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Original Research Article

Histological Study of Trachea Tissues of Rats Exposed to Cigarette and Waterpipe Smoking and the Ameliorating Effect by *Ammi visnaga*

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Abstract: Smoking remains a global health problem because it has negative impacts either on health or economy. Smoking has various forms or types of which cigarette and waterpipe smoking are mostly prevalent. The main objectives of the present study were to investigate the histological changes resulting from exposure to cigarette and waterpipe smoking on rat trachea, and to investigate the ameliorating effects of using *Ammi visnaga* in reversing the adverse effects of smoking. Animal smoking model was created based on male rats that were randomly assigned in to different groups (N=8). Groups included control group, cigarette smoking group, waterpipe smoking group, cigarette smoking group treated with *Ammi visnaga*, and waterpipe smoking group treated with *Ammi visnaga*. Smoking protocol was applied for 1 month. At the end of the experiment, animals were sacrificed, trachea was isolated and placed in formalin (10%) for fixation, and tissue sections were prepared using Hematoxylin and Eosin stain. Results showed that smoking either cigarette or waterpipe had negative impacts on tracheal tissue in terms of disruption of cilia, or amalgamation of cilia. Heavy infiltration of lymphocytes was observed. The use of *Ammi visnaga* showed health effects by lowering the infiltration rate of lymphocytes and partial recovery of epithelial cells and reversing impacts on cilia. Taken together, this study, May for the first time, showed the importance of using *Ammi visnaga* in the improvement of histological changes induced by smoking models on tracheal tissues of smoking rats.

Keywords: Waterpipe smoking, cigarette smoking, smoking model, rats, *Ammi visnaga*.

INTRODUCTION

The Trachea is a tube that transports air from the upper respiratory tract to the lower respiratory tract (Rokicki *et al*., 2016). The cervical trachea and the thoracic trachea are the two parts of the trachea. The trachea is made up of 15-20 hyaline cartilage rings (Aspinall, *et al*., 2009). A layer of pseudostratified columnar epithelium ciliated with goblet cells lines the trachea. Mucins are produced by goblet cells, which are unicellular glands that moisturize and protect the airways. Mucus coats the ciliated cells of the trachea, allowing the cilia to detect inhaled foreign particles and propel them to the larynx and finally the pharynx, where they are either ingested or ejected as phlegm**.** Mucociliary clearing is the name given to this mechanism. The ciliated cell has roughly 300 cilia, each with numerous mitochondria beneath them to supply energy. Brush cells, which have multiple microvilli connected to their apical surface, are another type of columnar cell (Young, *et al*., 2006).

Inhaling and exhaling gases from burning plant materials, particularly tobacco, is known as smoking. Cigarettes, cigars, chew, pipes, and water pipes are all used to consume it (Hoffmann and Wynder, 1986). There are two forms of smoke produced by tobacco combustion: mainstream smoke and sidestream smoke. The mainstream is a sort of smoke exhaled by a smoker while puffing on a tobacco product. The smoke released by a burning cigarette between puffs is known as the sidestream. The hazardous and carcinogenic chemicals in sidestream smoke are frequently higher than those in mainstream smoke (Stephen, 2010). The tracheal

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epithelium of albino rats exposed to cigarette smoke for three months showed dramatic histological alterations, including epithelial cell growth, cilia disruption, and the appearance of inclusion bodies (Ziad *et al*., 2013). The alveolar wall of the lung alveoli thickened significantly, with collapsed alveoli and blood extravasations. These findings suggest that nicotine-induced alterations akin to chronic tissue irritation may reduce alveolar gaseous exchange efficiency and predispose to neoplastic abnormalities.

Water pipe smoking (WPS) is a type of tobacco use that has been around for millennia but has recently exploded in popularity. WPS has long been a customary method of inhaling tobacco in Middle Eastern and southern Asian cultures. While cigarette use has decreased in Western countries in recent years, other tobacco products like as WPS have increased substantially over the previous two decades (Salloum *et al*., 2015; Jamal *et al*., 2018). WPS is often done in North America and Europe in cafés or bars located in most major cities (Darawshy *et al*., 2021).

Individual or group smoking of waterpipes is becoming a worldwide social phenomenon. Cafes and bars, for example. While the usage of cigarettes has decreased in recent decades, the use of non-cigarette tobacco products, such as waterpipe use, has increased (Jamal *et al*., 2018).

