Fumonisin Lung Toxicity: Gross and Microscopic Changes are Dose and Time Dependent

Gamal Abdel Salam[1], Esam Mehlab [1] and Mohamed El-Shishtawy[2]

[1]Department of Anatomy, Faculty of Medicine, Benha University
[2]Department of Forensic Medicine & Clinical Toxicology, Faculty of Medicine, Benha University
G_Abdelsalam@yahoo.com

Abstract: Objectives: To investigate the toxic insult of chronic fumonisin 1b (FB1) exposure on rats' lung using light (LM) and electron microscopic (EM) examination. **Materials and Methods:** The study comprised 60 normal healthy growing male albino rats divided into Control group (n=15) and four study groups. Two ratios of added *Fusarium* culture material were used: 10 mg/kg b.wt. of feed for Study group I and 30 mg/kg b.wt. of feed as a study heavy dose for Study groups II-IV. Mean animal weight was determined weekly and percentage of weight gain in relation to baseline weight was calculated. Five rates were randomly selected from each group to be sacrificed at 1, 4 and 8 weeks after start of study regimen. At necropsy, the wet lung weight was determined and lung/body weight ratio was calculated. The lungs were also evaluated grossly for evidence of pulmonary edema and specimens were examined using LM and EM. **Results:** Percentage of body weight gain in study groups III and IV showed significantly lower percentage of weight gain compared both to control group and to study groups I and II with significantly decreased weight gain in group IV compared to group III. Mean lung wet-weight to body weight ratio was significantly increased in study groups III and IV compared to control and study groups I and II. LM examination of lung specimens obtained from study groups showed a progressive dose and time-dependent affection of the lung in the form of mild pulmonary congestion and alveolar edema, focal areas of interstitial edema, pulmonary congestion with inflammatory cellular infiltration at 1-week. Specimens taken 4-w after starting the study, showed moderate interstitial edema, scattered areas of hemorrhage, proliferation of alveolar cells and thickening of the capillary wall. At 8 weeks lung specimens showed proliferation of alveolar lining cells with inflammatory cellular infiltration, alveolar septal edema and scattered areas of compensatory emphysema. Two specimens obtained from animals received high dose for 8 weeks showed scattered areas of atypia suggestive of starting malignant transformation giving a picture of well differentiated carcinoma. EM examination of specimens taken after 4-weeks showed thickening of the interalveolar septa and hemorrhage and interstitial fibrosis and increased collagen fiber deposition and after 8-weeks EM examination revealed endothelial cell damage and distortion of alveolar epithelium and showed increased alveolar macrophages with apoptotic changes in the form of nuclear fragmentation. **Conclusion:** Exposure to fumonisins induced variable gross and microscopic effects on lung tissue and severity and character of these pathological changes showed dose and time dependency.
[Gamal Abdel Salam, Esam Mehlab and Mohamed El-Shishtawy. **Fumonisin Lung Toxicity: Gross and Microscopic Changes are Dose and Time Dependent.** *J Am Sci* 2012;8(9):729-736]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 100

Keywords: Fumonisin, Rats, Lung toxicity, Light microscope, Electron Microscope

1. Introduction

The alveolar wall is very thin (25 nm) and formed by squamous epithelium (type I cells) covered by a thin film of surfactant fluid rich in hydrophilic phospholipid produced by type II cells (septal cells). This surfactant fluid keeps the alveoli open by reducing the surface tension of the interface between opposing alveolar surfaces, which reflects into reduced inspiratory work. The respiratory epithelium is composed mainly of type I cells (98%), along with some type II cells. The basal lamina is in intimate contact with the capillaries from the pulmonary vascular system, favoring the transfer of oxygen to the red blood cells and the release and transfer of carbon dioxide to the alveolar airway **(Sulkowska *et al.*, 1996)**.

Ultrastructurally, the epithelium of primary and secondary bronchioles consisted of four cell types: basal cells, intermediate cells, ciliated cells, and non-ciliated (Clara) cells, whereas the epithelium of terminal and respiratory bronchioles consisted of only two kind of cells: ciliated and Clara cells. The alveolar wall, including alveolar ducts and saccules, was covered by type I and type II pneumocytes **(Bouljihad & Leipold, 1994)**. In addition, the presence of pulmonary intravascular macrophages was a constant in most of the alveolar capillaries. Ciliated epithelial cells, goblet cells, microvillous cells, Clara cells, alveolar epithelial cells of type 1 and type 2, and alveolar macrophages could be distinguished by their universally characteristic surface morphologies **(Saari, 1995)**.

