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Biochemical Effect of Nicotine on Oxidative Stress and Inflammatory Markers in Lung tissue of rats.

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ABSTRACT

Studies regarding nicotine-related lung toxic effects are contradictory and limited. This study aimed to evaluate the nicotine toxic effect on the lung tissues of rats in the light of increased lipid peroxidation (MDA), disrupted antioxidants (GPx, GSH), elevated pro-inflammatory cytokines (TNF-α, NF-κB), and an inflammatory-mediated enzyme (Caspase-1). Twenty rats were divided into two groups: group I control and group II rats injected intraperitoneally with nicotine. After the experiment, nicotine injection significantly elevated ($P<0.0001$) MDA levels and, conversely, caused a significant reduction in antioxidant (GPx activity and GSH) pulmonary levels. Also, there was remarkably upregulated NF-κB mRNA expression (8.34-fold increase) in the lung tissues associated with nicotine injection. Also, the pro-inflammatory cytokine Tumer Necrosis Factor (TNF-α) and inflammatory-mediated enzyme Caspase-1 are significantly elevated. Therefore, it was concluded that nicotine has toxic effects on lung tissues as it elevates lipid peroxidation, increases pulmonary oxidative and inflammatory toxicity and abolishes antioxidant capacity..

1. INTRODUCTION

According to the World Health Organization (WHO), tobacco smoking is one of the main causes of mortality and is significantly linked to poor health outcomes and a shorter lifespan (Khaled et al., 2020; Wahbeh et al., 2024). According to Wahbeh et al. (2024), smoking prevalence will surpass 30% in 2025, despite efforts made worldwide to reduce tobacco use. When absorbed into the bloodstream, nicotine, the main tobacco alkaloid, is linked to damage to the liver and lungs (Moghbel et al., 2017; Khaled et al., 2020) .

Numerous studies have shown that tobacco and nicotine increase the induction of oxidative stress status and lower the antioxidant defense mechanism when compared to nonsmokers (Ahmadkhaniha et al., 2021). Oxidative damage to DNA, lipids, and proteins results from the generation of reactive oxygen species (ROS) linked to nicotine beyond the ability and capacity of the antioxidant defense mechanism (Caliri et al., 2021). Many proteins and enzymes, including CAT, SOD, GR, GPx, and GSH, can be disrupted by ROS when they react with polyunsaturated fatty acids (lipid oxidation) in cell membranes. (Juan et al., 2021; Endale et al., 2023). Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase/NOX) may be activated to induce ROS in nicotine (Shen et al., 2019; Seo et al., 2023). Additionally, an increase in DNA damage and protein and gene regulation leading to cancer, apoptosis, and inflammation is caused by lipid oxidation-induced oxidant/antioxidant imbalance (Aslan et al., 2023). In general, exposure to nicotine causes immunological and epithelial cells in the lung and upper airway to produce pro-inflammatory cytokines, such as interleukins and tumor necrosis factor-alpha (TNF-α) (Matsumoto et al., 2020; Park et al., 2022) .

Across studies, specific neutrophil signal patterns have been demonstrated and nicotine exposure was reported to activate neutrophils by the action of some enzymes (Reidel et al., 2018). Thus, nicotine has a direct role in many processes related to lung inflammation, including cytokine production (Hamza and El-Shenawy, 2017).

The overall goal of this research was to assess how nicotine affects lung inflammation and damage in the context of elevated lipid peroxidation (malondialdehyde (MDA)), disrupted antioxidant activity (GPx, GSH), increased pro-inflammatory cytokines (TNF-α, NF-κB), and inflammatory-mediated enzymes (Caspase-1).

2 .MATERIAL AND METHODS

2.1 .Materials

Nicotine (CAT: NI00200100, Scharlau, Scharlab S.L Barcelona, Spain) was commercially obtained.

2.2 .Ethics statement

The study protocol was approved by the Institutional Animal Care and Use Committee Research Ethics, Faculty of Veterinary Medicine, Benha University (BUFVTM12-11-22).

2.3 .Animals and experimental design

In hygienic cages at a temperature of 22 ± 2 ◦C, male rats (100–120 g) were housed and collected from the Nile Company for Pharmaceuticals & Chemical Industries, Egypt. Animals were given unrestricted access to potable water, a commercial pellet diet and a consistent 12-hour light/dark cycle. Rats in the experimental groups were distributed to two experimental groups (10 rats/group). Control group: normal rats were received orally; normal saline served as the control; Nicotine group: rats were

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intraperitoneally treated with nicotine (1 mg/kg/day) according to Okada and Matsuo (2023) for 3 weeks.

2.4 .Sample collection

At the end of the experiment, all rats were sacrificed with urethane anaesthesia (1.3–1.5 g/kg in a~1.5 g/ 5 mL solution) according to (Field et al., 1993) and, by puncture of the heart, blood samples (about 5 mL) were collected. Lung tissue specimens were fixed in formol saline (10%), trimmed off, washed, and dehydrated in alcohol ascending grades.

