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ORIGINAL ARTICLE

A Comparative Histological Study of Effects of Cigarette and Shisha Smoke on Lungs of Mice

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ABSTRACT

Background: The most popular way to take in tobacco is as cigarettes, which are now frequently accessible as vaporizers. When inhaled for an extended period of time, the smoke's respiratory tract irritants cause fibrosis, inflammation, and precancerous diseases. Nicotine is the main alkaloid found in both commercial and home-made tobacco products, making up 0.6-3.0% of the dry weight of the tobacco plant.
Aim: To compare the histological effects of cigarettes and shisha on the lungs of experimental animals.
Study design: Randomized control trial.
Methodology: A randomised control trial was used. In partnership with the National Institute of Health (NIH), Islamabad, the Department of Anatomy at the Islamic International Medical College in Rawalpindi developed and carried out this six-month study. 40 mature male BALB/c mice were allocated into three groups at random. Mice from the Control group C were housed in a fresh air- and smoke-exposed environment. Third Group CS was exposed to cigarette smoke, while Experimental Group SS was subjected to shisha smoke. Two exposures per day, five days per week, were administered. After a total of eight months of exposure, the subjects were dissected, the lung tissue was evaluated under a microscope, and the outcomes were compared across experimental groups. The quantity of carbon-loaded alveolar macrophages per unit was measured in the lung tissues. SPSS 20.0 was utilized to analyze the data. For quantitative histology data, mean and standard deviation were provided, while frequencies and percentages were provided for qualitative factors. The Pearson Chi Square test was used to determine the p value when comparing two groups and one way analysis of variance (ANOVA) was used to evaluate the mean differences between the control and experimental groups. Statistical significance was defined as a p-value of 0.05.
Results: In comparison to group CS, group SS showed significantly more fibrosis, peribronchiolar inflammation, and bronchiolar constriction. Additionally, there were more carbon-loaded alveolar macrophages in group SS than in group CS, and this difference was statistically significant.
Practical implication: As sheesha and vaping are now popular trends, this study will aid researchers in determining the risk linked with sheesha smoking.
Conclusions: Shisha use is not a risk-free substitute for cigarette use. Compared to cigarette smoke, it has a larger concentration of toxicants that alter tissue at a far higher rate.
Keywords: Shisha Smoke, Cigarette Smoke, Lungs Tissue, Mice and Histology.

INTRODUCTION

Despite anti-smoking programmes and awareness initiatives, tobacco consumption is rising globally. More than 8 million smokers die each year, and 7 million of those deaths are directly related to tobacco use, according to the WHO[1].

The most popular way to ingest tobacco is as cigarettes, which are now frequently accessible as vaporizers. When inhaled for an extended period of time, the smoke's respiratory tract irritants cause fibrosis, inflammation, and precancerous diseases. Nicotine is the main alkaloid found in both commercial and homemade tobacco products, making up 0.6-3.0% of the dry weight of tobacco[2]. It is the main factor driving many people to smoke tobacco since it causes dependence and addiction[3]. Recent studies on the use of electronic cigarettes, particularly after COVID infection, have revealed that smoking cigarettes is linked to an inflammatory storm that results in mucus hypersecretion, submucosal airway obstruction, bronchial epithelial hyperplasia, cilia and pulmonary connective tissue damage, gland enlargement, intraluminal, mucosal, and parenchymal inflammation[4]. Eosinophils, lymphocytes, macrophages and neutrophil levels have all increased. These cells discharge a range of proteases, including as cathepsins, matrix metalloproteinases, neutrophil and macrophage elastases, which break down the elastic and collagen fibres in the alveoli and cause emphysema[5].

Shisha smoking, sometimes referred to as water pipe smoking, is another popular method of tobacco use. Smokers frequently believe that because hazardous substances are filtered out by the water in which the smoke bubbles, it is less damaging than cigarette smoke[6]. Smokers of cigarettes take 8 to 12 puffs during the course of an average 6-minute period, inhaling a total of 500–600ml of smoke. Contrarily, water pipe sessions often last 30 to 60 minutes, during which the user may inhale 50 to 200 puffs, averaging 500 millilitres (ml) per puff. 50,000ml of smoke are produced throughout one session[7,8].

