Original Article

Evaluation the Effect of Nicotine Injection on the Lungs of Mice

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Abstract

Background: Nicotine is the chief addictive substance in cigarette smoking which conceder as the main cause of mortality worldwide. **Aims:** Through the current *in vivo* study, we tried to evaluate the harmful effect of nicotine injection on the lungs of mice. **Materials and Methods:** A total of 40 healthy male mice were divided into four groups of 10 mice each, injected subcutaneously with 0.1 ml of (1 mg/kg) nicotine for 5 days a week for (8, 12, and 16) weeks, whereas the control group injected with 0.1 ml of normal saline. The mice were sacrificed, lungs were isolated and divided into two parts, the first for measurement malondialdehyde (MDA) and glutathione (GSH), the second was subjected to histopathological examination. **Results:** The results showed that the levels of MDA were significantly elevated ($P \leq 0.01$) in all work groups and the mice of Group D had the highest MDA value (23.13 ± 3.4 nmol/ml) with statistically significant difference ($P \leq 0.01$), and GSH levels were significantly decreased ($P < 0.01$) in all workgroups, the Group D had the lowest value (6.77 ± 1.33 mM/ml) with statistically significant difference ($P \leq 0.01$), also the results clarified that nicotine injection was caused a pathological effect in mice lung tissues such as alveoli damage and emphysema, congestion of blood vessels, hemorrhage, alveolar edema, lung fibrosis, lymphocytes infiltration, and these effects were graded in terms of severity depending on the injection period of nicotine. **Conclusion:** It can be concluded that the nicotine injection causes significant changes in lung tissues and oxidant markers levels (MDA and GSH).

Keywords: *Glutathione, histopathological examination, lungs, malondialdehyde, mice, nicotine*

Introduction

Nicotine is the well-known chemical compound, that responsible for addiction in tobacco smoking, which conceder as the main cause of mortality worldwide, it is easily absorbed by lung, and has about 2 h as half-time in plasma before it is excreted through the kidneys,[1] and hence, it has the ability to change levels of oxidative stress markers in lung and blood.[2,3] Malondialdehyde (MDA) is a main oxidative marker considered a very reactive chemical compound which produced through arachidonic acid metabolism at prostaglandins production.[4] It can join with some important groups of molecules as ribonucleic acid, DNA, lipoproteins, and proteins and modification its vital function.[5] Estimation of MDA levels in vital fluids and tissues use as a significant signal of lipid peroxidation at different body disorders, exposure to toxins and chemicals like heavy smoking.[6,7] Glutathione (GSH) is a chief cellular antioxidant commonly called the master antioxidant.[8] It has vitally important role for the safety of lung and its ordinary function. The GSH in epithelial tissue of respiratory tract plays an important role of defense from oxidants and inflammatory damages;[9] hence, changes in alveolar and lung metabolism are a major characteristic of several inflammatory lung diseases.[10] A diminution in GSH levels in the lung lining fluid has been showed in acute respiratory distress syndrome, idiopathic pulmonary fibrosis (FS), human immunodeficiency virus patients, cystic FS, and smoking.[8] Therefore and due to the importance of the subject, we planned to estimate the effect of nicotine in the lungs of mice by histopathology examinations and measuring some oxidative stress markers such as MDA and GSH.

Materials and Methods

Forty male albino mice of 20 weeks age at weights ranged between (28 and 32 g) were obtained from Charles River Laboratories/USA, hosted in plastic cages at temperature (20°C–28°C) and dieted a basal diet according to Vodopich and Moore[11] and accordance with the

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guidelines approved by the Animal Ethical Committee of University of Baghdad. The mice were divided into four groups of ten mice each, and injected subcutaneously (5) days a week as follows:

1. Group A (control), was injected with (0.1 ml) of normal saline for (16) weeks
2. Group B, was injected with (0.1 ml) of (1 mg/kg) nicotine for (8) weeks
3. Group C, was injected with (0.1 ml) of (1 mg/kg) nicotine for (12) weeks
4. Group D, was injected with (0.1 ml) of (1 mg/kg) nicotine for (16) weeks. The mice were sacrificed by cervical dislocation after 3 days of last treatment; lungs were isolated and divided into two parts, the first for measurement MDA and GSH, the second was fixed in (10%) formaldehyde, then subjected to histopathology examination.