For example, a survey of 13–15-year-old in the Middle East found that frequent WPS was as high as 9–15 percent in this age range (Maziak *et al*., 2015). WPS has surpassed cigarettes as the most prevalent way of tobacco use among young adults and adolescents in the Middle East, and it is now second only to cigarettes in various other parts of the world (Maziak *et al.,* 2015).

Nicotine is a primary component found in all types of tobacco and is thought to be a major contributor to tobacco addiction. Nicotine concentrations vary depending on the type of tobacco used in waterpipe smoking, ranging from 67 to 713 mg per head. In addition to nicotine, waterpipe smoke contains nicotine-free dry particulate matter (known as tar) at a 10–200 fold higher concentration than regular cigarette smoke (Shihadeh *et al*., 2015).

In various organs, the effects of tobacco use on microvascular morphology have been regularly described. The thickening of arterioles in the trachea has been linked to tobacco smoke (Auerbach *et al.,* 1971; Darawshy *et al*., 2021).

Ammi visnaga L. (Visnaga daucoides Gaertn., Family Apiaceae) is an annual or biennial herb native to the Mediterranean region of North Africa, Asia, and Europe. It is also known as Khella Baldi or toothpick weed. Long ago, the herb was known to have been employed in traditional medicine. It is now utilized in modern medicine to treat a variety of ailments, including renal colic and cardiac insufficiency, and as an antioxidant, antifungal, and antibacterial, as well as having a larvicidal action on mosquito larvae. These pharmacological actions are attributed to the plant's valuable chemical contents, which include essential oil, polyphenolic compounds such as flavonoids, and -pyrones, primarily khellin and visnagin, according to peer-reviewed studies. Its essential oil is said to have antiviral, antibacterial, and larvicidal properties, and its flavonoid content is what gives it antioxidant properties. Its -pyrone component facilitates the transit of kidney stones and relieves renal colic, as well as having a relaxing effect on smooth muscle, especially that of the coronary arteries (Bhagavathula *et al*., 2015; Khalil *et al*., 2020).

Study objectives

The main objectives of the present study were to study the histologic changes associated with cigarette and waterpipe smoking on tracheal tissue and to study the impact of using *Ammi visnaga* in ameliorating the histologic changes of smoking.

METHODOLOGY

Experimntal Design

Male albino rats (*Rattus ratas*) weighted 50-180 g (6-8 weeks old) were obtained from animal house at the University of Science and Technology and maintained under optimal conditions of diet and temperature. Rats were randomly assigned to one of 5 groups (n= 8 per group), group 1 was negative control exposed only to fresh air, group 2 (cigarette smoking group), group 3 (cigarette smoking group and *Ammi visnaga* treatment), group 4 (waterpipe smoking group), and group 5 (waterpipe smoking group and *Ammi visnaga* treatment). Smoking programs were carried out as 1 cigarette/rat/day for 30 consecutive days. Water pipe smoking was carried out as exposure to flavored waterpipe coming from the complete burning of 20 g from one of moassal for a period of 30 days one session a day for whole body. Treatment by *Ammi visnaga* was carried out by giving 50 mg / kg *Ammi visnaga* seeds for one month.

At the end of the experiment, animals were sacrificed using ether inhalation for anesthetic purposes, and tracheal tissues were dissected and washed with normal saline. Tissues were then fixed in 10% formaldehyde for 24 hours. Tissue was then dehydrated in ascending grades of alcohol, cleared with xylene. Dehydration was achieved by passing tissues through a graded series of alcohol followed by two changes of xylene. After infiltration in paraffin wax, tissues were embedded in pure paraffin wax (Avti, *et al*., 2006).

Thin sections 5µm thick were obtained by microtome (Spencer 50). Finally, sections were

mounted on glass slides and stained with hematoxylin and eosin. Sections were examined and photographed using Zeiss photomicroscope1. Photomicrographs were taken using Moticam 2300 digital camera/3.0Megapixels.

RESULTS

Normal structure of rat tracheal tissue

As seen in figure 1, control sections had shown healthy ciliated pseudostratified columnar epithelium, and all other layers normally seen in tracheal tissue.

