Histological changes of Mice lungs after daily exposure to different concentration of Incense smoke

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Abstract: Since the discovery of Agarwood (Incense tree), many studies reported its characteristic effects and variable benefits, as either to produce Arabian Incense or as a traditional medicine against many diseases. Laboratory experiments were carried out on the effect of different concentrations of Incense smoke inhalation on the lung weight and tissue in female mice. This research derives its importance from the fact that Incense is heavily used in Saudi Arabia in the absence of thorough studies of its effects on health. Eighty animals are used in this study, and are divided into four groups, each is 20 animals. Three groups are exposed to different concentrations (2, 4 and 6 gm) of Incense smoke daily for three months, and the fourth group is the control. At the end of each month, five animals from each group were dissected. Obtained data showed an increase but not significant in animal body and lung weight, this results return to natural increase as a result of normal growth of animals. Light microscope reveals some changes in the lung tissue, such as focal emphysema, rupture in the alveolar walls, hemorrhage, congestion, edema and few peri-bronchial lymphoid cells. After continuous exposure to Incense smoke focal necrosis and degradation are observed in some cells of epithelial bronchioles. Also, fibrosis of peri-bronchial, thickening in alveolar walls and aggregation of lymphoid cells are demonstrated in some lungs sections . Conclusion: according to the above manifestations it could be concluded that exposure to Incense smoke causes pulmonary harmful effects. Therefore, we can recommend that Incense smoke will be used only in open places to reduce its harms.
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1. Introduction

Since the discovery of **Agarwood** (Incense tree), many authors studied the Incense smoke and its characteristic effects **[1-5]**. This wood have variable benefits, it used to produce Arabian Incense because of its good smelling odor **[6]**. Otherwise, some countries used Incense smoke as a traditional medicine against many diseases **[7]**.

Many previous studies demonstrated the effect of burning of Incense smoke on the surrounding environment where smoke that produce from the burning of considered as important factor of air pollution. They reported that Incense smoke produce a very small particles as similar as with those particles produced from the burning of tobacco **[8, 9]** In addition, they illustrated that Incense smoke containing a particular matters, carcinogenic material, and polyaromatic hydrocarbons (PAHs) **[10]**. Some countries used to use Incense wood in their temples where there are no good air circulation such as Bangladesh, Bhutane, India, Indonesia and most countries of south East Asia **[11]**. Incense smoke affect directly to the infants and children **[12, 13]**, where exposure to it's smoke were increasing the risk of some lung diseases including the asthma symptoms **[14]**. Daily use of Incense smoke at Saudi population consider as risk factor for chronic obstructive lung disease **[10]**. Continuous exposure to Incense smoke reported physiological and cellular changes that directly affected the efficiency of respiratory organs especially lungs **[15, 16]**. In addition, moderate inflammation, and lymphocytes infiltration changes in lungs of albino rats were observed after exposure to Incense smoke **[17]**, while other studies recorded a sever changes at the histological structure include lung carcinoma.

The aim of the present study is to investigate the histological changes occurs in lungs of mice that daily exposed to different concentration of Incense smoke. 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°C (± 2) with continuous supply of water. Its body weights were almost 25-30 g.

Incense smoke used in the experiment:

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

Exposure of mice to Incense smoke:

A total of 80 females BALB/c mice were divided into four groups. One group of twenty mice (group I) was left untreated with Incense smoke for control normal lung specimens. The remaining 60 mice were divided equally to three groups and were exposed daily (except Friday) for three months. Different concentrations of Incense smoke were used (2, 4, and 6 g). Group II was exposed to 2 g of Incense inhalation. Mice of group III was exposed to 4 g of Incense smoke. Mice of group IV was exposed to 6 g of Incense smoke. At the end of each month, five animals from each group are dissected.

