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The Possible Rescue Effect of Vitamin E or *Silymarin* on Lung Tissue of Male Albino Rats Exposed to Electro-Magnetic Field

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Abstract

Aim of the work: Investigation of the histological and histochemical changes in the lung of male albino rats exposed to mobile phone radiation and the possible protective role of vitamin E or Silymarin.

Material and Methods: The present study was carried out on 36 adult male albino rats (*Sprague dawly*); they were divided equally into 6 groups (C group: control rats; R group: rats exposed to 900 MHz (2h /day) of radiofrequency electromagnetic field (RF-EMF) radiation; S group: positive control rats given Silymarin; E group: positive control rats given Vitamin E; R+S group: rats treated with Silymarin post EMF irradiation and R+E group treated with vitamin E post EMF irradiation).

Results: Rats exposed to mobile phone radiation showed numerous histological and histochemical changes; these changes were ameliorated by using vitamin E or Silymarin. Vitamin E showed anti-damaging effect of lung tissue exposed to mobile phone radiation more than Silymarin.

Conclusion: The present study showed that the natural anti-oxidant Silymarin and vitamin E could protect the lung tissue from the damage produced after the exposure to mobile phone radiation. Vitamin E showed significant anti-damaging effect more than the anti-damaging effect of Silymarin.

Keywords: Mobile Phone, Radiations, Lung, Histology, Histochemistry, Albino rats.

Introduction

Nowadays, the rapid development of new technologies to support our modern life increases the level of electromagnetic fields (EMF) in our environment [1]. The modern electromagnetic (EM) equipment became a frequent source of danger with non-ionizing radiation [2]. All wireless technologies emit electromagnetic field (EMF) radiation that spreads worldwide and affects the human health [3]. Wireless technology includes personal computers, mobile phone, cordless telephone, local TV, and radio communication [4]. There is an increasing concern about the bio-hazard effects of radio frequency (RF)-EMF radiation on human health [5 &6]. The exposure to RF-EMF radiation could interfere with the body's own electromagnetic system and thus producing a variety of biological problems on the tissues and organs; this was previously demonstrated on the brain [2], tissues of the reproductive organs [4], liver [7], spleen [8], kidney [9] and pancreas [10]. With regards the respiratory system, humble studies were done to investigate the probable harmful effect of RF-EMF emitted from mobile phone. At the cellular level, Chinese hamster lung cells (CHLC) exposed to 1800 MHz of RF-EMF radiation showed DNA damage [11]. Also, tight junction proteins (ZO-1, actin and occludin) were significantly decreased in their expression in the lung of rats when exposed to electromagnetic pulse (200 kV/ m, 3h / d) [12]. Further, cancer could indirectly develop after the exposure to EMF of mobile phone radiation. The flow of continuous waves into the cell causes severe damage to the macromolecules that comprise the cell membrane and affects the cell functions; this leads to cancer [6 & 13].

Therefore, researchers are trying to deal with the current problem using natural anti-oxidant agents that can reduce the production of reactive oxygen species generated after the exposure to EMF radiation [14]. Among these agents are vitamin E and Silymarin which are effective anti-oxidant and free radical scavenger agent [14, 15 & 16].

Vitamin E (α-tocopherol) is found in virtually all cell membranes, especially in the inner mitochondrial membrane, the site of the electron-transport system [16]. Vitamin E is a lipid – soluble chain – antioxidant which protects the biological membranes from lipid peroxidation [16]. Silymarin (silybin) is found in the seeds of *Silybum marianum*[15]. Silymarin is natural flavonoids effective in preventing several diseases associated with environmental toxin exposure such as radiation [17].

Thus, the present study aimed to investigate two parameters. The first parameter is to detect the histopathological and histochemical changes in lung tissues exposed to RF-EMF at frequency equals 900 MHz. The second parameter is to

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investigate the possible protective roles of Vitamin E or *Silymarin* after the exposure to RF-EMF using histological and histochemical studies.

Materials and Methods

Experimental animals

In this study, thirty six healthy and active male albino rats (*Sprague dawly*) about 120 grams in body weight were used. The animals were housed in plastic cages under normal temperature, pressure, humidity and good ventilation condition during the whole period of experimentation. The animals were fed on a standard pellet diet and water.

Irradiation technique:

Irradiation was performed by using electromagnetic generator that produces frequency equals 900 MHz (RF-EMF) with constant power density about 1.4mW/ cm^2 (SAR, 1.2 W/kg). This generator was used at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. The animals were irradiated two hours per day, three days a week, for two months **[18].**

Animals were categorized into six main groups; each group consisted of six rats as follows:

1. Group C: this group served as the negative control rats.
2. Group R: rats were exposed to frequency equals 900MHz of RF-EMF/ two hours per day, three days a week, for two months.
3. Group S: positive control rats received orally Silymarin dissolved in distilled water (70mg/kg body weight) three days a week, day after day, for two months
4. Group E: positive control rats received orally vitamin E (60mg/kg body weight dissolved in corn oil) three days a week, day after day, for two months
5. Group R+S: rats were exposed to frequency equals 900MHz of RF-EMF/ two hours per day, for two months radiation. Then, Silymarin was administrated orally at a dose level of 70 mg / kg body weight to the animals of the R+S group three days a week, day after day, for two months **[19]**.
6. Group R+E: rats were exposed to frequency equals 900MHz of RF-EMF/ two hours per day, for two months radiation. Then, irradiated group received orally with vitamin E (60mg/kg body weight) three days a week, day after day, for two months **[20].**

