HISTOPATHOLOGICAL STUDY OF NICOTINIC IMPACT ON LUNG TISSUES OF RATS
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Abstract
Nicotine is the well-known chemical compound which responsible for addiction in tobacco smoking and consider as the main cause of mortality worldwide. The aims of the current study include evaluate the effect of nicotine on lung tissues and estimate the level of antioxidants and lipid peroxidation. Totally, 40 male rats were selected, acclimated and divided equally into two groups; G1 as control and G2 as an experimental group injected subcutaneously with 0.25 ml of nicotine daily for 28 days. Finally, the study animals were served to sampling of blood and lung samples. Results of CAT revealed a significant decreases in G2 (8.84 ± 0.52 pg/ml) when compared to G1 (18.36 ± 1.23 pg/ml). Concerning GPx, significant reduction was seen in G2 (215.91 ± 14.96 pg / ml) in comparison with G1 (418.48 ± 15.17 pg / ml). For SOD, value of G2 (3.37 ± 0.1 U / ml) was lowered significantly more than G1 (7.58 ± 0.17 U / ml). Significantly, higher MDA concentration were seen in G2 (126.25 ± 5.95 ng / ml) than G1 (53.8 ± 4.51 ng / ml). In contrast, the findings of G2 revealed presence of marked thickening in alveolar walls due to infiltration of mononuclear cells, complete abstraction of the bronchiole by the inflammatory exudate, hyperplasia of bronchiolar epithelia, sloughing, inflammatory cells inside the lumen, bronchitis and extensive hyperplasia. Also, there were severe pulmonary emphysema, blood vessel congestion and edema. In conclusion, we showed that nicotine is a harmful material for lung tissues and has serious systemic side effects which detected by reducing of the antioxidants and induces of furthermore MDA. Moreover studies are of great importance to detect the effect of nicotine on lung development as well as its effect on other body organs.

Keywords: Tobacco, Pulmonary tissues, Antioxidants, Lipid peroxidation, Iraq
Introduction
Cigarette smoking is a greater risk for diseases, and a common cause of early deaths in males and females (Koks et al., 2018). A part from the 21 known illnesses majorly correlated with smoking; many health risks still undifferentiated resulting in various systemic diseases (Carter et al., 2015). Association between the using of tobaccos and health status were initiated based on clinical data concerned to pulmonary tumors that represent 1st problem definitively related to using of tobaccos (WHO, 2015; Hou et al., 2019). European health experts identified that 7% and 24% of all female and male deaths in developed countries were caused by tobacco consumption (Sinha et al., 2018). In last 50 years, large volume of data was suggested the presence of approximately 4800 different chemical compounds in cigarette involving gases, 1010 particles of different sizes and several toxics, carcinogenic substances, tumor promoter and mutagenic (Ledda et al., 2017; Nlemedim, 2017). Although, there is a difficulty in determining the active substance, many researchers attributed the tobacco-related health risks to nicotine, nitrosamines and polycyclic hydrocarbons (Etman et al., 2023).
Nicotine is a naturally produced alkaloid originated most predominantly from Duboisia hopwoodii and tobacco, to be used in almost as an anxiolytic and stimulant (Wylie and Li, 2022). Gastrointestinal tract, respiratory system, urinary bladder and even skin can effectively absorb and metabolize nicotine (Khudhair, 2012; Jaber, 2013; Mishra et al., 2015; Panda and Albano, 2021). Also, nicotine has the ability to crossing the biological membranes as blood brain barrier with binding to the nicotinic cholinergic receptors at nerve terminal, and modulating the release of neurotransmitters like glutamate, serotonin, dopamine, norepinephrine and acetyl choline (Tega et al., 2018; Alhusban et al., 2023).

Materials and methods

Ethical approval
Scientific Committee in the College of Medicine (University of Al-Qadisiyah) was approved the work of this study.

