Vapor of Electronic Cigarettes Induces Histopathological Changes in the Rat Submandibular Gland

Original Article

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ABSTRACT

Introduction: Electronic cigarettes (E-cigs) are nicotine delivery devices that work by vaping the E-cigs liquid rather than burning tobacco. It was initially presumed that E-cigs usage was safe. As the popularity of E-cigarettes grows, it's vital to think about their potential dangers and risks. A paucity of studies concerning the hazards of E-cigs was a call to assess the effect of E-cigs on the structure of the submandibular salivary gland (SMG).

Aim of the Work: To clarify the structural changes that may take place in rat SMG after E-cigs exposure and the possible amelioration after its cessation.

Material and Methods: 30 adult male albino rats were allocated into three equal groups at random. Control group (Group 1). E-cigs exposed group (Group 2): was exposed to E-liquid smoke vapor (1ml/day) for 1 hour for 5 sequential days per week for four weeks. Withdrawal or recovery group (Group 3): was exposed to the same duration and dose of E-liquid smoke vapor as group 2 then left without exposure for another four weeks. At the end of experiment, the SMG were excised and processed for hematoxylin & eosin, Alcian blue-periodic acid Schiff's stain, Mallory's trichrome stain and Tumor Necrosis Factor-α (TNF-α) for immunohistochemical study. Also, serum malondialdehyde (MDA) and Glutathione peroxidase-1 (GPx1) enzyme activity were determined.

Results: SMG injury was caused by electronic cigarettes in rats, as shown by histological changes in the acini and ducts, including vacuolated cytoplasm, besides increased TNF-α immunoreactivity and its area percentage. Furthermore, E-cigs caused oxidative damage by increasing MDA and decreasing GPx1 enzyme levels. Interestingly, cessation of exposure resulted in some amelioration of the histological and biochemical changes.

Conclusion: Electronic cigarettes produce degenerative changes in the histological structure of the submandibular salivary gland and the withdrawal exhibits some degree of improvement.

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Key Words: Adult male rats, electronic cigarettes liquid, electronic cigarettes vaping, nicotine, submandibular salivary gland.

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INTRODUCTION

Electronic cigarettes (E-cigs) are battery-powered devices that generate an aerosol (vapor) of a nicotine-containing liquid that is often flavored[1]. This E-liquid is heated and evaporated, resulting in a smoke that the user inhales[2]. E-cigs are designed as smoking cessation tools rather than tobacco products. They are used as a satisfying alternative to traditional cigarettes for smokers in order to quit tobacco. Also, because E-cigs do not contain tobacco, they are seen to be safer than smoking combustible cigarettes (C-cigarettes). They have even been suggested as a way to quit smoking[3,4].

The vape consists of a tank filled with E-liquid, which includes vegetable glycerin, propylene glycol (PG), natural and artificial flavorings, nicotine, and other ingredients, but no tobacco. Fruit, candy, alcohol, and soda flavors are among more than 7000 E-liquid flavors available. Nicotine levels in E-liquids vary from non-nicotine (0 mg/nicotine-free) up to 36 mg/ml. Nicotine levels in E-liquids are most commonly 6, 12, 18, or 24 mg/ml[2]. In addition, despite having a lower total number of chemicals than C-cigarettes, E-cig liquid contains higher quantities of known carcinogens, including formaldehyde. E-cigarette fluids contain several other compounds known to cause cancer, such as diethylene glycol, according to the US Food and Drug Administration[3].

Actually, nicotine inhalation results in a high rate of absorption by the pulmonary system[5,6]. The enormous surface area of the alveolar capillaries in the lungs facilitates and increases the rate and amount of nicotine absorption[7]. These kinetic features show that nicotine inhalation delivery can affect the rate and amount of nicotine absorption, potentially leading to increased bioavailability. Furthermore, because nicotine is processed pre-systemically in the liver, oral intake has a decreased systemic bioavailability. This shows that nicotine absorption may be higher when using the inhalation approach[6].

Interestingly, according to a previous study investigating the distribution of nicotine at an organ-specific level, the

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absorbed nicotine was high in glands such as the adrenal gland and the salivary gland. This high distribution of nicotine lev¬el in the salivary glands may enhance the development of the patho¬logic process of these glands[8].