Measurement of malondialdehyde and the glutathione

Lung tissues were homogenized with ice-cold potassium chloride using tissue homogenizer (IKA ULTRA-TURRAX-Sigma-Aldrich-Germany), centrifuged at (3000 rpm) for (5 min), supernatant separated and taken to measure MDA and GSH levels by using a microplate reader, water was used instead the sample for blank. Levels of MDA were estimated by using thiobarbituric acid TBA and MDA assay kit (Abcam/USA) depending on methods of Ohkawa *et al*.[12] and Turguta *et al*.,[13] the levels were measured in nmol/ml, using colorimetric assay at (OD 532 nm) for normal values (5.5–12.5). While estimation of GSH levels was performed using 5-5′′-Dithio- bis (2-nitrobenzoic acid) by GSH assay kit (Abnova/Taiwan) depending to methods of Griffith[14] and Khan *et al*.,[15] the levels were measured spectrophotometrically in mM/ml, absorbance was read at (415 nm), normal values (11.5–20.5).

Histopathology examination

Preparation of paraffin blocks and deparaffinized was done according to Lillie.[16] Slides of lung tissues were stained with hematoxylin and Eosin (H and E) according to Lynch *et al*.[17] to detect the histopathological changes in tissue as a result of nicotine exposure.

Statistical analysis

Statistical analysis was performed using Statistical analysis System-SAS[18] program to evaluate the effect of different factors in work parameters, also the significant compare between means was done by using (ANOVA) or least significant difference test to estimate the significance of variability between work and control groups ($P < 0.01$) significant, the data were act as simple measure of mean ± standard deviation.

Results

The results of the measurement of MDA and GSH showed statistically significant alteration ($P < 0.01$) in MDA and GSH levels among all work groups as compared with control, and these levels were diverted according to the duration of nicotine exposure, and the mice whose injected with nicotine for 16 weeks as Group D had the highest MDA value (23.13 ± 3.4 nmol/ml) with statistically significant difference ($P \leq 0.01$) when compared with others work groups, as shown in Table 1; however, all work groups revealed significantly elevated values as they were compared with the control group.

On the other hand, results showed statistically significant decrease ($P < 0.01$) of GSH levels according to the duration of nicotine injection comparing with control group as shown in Table 2 Group D which mice injected with nicotine for 16 weeks had the lowest value (6.77 ± 1.33 mM/ml) with statistically significant difference ($P \leq 0.01$) as compared with others work for groups.

The result of the histopathological examination showed various changes according to duration of nicotine exposure, sections of lung tissue obtained from mice injected with normal saline for 16 weeks as Group A (control) had normal appearance of lungs which composed of thin walled alveolus of single layer of squamous epithelium, normal alveolar sacs [Figure 1].

Sections obtained from mice of Group B injected with nicotine for 8 weeks, showed an early damage alveoli (D), thickening wall (TW), alveoli emphysema, congestion (CON) of blood vessels and hemorrhage [Figure 2].

The microscopic appearance of lung tissue sections, obtained from mice injected with nicotine for 12 weeks

Table 1: The effect of nicotine injection on malondialdehyde in lungs of mice

Groups	MDA (nmol/ml), mean±SD
A	9.98±1.81
B	17.77±3.12
C	19.55±2.5
D	23.13±3.4
LSD value	5.664**
P	0.0001

**$P<0.01$. LSD: Least significant difference, SD: Standard deviation, MDA: Malondialdehyde

Table 2: The effect of nicotine injection on glutathione in lungs of mice

Groups	GSH (mM/ml), mean±SD
A	11.3±2.54
B	9.81±2.16
C	7.97±2.11
D	6.77±1.33
LSD value	2.894**
P	0.0001

**$P<0.01$. GSH: Glutathione, LSD: Least significant difference, SD: Standard deviation

as Group C, had a diffuse alveolar damage in the lung, which is the final prevalent pathway for a different of severe lung damages, alveoli which were filled with a smooth to slightly floccular pink material distinguishes for multifocal accumulation of pulmonary edema. There were hyaline membranes, as seen, lining alveoli. In areas of inflammation of the lung, there was a greater number of inflammatory cells (IC) as neutrophils and macrophages in alveoli and pulmonary FS were also seen as shown in Figure 3.

