numerical variables between two studied groups was done using Student t test and one way ANOVA (F) test was used for data analysis between all experimental groups. P values ≤ 0.05 was considered statistically significant.

RESULTS

Characterization of Silver nanoparticles:

AgNPs absorbance were measured using UV spectrophotometer analysis with a strong light absorption in the visible region= 420nm as shown in **Fig.(1)**

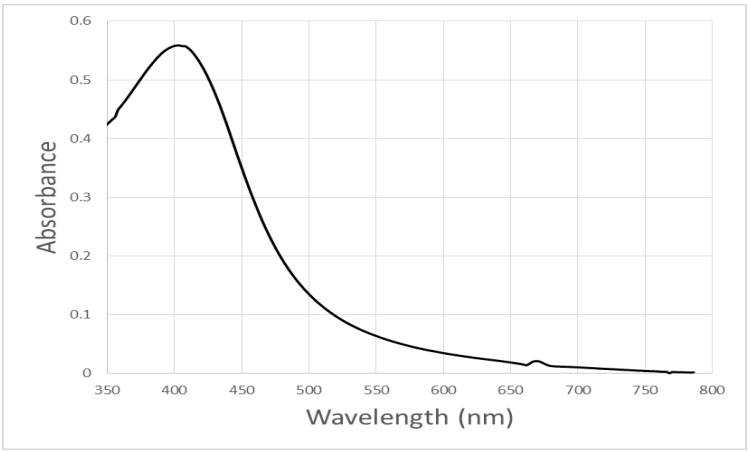

Figure (1): UV/Visible absorption of prepared silver nanoparticles at 420nm

Examination of the prepared AgNPs by transmission electron microscope (TEM) **Fig.(2),** revealed spherical shape and good particle dispersion of Ag NPs with average size at (35±8.5 nm)

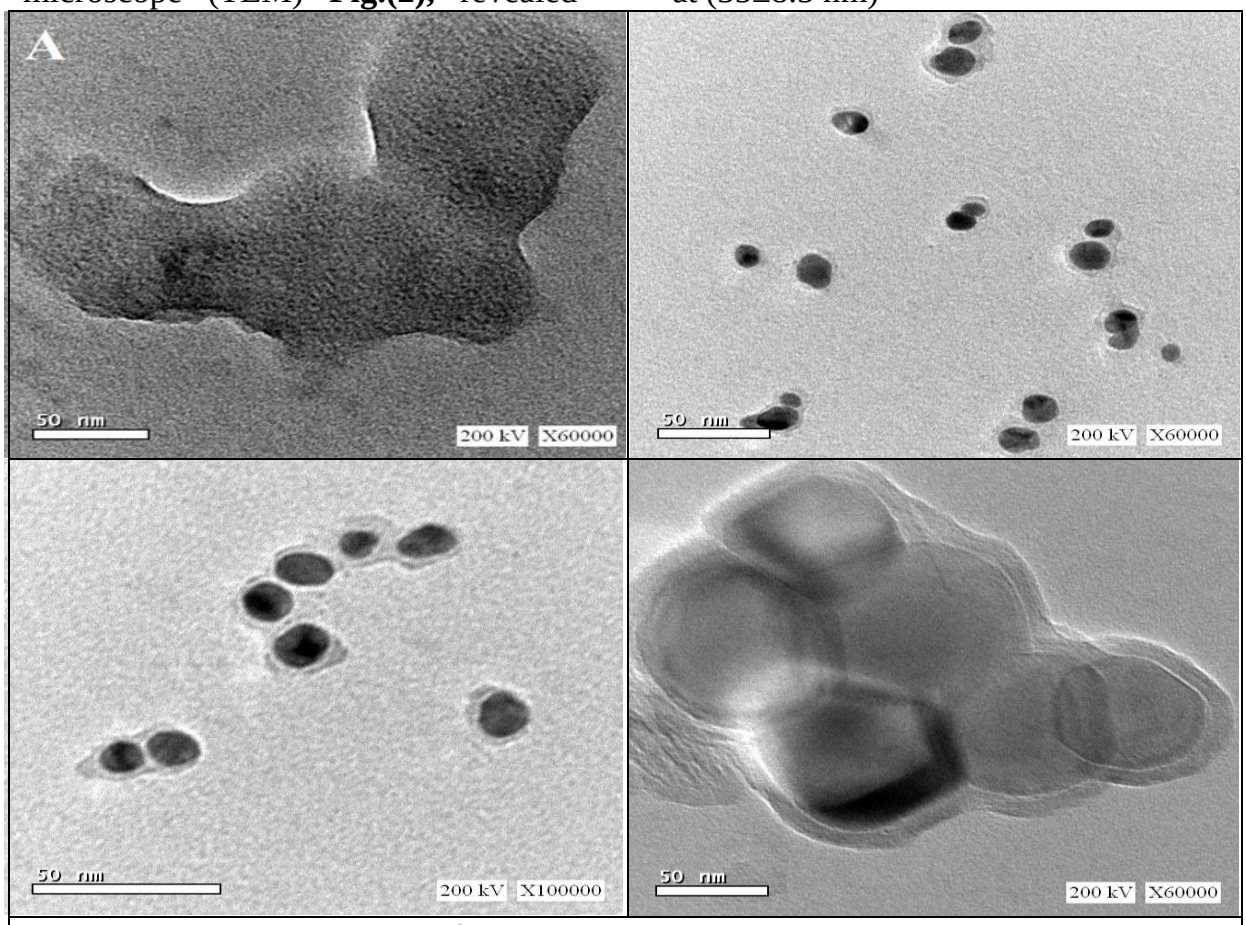

Figure (2): TEM images of prepared silver nanoparticles with low and high magnification power.

Animal observation after AgNPs and GT treatment:

Observation for mortality and general condition:

In the present study all animals survived and no mortality in both control and AgNPs treated groups with or without green tea throughout the study. Normal daily activity was observed in all groups, no deterioration of general condition, no signs of toxicity or possible illness was observed in any of the groups.

Changes in the mean body weight:

A statistically significant increase in mean body weight was detected in control group and all groups treated with AgNPs at the end of experiment as compared to the initial value. Nonsignificant increase in mean body weight was detected in green tea group and all groups treated with green tea in combination with AgNPs at the end of experiment as compared to the initial value **[Table 1]**.

Table (1) shows comparison between different studied groups as regard changes in body weight (gm) before and after the experiment:

Groups n=10rats/group	Total weight before Mean±SD	Total weight after Mean±SD
Group 1	186.75±5.377	345±31.091^{*a}
Group 2	186.25±9.464	191.25±16.52^{a*b}
Group 3	192.5±7.593	347.5±33.04^{*a}
Group 4	191.5±6.557	196.25±28.686^{a*c}
Group 5	187.75±7.41	345±34.156^{*a}
Group 6	191±8.041	195.75±31.287^{a*d}

Data are expressed as means±SD
Level of significance was set at P≤0.05*
Group 1 (ve control group), Group 2 (+ve control Green tea group) Group 3 (Ag NPs 0.5mg/kg/day), Group 4 (Ag NPs 0.5mg/kg/day +GT), Group 5 (Ag NPs 10mg/kg/day), Group 6 (Ag NPs 10 mg /kg/ day +GT).
a: compared change in body weight in each group before and after the experiment,
b: compared with group1,
c: compared with group3,
d: compared with group5,
Ag NP=Silver Nano Particles
GT=Green Tea

Lung weight and gross appearance:

Gross examination of the lungs showed no difference in the color and shape between control and all treated groups.

