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Histopathological Effects of Nicotine on Rats Pulmonary Cell Treated with Zinc and Vitamin D

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ABSTRACT: The present study sought to determine the role of vitamin D and zinc in protecting against nicotine stress by altering the alveolar response and modulating antioxidant mechanisms. Thirty mature adults Wistar rats, used as a model for mammals, were maintained at 23 ± 2 C°, randomly divided into five equal groups, and then given therapy for 14 days. G1: Nicotine was administered at a dose of 0.0015g/kg b.w. I.P., G2: zinc was administered orally at 0.06 g/kg b.w., G3: Orally administration vitamin D at 250 µg/kg b.w., and G4: both zinc and vitamin D were administered orally at the same dose as nicotine, 0.0015g/kg b.w. I.P. Rats were anesthetized with ketamine at the end of the treatment period. All groups of rats had lung samples extracted rapidly; alveolar groups of samples were cleaned by physiological solution and used 10% of neutral buffered formalin for tissue fixation. Then lung suction was repaired for histopathological study. The results showed highly significant protective effects of zinc and vitamin D in the tissues of the lung in G4. Zinc and vitamin D group G4 decreased the effectiveness of nicotine when compared with the G1 nicotine group. Protective effect of zinc and vitamin D by decreased mononuclear cells (MNCS) infiltration with thickening and slightly congested pulmonary blood vessels without emphysema and pulmonary edema

Keywords: Nicotine, zinc, vitamin D and lung histopathology.

1. INTRODUCTION

The alveolar is tightly surrounded by epithelium at a distance of 0.5 µm. There are 300 million human alveolar cells, and the entire surface of the alveolar wall connecting the capillaries equal to 70 square meters in the two lungs two different types of epithelial cells join the alveolar cells. (1). Type I squamous epithelial cells have large cytoplasmic growth cells that line the alveolar trunk line, occupying about 95% of the surface of the alveolar epithelium. Numerous lamellar inclusion bodies found in thicker cell called type II cells (granular pneumocytes) (2). Alveolar type I (ATI) function cells are responsible for the transcellular ion transport, alveolar fluid regulation, immune modulation and control of peptide growth factors metabolism. There are other specialized cells in the alveolar, such as mast cells, neuroendocrine cells, plasma cells, lymphocytes and pulmonary alveolar macrophages PAM (3). Surfactant active agents affect the immune system through indirect mechanisms by enhancing the activity of alveolar macrophages and lymphocytes (4, 5).

The generation of oxygen radicals by activated phagocytes causes lung surfactant lipid peroxidation, which leads to acute lung injury (6). These changes were linked to an increase in ROS and the antioxidant defense system's expression. Stress effects on lung surfactants and alveolar epithelial cells in humans lead to lipid peroxidation (7). Cytotoxic chemicals, which were produced by reacting ozone with surfactants, phospholipids, and cholesterol in cell membranes, stimulate second messenger systems like PGE_2, platelet-activating factor, and arachidonic acid (8). High levels of

neutrophils, along with activated macrophages and T cells, especially CD8 + T cells, are responsible for progressive chronic obstructive pulmonary disease in individuals with airway inflammation (9).

Tobacco smoke disrupts the barrier of alveolar epithelial cells, causing an increment in bronchial alveolar lavage fluid and edema protein in alveolar cells, as well as an increase in inflammation and hypoxia **(10).** Nicotine causes changes in the structural integrity of blood vessels, including increment severity, hyperplasia, and the development of atherosclerotic plaques. This abnormality is often accompanied by symptoms thrombosis risk is raised due to endothelial dysfunction and it impacts lipid metabolism lead to injry by releasing pro-inflammatory cytokines, cell adhesions with growth factors such as TGF-1 (11). Rat pulmonary cells exposed to nicotine were affected by the conversion of ANG I to ANG II, which was observed to be an activation of ACE expression. When ACE expression was high and ACE2 expression was low due to ANG II levels being high in the lungs. Decreased ACE2 expression in soft muscle cells of the pulmonary arteries of smoking mice was associated with increased proliferation **(12, 13).**

Zn^{2+} has been shown to prevent apoptosis at low concentrations **(14).** Apoptosis was suppressed by two major mechanisms: one produced a thiolate complex reduced from the sulfhydryl group of the Zn^{2+} protein and reduced the oxidation of the protein by reactive oxygen species two, zinc effected by inhibition of caspase-three activation **(15).** Macrophage/monocyte produced pro-inflammatory cytokines like IL-6 or TNF- in monocytes, this mechanism inhibits by vitamin D.