Histological and histochemical studies

The animals of the control and treated groups were sacrificed after two months and the lungs were immediately excised and fixed in 10% neutral formalin. Paraffin sections (5µm in thickness) were prepared for processing the histological and histochemical studies. For general histology, sections were stained with Harris' haematoxylin and eosin **[21].** Total proteins were detected by using mercuric bromophenol blue method **[22]**, polysaccharides were detected by using periodic acid Schiff's (PAS) reagent **[23]**, DNA materials were detected by using Feulgen method **[24]** and collagen fibres were stained by using Mallory's trichrome stain **[24].**

Image and statistical analysis

The mean red color intensity of DNA content in the different groups was measured using image analyzer software. The data was expressed as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA). Statistical significance level was defined at $P< 0.001$.

Results

The histological and histochemical observations of the lung

The control group (C): stained sections of the lung showed normal alveoli with thin alveolar septa, clear alveolar sacs and the bronchioles are lined with columnar epithelial cells and the pulmonary blood vessels are normal in appearance (Fig. 1). Also, lung tissues treated with vitamin E (E group) or Silymarin (S group) showed normal histological structure similar to the lung tissues of the C group.

The irradiated group (R): the lung of rats exposed to mobile phone radiation (900 MHz) for two months showed many pathological changes. The lungs had dilated bronchioles with corrugated walls and thickened epithelial lining (Figs. 2). Also, the lumen of the bronchioles contained degenerated epithelial cells (Figs. 2 & 3). The alveolar septa had congested and thickened walls (Fig. 3). Areas of lymphocytic infiltration were observed around the bronchioles (Fig. 3). The lung arteries of the irradiated animals were dilated and had corrugated walls (Fig. 2). Some alveolar blood vessels were obliterated and some arteries had thickened walls with narrow lumen (Fig. 3). Haemorrhagic areas were observed (Fig. 2).

Lung tissue of male albino rats received Silymarin (at a dose level of 70 mg / kg. body weight) after exposure to 900 MHz mobile phone radiation showed obvious recovery from

the damage produced by the exposure to EMW. However, some blood vessels were still congested and haemolysed blood cells appeared in their lumens (Fig. 4). However, lung tissue of male albino rats received vitamin E (at a dose level of 60 mg / kg body weight) after irradiation with mobile phone (900 MHz) showed somewhat normal architecture (Fig. 5). Many alveoli and bronchioles retained their normal appearance.

Mallory's trichrome stained sections of the lung tissue showed normal distribution of collagen fibres in the alveolar septa and around the alveolar sacs, bronchioles and blood vessels of the control group (Fig. 6). However, Mallory stained sections of the lung tissues of "R" group showed increased collagen fibres in both the fibrotic areas around the arteries and in the tunica intima of thickened walls of the pulmonary arteries (Figs. 7 & 8). Also, high incresed of the collagen fibres was observed around walls of the bronchioles and inside the thickened alveolar septa (Fig. 8). Lung tissues of "R + S" group showed somewhat normal content of the collagen fibres, but congested arteries and some alveolar sacs acquired red coloration indicating fibrosis (Fig. 9). However, lung tissues of "R + E "group showed reduced collagen fibres in some alveolar septae (Fig. 10).

Normal polysaccharides distribution in the lung tissues of the control group was observed in figure "11". Moderate PAS +ve reaction (magenta colour) appeared in the alveolar septa, and in the cytoplasm of epithelium lining of the bronchioles and pulmonary vessels. However, high increase of the PAS +ve materials in the thickened alveolar septa, and the walls of bronchioles and blood vessels were observed in the lung tissue of irradiated group (R) (Figs. 12 & 13). Also, the inflammatory cells infiltration forming areas of granuloma appeared densely stained (Fig. 12). Somewhat normal appearance of PAS + materials was observed in lungs of rats of "R+S" group, but the walls of bronchioles and the congested blood vessels were densely stained (Fig. 14). Normal appearance of PAS + materials was observed in lung tissue of male rats of group "R+E"(Fig. 15).

Normal distribution of total protein of lung tissue of the control group was observed in figure "16". However, increased bromophenol blue staining affinity was detected in the thickened walls of the blood vessels and inside them, alveolar septa and walls of bronchioles of lung tissue of "R" group (Figs.17 &18). Normal architecture of the lung tissue of "R+S" group was observed, but small areas of inflammatory cells infiltration appeared densely stained and debris of degenerated epithelium were moderately stained (Fig. 19). Normal appearance of total protein was observed in lung tissue of rats received vitamin E after irradiation in "R+E" group (Fig. 20).

Normal distribution of DNA was detected in the bronchioles epithelial cells, thin alveolar septa and walls of the blood vessels of the control group (Figs. 21& 22). However, lung tissue of R group stained with Feulgen reaction revealed a significant increase in red color intensity of DNA contents in the thickened walls of arteries, bronchioles, alveolar septa and areas of inflammatory cell infiltration (Figs. 23 a &b and 24) compared to DNA contents of the control group (Table 1 and Fig. 27). Normal distribution of DNA materials was noticed in the alveolar septa in lungs of R+S, but thickened arterial walls showed moderate staining affinity and small areas of inflammatory cell infiltration showed deep staining affinity, the mean red color intensity was (119.3±4.1) (Fig.25and Table 1). Non significant reduction in color intensity was detected in R+S group compared to color value of the R group (Table 1 and Fig. 27). Normal distribution of DNA materials was observed in the lung tissues of R+E group (Fig.26). Mean color intensity value in R+E group showed a significant reduction in DNA contents compared to color value of the R group (Table 1 and Fig. 27).