A statistically significant decrease in absolute lung weight was detected in group 2, 3, 5 as compared to control group and in group 4 as compared to group3. There was no significant increase in the absolute lung weight in group 6 as compared to group 5 **[Table 2]**

A statistically significant increase in relative lung weight was detected in group2 as compared to control group and in group4 as compared to group3. Finally a statistically significant increase was revealed in group6 as

compared to group5. Nonsignificant increase was observed in group3 and 5 when compared to control group **[Table 2]**

Table (2): shows comparison between different studied groups as regard changes in the absolute and relative lung weights.

Groups n=10 rats/ group	Lung weight	
	Absolute	Relative
	Mean±SD	Mean±SD
Group 1	1.874±0.089	0.478±0.071
Group 2	1.255±0.187*a	0.634±0.056*a
Group 3	1.516±0.141*[a]	0.480±0.0004[a]
Group 4	1.913±0.148*b	0.641±0.041*b
Group 5	1.658±0.064*[a]	0.484±0.038 [a]
Group 6	1.46±0.161*c	0.648±0.022*c

Data are expressed as means±SD
Level of significance was set at$P \leq 0.05^{*}$
a: compared with control group,
b: compared with group3,
c: compared with group 5

Biochemical Results:
(Antioxidant enzymes)

No statistically significant difference was observed between the control group and green tea group as regard the level of serum catalase. A statistically significant decrease in serum catalase was noticed in group 3 by 39% in comparison with control group. On comparing the level of catalase between group4 and group3, there was significant increase by 50.5%. A statistically significant decrease in serum catalase was detected in group5 by 73% when compared to control group. Whereas on comparing the level of catalase between group6 and group5, there was significant increase by184% **[Table 3]**

No statistically significant difference was observed between the control group and green tea group as regard the level of serum SOD. A statistically significant increase in serum SOD was noticed in group3 by 30% as compared to control group. While a statistically significant decrease in serum SOD was revealed in group4 by 40% when compared to group 3. A statistically significant increase in serum SOD was detected in group5 by 272.5 % as compared to control group. On comparing the level of SOD between group6 and group5, there was significant decrease by50% [**Table 3]**

Table (3): Shows a comparison between the different studied groups as regard the levels of serum Catalase and SOD

Groups n=10rats/group	Serum catalase		Serum SOD	
	(IU/L) Mean±SD	% of change	(IU/L) Mean±SD	% of change
Group1	778.908±37.339		21.955±1.163	
Group2	800.093±11.898 [a]		19.992±1.393 [a]	
Group3	472.006±23.341 [*a]	↓ by 39%	50.582±1.854 [*a]	↑ by 130%
Group4	710.509±13.035 [*b]	↑ by 50.5%	30.267±10559 [*b]	↓ by 40%
Group5	208.164±15.765 [*a]	↓ by 73%	81.794±2 [*a]	↑ by 272.5%
Group6	592.72±17.328 [*c]	↑ by 184%	40.693±1.306 [*c]	↓ by 50%

Data are expressed as means±SD
Level of significance was set at $P \leq 0.05$ [*]
a: compared with control group,
b: compared with group3,
c: compared with group5

Histological Results

H&E stain

Microscopic examination of lung sections of control and green tea groups, showed the lung alveoli (air sacs) with normal histological structure. The wall of the lung alveoli appeared thin and lined with thin wall of flat epithelial cells **(Fig.3, 4, 5)**

On the other hand, group3 showed that the wall of lung alveoli appeared slightly thickened, some alveoli are destructed. Also, some alveoli are dilated. There was mononuclear cellular infiltration and some areas of hemorrhage can be detected **(Fig.6)**

In group 4, treated with green tea there was partial improvement of the lung tissue. The wall of some lung alveoli appeared thin while others appeared slightly thickened. There were areas of hemorrhage and mononuclear cellular infiltration **(Fig.7, 8)**

Histological examination of group 5, showed marked thickening of the alveolar wall, dilated and congested blood vessel with thick wall. Multiple, large areas of hemorrhage and mononuclear cellular infiltration could be seen **(Fig.9, 10, 11)**

Treatment with green tea in group 6 revealed partial improvement of the lung tissue. The wall of some lung alveoli appeared thin while others appeared thickened. Some areas of hemorrhage could be seen **(Fig.12).**

Mallory trichrome stain

Light microscopic examination of lung sections of control and green tea groups showed few collagen fibers (which stained blue) **(Fig.13, 14).** On the other hand, there was marked collagen fibers deposition within the lung tissue in group 3, 5 if compared with control group **(Fig.15, 17).** There was moderate collagen fibers deposition in groups 4, 6 as compared with control group **(Fig.16, 18).**

Immunohistochemical results

Surfactant protein B expression appeared as brown granules. Strong positive immunoreaction was detected in control and green tea groups **(Fig.19,**

20). On the other hand, weak immunoreaction for Surfactant protein B was detected in groups 3, 5 **(Fig.21, 23).** Over immunoexpression of surfactant protein B could be observed in groups 4, 6 **(Fig.22, 24).**

Morphometric and statistical results

When comparing the means of area % of collagen fibers /µm² surface area in the lung tissue among the experimental groups revealed that the least mean was in control and green tea groups followed by groups4, 6. However, the highest mean was recorded in groups 3, 5. These findings were of statistically significant values ($P \leq 0.05$) **[Table 4]**

Statistical study of the means of the optical density of Surfactant protein B showed that the highest mean was recorded in groups4, 6 followed by control and green tea groups, while the least mean was found in groups 3, 5. All these findings were of statistically significant values ($P \leq 0.05$) **[Table 4]**

Table (4): Shows a comparison between the different studied groups as regard the area % of collagen fibers/um² and optical density of surfactant protein B in lung tissue

Groups n=10rats/group	Area % of collagen fibers Mean±SD	Optical density of surfactant protein B Mean±SD
Group1	1.9±0.18	1.2±0.13
Group2	1.8±0.21 [a]	1.1±0.22 [a]
Group3	13.23±0.45 [*a]	0.1±0.02 [*a]
Group4	3.51±0.17 [*b]	1.5±0.12 [*b]
Group5	15.24±0.41 [*a]	0.2±0.03 [*a]
Group6	5.73±0.38* [c]	1.3±0.14* [c]

Data are expressed as means±SD
Level of significance was set at$P \leq 0.05$ [*]
a: compared with control group,
b: compared with group3,
c: compared with group5.