Fumonisins are environmental toxins produced mainly by the molds *Fusarium moniliforme* (*F. verticillioides*), *F. proliferatum*, and several other *Fusarium* species that grow on agricultural commodities in the field or during storage **(Thiel *et al.*, 1992)**. Fusarium moniliforme is one of the predominant fungi associated with corn intended for human and animal consumption world-wide. Fumonisins, food-borne carcinogens that occur naturally in corn, were first isolated and chemically characterized in South Africa in 1988. The major metabolite, fumonisin B1 (FB1), was subsequently shown to cause leukoencephalomalacia in horses, pulmonary edema syndrome in pigs, and liver cancer in rats. Fumonisin B1 is also a cancer promoter and initiator in rat liver; hepatotoxic to horses, pigs, rats, and monkeys; cytotoxic to mammalian cell cultures; and phytotoxic to several plants. Fumonisins in home-grown corn have been associated with an elevated risk for human esophageal cancer **(Marasas, 1995)**.

There is a close structural similarity between fumonisin and sphingosine which is a principal constituent of cell biological membranes, and fumonisins are the first known naturally occurring inhibitors of sphingolipid biosynthesis. The natural occurrence of FB1, together with FB2 and FB3, has been reported in commercial corn and/or corn-based feeds and foods from multiple countries allover the world, so it is imperative that safe levels of fumonisins in human foods and animal feeds should be determined and realistic tolerance levels established as soon as possible **(Tardieu *et al.*, 2007)**.

The magnitude of the problem is that in many low-income countries mycotoxins affect staple foods, including groundnuts (peanuts), maize (corn), other cereals and nuts, such that exposure is continuous and often at high levels. It is in these same regions that agricultural practices and regulation to control human exposure to mycotoxins are the least adapted to do so. Another point of view is the occupational exposure during dealing with infected plants during field work which constitute a heavily exposure promoted through the respiratory tract which allows direct exposure and rapid absorption providing widespread organ affection **(CAST, 2003, Wild & Gong, 2010)**.

The current prospective experimental study aimed to investigate the toxic insult of chronic fumonisin exposure on rats' lung using light and electron microscopic examination.

2. Material and Methods

Animals

The study comprised 60 normal healthy growing male albino rats, weighing 100-200 gm. Rats were purchased from the laboratories of Ministry of Agriculture, and kept under standard conditions, temperature 20^{o}C, humidity 60% and 12-hs day/night cycle, and maintained on standard diet and free water supply till the start of study regimens.

Fumonisin

Fumonisins were produced by a toxigenic strain of *Fusarium moniliforme* (MRC 286) obtained from the laboratories of Ministry of Agriculture. Corn cultures of the fungus were prepared and incubated in a stove for 5 weeks at 25 °C. Cultures were dried at 50 °C for 12 h, ground to a powder, assayed for fumonisins, and stored at −18 °C. A ration was prepared from corn and *Fusarium* culture material containing 4.85 mg of FB_1/kg was added to feeds to achieve the desired concentrations of FB_1, never exceeding 0.62% of the basal diet. Two ratios of added *Fusarium* culture material were used: 10 mg/kg of feed for Study group I, a dose that provide the concentrations of FB_1 considered safe for pigs by **Ross, (1991)** and 30 mg/kg of feed as a study heavy dose for Study groups II-IV.

Study Protocol

The animals were divided into the following groups (each in a separate cage) according to diet regimen used:

1. Control group included 15 rats kept on normal diet without any supplements.
2. Study group I included 15 rats which were kept on the diet containing *Fusarium* culture in concentration of 10 mg/kg of feed.
3. Study group II included 5 rats which were kept on the diet containing *Fusarium* culture in concentration of 30 mg/kg of feed for one week.
4. Study group III included 10 rats which were kept on the diet containing *Fusarium* culture in concentration of 30 mg/kg of feed for four weeks.
5. Study group IV included 15 rats which were kept on the diet containing *Fusarium* culture in concentration of 30 mg/kg of feed for eight week.

Animals were observed twice daily and food consumption was monitored, and the mean animal weight was determined weekly and percentage of weight gain in relation to baseline weight was calculated as follows: [(Final weight-baseline weight)/(baseline weight))x(100)]. Five rates were randomly selected from each group to be sacrificed at 1, 4 and 8 weeks after start of study regimen. At necropsy, the wet lung weight was determined and lung/body weight ratio was calculated. The lungs were also evaluated grossly for evidence of

pulmonary edema.