Saliva is a complicated body fluid that is extremely necessary for dental health. It contains electrolytes, peptides, glycoproteins, and lipids that have antibacterial, antioxidant, and tissue-repair properties. Saliva is also necessary for oral mucosa protection, tooth remineralization, taste sensation, digestion, pH balancing, and phonation. Saliva is the first biological fluid to be exposed to smoking, and it contains a variety of hazardous components that cause structural and functional changes in saliva, as well as affect the mouth salivary flow rate (SFR), which contributes to the pathogenesis of oral and dental illnesses[9]. One of the most common complaints among smokers is xerostomia, or dry mouth[10].

To the best of our knowledge from the previous available literature that almost no studies have been conducted to assess the histological and immunohistochemical effects of E-cig vaping on the submandibular gland in experimental animals or in humans. This was an impetus to designate this study in which the effect of exposure to E-cigs vaping and the effects of their giving up on the cytoarchitecture of the submandibular gland in adult male albino rats.

MATERIALS AND METHODS

Materials

Chemicals

Electronic cigarette liquid (E-Liquid): was purchased from an Egyptian market (Dollar blends company, Egypt).

Each 1ml of E-Liquid contains: Nicotine 18 mg/ml, propylene glycol, vegetable glycerin, natural and artificial flavorings.

Portable electric Incense Burner

Was purchased from an Egyptian market (home electric company, china).

Design of the experiment

Thirty adult male albino rats, two months old and weighing 200-250 gm were gained from the Faculty of Medicine animal house at Zagazig University, provided with standard food and water ad libitum and kept in ventilated wide polypropylene cages (19× 26× 38 cm). The temperature was kept at 23±2°C. They were left to acclimatize for two weeks. All rats were treated in accordance with the standard guide for the care and use of laboratory animals. This study was authorized by the Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC) with the approval number (ZU-IACUC/3/F/10/2020).

Experimental animals

The rats were divided into three groups of ten rats each, as follows:

The control group: (Group I); exposed to fresh air for four weeks.

E-cigs exposed group: (Group II); exposed to E-liquid smoke vapor (1ml/day) for 1 hour for 5 sequential days per week for four weeks[11].

Withdrawal group or Recovery group: (Group III); exposed to E-liquid smoke vapor (1ml/day) for 1 hour for 5 sequential days per week for four weeks and then left without exposure for another four weeks.

Protocol of exposure to E-liquid smoke

Using a portable electric Incense Burner within the inhalation chamber (19 × 26 × 38 cm), the rats were exposed to E-liquid smoke vapor for one hour on 5 sequential days per week for four weeks. This chamber contained two holes; one for entry of E-liquid smoke vapor and the other for fresh air. Then, the animals were transferred to a new chamber of fresh air after exposure.

Necropsy, blood and tissue sampling

The animals were sedated by intraperitoneal injection of thiopental (75 mg/kg) at the end of the experiment, 24 hours after the final E-liquid smoke vapor exposure[12]. After that, venous blood samples from the retro-orbital plexus were taken, allowed to clot, and then centrifuged to extract the serum. For subsequent estimations, the serum samples were kept at -20°C for further estimations of malondialdehyde (MDA) and Glutathione peroxidase-1 enzyme activity (GPx1). Then, a midline upper cervical incision was made to expose and excise the submandibular salivary glands and they were processed for histopathological and immunohistochemical studies. Also, statistical analysis and morphometrical studies were done.

Biochemical assays

The blood levels of MDA and GPx1 were measured using enzyme-linked immuno-sorbent assay (ELISA) kits. MDA kits (Cat No: MBS268427) and GPx1 kits (Catalog No: MBS451149).

Histological study

Submandibular salivary gland specimens were fixed for 24 hours in neutral buffered formalin10%, dehydrated in rising grades of ethanol, cleaned with xylene and implanted in paraffin wax. The 5 μm thick paraffin sections were cut and stained with Hematoxylin and Eosin (H & E) (to evaluate the histological structure of the SMG[13], stained also with Alcian blue-periodic acid Schiff's stains (AB-PAS) (to assess the carbohydrate residue as it is specific for the secretory granules of the mucous acini as they contain salivary glycoprotein[14] and Mallory's trichrome stain (to detect the collagen fibers of the SMG[13]. Finally, in the Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt, the SMG sections were inspected using a light microscope (Leica Microsystems, Schweiz, AG, Heerbrugg, CH-9435, Switzerland) and photographed using a digital camera linked to that microscope.

