Ultrastructural Changes Occur In Mice Lungs after Cessation to Exposure of Incense Smoke

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Abstract: Background: Incense woods are special kind of trees called Agarwood, which characterized by good smelling odors and many medical benefits. Incense smoke is heavily used in Saudi Arabia although comprehensive studies of its effects on health are limited. The present study demonstrated lung ultrastructure changes of mice after exposure and cessation to Incense smoke. Eighty mice are divided equally into four groups, three groups are exposed to different concentrations of Incense smoke (2, 4 and 6 gm) for three months, while the fourth group is control one. At the end of each month, lungs of five animals from each group are gathered, while the last five animals from each group are kept for another 60 days without exposure to the Incense smoke to allow for recovery.
Results: Transmission electron microscope investigations of all exposed groups showed hypertrophy and hyperplasia in Clara Cells and some an enlargement of the macrophage to the point that it fills a large part of the alveolar lumen. Scanning electron microscope marks presence of mucus materials attached to the epithelial bronchioles. After prevention of exposure to the Incense smoke for 60 days, necrosis and degeneration in some cells of epithelial bronchioles, fibrosis of peri-bronchial, thickening in alveolar walls and aggregation of lymphoid cells were demonstrated. **Conclusion:** Based on the above findings and other related studies (not published), we conclude that exposure to Incense smoke causes harmful effects due to sever changes in pulmonary ultrastructure, such effects do not disappear even when Incense smoke inhalation was stopped. Therefore, we recommend that Incense smoke should use only in open places to reduce its harms.
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Key words: Incense smoke, lungs, ultrastructure of lungs, Agarwood

1. Introduction

Incense woods are special kind of trees called *Aquilaria agallocha* and *Aquilaria malaccensis*. These trees produce wood called Agarwood, which is characterized by good smelling odors [1]. This wood has variable benefits as producing Arabian Incense or as traditional medicine against many diseases [2]. Many countries used to use this kind of Incense wood in their temples whereas there are no good air circulation such as Bangladesh, Bhutan, India, Indonesia and most countries of South East Asia [3]. Previous study was conducted to examine the environmental situation and its impact on the health of workers employed in factories producing Incense in India. This study has recorded the presence of some symptoms such as pain in the chest and stomach, runny nose, cough, and quiver in hands, and this consider as the most common disease of workers factories producing Incense [4].

Burning of Incense smoke produced very small particles similar to those particles produced from the burning of tobacco [5, 6]. Many previous studies reported that Incense smoke containing particular matters, carcinogenic material, and polyaromatic hydrocarbons (PAHs) [7]. Smoke produced from Incense wood burning consider one of the most important factors of air pollution, which has a negative impact directly on infants and children [8, 9], caused increases the risk of some lung diseases such as asthma [10]. Daily use of incense smoke at Saudi population consider as risk factor for chronic obstructive lung disease [11]. In addition to, some studies conducted on both the cellular and physiological changes showed direct influence of respiratory organs especially lung as a result of persistent exposure to incense smoke [12, 13]. Furthermore, many histological studies discussed and recorded severity changes in lung tissues of rats after continuous exposure to Incense smoke. These changes ranged from, moderate inflammation, and lymphocytes infiltration [14], to severe changes including lung carcinoma [15].

In the present study, we investigated the ultrastructure changes due to continuous exposure to Incense smoke on mice lungs and its harmful effect. In addition, we planned to study if the observed ultrastructure changes enhanced by stopping exposed the same mice groups to Incense smoke for recovery. This research derives its importance from the fact that Incense is heavily use in Saudi Arabia in the absence of comprehensive studies of its effects on health.

2. Material and Methods

Mice:

Six to eight-week-old female BALB/c mice were raised and maintained throughout experimentation in the Center of King Fahed for Medical Research at King Abdel Aziz University, Jeddah, KSA, and maintained under standard laboratory conditions including diet and temperature of 22^{O}C (+/- 2) with continuous supply of water. Its body weights were almost 25-30 g.

Incense smoke used in the experiment:

Incense smoke was used in this study was bought from Jeddah local market, and it was burned using cubes of artificial coal.

Mice exposed to Incense smoke:

Eighty female BALB/c mice were divided into four groups. Group of twenty mice Group I was comprises of twenty mice and kept in open air along the experimental period. The remaining 60 mice were divided equally into three groups (II, III, IV), and were exposed daily (except Friday) for three months to different concentrations of Incense smoke (2, 4 and 6gm) respectively. A box was used to expose mice to different concentration of Incense smoke individually and to make them inhaled it.

Ultra structure studies:

Transmission electron microscopy:

Tissues samples were fixated in 2.5 % glutaraldehyde and 2% paraformaldehyde in 0.2 M sodium cacodylate buffer at pH 7 2. The lung slices were chopped into blocks, washed in 1 M sodium cacodylate buffer and post-fixed in 2 % osmium tetroxide for 30 minutes. After blocks specimens were washed by second buffer, they were dehydrated in a series of 70, 96 and 100 % ethanol solutions. After dehydration in graded alcohol solutions, samples were cleared in 2 changes of propylene oxide for 8 minutes and embedded in pure resin for 30 minutes. All samples sections were cut on an ultra microtome and the 1 micron thickness of the transverse sections of lungs were stained with 1% touledine blue stain at 95^{o}C for transmission ultra microscope investigation [22].

Scanning Electron Microscope:

Tissue samples were fixed in 3% Gluteraldehyde and 2% osmium tetroxide. Then they were dehydrated in graded series of ethyl alcohol, then immersed in acetone and infiltrated in a mixture of acetone and hexamethyldisilazana (1:1.v:v) The dried samples were fixed on the specimen stubs and were coated with a gold stain [22].

3. Results

Mice that are not exposed to Incense smoke (Group I)

Transmission electron microscope sections of group I showed lining epithelium composed of two types of cells; the first one is non-ciliated cells which are long cells characterized by large number of variable sized mitochondria and the cell membranes were mostly closely in contact. The second is ciliated cells, which are short cells with large vesicular nucleus and few cell organelles having cilia and embedded in between the non-ciliated cells (Fig. 1a). In addition, lung sections of control group showed the septal capillaries containing red blood cells and the other type is the alveolar cells which is characterized by large in size, bulging in the alveolar lumen having microvilli. The cells contained vesicular nucleus and the cytoplasm included moderate number of variable sized mitochondria as well as a few lamellar bodies (Fig. 1b). Scanning electron micrograph of the surface epithelium of the bronchiole showed that non ciliated cells have small microvilli while the ciliated cells have long cilia (Fig. 2a). Also, the surface of alveolar lumen of control group is showing smooth elongated cells (type I), and large cell with numerous variable size microvilli (type II) and the presence of red blood cell attached to the wall of the alveoli (Fig. 2b).

