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Effects of Cigarette Smoke and Electronic Cigarette Vapors on Lung Histomorphology: A Comparative Study in Rats

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A B S T R A C T

Background: The use of electronic cigarettes, or vaping, to obtain nicotine is gaining popularity. Numerous researches have demonstrated the potential health risks and development of nicotine dependency associated with smoking electronic cigarettes. The effects of traditional and electronic cigarettes on lung tissue were evaluated in this study.

Objective: The objective of the study was to determine the Lung Histomorphological changes exposed to Cigarette Smoke and Electronic Cigarette Vapors in Rats.

Methodology: This study was conducted at the Women Medical College Abbottabad and Ayub Medical College Abbottabad. The study duration was six months from July 2022 to December 2022. The study was conducted on, 30 male Wistar rats collected from NIH Islamabad. Three groups of animals were used: group C was the control group, meaning it did not get any nicotine; group A received liquid vapor from electronic cigarettes; and group B rats were exposed to regular smoking.

Results: Both experimental groups exhibited several abnormalities, including as collagen deposition, type II pneumocyte hyperplasia, parenchymal collapse, and an upsurge in macrophages inside a thicker alveolar septa. There was also evidence of an early elastolysis. In contrast to the group exposed to ordinary cigarette smoke, there were more α-SMA positive myofibroblasts and blood vessels, and the elastic fibers were thicker, less dense, irregular, and disordered.

Conclusion: Our study concludes that using electronic cigarettes causes fewer severe histomorphological changes than smoking regular cigarettes. However, the histopathological damage caused by vaping may lead to the formation of alterations in lung tissue that obstruct gas exchange.

Keywords: Histomorphological Changes; Nicotine; Smoking; Lung; Electronic Cigarettes

Introduction

It has been known for a long time that smoking cigarettes has harmful effects. There is an increased risk of lung fibrosis, asthma, cancer, cardiovascular disease, and metabolic problems with long-term smoking exposure, both actively and passively.[1-4] The most physiologically active ingredient in smoke is nicotine which is largely responsible for the harmful effects of cigarette smoking.[3] Nicotine has serious and complicated impacts on human health because its receptors are spread throughout the body.[1,5] The effects of nicotine on the lungs themselves are significant because most of the nicotine that is inhaled into the circulation is absorbed by the cells in the lungs.[6,7] Moreover, smoking cigarettes causes smoke residues to remain in the lung tissue, which causes inflammation over time.[4,8]

E-cigarettes, often known as electronic cigarettes, emit a tar-free, non-burning vapor. Because of this, it is seen to be a safer option for treating cigarette addiction than regular cigarettes.[3,9] Moreover, quitting smoking with e-cigarettes appears to be simpler than with nicotine gums or patches since their usage is comparable to that of traditional cigarettes.[9-12] A number of substances, when heated, combine to form vapor or aerosol, which mimics smoking, in e-cigarette liquids.[13] In addition to nicotine, other components that may improve mood include strong flavors (derived from plant or herb extracts) and humectants (glycerin, propylene glycol).[14] Recent years have seen the implementation of new rules in some countries regarding liquid contents; for instance, it is now illegal to combine sildenafil with psychiatric medications.[15-17] Vapour may also include additional dangerous substances such formaldehyde, carbonyls, and nitrosamines that are present in ordinary cigarettes.[8]

Given the rapid rise in e-cigarette use, it is crucial to assess their effects on lung histomorphology and compare them with those of conventional cigarette smoke. Animal models, particularly rats, serve as valuable tools for investigating the histological changes induced by exposure to various substances. By subjecting rats to controlled conditions of cigarette smoke and e-cigarette vapors, researchers can evaluate the structural alterations in lung tissue and gain insights into the potential risks associated with vaping.

Understanding the histomorphological changes induced by cigarette smoke and e-cigarette vapors in rats is essential for several reasons. Firstly, it can provide valuable data on the comparative toxicity of these two forms of nicotine delivery, informing public health policies and regulatory measures aimed at reducing tobacco-related harm. Secondly, elucidating the mechanisms underlying lung injury induced by e-cigarette use can guide the development of targeted interventions and safer alternatives for nicotine delivery.[7]

Previous studies have demonstrated that cigarette smoke exposure leads to a range of histological abnormalities in the lungs, including inflammation, epithelial cell damage, fibrosis, and emphysema. These changes are attributed to the complex mixture of toxicants present in cigarette smoke, including carcinogens, reactive oxygen species, and inflammatory mediators.[6] In contrast, research on the histopathological effects of e-cigarette vapors is still relatively limited but is rapidly expanding.

