A Study of the Effect of Aluminum Chloride on Pneumocyte Type II Cells of Albino Rats and Possible Protective Role of Propolis

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ABSTRACT

Background: Aluminum presents in many manufactured food, tooth paste and medicines. It is also add to the drinking water for purification purposes. However, aluminum has been proved to have toxic effects on many organs of the human body. Some studies suggested that AlCl (aluminum chloride) have detrimental effects on the histology of the lungs of Sprague Dawley rats, which was eminent in the congested blood vessels and hemorrhage. On the other hand, Propolis is a resinous wax-like bee product collected by honey bees from plant exudates and also known as bee glue. In addition, Propolis has also been found to have powerful anti-inflammatory properties and can counteract the damaging effects of aluminium. Moreover, Propolis is the focus of a large number of research projects.

Aim of work: Was to clarify the detailed histological changes in the lung resulting from aluminum toxicity and using propolis as a protective agent in albino rats.

Material and Methods: Thirty adult Sprague Dawley male albino rats, 200-250 gm body weight were used in the study. The animals were divided into three groups, ten rats each: Group I (control group): group Ia (5 rats) received distilled water and group Ib (5 rats) received propolis 50 mg/kg B.W. once daily. Group II (AlCl3 group): received 475mg/kg B.W., of aluminum chloride once daily. Group III (protected group): received 475mg/kg B.W., of aluminum chloride and 50 mg/kg B.W. of Propolis once daily. All the doses were given via oral gavage. After eight weeks, all rats were anaesthetized by thiopental sodium and the thoracic cage was opened and the lungs were taken out. Specimens were processed for both light and transmission electron microscopic examination.

Results: Examination of sections of lung tissue of group I (Ia and Ib) control groups showed almost the same findings, the lung had the appearance of fine lace because most of the tissue was composed of thin-walled alveoli. Examination of semithin sections revealed that the alveolar epithelium was formed of flattened squamous pneumocytes type I with its deeply stained nuclei and scanty cytoplasm. Pneumocytes type II were large irregular cuboidal cells with large rounded nuclei, prominent nucleoli and vacuolated cytoplasm. The ultrathin sections showed the pneumocyte type II with large heterochromatic nucleus and prominent nucleolus. The lungs of group II rats which received aluminum chloride showed diffuse affection. Alveoli were seen collapsed in many areas with thickening of the interalveolar septa. Congestion of the blood vessels together with extravasation of RBCs and heamosiderine granules were observed. Semithin sections showed cellular proliferation especially in pneumocytes type II was seen. Ultrathin sections revealed large areas of degeneration. Some nuclei appeared pyknotic. The cytoplasm lost most of the organelles except few attenuated mitochondria. Examination of the Propolis protected group III showed that administration of propolis minimized the cellular proliferation caused by aluminium. In addition the obliteration of the alveolar lumen seemed less. Semithin sections of this group revealed some congestion of blood vessels and macrophages scattered in between the alveolar cells. Ultrathin sections showed pneumocytes type II with heterochromatic nucleus and prominent nucleoli and regular nuclear membrane and organelles rich cytoplasm. However, some residual degeneration could be detected in some pneumocytes.

Conclusion: Based on the results of the present study, it can be concluded that aluminium chloride exposure had detrimental effects on the histology of the lungs of albino rats which could in turn negatively affect respiration. Therefore, caution should be taken in its usage. Administration of propolis combined with aluminium chloride can alleviate its harmful effects.

Key Words: Aluminum chloride, lung, propolis.

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INTRODUCTION

Aluminum presents in many manufactured food, tooth paste and medicines (Abbasali et al., 2005). It is also added to the drinking water for purification purposes (Newairy et al., 2009). However, aluminum has been proved to have toxic effects on many organs of the human body (cabus et al., 2015). Exposure to aluminum through breathing is significantly influenced by specific activities including industrial exposures (Polizzi et al., 2002) and habitual exposure such as smoking of cigarettes (Exley et al., 2006).