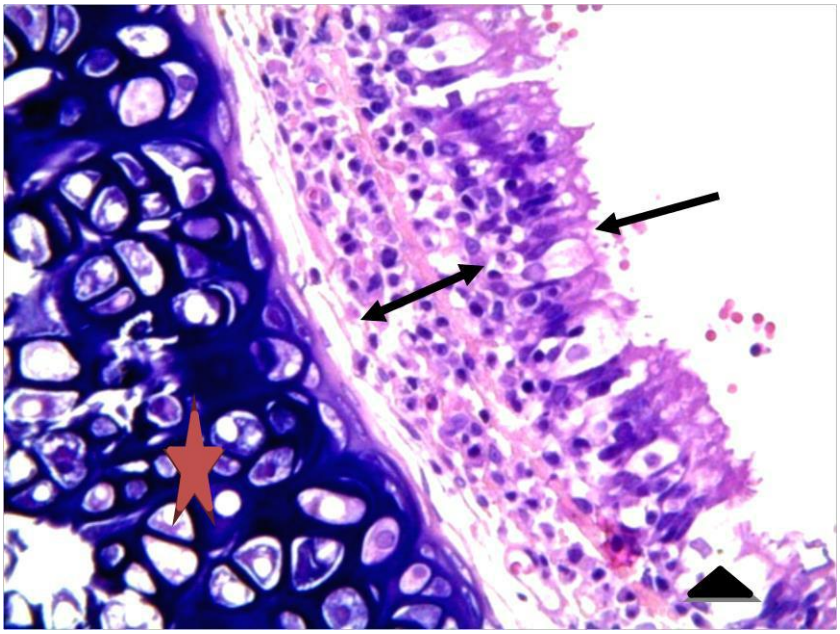

Fig-1: Normal tracheal tissue. Cilia (arrow) and goblet cell (triangle). Hyaline cartilage (star). Lamina propria (arrow with two heads). H&E stain. 400X.

Histological changes in rat's tracheal tissue by cigarette smoking

This group's tracheal mucosa was negatively damaged, with an increase in epithelial cells, cilia amalgamation, and the development of inclusion bodies. There was a lot of lymphocytic infiltration in the epithelial layer, which caused some structural alterations in columnar cells and goblet cells. The invasion of PMNL resulted in inflammatory conditions (Figure 2).

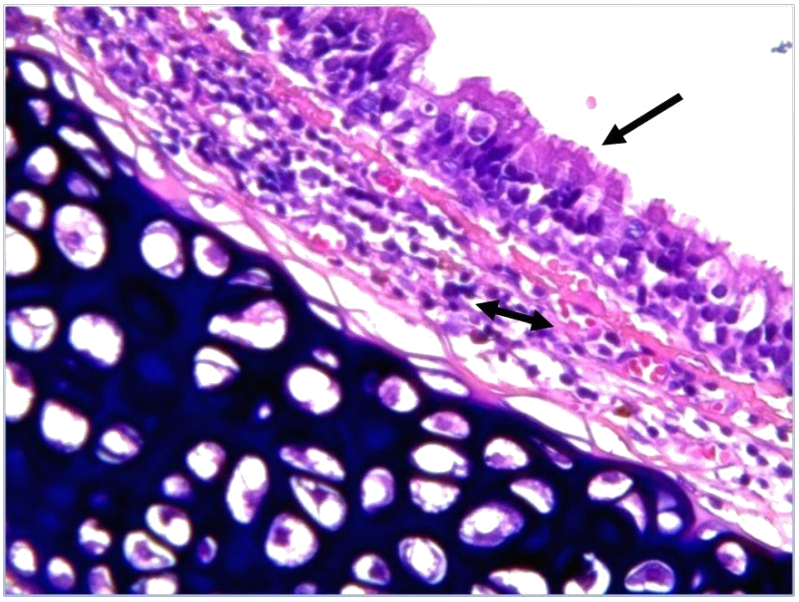

Fig-2: Tracheal tissue from Rat exposed to cigarette smoke. Infiltration of lamina propria with lymphocytes (arrow with two heads). And partially disrupted cilia (arrow). H&E stain. 400X

The effect of using *Ammi visnaga* treatment in the tracheal tissue of rats exposed to smoking

The treatment with *Ammi vinaga* seeds (figure 3) led to partial recovery of epithelial lumen but still associated with some inflammatory conditions as presented by the infiltration of PMNL and remaining congestion of capillary.