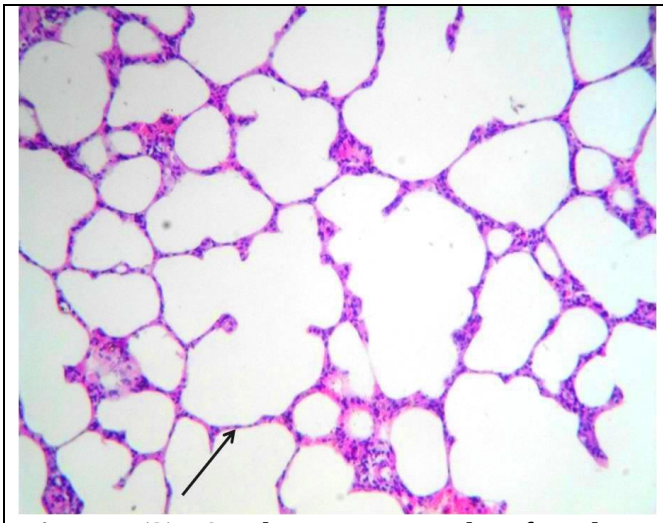

Figure (3) A photomicrograph of a lung section of an adult albino rat control group (Group 1), showing the lung alveoli (air sacs) with normal histological structure. The wall of the lung alveoli appears thin (arrow) **(H&E X 200).**

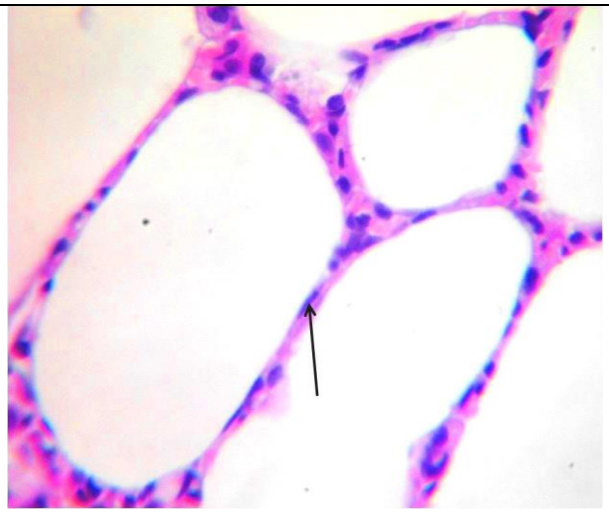

Figure (4) A photomicrograph of a lung section of an adult albino rat control group (Group 1), showing that the lung alveoli lined with thin wall of flat epithelial cells (arrow) **(H&E X 400).**

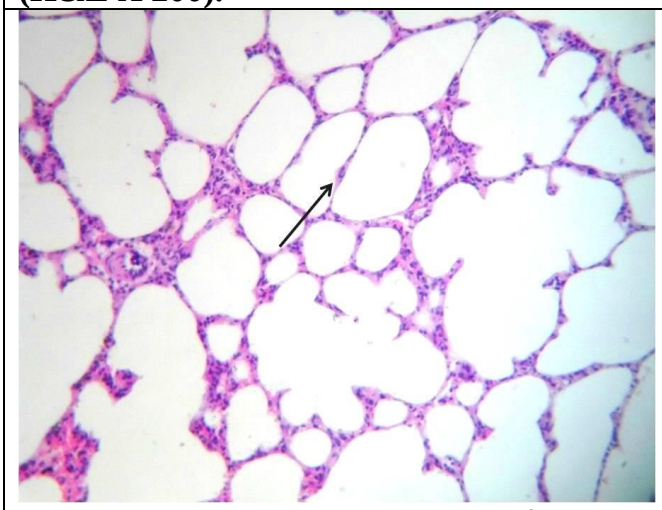

Figure (5) A photomicrograph of a lung section of an adult albino rat green tea group (Group 2), showing the lung alveoli (air sacs) with normal histological structure. The wall of the lung alveoli appears thin (arrow) **(H&E X 200).**

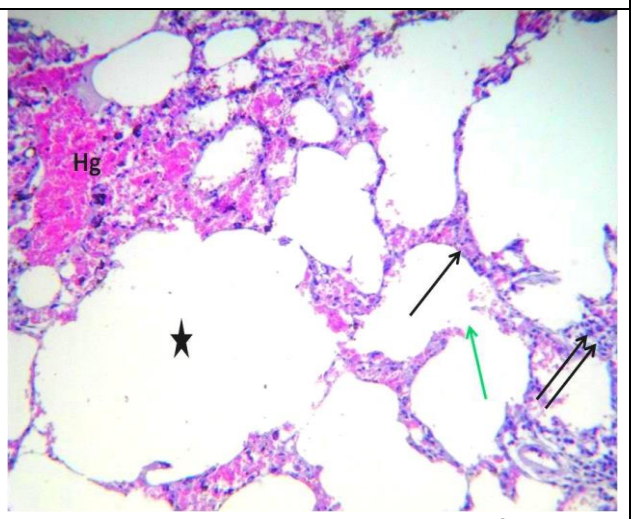

Figure (6) A photomicrograph of a lung section of an adult albino rat Group 3, showing that the wall of lung alveoli appears slightly thickened (arrow). Some alveoli are destructed (green arrows). Also, some alveoli are dilated (star). There is mononuclear cellular infiltration (double arrows). Some areas of hemorrhage (Hg) can be detected **(H&E X 200).**

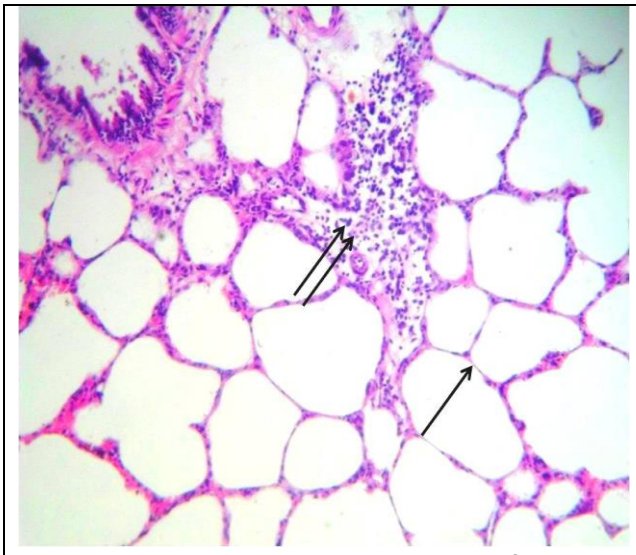

Figure (7) A photomicrograph of a lung section of an adult albino rat Group 4 treated with green tea, showing partial improvement of the lung tissue. The wall of lung alveoli appears thin (arrow). There is mononuclear cellular infiltration (double arrows) **(H&E X 200).**