Preparation of Histological and Cytological studies of mice lungs

All lung samples have been weighted and fixed at 10% formalin solution for one hour. After fixation each portion of specimen was dehydrated in a series of 80, 96 and 100 % ethanol solutions. Then the tissues were cleared in 2 changes of xylen solution and impregnated and embedded in paraffin. The tissue blocks were sectioned by rotary microtome (Leica). The 5-7 micrometer thicknesses of the transverse sections of lungs were stained with hematoxline and eosin for light microscopy examination **[18]**

Statistical analysis:

Statistical analysis was performed using t-test and ANOVA to determine significant differences in weight of mice lung between different exposure intervals.

3- Results:-

3.1-Weight of mice lungs after daily exposure to different concentrations of Incense smoke for 30, 60, 90 days

A slightly increase in weight of mice lungs were reported in all mice groups exposed to different concentration of Incense smoke comparing with the control group (Fig.1). The lungs weight was significantly increasing by increasing both of the concentration of the Incense smoke and the exposure time by using statistical analysis ANOVA. No significant differences were recorded by using t test (P value> 0.05).

3.2- Histological changes of mice lungs after exposure to different concentrations of Incense smoke

Control group (I)

The lung tissues of control group was formed of minute air spaces called alveoli. The histological investigation showed the presence of smooth muscle in the wall of air sac of the bronchi, bronchi final, bronchi respiratory, channels liposome, bags liposome and vesicles, and a blood vessel (Fig 2a). In addition to the normal mice lung illustrated the bronchi where lined with a single layer of epithelial cells, which contain cells of Clara (CC) (Fig 2b)

Mice that exposed to 2 gm of Incense smoke (Group II)

Histological studies on mice lungs exposed daily to 2 gm of Incense smoke for 30 days showed some dilation of the lung tissues associated with focal emphysema at specific site of lungs comparing with control group that not reported any of the previous changes (Figs. 2a, b). This emphysema leads to accumulation of the exhalation air at target lungs which cause a rupture in some alveoli (Fig. 3a). These changes were increased by increasing the exposure time to Incense smoke so by exposing mice to Incense smoke for 60 days changes were observed at the basement cells of the alveoli. Cell hyperplasia was reported with changes in the nucleus. Also congestion of lungs blood vessels was observed in many site of lung section (Fig. 3b). On the other hand, lung samples had been obtained from mice exposed to the Incense smoke for 90 days showed all the previous changes in addition to a severe congestion of lungs blood vessels. Moderate increases in the lymphoid cell were observed prebronchial as a reaction for all the previous changes. Secretions inside the alveoli cavities were reported. (Figs. 3c, d).

Mice that exposed to 4gm of Incense smoke for 90 days (Group III)

Histological investigation of sectors that have been obtained from the lung tissue of mice prone to 4 gm of Incense smoke daily, showed presence of severe changes in lung tissue which is similar to changes observed in group II. Lungs sections obtained from mice exposed daily to 4 gm of Incense smoke for 30 days showed an edema and changes at the basement layer of the alveoli tissue. Some alveoli reported decrease in their size while others showed size distension. (Figs. 4a, b). When time of exposure to Incense smoke was increased to 60 days, sever distension and lymphocyte infiltration observed in the alveoli of mice lungs sections. (Figs. 5a, b). Hyperplasia proliferation and sever hemorrhage was observed in all lung sections obtained from mice after the exposing to Incense smoke for 90 days. Also, both alveoli congestion and distension were reported at the same sections. (Figs. 6a, b).

Mice that exposed to 6g of Incense smoke for 90 days (Group IV)

Sections of mice lungs that exposed to 6 g of Incense smoke showed histological change as similar as those changes that have observed and reported in

the two previous cases to mice (groups II and III). Mice have exposed daily to 6g of Incense smoke for 30 days showed an accumulation of inflammatory cells inside alveoli cavities where alveoli seems to be smaller in size than others. In addition, severe hemorrhage, Edema, and alveoli rupture were reported as well as metaplasia was observed due to an increasing in the thickness of the alveoli wall (Fig. 7). Furthermore, by increasing the interval time of exposing to Incense smoke to 60 days, mice lungs showed necrobiotic changes of the bronchial epithelium with presence of mucinous secretions and some necrotic cells in both the lumen of the alveoli and bronchioles. Peri-vascular alveolar emphysema with fibrosis of the septal wall was reported. (Fig. 8). Simultaneously, exposure to Incense smoke for 90 days, histological changes recorded an increasing in the proliferation of the cells lined alveoli associated with hyperplasia. In addition, congestions, an increase in the secretions and dark brown granules were filled the alveoli cavities, (Figs. 9a, b).