Preliminary evidence suggests that e-cigarette aerosols contain fewer toxicants than conventional cigarette smoke, but they are not devoid of harmful substances. Studies have reported inflammatory responses, oxidative stress, and alterations in immune cell populations in the lungs of animals exposed to e-cigarette vapors. However, the long-term consequences of these changes and their implications for respiratory health remain to be fully elucidated.

In this context, conducting a comparative histomorphological analysis of lung tissue in rats exposed to cigarette smoke and e-cigarette vapors can provide valuable insights into the relative risks associated with these two forms of nicotine delivery. By evaluating parameters such as inflammation, epithelial integrity, fibrosis, and alveolar damage, researchers can assess the extent of lung injury induced by each exposure modality and identify potential differences in their pathological mechanisms.

Objective

The objective of the present study was to determine the Lung Histomorphological changes exposed to Cigarette Smoke and Electronic Cigarette Vapors in Rats.

Methodology

This was an experimental study conducted at the Women Medical College, Abbottabad and Ayub Medical College, Abbottabad. The study duration was six months from July 2022 to December 2022. This study included 30 male Wistar rats weighing an average of 180 to 220g were employed in our experiment at initial. The rats were categorized in three groups (A, B, and C). The rats in group A, were in contact with unscented e-cigarette vapors. Housed in a PCV cage of 0.13 m^3 and driven by a 0.18 kW pump running at 1.4/1.6 A, 230 V, 50/60 Hz, the rats were exposed to the vapours for 10 minutes. On one side of the box, it was mounted, and on the other, an aerosol for electronic cigarettes was blasted. This setting allowed airflow in the cage. Each time, five-animals hermetically enclosed in cage, the only two apertures left exposed were the e-cigarette and pump connection points. An e-liquid purchased from local market consist of

Table 1. The thickness of the blood-air barrier-forming membrane as measured by PAS staining, the quantity of blood vessels visible in a single field of view at 100X magnification, and the Masson's trichrome staining optical density score

Parameters measured	Groups			P-value
	A (n = 300)	B (n = 300)	C (n = 300)	
Optical density score of Masson staining's trichrome	0.19 ± 0.04	0.23 ± 0.07	0.14 ± 0.03	A:C, P = 0.0005; B:C, P = 0.0003; A:B, P = 0.0070;
Thickness of blood-air barrier forming membrane measured in PAS staining, µm	0.38 ± 0.06	0.42 ± 0.17	0.19 ± 0.05	A:C, P = 0.040; B:C, P = 0.030; A:B, P = 0.040;
Number of blood vessels observed in one field of view at x100 magnification	5.99 ± 1.94	8.18 ± 2.2	2.99 ± 1.19	A:C, P = 0.008; B:C, P = 0.004; A:B, P = 0.022;

12.1 mg/ml of propylene glycol, nicotine, and water was given to the animals at a dose of 0.6 ml each day. One treatment cycle consisted of five minutes of puffing and twenty minutes of pausing. For the experiment, an e-cigarette with a voltage of 5.6 Volts was utilized. When the cycle was over, the animals were moved to a sanitized cage. The animals were subjected to a single cycle every day for a consecutive period of six weeks .[18] Ten regular cigarettes with the same amount of nicotine as in group A were used to introduce the rats in group B to smoking. A sum of 210 mg of nicotine were given to one group of rats. The amount of nicotine in each rat's serum was not examined. Consequently, it is hard to determine the precise dosage of nicotine that a given rat received. As the control group, group C's rats went through the identical stressors related to inhalation as the other rats, but without the addition of nicotine. The rats had their lungs removed and were decapitated without anesthesia 24 hours after the prior exposure. At the time of sacrifice, the body weights of the rats were 285.55 SD 20.43g (group B), 325.39 SD 19.9g (group C), and 289.73 SD 14.69 g (group A). Rats in group (C) experienced the same inhalation-related stressors as the other rats, but without the addition of nicotine, serving as the control group. Twenty-four hours following the previous exposure, the animals were decapitated without anesthetic and had their lungs taken. The rats' body weights were 285.67 SD 20.34g (group B), 325.38 SD 20.16 g (group C), and 289.73 SD 14.69 g (group A) at the time of sacrifice. Moreover, it alters the blood's molecular markers in a way similar to CO2 .[19] Enflurane, halothane, isoflurane, and sevoflu-rane all have negative effects on rat sperm .[20] Moreover, the liver's metabolism is significantly impacted by isoflurane euthanasia, and the quantity of glycogen may change.[21] Cervical dislocation was refused since it requires anesthetic prior to surgery. However, since concussions can result in significant brain damage, they were not taken into account. To sum up, decapitation was selected because, unlike different methods approved by EU Directive 2010/63/EU, it will not affect the biochemistry or significantly change any organs other than the neck. By accepting responsibility, we were able to obtain approval from the Bioethical Committee for the complete trial, which included the decapitation. The material was fixed in formalin buffered 10% before the organs were inserted in paraffin blocks.

