

## Protective Effect Of Garlic Against Lead Acetate Toxicity In Lung Alveoli Of Rabbit: Ultrastructural Studies.

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**Abstract:** Lead is a heavy metal which is reported to have toxicological effects on various organs in humans and animals. In the present study, the main goal was to investigate the ultrastructural changes in the lung alveoli of adult male rabbits after long-term exposure to lead acetate and the possible protective antioxidant effect of garlic (*Allium sativum*). **Material and Methods:** Twenty mature New Zealand male rabbits were used and divided into four groups, First group: served as control, Second group: served as the positive control group administered only aqueous garlic extracts. Third group: was orally administered lead acetate dissolved in distilled water at a dose of 15 mg/kg body weight for two months (5 days per week). Fourth group: was orally administered garlic once daily (5 days per week) one hour prior to Lead Acetate administration at a dose of 15 mg/kg body weight for two months (5 day per week). **Results:** The histological changes were observed in the lungs are proliferation and hypertrophy of type II pneumocytes, some of them showed sign of degeneration, infiltration of macrophages and mast cells in the interalveolar septa, and inside the alveoli. Dilatation and congestion of blood capillaries as well as deposition of collagen fibers were observed. Treatment with combination of aqueous garlic extract and lead acetate improving to some extent the structure of lung alveoli as well as ameliorating the toxicity of lead acetate due to oxidative stress and the antioxidant properties of aqueous garlic extract. **Conclusion:** It can be concluded that lead acetate has harmful effect on the lung alveoli of experimental male rabbits. Therefore the present work advise people to prevent exposure to the lead compound to avoid injurious hazard risk.

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### 1. Introduction

Air pollution produces adverse health effects. A complex mixture of gases, chemicals, and particulate matter (PM) is present in the ambient air in polluted urban and industrial areas. Epidemiological studies strongly suggest that children and adults have increased morbidity and mortality from photochemical smog and PM (Woodruff *et al.*, 1997; Abbey *et al.*, 1999).

The consequences of lifelong daily exposures to atmospheric pollutants to the respiratory and cardiovascular apparatus of healthy children are of considerable clinical and epidemiological importance. (Caldero'n-Garciduen̄as *et al.*, 2000).

Lead (Pb) has been one of the most important heavy metals with wide applications for many centuries. Because of its broad industrial usage in the manufacture of batteries, fuel additives, pipes, pigments, solders, shielding, etc., Pb is a common occupational and environmental hazard throughout the world. The toxicity of lead remains a matter of public health concern (Duzgoren-Aydin, 2007) due to its pervasiveness in the environment and the awareness

about its toxic effects (Saleh *et al.*, 2003) at exposure levels lower than what was previously considered harmful (Sandhir *et al.*, 1994).

Domestic and wild animals living in polluted environments represent an important biological source to obtain data useful for assessing risks to human health (Schilderman *et al.*, 1997). Rabbits are often the species of choice as an experimental model for the study of pulmonary responses to long-term exposure to air pollutants (Heyder and Takenaka, 1996). There are multiple similarities between the rabbits and the human lungs in terms of anatomy, patterns of development, pulmonary function, and cellular composition (Kamaruzaman, *et al.*, 2013).

Antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Li *et al.*, 2007). Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells. Increasing the intake of dietary antioxidant is

believed to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system. According to Tepe *et al.* (2005), antioxidants have great importance in terms of preventing oxidative stress that may cause several generative diseases.

Garlic (*Allium sativum* L.) is among the important part of diet in many world populations, and there is also a long-held belief in their health enhancing properties. Among the oldest cultivated plants, garlic is used as food and for medicinal application as they have been proven to convey many benefits to human due to their long storage and portability. One of the advantages of these *Allium* species is that they could be dried and preserved for several months. Garlic, for instance, has been applied as culinary spice and medicinal herb, and it is an important constituent of the traditional Chinese medicine (Tepe *et al.*, 2005).

According to Benkeblia (2005), *Allium* species are revered to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries. During the last 20 years, *Allium* spices have been among the most studied vegetables and aroused great interest.