Ultrastructure changes on mice lungs after exposure to different concentrations of Incense smoke

Manifestations in mice lungs after daily exposing to 2 gm of Incense smoke have been illustrated by both transmission and scanning electron microscope. Transmission electron microscope sections illustrated that most epithelial cells were Clara cells (non-ciliated cells). The Clara cells were greatly hypertrophied and contained large number of variable shape and size mitochondria. Numerous electron dense granules as well as numerous dilated smooth endoplasmic reticulums were present at the periphery of Clara cells. Other lining cells were ciliated and appeared embedded in between the hypertrophied Clara cells. Fibroblast cell were present under the bronchiolar basement membrane and the bronchiolar capillaries (Fig. 3a). In addition, macrophage were containing numerous elongated cytoplasmic processes and the septal capillaries contain RBCs were lining by endothelial cells (Fig. 3b, 3c). Furthermore, alveolar cells types II have surfactant granules, large macrophage and numerous crystals beside other cell organelles embedded in mucin matrix (Fig. 3c). Scanning electron micrograph of lung sections of group II that continuously exposed to 2gm of Incense smoke for 90 days showed presence of mucinous secretion attached to the epithelial lining in mice lung sections. Swollen and separation from the neighboring cells

were demonstrated in non-ciliated cells while the cilia of the ciliated cells adhered to each other and non-erected (Fig. 4).

Exposing mice daily to 4gm of Incense smoke for 90 days (Group III) illustrated the same changes of group II in addition, the presence of basal cell and ciliated cells, which embedded in a numerous hypertrophied Clara cells (Fig. 5a). Also, alveolar lumen showed presence of large elongated, large number of variable sized mitochondria, some phagosomes, membranous vacuoles and rough endoplasmic reticulum. The cells have large cytoplasmic processes and alveolar capillary contains RBCs (Fig. 5b). Lung alveoli showed some macrophage cells characterized by presence of large number of cytoplasmic processes and the cytoplasm contains numerous light electron dense needle like crystals (Fig. 5c).

Moreover, mice exposed to 6 gm of Incense smoke for 90 days (Group IV) showed numerous changes of the lung bronchiole as rupturing of some Clara cells associated with releasing the cytoplasmic contents in the lumen which having electron dense granules. Other Clara cells were very rich with secretory endoplasmic reticulum, mitochondria that found mainly related to the cell membrane (Fig. 6a). Also some peri-bronchial fibrosis was manifested by the presence of fibroblast cells and large amount of collagenous fiber bundles (Fig. 6b). In addition, the hypertrophied Clara cells filling the alveolar lumen was observed in some sections. The mitochondria of the Clara cells were swollen and the cytoplasm contains a numerous membranous vacuoles having electron dense material (Fig. 6c).

Ultrastructure changes in Mice lungs left for recovery for 60 days

Mice lung sections, which daily exposed to 2 gm of Incense smoke for 90 days and left for 60 days for recovery showed numerous ciliated cells that adhered to the basement membrane in micrograph of the terminal bronchiole that have shrunken nucleus with condensation of the nuclear chromatin. The cytoplasm contains large number of variable sized vesicle. The non-ciliated cells were large and contain large number of variable sized and shaped mitochondria. Some cells appear to have three nuclei one of them is large and vesicular, while the other is compact banana shape and the third appears spherical contains nucleolus but the nuclear sap appears more electron dense than normal. The lumen contains mucinous secretions. The collagenous connective tissue forming the lamina propria of the bronchiole was observed under the basement membrane (Fig. 7a, 7b). By using Scanning electron micrographs of the surface of bronchioles showing the non ciliated cells have microvilli and some of them appeared finger like separate from the other cell and some of them adhered to each other (Fig. 8a). Otherwise, surface of bronchioles show some non ciliated cells appeared as tongue shaped while other ciliated cells have large number of non-erected cilia. Several spherical bodies adhered to the surface of both ciliated and non ciliated cells on the surface of the bronchioles (Fig. 8b). Micrograph of the alveolar surface of the lung showing a wide lumen and the lining cells were flat and branched with thickening of the alveolar septa (Fig. 8c).

Further studies was carried out on lung sections belonging to group III that left for 60 days for recovery were reported that the lining epithelium of terminal bronchioles were varies greatly in height while the non-ciliated cells or Clara cells were long and contain vesicular nucleus. The cytoplasm contains large number of mitochondria, which varies greatly in size and shape. In addition, the same group is showing the presence of some electron dense secretory granules at the periphery of the cells. Ciliated cells seemed to be short and contain numerous vesicles and characterized by the presence of peri-nuclear spaces with dentations of the nucleus. The sub-epithelial layer formed by collagenous connective tissue (Fig. 9). Scanning electron micrograph of the alveoli shows compression of the lumens and the septa show thickness. The alveolar cells formed by long and flat pneumocytes having variable size and long microvilli (Fig. 10).

Ultrastructure study of bronchial epithelium belonging to mice that daily exposed to 6 gm of Incense smoke and left for 60 days showed degenerative changes represented by shrinkage and condensation of the nuclear chromatin in the nuclei of some ciliated cells as well as non-ciliated ones. The lumen contains mucin secretion, secretory granules and deciliated cilia (Fig. 11a). Furthermore, the bronchial epithelium belonging to the same group is showing some Clara cells in state of necrobiosis associated with fragmentation of the nuclear chromatin and the cytoplasm contains large number of minute vesicles. Other cells appear electron dense either in the nucleus or in the cytoplasm with presence of numerous light electron granules seemed to be mucin granules. The other cells either ciliated or non-ciliated showing the presence of large number of vesicles varies in size (fixation artifact) all nuclei having pei-nuclear spaces (Fig. 11b). Also the lamina propria formed by a thick layer of collagenous connective tissue in the epithelial lining showing both the non-ciliated and ciliated cells in state of necrobiosis which manifested by presence of large inter-cellular vesicular spaces and presence of numerous SE vesicles in the cytoplasm as well as

mucinous granules. The ciliated cells showed compact and more electrons dense with deciliation of the cilia (Fig. 11c), and the septal wall belonging to group IV showing bundles of collagen fibers in the septal wall (Fig. 11d). In addition, high magnification of the previous photo belonging to group IV showing thickening of the basement membrane with light electron dense deposit in some areas appears fibirillar. The pneumocyte type II, Clara cells have greatly swollen mitochondria, which occupy all the cells and some of them is destructed and change to vesicles (Fig. 11e). Otherwise, scanning electron micrograph belonging to this group showed great thickening of the septal wall of some alveoli with dilatation of the lumen and compression of others (Fig. 12a). Also, number of ciliated cells is observed as they increase in relation to non-ciliated cells. Furthermore, the presence of wide spaces between the lining cells, with presence of some spherical bodies adhered to the surface of the non-ciliated cells was observed (Fig. 12b). High magnification of the bronchiole belonging to the same group are showing the ciliated cells long non erected cilia and the non ciliated cells have variable sized and shaped microvilli with presence of spherical bodies attached to the surface, while, wide werea of peri-bronchial collagen fibers in the lung lobule is reported in some sections (Fig. 12c).

