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# Histological Changes in Tissues of Trachea and Lung Alveoli of Albino Rats Exposed to the Smoke of Two Types of Narghile Tobacco Products

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**Abstract**

Undoubtedly, narghile smoking has become a common social practice in the Arab region, and a growing phenomenon in the whole world. This study is an attempt to reveal the effects of narghile smoking on the cellular level, through exposing a group of experimental albino rats to the smoke of two types of narghile tobacco-derived products: flavored (moassal) and unflavored (tumbak), for three months on a daily basis, using a specially designed smoking machine. The most prominent histological changes were an abnormal proliferation in the epithelium of trachea, disruption of its cilia, and a marked hyperplasia in the connective tissue of lung alveoli. Finally, further research should be done to give definitive conclusions about the product with an overall stronger effect. However, based on our experiment, smokers could be advised not to smoke on a daily basis and in poorly ventilated areas.

**Keywords**: Narghile smoking, trachea, lung alveoli, moassal, tumbak, cilia.

## 1. Introduction

Water-pipe (narghile) is a generic name which refers to any apparatus involves the passage of smoke through water before inhalation (Al-Safi *et al*., 2009). Water-pipe smoking is currently considered a fashionable way of tobacco leaves consumption, especially among the present-day water-pipe smokers including trendy youth, university students, and even high-school-aged children, although it used to be as a pleasurable pastime of older and retired people (Onder *et al*., 2002; Knishkowy and Amitai, 2005; Neergaard *et al*., 2007).

Primarily, there are two types of narghile tobacco products: the flavored one which could be either moassal (also known as tobamel) or jurak, and the unflavored type, called tumbak (or ajamy). Both types are tobacco-based; the flavored type contains lesser amount of tobacco than tumbak. Tumbak is the one that is purely made of moistened shredded tobacco leaves, usually soaked in water before being squeezed and packed in the bowl of the narghile (Chaouachi, 2009). In addition to tobacco, moassal contains molasses, honey, or other syrups, together with glycerol, and flavoring essences (Chaouachi, 2009; Chaouachi, 2010).

Upon passing over the charcoal and through the tobacco, the heated air becomes loaded with the combustion products of charcoal, as well as a variety of products from the heated tobacco, forming the mainstream smoke (MSS) aerosol, that consists of both gaseous and suspended particles in the form of liquid droplets, containing a wide variety of condensed organic compounds (Knishkowy and Amitai, 2005). Then, the smoke will bubble into the water jar, being cooled and diluted there, and finally a post-bubbling MSS is carried through the hose to the smoker (Knishkowy and Amitai, 2005).

Although a number of adverse health consequences have been epidemiologically associated with the use of narghile smoking as heart disease and oral cancer, other fields of research, especially histopathology is very limited (Akl *et al*., 2010). However, most of the corresponding studies were led in the past decades, and the authors of recent reviews have not realized that the products were either not detailed or not the one of growing concern (flavored moassal with a certain type of charcoal (quicklighting). This has resulted in a growing global confusion, including in meta-analysis (Chaouachi, 2011; Neergaard *et al*., 2007).

Hence, among the scarcity of histopathological research about narghile smoking, this study may increase the public concern about the narghile use, hoping it would help to uncover the negative face of this smoking method, through revealing the potential adverse effects of narghile smoke from two tobacco products, that differed principally in their components, on some histological parameters, that weren't highlighted by literature on selected tissues in an animal model (albino rat).

## 2. Materials and Methods

### *2.1. Experimental animals*

Wistar albino male rats, *Rattus norvegicus*, with an average weight 215±2.5g were used. The animals were obtained from the University of Jordan colony and

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maintained under optimal conditions including diet and temperature.

*2.2. Histological study design*

This study is based on the chronic exposure of thirty experimental albino rats to the post-bubbling narghile MSS, coming from the complete heating/burning of 20 g from one of either two different narghile tobacco products: moassal or tumbak, for a period of 3 months, one session a day. An automated smoking machine as discussed in (Shraideh *et al.*, 2011) was used to expose the rats to narghile MSS. Each cycle of the smoking regimen lasts for 90 seconds and consists of three successive steps, operating as follows: Narghile smoke is drawn through the inhalation chamber continuously for 30 sec. An inlet to fresh air is then opened, allowing fresh air to be introduced instead of smoke, which will be washed out of the chamber. The washing out process will also take 30 sec. In the last 30 sec, the vacuum pump will be turned off, and rats will be allowed to breathe fresh air, normally (Shraideh *et al.*, 2011).