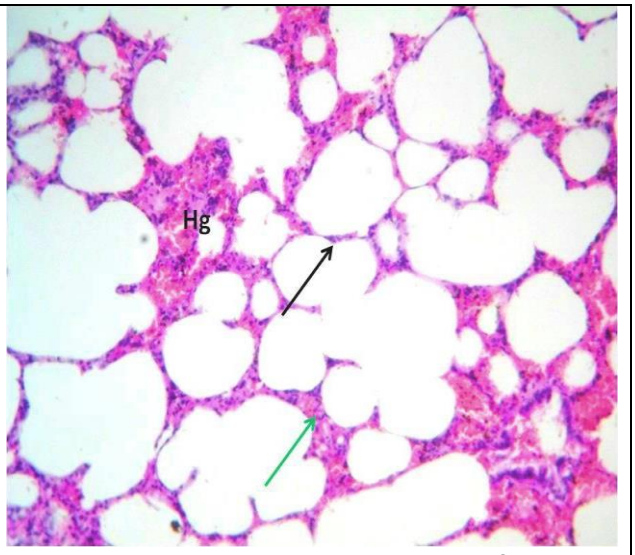

Figure (8) A photomicrograph of a lung section of an adult albino rat Group 4 treated with green tea, showing moderate improvement of the lung tissue. The wall of some lung alveoli appears thin (black arrow) while others appears slightly thickened (green arrow). There are areas of hemorrhage (Hg) **(H&E X 200).**

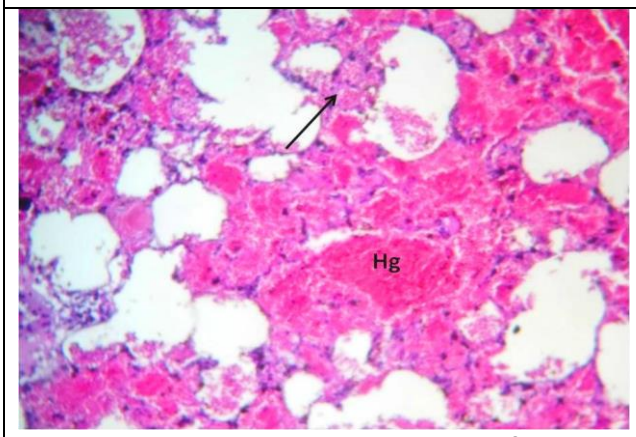

Figure (9) A photomicrograph of a lung section of an adult male albino rat Group 5, showing marked thickening of the alveolar wall (arrow). Multiple, large areas of hemorrhage (Hg) can be seen. **(H&E X 200).**

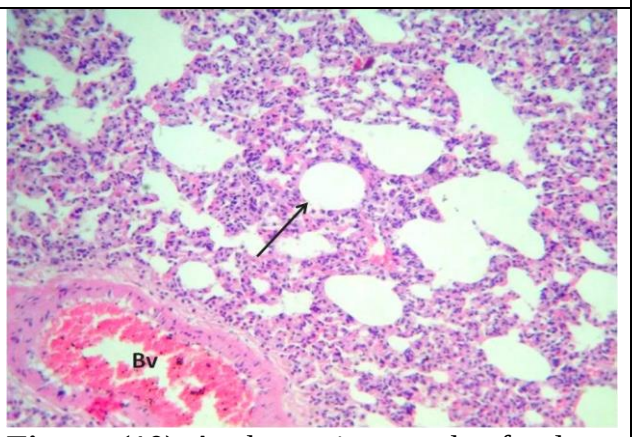

Figure (10) A photomicrograph of a lung section of an adult male albino rat group 5, showing marked thickening of the alveolar wall (arrow). Dilated and congested blood vessel with thick wall (Bv) can be seen **(H&E X 200).**

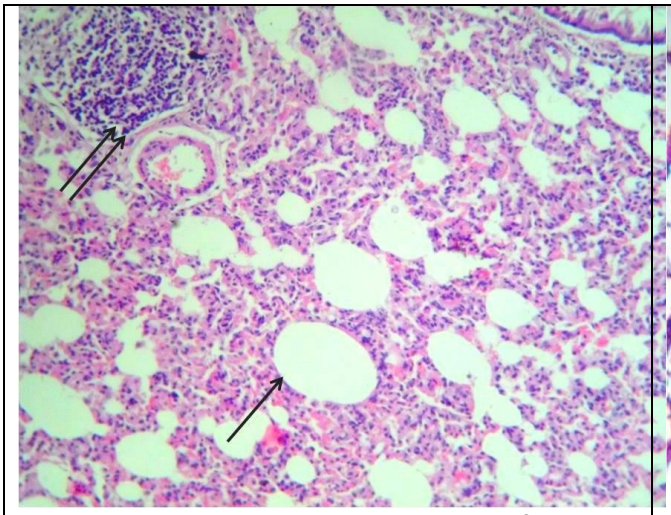

Figure (11) A photomicrograph of a lung section of an adult male albino rat group 5, showing marked thickening of the alveolar wall (arrow). There is marked mononuclear cellular infiltration (double arrows) **(H&E X 200).**

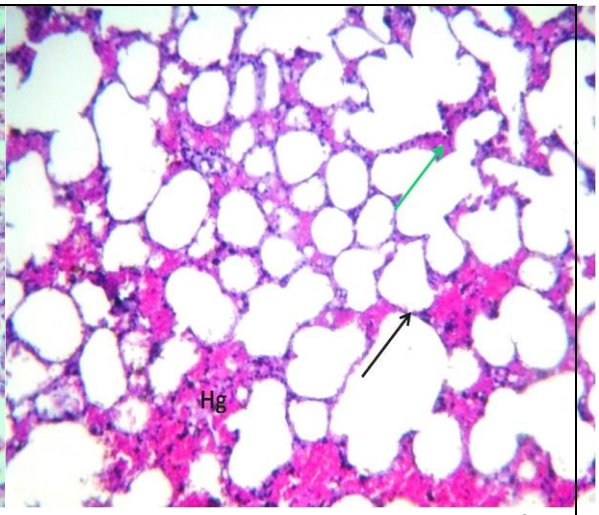

Figure (12) A photomicrograph of a lung section of an adult male albino rat Group 6 treated with green tea, showing partial improvement of the lung tissue. The wall of some lung alveoli appears thin (black arrow) while others appears thickened (green arrow). Some areas of hemorrhage (Hg) can be seen **(H&E X 200).**

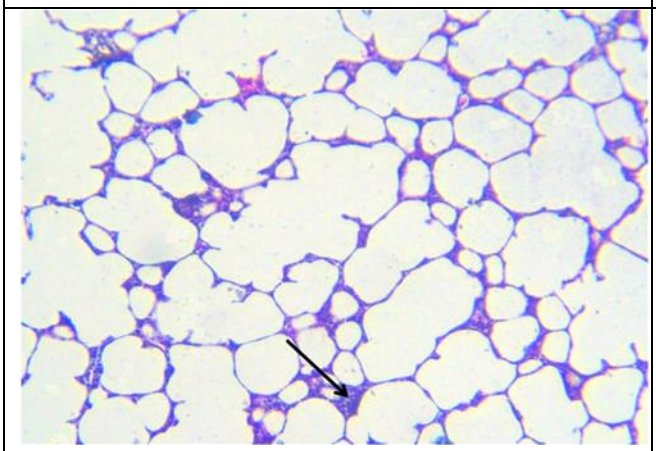

Figure (13) A photomicrograph of a lung section of an adult male albino rat control group (Group 1), showing minimal collagen fibers in between the lung alveoli (arrow). Notice, the collagen fibers are stained blue **(Mallory trichrome x 200).**