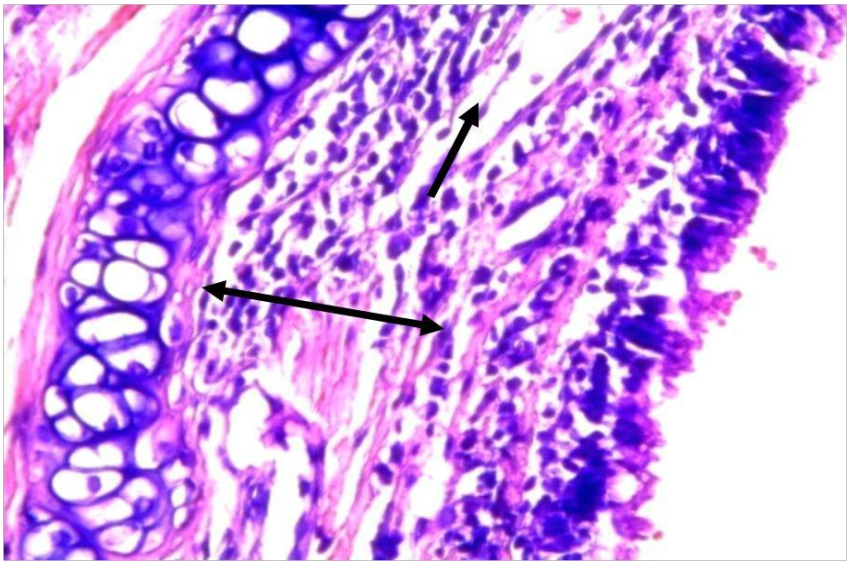

Fig-3: Tracheal tissue of rat exposed to cigarette smoke and treated with *Ammi visnaga* seeds. Congestion (arrow) and partially disrupted laminapropria (Arrow with two heads). H&E stain. 400X

The impacts of waterpipe smoking on the rat's tracheal tissue

This group's tracheal sections showed disruption and extensive epithelial cell growth, as well as substantial lymphocytic infiltration. Cilia were either merged or nearly obliterated in other sections. In the submucosal layer, there was also an aberrant rise in cell number. Blood extravasations and the spilling of blood from a vessel into the tissues around it are caused by capillary congestion diluted by blood (Figure 4).

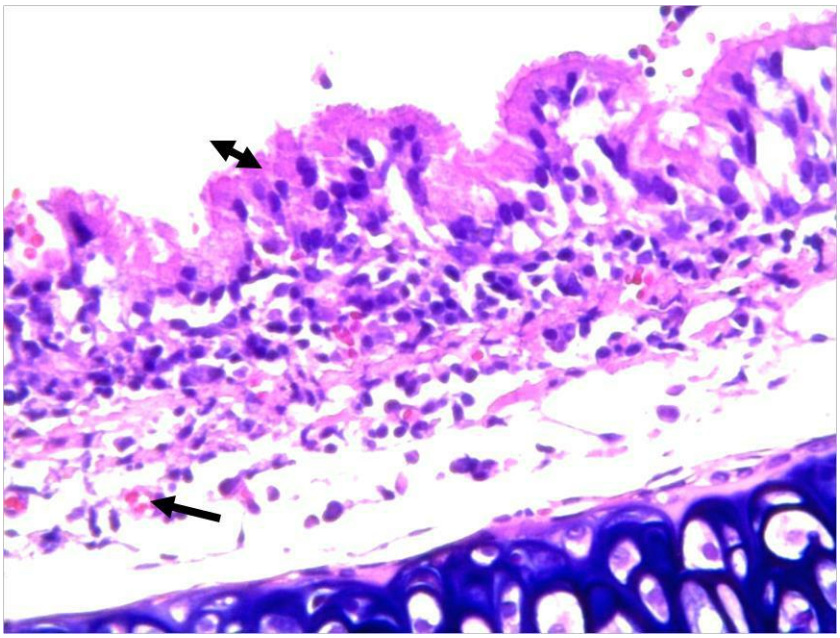

Fig-4: Tracheal tissue of waterpipe smoke exposed rat. Arrow indicates blood extravasations. Disrupted of cilia shown (Arrow of two heads). H&E stain. 400X.

The effect of using *Ammi visnaga* treatment in the tracheal tissue of rats exposed to waterpipe smoking

As seen in figure (5), the exposure of trachea to waterpipe smoking and *Ammi visnaga* seeds (figure 5) showed slight recovery with less inflammatory conditions as indicated by less infiltration of PMNL and eosinophilia but still had lost of cilia and congestion of capillary present.