2. EXPERIMENTAL DESIGN

Thirty (30) adult male rats were kept at (23±2Cº) and randomly divided into five groups that were equally divided for the experiment and treatment over 14 days. Rats. Control group: administered orally and injected) with sterile distilled water, G1: injected with nicotine 0.0015g/kg b,w. I/P., G2: administered 0.06g/kg b.w. of zinc orally, G3: administered 250 µg/kg b.w. vitamin D orally, and G4: administered both zinc and vitamin D with the same doses orally and injected with nicotine 0.0015g/kg. I.P. at fourteen days. Rats were anesthetized with ketamine and xylazine at the end of the treatment period. (kitamine 0.1g /kg I.P.) with xylazine (0.01g / kg I.P.) (16). Lung sample of each group were cleaned, placed in physiological normal saline. Used10% of neutral buffered formalin for tissue fixation, and changed after 24 hour lung tissue sample was put in various concentration of ethanol for tissue dehydration,then clearing, impregnation with paraffin wax, blocking and sectioning then staining. With hematoxylin and eosin (H&E) stain, tissue sections were prepared by microtome in thickness 6µ and stained (H&E) stain**.**

3. RESULT

The histological sample of control group showed normal histological structures with complete alveolar cells supported by basement membrane together with interstitial space figure 1 (1A and 1B).

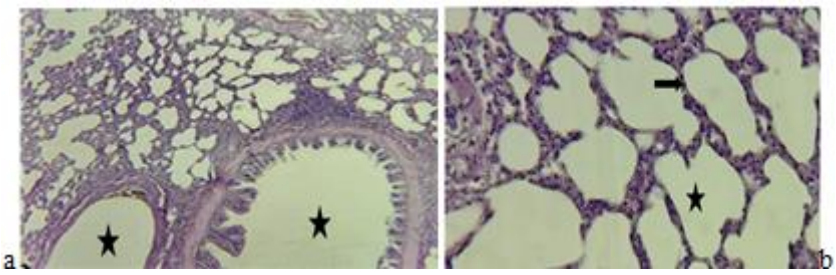

Figure 1 C: Histological sections of rats lungs in the group of control show histological structures are normal, and the bronchial lumen is normal BALT (★) and normal alveolar sac (★) with normal alveolar septa ➡(H&E stain, 20X).

Administration nicotine at 0.0015 g /kg caused marked changes in the alveolar cells' histology. It is characterized by the majority showing marked thickening of the alveolar wall due to mononuclear cells' infiltration and complete abstraction of the bronchiole by the inflammatory exudate with interstitial pneumonia in G1 figure 2 (A). In the G1 figure 2 (B), histopathological lesion includes hyperplasia and sloughing epithelia of bronchiolar epithelia, and presence the inflammatory cells inside their lumen with bronchitis and extensive BALT hyperplasia. Whereas, severe pulmonary emphysematous pneumonia is characterized by thickening of the alveolar wall, blood vessel congestion, and edema G1 figure 2 (C and D). Furthermore, they showed bronchitis, severe thickening in inter-alveolar septa with atelectasis, due to severe MNCS infiltration, pulmonary emphysema with extensive inflammatory cell infiltration figure 2 (E, and F).