Discussion

The everyday exposure to radio frequency (RF) - electromagnetic field (EMF) radiation emitted from cell phones and other wireless devices became a worldwide concern about their risk of producing of cancer and other diseases [1, 6 &25]. Microwaves generated from cell phones affect biological systems by modulating the cellular activities of Ca^{+2} channels located on the cell membranes [1], by increasing reactive oxygen species (ROS) which may change anti-oxidative activities leading to DNA damage and apoptosis [26]. The increase in the reactive oxygen species after EMF radiation could also lead to inhibition in cell growth in mice during embryonic development [27], decrease the anti-oxidative activity of superoxide dismutase that

detoxify free radicals [28 & 29].

Many therapeutic studies are aimed to reduce the injuries caused by EMF radiation using natural anti-oxidants that can reduce the production of reactive oxygen species [14, 19, 20 &30]. Thus, this work investigated the role of vitamin E and Silymarin which have antioxidant properties against 900 MHz mobile phone radiation. RF-EMF of 900 MHz radiation selected for this experiment because it is a frequency of the Global System Mobile (GSM) signal modulation used for all mobile communication. [31] In the present study, lung tissue of male albino rats (120 g) exposed to RF-EMF (900 MHz) emitted from mobile phone radiation three days a week, day after day, for two months showed the deleterious effect of radiation. The lung was selected because it is a very sensitive organ that has a vital role during respiration and gases exchange.

Lungs of irradiated rats (R group) showed severe changes when compared to the control group represented in dilated and congested blood vessels with haemolysed blood cells, presence of areas of lymphocytes infiltration around the bronchioles and thickened alveolar septa. Also, the lumen of the bronchioles contained debris of degenerated epithelial cells. These results are in agreement with the findings of **El-Salk** [32] who observed numerous pathological changes in the lung tissue of mice exposed to EMW radiation. These changes include**:** highly thickened arterial walls, dilated bronchioles with degenerated epithelial cells and congested alveolar septa. The exposure to radiofrequency pulse caused severe damage to the lung tissue of rats because of producing high level of toxicity from nitric oxide and lipid peroxidation and suppressing glutathione level, the antioxidant defense mechanism [33].

The present study showed that many alveoli and bronchioles retained their normal appearance in "R+E" and "R+S" groups. This result agreed with numerous studies demonstrating that the antioxidants can protect the cells from radiation by ameliorating the deleterious effects of free radical reactions [34& 35]. Rat endometrium exposed to 900 MHz RF-EMW had severe damages including the presence of apoptotic cells in the endometrial epithelial and glandular cells, and inflammatory cellular infiltration was increased. Whereas, after the treatment with vitamin E and C, the antioxidant, reduced the damage caused by mobile phone radiation [14&34]. The present results revealed increased collagen fibres around walls of the bronchioles and inside the tunica intima of the blood vessels in lung of rats of the irradiated group (R) when compared to the control group. This result is in line with that of **Seyhan and Canseven** [36] who concluded that 50Hz extremely low frequency EMFs (2& 3mT 4hrs/day for 5 days) increased collagen synthesis in Guinea pigs. The increase of collagen fibres observed in the present study might lead to rapid healing as discussed by **Suvik and Effendy**[37] who reported that increased collagen might stimulate cell differentiation causing cell healing. On the other hand, normal distribution of collagen fibres was detected in the lung bronchioles and alveolar septa of the control rats and those treated with Silymarin and Vitamin E. These results could reflect the indirect anti-oxidative role of Silymarin and Vitamin E in reducing the inflammation of lung tissue and the congestion of blood vessels caused by EMW radiation.

Results of the present study showed increased PAS +ve materials in the thickened alveolar septa, walls of bronchioles and walls of the blood vessels of the irradiated group (R) when compared to the control group. The increase in the staining affinity of polysaccharides post-irradiation in this work was previously detected in the lung tissue of pregnant rats and their fetus exposed to 2Gy of gamma rays [38]. They concluded that the increase in the staining affinity due to the increase in the RBCs post-irradiation with gamma rays [38]. The present study showed that the lung tissue of male rats received vitamin E or Silymarin after irradiation restored their normal polysaccharides content due to the ability of both the antioxidants to inhibit free radical formations [34 & 35].

The present results showed that lung of irradiated rats increased the staining affinity of DNA and total protein content around the walls of the bronchioles, alveolar septae, areas with inflammatory cells infiltration and thickned arterials walls when compared to the control group. The exposure to RF-EMF may alter DNA and protein expression as demonstrated in various cell lines [11 &39]. **Zhang*et al.*** [11] found that 1800 MHz (SAR, 3.0 W/kg) for 24h caused DNA damage in Chines hamster lung cells. Similar results was recorded when neuroblastoma cells exposed to 872 MHz radiofrequency radiation[39].Evidence from biochemical studies suggested that EMF radiation can accelerate electron transfer to

interact with the DNA [40]. The initial electron interaction could result in displacing the electrons present in the H bonds holding the DNA together. This could lead to separate the base pairs and DNA chain and initiate transcription and translation [40]. Treating irradiated rats with vitamin E or Silymarin improved the pulmonary tissue architecture. Values of the mean color intensity confirmed the histological results. In this respect **White *et al.*** [41] stated that vitamin E is a lipid soluble concentrated in the hydrophobic interior site of cell membrane and is the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. α-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations [41].