For light microscopic examination, lung specimen were obtained, fixed in 10% buffered formalin, (pH 7.8) and, then thin sections (4 µm) were stained with hematoxilin-eosin (HE) and examined by light microscopy. Specimens for electron microscopic examination were immersed in 2.5% gluteraldehyde buffered with 0.1 M phosphate buffer for 2 hours at room temperature, then post-fixed in 1% osmium tetroxide for 2 hours at 4°C. After fixation, dehydration with ascending grades of ethanol was performed; specimens were cleared in propylin oxide, embedded in epoxy resin and sectioned with ultramicrotome **(Hayat, 1989)**.

3. Results

Gross appearance changes

Percentage of body weight gain in study groups I and II was non-significantly ($p>0.05$) lower compared to control group. However, rats included in study groups III and IV showed significantly ($p<0.05$) lower percentage of weight gain compared both to control group and to study groups I and II with significantly ($p<0.05$) failure to thrive in group IV compared to group III, (Table 1, Fig. 1).

The mean lung wet-weight to body weight was increased in all FB_1-treated rats, irrespective of dose used or duration of exposure, compared to control ratio. Moreover, mean lung/body weight ratio was significantly higher in study groups III and IV compared to control and study groups I and II. However, the difference between study groups I and II was non-significant and similarly, the difference between study groups III and IV was also non-significant (Fig. 2).

Table (1): Body weight and percentage of weight gain in the studied rats categorized according to time of scarification among groups

		Control	Study groups			
			I	II	III	IV
Baseline		161.7±10.8	163.2±10.5	160±14.3	168±9.6	162.7±12.4
1-week	Weight	167.5±10.6	168.2±10.3	164.7±14.3	170.9±9.6	163.2±12.3
	% of change	3.7±2	3.1±1.7	3.1±1.9	1.8±0.3*†‡	1.55±0.5*†‡
4-week	Weight	169.1±13.3	168.1±12.3		174.2±9.4	164.1±12.7
	% of change	5.1±3.2	4.3±2.7		2.8±0.6*†	1.85±0.5*†#
8-week	Weight	172.2±13.6	170.6±12.3			165.9±10.7
	% of change	8.5±4.9	7.2±4.1			2±0.3*†

*: Significant versus control group
†: Significant versus Study group I
‡: Significant versus Study group II
#: Significant versus Study group III

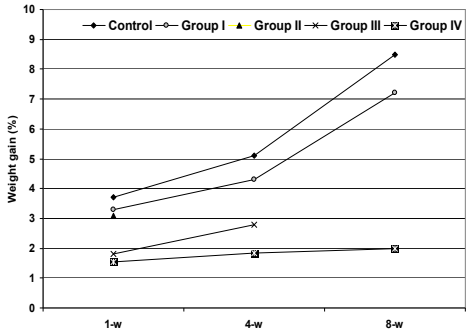

Fig. (1): Percentage of weight gain determined in study groups compared to control group

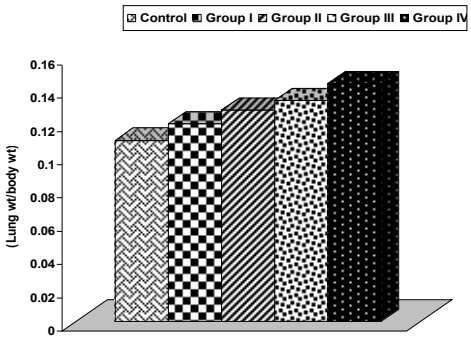

Fig.(2): Mean lung weight/body weight ratio in the studied groups

LM microscopic appearance

LM examination of lung specimens obtained from control animals showed no pathological changes. The alveolar walls are thin and delicate. The alveoli are well-aerated and contain only an occasional pulmonary macrophage (type II pneumonocyte), (Fig. 3).

Microscopic examination of lung specimens obtained from study groups' animals showed a progressive dose and time-dependent affection of the lung. In specimens taken 1-w after dieting regimen, there were mild pulmonary congestion and alveolar edema, (Fig. 4) and in other specimens, there were focal areas of interstitial edema, pulmonary congestion with inflammatory cellular infiltration

(Fig. 5). Specimens taken 4-w after starting the study, showed moderate interstitial edema, scattered areas of hemorrhage, proliferation of alveolar cells and thickening of the capillary wall, (Fig. 6). However, the effect was more manifest in the animal treated for 4 weeks, some specimens showed moderate interstitial edema, areas of hemorrhage, proliferation of alveolar cells, pulmonary venous congestion and thickening of the vessels, (Fig. 7). At 8 weeks after starting dieting regimen, the obtained lung specimens showed proliferation of alveolar lining cells with inflammatory cellular infiltration, alveolar septal edema and scattered areas of compensatory emphysema, (Fig. 8). Unfortunately, two specimens obtained from animals received high dose for 8 weeks showed scattered areas of atypia suggestive of starting malignant transformation giving a picture of well differentiated carcinoma, (Figs. 9 & 10).