Garlic (*Allium sativum* L.) is used as a spice and medicinal herb. Most recent research on garlic has used garlic in the form of tablets, flesh, raw, boiled, cooked and dried (Gorinstein *et al.*, 2006). Commercially available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (Elkayam *et al.*, 2003). Garlic exhibits a wide range of properties including immunomodulatory hepatoprotective, antimutagenic and anticarcinogenic effects (Uma *et al.*, 2007). Garlic and garlic extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (Steiner and Li, 2001; Rahman and Lowe, 2006) and modulates lipid metabolism (Steiner and Li, 2001).

Pulmonary toxicity of lead was studied in rats after an intraperitoneal administration of lead acetate at a dose of 25 mg/kg (Kaczynska *et al.* 2011).

In the present study, the main goal was to investigate the ultrastructural changes in the lung alveoli of adult male rabbit after long-term exposure to lead acetate and the possible protective antioxidant effect of garlic.

## 2. Materials and Methods:

**Preparation of garlic extract:** Fresh garlic bulbs (*Allium sativum* L.) were purchased from the local market in AL-Dawadmi, Saudi Arabia. Dried and ground bulbs (about 100g) were submitted to

extraction with 300ml ethanol (80%) in a Soxhlet apparatus for 72 hours. After extraction, the solvent was filtered and made to evaporate by Rota vapor. The obtained garlic alcoholic extract was stored at -20 °C until use. Garlic extract was suspended in distilled water to give 0.4g garlic extract per one ml of the suspension and administered orally through orogastric tube (Khalid, 2009). The volume of administrated extract was 0.4g/kg body weight daily.

**Lead acetate** was purchased from Sigma (Sigma Chemical Co., St. Louis, USA) and dissolved in distilled water.

**Animals:** Twenty mature New Zealand male rabbits were used and maintained in individual wire cages, given limited food and water. Rabbits were reared in the animal house, College of Applied Medical Sciences – AL- Dawadmi. The animals had 2500-3000 gm body weight and were divided into four groups, each of five animals.

**Group I:** served as vehicle control group, was given distilled water once daily for two months (5 day per week).

**Group II:** served as positive control group was given orally aqueous solution of garlic extract only (400mg / kg B.W.) once daily for two months (5 day per week).

**Group III:** served as the first experimental group, was administered lead acetate (LA) solution orally at a dose of 15 mg/kg body wt/day for two months (5 day per week).

**Group IV:** served as the second experimental group received combination of aqueous solution of Garlic orally (400mg / kg B.W.) and lead acetate 15 mg/kg body weight orally for two months (5 day per week).

The aqueous solution of garlic was administered one hour prior to Lead Acetate administration.

The animals were anesthetized with chloroform, the lungs were excised; a small pieces from the lung were taken (1 mm<sup>3</sup>) from each animal and fixed in 5% cold gluteraldehyde for at least 24 hours then washed in 3-4 changes of cacodylate buffer (pH 7.2) for 20 minutes in each change and post fixed in cold osmium tetroxide for 2 hours. The specimens were washed in four changes of cacodylate buffer for 20 minutes for each. Dehydration was done by using ascending grades of ethyl alcohol (30, 50, 70%) each for 2 hours and then 90%, 100% two changes 30 minute each. Embedding was done in Epon 812 using gelatin capsules for polymerization. The embedded samples were kept in incubator at 35°C for one day, at 45°C for another day and for three days at 60°C (Gupta, 1983). Then semi thin sections (0.5-1 microns) were prepared by using LKB ultra microtome. The sections were stained with Toluidine blue, examined with light microscope and photographed. Ultrathin sections (50-80 nm) from selected areas of the trimmed blocks were made and collected on copper grids. The

ultrathin sections were contrasted with uranyl acetate for 10 minutes, lead citrate for 5 minutes. Finally, the sections were examined and photographed by transmission electron microscope (Jeol 100x) in Assiut University- Electron Microscope Unit.

### 3. Results and Discussion:

Many studies were concerned with the antioxidant effect of garlic in various stressful conditions. Less is known about the effects of lead toxicity on the lung and the role of antioxidant garlic in ameliorating these effects.

The histology and Ultrastructural architecture of the lung alveoli of positive control group administered only aqueous solution of garlic extract resemble that observed in the vehicle control group. These findings are in line with the results of Kilikdar *et al.* (2011) who found that the hepatic tissue morphology in aqueous garlic only treated rats resembles that observed in the control rats.