In conclusion, the present histological and histochemical study showed that both Silymarin and vitamin E showed anti-damaging effect of lung tissue exposed to mobile phone radiation. Vitamin E showed significant anti-damaging effect more than Silymarin, but both could be useful natural supplemental remedy for EMF radiation toxic effect. There could be several mechanisms of action of Silymarin and Vitamin E for their effectiveness during irradiation. The antioxidant mechanism seems to be highly significant. However, further studies on their mechanism are needed.

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Figures (1-5) Photomicrographs of lung tissue of the control and treated groups. (H&E X200)

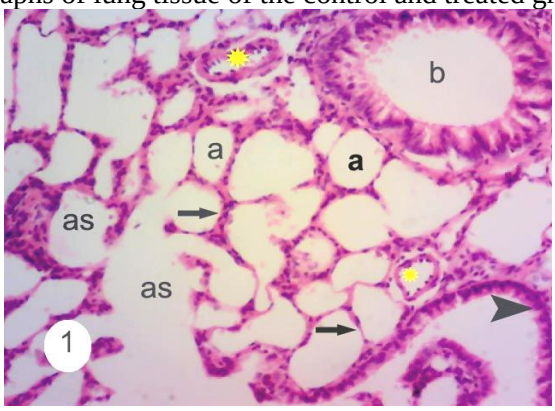


Fig. (1) Lung tissue of the control group shows well developed tissue which contains lung bronchioles (b) with columnar epithelial cells (arrowhead), alveoli (a), alveolar sacs (as), alveolar septa (arrow) and pulmonary vessels (yellow stars).

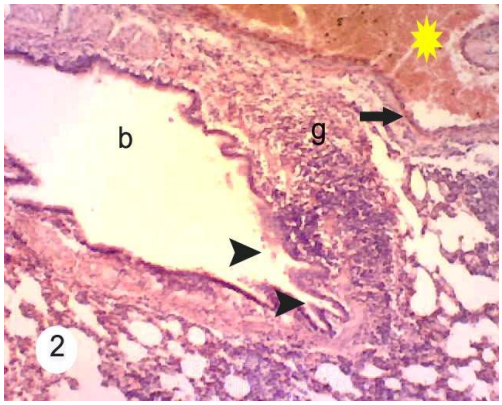


Fig. (2) Lung tissue of the irradiated group (R) shows thickened and corrugated walls of the bronchiole which contains debris of degenerated epithelial cells (arrowhead), presence of inflammatory cells infiltration (arrow) forming granuloma (g) and highly dilated and congested arteries which contain haemolysed blood cells (yellow star).

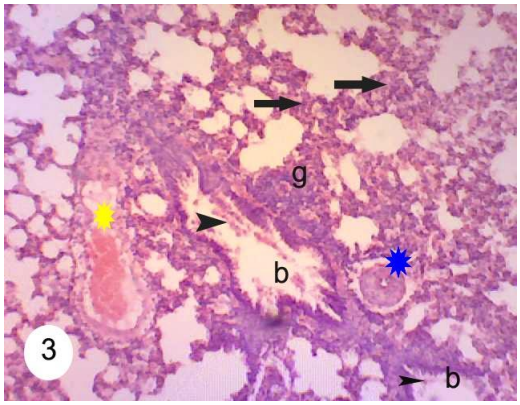


Fig. (3) Lung tissue of the irradiated group (R) shows lumens of the bronchioles (b) with debris of degenerated epithelial cells (arrowhead), presence of granuloma (g), thickened alveolar septa (arrows) and thickened arterial wall with narrow lumen (blue star), and haemolysed blood cells in the lumens of the vein (yellow star).

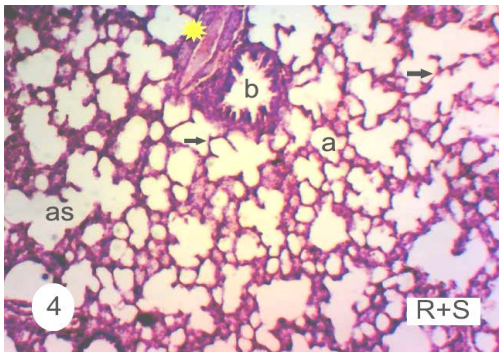


Fig. (4) Lung tissue of R + S group shows normal architecture of lung tissue including bronchioles (b), alveolar sacs (as), thin alveolar septa (arrows) ; but the vein is still congested (yellow star).

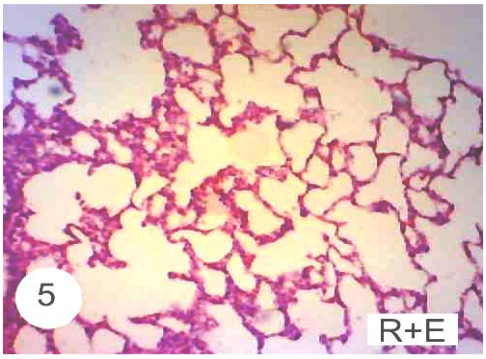


Fig. (5) Lung tissue of R + E group shows normal appearance of lung tissue.