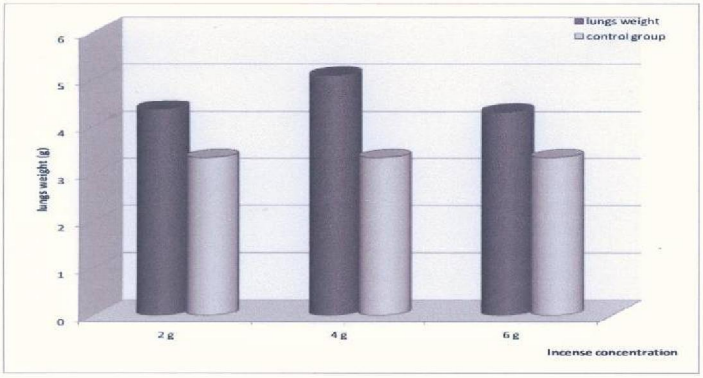

Fig 1: Differences between mice lungs weight daily exposed to different concentration of Incense smoke for 90 days.

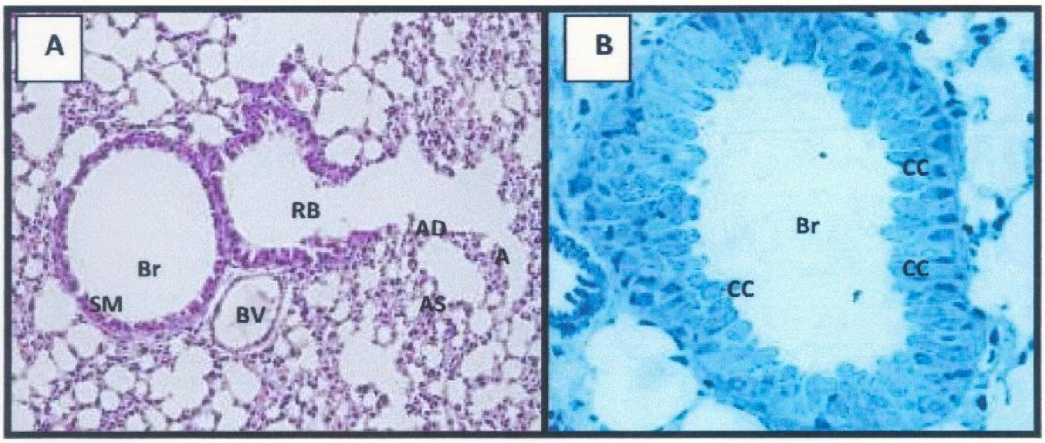

Fig. 2:- Semithin lung section lungs from the control group (A) showed smooth muscle (SM) in the wall of SAA air (Br), bronchi final (TB), bronchi respiratory (RB), channels liposome (AD), bags liposome (AS) and vesicles (A), also showed a blood vessel (BV), H & E (× 400).

(B) Showed SAA air (Br) of normal mice lung, where lined with a single layer of epithelial cells, which contain cells of Clara (CC), Toleudin blue (× 40).