Using semi thin sections stained with Toluidine Blue reveals that the lung alveoli of control rabbits is formed of wide air spaces and the alveolar wall is lined with thin epithelium formed of two types of cells. Type I pneumocytes (squamous alveolar cells) and type II pneumocytes (great alveolar cells). The type I pneumocytes are squamous cells with pale cytoplasm and vesicular oval nuclei (Fig.1). They are few in number. The type II pneumocytes are large rounded or cuboidal cells with large vesicular nuclei and prominent nucleoli. Their cytoplasm contains deeply stained small granules. The blood capillaries are observed in the interalveolar septum and lined with flat endothelial cells. Also macrophages are observed in the interalveolar septa and inside the alveolar lumen (Figs.1 & 2). These results are similar to those observed in animals treated only with garlic. Our results are in agreement with several other studies (El-Nouri, 2009 and Rania,2011).

Ultrastructurally, the alveolar cells of control rabbits reveals that the Type II Pneumocytes are large cells, have few apical short microvilli and their cytoplasm contains large euchromatic nucleus, free ribosome's, mitochondria, RER and few multilamellar bodies (Fig.3). The type I pneumocytes are squamous cells, their cytoplasm is deficient in the cytoplasmic organelles. The air (gas exchange) barrier between blood and alveolar air is extremely thin and consists of thin cytoplasm of a type I pneumocytes, fused basement membrane (epithelial and endothelial basement membranes) and the endothelial cells of the blood capillary (Fig.3). Few collagen fibers and lymphocytes are observed in the interalveolar septum (Fig.3). The alveolar macrophages are observed within the alveolar lumen as well as in the interalveolar septa (Fig. 4). Their cytoplasm contains irregular nucleus, numerous vacuoles, electron dense inclusions and

ingested lamellar bodies. These results are in agreement with the results of Rania (2011) in rat lung.

Treatment with lead acetate for two months caused obvious histological changes in the rabbit lung alveoli. Type II pneumocytes showed proliferation and hypertrophy with pale vacuolated cytoplasm. Desquamated type II pneumocytes and numerous giant macrophages were observed in the air spaces (Fig.5). Alveolar macrophages lie free in alveolar spaces to phagocyte foreign substances that enter pulmonary alveoli and digest it (Bils and Christie, 1980). Also, the observed detached alveolar macrophages seemed to be activated to phagocytose the degenerated pneumocytes. Numerous dilated blood capillaries were observed in some specimens (Fig, 6). Also mast cells with numerous dense granules in their cytoplasm were seen in the interstitial connective tissue (Fig.7). These observations are similar to the results noticed by El-Nouri, (2009) in mice and Rania (2011) in rat, who demonstrated that Alveolar macrophages with large, indented nuclei and many lysosomes were frequently encountered within the alveolar lumen with congested blood capillaries after treatment with lead acetate and amiodarone respectively.

The observed pneumocytes hyperplasia is considered as an early response of the alveolar wall to injury (Adamson and Bowden, 1980). It is suggested that cellular hyperplasia has taken place as a regenerative trial to replace the damaged alveolar cells. Type II pneumocytes are known to be the progenitors of type I cells (membranous pneumocytes) (Masubuchi *et al.*, 1998).

Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules. Therefore, the toxicities associated with these metals might be due to oxidative tissue damage (Mitra *et al.*, 2012).

At the ultrastructural level, in the present study, rabbits exposed to lead acetate revealed significant ultrastructural changes in their lung alveoli including capillaries, interstitium, and alveolar lining layer. The lung alveoli showed an increased number and hypertrophy of Type II pneumocytes (Fig.8), macrophages, (Fig. 11) and fibroblasts in the alveolar walls (Fig. 9). Deposition of collagen fibrils in the alveolar walls was also observed. Accumulation of neutrophils was found within capillaries (Fig.10). Our results are in agreement with the results of (Saud *et al.*, 2004) which observed that exposure of lung alveoli to Arabian incense (Bakhour) led to hyperplasia of type II alveolar cells and neutrophils

were recognized infiltrating pulmonary alveoli and accompanied with degenerative and necrotic changes of the alveolar cells.