Immunohistochemical investigation

Sections of 4m thick were cut and mounted on positively charged slides from paraffin blocks of SMG specimens, deparaffinized and rehydrated, then blocked with 5 percent BSA (Bovine Serum Albumin) in tris-buffered saline for 2 hours, then incubated with the primary antibody anti TNF-α [polyclonal IgM to rat TNF-α and its catalog number: RA0316-C.5] at a concentration of 1µg/ml with 5 percent BSA in tris-buffered saline, then incubated at 4°C overnight. The slides were then rinsed in tris buffered saline, diluted in 5 percent BSA in tris buffered saline (1:2000), and incubated for 2 hours with secondary antibodies (Bangalore Genei, India). Slides were treated with 0.02 percent diaminobenzidine with 0.01 percent hydrogen peroxide for 5–10 minutes after being washed with tris-buffered saline[15]. Finally, they were stained with haematoxylin. In the Anatomy Department, Faculty of Medicine, Zagazig University, Egypt, immunostained sections were photographed using a Leica DM500, Microsystems, AG, Heerbrugg, CH-9435, Switzerland.

Morphometric studies

Using the public domain image-processing software "Image J 1.49v/Java 1.6.0_244", the area percentage of TNF- α in immunostained sections at magnification X 400 and the area percentage of collagen in Mallory trichrome stained sections at magnification X 400 were calculated (National Institutes of Health, USA). Prior to usage, the image analyzer was calibrated for measurements by automatically converting picture pixels into micrometer units and the data was presented in the form of a mean and standard deviation (SD).

Statistical analysis

Using the SPSS software, the collected data was statistically evaluated (Statistical Package for Social Science, version 19.0. Inc, Chicago, IL, USA). The data was presented as a mean and standard deviation. The difference between quantitative variables in more than two groups in normally distributed data was calculated using analysis of variance (ANOVA), followed by the least significant difference (LSD) multiple comparison post hoc test to determine the significance difference between the two studied groups. When the *P value* was less than 0.05, the results were considered statistically significant.

RESULTS

Biochemical results

In oxidant/antioxidant parameters, Group II (exposed group) exhibited a highly statistical increase ($p<0.001$) in MDA compared with Group I (control group). Also, Group II was highly statistically increased ($p<0.001$) than Group III (withdrawal) (Histogram 1a). Regarding GPx1, Group II (exposed group) was highly statistically decreased ($p<0.001$) than Group I (control group), also Group II was highly statistically decreased ($p<0.001$) than Group III (withdrawal group) (Histogram 1b).

Light Microscopic Examination

H&E stain

The normal histological structure of the SMG was seen in H & E-stained sections taken from the control group. The gland was divided into numerous lobules separated by thin connective tissue septa which conveyed interlobular excretory ducts. Each lobule contained closely packed mixed acini (serous & mucous acini) as well as intralobular ducts (Figure 1a).Serous cells were pyramidal shaped, with spherical nuclei. Mucous cells were more columnar in shape with nuclei that were compressed at the base. Striated ducts that were lined by columnar cells having spherical nuclei were noticed. The well-developed granular convoluted ducts were lined by columnar epithelial cells having acidophilic cytoplasm and rounded nuclei (Figures 2a,2b).

Histological examination of the submandibular salivary glands of the exposed group revealed severe structural changes in the acini and ducts. The acini and ducts are widely spaced. Thickening of the connective tissue septa was detected. The excretory ducts appeared dilated with degenerated epithelial lining and retained secretion (Figure 1b).

The cytoplasm of the cells lining the serous acini was vacuolated with small deeply stained and irregular nuclei compressed by their cytoplasmic vacuoles. Some ducts showed reduced acidophilic content of the cytoplasm with vacuolation (Figure 2c). Numerous congested blood vessels engorged with red blood cells and mononuclear cellular infiltrations were also detected in the connective tissue septa (Figure 2d).