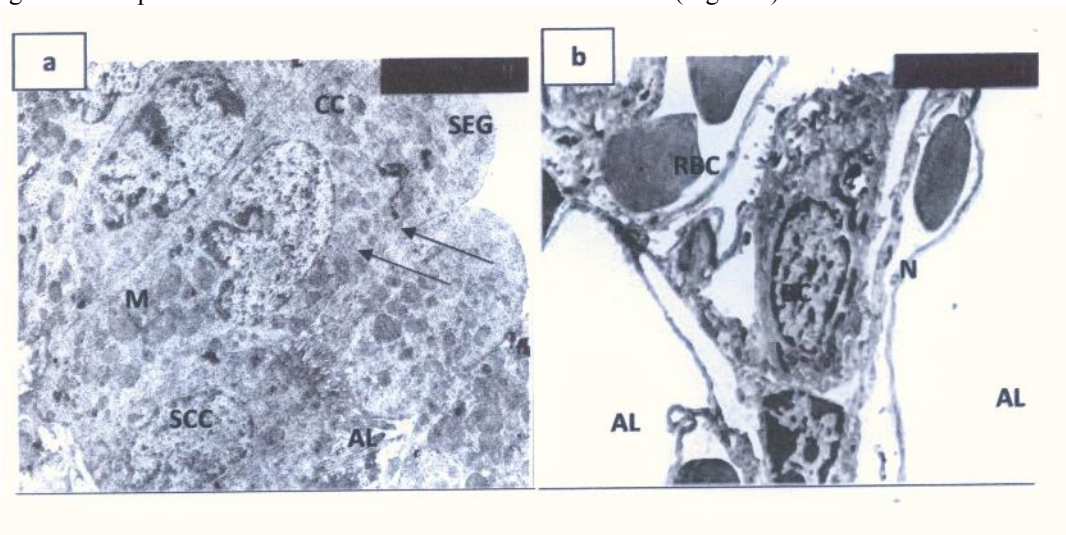

Fig. 1. Transmission electron micrographs of the terminal bronchiole of lung belonging to control group
(a) The lining epithelium formed by two types of cells non-ciliated cells (CC). Large number of variable sized mitochondria (M). The cell membranes ( ) are mostly closely in contact with each others. Ciliated cells are short cells with large vesicular nucleus (SEG) and few cell organelles having cilia and embedded in between the non-ciliated cells (SCC). Mag. 8800X.
(b) The septal capillaries containing red blood cells (RBC) and alveolar lumen cells (AL), the nucleus of the cell (N) is vesicular and the cytoplasm contain both moderate number of variable sized mitochondria (M) and few lamellar bodies (LB). Mag. 8800X.

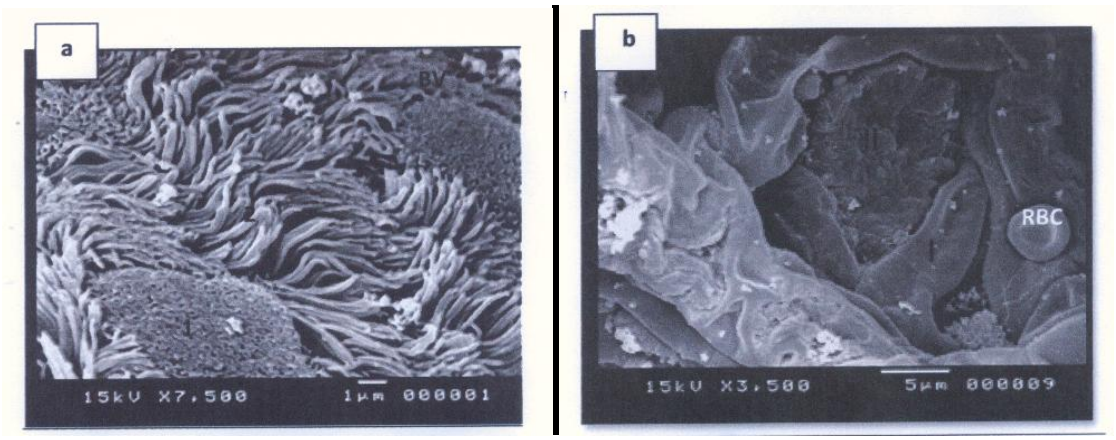

Fig. 2. Scanning electron micrographs of the surface epithelium of the bronchiole belong to control group.
(a) Non ciliated cells contain small microvilli (I) and ciliated cells have a long cilia (II).
(b) Smooth elongated cells (type I), and large cell with numerous variable size microvilli (type II). Note presence of red blood cell (RBC) attached to the wall of the alveoli and Alveolar wall and the alveolar lumen (AL).