Ultrastructural EM appearance

On contrast to normal EM appearance of alveoli detected in specimens harvested from control animals, (Fig.11), EM examination showed normal alveolar macrophages with highly vacuolated cytoplasm in specimens obtained after 1-w of starting the study, (Fig. 12), while in specimens taken after 4-w EM showed thickening of the interalveolar septa and hemorrhage, (Fig. 13) and interstitial fibrosis and increased collagen fiber deposition, (Fig. 14) and in specimen taken 8-w after start of study EM examination revealed endothelial cell damage and distortion of alveolar epithelium, (Fig. 15) and showed increased alveolar macrophages with apoptotic changes in the form of fragmentation of the nuclei in other specimens, (Fig. 16).

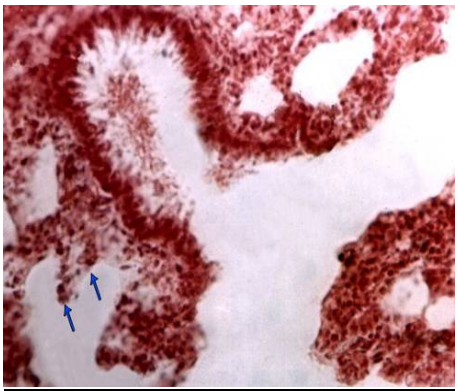

Fig. (3): A photograph of lung tissue of control animals showing no pathological changes with normal alveolar septa. The alveoli are well-aerated and contain only an occasional pulmonary macrophage (type II pneumonocyte), (arrows), (Hx & E, x200).

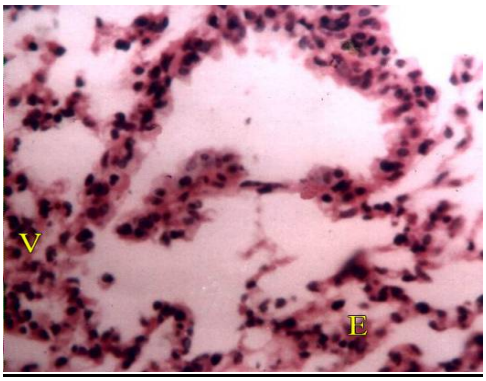

Fig. (4): A photograph of lung tissue of study animals (1-w after starting study) showing mild pulmonary vascular (V) congestion and alveolar edema (E) (Hx & E, x400).

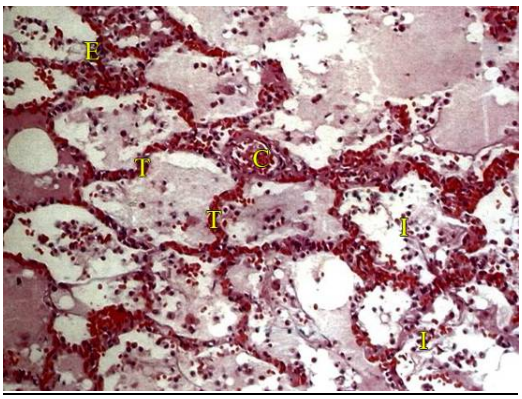

Fig. (5): A photograph of lung tissue of study animals (1-w after starting study) showing focal areas of interstitial edema (E) and thickened alveolar wall (T), pulmonary vascular (C) congestion with inflammatory cellular infiltration (I) (Hx & E, x400).

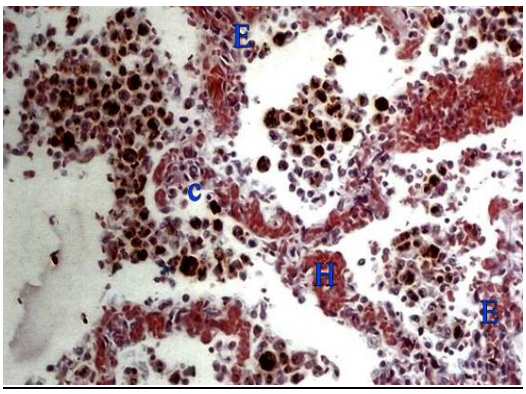

Fig. (6): A photograph of lung tissue of study animals (4-w after starting study) showing moderate interstitial edema (E), scattered areas of hemorrhage (H), proliferation of alveolar cells and thickening of the capillary (C) wall (Hx & E, x400).

