

Lung histopathological changes in Swiss mice exposed to narghile smoke

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ABSTRACT

Objective: To investigate the histological influence of waterpipe smoke exposure on lung tissues of Swiss mice during the periods of 7, 15, 30, 60, and 90 days.

Methods: The sample consisted of 60 animals, divided into 6 groups, one control group, exposed only to air, and the other experimental groups, daily submitted to water pipe smoke for 30 minutes through the whole body system, for 7, 15, 30, 60 and 90 days. After these periods, the mice were euthanized to obtain the tissue samples and subsequent preparation and analysis of histological slides.

Results: In the slide microscopy, the control group presented normal aspects. In experimental groups, exacerbation of inflammation was observed, there was an increased thickness of intra-alveolar septa, reduced alveolar lumen, areas of ciliary loss, and neovascular formation. And as the animals' exposure to smoke was extended, the progressive exacerbation of these pulmonary changes was noted.

Conclusions: The exposure to waterpipe smoke stimulates inflammation and cellular changes in lung tissues of Swiss mice and suggests that the longer the animals' exposure period, the more exacerbated this picture will appear.

Key words: waterpipe smoking; lung; inflammation; carcinogenesis

INTRODUCTION

The hookah, also known as “waterpipe smoke”, “hubble bubble”, “shisha” or “narghile”,^(1,2) consists of a smoking device composed of three main parts: a glass vase, a hose, and support for burning tobacco. The vessel contains water, the vertical tube is immersed in this water and the upper end of this tube is connected to the tobacco holder lit with charcoal. The vase is connected to another tube that reaches the hose through which the smoke is expelled by a series of puffs after bubbling into the water⁽³⁻⁵⁾.

Narghile smoking is a traditional method of tobacco consumption commonly practiced in the Arabian Peninsula, Turkey, India, and China. However, the popularity of its use has been increasing globally, especially among adolescents and young adults^(6,7). In Brazil, narghile is the tobacco product most used by young people except for conventional cigarettes⁽⁷⁾.

This growing practice is partly related to the belief that hookah use is less harmful than other forms of smoking, such as conventional cigarettes. This stems from the common and erroneous perception of users that the water present in the hookah device “filters” the smoke, making it less harmful and less likely to cause dependence when compared to cigarette smoke, although studies have shown that the use of water in the hookah does not change the nicotine level of the smoke. Another factor associated with this popularity is due to the type of tobacco used in the hookah, called “ma’assel”, which is sweetened and available in many flavors⁽⁸⁻¹¹⁾. The easy access to the hookah and its availability in social environments also contribute to the widespread use of this device⁽¹²⁾.

Taking into account the increasing consumption of hookah tobacco associated with the scarce amount of existing literature, the limited information, and the little public knowledge about the

repercussions generated by the hookah smoke^(8,13,14). The question arises of what would be the effects on the lung tissues caused by the smoke from this device. Given the limited studies available, which report that the hookah is not as harmless as is believed⁽¹⁵⁾, therefore needing more depth on the subject and transmission of this information to the population.

Regarding the effects caused by hookah smoke, there is a concern about possible harm, since tobacco smoke contains highly toxic chemicals and carcinogens, increasing the risk of developing cardiovascular diseases and cancer in the lung, oral cavity, esophagus, stomach, and other organs; also facilitating the transmission of infectious diseases since in one session, a single mouthpiece is shared with everyone^(2,11,16).

In this context, the objective of this study was to investigate the histological alteration caused by exposure to hookah smoke in the tissues of the lungs of Swiss mice during the periods of 7, 15, 30, 60 and 90 days.

MATERIALS AND METHODS

This study was previously approved by the Ethics Committee on the Use of Animals at the *University of Vale do Itajaí* - CEUA / UNIVALI, under No. 063/17.

In this experimental study, 60 female Swiss mice were housed in conventional cages, maintained in a 12-hour light-dark cycle, and with a daily change of food and water. Data collection took place from May 20 to July 18, 2018, at the *University of Vale do Itajaí* Educational Institution, in the histological research laboratory of block C5. The animals were removed from the central vivarium of UNIVALI one week before the experiment for the setting. Then, the animals were randomly divided into six groups of ten animals each, one being the control group and the other five groups, the experimental groups, which were exposed to conventional hookah smoke. The number of animals for each group was decided based on previous experimental literature⁽¹⁷⁾. The survey data were collected after the periods of 7, 15, 30, 60, and 90 days of exposure.