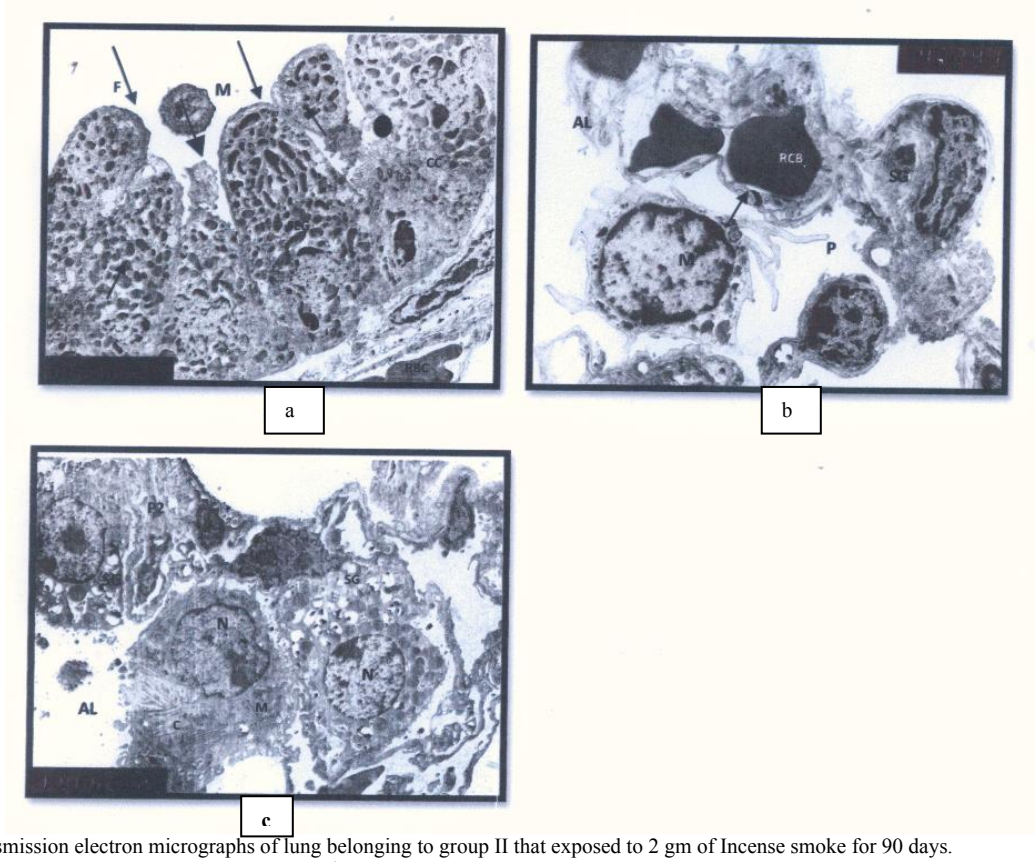

Fig.3: Transmission electron micrographs of lung belonging to group II that exposed to 2 gm of Incense smoke for 90 days.
(a) Clara cells (CC) are greatly hypertrophied (←) containing large number of variable shape and size mitochondria (M) with presence of numerous electron dense granules (SEG). Ciliated lining cells (↘) are appeared embedded in between the hypertrophied Clara cells. Fibroblast cells (F) observed under the bronchiolar basement membrane and the bronchiolar capillaries. Mag. 6000X.
(b) Macrophage (M) contain numerous elongated processes. The septal capillaries contain RBCs and lined by endothelial cells (P). Alveolar cell type II with surfactant granules (SG) is present. Mag. 8800X.
(c) Alveolar cells type II which contain numerous surfactant granules (SG) and the lumen of the alveoli (AL) contain large macrophage (M). Numerous light electron dense crystals (C) beside the other cell organelles embedded in mucin matrix demonstrated in alveolar cells (P2). Mag. 8800X.

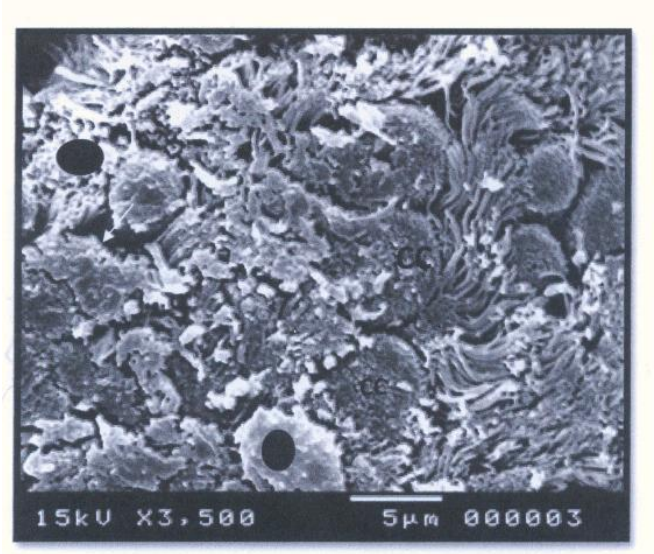

Fig. 4: Scanning electron micrographs of the surface epithelium of the bronchiole belong to group II showed the presence of mucinous secretion (←) attached to the epithelial lining, the non-ciliated cells (CC) swelled and separated from the neighboring cells while the cilia of the ciliated cells (●) are observed. Mag.6000 X.

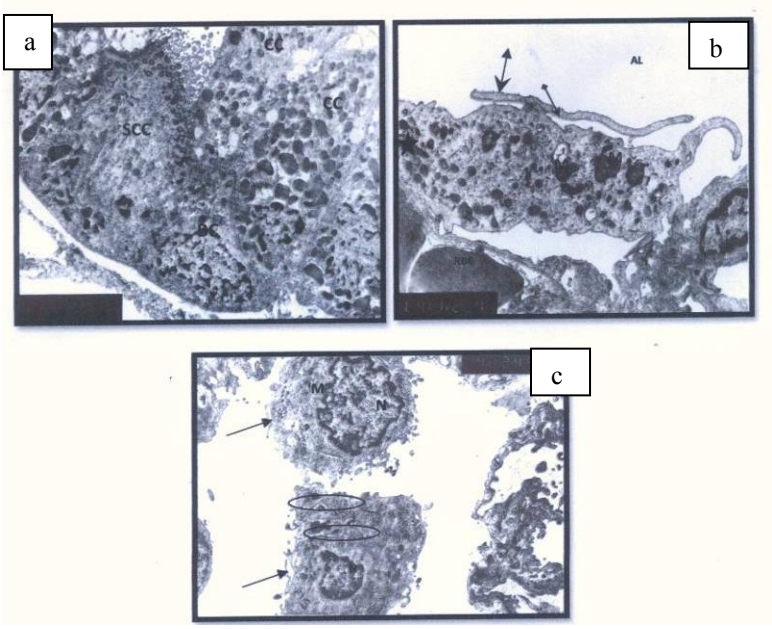

Fig. 5 Transmission electron micrographs of the lung bronchiole belonging to mice that exposed daily to 4gm of Incense smoke (Group III).
(a) Basal cell (BC) and ciliated cells (CC) embedded in a numerous hypertrophied Clara cells (SCC). Mag. 8800X.
(b) Large elongated macrophage (★) in the alveolar lumen which contains a large number of electron dense variable sized mitochondria (M) and some phagosomes (↙↗).
The macrophage has large cytoplasmic processes (↙↗). The alveolar capillary contains RBCs. Mag. 15000X.
(c) Macrophage cells (M) characterized by presence of large number of cytoplasmic processes (↗) and the cytoplasm contains numerous needle like crystals (⬭). Mag. 8800X.