The current study presented an evidence that many alveolar capillaries were distended and contained several rows of erythrocytes (Fig.9) reflecting the hyperemia of the pulmonary vasculature. Cytoplasm of the capillary endothelial cells showed surface blebblings of the endothelial cells. Lodged leukocytes, obliterating considerable number of alveolar capillaries, were an outstanding feature (Fig.10). These leukocytes were mostly neutrophils which had electron dense granules which represent lysosomes. In some alveoli, neutrophils that exuded from the distended alveolar capillaries were found free in interalveolar septum. These accumulations of inflammatory cells could be due to the harmful effect of lead acetate to alveoli which lead to acute inflammation reaction (Adamis *et al.*, 1999; Agius, 2001).

The present results are in line with the findings of Rania (2011) who demonstrated that, treatment with amiodarone for 12 weeks in the rats showed alveolar macrophages with large, indented nuclei and many lysosomes were frequently encountered within the alveolar lumen with congested blood capillaries. The interalveolar septa were thickened with cellular infiltration of neutrophils and eosinophils. Other septal cells were also observed.

Fibroblast cell with irregular nucleus and extraordinary increase in the collagen fibers were observed in the interalveolar septum (Fig. 9). Mast cell with numerous large granules in their cytoplasm was also observed in the interstitial connective tissue (Fig.12). The deposited collagen fibrils in the alveolar walls was an evidence of alveolar fibrosis. The increase in the amount of collagen fibers shown in the present study may be a result of intense inflammation symptoms, evidenced as accumulation of macrophages, neutrophil and mast cells, which we observed after lead intoxication. Activated macrophages release profibrotic substances in the process of inflammation (Martinez *et al.*, 2009), as well as mast cells, which via various mediators ~mast cell tryptase! Stimulates proliferation of lung fibroblasts and collagen synthesis (Akers *et al.*, 2000).

The present results are in accord with the results of Kaczynska *et al.*(2011) who demonstrated that the effects of lead toxicity were observed in lung capillaries, interstitium, and alveolar lining layer. Accumulation of aggregated platelets, leucocytic elements and monocytes was found within capillaries. Interstitium comprised a substantial number of collagen, elastin filaments and lipofibroblasts. Pulmonary alveoli were filled with macrophages.

Oxidative stress, induced by heavy metal intoxication may be responsible, at least in part for lead toxic effects and morphological changes (Alcaraz-Contreras *et al.*, 2011). Emphysematous lung alveoli were accompanied with fibrotic interstitium consisting of elastic, collagen fibers, and fibroblasts rebuilding the alveolar septum. Fibrotic damages caused by oral lead acetate exposition was also demonstrated in the renal tissue of rabbits (Bazzy *et al.*, 2012). Another metal, zinc, instilled as a soluble salt to lungs induces focal areas of fibrosis after treatment (Adamson *et al.*, 2000).

Vascular congestion and cellular infiltration of the lung tissue observed in this study could be referred to changes of the vascular integrity of the lung vessels causing disruption of the endothelial barrier and increased capillary permeability evoking an inflammatory response through activation of oxidative stress-sensitive signaling pathways (Adamson *et al.*, 1990).

Lead poisoning is one of the oldest occupational and environmental disease in the world. Lead exerts multisystemic toxic effects through several mechanisms: by inhibiting enzyme activity, sometimes a consequence of binding to sulfhydryl group, also by altering the structure of cell membranes and receptors and by binding with proteins necessary for cellular functions (Kosnett, 2004; Hurst and Martin, 2004).

Our current study revealed that, lead acetate stimulated the formation of new blood vessels. It has previously been shown that some toxic metals like cadmium and arsenic promote angiogenesis (Prozialeck *et al.*, 2008). In pathological states angiogenesis is often induced by inflammation. Inflammatory cells, including macrophages, platelets, mast cells, and other leukocytes are attracted to sites of inflammation and wound healing, in part by angiogenic factors such as vascular endothelial growth factor. Infiltrated cells, in turn, produce angiogenic and arteriogenic growth factors that attract endothelial cells that can contribute to formation of new blood vessels (Carmeliet, 2003). We have shown for the first time that exposure to metal such as lead may contribute to vascular remodeling.

We have tested the efficacy of the aqueous solution of garlic extract against lead acetate-induced changes in the lung alveoli of rabbits. In the present study, Treatment with combination of aqueous garlic extract and lead acetate showed to some extent improvement in the histological and ultrastructure of lung alveoli (Fig. 13) and also showed evident reduction of all alveolar changes.