The animals in the experimental groups were submitted to the whole-body exposure system^(10, 11), through confinement in a chamber with a glass lid sealed with silicone, measuring 175x170x270mm and connected to a common hookah apparatus by a suction device, which blew the smoke into that chamber through a manual flow control⁽¹⁸⁾. The animals were exposed to the smoke of 4 grams of tobacco for conventional hookahs of the Mizo brand apple flavor (Apple Waterpipe Tobacco) with 0.5% percentage of unwashed tobacco and the bamboo charcoal

brand Bamboo Brasil with 33mm in diameter. The duration of the session was 30 minutes/day for each of the experimental groups⁽⁴⁾, during the periods of 7, 15, 30, 60, and 90 days. The concentration of tobacco smoke applied to the animals in the exposed groups was 35 ml for two seconds, while the other 58 seconds were for fresh air⁽⁶⁾, being distributed in total in 210mL for each mouse. The flow rate of the pump was manually adjusted to maintain the volume of 530mL / 2sec - breath specified by the Beirut Method - chosen regime because it approximates, on average, the topography of the human breath during the use of the hookah apparatus⁽⁴⁾. The exposure time of animals to smoke was selected from recently published studies that evaluated the cardiorespiratory effects of hookah smoke in humans^(19,20). The animals in the control group were exposed to air only, with the same conditions as the experimental groups.

To obtain tissue samples and later make histological slides, the animals in each experimental group were euthanized after the last exposure session of their respective period, with the control group euthanized together with the 90-day group. Euthanasia was performed using an anesthetic dose composed of 50µl of Xylazine (0.23g/ml) and 210µl of Ketamine (0.1g/ml) for every 10 grams of the animal's weight, that is, if the animal was weighing 20 grams, 100µl of Xylazine with 420µl of Ketamine was used (Figure 1). Tissue samples were obtained through surgery on mice to remove the lungs. The tissues were fixed in paraformaldehyde 4% in phosphate buffer pH 4 to 7 and properly processed and included in paraffin. The sections for making the histological slides were stained with hematoxylin and eosin (H.E.). The microscopic analysis of the slides was performed blindly and through it was performed a descriptive analysis of the characteristics observed in the slides.

RESULTS

In the histological analysis of the lung of the control group, alveolar sacs were found, constituted by alveoli and terminal bronchioles covered by epithelium that varies from cylindrical to cubic, from stratified to simple, ciliated or not, without alterations, presence of discrete chronic inflammatory infiltrate in some areas and respiratory alveoli with walls covered by simple pavement epithelium with cells called pneumocytes, within normal standards.

In the lung microscopy of the experimental groups, it was observed an increase in the thickness of the walls of the intra-alveolar ducts/septa, a reduction in the light of the alveoli, the formation of new cells, increasing the number of red blood cells in the region, areas of ciliary loss in the terminal bronchioles