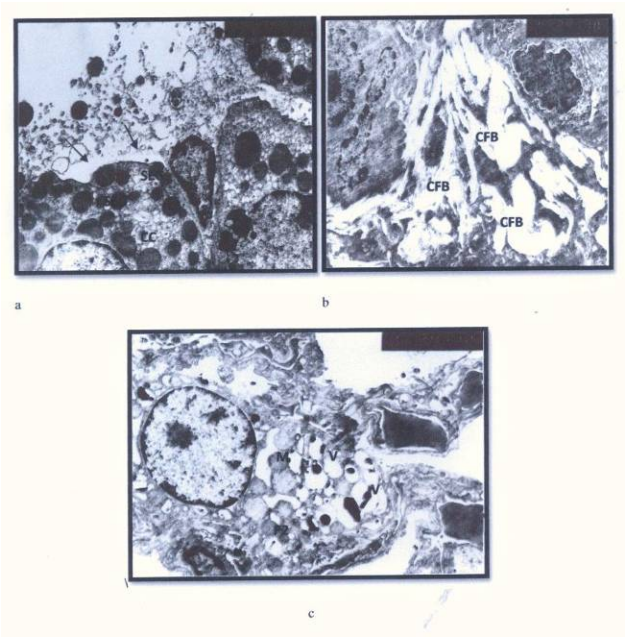

Fig. 6: Transmission electron micrographs of the lung bronchiole belonging to mice that exposed to 6 gm of Incense smoke (Group IV).
(a) Clara cells (CC) rupture and release the cytoplasmic contents in the lumen, which have electron dense granules (↘). Secretory endoplasmic granules (SEG), mitochondria and electron dense granules found mainly related to the cell membrane of Clara cells. Mag. 15000X.
(b) The presence of fibroblast cells and large amount of collagenous fiber bundles (CFB) Mag. 8800X.
(c) The hypertrophied Clara cells (P2) contain the swollen mitochondria (M), and the cytoplasm contain a numerous membranous vacuoles (V) have electron dense material. Mag. 11000X.

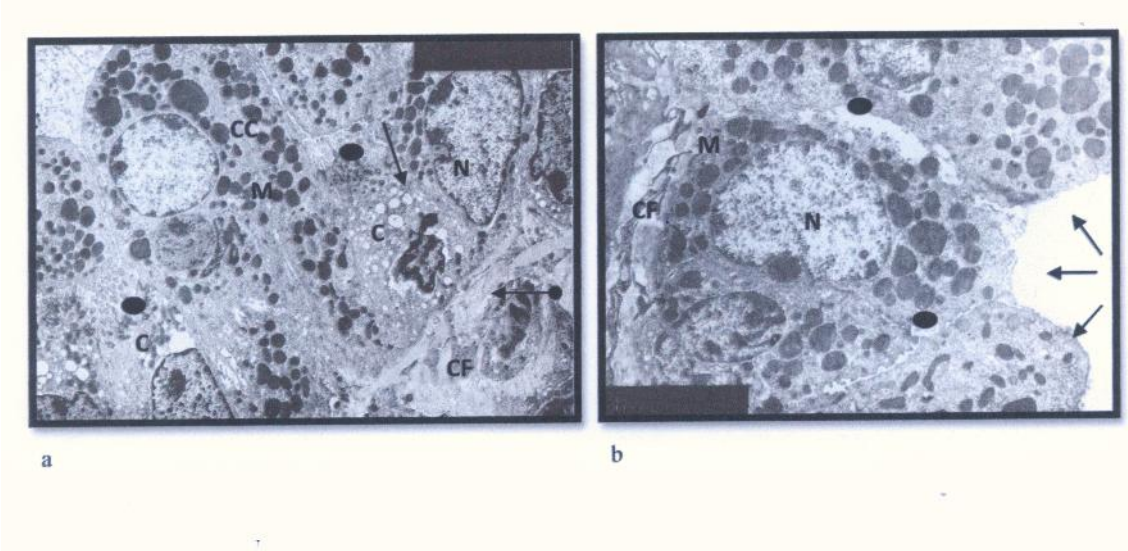

Fig7: Transmission electron micrographs of the lung bronchiole belonging to group II after prevention from exposure to Incense smoke for 60 days.
(a) Ciliated cells (C) are numerous, short closely adhered to the Basement membrane (←●) and have shrunken nucleus (N). The cytoplasm contains large number of variable sized vesicle (↖). The cilia embedded in between the non-ciliated cell not reaching the surface (●). The non-ciliated cells (CC) are large and containing large mitochondria (M). The lumen contains mucinous secretions, and collagenous connective tissue (CF) observed under the basement membrane. Mag. 6000X.
(b) Different lengths of non-ciliated cell (↖) and presence of both intercellular spaces (●), vesicular nucleus (N) and large numbers of mitochondria (M). Sub-epithelial collagenous connective tissue is found (CF) Mag. 8800X.