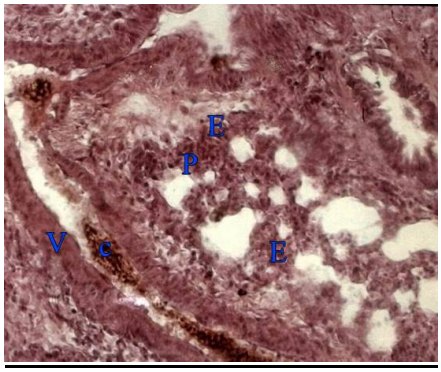

Fig. (7): A photograph of lung tissue of study animals (4-w after starting study) showing moderate interstitial edema (E), proliferation of alveolar cells (P), pulmonary venous congestion (C) and thickening of the vessels (V) (Hx & E, x100).

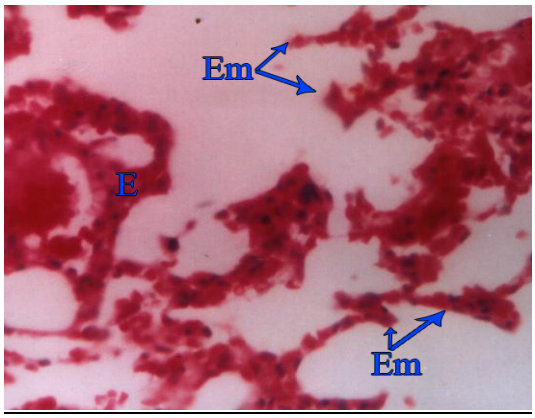

Fig. (8): A photograph of lung tissue of study animals (8-w after starting study) showing alveolar septal edema (E) and scattered areas of compensatory emphysema (Em) (Hx & E, x400).

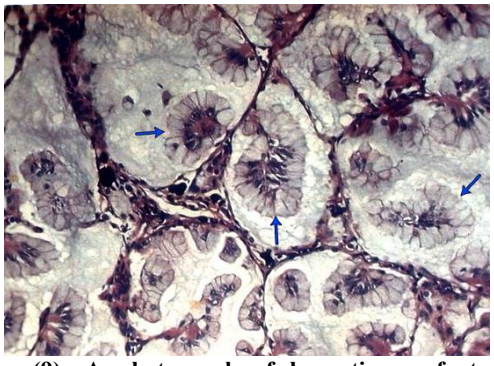

Fig. (9): A photograph of lung tissue of study animals (8-w after starting study) showing a picture of well differentiated carcinoma (arrows) (Hx & E, x400).

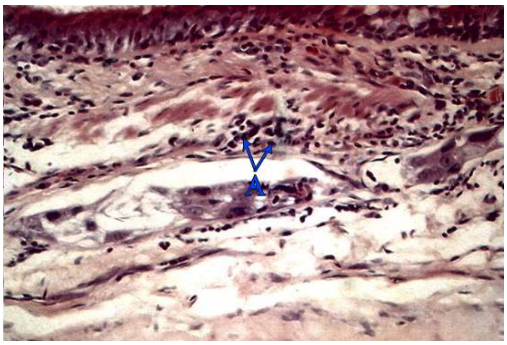

Fig. (10): A photograph of lung tissue of study animals (8-w after starting study) showing scattered areas of mild atypia (A) and hyperchromatic nuclei (arrows); a picture suggestive of starting malignant transformation (Hx & E, x100).

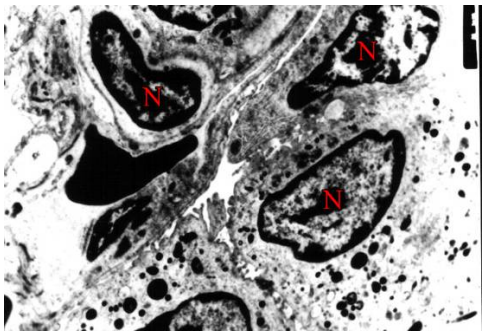

Fig. (11): A photograph showing normal EM appearance of the normal nuclei (N) of alveolar cell detected in specimens harvested from control animals (x2000).

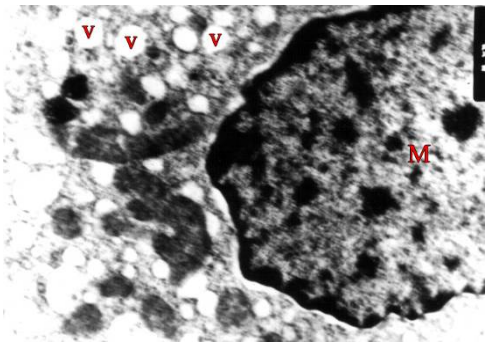

Fig. (12): A photograph showing EM appearance of specimens obtained after 1-w of starting the study, showing normal alveolar macrophages (M) with highly vacuolated (v) cytoplasm and normal nuclei (x6000).