Figures (6 – 10): photomicrographs showing distribution of the collagen fibers in the lung issues of the control and treated groups (Mallory's trichrome stain X 200).

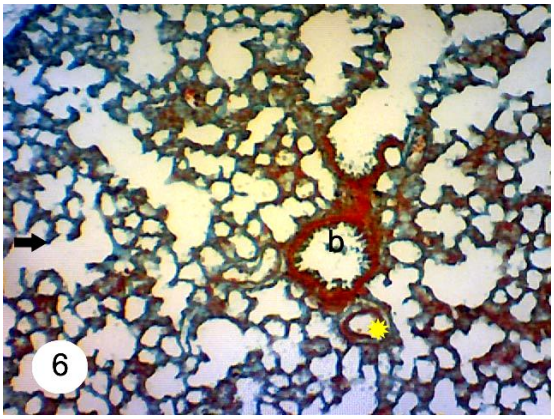


Fig. (6) Normal distribution of collagen fibres in the lung tissue of the control group (C) which support the bronchioles (b), wall of the blood vessel (yellow star) and alveolar septa (arrow).

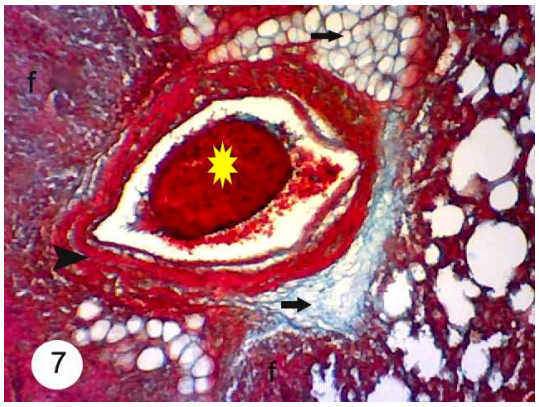
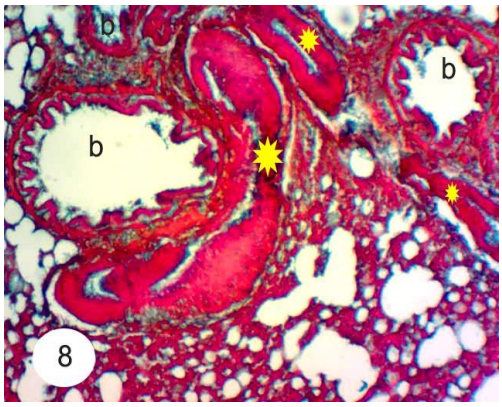


Fig. (7) Lung tissue of the R group shows increased collagen fibres in the tunica intima of the arterial wall (arrowhead), haemolysed RBCs inside the lumen of the artery (star) and areas of fibrosis around them (f). Areas of fatty degeneration appear faintly stained (arrows).



Figs. (8) Lung tissue of the R group shows increased collagen fibres around the thickened walls of the bronchioles (b) and blood vessels (stars). Some alveolar septa are densely stained.

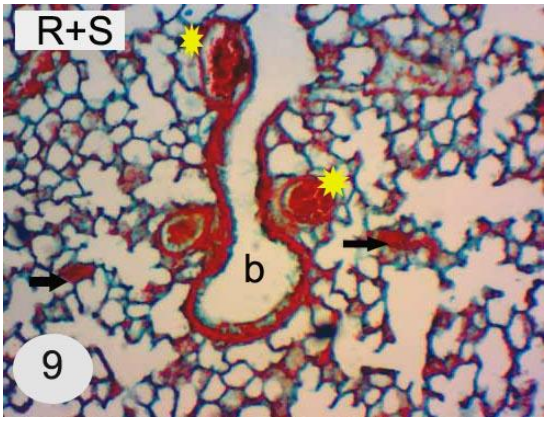


Fig. (9) Shows somewhat normal appearance of collagen fibres in lung of R+S group, but congested arteries (stars) and some alveolar septa (arrows) acquire red colour.

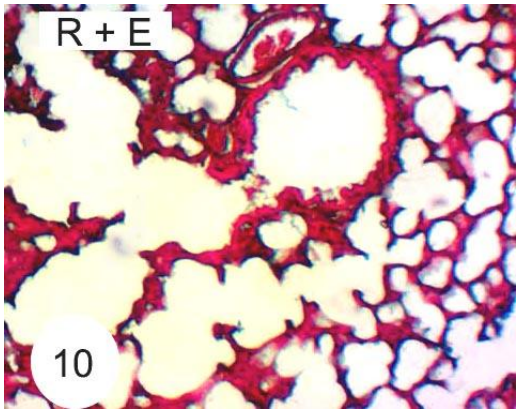


Fig. (10) Shows reduced collagen fibres in some alveolar septa of R+ E group.

Figures (11-15): The photomicrographs show the distribution of PAS +ve materials in the lung tissue of the control and treated groups (stained with PAS X 200).