Masson's trichrome staining, periodic Acid-Schiff (PAS), and hematoxylin and eosin (H&E) staining

Using a light microscope fitted with x10, x40, and x100 lenses, the H&E stained tissues were investigated histomorphologically. Masson's Trichrome was used to stain the samples in order to measure the degree of fibrosis and collagen deposition. Using PAS staining, the blood-air barrier was visible. We stained according to regular procedure. The PAS-stained sections were examined using a microscope fitted with an Olympus BX41 digital camera, lens x100, and Cell Sense software to determine the thickness of the membrane forming the blood-air barrier. The optical density of the picture of the trichrome-stained area was identified and quantified as previously reported [22], using ImageJ and the associated color deconvolution

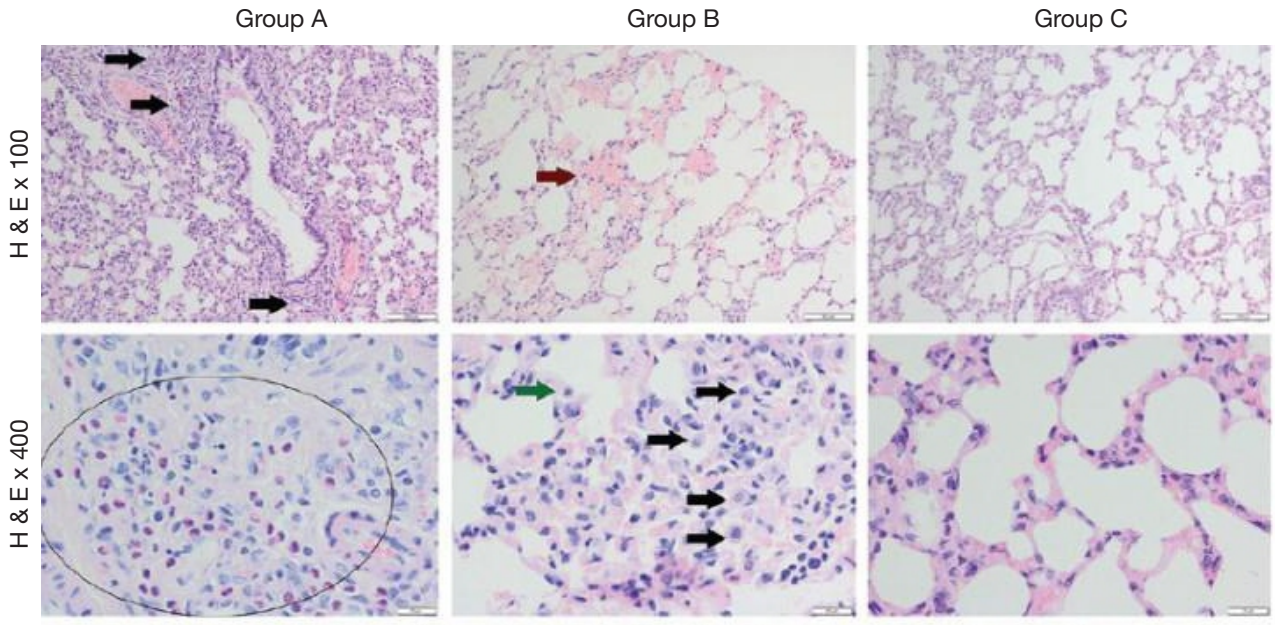

Figure 1. Histological characteristics of various lung groups. The control group (group C) showed normal histological organization of lung tissue with collagen fibers surrounding the bronchioles. Black arrows, thicker alveolar septa, and hyperaemia were indicative of eosinophil, erythrocyte, and mononuclear cell infiltration in the e-cigarette group. Additionally, collagen deposits were seen within the alveolar septa. The traditional cigarette group (group B) had thicker alveolar septa, macrophages shown by green and black arrows, and hemorrhage indicated by a red arrow. Amplification, either x100 or x400. Hematoxylin and eosin, or H&E

plugin.[23]

Immunohistochemical (IHC) and orcein stainings

α-Smooth Muscle Actin (α-SMA, Elabscience, polyclonal rabbit anti-human, -mouse and -rat antibody, E-AB-33323, dilution 1:200) was the target antibody used in this immunohistochemical staining. Li et al. (36) used it for the first time to evaluate myofibroblasts, which are the cells that make blood vessel walls and collagen fibers. In an 800 W microwave oven, the antigenic sites were thermally exposed over three 5-minute incubation cycles in a pH=6 citrate