The charcoal used to burn water pipe tobacco may also cause the concentration of carbon monoxide to rise. Compared to cigarette smoke, which has a carbon monoxide to nicotine ratio of 16:1, water pipe smoke has a ratio of about 50:1. As a result, those who smoke water pipes are exposed to much more carbon monoxide while doing so[9].

Any type of tobacco consumption causes oxidative stress because it increases levels of reactive oxygen and nitrogen species (ROS & RNS). After repeated use, the free radicals cause irritation in the respiratory system, which progresses to precancerous and cancerous phases[10]. In addition, certain components of water pipes have been linked to respiratory illnesses, cardiovascular ailments, and malignancies (polycyclic aromatic hydrocarbons). unsteady aldehydes[11]. Smokers who use a water pipe have a 6-fold increased risk of getting lung cancer because nicotine causes apoptosis in bronchial epithelial cells and aortic endothelial cells and abnormalities in the cell cycle [12]. Mucous cell hyperplasia, sub mucosal glandular hypertrophy, neutrophil, eosinophil, macrophage, and lymphocyte infiltration, thickening of the basement membrane, and a significant loss of cilia are among the effects of chronic exposure on the trachea[13].

In their study of the harmful effects of two types of Shisha tobacco on the trachea and lung alveoli, Shraideh and Najjar noted significant lymphocytic infiltration and epithelial cell growth in

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tracheal sections. Cilia were either combined with other cilia or nearly eliminated in other areas. Alveolar wall thickening, extravasated erythrocytes, bronchoconstriction and increased alveolar diameter were among the negative consequences observed in the lungs[14].

The purpose of this study was to compare the negative health consequences of these two types of tobacco on experimental animals under controlled conditions at the tissue level. This was done in light of the rise in water pipe tobacco usage around the world, the lack of information on the exposure of water pipe smokers to toxicants, and the changes in smoking processes that result in different chemical compositions of water pipe smoke compared to cigarette smoke.

METHODOLOGY

After receiving approval from Riphah International University's Institutional Review Committee, the study was conducted at the Islamic International Medical College's anatomy department in Rawalpindi. Mice were exposed to smoke in the National Institute of Health (NIH) animal house in Islamabad. 40 mature male BALB/c mice, aged 10 to 12 weeks and weighing 35 to 45 g, were purchased from the NIH's animal home and housed in typical lab settings. Ten mice were housed in one cage, and they were all given a week to get used to it. They were maintained in a chamber that ranged in temperature from 22 to 240C and were allowed access to unlimited amounts of food and water. Mice with any form of disease, those younger than three months, and those weighing more than or less than 35g were excluded.

Three groups of mice were formed at random: Group C (control), which had 10 mice; Group SS, which contained 15 mice; and Group CS, which contained 15 mice. Groups SS and CS received whole-body inhalation smoke exposure for 8 weeks. (Fig.1 a & b). For Group SS, commercial Ma'assel with strawberry flavouring (30% tobacco and 70% honey or molasses) was used, whereas Group CS utilised nonfiltered cigarettes of a local brand. All the mice were given anaesthesia, placed in glass containers with cotton balls saturated in chloroform, and sealed after 8 weeks. This was done 24 hours after the final dosage of smoke. The lungs were removed after being dissected and stained histologically with hematoxylin and eosin, Giemsa stain for inflammatory cells, and mason trichome stain for lung fibrosis (Fig. 2).

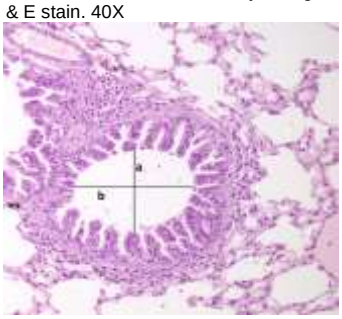
Fig. 1:

Figure 2: Photograph of an opened chest cavity of the dissected mouse Trachea (a), left lung (b) and right lung (c)

The number of carbon-laden alveolar macrophages per unit area, the terminal bronchiolar diameter in micrometres, and the presence or absence of peribronchiolar fibrosis were all examined in the lung tissues of experimental groups SS and CS. The ocular micrometre was used to measure the quantitative parameters.