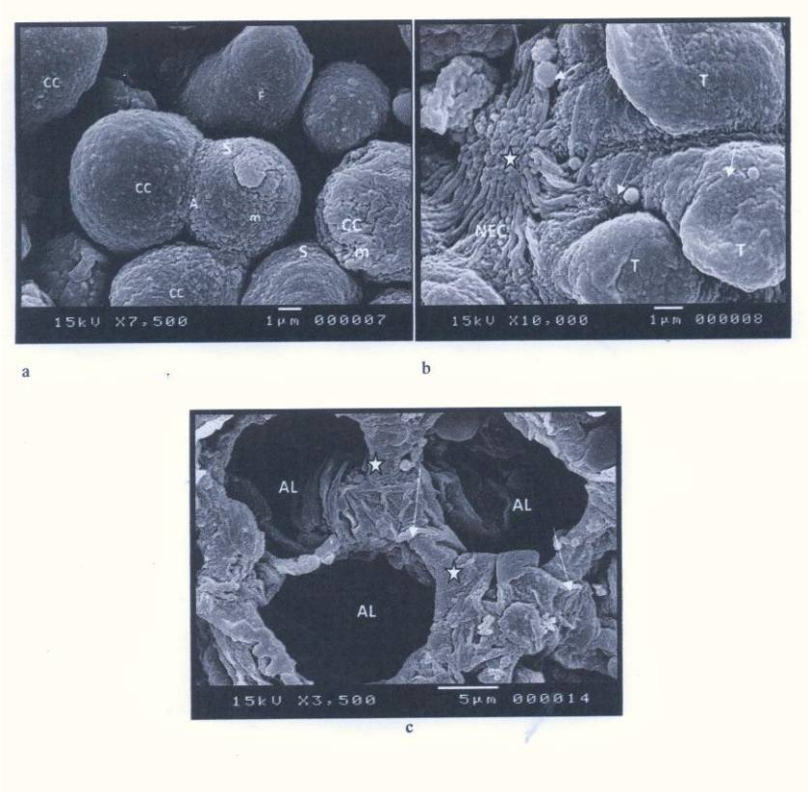

Fig. 8: Scanning electron micrographs of the surface of bronchioles belonging to group II that left for recovery for 60 days.
(a) Non-ciliated cells have microvilli (CC). Some non ciliated cells appear finger like (F) separated from each other cells (S) and some of them adhered together.
(b) Tongue shaped non-ciliated cells (T) while ciliated cells have large number of non-erected cilia (NEC). Several spherical bodies (←) adhered to the surface of cells
(C) Presence of wide lumen (AL). The lining cells are flat (★), also thickening of the alveolar septa (←) is observed

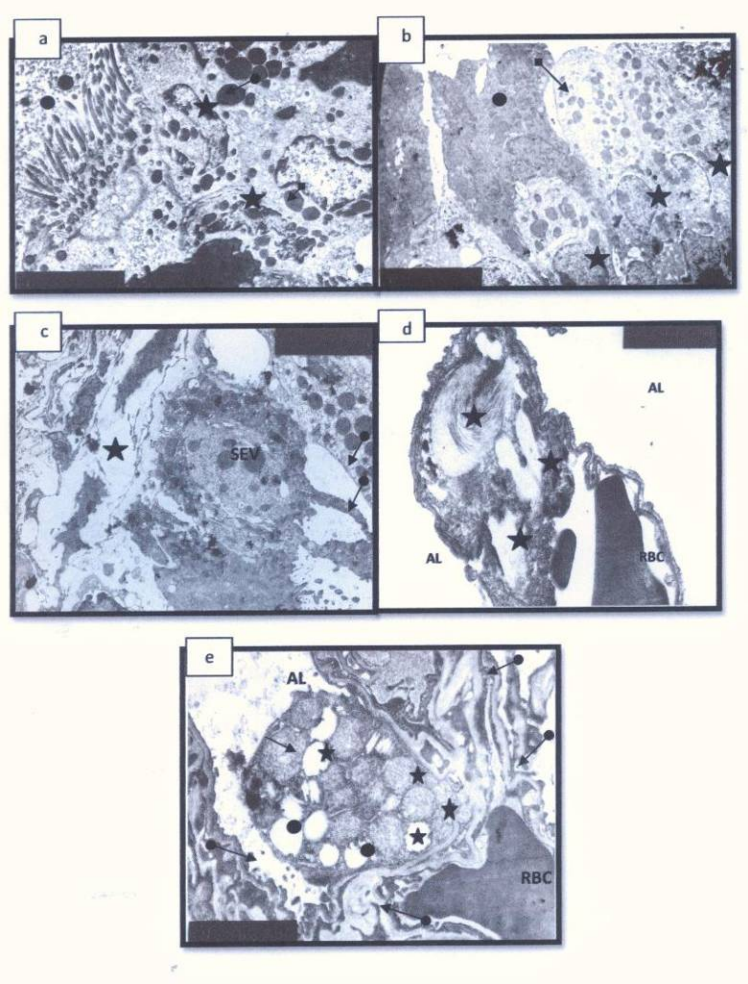

Fig. 9:- Transmission electron micrographs of bronchial epithelium belonging to group IV and left for 60 days for recovery.
(a) Degenerative changes of the nuclear chromatin ( ) in the nuclei of both ciliated cells and non-ciliated ones. Some nuclei appear elongated (★) and other having dentations (●). The lumen contains mucin secretion, secretory granules and deciliated cilia Mag. 11000X.
(b) Fragmentation of the nuclear chromatin of Clara cells (●). The presence of numerous light electron granules ( ) seems to be mucin granules in the cytoplasm. The other cells either ciliated or non-ciliated having intercellular vesicular spaces (★) Mag. 6000 X.
(c) The thick layer of lamina propria characterized with by thick layer of collagenous connective tissue (★). Clara cells epithelial lining showed presence of large inter-cellular vesicular spaces ( ) and presence of numerous vesicles in the cytoplasm (SEV) Mag. 8800X.
(d) Bundles of collagen fibers in the septal wall (★) Mag. 6000 X.
(e) High magnification of the previous photo (D) showed thickening of the Basement membrane (●) with light electron dense deposit in some areas appears fibirillar ( ). The pneumocyte type II, Clara cells have greatly swollen mitochondria ( ) which occupy all the cells and some of them is destructed and change to vesicles (★) Mag. 8000X.

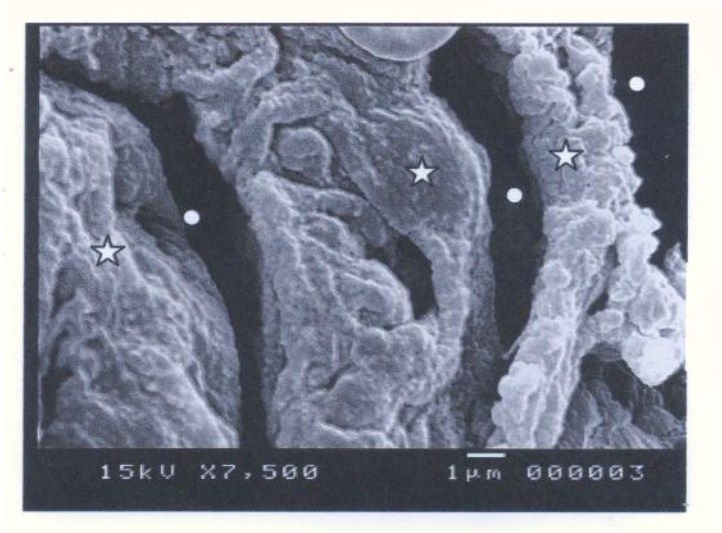

Fig. 10: Transmission electron micrograph of the terminal bronchioles belonging to group III and left for 60 days for recovery showed the non-ciliated cells (SCC) contain vesicular nucleus (N). The cytoplasm contains large number of mitochondria (M) and some electron dense secretory granules (SG) at the periphery of the cells. The sub-epithelial layer formed by collagenous connective tissue (★) Mag. 6000X.