Narghile water was changed, and the tube was cleaned with distilled water after every experiment.

Albino rats were divided into 3 equal groups. The first group was the air-exposed control one, the second contains rats that have been exposed to moassal smoke, and the third group exposed to tumbak smoke. Following the exposure period, a histological examination by light microscopy of tissue pieces from the middle of tracheal segments and the anterior aspect of the right middle lobe of lung tissue was done. For each type of tobacco product, tissue pieces were taken from three randomly-selected albino rats.

*2.2.1. Protocol of light microscopy*

After overnight recovery from the last smoke exposure, rats were sacrificed by ether anaesthesia, and tissues of trachea and lung alveoli were gently dissected out, washed well with normal saline (0.9% NaCl), and fixed in 10% salined formalin. Using an automated tissue processor, tissues were fixed, dehydrated, cleared, and finally infiltrated by a hot liquid paraffin wax. To be ready for sectioning, tissues were embedded in paraffin. A ribbon of tissue sections were then obtained on a manual rotary microtome (Spencer 50) at 5 µm thickness. Two baths of 30% ethanol and a hot tap water were used to overcome the folding tendency. Thereafter, tissue sections were loaded on a glass slide meshed with egg albumin, dried, stained with classical haematoxylin and eosin stain (H&E), and finally mounted using Distyrene, Plasticizer, and Xylene (D.P.X.) mountant.

## *3.* Results

*3.1. Effect on the trachea*

Control sections showed healthy ciliated pseudostratified columnar epithelium, mucosal and fibroelastic layers normally seen in tracheal tissue (Figure. 1).

*3.2. Moassal smoke-exposed group*

The tracheal mucosa of this group was adversely affected; showing an increase in the number of epithelial cells, amalgamation of cilia, presence of inclusion bodies, and lymphocytic infiltration (Figure. 2).

*3.3. Tumbak smoke-exposed group*

Profound epithelial cell proliferation and lymphocytic infiltration were observed in tracheal sections of this group. Cilia were either amalgamated or almost lost in other sections (Figure. 3).

*3.4. Effect on alveoli of the lung*

Photomicrographs of lung alveoli from control animals revealed the normal appearance of their characteristic simple squamous epithelium (Figure. 4).

*3.5. Moassal smoke-exposed group*

Lung alveoli of this group showed clear thickening in the connective tissue, and lymphocytic infiltration (Figure. 5).

*3.6. Tumbak smoke-exposed group*

Lung alveoli of this group showed areas with profound thickening in the connective tissue (Figure. 6).

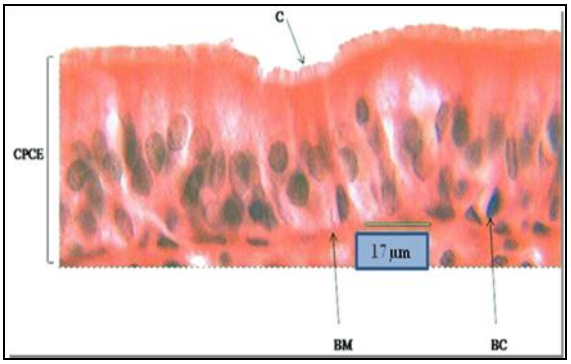


Figure 1. Section of normal tracheal tissue. CPCE: ciliated pseudostratified columnar epithelium, C: cilia, BM: basement membrane, BC: basal cell. H&E stain.

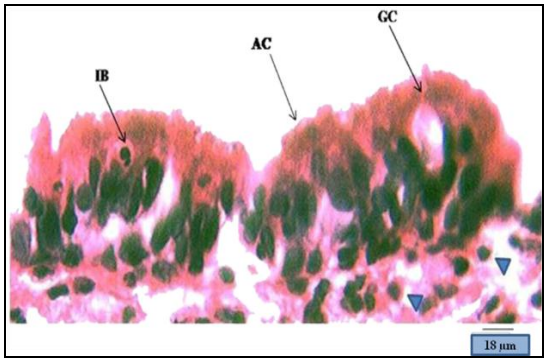


Figure 2. Section from the tracheal mucosa of moassal smoke-exposed rat, showing an increase in the number of epithelial cells, and lymphocytic infiltration (triangles). IB: inclusion body, AC: amalgamated cilia, GC: goblet cell. Triangles indicate lymphocytes. H&E stain.