2.5 .Biochemical measurements

Commercial biochemical kits (Bio-Diagnostic Company, Cairo, Egypt) were used to measure MDA levels (Kie, 1978), as well as antioxidant parameters GSH (Beutler et al., 1963) and GPx (Paglia and Valentine, 1967) procured from. Also, according to the manufacturer's guidelines, lung tissue levels of TNF-α (Cat#MBS924824) and caspase-1 (Cat#MBS2019421) were obtained by commercial ELISA kits from My BioSource, San Diego, USA (Trevejo, et al., 2001) .

2.6 .NF-κB relative gene expression

To find NF-κB mRNA expression, total RNA was taken from lung tissues using a purification kit (#K0731, Thermo Scientific, Fermentas, USA). After that, complementary DNA (cDNA) was obtained using reverse transcription kits (#EP0451, Thermo Scientific, Fermentas, USA). Quantitative real-time PCR (RT-PCR) was established using a Step OnePlus thermal cycler (Applied Biosystems, Life Technology, USA) and SYBR Green PCR Master Mix (# K0221, Thermo Scientific, USA). To normalize NF-κB expression, β-actin was used as an internal reference. β-actin is one of the most commonly used reference genes because it has more stable expression levels compared with other internal controls (Biederman et al., 2004). Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The used primers were as follow:

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
NF-κB	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
B-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

2.7. Statistical analysis

GraphPad Prism 8 (GraphPad, San Diego, CA, USA) was used to produce various charts, and SPSS 20 (SPSS Inc., USA) was used to analyze the data. The means and standard error of the mean (SEM) were used to express the results. Using the student t test, several comparisons between groups were evaluated. $P \leq 0.0001$ indicated significance.

3. RESULTS

3.1. Effect on redox status

Nicotine effect on lipid peroxidation and redox status impairment was evaluated by measuring the lung tissue contents of MDA, GPx activity, and GSH. As shown in Table 1, nicotine injection significantly elevates ROS levels and causes a marked (P<0.0001) increase in MDA (Fig. 1A) and a reduction in GPx activity (Fig. 1B) and GSH (Fig. 1C) pulmonary levels. This suggests that the cell eliminated its antioxidant capacity and caused pulmonary oxidative toxicity.

3.2. Impact on the inflammatory markers

The NF-kB signaling pathway is crucial for controlling cellular redox balance and the inflammatory response. In this study, NF-κB mRNA expression was significantly increased (8.34-fold) in the lungs of rats that were given nicotine (Table 1, Fig. 2). Also, nicotine injection was associated with a significant elevation of the pro-inflammatory cytokine TNF-α (Fig. 3A) and the inflammatory-mediated enzyme Caspase-1 (Fig. 3B).

Table 1. Effect of nicotine exposure on oxidative stress and inflammation associated markers

Variable	Controls	Nicotine exposed rats	*P* value
MDA (nMole/g tissues)	18.88 ± 0.85	128.04 ± 5.84	0.0001
GPx (U/g tissues)	137.26 ± 5.29	55.63 ± 2.05	0.0001
GSH (mg/g tissues)	62.10 ± 3.10	17.20 ± 0.51	0.0001
TNF-α (pg/g tissues)	319.29 ± 15.64	1210.7 ± 33.45	0.0001
Caspase-1 (ng/g tissues)	5.60 ± 0.20	14.0 ± 1.02	0.0001
NF-kB (Fold-increase)	1.0±0.0	8.34±0.36	0.0001

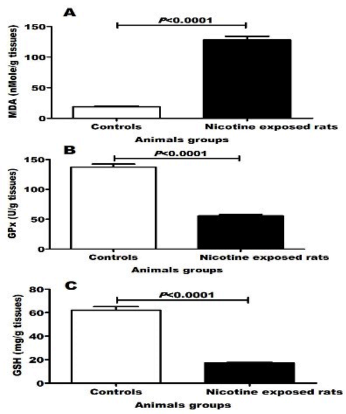

Figure 1. Impact of nicotine on lipid peroxidation and antioxidant parameters. Nicotine injection significantly elevated (A) MDA levels and decreased both pulmonary (B) GPx activity and (C) GSH levels. Values were expressed as mean ± SEM.

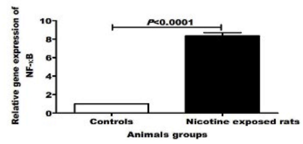

Figure 2. Fold change in NF-κB expression between rats treated with nicotine and control rats.

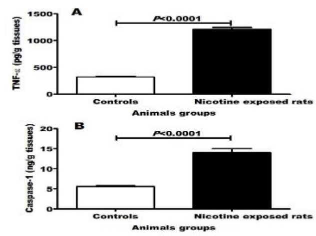

Figure 3. Effect of nicotine on lung tissues inflammatory markers. Nicotine injection significantly increase (A) TNF-α and (B) Caspase-1 lung levels. Values were expressed as mean ± SEM.