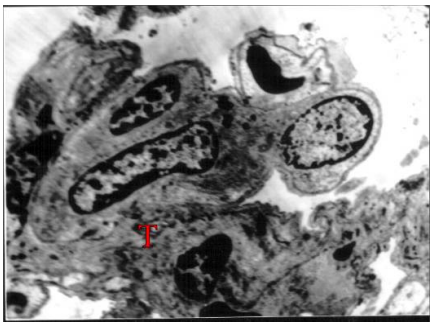

Fig. (13): An EM picture showing thickening of the interalveolar septa (T) and hemorrhage in specimens obtained after 4-w of starting the study (x6000).

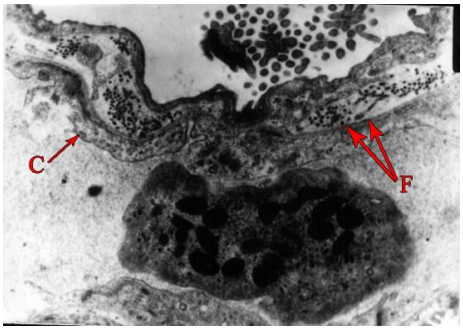

Fig. (14): An EM picture showing interstitial fibrosis (F) and increased collagen (C) fiber deposition in specimens obtained after 4-w of starting the study (x10, 000).

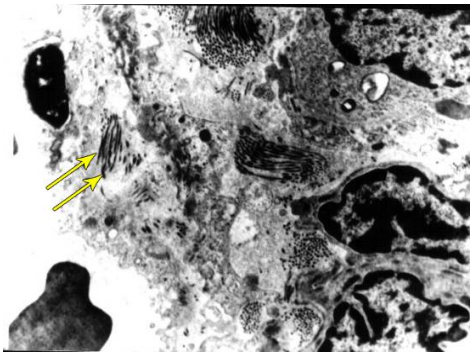

Fig. (15): An EM picture showing endothelial cell damage and distortion of alveolar epithelium (arrows) in specimens obtained after 8-w of starting the study (x6000).

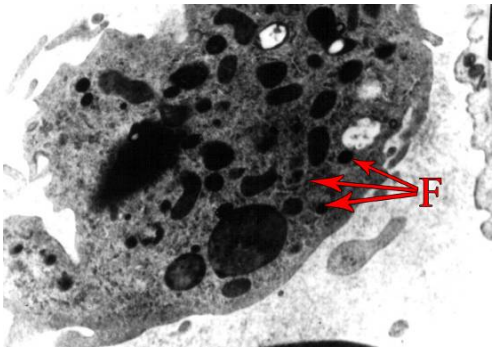

Fig. (16): An EM picture showing increased alveolar macrophages with apoptotic changes in the form of fragmentation (F) of the nuclei in specimens obtained after 8-w of starting the study (x6000).

4. Discussion

Chronic fumonisin exposure induced multiple deleterious effects; as regards general effects, body weight gain of animals exposed to high dose of fumonisin for 4-8 weeks was significantly lower compared to those exposed to the same dose for one week or the small dose for similar duration. However, animals exposed to low-dose or high-dose for short duration showed non-significantly lower weight gain compared to control animals. These data indicated that chronic heavy exposure to fumonisin induced a state of failure to thrive and decreased body weight could be attributed to effects of fumonisin on appetite with concomitant decrease in food consumption or to impaired protein synthesis secondary to induction of DNA alterations.

In hand with these data, **Carratù *et al.* (2003)** reported that exposure to FB1-contaminated diets produces a significant reduction of body weight gain and attributed this effect to the suppressant effect of FB1 on protein synthesis and to the fact that FB1 adversely affect folate uptake and potentially compromise cellular processes dependent on this vitamin. **Tessari *et al.* (2006)** found that irrespective of dose of fumonisin, at 41 day of exposure, all mycotoxin-treated groups had lower body weight and weight gain compared with controls. **Gbore (2009)** reported that in comparison to weight gain by control animals; the daily and the final weight gains of animals fed diet containing 15 mg fumonisin/kg were 75.8% and 90.6%, respectively and these animals exposed to fumonisin attained puberty 30 days after the control animals attained puberty. **Gbore (2009)** concluded that chronic heavy exposure to fumonisin not only affect the general body built progress but also retards puberty.

In support of the effect of fumonisin on appetite and ration consumption; **Dilkin *et al.* (2003)** reported that animals fed 30 or 50 mg of FB1/kg of feed for 4

weeks, partially refrained from consuming the rations with subsequent significant reduction in mean feed consumption as well as in final body weight gain during the last week of intoxication.