The eyepiece micrometre was superimposed on the terminal bronchioles to determine the bronchiolar diameter. For bronchioles that were sliced transversely or almost transversely, The number of divisions between one basement membrane and the opposing basement membrane were counted in the eyepiece. The diameter of the bronchiole in micrometres was calculated by multiplying the average of these two values by 2.5. In each slide, this was done for four bronchioles that were chosen at random, and the mean was calculated (Fig. 3).

Figure 3: Photomicrograph of lung of mouse showing a terminal bronchiole. The diameter of the bronchiole was calculated by taking the average of reading a and b H & E stain. 40X



RESULTS

The control group's lungs displayed typical gross characteristics, including normal size, texture, and colour, as well as no evident abnormality. The lung parenchyma in the histological sections was normal, with homogeneous alveoli and a clean bronchiolar lumen. Alveolar macrophages were dispersed throughout the narrow interalveolar septa. The intrapulmonary bronchial mucosa had a well-developed muscular layer and hyaline cartilage plates that gave it a folded appearance. Simple epithelium without goblet cells lined the small bronchioles of the lungs.

Compared to the control group, the group CS mice's lungs were darker in colour and seemed mottled. The severe fibrosis in the pleural cavity was the most startling finding in the group SS of mice. To prevent damaging the delicate lung tissue, the cavity was carefully opened, and the lungs were carefully dissected out. They were also speckled and dark. Histological alterations were checked for on the slides of lung tissue.

Slides of the lungs from control group C did not contain any carbon-loaded alveolar macrophages. They were present in the CS and SS experimental groups. There were clusters of macrophages and inflammatory cells that were stuffed with tarry, black carbon particles. They were mostly seen in the parenchyma, near blood arteries, bronchioles, and alveolar walls. Compared to group CS, they were more prevalent in group SS. (Fig. 4 & 5).

In group CS mice, carbon-laden alveolar macrophages were lacking in 8 mice (53.3%) and identified in 7 mice (46.7%). Of the mice in group SS, 12(85.7%) had macrophages, while only 3(14.3%) did not (Fig. 6). (p=0.020) The difference was statistically significant. In the experimental groups CS and SS, the median number of carbon-laden alveolar macrophages/unit area was 3.0 (IQR: 2-4) and 5. (IQR: 3-5) respectively. Significant difference existed between the two groups (p=0.021) (Table 1). In contrast to group CS, group SS demonstrated a larger recruitment of carbon-laden alveolar macrophages. When compared to the control group C, the diameter of the groups CS and SS was much lower. On the other hand, group SS was shown to have a smaller bronchiolar diameter than group CS. The terminal bronchial's average diameter was 110.46 mm in group SS, 131.17 mm in group CS, and 167.95 mm in control group C (Fig. 7). Significant differences between each group were found across the board (p 0.001). In comparison to groups CS and SS, the control group's average terminal bronchial diameter was considerably larger (p = 0.001)

and smaller (p 0.001). Although there was a significant difference in terminal bronchial diameter between groups CS and SS (p=0.044) (Table 2).

Figure 4: Photomicrographs of lung of group CS (a-mouse no 7) showing few carbon laden alveolar macrophages **(a)** and lung of group SS (b-mouse no6) showing many carbon laden alveolar macrophages **(b**) H & E stain. Photomicrograph 40X

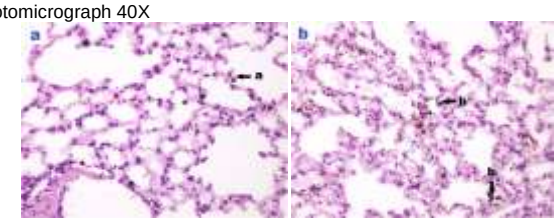


Figure 5: Photomicrographs of lung of group CS (a-mouse no 3) showing inflammation in parenchyma (a) and lung of group SS (b-mouse no 6) showing carbon laden alveolar macrophage (b) Giemsa stain. 40X

