Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2024, 37(4)

Attenuation Acute Effects of Passive Smoking on Pulmonary Tissues in Rats by Bee Honey

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Received: 21 March 2024 Accepted: 9 June 2024 Published: 20 October 2024
doi.org/10.30526/37.4.3955

Abstract

The recent study's goal was to use honey to reduce the acute effects of passive smoking on rats' pulmonary tissues. A total of 28 adult male rats were categorized into four groups: (1) control group; (2) rats were given honey (100 mg/kg BW/30 days); (3) rats were exposed to the cigarette smoke for 30 days; and (4) rats were given honey (100 mg/kg BW/14 days); previously, the rats were exposed to cigarette smoke for 30 days. The mean Wet/Dry values were significantly higher in the KAR group than in the NC rats. While the PTV rats showed a notable decline in comparison to the KAR rats, the PTV rats showed no change with the NC rats. Also, after 30 days of exposure to KAR, we observed numerous changes in the rats' gross lung lesions and more histological alterations as compared to all other rats' lungs. Furthermore, the lung tissue of the PTV rats had some histological changes and a moderate improvement in tissue structure when compared to KAR rats. In conclusion, the study demonstrated the beneficial effects of honey in lowering lung tissue damage. It is also a better treatment choice, which may lead to better results in terms of tissue damage caused by CS.

Keywords: Lung tissues, honey, histological, rat, cigarette smoke.

1. Introduction

Inhaled tobacco smoke (TS) is known as passive smoking (PS), and it is a common source of indoor air pollution worldwide [1]. Non-smokers view passive smoking (PS) as the inhalation of smoke from tobacco compounds [2]. Moreover, homes and workplaces are the primary areas where people commonly encounter PS exposure. Additionally, PS is a complex mixture of various toxins, some of which originate from burning side stream smoke (TS) [3]. Moreover, cigarette smoke (CS) contains more than 400 chemical components, of which 250 originate from treated tobacco, and the remaining components are associated with the addition of pesticides, biological complexes, and metallic compounds [4]. Additionally, PS exposes people to the same carcinogens as heavy smoking, which is the main cause of lung cancer [5], respiratory and cardiovascular diseases [3]. Moreover, smoke from tobacco products causes lung oxidative stress, inflammation, and cancer [6]. On the other hand, CS byproducts lead to structural abnormalities in tissues due to microcirculation deficiencies, low oxygen concentration,

inflammatory processes, and tissue repair mechanisms [7]. In addition, CS is one of the main causes of death in many countries. It damages tissues and lets many chemicals into the body, which can directly cause the production of free radicals (FR) and inflammatory cells, as well as reactive oxygen species (ROS) [8].

By neutralizing FR, antioxidants (ANT) prevent damage to the constituent parts of cells. As a result, eating antioxidants helps prevent cell damage [9]. Also, foods high in flavonoids and ANTs provide protection against a variety of illnesses [10]. Among honey (HY), many constituents are organic acids, minerals, polyphenols, flavonoids, proteins, carotenoid derivatives, vitamins, aroma compounds, and amino acids [11, 12]. Researchers have reported that HY, a nutritionally advantageous natural substance, is also therapeutic and an antioxidant [14]. Moreover, it possesses immunomodulatory, anticancer [13], antibacterial, anti-inflammatory, and antioxidant qualities [11]. Accordingly, HY may reduce oxidative stress in a variety of organs and, whether taken, may help avoid chronic diseases [15]. As a result, the current investigation aimed to use honey treatments to lessen the acute lung damage caused by passive smoking in albino rats.

2. Materials and methods

2.1. Materials

From the local market came the natural honey (HY), and the Karelia red cigarettes (KAR). The HY was examined in a lab at the Omar Al-Mokhtar University Center in El-Beyda, Libya.

2.2. Housing of the animals

There were 28 adult male rats that weighed 180–200 gm. The University of Omar Al-Mokhtar, El-Beyda, Libya, provided rats for the experiment through the Zoology Department of the Faculty of Science. Following three weeks of acclimatization, the animals were kept in cages at room temperature (22±2°C), following conventional laboratory procedures. Normal rat food and unlimited water were provided to the rats.