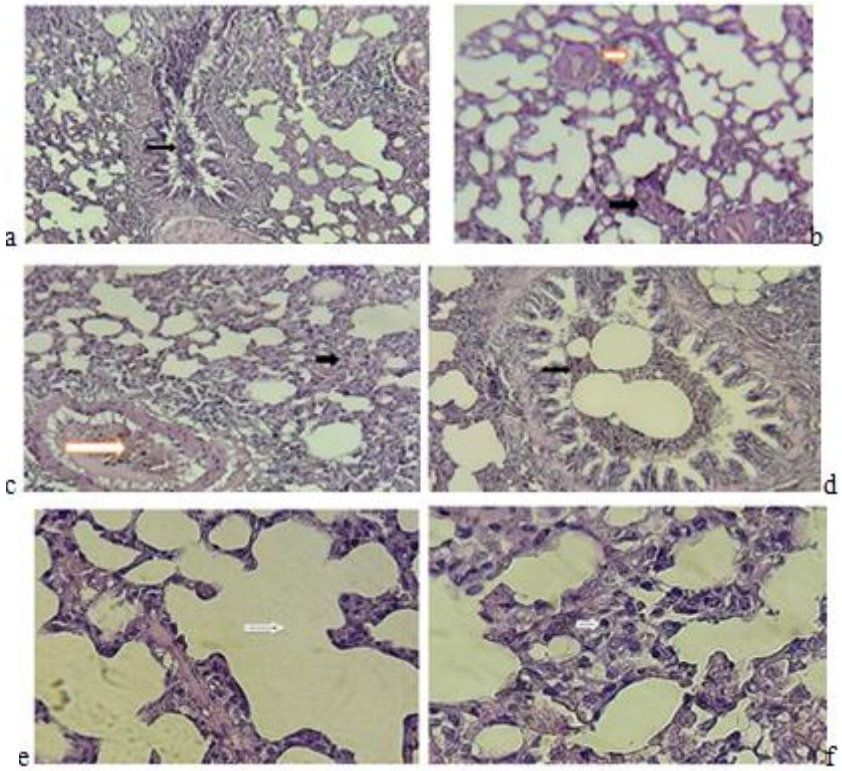

Figure 2: Histological section of rat's lung in the G1 A group treated with nicotine 1.5 mg/kg b.w shows marked thickening of alveolar wall due to mononuclear cells infiltration (black arrow) and sloughing epithelia and inflammatory cells inside their lumen (white arrow) (H&E stain,10x). In slid B shown inflammatory cells inside their lumen (black arrow). Slid C shown sever thickening in inter-alveolar septa due to sever MNC_S infiltration (black arrow) and congestion (white arrow) and slid D shown bronchitis COPD (black arrow) (H&E stain, 20X). Slid E shown sever pulmonary emphysema (white arrow) and slid F shown sever thickening in inter-alveolar septa due to sever MNC_S infiltration (white arrow) (H&E stain, 40X).

Normal histological sections in the G2 and G3 are characterized by normal alveolar septa, bronchial epithelial cells with normal BALT figures 3 (A, B) and figure 4 (A, B).

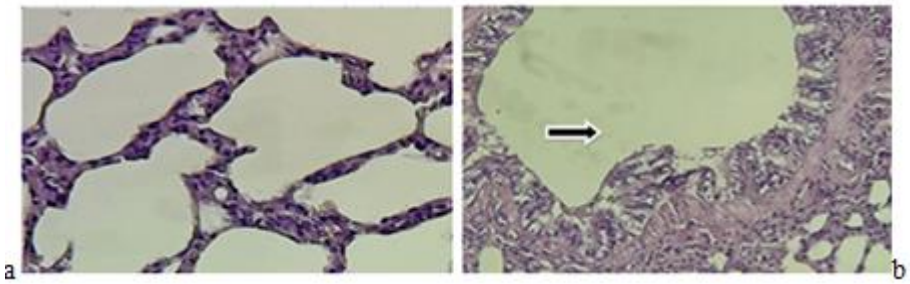

Figure 3: Histological section of rat's lung in the G2 3A slid treated with zinc 60 mg/kg shown normal histological structures and shown normal of bronchial epithelia with normal BALT in slid 3B (H&E stain and 40X).