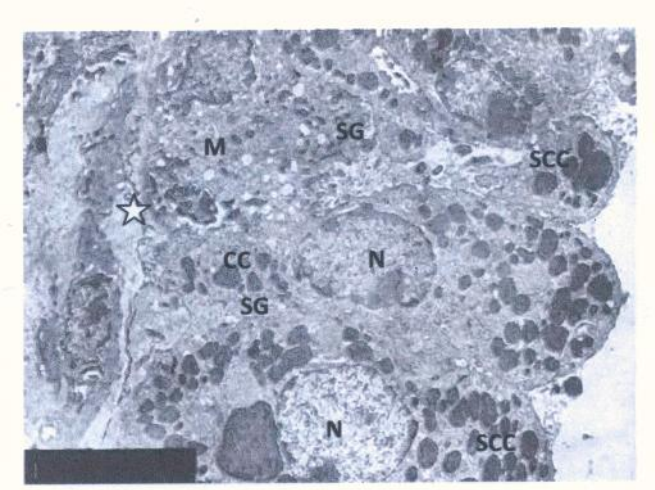

Fig. 11: Scanning electron micrograph of the alveoli belonging to group III and isolated for 60 days for recovery showed compression of the lumen and thickened septa (●). The alveolar cells formed by long and flat pneumocytes that had variable size (★) and long microvilli.

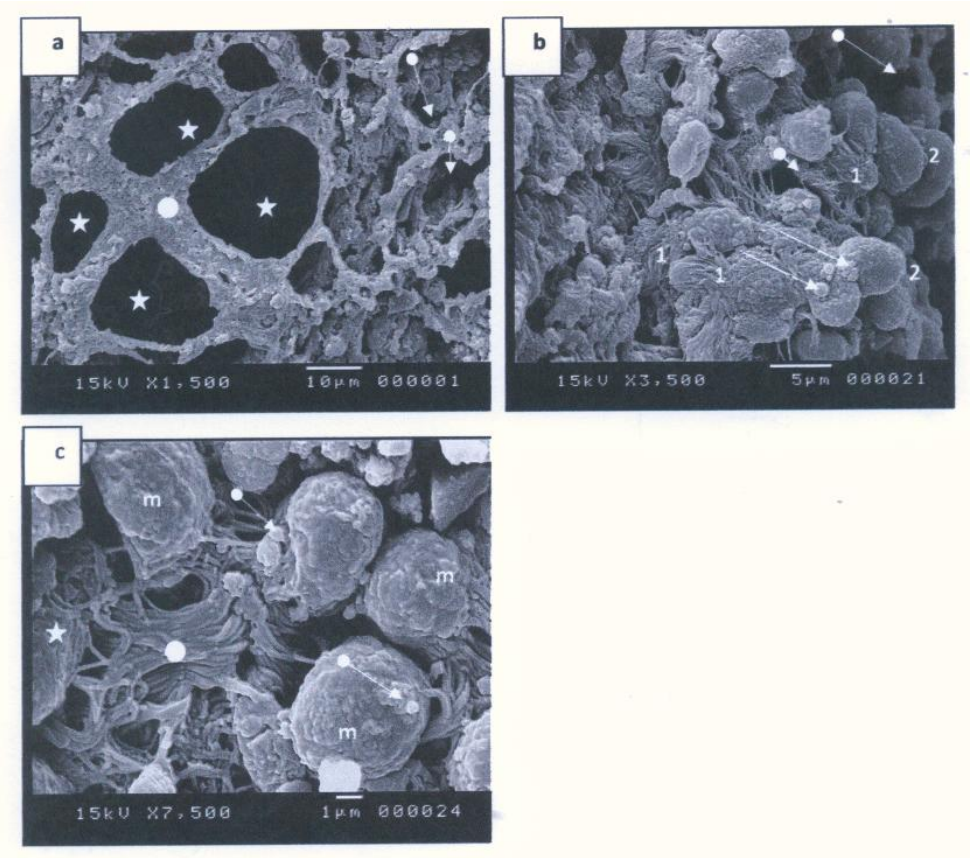

Fig. 12: Scanning electron micrograph belonging to group IV after left for 60 days as a recovery time.
(a) Increasing in thickens of the alveoli septal wall (●) with dilatation of the lumen (★) and compression of others (●→).
(b) Increasing the numbers of ciliated cells (1) in relation to non-ciliated cells (2), and presence of wide spaces (→) between the lining cells. Some spherical bodies (↘) is reported.
(c) High magnification of the bronchiole section showing the variable size and shape of microvilli (m) of non-ciliated cell. The ciliated cells long and have non erected cilia (★) with presence of spherical bodies (→) attached to the surface of the cells. In addition, wide area of peri-bronchial collagen fibers (●) in the lung lobule is recorded in the same section.

4. Discussion

Many previous studies had been reported the harmful effect of the Incense smoke on population especially children and infants [8, 9, 11]. The present ultrastructure findings of current search demonstrated sever changes in mice lungs exposed to different concentration of Incense smoke and continue with the same changes, even after cessation of exposure to Incense smoke. The results of transmission electron microscope of mice lungs exposed daily to 2, 4, and 6 gm of Incense smoke for 90 days recorded the presence of Clara cell hypertrophy due to excessive growth and an increase in the size of these cells associated with an increase in the number hyperplasia. Also, appearance of different size and shape of mitochondria were reported after exposure to either 2 or 4 gm of Incense smoke while giant vesicular macrophage observed after exposure to 6 gm of the Incense smoke whereas they fill out a large part of alveoli cavity. The alveoli cavity

characterized by the presence of numerous elongated processes, and some crystals in form of needle shape. These crystals may perform from metal deposition, or as a remaining due to an interaction of cell organelles, and fibrosis in the tissue around the bronchi (Peri-bronchial fibrosis) was observed. The present results were confirmed with the findings of (Alarifi *et al*., 2004a) study that noticed blockage of alveoli cavity due to presence of large size of the vesicular macrophages in mice lungs sections daily exposed to 4 gm of Incense smoke for 14 weeks, but disagree as they didn't mention any changes occur in the epithelia bronchi layer as hyperplasia or hypertrophy. White collagen fibers have been recorded in liposome walls. Also, they reported an increase in vesicular cell size (P2) which cause cell hypertrophy, associated with mitochondrial overgrown that contains a large amount of Alservictant [13, 16-18]. This difference in results may be due to different concentrations of Incense smoke, which experimental animals were exposed, or perhaps due to the duration and the time of the exposure to Incense smoke.