In addition, aluminum is an important component of many aerosol formulations of cosmetics, and particularly antiperspirants, and these, especially through regular use, will contribute significantly to exposure to aluminum through breathing. Topically applied cosmetics and related skin, hair and hygiene products are often significant sources of aluminum (Al-Dayel et al., 2011).

Dougall (2004) added that although aluminum is present in cans, foils, containers, baking powder, cake mixes, frozen dough, pancake mixes, self-rising flour, grains, processed cheese but the healthy human body has effective barriers such as skin, lungs, and gastrointestinal tracts, against aluminum.

The lung, consisting of both conducting and respiratory airways, presents a considerable surface area for interactions with air-borne materials. Both airway and alveolar epithelia are ‘serviced’ by dynamic layers of mucus which may both help in removing aluminum from the lung and offer a substrate for the capture and dissolution of more labile forms of incipient aluminum. The lung epithelia are diverse in respect to their composition of different cell types and, in the alveolar epithelium in particular. The highly dynamic nature of the lung epithelium means that it must be a site for the accumulation of aluminum and a surface for the uptake of aluminum into lung tissues and access to the systemic circulation (Riihimaki & Aitio, 2012).

Buraimoh and Ojo (2013) suggested that AlCl (Aluminum Chloride) have detrimental effects on the histology of the lungs of Wistar rats, which were eminent in the congested blood vessels and hemorrhage.

On the other hand, propolis is a resinous wax-like bee product collected by honey bees from plant exudates and also known as bee glue. Chemical properties of Propolis are not only beneficial to bees but have general pharmacological value as a natural mixture (Orsolic, 2010). Propolis has been used as antioxidant and immune-stimulating (Hendi et al., 2011). Several studies point to the fact that Propolis may be effective against environmental pollutes like Lead (Al-Qayim et al., 2013). In addition, Propolis has also been found to have powerful anti-inflammatory properties and can counteract the damaging effects of aluminium (Mahmoud and Elsoadaa, 2013). Moreover, propolis is the focus of a large number of research projects (Sforcin & Bankova, 2011).

AIM OF WORK

So, it became the aim of the present work to clarify the detailed histological changes in the lung resulting from aluminum toxicity using albino rat as an experimental model. Moreover, a trial to protect the lung from aluminum toxicity was done using propolis.

MATERIAL AND METHODS

Animals:

Thirty adult male albino rats Sprague Dawley weighing 200 – 250 gm were used in the present study and obtained from the Animal House of the Medical Research Centre, Faculty of Medicine, Ain Shams University. All experiments were carried out in accordance with the guide of the Committee of Animal Research Ethics (CARE)-Faculty of Medicine - Ain Shams University. The animals were housed in conventional wire-mesh cages in a room temperature regulated at 21±10c and light/dark cycles (12h). Rats were fed on standard rat diet and allowed free water access. Animals were allowed to acclimatize to experimental conditions by housing them for 10 days prior to experiment.

Drugs:

Aluminum chloride powder was purchased from El Gomhoreya Company and dissolved in distilled water. The dose of aluminum chloride was 475mg/kg B.W. (Buraimoh and Ojo, 2013). Each rat received 1 ml distilled water containing
95 mg aluminium chloride. Propolis was purchased from Emtinan Company as a sticky powder and dissolved in warm distilled water. The dose of propolis used in this experiment was 50 mg/kg (Türkez et al., 2010). Each rat received 1 ml distilled water containing 10 mg propolis.

**Experimental protocol:**

Thirty rats were divided into three groups, ten rats each:

**Group I (control group):** group Ia (5 rats) received distilled water and group Ib (5 rats) received propolis 50 mg/kg B.W. once daily through oral gavage for period of eight weeks.

**Group II (AlCl₃ group):** received 475mg/kg B.W. once daily, of aluminum chloride through oral gavage for period of eight weeks.

**Group III (protected group):** received 475 mg/kg, of aluminum chloride and 50 mg/kg B.W. of Propolis through oral gavage for period of eight weeks.