Histological examination of the submandibular salivary glands of the withdrawal group revealed some improvement in the histological structure. The acini and ducts were slightly spaced (Figure 1c). Many intralobular acini and ducts appeared normal, but some acinar cells still showed vacuolated cytoplasm with small deeply stained or irregular nuclei compressed by their cytoplasmic vacuoles (Figure 2e). The connective tissue septa appeared slightly thickened and the excretory ducts appeared slightly dilated. Some congested blood vessels engorged with red blood cells were detected in the connective tissue septa (Figure 2f).

Alcian blue-periodic acid Schiff's stains (AB-PAS)

The AB-PAS stained section of the control group revealed normal appearance of the submandibular salivary gland with normal appearance of salivary glycoprotein in the form of lightly stained blue secretory granules in the mucous acini (Figure 3a). Section of the exposed group revealed vacuoles with a decrease in their salivary glycoprotein blue stained secretory granules of the mucous acini (Figure 3b). While section of the withdrawal group revealed some normal mucous acini with their secretory granules, and others revealed vacuoles with a slight decrease in their acinar secretory granules (Figure 3c).

Mallory's trichrome stain

In the control group Mallory trichrome-stained sections, the collagen fibres in the connective tissue septa between the lobules were few (Figure 4a). Sections of the exposed group revealed deposition of abundant collagen fibers in the connective tissue septa between the lobules. These collagen fibers extended to surround the acini and intralobular ducts (Figure 4b). While sections of the withdrawal group revealed a slight increase in the amount of collagen fibers in the connective tissue septa between the lobules and around the acini and intralobular ducts (Figure 4c).

Immunohistochemical staining with TNF-α

Immunostaining of TNF-α was negative in the control group. It showed negative reaction in acinar and ductal cells (Figure 5a). While immunohistochemical staining of the exposed group revealed extensive positive cytoplasmic reaction to TNF-α in the acinar, ductal cells and in the interstitium (Figure 5b). However, immunohistochemical staining of the withdrawal group revealed positive cytoplasmic reaction in a few acinar, ductal cells and in limited areas of the interstitium (Figure 5c).

Morphometric studies

There was a highly statistically significant difference ($p<0.001$) among the studied groups. Using the LSD post hoc multiple comparison test to find the difference between each two groups, it was found that in Mallory trichrome (Figure 4d) & TNF-α (Figure 5d);

Group II (exposed group) was highly statistically increased ($p<0.001$ and $p<0.001$, respectively) than Group I (control group). Also, Group II (exposed) was highly statistically increased ($p<0.001$ and $p<0.001$, respectively) than Group III (withdrawal).