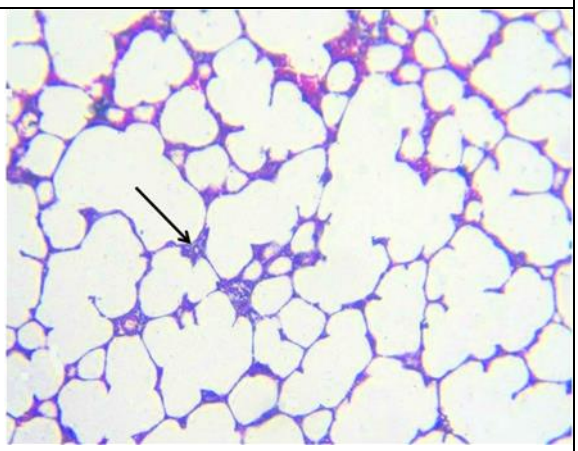

Figure (14) A photomicrograph of a lung section of an adult male albino rat green tea group (Group 2), showing minimal collagen fibers in between the lung alveoli (arrow) **(Mallory trichrome x 200).**

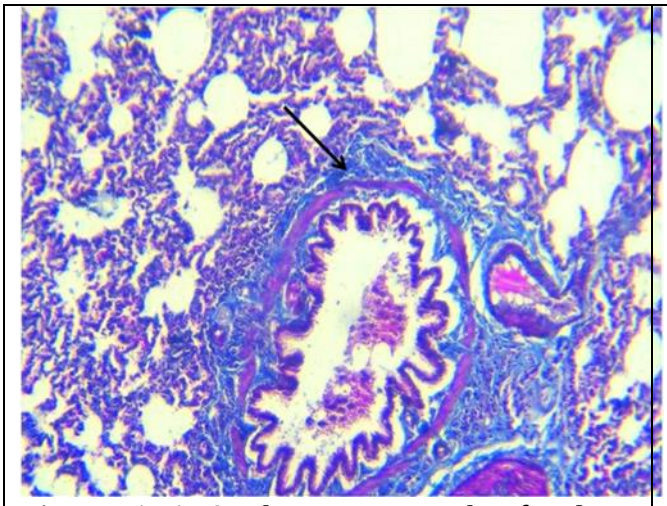

Figure (15) A photomicrograph of a lung section of an adult male albino rat Group 3, showing marked collagen fibers deposition in lung interstitium (arrow) **(Mallory trichrome x 200).**

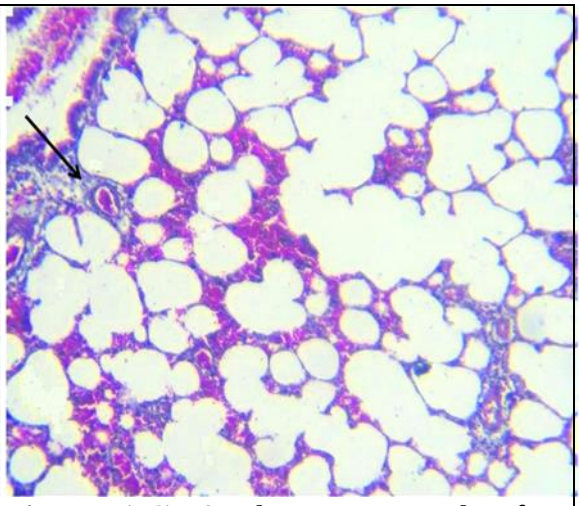

Figure (16) A photomicrograph of a lung section of an adult male albino rat Group 4 treated with green tea, showing moderate collagen fibers deposition in lung interstitium (arrow) **(Mallory trichrome x 200).**

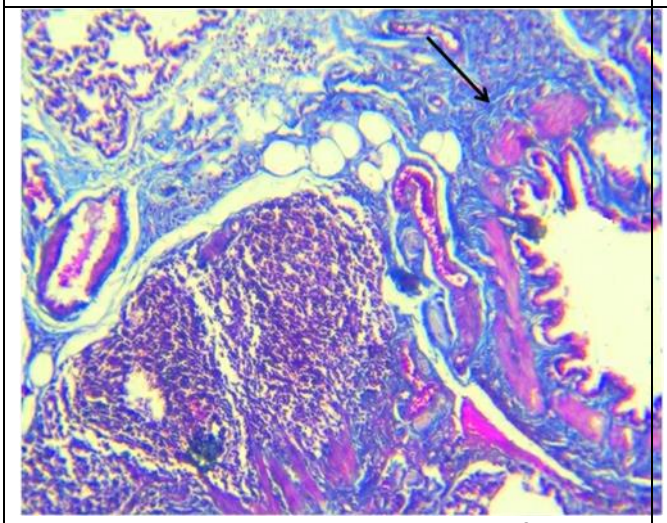

Figure (17) A photomicrograph of a lung section of an adult male albino rat Group 5, showing excessive collagen fibers deposition in lung interstitium (arrow) **(Mallory trichrome x 200).**

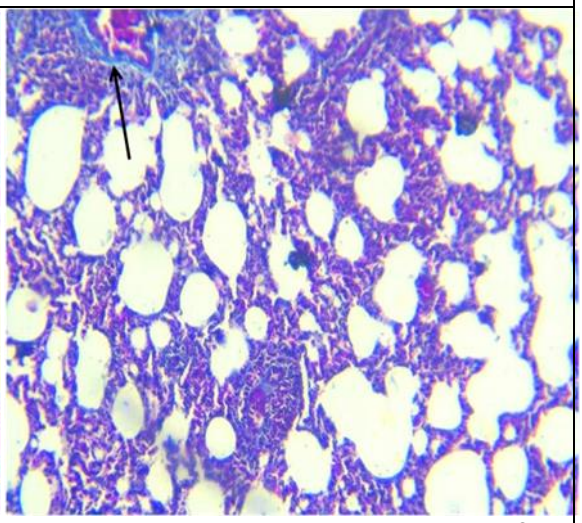

Figure (18) A photomicrograph of a lung section of an adult male albino rat Group 6 treated with green tea, showing moderate collagen fibers deposition in lung interstitium (arrow) **(Mallory trichrome x 200).**

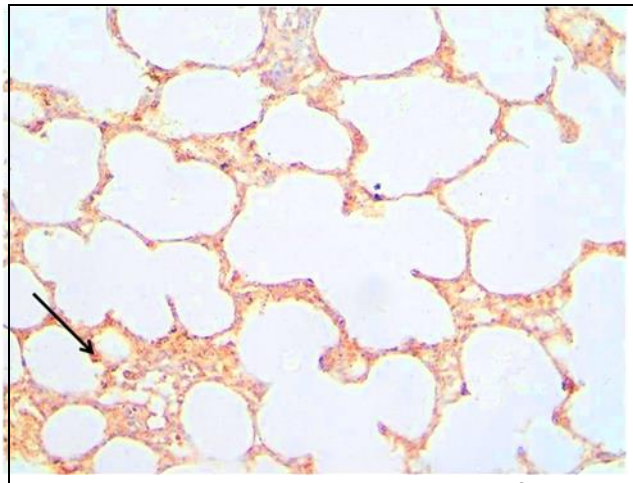

Figure (19) A photomicrograph of a lung section of an adult male albino rat control group (Group 1), showing strong positive immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