4. DISCUSSION

Compounds and hazardous substances that cause intracellular oxidative stress are considered to be major agents that cause damage to biological molecules (Olufunmilayo et al., 2023). Initially, tobacco was distilled to extract nicotine, which was then given to treat ulcers and constipation in rodents (Barr et al., 2007). Later, it was realized that it was toxic to humans (Mishra et al., 2015; Chioran et al., 2022). Reports on nicotine-related toxic effects are contradictory and limited (Xu et al., 2023). For example, some research has discovered that nicotine is not the cause of DNA changes brought on by tobacco use (Mizusaki et al., 1977), and that nicotine and its metabolites have no genotoxic effects (Doolittle et al., 1995). As a result, research on nicotine toxicity, the main alkaloid found in tobacco and cigarette smoke, is very thorough (Barr et al., 2007). In the present study, we address nicotine toxic impacts in lung tissues in light of lipid peroxidation indicated by MDA levels, impairment of antioxidants (GPx, GSH), activation of NF-κB signaling pathway and induction of inflammatory markers (TNF-α, Caspase-1) in rats injected with nicotine.

The obtained findings in this study revealed that nicotine injection significantly ($P < 0.00001$) triggered pulmonary oxidative stress, causing enhanced lipid peroxidation (evidenced by high MDA pulmonary values) and impaired redox status (indicated by a reduction in activities of pulmonary GPx and GSH). Nicotine exposure has been shown to induce oxidative stress and inflammation in alveolar cells, which can compromise surfactant synthesis and decrease regeneration potential (Cha et al., 2023). The generation of ROS is one of the primary mechanisms that connects cigarette smoking to lung ageing (Morsch et al., 2019). According to Cha et al. (2023), the metabolism of nicotine and other harmful components of cigarette smoke produces ROS, which in turn causes oxidative stress in lung cells. Elevated ROS have the potential to harm cellular constituents, including proteins, lipids, and DNA, ultimately leading to oxidative stress, malfunction, and death of the cell (Su et al., 2019). When GPx, GSH, CAT, and SOD levels were compared to the control. in a study conducted by Oyeyipo et al., (2014), the effects of nicotine on serum antioxidant levels revealed a substantial drop in the nicotine group. Conversely, they found that MDA was significantly elevated (Oyeyipo et al., 2014). Mahmoud et al. (2021) examined the impact of nicotine on oxidative stress indicators in the lungs of rats, which is also consistent with our findings. They discovered that, in comparison to a control, eight weeks of consecutive nicotine injections significantly increased oxidative stress in lung tissues.

Apart from oxidative stress, exposure to nicotine may also trigger inflammation in the lungs by elevating levels of pro-inflammatory cytokines, including TNF-α (Cha et al., 2023). NF-κB is an inducible transcription factor found in neurons that has been linked to several biological processes, including development, innate immunity, antiapoptosis, and inflammation (Widera et al., 2006). Here, there was a remarkable upregulation in NF-κB mRNA expression (8.34-fold increase) in the lung tissues of rats injected with nicotine. Also, nicotine injection was associated with a significant elevation of the pro-inflammatory cytokine TNF-α and the inflammatory-mediated enzyme Caspase-1.

The NF-kB signaling pathway is crucial for controlling cellular redox balance and the inflammatory response. Nicotine exposure was reported to cause activation of airway epithelial cells and alveolar macrophages that released pro-inflammatory cytokines and infiltrated the lungs by inflammatory cells (Lugg et al., 2022; Cha et al., 2023).

Many studies have demonstrated that nicotine exposure induces oxidative stress, and inflammation and activates NF-κB via the ROS/NF-κB signaling pathway (Barr et al., 2007; Wang et al., 2019; AlQasrawi et al., 2021). Moreover, the interaction between activated NF-κB and forkhead box O1 (FOXO1) provoked pro-inflammatory mediator production such as NLRP3, which in turn enhanced caspase-1 activation and eventually mediated pulmonary injury (Wu et al., 2019). Zhong et al. (2008) discovered that exposure to tobacco smoke activated initiator caspases for the mitochondrial pathway (caspase 9), the death receptor pathway (caspase 8), and effector caspase 3.

TNF-α, as a pro-inflammatory cytokine, may play an important role in the nicotine-associated inflammatory response. Liu et al. (2017) results suggested that nicotine aggravates cardiovascular effects, including inflammation, oxidative stress, and endothelial dysfunction, by targeting the endothelium through the enhancement of macrophage-produced TNF-α (Liu et al., 2017). Similarly, Wang et al. (2004) found that in human endothelial cells, nicotine could augment adhesion molecule expression via macrophages producing TNF-α.

5. CONCLUSIONS

Our study demonstrated that nicotine could augment lung injury through aggravating lipid peroxidation, imbalance of redox status, antioxidant defense system impairment, enhancing pulmonary inflammation, and pro-inflammatory cytokine production.

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