The lung section of Group D mice injected with nicotine for 16 weeks showed fibroblast proliferation and collagen deposition was progressed and pulmonary FS. The alveolar walls were damaged (D) with TW, congested (CON) blood vessels and hemorrhage with many red blood cells, in addition to lymphocytes proliferation and infiltration (LI) [Figures 4 and 5].

Discussion

Nicotine is one of the main substances contained in cigarette smoke and due to its toxicity, it was evaluated in this study *in vivo*. The current study showed significant alterations ($P < 0.01$) in MDA and GSH levels among all work groups when compared with control, MDA is a very reactive chemical formed naturally as a lipid peroxidation by-product of poly-unsaturated fatty acid, which is a well-known mechanism of cellular damage in humans, animals and plants, MDA is used as index of oxidant stress in biological systems, as well, estimation of MDA levels in vital fluids and tissues, is used as a significant signal of lipid peroxidation *in vitro* and *in vivo* for different disorders, MDA has been showed to be produced in chronic disease and heavy smoking[7,19] Bamonti *et al*.[20] suggested that the smoking conceder as a risk factors to oxygen-free radical formation, which it leads to significant elevated of free MDA in smoker's serum. However, GSH regard as a main cellular antioxidant, usually called the body's own master antioxidant,[8] it has a vitally essential for the safety of the lung and its normal role, and achieves many others important roles,[21] such as DNA synthesis repair, protein synthesis, regulation of cell growth and division, enzyme activation, transport of amino acid, enzyme catalysis, conjugation to heavy metals and xenobiotics, metabolism of toxins, carcinogens and xenobiotics[22] enhancement of systemic and humoral immune function, defense from ultraviolent radiation, reduction-free radical, oxyradical, and radiation damage.[23,24] Furthermore, Ballatori *et al*.[25] suggested that the cigarette smoking was reduced GSH, consequence has been involved in smoking pathogenesis and related lung cancer diseases. In addition, the interaction of free radicals with DNA causes DNA strand fractures, and this appears as a chief response which leads to cancers.[26,27] likewise, oxidant stress can stimulate the alteration in gene expression.[28] However, GSH in epithelial tissue of respiratory tract, plays an important

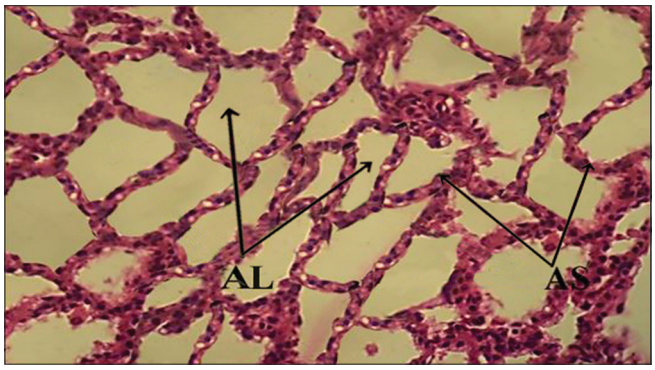

Figure 1: Lung tissues of Group A showing normal alveolar sac and alveoli (H and E, ×400)

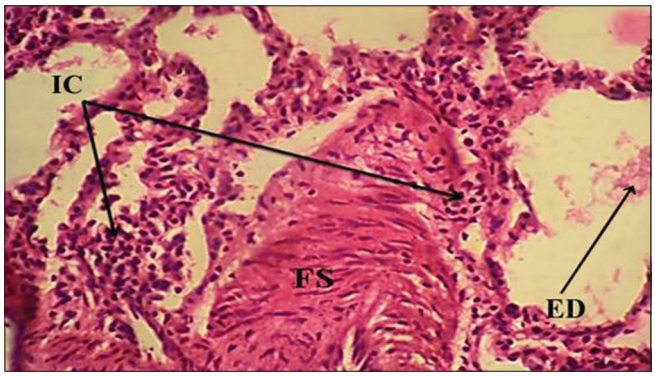

Figure 3: Lung tissues of Group C showing pulmonary fibrosis, edema with inflammatory cells more likely to be interstitial (within tissues), an inflammatory infiltrate extends from the bronchial lumen (H and E, ×400)