2.3. The study's design

We randomly assigned four groups of eight male rats, as follows: Group 1: We did not expose the rats in the normal control group (NC) to KAR and maintained them in a standard laboratory setting with ventilation. Group 2: For 30 days, rats in the honey group (HY) received natural honey (100 mg/kg BW) through oral feeding [15]. Group 3: The Karelia Red (KAR) cigarette group connected the bee smoker machine to a glass box via a connection pipe, resulting in 30 days of KAR exposure [16]. This box was made by the Zoology Department at the University of Omar Al-Mokhtar in El-Beyda, Libya (**Figure 1**). The rats in Group 4 were in the protective group (PTV) and were given HY (100 mg/kg BW) by mouth for 14 days. As a result, they exposed the rats to KAR and administered the HY for 30 days [17]. All animal experiments adhered to the ethical criteria for animal research.

Figure 1. The process box of smoke exposure.

2.4. Macroscopic evaluation of the lungs' gross lesions

Both the comparative control and treatment groups showed macroscopic lung pathology [18]. The histopathological lesion scores were determined using a dissecting microscope and ranged from (- to +++) in all rats, as shown in **Table 1** [12].

Table 1. Histopathological ailment scoring design.

Scoring of lesions	Description
-	no lesion
+	Slight lesions in 25%
++	Reasonable lesions in 25% -75%
+++	Severe lesions in more than 75%

2.5. Lung water content

The water content of the rats' lungs, a gauge of the severity of pulmonary oedema, was determined by calculating the Wet/Dry weight ratio of lung tissues. The lung was sliced, and the wet weight was noted after filter paper was used to remove the blood and water stains from the lung tissue surface. After the same lung was dried for 48 hours at 80 ^{o}C to calculate its dry weight, the wet/dry ratio was calculated [19].

2.6.Tissues study

The best ways to fixate the lungs were to inject 10% formalin into the trachea and embed 10% formalin in paraffin. We then dehydrated the lungs in a series of ethanol chemicals and fixed them with xylene. Sections (5 μm) were produced using a rotary microtome [20]. We applied hematoxylin and eosin to the sections following established protocols [21].

2.7. Analysis of data

Minitab version 17 was used for data analysis, and all data were represented as the (M ±SE). The means of the treated and control groups were compared using the One-Way ANOVA statistical test, with a significance level of $P \leq 0.05$. Additionally, Turkey's test was used to differentiate the means. Additionally, the T test was employed to compare the two means.

3. Results

3.1. Lung water content

Table 2 and **Figure 2** display the average wet/dry weight ratio of the experimental and control groups' lungs. The KAR group had a significant ($P \leq 0.05$) increase in the mean Wet/Dry values

(5.644±0.362) compared to the NC rats (4.44±0.239). On the other hand, as compared to KAR rats, the PTV rats exhibited a significant drop (5.188±0.375), while no change was observed with NC rats.

Table 2. Average of mean values of the Wet/ Dry weight lungs in the all groups.

Parameters	NC mean ± SE	HY mean ± SE	KAR mean ± SE	PTV mean ± SE
Wet/Dry weight ratio	4.44±0.23 B	4.72±0.188 B	5.644±0.361 A	5.188±0.375 B