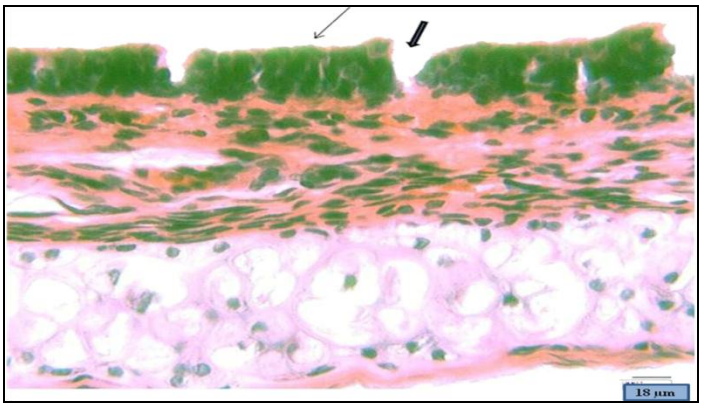


Figure 3. Trachea of tumbak smoke-exposed rat. The thick arrow indicates an area where the epithelium was disrupted. The thin arrow indicates a profound loss of the cilia. Epithelial cells are highly proliferated. H&E stain.

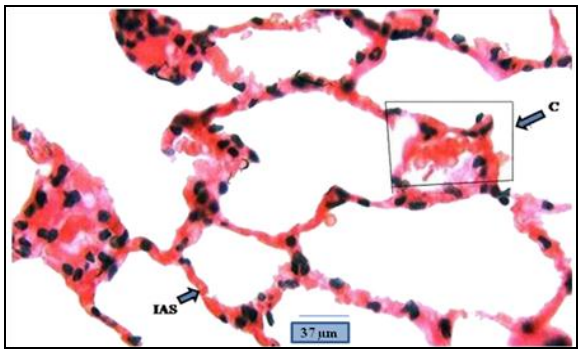


Figure 4. Control lung alveoli. IAS: interalveolar septum, C: capillary. H&E stain.

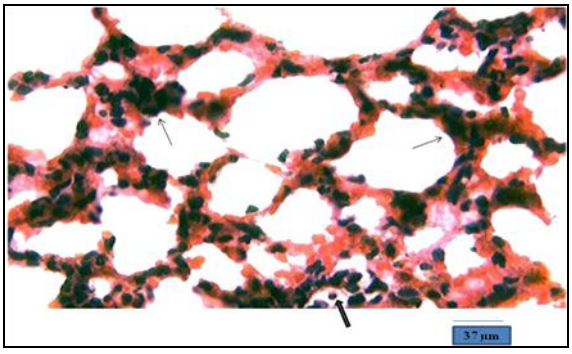


Figure 5. Lung alveoli of moassal smoke-exposed rat. Thin arrows denote areas with obvious alveolar wall thickening. The thick arrow indicates a lymphocytic cell. H&E stain.

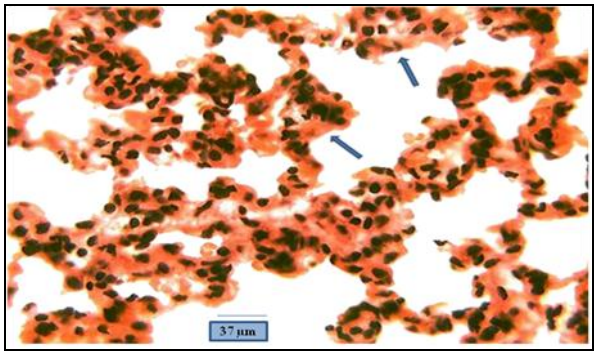


Figure 6 Lung alveoli of tumbak smoke-exposed rat. The arrows indicate obvious alveolar disruption. H&E stain.