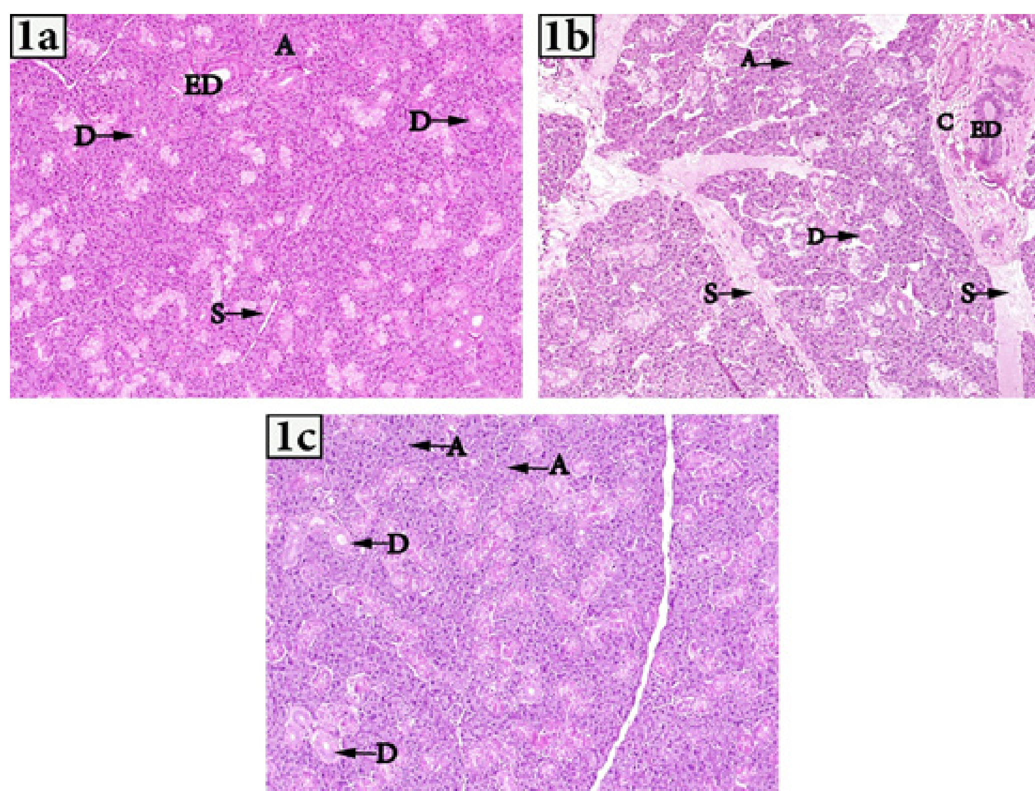


Fig. 1: Photomicrographs of adult male albino rat SMG sections of different groups: 1a) Control group showing some gland lobules are separated by thin connective tissue septa (S). Each lobule has closely packed acini (A), intralobular ducts (D), and also, excretory duct (ED). 1b) Exposed group showing widely spaced acini (A), ducts (D), thickened septa (S), with collagen fibers (C), and the excretory ducts appear dilated with retained secretion (ED). 1c) Withdrawal group showing some spaced acini (A), and ducts (D) [H & E x 100]