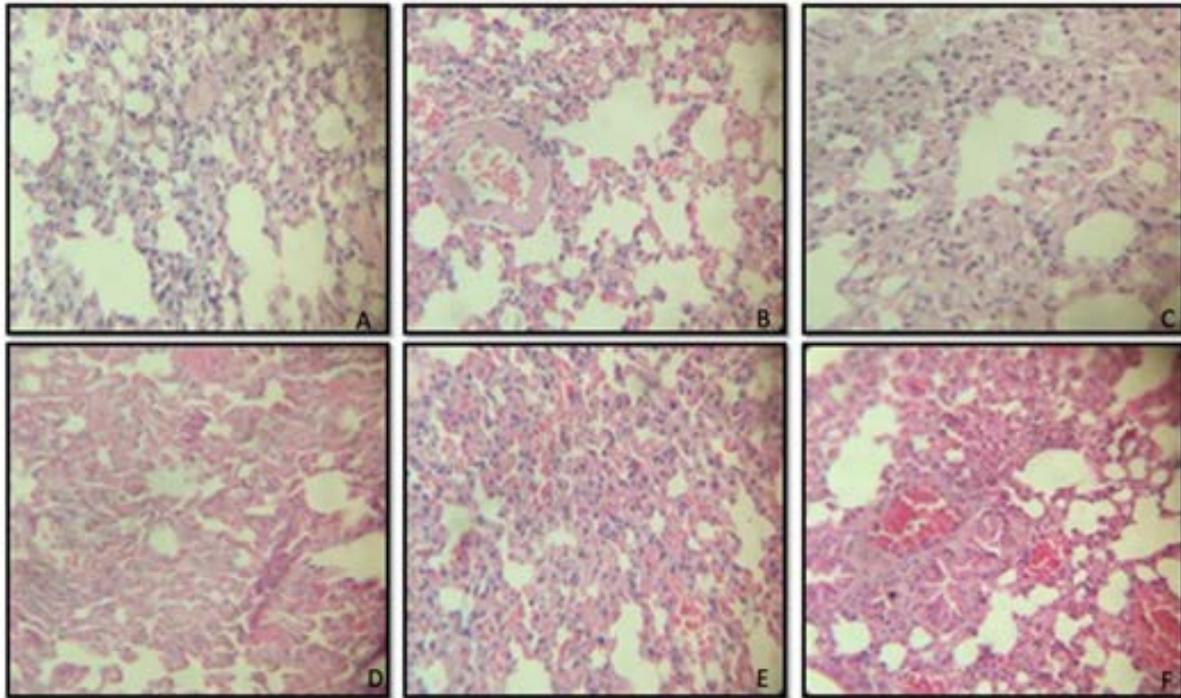


FIGURE 1 – Microscopy of a lung section stained with hematoxylin and eosin (H.E.) at 100 X magnifications. In the control group (A) alveolar sacs were found, without alterations and presence of discrete chronic inflammatory infiltrate. In the experimental groups at 7 (B), 15 (C), 30 (D), 60 (E) and 90 days (F) exposure period it was observed an increase in the thickness of the walls of the intra-alveolar ducts/septa, a reduction in the light of the alveoli, the formation of new cells, increasing the number of red blood cells in the region, areas of ciliary loss in the terminal bronchioles and presence of inflammatory infiltrate. And, as the period of exposure of the animals to smoke was extended, a progressive exacerbation of the pulmonary changes was noted.

and presence of inflammatory infiltrate with mononucleated cells (lymphocytes and mast cells) and other defense cells. And, as the period of exposure of the animals to smoke was extended, a progressive exacerbation of the pulmonary changes was noted.

DISCUSSION

Narghile smoking is a method of tobacco use traditionally associated with the Middle East. However, its use is becoming increasingly prevalent in the US and Western societies, especially among young people^(2,21). This consumption is driven by the scarcity of knowledge related to the harmful effects of hookah smoke and popular belief that it is less harmful to health than that of cigarettes, as it is “filtered” when passing through water^(2,8,14). And yet, there is the use of flavorings in the hookah tobacco that work as an attraction for its use⁽²²⁾.

Compared to ordinary cigarettes, studies show that the use of hookah is associated with the exposure of a greater amount of smoke, as well as the inhalation of a higher value of carbon monoxide and similar nicotine^(1,9,23). The narghile smoke contains more than 4800 different chemicals and particulate material resulting from its burning, in addition to a large amount of

carbon monoxide and nicotine, the presence of tar, chrysene, fluoranthene, anthracene, pyrene, and phenanthrene. Several of these substances are considered to be toxic and causal factors of cancer, cardiovascular disease, and addiction^(2,24). Thus, it appears that the hookah has a considerable variety of toxic chemicals and, in this sense, it is not as harmless as it is popularly known. And yet, it exposes its users to some of the same toxics as cigarettes, and therefore both methods of smoking probably share some of the same health risks.