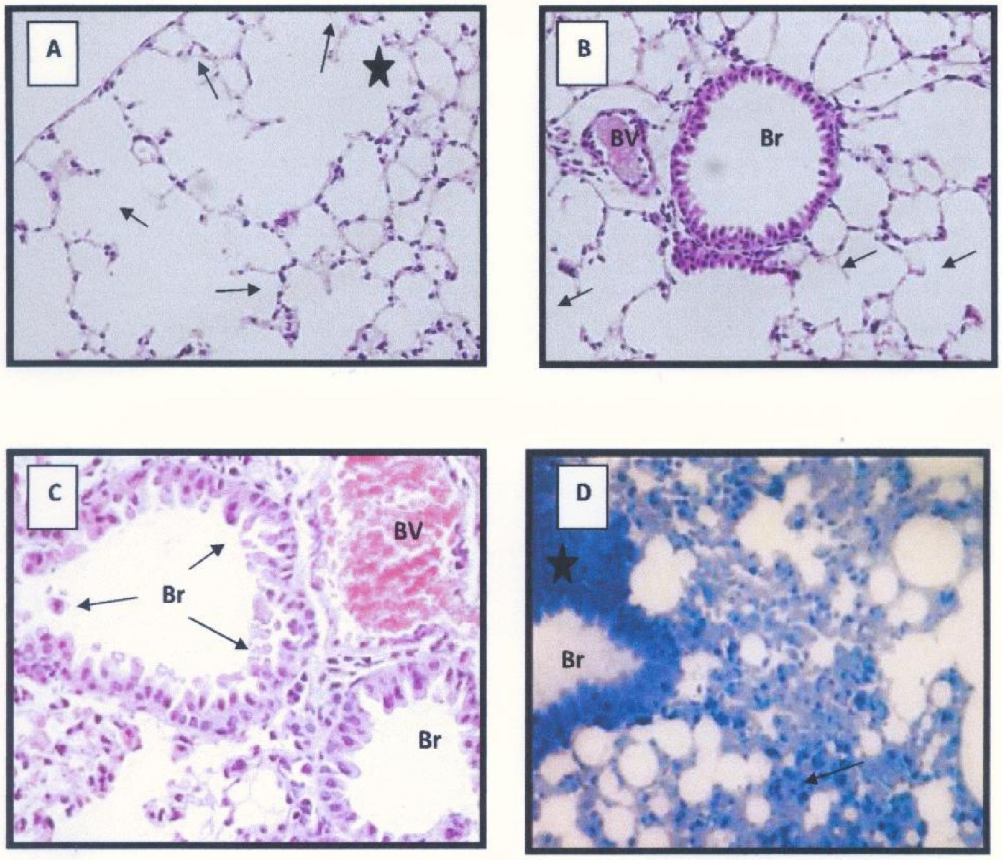

Fig. 3:- Semithin lung sections lungs from mice that exposed daily to 2gm of Incense smoke (A) Semithin section of the terminal bronchioles after 30 days from exposing to Incense smoke, showed over- distension (→) of the alveoli and some of the alveolar cells type II showed hypertrophied with numerous vacuoles (★). Mag. 40 X. **(B) Semithin section of mice lung exposed to Incense smoke for 60 days** indicate a change in the form of cells lining the bronchi (Br) and the emergence in some cells and change the shape of nuclei ,and note the occurrence of congestion in the blood vessel (BV), It also shows the breadth of rupture and in some vesicles (↗) H & E (× 400). **(C) A microscope optical sector in the lungs of mice in the group II exposed to incense daily for 90 days** shows the presence of congestion in the blood vessel (BV), and some cells are separated cells and filled (↗) the bronchioles cavity (Br). H & E. 400X. **(D)** Also, same group showed mild to moderate bronchial epithelial proliferation (★), the lumen is filled with mucinous secretions, peri-bronchial mild lymphoid reaction (↗), congestion of septal capillaries, patches of alveolar collapse and other showing over distention. Mag. 400 X.