Ultrastructure study have been carried on mice lungs after cessation for 60 days to exposure of different concentration of Incense smoke for 90 days have reported the continuation of histological changes with the increase in intensity where there is no improvement in lung tissue. Many findings were observed in lung tissues, as necrosis of some epithelial bronchi cells and accumulation and aggregation of lymphoid cells in different locations. Also, lung tissues were recorded changes that occur due to degeneration such as the existence of smooth endoplasmic vesicles in epithelial cells of the bronchioles, the contraction and the concentration in the chromatin nuclei of some epithelial cells. In addition, we noted swelling in cells vesicular P2 that contain swollen and destructive mitochondria These results confirmed with previous studies who demonstrated the ultrastructure of rats lungs exposed to Incense smoke for 14 weeks and without giving the animals a rest period. They have been noticed cells necrosis and accumulation of lymphoid cells, and the cells contain swollen vesicular P2 and mitochondria [14, 19-21].

Current study reported severe changes in mice lungs after cessation for 60 days exposure to different concentration of Incense smoke for 90 days using scanning electron microscope. Scanning electron micrographs for the interior surface of the bronchioles demonstrated an increase in the thickness of some septal walls of alveoli, and the appearance of spherical bodies attached to the surface of the bronchioles lining cells. IM addition, Clara cells appeared in different shapes and sizes and they either connected together or separated from each other. As well as this current study was demonstrated the appearance of white collagen fibers in the lungs lobule of mice exposed to 6gm of Incense smoke for 90 days and left for recovery for 60 days.

Conclusion:

Based on the recent findings and other related studies, we conclude that exposure to Incense smoke causes harmful effects due to sever changes in pulmonary ultrastructures, such effects do not disappear even when Incense smoke inhalation was stopped. Therefore, we recommend that Incense smoke should use only in open places to reduce its harms.

Competing Interests: "The authors declare that they have no competing interests"

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References

[1] Iu KC(1983): The Cultivation of the "Incense Tree" (Aquilaria sinensis), Hong Kong. J. Hong Kong Branch of The Royal Asiatic Society, 23: 247-249.
[2] Meng-ling H, Shu-Yuan Q, Lan-Juan H. (2005): Rapid *in vitro* propagation of medicinally important Aquilaria agallocha. J. Zhejiang Univer. Sci., 6(8): 849-852.
[3] Rathnakara UP, Krishna V, Rajmohan, HR. (1992): An enquiry into mork environmental status and health of workers involved in production of Incense stickes in city of Bangalore. Indian J. Puplic Health, 36(2): 38-44.
[4] Lung S, Hu S. (2003): Generation rates and emission factors of particulate matter and particle-bound polycyclic aromatic hydrocarbons of incense sticks, Taichung, Taiwan Chemosphere., 50(5): 673-679
[5] Chiang K C, Liao CM. (2006): Heavy incense burning in temples promotes exposure risk from airborne PMs and carcinogenic PAHs, Taipei, Taiwan: Science of the Total Environment, 372: 64-75.
[6] Lin T, Krishnaswamy G, Chi D. (2008): Incense smoke: clinical, structural and molecular effects on airway disease, Taiwan. Clinical and

Molecular Allergy, 6:3, doi:10.1186/1476-7961-6-3.

[7] Lin T, Chang F, Hsieh JU, Chao H, Chao M. (2002): Characteristics of polycyclic aromatic hydrocarbons and total suspended particulate in indoor and outdoor atmosphere of a Taiwanese temple, Taiwan. J. Hazardous Materials, 95(2):1-12.

[8] Dawod ST, Hussain AA. (1995): Childhood asthma in Qatar, Doha, Qatar: Ann Allergy Asthma Immunol., 75(4): 360-364.

[9] Hijazi Z, Ezeamuzie CI, Khan M, Dowaisan AR. (2002): Characteristics of Asthmatic Children in Kuwait, Kuwait: J. Asthma., 39(7): 603-609.

[10] Al-Rawas O, Al-Maniri A, Al-Riyami B. (2009): Home exposure to Arabian incense (bakhour) and asthma symptoms in children: a community survey in two regions in Oman, Muscat, Sultanate of Oman: BMC Pulmonary Medicine, 9:23. doi:10.1186/1471-2466-9-23.

[11] Dossing M, Khan J, Al-Rabiah F. (1994): Risk factors for chronic obstructive lung disease in Saudi Arabia, Copenhagen, Denmark. Respir. Med., 88(7): 519-522.

[12] Alarifi A, Mubarak M, Alokail S. (2004a): Ultrastructural changes of pneumocytes of rat exposed to Arabian incense (Bakhour), Riyadh, Kingdom of Saudi Arabia. Saudi Med. J., 25(11): 1689-1693.

[13] Alarifi A, Mubarak M, Alokail S. (2004b): Ultrastructure of the Pulmonary Alveolar Cells of Rats Exposed to Arabian Mix Incense (Ma, amoul), Riyadh, Saudi Arabia. J. Bio. Sci., 4(6): 694-699.

[14] Alokail S, Alarifi A. (2004): Histological changes in the lung of Wistar albino rats (Rattus norvegicus) after exposure to Arabian incense (Genus Boswellia). Ann. Saudi Med. J., 24(4): 293-295.

[15] Friborg T, Yuan J, Wang R, Koh W, Lee H, Yu C. (2008): Incense use and respiratory tract carcinomas. Cancer Journal for Clinicians., 113(7): 1676-1684.

[16] Madany I.(1994): The burning of incense as an indoor source of Volatile Organic Compounds. Indoor and Built Environment, 3(5): 292-298.

[17] Lee S, Wang B. (2004): Characteristics of emissions of air pollutants from burning of incense in a large environmental chamber, Hong Kong. Atmospheric Environment., 38(7): 941-951.

[18] Yang T, Lin T, Chang M. (2007): Characteristics of emissions of volatile organic compounds from smoldering incense. Bull. Env. Contam. Toxical., 78: 308-313.

[19] Alarifi A. (2005a): Morphological evidence of apoptosis in hepatocytes of rats (Rattus norvegicus) exposed to Arabian Incense, Riyadh, Kingdom of Saudi Arabia. J. Med. Sci., 5(3): 222-227.

[20] Alarifi A.(2005b): Histological alterations in liver of rats (RATTUS NORVEGICUS) exposed to Arabian mix incense (ma amoul). J. Egypt: Ger. Soc. Zool.,.47C: 253-266.

[21] Tran C, Marriott J.(2007): Characterization of incense smoke by solid phase microextraction-Comprehensive two-dimensionl gas chromatography (GC×GC). Aust. Atmospheric Environment., 41(27): 5756-5768.

[22] Waynforth HB (1980): Experimental and surgical technique in the rat. London; Academic press INC..