Ultra structurally, the lung tissue of this group showed a considerable degree of preservation of alveolar architecture. Pneumocytes type II appeared

rounded with euchromatic nuclei with peripheral clumps of heterochromatin and their cytoplasm contained lamellar bodies, mitochondria, RER and vacuoles (Fig.14). These results are in line with the results of Kilikdar *et al.*(2011 & 2013) who found that the damage in the hepatic and myocardial tissue architecture due to treatment with lead acetate was prevented from occurring when the rats were pretreated with the aqueous garlic extracts for the 7 consecutive days.

Combined treatment with lead acetate and aqueous garlic extract led to decrease the deposition of collagen fibers in the interalveolar septum (Fig.14) indicating the protective effect of garlic extract against the toxicity of lead acetate. Jarad (2012) observed that, using garlic as a protective agent with lead acetate in liver and kidney appear nearly like control. Garlic can decrease the damage of liver cells and kidney from oxidative effect induced by lead, and that related to their antioxidant effects.

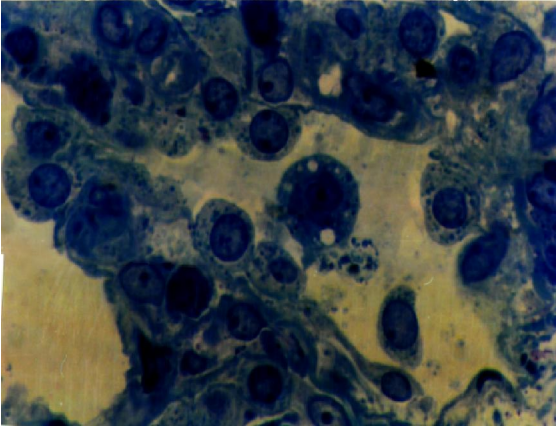


Fig. (1): A semi thin section in the lung alveoli of control rabbit showing; large type II alveolar cells(II) with rounded vesicular nuclei and dense granules inside their cytoplasm. Note: squamous type I alveolar cell(I) and the macrophages inside the alveolar lumen(mac.). (Toluidine Blue Stain X 1000)

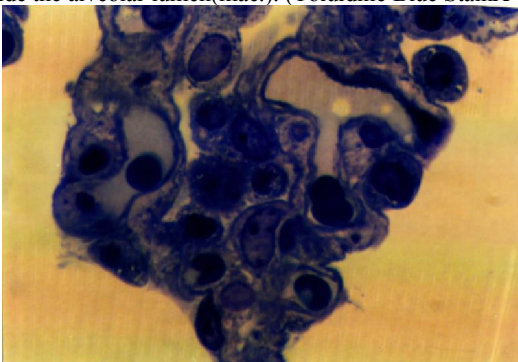


Fig. (2): A semi thin section in the lung alveoli of control rabbit showing; rounded or cuboidal type II alveolar cells(II) with rounded vesicular nuclei and dense granules inside their cytoplasm; type I alveolar cell(I) and blood capillaries(c). Note: macrophages(mac.) in the interalveolar septum.(Toluidine Blue Stain X 1000).

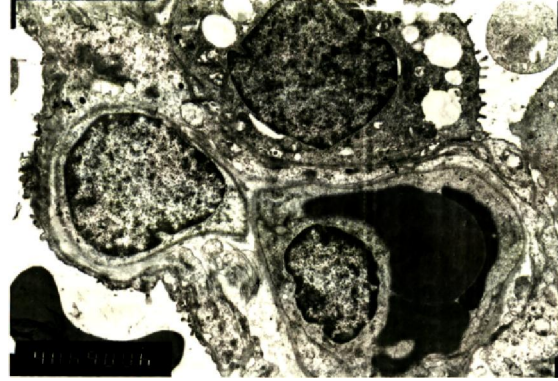


Fig. (3): An electron micrograph of control lung alveolus showing; type II alveolar cell(II) and the blood air barrier(arrow); the type II cell appeared rounded in shape with short apical microvilli(m) and its cytoplasm contained rounded euchromatic nucleus, few multilamellar bodies, vacuoles, mitochondria, RER, and free ribosomes. Note: The blood capillary engorged with RBCs, lymphocytes and few collagen fibers in the interalveolar septum.(X 4000)