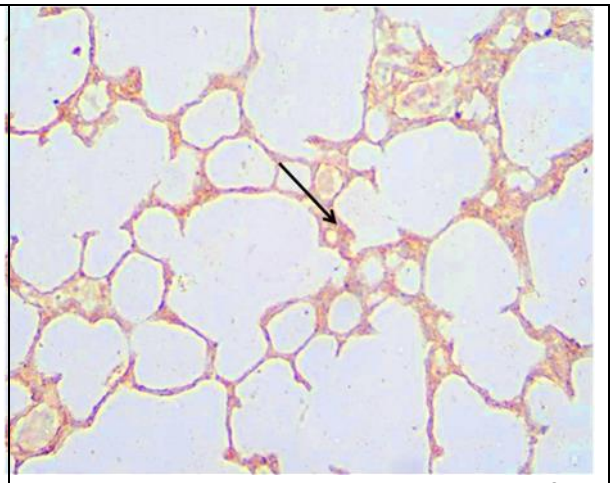

Figure (20) A photomicrograph of a lung section of an adult male albino rat green tea group (Group 2), showing strong positive immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

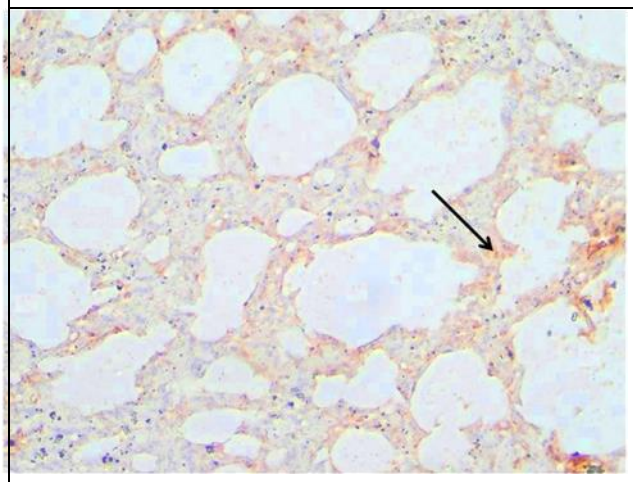

Figure (21) A photomicrograph of a lung section of an adult male albino rat Group 3, showing weak immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

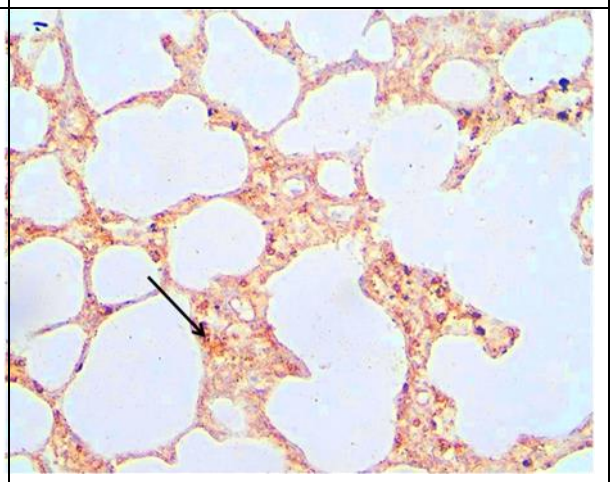

Figure (22) A photomicrograph of a lung section of an adult male albino rat Group 4 treated with green tea, showing very strong immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

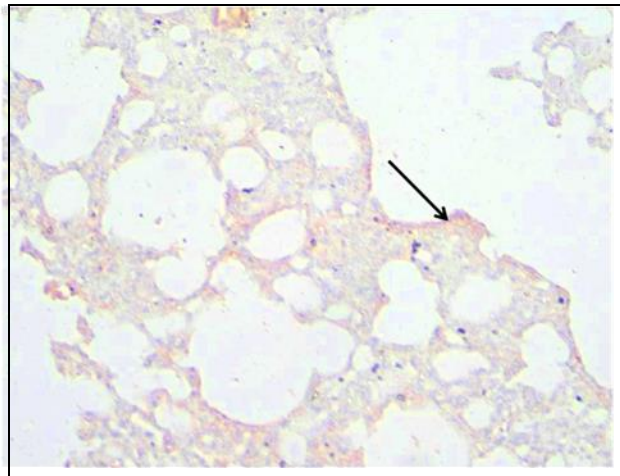

Figure (23) A photomicrograph of a lung section of an adult male albino rat Group 5, showing weak immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

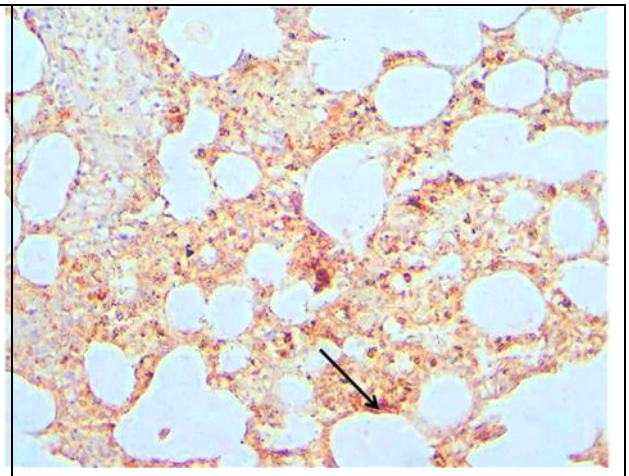

Figure (24) A photomicrograph of a lung section of an adult male albino rat Group 6 treated with green tea, showing very strong immunoreaction for surfactant protein B (arrow) **[Avidinbiotin peroxidase stain with Hx counter stain x 200].**

DISCUSSION

In the present study the animals in all groups survived. No deterioration of general condition, no signs of toxicity or possible illness were observed in any of the groups. This result was supported by **Hritcu et al., (2011)** who found no significant differences regarding daily behaviors such as feeding, drinking and physical activity between AgNPtreated groups and the control group. **Genter et al., (2012) and Yousef et al., (2012)** approved the same findings.

Concerning the results of body weight gain, the present study revealed a statistically significant increase in mean body weight in control group and groups treated with AgNPs (groups3, 5) at the end of experiment as compared to the initial value. But no significant difference in the total body weight between different groups treated with different doses of silver nanoparticles and control group.

This result was in agreement with **Hendi (2011)** who found no significant difference in the total body weight between the groups treated with AgNPs and control group. The same findings were supported by (**Hritcu et al., 2011; Genter et al., 2012 and ElMahdy et al., 2014)**

In the present study, there is nonsignificant increase in mean body weight in green tea group and all groups treated with green tea in combination with AgNPs at the end of experiment as compared to the initial value (group 2, 4, 6).

The mechanism of weight reduction induced by green tea treatment may be due to the increase in energy expenditure and fat oxidation; however, there is another possible mechanism involved, i.e., suppression of the lipogenic enzyme fatty acid synthase, so antiobesity effect of tea polyphenols could be also observed in the normal (nonobese) people **(Cooper**

et al., 2005).