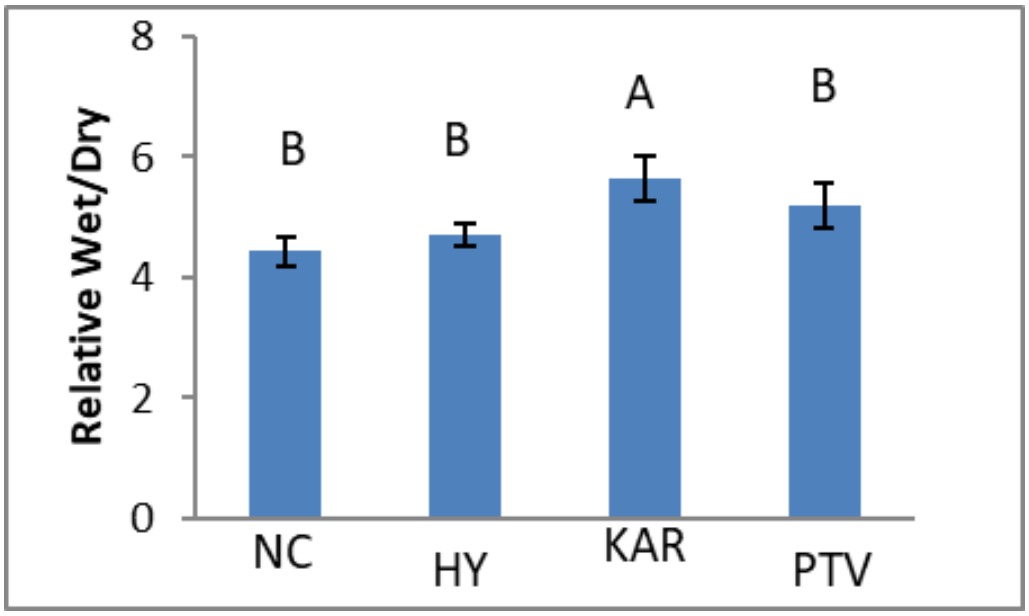

Figure 2. Comparative the Wet/Dry lung in the experimental and normal control groups.

3.2. Macroscopic assessment of the gross lesions' lungs

The gross lung lesions of rats, which illustrate the 30-day lung health in NC and HY rats (**Figures 3A, B**), are shown. The typical injuries in rats exposed to KAR are shown in Figure 3C. These include severe red pleural discoloration, dark red pleural discoloration, gray hepatization, and bulge nodules. In contrast, Figure 3D shows that there was no significant change in the overall appearance of the lung in the PTV after 30 days, with only a small amount of red spots. In addition, Table 3 shows the histopathological illness scores for all animals. The NC and HY rats had no lesions, while the KAR rats had severe lesions. The PTV rats, on the other hand, had fewer lesions than the NC rats.

Table 3. Histopathological illness scoring for all groups.