The mean lung wet-weight to body weight was increased in all FB_1-treated rats, irrespective of dose used or duration of exposure, compared to control ratio. Mean lung/body weight ratio was significantly higher in study groups III and IV compared to control and study groups I and II. However, the difference between study groups I and II was non-significant and similarly, the difference between study groups III and IV was also non-significant.

The reported significantly higher wet weight of lung of animals heavily exposed to fumonisin could be attributed to development of pulmonary edema which showed time-course dependency. In support of such attribution histological examination of lung specimens obtained from animals exposed to FB_1 in a dose of 30 mg/kg of feed for one week showed only mild pulmonary congestion and alveolar edema in some slices and focal areas of interstitial edema with inflammatory cellular infiltration in others, whereas in animals exposed for 4 weeks there was moderate interstitial edema, areas of hemorrhage, proliferation of alveolar cells, pulmonary venous congestion and thickening of the vessels. The picture was progressed more intensely after 8-w exposure where microscopic examination showed proliferation of alveolar lining cells with inflammatory cellular infiltration, alveolar septal edema and scattered areas of compensatory emphysema. These data illustrated the effect of dosing and signified that the more the dose of FB_1 in contaminated diet the more effect on lung tissue.

These data agreed with **Smith *et al.* (1996 & 1999)** who reported that culture material containing fumonisins at <20 mg/kg/day induce no evidence of pulmonary edema formation either histologically or by altered lung wet/dry weights. **Zomborszky-Kovács *et al.* (2002)** reported that in case of acute toxicosis, pulmonary edema was observed and histopathological examination revealed interlobular and subpleural pulmonary edema, while in case of chronic toxicosis the pathological changes turned to irreversible fibrosis, so the widening of the septa was due to the increase of reticular fibers in the connective tissue. The proliferation of the connective-tissue fibers (collagenic fibers, elastic fibers) was seen (fibrosis, elastosis, fibro-elastosis) in the subpleural and interlobular connective tissue of the lungs, extending to the peribronchial and peribronchiolar areas. New formation of elastic fibers in the alveolar walls was also observed (alveolar elastosis).

Liu *et al.* (2002), found swine alveolar macrophages treated with 50 ng/ml of FB1 for 24 h led to a reduction in phagocytic ability to approximately 55% of the control levels and dramatically decreased the mRNA levels of interleukin-1beta and tumor necrosis factor-alpha; a finding suggesting that FB1 is immunotoxic to swine alveolar macrophage.

EM examination illustrated the time course affection of lung ultrastructure in the form of highly vacuolated cytoplasm of normal macrophages after 1-w, thickening of the interalveolar septa and hemorrhage and interstitial fibrosis and increased collagen fiber deposition after 4 weeks and endothelial cell damage and distortion of alveolar epithelium after 8 weeks with increased alveolar macrophages and appearance of apoptotic changes. The reported fibrosis and increased collagen fiber deposition explain the occurrence of compensatory emphysema detected by light microscopy. Such picture of emphysema coincided with that previously reported using variant toxins and point to a diagnosis of FB1-induced toxic emphysema. In line with such assumption; **Gumprecht *et al.* (2001)** reported numerous large endocytic vesicles and distortion of endothelial cells with numerous channels continuous with the membranous material in models of fumonisin toxicity.

Unfortunately, two specimens obtained from animals received high dose for 8 weeks showed scattered areas of atypia suggestive of starting malignant transformation giving a picture of well differentiated carcinoma. These findings indicated the disruptive effect of fumonisin on cellular texture and induction of carcinogenesis. These changes could be attributed to the documented effects of myotoxins on DNA and the disturbances of the cellular redox milieu depending on the experimental evidences provided by **Kouadio *et al.* (2005 & 2007)** who found fumonisin B1 display synergistic effects in lipid peroxidation and inhibit DNA synthesis by 43% and concluded that Fusarium toxins are able to induce lipid peroxidation, DNA damage, DNA fragmentation, DNA methylation, and cytotoxicity in examined cells. Also, **Gelderblom *et al.* (2008)** documented that the ability of different stimuli to selectively promote the outgrowth of fumonisin B1 initiated cells further verifies the cancer initiating potency of this apparent non-genotoxic mycotoxin.

The obtained data and review of literature allowed concluding that exposure to fumonisins induced variable gross and microscopic effects on lung tissue and severity and character of these pathological changes showed dose and time dependency.