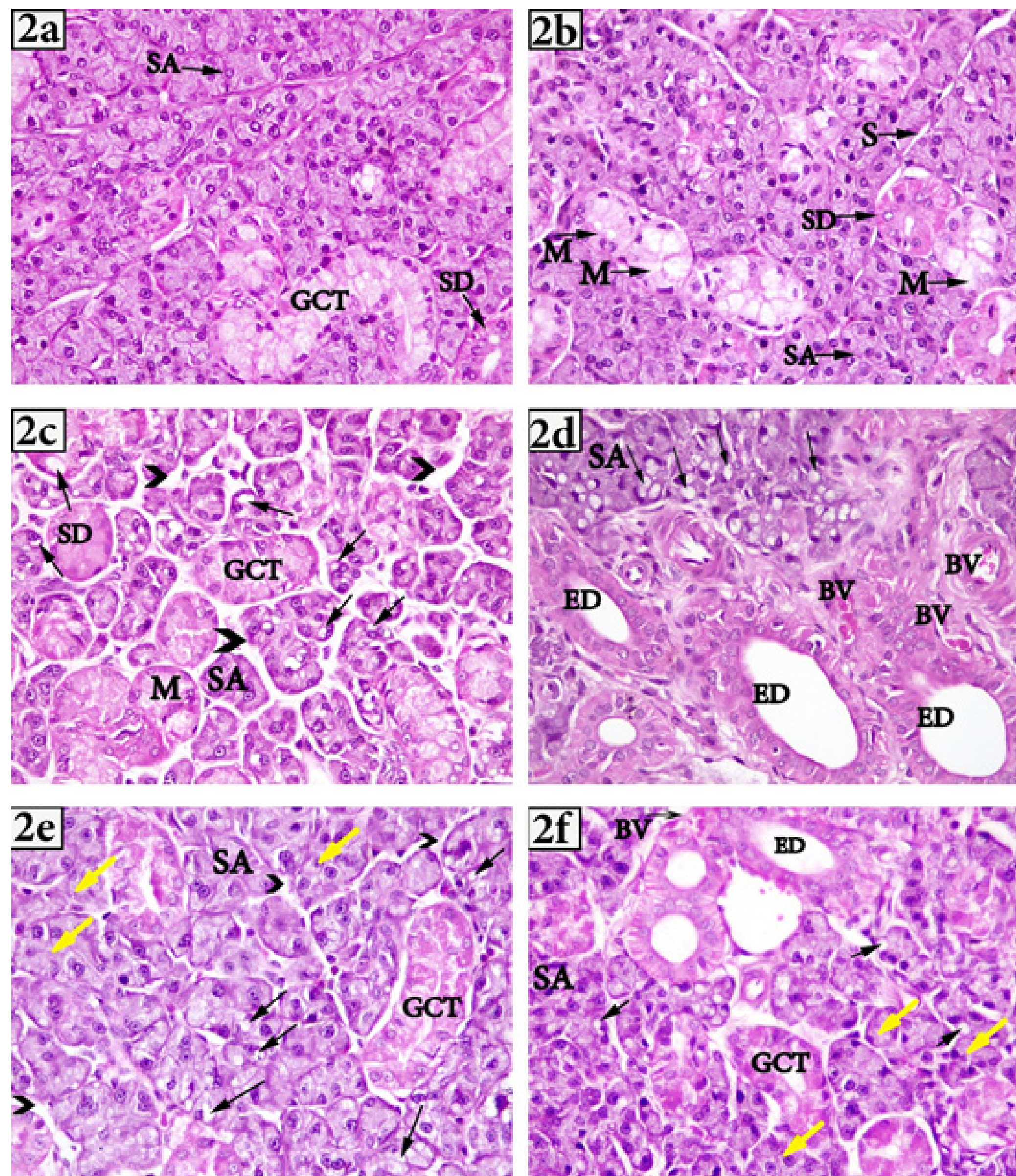


Fig. 2: Photomicrographs of adult male albino rat SMG sections of different groups: 2a) Control group showing closely packed serous acini (SA) that are lined by pyramidal shaped epithelial cells with rounded nuclei, striated duct (SD) that is lined by columnar epithelial cells having large, spherical and centrally located nuclei, and granular convoluted tubule (GCT) having tall columnar epithelial cells. 2b) Control group showing closely packed serous acini (SA), mucinous acini (M) that are lined by columnar shaped epithelial cells with basal compressed nuclei, striated duct (SD), and thin connective tissue septa (S). 2c) Exposed group showing wide spaces (black arrowheads) in between the serous acini (SA), mucinous acini (M), striated duct (SD), and granular convoluted tubule (GCT) with vacuoles compressed the nuclei (black arrows). 2d) Exposed group showing serous acini (SA), with vacuolation (black arrows), dilated excretory ducts (ED), and dilated congested blood vessels (BV). 2e) Withdrawal group showing some spaces (black arrowheads) in between serous acini (SA), and apparently normal granular convoluted tubule (GCT). Some acini appear normal (yellow arrows), and others are vacuolated (black arrows). 2f) Withdrawal group showing some spaced serous acini (SA), apparently normal granular convoluted tubule (GCT), with some acini appear normal (yellow arrows), and others are vacuolated (black arrows). Slightly dilated excretory duct (ED) and some congested blood vessels (BV). [H & E x 400]

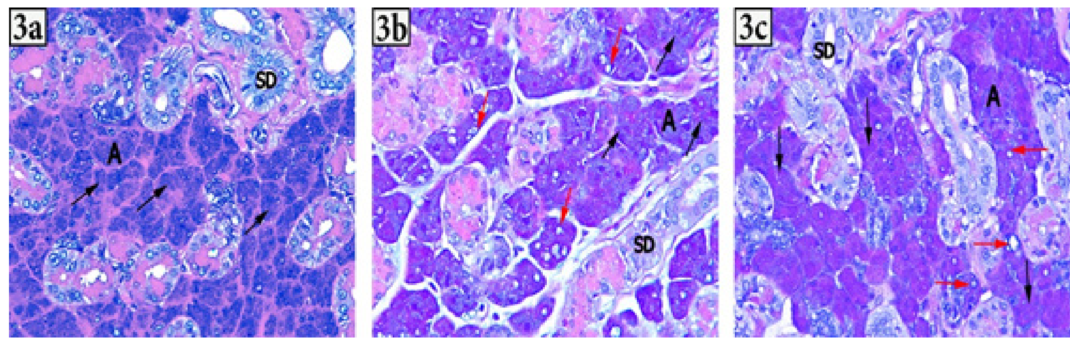


Fig. 3: Photomicrographs of adult male albino rat SMG sections of different groups: 3a) Control group showing closely packed acini (A) & striated duct (SD) with normal salivary glycoprotein in the form of lightly stained blue secretory granules in the mucous acini (black arrows). 3b) Exposed group showing widely spaced acini (A), striated ducts (SD) with vacuolations (red arrows), and a decrease in their salivary glycoprotein blue stained secretory granules in the mucous acini (black arrows).3c) Withdrawal group showing slightly spaced acini (A), striated ducts (SD), and some acini are vacuolated (red arrows), with a slight decrease in their salivary glycoprotein blue stained secretory granules in the mucous acini (black arrows). [AB-PAS x 400]