Groups	NC	HY	KAR	PTV
Scoring of lesions	-	-	+++	+

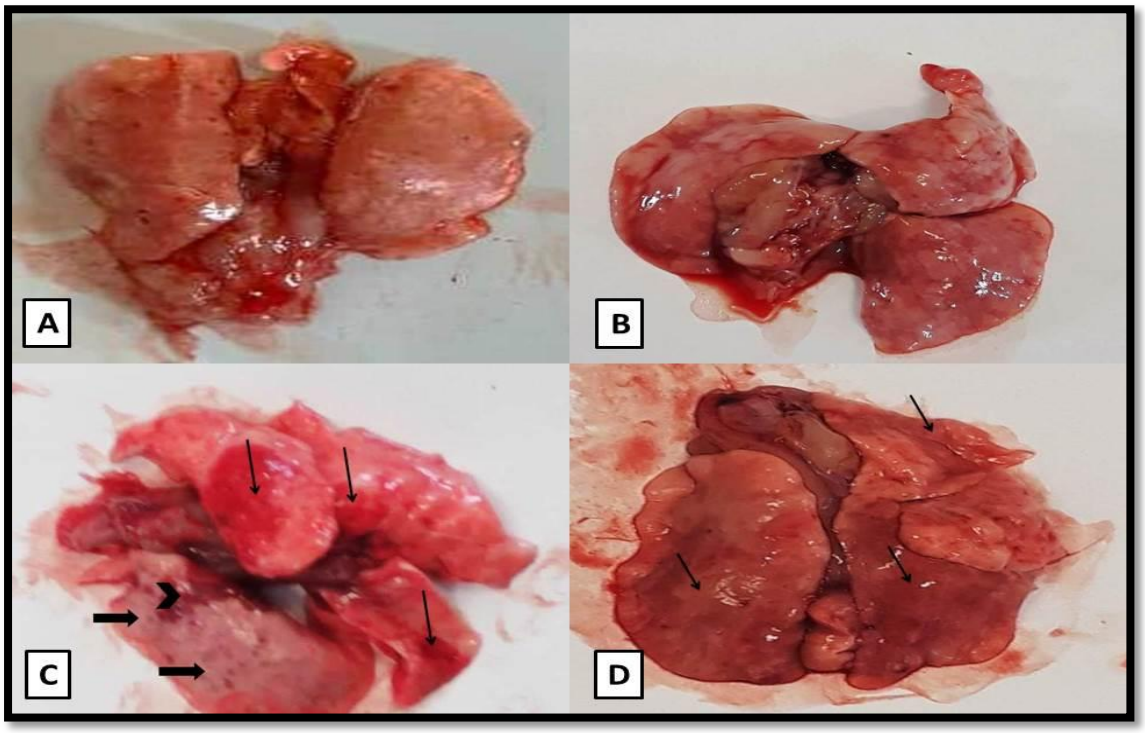

Figure 3. Gross lesions of lungs of rats, showing, (A): Healthy lung NC rats; (B): Healthy lung in rats that received HY; (C): Severe red pleural discolouration by indicates typical lesions (arrows), rats treated to KAR showed dark red pleural discoloration (head arrows), gray hepatization, and bulging nodules (thick arrows); (D): There was obviously no discernible change in the PTV rats' lungs overall appearance, with just a tiny quantity of red spot (arrows).

3.3.Histopathological studies

Under a microscope, the lung sections from the NC and HY rats revealed normal blood vessels, mucous-secreting columnar respiratory epithelium-lined bronchioles, classical inter-alveolar septa, normal lung parenchyma, and normal alveoli (**Figures 4-7).** On the other hand, animals exposed to KAR alone for 30 days showed more significant histological alterations compared to the lung sections of NC rats. The bronchiole wall shows a lot of inflammatory cells penetrating it, a blood vessel that is deviated and has a thick wall, persistent inflammatory peribronchiolar penetration, and bronchiole degeneration (**Figure 8**). Furthermore, **Figure 9** shows lung parenchyma shrinkage, degeneration, and necrosis, as well as inflammatory cells permeating congested vessels. **Figure 10** illustrates the bronchiolar epithelium's desquamation. **Figure 11** illustrates diffuse extravasation of RBCS, lung parenchyma deterioration, and inter-alveolar septa narrowing, all of which are associated with vascular congestion and large cellular infiltration. Additionally, the deformed basement membrane and degeneration of the bronchiolar epithelium with desquamation into the lumen are shown in **Figure 12.** Accordingly, the data show the various histological alterations in the lung tissue of the rats receiving KAR treatment. On the other hand, the lungs of PTV rats had thicker inter-alveolar septa, vascular congestion, breakdown of the bronchiolar epithelium, and a clear presence of inflammatory cells (**Figures 13, 14**). The lung sections from the shielded rats showed some histological changes in tissue content and a moderate improvement in tissue structure when compared to KAR rats.

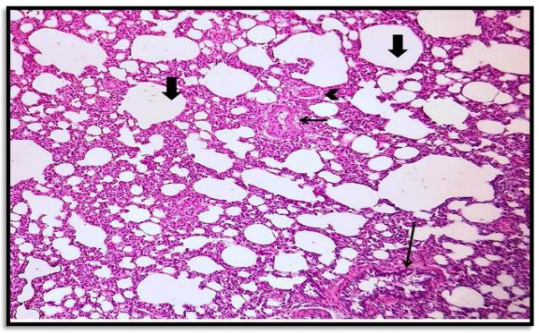

Figure 4. Section in lung of NC rats' reveal a normal lung parenchyma with normal alveoli (thick arrows), mucous-secreting columnar respiratory epithelium lined bronchioles (long arrow), a normal vein, and head artery (small arrow). (H&E, 10x).