At the end of the experiment, all rats were anaesthetized by subcutaneous injection of thiopental sodium 25 mg/kg B.W. The thoracic cage was opened and the lungs were taken out. Small pieces from both lungs were excised and fixed in 10% neutral formalin and processed for light microscopic examination. Sections were cut 5µm in thickness and stained with Haematoxylin and Eosin (Bancroft & Gamble, 2002). Other specimens 1mm³ were immediately fixed in 4% glutaraldehyde and processed for transmission electron microscopic examination. Semithin sections were stained with Toluidine blue, while the ultrathin sections were stained with Uranyl Acetate and Lead Citrate (Graham & Orenstein, 2007). The sections were examined and photographed with Philips 201- transmission electron microscope at the Medical Military Academy in Cairo.

**RESULTS**

The Examination of sections of lung tissue of group I (Ia and Ib) control groups showed almost the same findings, the lung had the appearance of fine lace because most of the tissue composed of thin-walled alveoli. Each alveolus was lined by a single layer of squamous epithelium. Between the alveoli, there was a thin layer of connective tissue with numerous blood vessels (interalveolar septum). Terminal bronchioles lined by cuboidal epithelium were seen leading to alveoli and surrounded by few layers of smooth muscle cells (Fig. 1).

Semithin sections revealed more details of the alveolar epithelium which was seen formed of flattened squamous cells in pneumocytes type I, lining almost the whole alveolar surface with its deeply stained nuclei and scanty cytoplasm. Pneumocytes type II lined a part of each alveolus and were large irregular cuboidal cells with large rounded nuclei, prominent nucleoli and vacuolated cytoplasm (Figs 2,3). Alveolar macrophages were seen with its characteristic eccentric kidney shaped nuclei, irregular outline and cytoplasmic granules. In some sections, groups of alveoli clustered around a common air space (alveolar sac) (Fig. 3).

The ultrathin sections showed the pneumocyte type II with large heterochromatic nucleus and prominent nucleolus. The nucleus was surrounded by a considerable amount of cytoplasm rich in organelles including dense matrix mitochondria with prominent cristae (Fig. 4), rough endoplasmic reticulum (Fig. 5). Intracellular lamellar bodies of different shape and size and lysosomes were scattered throughout the cytoplasm (Figs. 4&5).

The lungs of group II rats which received aluminum chloride showed diffuse affection. Alveoli were seen collapsed in many areas with thickening of the interalveolar septa (Fig. 6). In addition, some air passages filled with vacuolated acidophilic exudate could be detected. Some alveoli containing desquamated cells (Fig.7) and other alveoli are interconnected (Fig. 6). Congestion of the blood vessels and dark haemosidrine granules were observed (Figs. 6&8).

Semithin sections revealed loss of the normal alveolar architecture with thickened interalveolar septa and some collapsed alveoli. Cellular proliferation especially in pneumocytes type II was seen (Fig. 9). In addition, some pneumocytes type II cells were seen extruded in the alveolar lumen, while others showed pale nuclei with lost chromatin. Extravasation of RBCs and hemorrhage were also seen in some sections (Fig. 10).
Ultrathin sections revealed large areas of degeneration (Figs 11, 12). Pneumocytes type II showed pyknotic nuclei with peripherally condensed chromatin, irregular nuclear membrane and widened perinuclear space (Fig. 11). The cytoplasm lost most of the organelles except few attenuated mitochondria. (Figs 11, 12). Some lamellar bodies were observed with nearly empty lumen (Fig. 13). Polymorphonuclear leukocyte was seen in some sections (Fig. 12).

Examination of the propolis protected group III showed decrease in the obliteration of the alveolar lumen. The interalveolar septa seemed more or less as the control. No exudation could be seen in the lung sections. However, some residual congestion and desquamated cells could still be detected in some alveoli and some alveoli appeared interconnected (Fig. 13).