Organ weight is one of the most sensitive indicator of toxic agents that reflect impact on the metabolism due to effects on the health and immunological status of the body **(Bailey et al., 2004).**

Pariyani et al., (2015) concluded that the relative organ weight index is used as basic indicator to assess the deleterious effects of the toxic metabolites. The effect of toxic substances on the internal organs could be identified by assessing the relative organ weight as the index gives a preliminary insight to the swelling or damage caused by any harmful agent.

Concerning the results of relative lung weight, the present work showed a nonsignificant increase in groups treated with AgNPs (groups 3,5) when compared to control group.

These observations were in agreement with **Sung et al., (2009)** who demonstrated that no significant dose related changes were noted in organ weight values in rats exposed to silver particles. Whereas **Amin et al., (2015)** concluded that oral administration of AgNPs didn't significantly affect the ratio of liver, kidney, lung, testis weight to total body weight as compared to vehicle control.

The result of the current study also demonstrated that a statistically significant increase in relative lung weight in green tea group as compared to control group and in groups treated with green tea in combination with AgNPs (groups 4, 6) as compared to groups treated with AgNPs alone (groups 3, 5).

The observed increase in relative lung weight following administration of green tea in the present study could be attributed to the reduction in body weight gain of experimental animals **(Heikal et al., 2011).**

Many studies have shown the role of oxidative stress in AgNPs toxicity **(Choi et al., 2010).** The present study revealed a statistically significant decrease in the level of catalase (CAT) in groups treated with silver nanoparticles (groups 3, 5) which were more pronounced in high dose as compared to control.

In agreement with this result **Shayesteh et al., (2014)** who found a decrease in CAT in rats treated with silver nanoparticles. Also, **Adeyemi and Faniyan, (2014)** detected inconsistent decrease in the levels of catalase relative to controls.

These results could be explained according to **Wu and Zhou, (2013)** as they concluded that dosedependent decrease in CAT activity after AgNPs exposure suggested an excessive consumption of this antioxidant enzyme in the tissue. The reduced CAT activity ultimately failed to catalyze the transformation of oxyradicals. These data indicate that the ability of antioxidant defense was significantly depressed and that a potential enhancement of reactive oxygen species (ROS) was produced.

On the other hand, the present study showed a statistically significant increase in the level of SOD activity in groups 3, 5 treated with AgNPs alone this increase were more pronounced in high dose in relation to control group. This result coincided with **Adeyemi and Faniyan, (2014)** as they found increase in the serum level of superoxide dismutase relative to the control group. The increased serum level of SOD in the present study might be explained by **Piao et al., (2011)** as they stated that SOD is an inducible

enzyme, and elevated levels may indicate the presence of reactive species and the alterations in the levels of these enzymes may represent an adaptive mechanism to offset the stress of exposure.

In the current research, coadministration of green tea and AgNPs caused an improvement in the alterations of CAT and SOD activities in groups 4 and 6. This result coincided with **Hamden et al., (2008).** Also, **Chakraborty et al., (2015)** demonstrated that green tea extract treatment resulted in significant improvement in SOD and CAT activity.

The protective effect of green tea is due to its antioxidant properties that scavenging free radicals. It has been shown that green tea contains volatile oils, antioxidant vitamins like (B, C, E and folic acid); tannins and amino acid (theanine) which is a major amino acid present in green tea. Additionally, polyphenols may also function indirectly as antioxidants through: (a) Inhibition of the redoxsensitive transcription factors, the antioxidative activity of green tea catechins is related to its ability for chelating redoxactive transition metal ions like iron, copper and prevents their participation in Fenton and HaberWeiss reactions **(Higdon and Frei, 2003)**. (b) Inhibition of "prooxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase. (c) Induction of phase I and phase II metabolic enzymes, which increase the formation and excretion of detoxified metabolites resulting from xenobiotic metabolism. (d) Induction of antioxidant enzymes such as catalase, superoxide dismutases and glutathione Stransferases **(Mohammed et al., 2011).**

Histological examination of lung tissue in groups treated with different doses of AgNPs revealed variable degrees of histological alterations which were more pronounced in high dose group, these alterations including; thickening of the alveolar wall (inter alveolar septum), destruction of the alveoli, dilated alveoli, mononuclear cellular infiltration and areas of hemorrhage. Also, there was marked deposition of collagen fibers in the lung intrestitium.

This histological picture is consistence with the picture pulmonary fibrosis. Pulmonary fibrosis is a chronic, and progressive disease of the lungs interstitium characterized by inflammation and deposition of collagen in the alveolar septa **(Asghari et al., 2015).**

These observations were in agreement with **Cho et al., (2013)** and **Najjaran et al., (2014)** who reported that there were cell infiltrate, alveolar wall thickening and abnormal enlargement of the air spaces in lungs of male rats exposed to silver nanoparticles. Moreover, **Doudi and Setorki, (2014)** and **Amin et al., (2015)** confirmed these findings.

Qian et al., (2015) and **Zhu et al., (2016)** demonstrated that there was correlation between nanopatricles exposure and lung fibrosis.

These results of the present study could be explained according to **Levard et al., (2011) and Fen et al., (2013)**, they indicated that Ag+ ion release is a major pathway underlying AgNPs bioreactivity and toxicity. Ionic silver (Ag+), a wellknown oxidation catalyst, is implicated in protein damage by desulphurisation, generates reactive oxygen species (ROS) and

may interfere with nitric oxide (NO) redox equilibria in the lung. This oxidative potential may increase the permeability of the lung epithelium to AgNPs resulting in DNA damage, lipid membrane damage, chromosomal aberrations, and cellcycle arrest. Through these biochemical pathways, release of Ag+ ions is a critical process that will determine the downstream effects of AgNPs on human health.

These explanations are confirmed by **Amin et al., (2015)** who hypothesized that the toxic effects of silver are proportional to free silver ions, but it is unclear how this relates to silver nanoparticles. AgNPs in the blood, indicate that the orally absorbed silver from nanoparticles is able to enter the blood circulation and be distributed to other organs. The damage illustrated in the histological examination in lung may be related to AgNPs deposition in the tissue.

On the other hand, histological examination of lung tissue in groups treated with green tea in combination with AgNPs showed amelioration in histological alterations as compared to groups treated with AgNPs alone, indicating the protective effect of green tea against lung complications of silver nanoparticles. The degree of beneficial effects of green tea against AgNPs toxicity was more pronounced in low dose of AgNPs treatment (0.5mg/kg/day).

These results generally agree with **Gawish et al., (2012)** who found that green tea decrease most of the pathological lesions in lung tissue induced by oxidative stress. This was manifested by almost normal appearance of most air sacs and interalveolar septa due to decrease in thickening of interalveolar septa and most of air sacs returned to normal shape and size. The amelioration effect of green tea may be attributed to antiinflammatory and antioxidant properties.

As regard the immunohistochemical expression of surfactant protein B, there was weak expression in AgNPs treated groups when compared to control group. In green tea treated groups, over expression could be detected. Surfactant protein B is considered as a marker for alveolar epithelial cells type II **(You et al., 2014)**.