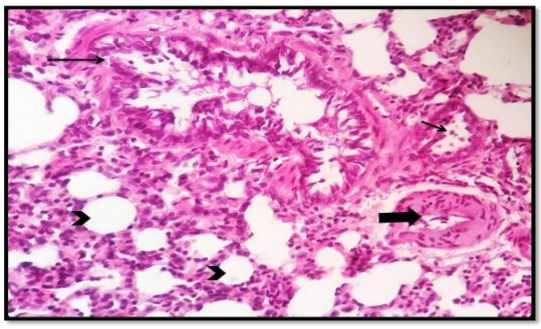

Figure 5. Section in lung of NC rats' reveal the ordinary alveolar duct (head arrows), classical inter-alveolar septa with typical alveoli (head arrows), mucous-secreting columnar respiratory epithelium lining the bronchioles (long arrow), standard arteries (thick arrow), and capillaries (small arrow). (H&E, 40x).

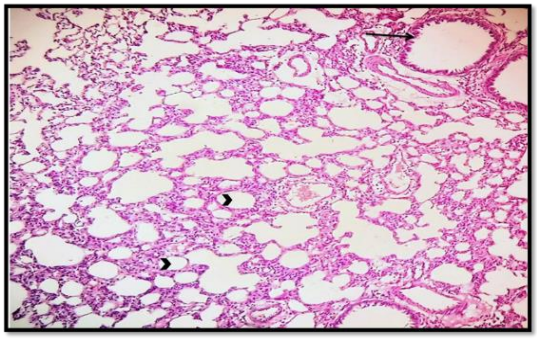

Figure 6. Section in lung of HY rat displaying the typical lung parenchyma with typical alveoli (head arrows), mucous-secreting columnar respiratory epithelium lined bronchioles (long arrow). (H&E, 10x).

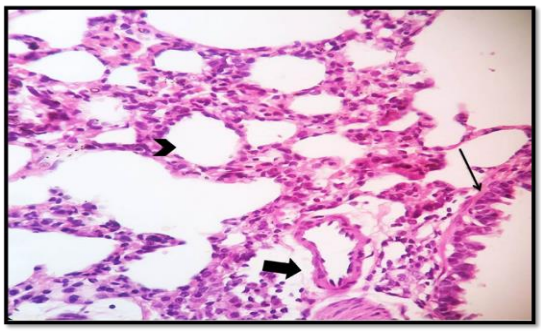

Figure 7. Section in lung of HY rat displaying the blood vessels (thick arrow), mucous-secreting columnar respiratory epithelium (long arrow), and regular alveoli with classical inter-alveolar septa (head arrow). (H&E, 40x).

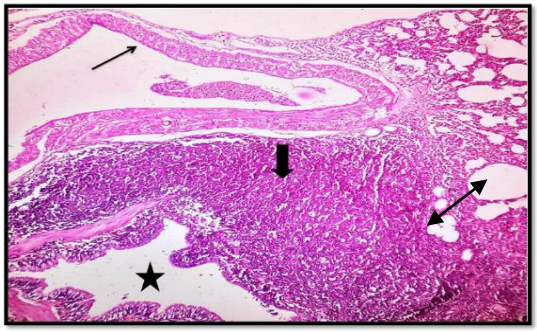

Figure 8. Section in lung of rats exposed to KAR, demonstrated deterioration of the bronchioles, an obvious intrusion of inflammatory cells (star), necrosis (↕), and chronic inflammatory peribronchiolar intrude (thick arrow). (H&E, 10x).

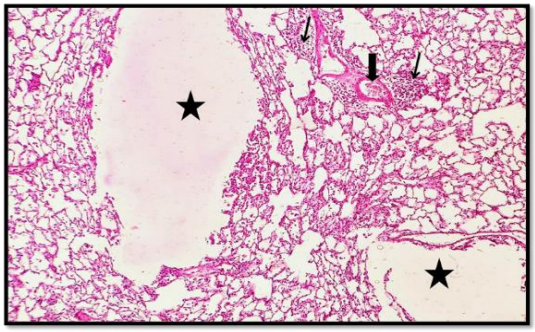

Figure 9. Section in lung of rats exposed to KAR exhibit atrophy, degradation, and necrosis of the lung parenchyma (stars), surrounded by congested blood vessels (thick arrow) and permeation of the inflammatory cells (arrows). (H&E, 10x).