Corresponding author
Gamal Abdel Salam

Department of Anatomy, Faculty of Medicine, Benha University
G_Abdelsalam@yahoo.com

References

1. Bouljihad M, Leipold HW: An ultrastructural study of pulmonary bronchiolar and alveolar epithelium in sheep. Zentralbl Veterinarmed A. 1994; 41(8):573-86.
2. Carratù MR, Cassano T, Coluccia A, Borracci P, Cuomo V: Antinutritional effects of fumonisin B_1 and pathophysiological consequences. Toxicol. Letters, 2003; 140:459-63.
3. Council for Agricultural Science and Technology (CAST). Mycotoxins: Risks in Plant, Animal, and Human Systems. Task Force Report No. 139. CAST, Ames, IA: 2003.
4. Dilkin P, Zorzete P, Mallmann CA, Gomes JD, Utiyama CE, Oetting LL, Corrêa B: Toxicological effects of chronic low doses of aflatoxin B_1 and fumonisin B_1-containing Fusarium moniliforme culture material in weaned piglets. Food Chem. Toxicol., 2003; 41: 1345-53.
5. Gbore FA: Growth performance and puberty attainment in growing pigs fed dietary fumonisin B(1). J Anim Physiol Anim Nutr (Berl). 2009; 93(6):761-7.
6. Gelderblom WC, Marasas WF, Lebepe-Mazur S, Swanevelder S, Abel S: Cancer initiating properties of fumonisin B1 in a short-term rat liver carcinogenesis assay. Toxicology. 2008; 250(2-3):89-95.
7. Gumprecht LA, Smith GW, Constable PC, Haschek WM: Species and organ specificity of fumonisin-induced endothelial alterations: Potential role in porcine pulmonary edema. Toxicology 2001; 160(1-3): 71-9.
8. Hayat MA: Principles and techniques of electron microscopy. In: Biological Applications, Hayat MA, (ed), 3rd ed., McMillan Press, London, 1989.
9. Kouadio JH, Mobio TA, Baudrimont I, Moukha S, Dano SD, Creppy EE: Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. Toxicology. 2005; 213(1-2):56-65.
10. Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE: Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon. 2007; 49(3):306-17.
11. Liu BH, Yu FY, Chan MH, Yang YL: The effects of mycotoxins, fumonisin B1 and aflatoxin B1, on primary swine alveolar macrophages. Toxicol Appl Pharmacol. 2002; 180(3):197-204.
12. Marasas WF: Fumonisins: their implications for human and animal health. Nat Toxins. 1995; 3(4):193-221.
13. Ross PF, Rice LG, Platter RD, Osweiler GD, Wilson TM, Owens DL, Nelson HA, Richard JL: Concentrations of fumonisin B_1 in feeds associated with animal health problems. Mycopathologia, 1991; 114: 129–35.
14. Saari S: An SEM study of the morphology of the lower respiratory-tract surface of the reindeer (Rangifer tarandus tarandus L.). Anat Histol Embryol. 1995; 24(3):165-9.
15. Smith GW, Constable PD, Bacon CW, Meredith FI, Haschek WM: Cardiovascular effects of fumonisins in swine. Fund. Appl Toxicol. 1996; 31: 169–72.
16. Smith GW, Constable PD, Tumbleson ME, Rottinghaus GE, Haschek WM: Sequence of cardiovascular changes leading to pulmonary edema in swine fed fumonisin-containing culture material. Am. J. Vet. Res., 1999; 60: 1292–300.
17. Sulkowska M, Sulkowski S, Nowak HF, Terlikowski S: Type II alveolar epithelial cells and free alveolar cells after intratumor TNF-alpha administration. Histol Histopathol. 1996; 11(3):633-40.
18. Tardieu D, Bailly JD, Skiba F, Meayer JP, Grosjean F, Guerre P: Chronic toxicity of fumonisins in turkeys. Poult Sci. 2007; 86(9):1887-93.
19. Tessari EN, Oliveira CA, Cardoso AL, Ledoux DR, Rottinghaus GE: Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Br Poult Sci. 2006; 47(3):357-64.
20. Thiel PG, Marasas WF, Sydenham EW, Shephard GS, Gelderblom WC: The implications of naturally occurring levels of fumonisins in corn for human and animal health. Mycopathologia 1992; 117: 3-9.
21. Wild CP, Gong YY: Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010; 31(1): 71–82.
22. Zomborszky-Kovács M, Kovács F, Horn P, Vetési F, Repa I, Tornyos G, Tóth A: Investigations into the time- and dose-dependent effect of fumonisin B_1 in order to determine tolerable limit values in pigs. Livestock Prod. Sci. 2002; 76: 251-6.

8/10/2012