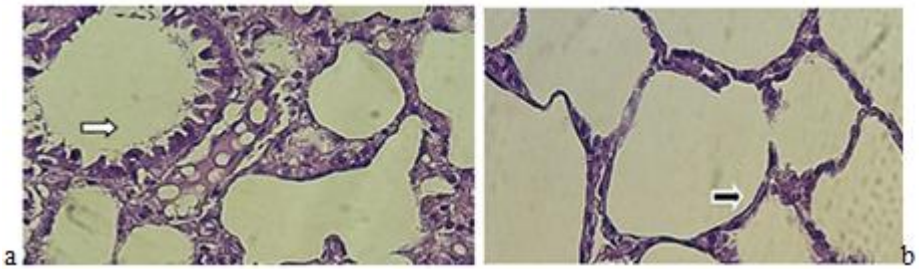

Figure 4: Histological section of rat's lung in the G3 4A slidtreated with vitamin D 250 µg/kg b.w shown normal histological structures and normal of bronchial epithelia with normal BALT (white arrow) and in slid 4B shown normal histological structures with normal alveolar septa and spac (H&E stain, 40X).

In contrast, highly decreased the effectiveness of nicotine in compression between G1 and G4 treated by zinc and vitamin D, showed moderately markedly thickened inter-alveolar septa due to mild MNCS infiltration with thickening and slightly congested pulmonary blood vessels without emphysema and pulmonary edema figures 5 (5A, 5B).

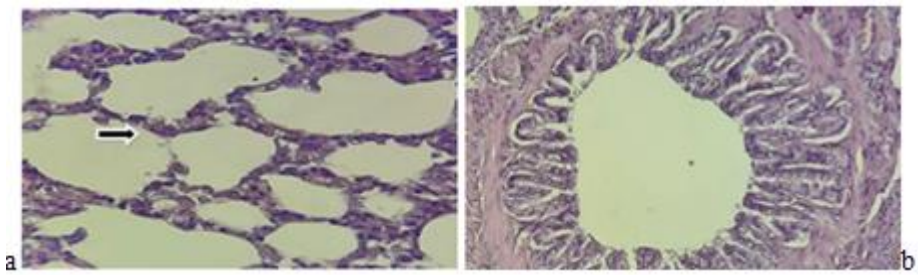

Figure 5: Histological section of rat's lung in the G4 group treated with zinc 60 mg/kg and vitamin D 250µg/kg with nicotine i/p shown slightly thickening of inter-alveolar septa (black arrow) in slid 5A (H&E stain, 40X). Slid 5B shown normal bronchial lumen (20X).

4. DISCUSSION

Histopathological alterations of lung architecture were obvious in sections of rat exposure to 1.5mg/kg b.w intraperitoneal nicotine such as decrease in the thickness of alveolar wall and basement membrane of alveolar with severe diminution of the alveolar cells in lung tissue, cell debris, of group G1 compared to other experimental groups. Similar results were reported by (17). As mentioned previously, these findings are well correlated with the nicotine induced alterations of oxidant/antioxidant status. Moreover, high effects of free radicals induced by nicotine on the alveolar cells characterized with high decreased in serum GPx and increased in lipid peroxidation levels. Nicotine causes liberation of H_2O_2 in the alveolar cells and accumulation in its, may be important in oxidative stress induced apoptosis. These results are consistent with the previous findings of (18, 17, 10, 19, 7).

Kanithi, *et al.,* (2022) revealed that cigarette and e-cigarette smoke trigger abnormal intercellular responses, which enhance ROS and increase oxidative mitochondrial damage, consequently disrupting mitochondrial morphology and initiating mitochondrial apoptosis associated with the release of cytochrome-C and stimulation of the caspase cascade, resulting in apoptosis via both Fast and Fast. These results disrupt the homeostasis of the cellular organelles'(21, 20), as well as NADH-ubiquinone reductase activity inhibited by nicotine, whose binding with complex I leads to an effect on electrons flowing from NADH to complex I, which results in decreased oxygen consumption by mitochondria (22, 23). Inflammatory cell infiltration and an increase in (BALF), differential cell counts, as well as content of total protein in the lung and liver tissues of nicotine stressed rats reflect the effect of nicotine **(**21), with another sign of pulmonary tissue injury, the nicotine group enhanced MDA activity in the BALT (6).