buffer solution. Slides were treated with 0.3% perhydrol (H2O2) in methanol for 30 minutes in order to lower endogenous peroxidase activity. To avoid non-specific bindings, the samples were treated in normal serum for one hour. The material was treated with diluted primary antibody and kept at 4˚C for 17 hours. The reaction was seen using hematoxylin coloring and the diaminobenzidine solution (DAB) for five minutes. The lack of the specific primary antibody was the only modification made to the negative control manufacturing process. Using x10 and x40 lenses on a light microscope, the material was investigated. The number of vessels may be measured thanks to the imaging of Vessel wall cells contain α-SMA muscle actin. A 100x lens was used to count the blood vessels in 50 fields of vision.Orcein stain was used to evaluate elastic fibers.

The test results that were gathered were statistically analyzed using SPSS 20. In this case, a probability (P-value) of less than 0.05 was considered statistically significant.

Results

In the current study, a total of 30 male Wistar rats weighing an average of 180 to 220 g were employed in our experiment at initial.

There was an increase in macrophages, parenchyma, hyperhagia, and a collapse of type II pneumocyte hyperplasia in both experimental groups. The e-cigarette group also had larger alveolar septa, hyperaemia, intrabr-onchiolar erythrocytes, and an infiltration of mononuclear cells and eosinophils in addition to increased mucus production. Emphysema signs, such as cell vacuolization in the alveolar septa, mucus intrabronchioles, and bleeding into the bronchiole and alveolar lumen, were seen in the group of smokers who smoked ordinary cigarettes. They also showed abnormalities in the alveolar lumen.

When Masson's Trichrome staining was used, both experimental groups had regions of increased collagen deposition within larger alveolar septa as well as early fibrosis. According to Table 1, the group of regular cigarettes had the highest optical density score. The