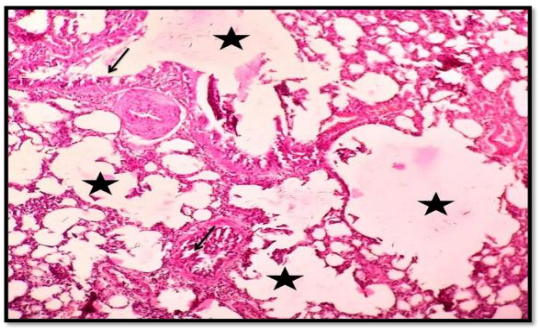

Figure 10. Section in lung of rats exposed to KAR showed bronchial epithelial desquamation (arrows) and lung parenchyma shrinkage, degradation, and necrosis (stars). (H&E, 20x).

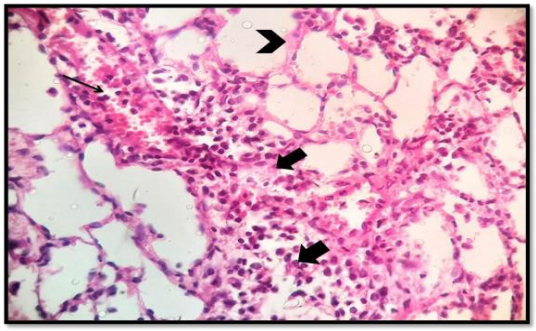

Figure 11. Section in lung of the rats exposed to KAR exhibit retreating inter-alveolar septa and deteriorating lung parenchyma (head arrow). Diffuse of RBCs is observed with vascular congestion (arrow), and massive cellular inflammatory penetration (thick arrows). (H&E, 40x).

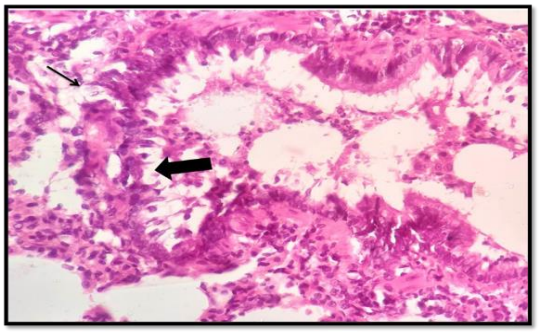

Figure 12. Section in lung of the rats exposed to KAR demonstrated deformed basement membranes (arrow) and deteriorating bronchiolar epithelium (thick arrow), with desquamation visible into the lumen. (H&E, 40x).

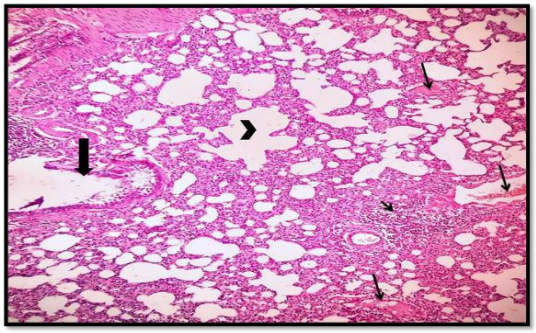

Figure 13. Section in lung of the PTV rats, showing the degradation of lung parenchyma (head arrow), vascular congestion (long arrows), erosion of bronchiolar epithelium (thick arrow), and inflammatory cell permeation (short arrow). (H&E, 10x).

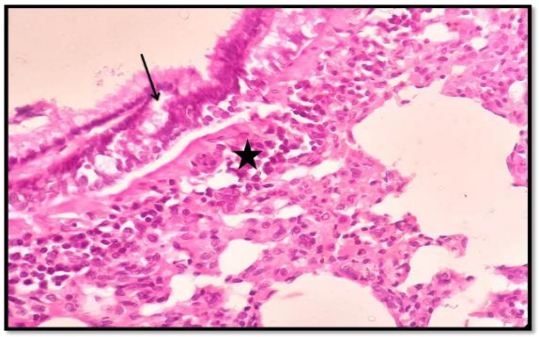

Figure 14. Section in lung of the PTV rats, display thicker of inter-alveolar septa, inflammatory cell penetration (star), and a disintegrated bronchiolar epithelium (long arrow). (H&E, 40x).