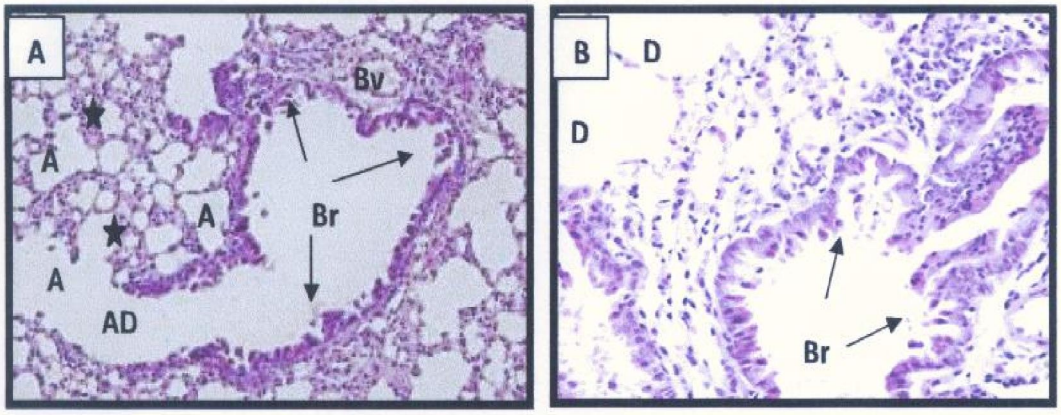

*Fig 4: Semithin section of mice lungs exposed daily to for 4 g of Incense smoke daily for 30 days showed the presence of SAA air (Br), channel liposome (AD), vesicles (A) and blood vessel (BV). Also recorded decreasing in the size of some alveoli (★) and presence of some falling cells (↗) in the cavity of bronchioles **(A)** H & E. 200X. **(B)**. showed swelling in some alveoli (D). H & E 200X.*

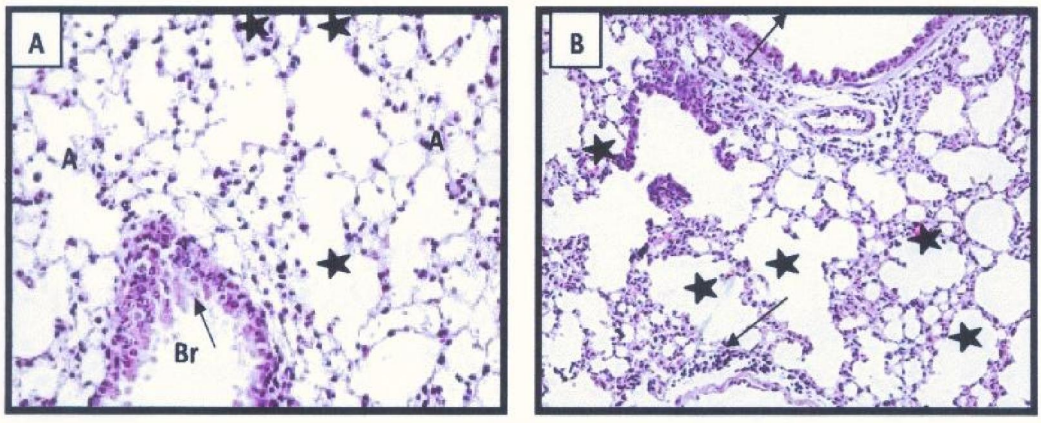

***Fig. 5:- Semithin sections of lungs mice belong to group III which daily exposed to 4 g of Incense smoke for 60 days. (A)** Showed emergence of some falling cells and clusters of mucous secretions (↗) in the alveoli (A), also shown by swelling Distension in some vesicles (★) H & E .400X. **(B)** Showed swelling of alveoli (★) associated with lymphocytes infiltration due to an inflammation inside lung alveoli (↗). H & E. 200 X.*