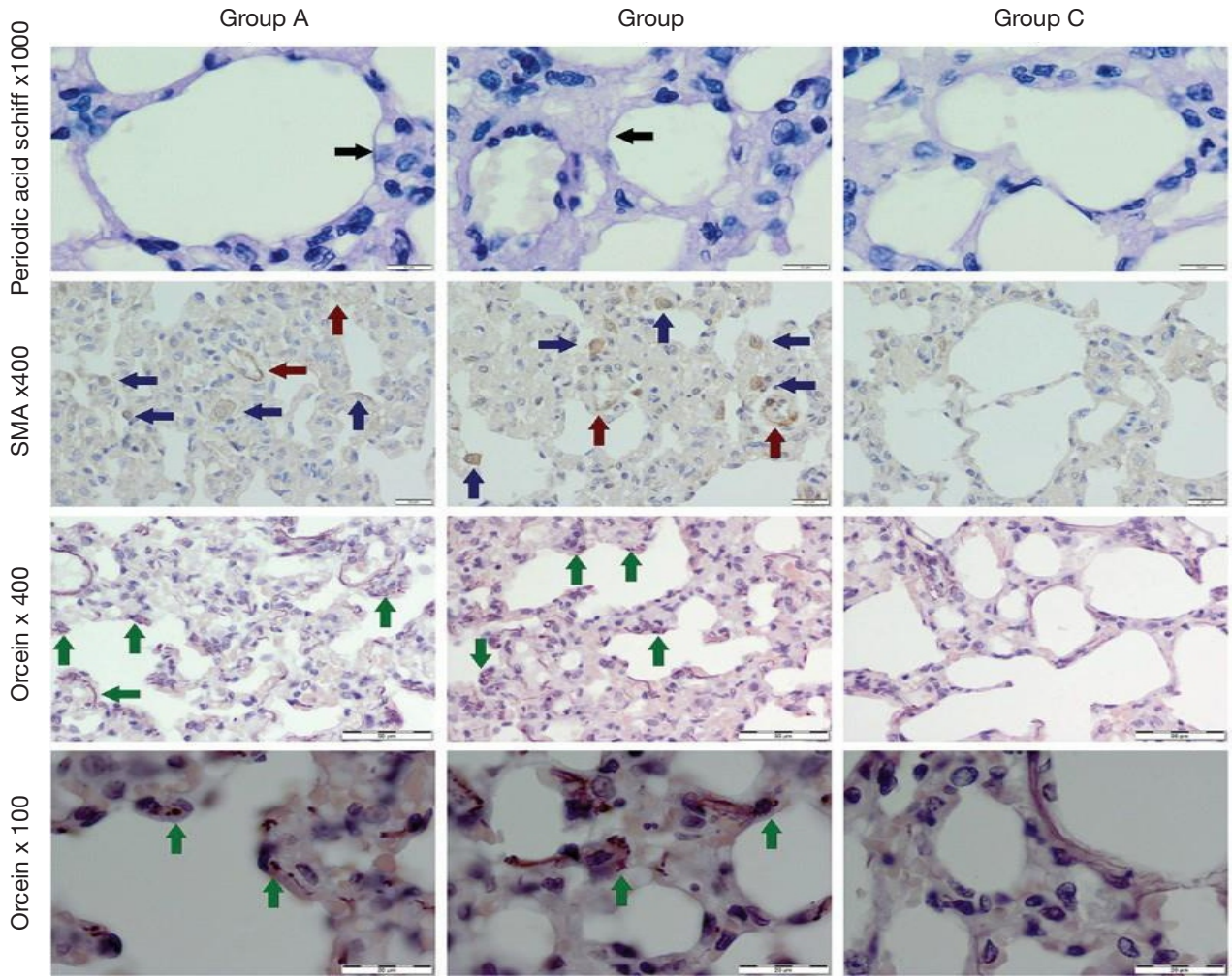

Figure 2. Control group (group C) exhibited normal alveoli, single myofibroblast and blood vessels with positive expression of α-SMA, and delicate normal elastic fibers within alveolar septa in orcein staining (green arrow). In the e-cig group (group A) and the conventional cigarette group (group B), thickened basement membrane (black arrows), vacuolization of cells in alveolar septa, more numerous α-SMA positive myofibroblasts (blue arrows) and blood vessels (red arrows), and thicker, disrupted, sparse elastic fibers (green arrows) were observed. Original magnification, x400 or x1,000. SMA, smooth muscle actin

control group's collagen fibers were restricted to the region around the bronchioles and generally had typical lung tissue appearance.

Both experimental groups showed an increase in the thickness of the blood-air barrier-forming membrane in the PAS staining (Table 1). In the orcein staining, the control group had indications of brittle elastin fibers. An early elastolysis was seen in both experimental groups, with four elastic fibers becoming fragmented, sparse, irregular, and thickened. The group B that smoked conventional cigarettes showed the highest level of α-SMA expression (Figure 2). The most myofibroblasts (20 ± 5 in one field of view) were seen in the parenchyma of that same group; the e-cigarette group A had less (17 ± 6 in one field of view); and the control group had very little. The greatest number of blood vessels that tested positive belonged to the group that smoked traditional cigarettes for α-SMA (Table 1).