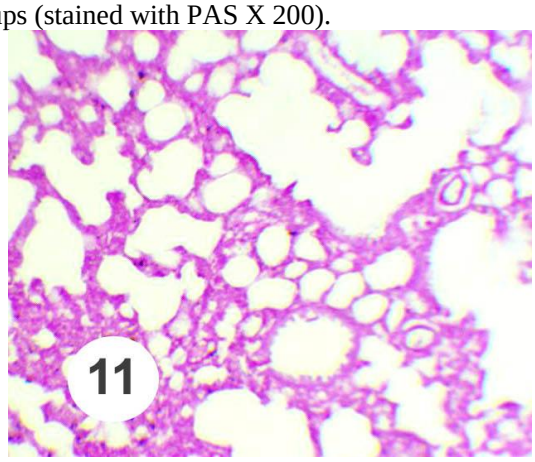


Fig. (11) Normal distribution of PAS + ve materials in the lung tissue of the control group.

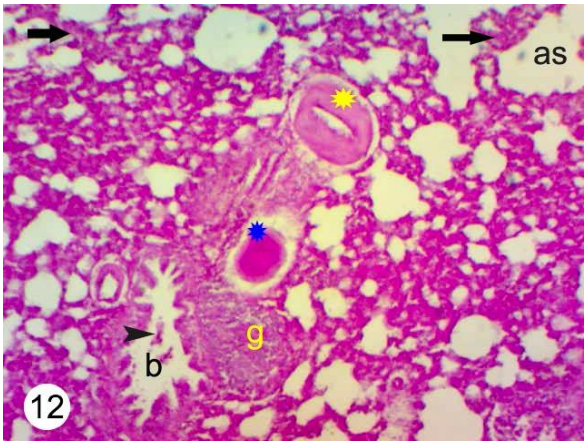


Fig. (12) Shows increased PAS +ve materials in the lung tissue of R group including the thickened alveolar septa (arrows), thickened walls of the bronchioles (b) and walls of the blood vessels (yellow star) with densely stained granuloma areas (g).

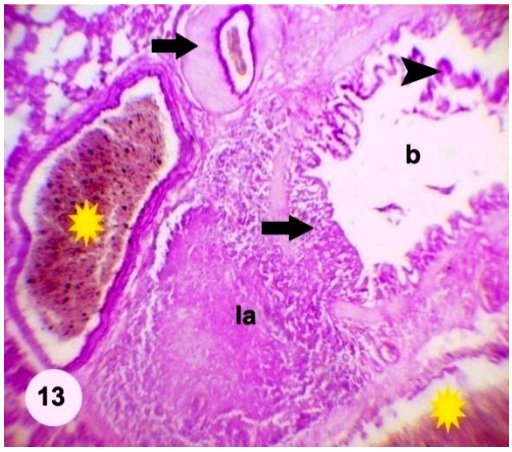


Fig. 13: Shows increased PAS + ve materials in the lung tissue of R group including the thickened walls of the pulmonary blood vessels (star) and walls of the bronchioles (b). Areas of infiltration with inflammatory cells (Ia) forming granuloma appear densely stained.

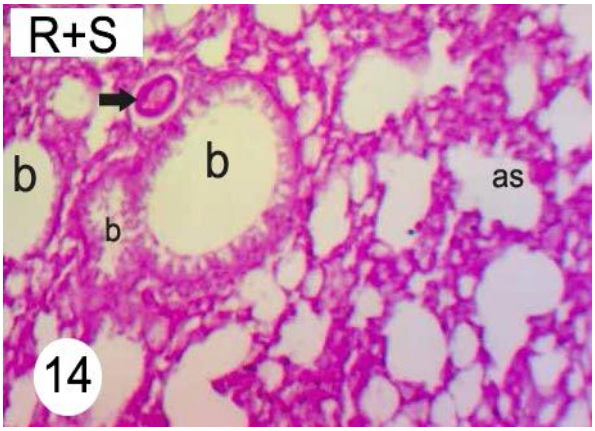


Fig. (14) Somewhat normal appearance of PAS +ve materials within the lung tissue of R + S group, but walls of bronchioles (b) are densely stained, and some congested blood vessel appeared densely stained (arrow).

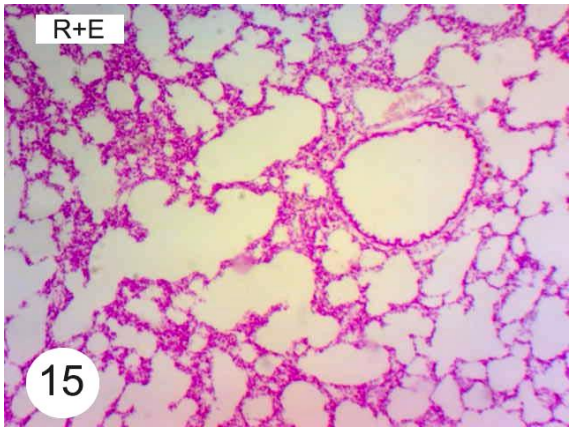


Fig. (15) Normal distribution of PAS +ve materials in the lung tissue of the R+E group

Figures (16-20) Photomicrographs showing total protein distribution in the lung tissues of the control and treated groups (stained with Bromo-phenol blue X200