In the lung microscopy of the animals in the control group of the present study, an aspect of normality was observed with areas of mild inflammation. Such result, according to Junqueira and Carneiro (2013)⁽²⁵⁾, can be explained by the air not being completely clean and, in this way, it is normal in the histological visualization of pulmonary tissues the presence of defense cells such as chronic inflammatory infiltrate, macrophages, neutrophils, and lymphocytes, as well as the areas of well-defined intra-alveolar septa.

From the histological analysis, the groups exposed to the hookah showed greater infiltration of inflammatory cells when compared to the control group, which coincides with the findings of other authors^(6,26,27). Inflammation is an early marker of hookah

smoke exposure that precedes airway dysfunction⁽⁶⁾ and, according to the increase in the exposure periods in the experimental research groups, there was a progressive exacerbation in the intensity of this inflammatory condition, which suggests that the longer the exposure time to smoke, the greater the severity of the damage.

In the analysis of the pulmonary inflammatory infiltrate of the experimental groups, the presence of mononucleated cells (lymphocytes and mast cells) and other defense cells was found, which is consistent with other studies in which they mainly identified neutrophils, lymphocytes, and macrophages^(10,26,28).

Histologically, an increase in the thickness of the walls of the intra-alveolar ducts/septa, a reduction in the light of the alveoli, in addition to the formation of new cells, and an increase in the number of red blood cells was observed in the lungs of the experimental groups. In this sense, in experimental studies by other authors, alveolar spaces and dilated alveolar ducts, thickening of the interalveolar septa, alveolar damage, and impaired lung function were observed^(28,29). Thus, the present study partially agrees with the findings of these authors, since they observed the dilation of the alveolar spaces instead of the reduction of the alveolar light. This divergence can be explained by the longer period of exposure of the animals.

In the lung microscopy of the experimental groups, areas of ciliary loss were also observed in the terminal bronchioles, which agrees with the existing literature, which mentions that the epithelium of the small airways of hookah smokers presented a higher percentage of secretory cells and cells. intermediate and a lower percentage of ciliated cells and basal cells⁽³⁰⁾.

In addition to the changes described in this lung study, the literature points out that long-term use of hookah increases the risk of cancer and cardiovascular diseases, generates chromosomal changes, and may also be associated with cases of alveolitis after tooth extraction, impacts to the fetus in the case of use during pregnancy and increase of respiratory diseases in non-smokers who live in the same environment. And, at the same time, the popular culture of sharing the same mouthpiece between multiple users can lead to the spread of diseases such as tuberculosis, herpes, and hepatitis^(2,31,32). It also suggests that the mutation in p53 (tumor suppressor protein) is associated with the use of hookah in squamous cell carcinomas, in premalignant lesions, and the normal mucosa of the oral cavity⁽³³⁾.

Finally, studies show that the prevalence of conventional

cigarette consumption is decreasing worldwide due to public control policies and that, in contrast, other tobacco products, such as hookahs, have become an international phenomenon when they appear on the market^(7,34,35). Therefore, further studies on the subject are necessary, as these are important to alert this public to the harmful effects that result from the use of hookah smoke, as well as serving as a parameter for the implementation of public health policies and for the creation of its legislation aimed at restricting and regulating the consumption of hookah tobacco.

CONCLUSION

The results of this study indicate that exposure to hookah smoke stimulates inflammation and cellular changes in lung tissues of Swiss mice and suggest that as the animals' exposure period is extended, that is, during 7, 15, 30, 60, and 90 days, the more exacerbated alterations will appear.

AUTHOR'S CONTRIBUTION

Carina Demarchi: work project and design or acquiring, analyzing and interpreting the data; initial essay writing and critical review of its content; final approval of the version for publication; responsibility for all aspects of the work.

Morgana de Souza: work project and design or acquiring, analyzing and interpreting the data; initial essay writing and critical review of its content; final approval of the version for publication; responsibility for all aspects of the work.

Laura Sagás de Souza: work project and design or acquiring, analyzing and interpreting the data; initial essay writing and critical review of its content; final approval of the version for publication; responsibility for all aspects of the work.

Fernando Lacerda Galli: work project and design or acquiring, analyzing and interpreting the data; initial essay writing and critical review of its content; final approval of the version for publication; responsibility for all aspects of the work.

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