Discussion

Teenagers and young adults who have never smoked are increasingly reaching for e-cigarettes. Their understanding of these items is, at most, limited.[18,24–26] Furthermore, using e-cigarettes can have a negative influence on one's health and result in nicotine

dependency.[22,27,28] It has been demonstrated that exposure to e-cigarette vapours causes oxidative stress, an inflammatory response, and morphological alterations in human lung fibroblasts in the lungs of mice.[7,14,21] An instance of acute alveolitis with intra-alveolar fibrosis and neutrophil, eosinophil, and macrophage infiltration was described by Itoh et al. [29] In both healthy and ill individuals, the vapour produced by e-cigarettes likely influences the gene expression of proteins linked to the circadian rhythm.[30]

The probable impacts of e-cigarette exposure include decreased expression of genes involved in cilia assembly and movement in human bronchial epithelial cells, increased production of reactive oxygen species, and activation of genes implicated in oxidative and xenobiotic stress pathways.[31] Rats were employed in this study as a realistic model for human exposure to both traditional and e-cigarette smoke. The approach used in this investigation was similar to that suggested by Canistro et al. in terms of e-cigarette vapour exposure in animals.[27]

As far as the authors are aware, this is the first research to show that breathing in smoke or vapour damages the structure of the lung tissue. Rats exposed to vapour showed several pathological alterations in their lungs, including bronchial bleeding, thickening of the alveolar septa, and infiltration of macrophages and eosinophils. However, the lungs of rats exposed to conventional smoke showed more substantial detrimental alterations within the characteristics of fibrosis and emphysema. In both experimental groups, there was disruption to the elastic fibers that control the thickness of the bronchiole lumen and alveolar wall.

Our findings are consistent with a previous study,[32] where rats given intraperitoneal nicotine showed infiltration of inflammatory cells and disorganized parenchyma. Additionally, there was a disruption of elastic fibers, an increase in parenchymal arteries, and a thickening of alveolar septa.[32] The thickening of the alveolar septa may be caused by oedema, inflammatory infiltration, and an increase in blood capillary volume. Additionally, smoking cigarettes activates the elastases of neutrophils and macrophages, damaging elastic fibers and resulting in emphysema.[32]

The damaging substances included in smoke or vapour are thought to cause oxidative stress and blood-air barrier dysfunction, which is likely connected to the observed alterations to the lung tissues.[3,33] A previous study found that nicotine from e-cigarette juice broke down the endothelium barrier in monolayers of cultivated cells and made animal lung inflammation worse by producing oxidative stress.[33] According to another study, rats' lung tissue may experience considerable interstitial fibrosis, respiratory epithelial proliferation, intraparenchymal hemorrhage, and empchysematous changes if nicotine is administered subcutaneously at a daily dosage of 1.5 mg/kg for four weeks.[3]

According to a recent study, e-cigarette vapour can harm lung tissue in a manner similar to that of traditional cigarette smoke.[34] In contrast to our research, animals exposed to smoke or vapour showed emphysematous alterations and a reduction in the number of lung capillaries, just like rats treated to subcutaneous nicotine administration.[34] Pulmonary fibrosis and changes to lung architecture resulted from a chronic disease brought on by acute disruption, inflammation, and inefficiency of regeneration processes. Nicotine promotes the growth of fibroblasts and the synthesis of collagen type I.[5] Moreover, nicotine may induce the differentiation of fibroblasts into myofibroblasts. The latter are in charge of secreting the extracellular matrix proteins that promote fibrosis.

As a result, the build-up of collagen in lung tissue causes fibrosis and reduces the area used for gas exchange.[22,35] The current study found that both experimental groups had overexpressed levels of α-SMA expression, a marker for myofibroblasts, while the traditional cigarette group had greater levels of expression. In summary, this study demonstrates that, in comparison to smoking traditional cigarettes, using e-cigarettes causes less severe pathological alterations. But it's not safe to say that e-cigarettes are 100% safe.[28,36] It seems that smoking electronic cigarettes exposes users to nicotine, which causes lung tissue to deteriorate, collagen deposits to develop, eosinophils, myofibroblasts, and angiogenesis to be activated.

Additionally, such modifications may result in changes to the architecture of the lungs, which would further impede gas exchange in specific regions. For a brief period of six weeks, laboratory animals were used in the current investigation. To maintain the homogeneity of the groups under study, only male rats were employed; intersex comparisons should be included in future research.

Conclusion

Our study concludes that using electronic cigarettes causes fewer severe histomorphological changes than smoking regular cigarettes. However, the histopathological damage caused by vaping may lead to the formation of alterations in lung tissue that obstruct gas exchange.

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