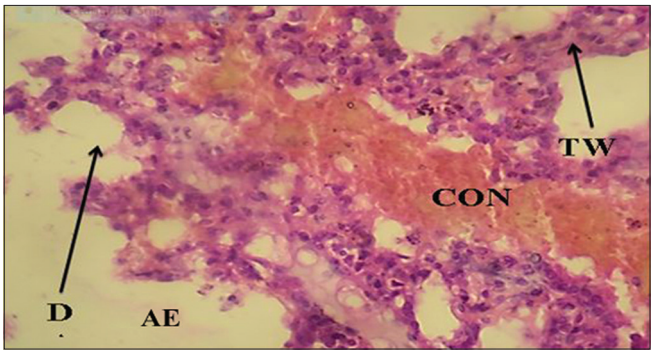

Figure 2: Lung tissues of Group B showing damage alveoli (D), thickening wall alveoli emphysema and congestion of blood vessels (H and E, ×400)

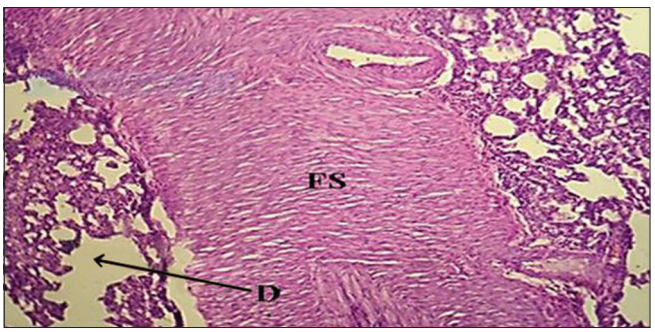

Figure 4: Lung tissues of Group D showing damage alveoli (D) with fibrosis (H and E, ×100)

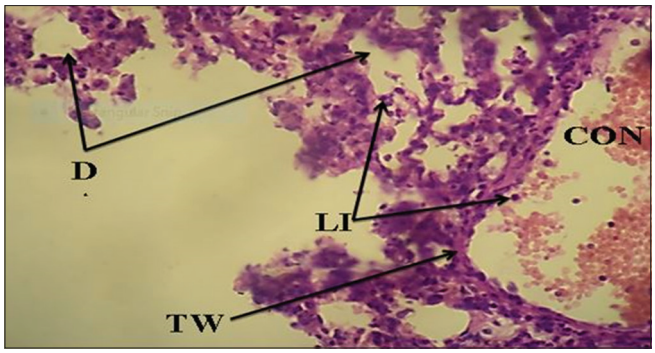

Figure 5: Lung tissues of Group D showing damage alveoli (D), thickening wall and congestion of blood vessels with lymphocytes infiltration (H and E, ×400)

role of the defense from oxidative and inflammatory damages;[9,29] hence, the changes metabolism of lung GSH are a major of several lung diseases,[10] decrease the level of lung GSH lining fluid has been showed in idiopathic pulmonary FS, cystic FS, several virus infections as human immunodeficiency virus patients, and smoking.[22] The present study revealed that treatment of adult male mice with nicotine induced lung tissue injury represented by degenerative changes such as damage of alveolar, thickening walls and CON of blood vessels, then infiltration of IC such as lymphocytes that caused by chronic nicotine toxicity exposure, in accordance with that mention by Piipari *et al*.[30] who study the histological effect of smoking on the bronchoalveolar and lungs tissues. Many studies have addressed the histopathological examination of the lungs, such as the study of Duniho *et al*.[31] who investigated acute lung histopathological alterations and isolated fluid of broncho alveolar lavage factors to diagnose the initial signals in mice exposed to cigarette smoke, also study of Thun *et al*.[32] who showed histopathologic variations in microscopic appearance, and found about 50% of lung tumors showed more than one histologic types when estimated the effect of cigarette smoking on histopathological alterations of lung cancer. Furthermore, because of the low sensitivity of laboratory animals, especially mice, Waldum *et al*.[33] suggested that the dose used in laboratory animals to estimate the carcinogenic probability of nicotine should usually more than of those that human might be exposed to, when they investigated nicotine carcinogenicity *in vivo* on 68 female Sprague-Dawley rats which were treated with nicotine inhalation for 20 h at 5 days a week and estimated nicotine concentrations in plasma.

Conclusion

The current study revealed that the nicotine injection could cause significant alteration in lung tissues as well as changes in oxidant markers levels such MDA and GSH which necessitates finding ways to protect individuals against this bad habit to maintain the public health.

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Conflicts of interest

There are no conflicts of interest.

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