Semithin sections of this group revealed interalveolar septa with the same thickness as the control group in some areas, in other areas partially thickened interalveolar septa were observed. Pneumocytes type I were seen more or less similar to the control group with flat nuclei. Pneumocytes type II were irregular cuboidal with large rounded nuclei. Macrophages appeared scattered in between these cells. It was observed that propolis minimized the cellular proliferation caused by aluminum (Figs. 14, 15).

Ultrathin sections showed some details of the control group. Pneumocytes type II appeared with heterochromatic nucleus with regular nuclear membrane (Fig. 16) in some nuclei and slightly irregular nuclear membrane (Fig. 17) in other nuclei. Cytoplasm showed some organelles especially lamellar bodies (Figs 16,17) and mitochondria (Fig. 16). However, some residual degeneration could be detected in some pneumocytes (Fig. 17).
EFFECT OF ALUMINUM CHLORIDE ON PNEUMOCYTE TYPE II CELLS

Fig. 5: Transmission electron micrograph of a section of lung from the control group showing pneumocyte type II containing nucleus (N). Note the mitochondria (m), lysosome (l) and rough endoplasmic reticulum (r). Uranyl acetate and lead citrate; X 10,000.

Fig. 6: Photomicrograph of a section of the lung from the aluminium chloride group showing collapsed alveoli (↑) in many areas with thickening of the interalveolar septa (S). Congestion of the blood vessels (C) together with extravasation of RBCs. Many dark haemosidrine granules are observed. Note some alveoli are interconnected (↑↑). Hx.& E.; x100.

Fig. 7: Photomicrograph of a section of the lung from the aluminium chloride group showing some air passage filled with vacuolated acidophilic exudate (*). Some alveoli (A) containing desquamated cells (↑) Most of the alveoli are collapsed. Hx.& E.; x100.

Fig. 8: Photomicrograph of a section of the lung from the aluminium chloride group showing congestion (C) of the blood vessels. Many dark haemosidrine granules are observed. Hx.& E.; x100.

Fig. 9: Photomicrograph of a semithin section of the lung from the aluminium chloride group showing some collapsed alveoli (A) with thickening of inter alveolar septa (S). Pneumocytes type II (P2) are frequently observed. Toluidine blue; x1000.

Fig. 10: Photomicrograph of a semithin section of the lung from the aluminium chloride group showing some pneumocytes type II cells (P2) are extruded in the alveolar lumen (L), while others show pale nuclei (N) with lost chromat. Extravasation of RBCs (R) and hemorrhage (H) are observed. Toluidine blue; x1000.
Fig. 11: Transmission electron micrograph of a section of lung from the aluminium chloride group showing large areas of degeneration (D). Pneumocytes type II nuclei (N) appear with dark peripherally condensed chromatin (*), highly irregular nuclear membrane (nm). Lamellar bodies appear with nearly empty lumen (↑). Attenuated mitochondria (m). Uranyl acetate and lead citrate; X 8000.

Fig. 12: Transmission electron micrograph of a section of lung from the aluminium chloride group showing polymorphonuclear leukocyte (*) with bilobed nucleus. Few attenuated mitochondria (m) and lamellar bodies (↑) are observed. Note area of degeneration (D). Uranyl acetate and lead citrate; X 10,000.

Fig. 13: Photomicrograph of a section of the lung from the propolis group showing decrease in the obliteration of alveolar lumen (L), with thin interalveolar septa (S). No exudates can be seen. Note some residual congestion (C) and desquamated cells (?) can still be detected in some alveoli. Note some alveoli appear interconnected (*). Hx.& E.; x100.

Fig. 14: Photomicrograph of a semithin section of the lung from the propolis group showing interalveolar septa (S) similar in thickness as the control group in some areas and partially thickened septa (?) in other areas. Pneumocytes type I (P1) are seen more or less similar to the control group with flat nuclei. Pneumocytes type II (P2) are irregular cuboidal with large rounded nuclei. Macrophage (M) is observed. Note pneumocytes type II are less frequently seen. Toluidine blue; x1000.