Study animals
Totally, 40 male Wistar albino rats of 2 months old and 180-229 gm weight were purchased from the local markets, acclimated for 1 week, and then divided equally and randomly into two groups; G1, in which the study rats were received only normal saline; while rats of G2 were injected subcutaneously the nicotine daily for 28 days at a dose of 0.25 ml. During the acclimation and experimental periods, the study rats were fed pellet, received tap water and exposed to 12/12 dark / light.

Samples
At the end of experiment, the study rats were euthanized with chloroform and subjected for direct blood sampling into glass gel-free anticoagulant tubes. Post centrifugation (5000 rpm / 3 minutes), the sera were saved frozen in darkened containers for serology, and the lung tissues were collected into plastic containers contain 10% neutral buffered saline (NBF) for histology.

Serology
Following the manufacturer instructions (SunLong Biotech, China) of the CAT (Cat.No: SL1084Ra), GPx (Cat.No: SL1033 _Ra), SOD (Cat.No: SL1341Ra) and MDA (Cat.No: SL0475Ra) kits, the samples and Standards of each kit were prepared, processed, and the optical density (OD) were measured at 450 nm using the Microplate Reader (BioTek, USA). After setting the blank control at zero, the concentrations of the samples in each parameter were detected using the Standard Curve.

Histology
The lung tissues saved in 10% NBF were dehydrated, cleared, infiltrated, embedded, sectioned at (4-5 μm), and loaded on the slides that stained with the Hematoxylin and Eosin and examined under the light microscope (MEIJI, Japan) at 40X (Gharban et al., 2023).

Statistical analysis
The GraphPad Prism Software was served for identification of significant differences in values of serology among G1 and G2 groups at P<0.05 (Gharban, 2022). Values were represented as Mean ± Standard Errors (M±SE).

Results

Serology
The findings of CAT revealed a significant decrease (P < 0.0001) in values of G2 (8.84 ± 0.52 pg/ml) when compared to G1 (18.36 ± 1.23 pg/ml), (Figure 1). Concerning GPx, there was significant reduction (P < 0.0001) in value of G2 (215.91 ± 14.96 pg/ml) in comparison with those of G1 (418.48 ± 15.17 pg/ml), (Figure 2).

For SOD, value of G2 (3.37 ± 0.1 U/ml) was lowered significantly (P < 0.0001) more than value of G1 (7.58 ± 0.17 U/ml), (Figure 3). Significantly, higher value of MDA was seen in G2 (126.25 ± 5.95 ng/ml) than G1 (53.8 ± 4.51 ng/ml), (Figure 4).

Histology 
Normal histological sections of G1 were characterized by normal alveolar septa and epithelial cells (Figure 5). In contrast, the findings of G2 revealed the presence of marked thickening in alveolar walls due to infiltration of mononuclear cells, complete abstraction of the bronchiole by the inflammatory exudates, hyperplasia of bronchiolar epithelia, sloughing, inflammatory cells inside the lumen, bronchitis and extensive hyperplasia. Also, there were severe pulmonary emphysema, blood vessel congestion and edema (Figure 6).
Figure (6): Histological sections of lung tissues of G2 show abnormal tissues stained with the Hematoxylin and Eosin and examined under light microscope at 10X (A) and 40X (B & C).
Discussion

Nicotine is one of the greatly toxic chemical substances which identified as a major risk factor for lung-related diseases and can potentially cause adverse effects or even death at high dose; 6 mg for children and 50-60 mg for adult (Mayer, 2014; Rowell and Tarran, 2015). The damage to the tissues by nicotine is evidenced in this study by the reduction of antioxidants (CAT, GPx and SOD) and increasing lipid peroxidation (MDA) in blood. Sies (1997), who the first researcher introduced the phrase of “oxidative stress” and defines it as a disturbance in the pro-oxidant-antioxidant balance that causes either reduction for antioxidant concentrations or increasing the production the highly diffusible reactive oxygen species (ROS) which manifests its deleterious effects (Al-Khafaji, 2015; Bardaweel et al., 2018). Some authors have reported that smokers have poorer dietary habits and this result in observable consuming of ascorbic acid which may be the reason for the significant increase of oxidative stress (Shah et al., 2015; Ahmadi-Motamayel et al., 2017; Singh et al., 2019).