Lung alveoli (air sacs) were lined with two types of cells, Type I alveolar epithelial cells (or type I pneumocytes) and Type II alveolar epithelial cells (type II pneumocytes or septal cells). Type I cells maintain the alveolar side of the bloodair barrier and cover about 95% of the alveolar surface. Type II cells interspersed among the type I alveolar cells and bound to them. Type II cells divide to replace their own population after injury and to provide progenitor cells for the type I cell population. They secreted material spreads over the entire inner alveolar surface as a film of complexed lipoproteins and water (pulmonary surfactant). The surfactant film lowers surface tension at the airepithelium interface, which prevents alveolar collapse at exhalation and allows alveoli to be inflated with less inspiratory force, easing the work of breathing **(Mescher, 2013).**

A highly significant increase in immunoexpression of surfactant protein B in the groups treated with green tea was in harmony with the result of **(Miller et al., 1987 and Mansour and Seleem, 2012)** who reported hyperplasia and hypertrophy of type II

cells. They added that these cells have the ability to divide by mitosis to replace their own population and also type I population.

So, from the above findings alveolar epithelial cells type II were decreased in lung fibrosis and were abundant in alveolar walls of the lung tissue of green tea treated rats. These result suggested that green tea protected alveolar epithelial cells type I and II from free radical damage. These findings were supported by **(You et al., 2014).**

CONCLUSION

Silver nanoparticles treatment caused oxidative damage, biochemical alterations and lung fibrosis. The green tea provided strong, persistent antioxidant, antiinflammatory, and antiproliferative effects that protected against AgNPs induced pulmonary fibrosis in rats. The findings suggested that these effects were mediated by inhibiting the synthesis and secretion of free radicals that caused extensive oxidative damage to lung interstitium. Green tea also decreased collagen fibers deposition and prevented alveolar epithelial cells type I and II damage.

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الملخص العربى

دراسة التأثيرات السامة والنسيجية لجسيمات الفضة النانوية على الرئة في ذكور الجرذان البيضاء البالغة والدور الوقائي لمستخلص الشاي الأخضر

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تدرج الفضة النانوية في عدد كبير من المنتجات الاستهلاكية والطبية و أفادت التقارير أن الفضة النانوية من المواد ذات السمية العالية خاصة بعد الاستخدامات الجهازية. يهدف العمل الحالي إلى دراسة التأثيرات السامة والنسيجية لجسيمات الفضة النانوية (8.5 ± 35) نانومتر في جرعتين مختلفتين 0.5مج / كغ / يوم و 10مج / كغ / يوم، والدور الوقائي لمستخلص الشاي الأخضر(تركيز 1,5%) عن طريق الفم لمدة 30 يوم. أجريت هذه الدراسة علي ستين من ذكور الجرذان البيضاء البالغة حيث تم تقسيمهم إلى ست مجموعات (عشرة / مجموعة)؛ المجموعة 1: مجموعة ضابطة وتم اعطاؤها ماء مقطر. المجموعة 2: تم اعطاؤها مستخلص الشاى الاخضر. المجموعة 3: تم اعطاؤها مادة الفضة النانوية بجرعة (0,5مج / كجم من وزن الجسم) المجموعة 4: تم اعطاؤها الفضة النانوية بجرعة (0,5مج / كجم من وزن الجسم) بالإضافة الي مستخلص الشاى الاخضر. المجموعة 5: تم اعطاؤها الفضة النانوية بجرعة (10مج / كجم من وزن الجسم). المجموعة 6: تم اعطاؤها الفضة النانوية بجرعة (10مج / كجم من وزن الجسم) بالإضافة الي مستخلص الشاى الاخضر. تم إجراء هذا الهدف من خلال اعتماد معايير معينة منها مراقبة الحيوانات والدراسات البيوكيميائية للانزيمات المضادة للأكسدة (فوق أكسيد ديسميوتاز والكاتلاز)، كذلك التغيرات في وزن الجسم، ووزن الرئة المطلق والنسبي. أيضا تم فحص النسيج الرئوى باستخدام اصباغ مختلفة؛ منها صبغة الهيماتوكسيلين والايوسين: كصبغة روتينية ومالوري ثلاثي الألوان، بالإضافة إلي دراسة الكيمياء المناعية للبروتين السطحي ب في الرئة والدراسات الإحصائية.

النتائج : لم يلاحظ أي تدهور في الحالة العامة في أي من المجموعات طوال فترة الدراسة. أظهرت النتائج زيادة ذات دلالة إحصائية في متوسط وزن الجسم في المجموعة الضابطة وجميع المجموعات التي عولجت بالفضة النانوية، في حين كان هناك زيادة غير ملحوظة في المجموعة التي أعطيت مستخلص الشاي الأخضر و المجموعات التى تم اعطاؤها الشاى الاخضر بالإضافة الى الفضة النانوية بالمقارنة مع القيمة الأولية لوزن الجسم. كان هناك انخفاض ذو دلالة إحصائية في مستوى كاتلاز، في حين كانت هناك زيادة ذات دلالة إحصائية في مستوى فوق اكسيد ديسميوتاز في المجموعات التى تم اعطاؤها الفضة النانوية مقارنة بالمجموعة الضابطة. أظهرالوزن النسبي للرئة زيادة ذات دلالة إحصائية في مجموعة الشاى الاخضر في حين وجدت زيادة غير كبيرة في جميع المجموعات التى تم اعطاؤها الفضة النانوية مقارنة بالمجموعة الضابطة. أظهرت نتائج فحص أنسجة الرئة في المجموعات التى تم اعطاؤها الفضة النانوية تغييرات نسيجية متنوعة والتي كانت أكثر وضوحا في الجرعة العالية وتشمل هذه التغيرات زيادة في سمك الجدر المحيطة بالحويصلات الهوائية، تلف واتساع الحويصلات الهوائية مع ارتشاح الخلايا المضادة للالتهابات مصاحب بترسب ألياف الكولاجين وضعف في التعبيرالمناعي الكيميائي للبروتين السطحي ب في الرئة. من ناحية أخرى، المجموعات التى تم اعطاؤها الشاى الاخضر بالإضافة إلي الفضة النانوية أظهرت النتائج تحسنا كبيرا في التغيرات البيوكيميائية للانزيمات المضادة للأكسدة، كذلك النسيجية والمناعية الناتجة عن الفضة النانوية .

الاستنتاج : تسبب جسيمات الفضة النانوية ضررالأكسدة، والتغيرات البيوكيميائية كذلك التغييرات النسيجية في الرئة في ذكورالفئران .وتوضح هذه الدراسة فوائد الشاي الأخضرلأنه يقلل من اضرارالأكسدة بحكم خصائصه المضادة للأكسدة وبالتالي تحسين مستوي مضادات الأكسدة و تحسين سلامة البنية الهيكلية للأنسجة الرئة، وأخيرا يقلل من التغيرات النسيجية الناجمة عن الفضة النانوية.

الكلمات المفتاحية : الفضة النانوية، إنزيمات مضادة للأكسدة ،أنسجة الرئة، علم الأنسجة المناعية، مستخلص الشاي الأخضر، الجرذان.