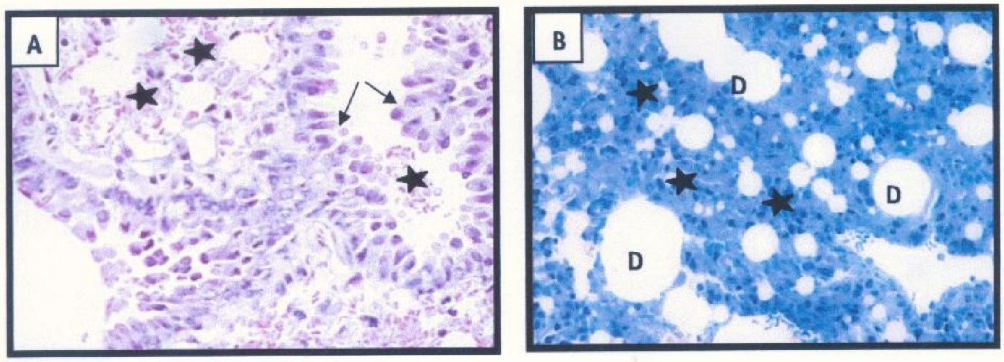

Fig. 6:-Semithin sections of lung mice belong to group III that exposed daily to Incense smoke for 90 days. *(A) Showed a moderate proliferation of the Clara cells (→) with presence of hemorrhage (★) either in the lumen of the bronchioles or in the alveolar lumina proparia, H & E.400X* ***(B)*** *Showed a congestion of the septal capillaries (★) with presence of mucinous secretion in the alveolar lumen of some alveoli and over distension (D) of other alveoli,* ***T.B****. Mag. 400X.*

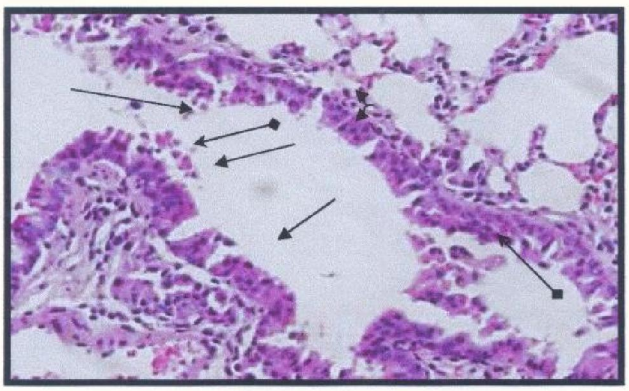

Fig. 7: A sector of mice lung exposed daily to 6 gm of Incense smoke daily for 30 days showed an increase in the thickness of bronchi wall (⟶) and changes in the shape of the cells during their growth and development of metaplasia (⟶), as there are cells falling inside the alveoli cavity (⟵) H & E 400 X.

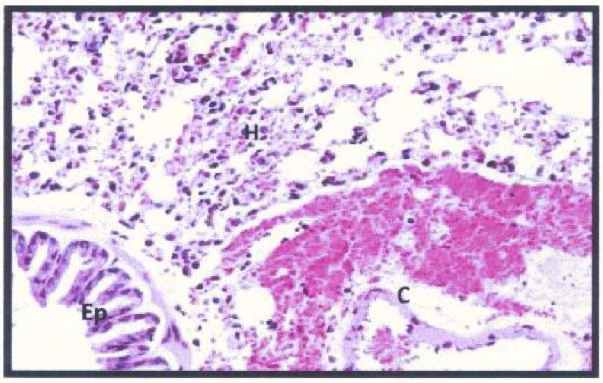

Fig. 8:- Sector of mice lungs exposed daily to 6 gm of Incense smoke for 60 days showing a sever hemorrhage (H) in the lung tissue and congestion of blood in the lung tissue (C), as it appears part of the lining of SAA air was noted where an increase in Flections epithelial layer (Ep). H & E (× 400)

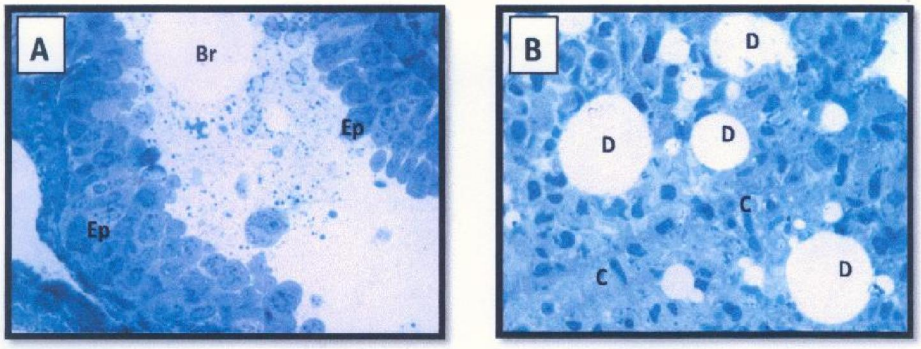

Fig. 9: Sections of mice lung belonging to group IV exposed for 90 days to Incense smoke. (A) showed prominent (Ep) proliferation of the bronchial epithelium where it formed by more than five layers, the lumen of the bronchioles (Br) containing moderate amount of mucin secretion containing deeply stained granules similar to that observed in the cytoplasm of the bronchial epithelium (Clara cells) and some of detached epithelial cells and most of the alveolar lumen filled with large reacting cells similar to the macrophage cells. **(B).** showed other alveolar lumina were distended with air (D), also the septal capillaries are congested (C).