CAT is one of the most important antioxidant enzymes, which represent in almost all aerobic organisms. This enzyme is produced by numerous metabolic reactions and decompose the hydrogen peroxide to oxygen and water (Gebicka and Krych-Madej, 2019). Several studies demonstrated that catalase deficiency or malfunctioning is associated with many diseases and can occur as a result of inherited or genetic disorders (Dai et al., 2017; Maciejczyk et al., 2017; Nandi et al., 2019). Other authors found that CAT activity decreased significantly in the blood of smokers attributing this effect to increasing the ROS production and inhibition of CAT activity by nicotine which can result later in mutagenic and cytotoxic effects (Rickert et al., 2011; Crooks et al., 2018; Smart et al., 2019). Raddam et al. (2017) suggested that the decreased activity of CAT is attributed to inactivation of CAT by the cross linking or impairment of nitric oxide synthesis which can bind reversible to ferric iron and inhibiting afterwards CAT activity (Noichiri et al., 2013; Silambbarasan et al., 2014). Our results were in contrast with previous studies which detected an increasing of CAT levels in smokers (Garg et al., 2006; Reejamol and Swaminathan, 2013).

GPx is a systolic enzyme that catalyzes the reduction hydrogen peroxide radical to alcohol and oxygen. This enzyme has the ability to cure several diseases since increasing the endogenous levels of GPx and resolving of ROS-induced pathology (Prasad et al., 2018; Joshi et al., 2020; Zhang et al., 2021). We agrees the major worldwide reports that revealed the reduction of GPx levels in smokers (Agarwal et al., 2019; Oladunjoye et al., 2022). This reduction could be resulted by fluxing of more hydroxyl radicals and hydrogen peroxide as a result of smoking. In normal conditions, SOD considers as one the most active important antioxidant enzyme as free radical scavenger. Our results showed a significant reduction in concentration of SOD in experimentally G2 when compared to control G1 which agrees with other findings by other authors (Jain and Flora, 2012; Oyeyipo et al., 2014). Also, we agreed with Mohammed and Al-Thwani (2019) who detected that the levels of GPx were decreased significantly while MDA was increased significantly in experimentally mice injected nicotine subcutaneously. In human, Abdul-Rasheed and Al-Rubayee (2013) showed that the plasma MDA level was significantly increased in smokers compared with non-smokers; however, the plasma SOD AND GPx were differed insignificantly.

Mohammed and Al-Thwani (2019) observed that injection of nicotine causes different pathological changes in lung tissues of mice such as lymphocytes infiltration, fibrosis, alveolar edema, hemorrhage, congestion of blood vessels, emphysema and damage to alveoli.

Structural modification in cellular proteins due to oxidative stress was also showed in smokers (Stangenberg et al., 2015; Caliri et al., 2021); while others mentioned that the damage in tissue is limited to few protein molecules (Zong et al., 2019). As observed by other researchers, the exposing of lung tissues to great flux of hydroxyl radicals and hydrogen peroxide could reflect in absence of balance in antioxidant production (Tribble and Jones, 1990; Rahal et al., 2014). Raddam et al. (2017) mentioned that nicotine play a role in increasing the generation of hydrogen peroxide and superoxide anion which in turn elevate significantly the production of ROS and cause deleterious injury to alveolar macrophage by cause releasing of proteolytic enzymes.

Conclusion

This study showed that nicotine is a harmful material for lung tissues and has serious systemic side effects which detected by reducing of the antioxidants and induces of furthermore MDA. Moreover studies are of great importance to detect the effect of nicotine on lung development as well as its effect on other body organs.

Conflict of interest

No.

Authors’ contributions

MAS: Experimental study, collection of blood and tissue samples, and statistical analysis. NED: Tissue processing and serology. Both authors contributed equally in microscopic examination of slides, reading and approving the final copy of the manuscript.

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