4. Discussion

The mean wet/dry values were significantly ($P \leq 0.05$) higher in the KAR group than in the NC rats. On the other hand, the PTV rats showed a notable decline in comparison to the KAR rats. The PTV rats showed no change compared to the NC rats. [22] corroborates this by finding that CS exposure increased the amount of water in the lungs of CS rats compared to the control group. They discovered that inhalation of the CS resulted in pulmonary edema. Moreover, a surplus of extravascular lung water and a significant increase in the permeability of the endothelial barrier demonstrated a higher rise in pulmonary oedema in mice exposed to CS [23]. However, after 30 days of exposure to KAR, the researchers saw many differences in the rats'

lung lesions compared to the other groups. These differences included severe red pleural discoloration, dark red pleural discoloration, gray hepatization, and bulge nodules. KAR produced a variety of histopathological alterations in lung tissues, which could account for these findings. According to [23], animals exposed to CS were more likely to experience inflammatory lung damage and had higher concentrations of IL-6 and CXCL-9. Additionally, rats exposed to KAR alone for 30 days showed more significant histological alterations compared to lung rats from NC rats. These alterations included substantial penetration of inflammatory cells into the bronchiole wall; a diluted blood vessel with a thick wall, persistent inflammatory peribronchiolar penetration, bronchiole degeneration, shrinkage and degeneration of the lung parenchyma, necrosis with inflammatory cell permeation around congested vessels, diffuse extravasation of RBCS along with lung parenchyma deterioration, vascular congestion, and large cellular infiltration Desquamation into the lumen is also caused by the deformed basement membrane and the breakdown of the bronchiolar epithelium. [24, 25] confirmed similar outcomes. Exposure to CS vapors has triggered morphological changes in lung fibroblasts, inflammatory responses, oxidative stress, intra-alveolar fibrosis, acute alveolitis, and the penetration of neutrophils, macrophages, and eosinophils in the lungs [24]. Also, [26] said that inflammatory cells might be hurting the interstitial and alveolar lung tissues by releasing lytic enzymes and oxygen-free radicals. [27] also found that the CS increases oxidative stress through a number of pathways, such as explicit radical species damage and the inflammatory reaction. In addition, [28] confirmed that rats exposed to CS develop fibrosis and hyperplasia of the tiny airway wall muscles, along with vascular abnormalities linked to pulmonary hypertension. These alterations are likely the result of inflammation-induced arterial wall thickening. Furthermore, there was extensive erythrocyte extravasation and partial bronchiole erosions, with missing cilia from the CS exposure [25]. The thickening of the alveolar septa may be caused by oedema, inflammatory penetration, the secretion of pro-inflammatory cytokines, and an increase in blood capillary volume [24]. Inhalation of CS caused acute effects of oxidative stress in animal lung tissue, reduced lung function, and increased airway inflammation [29]. However, [30] demonstrated that the oxidative damage caused by CS exposure increased the amount of ROS in the lung tissue. Moreover, inflammation within the pulmonary alveoli after exposure to CS may also harm the alveolar walls, which may impair the alveolar walls' capacity to exchange gases effectively under oxidative stress [27].

In addition, the current investigation found that the lung tissue of PTV rats exhibited some histological changes in its composition, and showed a moderate improvement in its structure compared to KAR rats. These results could be attributed to the antioxidant properties of HY [31], the presence of phenols and vitamins [32], its ability to inhibit oedema [15], and its protective effects on oxidative stress in rat tissues exposed to CS [17]. Numerous compounds and distinct physicochemical features of HY, like flavonoids [11], contribute to its anti-inflammatory and FR scavenging properties, and it also fosters healing in rats' injured tissue [17].

5. Conclusions

The current findings unequivocally show that honey has a positive impact on reducing lung tissue damage. It is also an improved therapeutic option that has the potential to improve outcomes when it comes to tissue damage caused by CS.

Acknowledgment

Many thanks to editor-in-chief and members of the editorial committee in Ibn Al-

Haitham Journal for Pure and Applied Sciences.

Conflict of Interest

There is no conflict of interest.

Funding

There is no funding for the article.

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