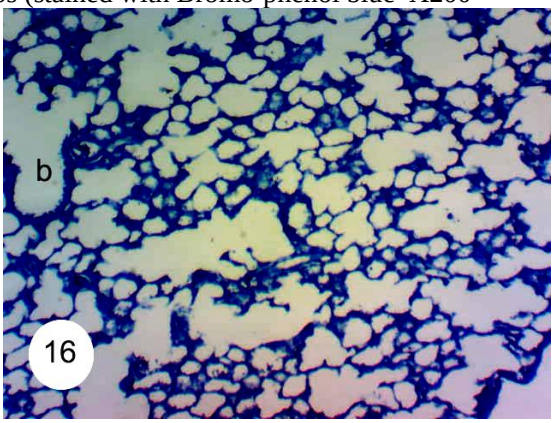
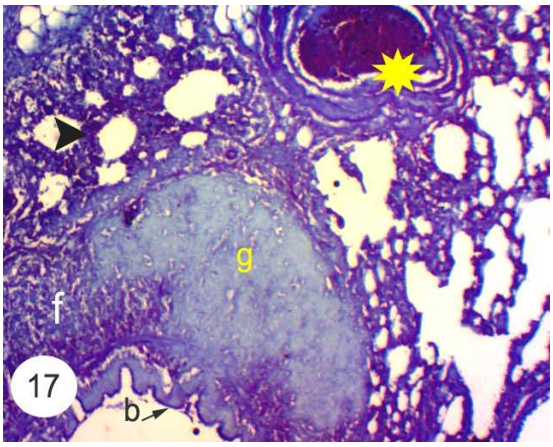
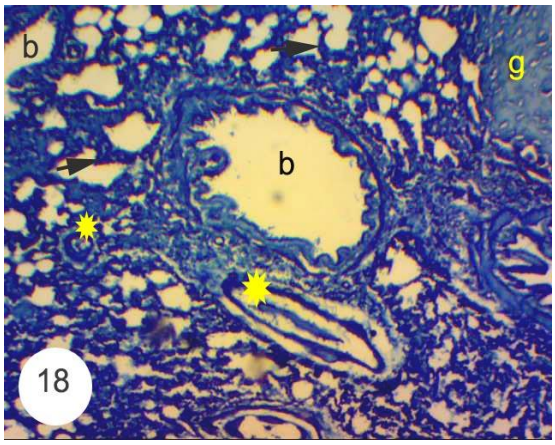


Fig. (16) Normal distribution of total protein in lung tissue of the control group (C).





Figs.(17& 18) shows increased total protein in walls of the bronchioles (b), alveolar septa (arrowhead), granuloma areas (g) and thickened walls of the pulmonary blood vessels and inside them (stars).

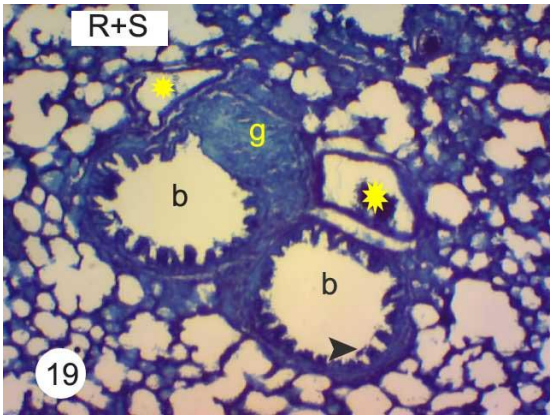


Fig. (19) Somewhat normal protein content in lung tissue of the R+S group. Notice: moderately stained bronchioles (b), degenerated epithelium (arrowhead), pulmonary blood vessels (stars), but small granuloma areas (g) are densely stained.

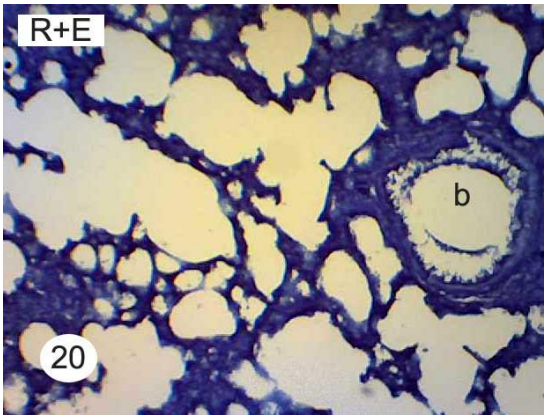
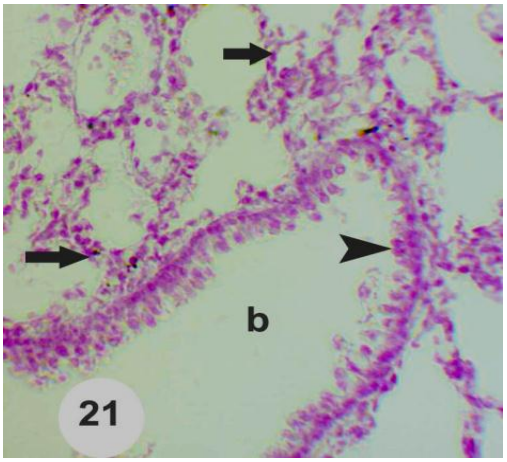
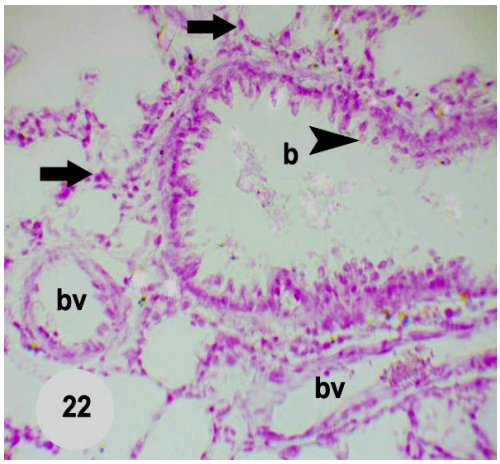