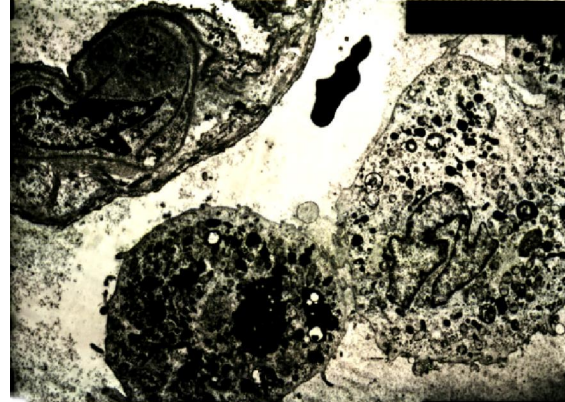


Fig. (4): An electron micrograph of control lung alveolus showing; two macrophages (mac.) in the alveolar lumen. The macrophage cell appears large with irregular cell membrane and their cytoplasm contains irregular nucleus and numerous dense granules. (X 4000)

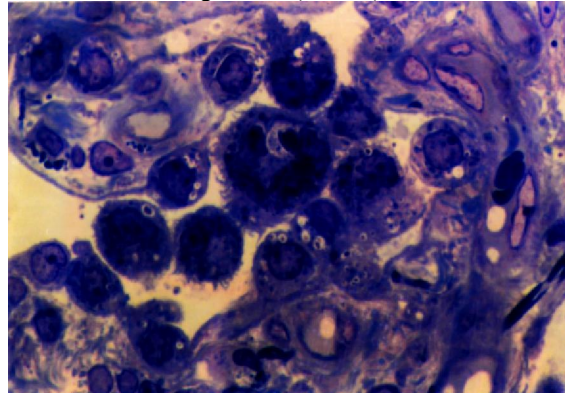


Fig. (5): A semi thin section in the lung alveoli of rabbit treated with lead acetate showing; numerous hypertrophied type II alveolar cells, some of them appeared exfoliated inside the lumen. Note: numerous macrophages (mac.) inside the alveolar lumen and the collagen fibers in the interalveolar septum. (Toluidine Blue Stain X 1000).

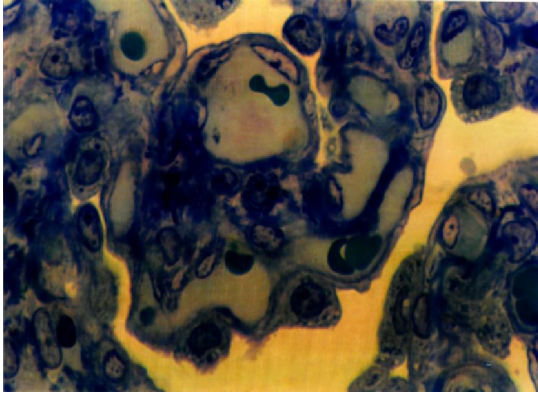


Fig. (6): A semi thin section in the lung alveoli of rabbit treated with lead acetate showing; numerous blood capillaries (bl.c.) Note: Type II and type I alveolar cells (Toluidine Blue Stain X 1000).

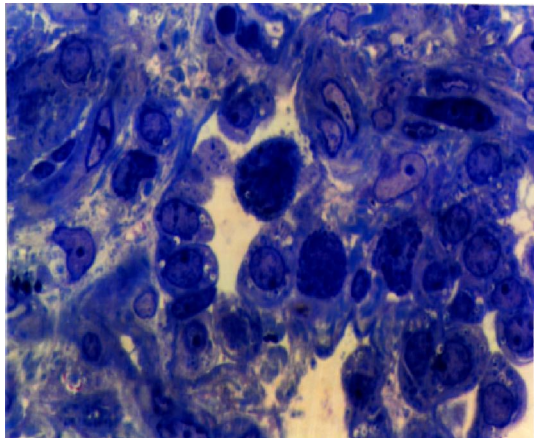


Fig. (7): A semi thin section in the lung alveoli of rabbit treated with lead acetate showing; numerous type II alveolar cells with vacuolated cytoplasm containing dense granules (II). Note: macrophages inside the alveolar lumen (mac.) and mast cells in the interalveolar septum(arrow). (Toluidine Blue Stain X 1000)