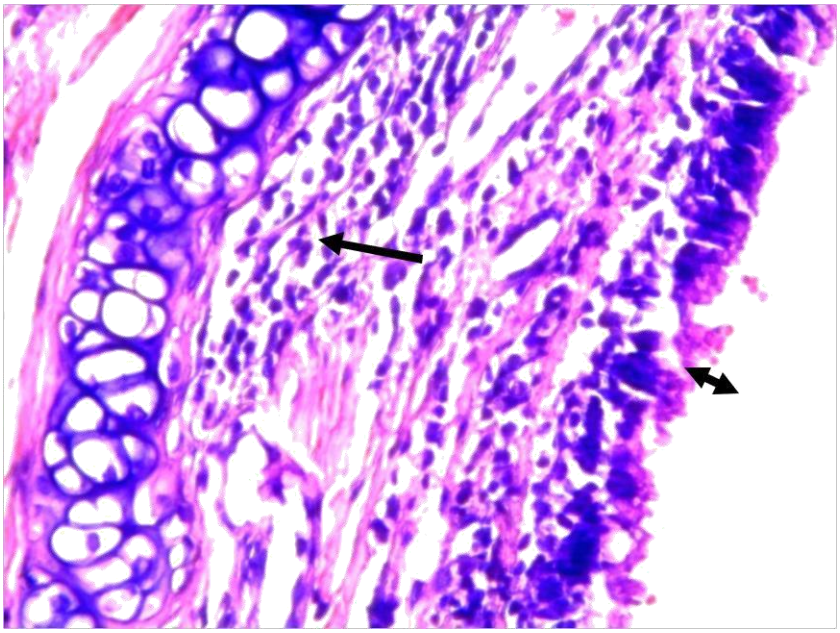

Fig-5: Tracheal tissue of waterpipe smoke exposed rat treated with *Ammi visnaga* seeds. Heavenly infiltration with lymphocytes cells (arrow of two heads) and disrupted of cilia. H&E stain. 400X

DISCUSSION

The exposure to smoking has been associated with adverse mucosal effects in the trachea. These changes included proliferation of epithelial cells, amalgamation of cilia, presence of inclusion bodies, and lymphocytic infiltration within the epithelial layers. Structural Changes in columnar cells were also observed as well as in goblet cells. These findings are consistent with other studies. Liao, *et al*. (2015) showed that passive exposure to cigarette smoking in rats induced inflammatory conditions in trachea. The study of Shraideh, *et al*. (2013) reported similar findings in which the exposure of albino rats for 3 months to cigarette smoke induced drastic histological changes in the tracheal epithelium such as epithelial cells proliferation, disruption of cilia, and presence of inclusion bodies.

When animals in cigarette smoking groups were treated with *Ammi vinaga* seeds, we observed partial recovery of epithelial widening of the lumen with remaining of some inflammatory conditions as congestion of capillary. The recovery from smoking with continuous intake of *Ammi visnaga* seeds further improved the inflammatory status. The present study showed protective effects of *Ammi visnaga* seeds against the adverse effects of cigarette smoking which were not described before (up to the best knowledge of the author). It is plausible to explain the protective effects of *Ammi visnaga* seeds due to its anti-oxidative effects which have been reported in several studies (Rauwald, 1994; Vanachayangkul, 2008).

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The effects of waterpipe smoke on tracheal histology included increased proliferation of epithelial cells as well as heavy lymphocytic infiltration. Loss of cilia was observed. It was also observed that the submucosal layer is highly cellular. Congestion of capillaries and leakage of blood from vessels into surrounding tissues was noted it was noted. In the recovery period, most of these changes were reverted. These findings are consistent with another study by Shraideh and Najjar, (2011) who found that the exposure to waterpipe smoking in rats induced similar histological changes of trachea with features such as

increased proliferation of epithelial cells and loss of cilia. It is plausible to explain the damaging effects of waterpipe smoke on trachea by considering that the high content of nicotine in waterpipe smoke and its effects on microtubules; polymerization / depolymerization of tubulin (Zenzes and Bielecki, 2004).

The exposure of trachea to water pipe smoking and *Ammi visnaga* seeds showed slight recovery with less inflammatory conditions. Although the results of the present study in relation to the protective effects of treatment with *Ammi visnaga* seeds were not reported before, we can explain its protective effects through its antioxidative and anti-inflammatory properties (Vanachayangkul, 2008).

CONCLUSIONS

Cigarette smoking and waterpipe smoking have adverse effects on trachea including amalgamation of cilia and infiltration of lymphocytes. These changes are likely to be reversed using *Ammi visnaga*.

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