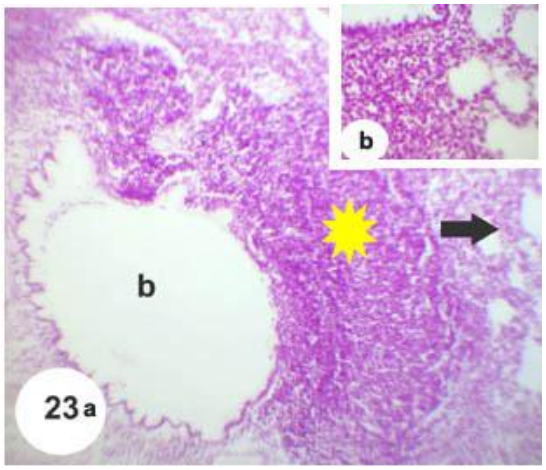
Fig. (20) Normal appearance of total protein in bronchioles (b) and lung tissue of the R+E group.

Figures (21-26) Photomicrographs showing distribution of DNA content in the lung tissues of the control and treated groups (Feulgen reaction X200 & X400).





Figs. (21 & 22) Normal distribution of DNA content in epithelial cells (head arrows) of walls of the bronchioles (b), thin alveolar septa (arrows) and blood vessels (bv) of rats of the control group (21&22 x 200).



Figs. (23a & b) Highly increased DNA content in the thickened, alveolar septa (arrow) and lymphocytic infiltrated areas (star) arround the bronchioles (b) of R group (Figs23a,bx200).

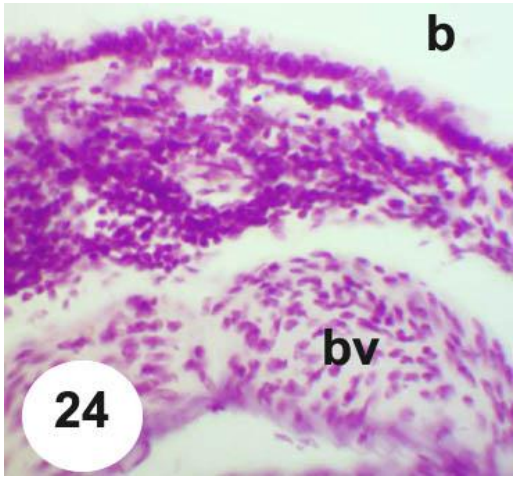


Fig. (24) Highly increased DNA content in the thickened walls of bronchioles (b) and pulmonary blood vesseles (bv) of R group (x400).

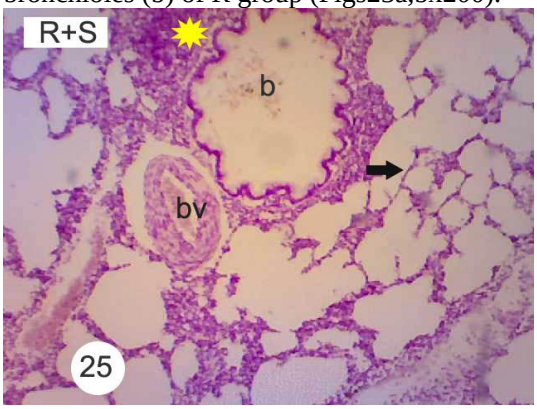


Fig. (25) Normal distribution of DNA content in the alveolar septa (arrow) and bronchioles (b), but the thickened walls of blood vesseles (bv) show moderate staining affinity. Small granuloma areas (star) show deep staining affinity in R+S group (x200).

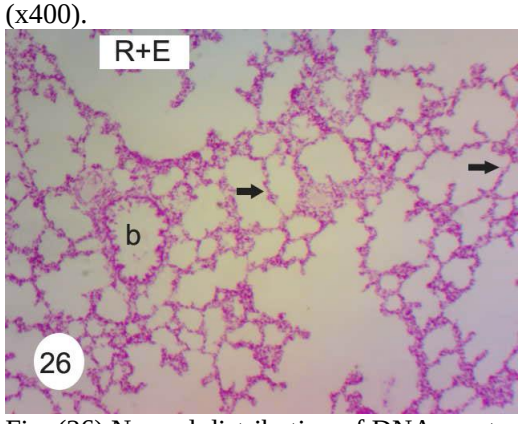


Fig. (26) Normal distribution of DNA content in the bronchioles (b), thin alveolar septa (arrows) in the R+E group (x200).

Table (1): Value of mean color intensity of DNA contents in lungs of the control and treated groups

Groups	Mean Red		ANOVA	
	Range	Mean ± SD	F	P-value
Controls	83.280 - 121.000	104.286 ± 10.290	235.959	<0.001*
R group	99.130 - 127.000	120.908 ± 4.514		
(R+ S) group	109.200 - 127.000	119.380 ± 4.192		
(R+ E) group	82.840 - 117.000	105.066 ± 7.759		

Tukey's test					
Controls & R	**Controls& (R+S)**	**Controls&(R+ E)**	**R group& (R+ S)**	**R group&(R+E)**	**(R+ S) group&(R+E)**
<0.001*	<0.001*	0.781	0.249	<0.001*	<0.001*

Fig. (27) Showing the mean color intensity of DNA contents in lungs of the control and treated groups