## 4. Discussion

Cells of trachea and lung alveoli showed an adaptation by altering their pattern of growth, resulting in a hyperplasia. However, the limit of this adaptive response was exceeded, resulting in cell injury, which may be due to an oxidative stress; following the statement by (Ben Saad *et al*., 2010), that oxidative stress was increased significantly by regular water-pipe smoking, and the observation by (Sharma *et al*., 1997) of the presence of elevated levels of free radicals in peripheral blood neutrophils of water-pipe smokers. However, further support to the occurrence of oxidative stress associated with water-pipe smoking, which can lead to an imbalance in the production/ consumption level of reactive oxgyen species (ROS), would come from the following two studies: The first study, done by (Al-Numair *et al*., 2007),showed a significant increase in malondialdehyde -a biomarker for oxidative stress-, and a significant decrease in vitamin C - a potent antioxidant- in water-pipe smokers. The second study, done by) Wolfram *et al*., 2003), investigated the potential effect of smoking narghile on oxidation injury, by monitoring parameters of the (iso) eicosanoid system in narghile smokers. Two biomarkers of *in vivo* oxidative stress: 8-Epi-prostaglandin F2 alpha (8-epi-PGF2 alpha) and malondialdehyde were significantly increased after a single smoking session, and that repeated daily smoking induced a persistent long-lasting oxidation injury.Based on these facts, the following discussion is actually revealing the degree of cellular injury in two of the primary routes for smoke exposure (trachea and alveoli of the lung).

**Trachea:** The obvious disruption of the tracheal epithelium occurred by exposure to either moassal or tumbak smoke which caused powerful epithelial damage; will paralyze the cilia, enabling harmful foreign particles as dust or bacteria to remain in contact with the respiratory membranes for a prolonged periods, easily reach the lamina propria, where they can invade blood capillaries or lymphatic vessels, increasing the risk of toxic damage. Cracks that frequently observed within the tracheal epithelium are due to cell degeneration.

Ciliary amalgamation that can be viewed as part of epithelial disruption, may result from the hyperplasia of mucus-secreting submucosal glands, and may affect the airway clearance mechanisms. Inclusion bodies were observed, and they referred to any small amorphous blackish aggregate of smoke toxicants, primarily tar components.

The observed loss of cilia especially in tumbak-treated sections may be related to the high degree of nicotine it contains, through its effect on microtubules; polymerization / depolymerization of tubulin (Zenzes and Bielecki, 2004). Acetaldehyde and acrolein are suspected to play a role in the damage of cilia. Acetaldehyde was able to impair the ciliary function and beat frequency, by inhibiting ciliary dynein ATPase activity, and binding to ciliary proteins critical in the functioning of dynein and tubulin, whereas acrolein was found to adversely perturb the cilia by reducing its beat frequency, in cultured bovine bronchial epithelial cells (Dye and Adler, 1994).

**Alveoli of the lung:** Concerning the layers of cells lining the alveoli and the surrounding capillaries, are each only one cell thick, and are in very close contact with each other to facilitate gas diffusion between them; it is expected that the prominent thickening of the alveolar wall (pulmonary hyperplasia and hypertrophy) will compromise its capacity for gaseous exchange, resulting in a reduced gas transfer. According to the presence of extravasated erythrocytes, it could be simply justified by vascular injury. Lymphocytic infiltration may mediate the occurrence of inflammation. However, lymphocytes together with other inflammatory cells are frequently found in the bronchoalveolar lavage (BAL) of narghile smokers. For instance, Ourari, *et al*. (2006), have compared the cytology of the bronchoalveolar lavage BAL fluid (macrophages, lymphocytes, neutrophiles, and eosinophiles), and the lung function in 30 narghile users and 10 cigarette smokers. Researchers found that regular use of narghile induces a rise in the overall cell number in BAL. The increase does not seem to bring about significant changes in a number of lung function parameters when compared to cigarettes. The FEV1 and lung capacity were significantly higher. These results were also reported in the comprehensive critical review drawn by (Ben Saad *et al*., 2010).

## 5. Conclusion

No definitive conclusions can be drawn because, first, it is only an animal experimentation; second, it is based on exposing animals to smoke in a closed chamber. However, based on this experiment, smokers could be advised not to smoke on a daily basis and in poorly ventilated areas.

Regarding the smoking machine, we can suggest that the method of smoke exposure should be improved in a future study based on the same machine, but it could be set with different

parameters (for instance reducing puffing period, even if the animals may be exposed to a longer duration).

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