Results of histopathological examination revealed that in the second week of treatment with nicotine (0.0015g /kg/day) I/P, thickening of inter-alveolar septa caused alveolar wall extensive destruction, irregular airspace and the formation of enlarged. As a result of nicotine, changes in lung architecture, a rise in alveolar septal thickening were dominant in lung tissue inflammatory infiltrations, abnormal air spaces, and these findings are consistent with previous research (24). Histopathological findings support biochemical mutations by identifying significant morphological changes in the lungs of nicotine-stressed mice. The results referred to secreted different types of cytokines, TNF-α and free radicals, which

cause lung congestion due to damage to the alveolar structure. (11). The presence of neutrophils in saliva has been shown to be directly associated with pulmonary dysfunction, suggesting a strong association between neutrophil inflammation and airway obstruction (21).

This finding showed that intraperitoneal injection of nicotine at 1.5 mg/kg causes increased oxidative stress by increasing lipid peroxidation (MDA) and IL-6, which is linked to lower levels of GPx and other antioxidant enzyme activity in the lung tissue. Lipid peroxidation causes pulmonary cell membrane disruption and cytoplasmic enzyme leakage, such as lactate dehydrogenase, resulting in a significant increase in oxidative stress, as well as, histological findings suggest that nicotine is toxic due to oxidative stress and increased ROS production from microvessels and alveolar structures such as lung cells and endothelial cells (25). In the model rats' studies, of nicotine-induced lung injuries, the epithelial cells of the alveolar system are necrotizing, with serous injection into the lung cavity, inflammation of the alveolar wall, and damage to the lung tissue. Measurement of arterial blood gas exchanges was an effective indicator of alveolar wall damage and gas exchange, confirming the construction of a lung injury model in rats (21, 17).

Activated macrophages and lymphocytes release many pro-inflammatory cytokines that stimulate cells to act as internal signals that promote the inflammatory process. Infiltration of lymphocytes and macrophages showed a strong acute response to sub chronic inflammatory conditions (26). Chronic elevation of nicotine causes serum fibrin fluid to flow into the alveolar region, flooding alveolar spaces and expanding into bronchioles. Damage to the alveolar, increased vascular permeability, and loss of pulmonary capillary endothelial cells cause emphysema, pulmonary microvascular flooding (edema, fibrin), and bleeding (26, 6).

14 days after injecting nicotine (1.5 mg / kg body weight, I.P) with zinc (60 mg / kg body weight, oral) and vitamin D (250 g / kg, p.o) in equal doses at very high doses we have to. Lipid peroxide and antioxidants in lung tissue increased levels of malondialdehyde (MDA), interleukin-6 (IL-6) activity, and increased apoptosis of alveolar cell damage in the nicotine group. Histological examination of the lung part revealed the occurrence of lung lesions in both treatment groups. Emphysema lesions, perivascular and lymphocyte infiltration in the interstitial area (25). However, abnormalities were more obvious in the nicotine group than in the nicotine G4 group and zinc and vitamin D. the possible reduction effects of nicotine by zinc and vitamin D due to regulation mechanism of the alveolar cells, counteractive interactions between nicotine with zinc and vitamin D may attenuate the effect of nicotine due to their antioxidant properties (27, 28). All these abnormalities can be a sign of the onset and progression of lung disease, as confirmed by histopathological studies (29, 30).

Taken together, our results highlight that nicotine induces extensive tissue damage in the lung and alters plasma oxidative stress profiles. These findings also revealed that supplementing vitamin D and zinc ameliorates these pathologies criteria and improves respiratory performance in rats and proposes the use of these supplementations for nicotine-induced stresses. Oral administration of zinc and vitamin D with intraperitoneal nicotine to adult male rats showed positive effects on alveolar cell by decreased oxidative stress, upregulation of tight junction protein expression, modulate immune response. In spite of all these changes for vitamin D and zinc, it was found that when they give with no stress there was an iron deficiency anemia.

5. CONCLUSION

It can be concluded that vitamin D and zinc combination have an efficient role as antioxidant, anti-inflammatory, and immunomodulatory responses of mammals under nicotine induced oxidative stress.

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