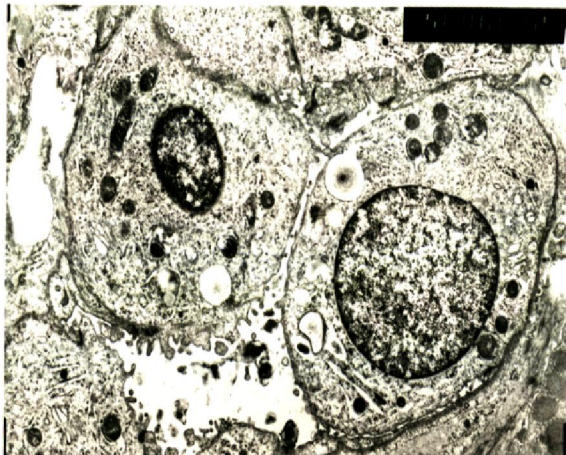


Fig. (8): An electron micrograph of treated lung alveolus showing; two adjacent hypertrophied type II alveolar cells with pyknotic nuclei and electron lucent cytoplasm containing few multilamellar bodies, vacuoles, and dilated RER. (X 4000)

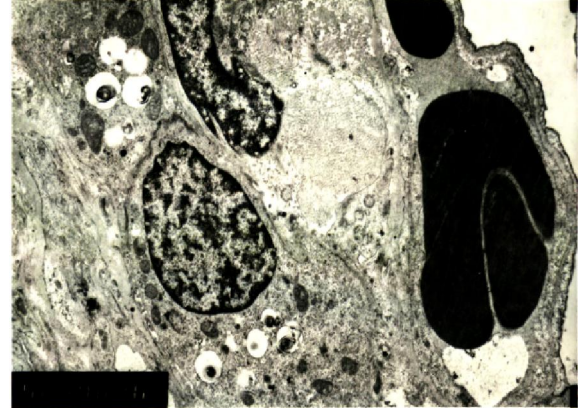


Fig. (9): An electron micrograph of lung alveolus of treated rabbits showing; type II alveolar cell(II) with short apical microvilli(m) and its cytoplasm contained multilamellar bodies, mitochondria,RER, and free ribosomes. Note: The dilated blood capillary congested with RBCs and fibroblast cell (F) with numerous collagen fibers (C.F) in the interalveolar septum. (X 4000)

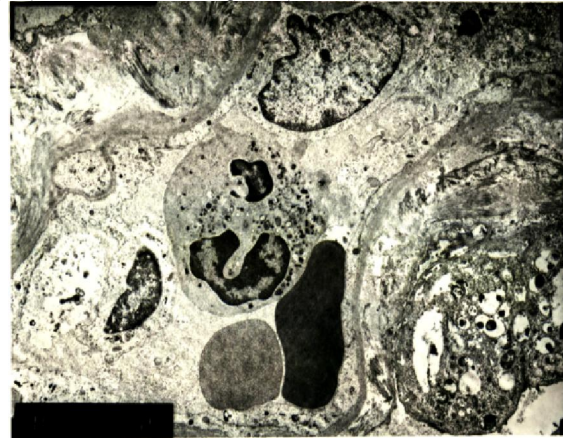


Fig. (10): An electron micrograph of treated lung alveolus showing; part of type II cell and large dilated blood capillary with hypertrophied endothelial cells engorged with neutrophil and RBCs. (X 4000)

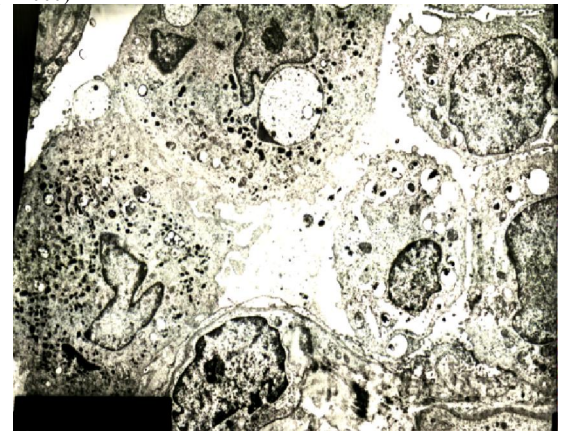


Fig. (11): An electron micrograph of treated lung alveolus showing; two large macrophages (mac.) with irregular cell membrane in the alveolar lumen. Their cytoplasm contained irregular nuclei, numerous dense granules and vacuoles. Note: the exfoliated type II alveolar cell (II) and enlarged type I pneumocyte (I). (X 4000)