Fig. 15: Photomicrograph of a semithin section of the lung from the propolis group showing pneumocytes type I (P1) and pneumocytes type II (P2). Note some macrophages (M) are scattered in between these cells. Toluidine blue; x1000.

Fig. 16: Transmission electron micrograph of a section of lung from the propolis group showing pneumocytes type II with its nucleus (N) and cytoplasm containing organelles especially lamellar bodies (?) and mitochondria (m). Uranyl acetate and lead citrate; X 10,000.
**DISCUSSION**

The healthy human body has effective barriers such as skin, lungs, and gastrointestinal tracts, against aluminium. The present work revealed diffuse affection of the lung tissue and loss of the normal alveolar architecture in response to aluminum administration. Riihimäki & Aitio (2012) declared that lung epithelium may be a site for the accumulation of aluminum and a surface for its uptake into lung tissues and access to the systemic circulation. This access could be explained by the fact that both airway and alveolar epithelia are 'serviced' by dynamic layers of mucus. This mucus helps in removing aluminum from the lung and offers a substrate for the capture and dissolution of more labile forms of incipient aluminum.

The current work showed collapsed alveoli and thickened interalveolar septa after aluminium administration. Lassus et al. (2001) and Sahar & Tarek (2013) declared that variation of the alveolar shape between obliteration & dilatation, with thickened alveolar septa were described with severe airway injury, fibrosis and chronic inflammatory lung diseases. John et al. (1997) added that chronic obstructive air way disease is characterized by progressive and irreversible narrowing of airway lumen diameters that develops as a result of varying perturbations in both airway and interstitial lung tissue.

In addition, considerable evidence now links chronic obstructive airway diseases with increased oxidative stress. Aluminum intoxication has been reported to cause oxidative stress and a decrease in the intracellular levels of reduced glutathione (Gonzalez et al., 2007). Al Kahtani (2010) suggested that Aluminum was able to induce oxidative stress in many tissues which is further described by EL-Dermerdash, (2004) who stated that aluminum chloride generates reactive oxygen species, resulting in oxidative deterioration of lipids, proteins and DNA.

In the present study proliferation of pneumocytes type II with pale vacuolated cytoplasm were detected. This was in accordance with Wu et al. (2011) who declared that chronic inflammatory process induces conversion of mesenchymal stem cells into type II pneumocytes, leading to increase their number. Congested blood vessels with extravasations of RBCs and hemorrhage were also seen in some sections in the present work. Buraimoh & Ojo, (2013) as well observed blood vessels engorged with blood in aluminum subjected rats.

In the present study, the cytoplasm of many pneumocytes lost most of its organelles with areas of degeneration. The mitochondrial cristae in these cells exhibited obvious signs of attenuation and the lamellar bodies were nearly empty. This was documented by Majeda & Sawsan (2014) who studied the effects of aluminium on different cellular organelles like mitochondria, endoplasmic reticulum, lysosomes and cell membrane. Saleh et al. (2013) declared that intraperitoneal injection of aluminum chloride caused vacuolization of cellular organelles leading to structural and biochemical changes at the cellular level. Anane & Creppy, (2001) added that aluminum toxicity might lead to the accumulation of calcium in the mitochondria and resulted in irreversible damage to its membrane.

Examination of Propolis group showed preserved lung architecture, decrease of cell proliferation and obliteration of alveolar lumen. The interalveolar septal thickness seemed more or less as that of the control. Some studies declared that propolis has beneficial influences and could be able to antagonize AlCl toxicity (Mahmoud & Elsoadaa, 2013). This was described by Yousef & Salama (2009) who reported that propolis

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**Fig. 17:** Transmission electron micrograph of a section of lung from the propolis group showing heterochromatic nucleus (N) with slightly irregular nuclear membrane (↑) of pneumocytes (P2) type II. Some residual degeneration (D) can still be detected in some pneumocytes. Note lamellar bodies (↑↑). Uranyl acetate and lead citrate; X 10,000.
is an important antioxidant that antagonize aluminum free radical-mediated cytotoxicity. Newairy et al. (2009) added that the antioxidant enzymes were decreased after aluminum chloride administration and then increased after propolis administration. This was further described by Sun et al. (2000) and Newairy et al. (2009) who declared that administration of propolis decreased lipid peroxidation of cellular membrane so it can play a prevention role against the free radical reaction. Other explanation was suggested by Ferrali et al. (1997) who described that the antioxidant activities of propolis and its components are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite.