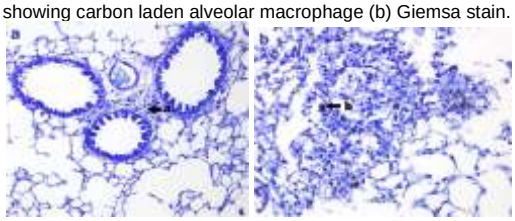


Figure 6: Qualitative parameters in lungs of group CS and SS

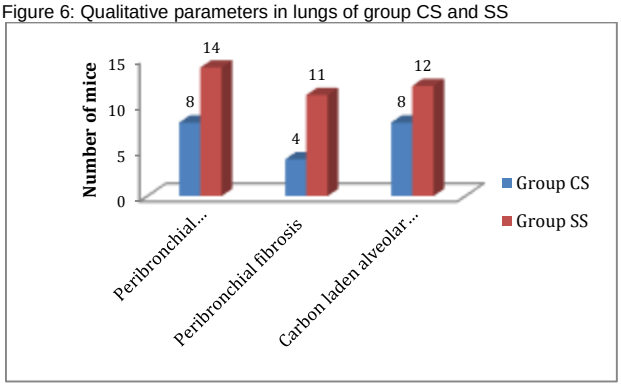


Table 1: Comparison of the groups' carbon-laden alveolar macrophage counts per unit area

Groups	Number of Carbon Laden Alveolar Macrophages/ Unit Area
Group CS(n=15)	3.00 (IQR: 2 – 4)
Group SS(n=15)	5.00 (IQR: 3 – 5)
p-value	0.021*

*p<0.05= significant

Figure 7: Mean diameter of terminal bronchioles (µm) in the group

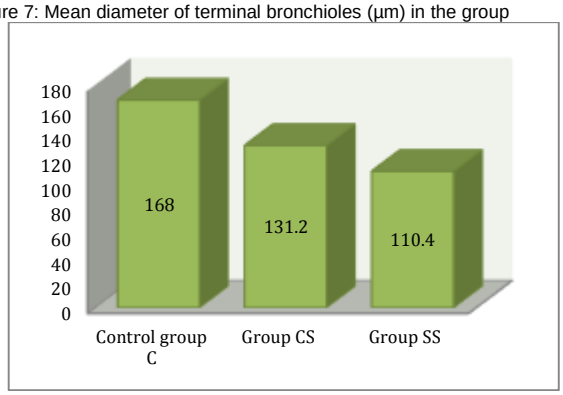


Table 2: Post hoc comparison of diameter of terminal bronchioles (µm) between the groups.

Groups	Mean Difference	p-value
Control group C vs.Group CS	36.782	0.001*
Control group C vs.Group SS	53.928	< 0.001*
Group CS vs.Group SS	17.145	0.044*

DISCUSSION

This investigation looked at how water pipe and cigarette smoke on the lung tissue of mice were compared. Histological lung sections stained with H&E and Giemsa revealed pulmonary inflammation in the experimental groups, with peribronchiolar and perivascular inflammatory cell infiltrates in the airways that were noticeably more pronounced in the water pipe group. Additionally, compared to the control group, the smoke-exposed groups had a significantly higher number of macrophages, neutrophils, and lymphocytes, according to the results. Several in vivo studies have observed these histological alterations[15-21].

As opposed to group CS, group SS had more pronounced peribronchiolar fibrosis, and this difference was statistically significant (p=0.011). There have been reports of minor airway narrowing brought on by fibrosis in the past.[14,19] In peri bronchial regions, type III collagen is said to have been deposited. Other signs of fibrosis were persistent inflammation accompanied by lymphocyte infiltration, mucinous purulent plugs, disrupted elastic fibres in the bronchial wall, and mucous purulent plugs. This supports the study's result that the area around small airways, particularly the terminal bronchioles, showed an increase in inflammation and collagen deposition.

The particles in cigarette smoke, according to Sangani[22], cause a rise in their chest X-rays, smokers' smaller airways have collagen deposition, and irregular opacities indicating this fibrosis are visible. According to Skold[23], the activation, proliferation, and contraction of fibroblasts in the connective tissue, as well as an increase in the formation of extracellular matrix, are the results of a bronchiolar epithelium's poor ability to heal after injury. Small airway fibrosis develops as a result of this. In his work on rats exposed to water pipe smoke, Shraideh[24] noted similar alterations, including a significant thickening of connective tissue in the exposed animals' lungs**.** Shisha smoke includes a significantly larger percentage of toxicants than cigarette smoke, claims Shraideh[25]. These toxicants harm the bronchiolar epithelium and increase inflammation, which leads to fibrosis because of aberrant type III collagen deposition.