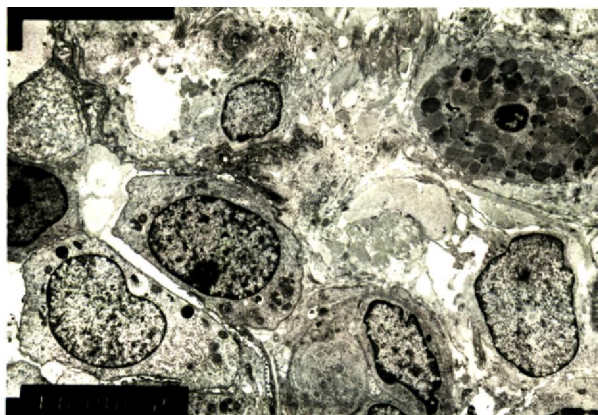


Fig. (12): An electron micrograph of treated lung alveolus showing; two adjacent type II alveolar cells. The interalveolar septum contained mast cell (mast), fibroblast cell with surrounding collagen fibers(CF) and blood capillary. (X 2700)

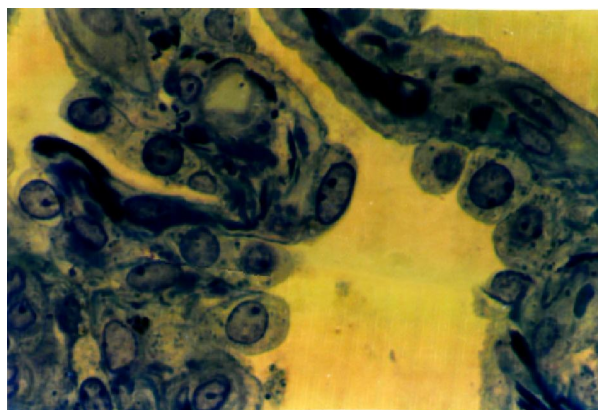


Fig. (13): A semi thin section in the lung alveoli of rabbit treated with lead acetate and garlic showing; type II alveolar cells appeared nearly more or less similar to the control. (Toluidine Blue Stain, X 4000)

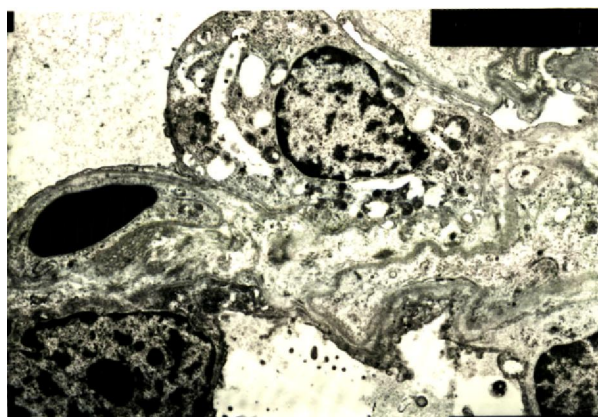


Fig. (14): An electron micrograph of lung alveolus treated with combination of lead acetate and garlic showing; type II alveolar cell appeared more or less similar to the control Note: part of type I alveolar cell (I) and the underlying blood capillary (bl.c.) forming the blood air barrier. (X 4000)

Garlic contains certain compounds such as germanium and selenium that play an important role in normalizing the oxygen utilization in the cells (Hussein *et al.*, 2007).

However, treatment of another set of rabbits with aqueous garlic extract only was found to have no effect on lung alveoli. These results indicate that the extract not only has the ability to protect the tissues in the face of oxidative stress but also do not injure the tissue in control animals as well. This indicating that the aqueous extract may be considered safe for future human consumption (Kilikdar *et al.*, 2011).

#### Conclusion:

It can be concluded that lead acetate has harmful effect on the lung alveoli of experimental male rabbits. Therefore the present work advice people to prevent exposure to the lead compound to avoid injurious hazard risk.

Garlic can decreased the damage of lung alveoli from oxidative stress induced by lead acetate and exhibits the capability to inhibit the metabolic pathway of ROS generation. The results raise the possibility of garlic being considered as one of the component of the regular diet of the people in the areas who may have chances of exposure to lead occupationally or environmentally.

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