In the present work, some macrophages were detected scattered in between the cells in propolis group. In that respect Araujo et al. (2012) stated that the polyphenol components derived from propolis activate macrophages and that may be responsible for increasing macrophage capacity to phagocyte and stimulate lymphocytes to kill microorganisms and tumors.

CONCLUSION

Based on the results of the present study, it can be concluded that aluminum chloride exposure had detrimental effects on the histology of the lungs of albino rats which could in turn negatively affect respiration. Therefore, caution should be taken in its usage. Administration of propolis combined with aluminum chloride can alleviate its harmful effects.

REFERENCES


دراسة لتأثير مادة كلوريد الألومنيوم على النوع الثاني من الخلايا الرئوية في الجرذان البيضاء والدور الوقائي المحتمل لصمغ النحل (البروبوليس)

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ملخص البحث

توجد الألومنيوم في العديد من المواد الغذائية المصنعة، ومعجون الأسنان والأدوية، كما يتم إضافته أيضا إلى مياه الشرب لأغراض التنقية. وقد ثبت أن الألومنيوم له آثار سامة على العديد من أجهزة الجسم البشري. وتشير الدراسات إلى أن كلوريد الألومنيوم له ضرر على تأكسد العروق والنزيف. من ناحية أخرى، فإن البروبوليس هو مادة شمعية، يجمعها النحل من الافرازات النباتية ويدعوها (صمغ العسل). وقد أوضح المؤلفون أن البروبوليس له خصائص قوية مضادة لالتهابات كما يمكن أن يخفف من الآثار الضارة الناتجة عن الألومنيوم.

هدفمن العمل: يتضمن الهدف من البحث تحديد التغييرات السجية في رئة الجرذان البيضاء الناجمة عن سمية كلوريد الألومنيوم وتفحص مدى كفاءة حماية الرئة باستخدام البروبوليس.

مواد والطرق المستخدمة: استخدمت ثلاثي من ذكور الجرذان البيضاء البالغة التي تزن 200-250 جرام. تم تقسيم الجرذان إلى ثلاثة مجموعات: مجموعة الضابطة، المجموعة التي تلقت الماء المقطر، وخمسة جرذان تلقوا كلوريد الألومنيوم لكل كجم من وزن الجسم. المجموعة الثانية، المجموعة التي تلقت كلوريد الألومنيوم لكل كجم من وزن الجسم. المجموعة الثالثة، المجموعة التي تلقوا البروبوليس لكل كجم من وزن الجسم. تم استخدام البروبوليس في مراحل مختلفة من تضخيم الحويصلات الهوائية، وتشير النتائج إلى أن البروبوليس يمكن أن يخفف من آثار الألومنيوم الضارة.

النتائج: أظهرت الفحوصات أن التمتص البالغة التي تلقت كلوريد الألومنيوم تسبب تغيرات سجية في رئة الجرذان، بما في ذلك قصر الحويصلات الهوائية، وارتفاع نسبة الخلايا الرئوية النوع الثاني، وانخفاض نسبة الخلايا الرئوية النوع الأول. وعند استخدام البروبوليس، تم الحفاظ على البنية الطبيعية للرئة وانخفاض نسبة الخلايا الرئوية النوع الثاني، وارتفاع نسبة الخلايا الرئوية النوع الأول.

الاستنتاج: يظهر أن استخدام كلوريد الألومنيوم يمكن أن يسبب آثارا ضارة على أنسجة الرئتين في الجرذان البيضاء، ولكن البروبوليس يمكن أن يحمي هذه الأنسجة من هذه الآثار.