Alveolar macrophages that had been exposed to carbon revealed both qualitative and quantitative changes. When compared to group CS, the presence of carbon-laden alveolar macrophages was considerably higher in group SS (p=0.02). The alveolar macrophage is a crucial link in the immunologic chain since it is a component of the body's mononuclear phagocytic system. In mice exposed to cigarette smoke, previous study documented an accumulation of pigmented macrophages[16]. Mice exposed to secondhand cigarette smoke had macrophage recruitment in their lungs, according to a different study by Woodruff et al[21].

The effective burden of tiny particles that reach the alveoli appears to be precisely adjusted by the macrophage delivery system[26]. The amount of the load, its chemical makeup, and the size of the individual particles can all have an impact on the output. Carbon particles and mucus exudates were commonly seen in the airway lumen of group SS animals. The source of the carbon particles in the lung parenchyma are these inhaled particles. According to Bowden[27] who claims that carbon particles have been demonstrated to cross Type I epithelial cells within cytoplasmic vesicles, this fact is corroborated. Interstitial macrophages phagocytose the free particles after they have passed the barrier.

Additionally, it appears that the effective stimulus that sets off these reactions is more closely connected to the quantity of particles introduced than to the total load[27].Compared to comparable doses of much bigger particles, little carbon particles

cause a substantially stronger macrophage response. In a comparison of the particle sizes produced by cigarette and water pipe smoke, when compared to cigarette smoke, Daher et al[28] found that ultrafine carbon particles in water pipe smoke were substantially smaller in size. This explains why group SS in the current study had a larger recruitment of alveolar macrophages. These tiny carbon particles cause more inflammation and alveolar macrophage recruitment. Bronchiolar diameter measurements revealed variations across study groups. The diameter of the experimental groups decreased as compared to the control group, but there was a significant difference between groups CS and SS (p=0.044). Smaller airway fibrosis or bronchiole-specific smooth muscle hyperplasia and hypertrophy are two causes of bronchiole constriction. According to this study, several bronchioles had fibrosis around them although the smooth muscle layer seemed healthy. Martin[29] and Skold's[23] research both came to similar conclusions.

Martin[28] states that, and the smaller overall cross-sectional area of the tiny airways is implied by the increase in expiratory resistance to airflow. Smoking-induced COPD appears to have airflow limitations in small conducting airways. Peribronchiolar fibrosis has been linked in numerous studies to the constriction of tiny airways in COPD patients. This backs up the study's conclusion. According to Skold[23], airway constriction in COPD patients is not well-established to be caused by smooth muscle hypertrophy. Mullen's[30] research has emphasised the significance of goblet cell metaplasia and mucus hypersecretion in the development of minor airway constriction. In this study, mucus exudates were discovered in the airways, but due to the short exposure time, no goblet cell metaplasia was observed.

After 8-week exposure period, Al Easawi[31] found that the bronchiolar width of the Shisha-exposed mice was smaller than that of the controls. This backs up the study's conclusion that group SS had significantly constricted bronchioles compared to the control group. This discrepancy between experimental groups can be attributed to group SS's more severe peribronchiolar fibrosis, which resulted in narrowing of the lumen. The results of the current investigation unequivocally show that shisha smoke has significant inflammatory consequences. In group SS, there were noticeable histological alterations in the lungs, including more fibrosis, bronchiolar constriction, inflammation, and the recruitment of carbon-loaded alveolar macrophages. This demonstrates that smoking shisha is not a risk-free substitute for cigarettes and is linked to pronounced lung inflammatory alterations.

Limitations of study: This study was only conducted on a small group, hence a larger study is required to extrapolate the results to larger populations. One of the restraints is financial limitations.

Conclusions: It was concluded that shisha use is not a risk-free substitute for cigarette use. Compared to cigarette smoke, it has a larger concentration of toxicants that alter tissue at a far higher rate.

Author's contribution: SI&HB: Overall supervision and Write up and literature review. **KA:** Statistics application, analysis literature review, help in write up. **HZ:** Literature review help in write-up.

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