4. Discussion

Many studies reported that Incense smoke composed of both volatile organic compounds (VOC) [1-3] and polyaromatic hydrocarbons [4, 5] that caused a harmful effect on population especially children and infants [13-15]. Our current research studying the effect of daily exposure to different concentrations of Incense smoke on weight of mice lungs where no significant increase in mice lungs weight had been reported either after daily exposure to 2, 4 or 6 g of Incense smoke in comparing with control group. This increase may related to normal growth of mice.

Current study examined the influence of the Arabian Incense on the risk of histological alteration of lung tissue in the experimental groups which exposed to different Incense concentrations (2, 4 and 6 g). Changes intensity gradually increased by increasing both or either period time of exposure and concentration of Incense smoke inhaled by female mice. Mice lungs tissues have been demonstrated an expansion and swelling of some lung alveoli combined with focal emphysema (distension). Although this distension causes decreasing size in some vesicles, it cause increasing sizing of others that lead to rupture of alveoli wall. Furthermore, tissue lungs showed an occurrence of hyperplasia in epithelial bronchi which are composed of several layers and as a result raise the epithelial cells bronchi due to chemical irritants resulting from the burning of Incense. Sever hemorrhage and congestion of both blood vessels and capillaries have observed inside the alveoli and some areas of the lung tissue combined with infiltration Edema. This current observation were confirmed with some studies carried out on male albino rats exposed daily to 4 grams of Incense smoke for 14 weeks, where many alveolar capillaries were distended and contained several raw of erythrocytes reflecting the hyperemia of the pulmonary vasculature. Also they noticed the occurrence of the hyperplasia in P2 cells by the crowds of nuclei of the proliferated cells and identified by the irregularly shaped nuclei which had condensed chromatin. Cellular hyperplasia caused thickening of the alveolar walls [19]. Confirmed to our results, another study were reported a distended capillaries, as a result of daily exposure of male albino rats to 4 gm of Incense smoke for 14 weeks every day. On the other hand, point of difference was noticed that vesicles cavity was containing red blood cells leaking from capillaries bloated [20].

Current study, have demonstrated an increase in lymphoid tissue around the bronchi where an accumulation of lymphoid cells have observed peribronchial and this indicates the stimulation of inflammatory cells of the lung. In addition, presence of mucous secretions, cells separation and falling have reported inside the cavity of bronchi. This current result were supported study explained that the Incense is causing the pneumonia and the emergence of clusters of lymphocytes in the site of infection in male rats when exposed to Incense smoke Ghali and another cheap one called Buzoilia Boswellia tree. Also, they noticed some of the bronchi contain the debris and the remnants of the cells falling after exposure to the incense smoke [21-22]. Other study was reported that stressed the high level of risk increase the symptoms of acute inflammation on the respiratory tract in workers temples who are exposed continuously to a high percentage of contaminated material, the various emissions from the burning of Incense [23-25].

Conclusion:

It was clear when you return to the global search engines and databases, that this study is the first of its kind and there are no previous studies for comparison. Based on the above findings, we conclude that continuous exposure to Incense smoke causes harmful effects in pulmonary system specially lungs alveoli. Such harmful effects increase by increasing both period time of exposure and the concentration of Incense smoke. Therefore, we recommend that Incense should be used only in open places to reduce its harms. This research derives its importance from the fact that Incense is heavily used in Saudi Arabia in the absence of thorough studies of its effects on health. Hopefully, this study would fill this gap.

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