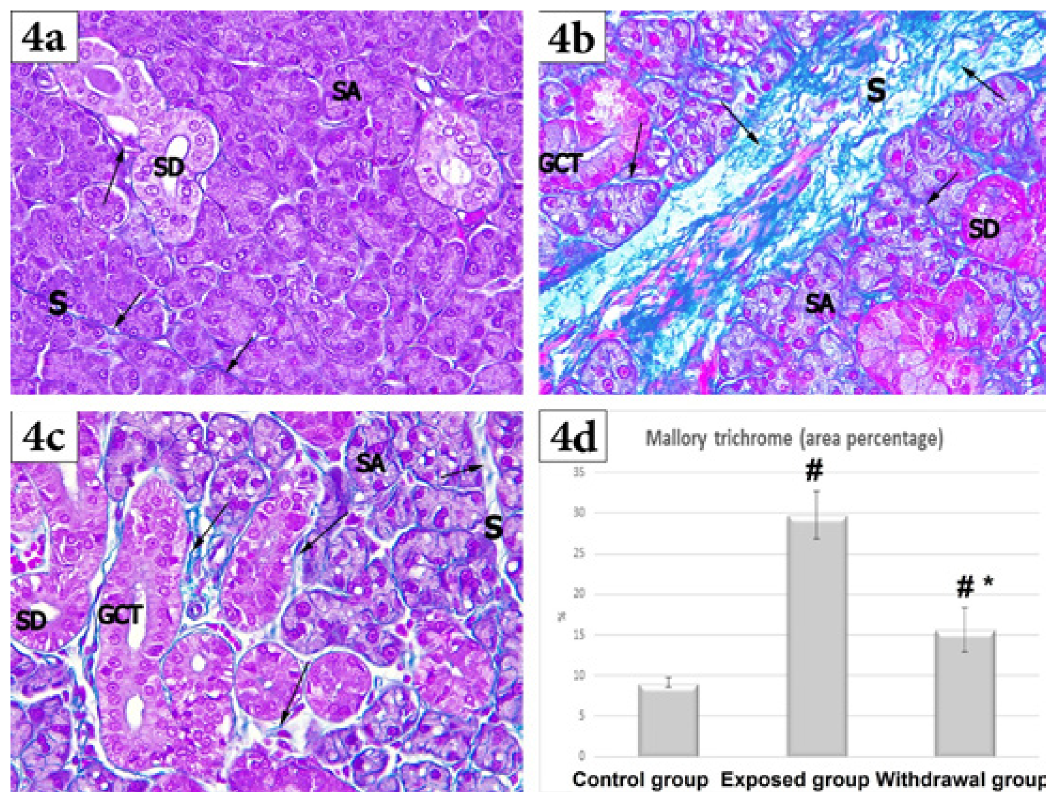


Fig. 4: Photomicrographs of Mallory trichrome staining of rat SMG sections of different groups: 4a) Control group showing closely packed serous acini (SA), striated duct (SD), and thin connective tissue septa (S), with scanty collagen fibers (black arrows). 4b) Exposed group showing widely spaced serous acini (SA), striated duct (SD), granular convoluted tubule (GCT), and thickened broad septa (S), with deposition of abundant collagen fibers that extended to surround the acini and intralobular ducts (black arrows). 4c) Withdrawal group showing slightly spaced serous acini (SA), striated duct (SD), granular convoluted tubule (GCT), slightly wide septa (S) with a moderate amount of collagen fibers in the connective tissue septa between the lobules that extended to surround the acini and intralobular ducts (black arrows). 4d) Bar charts showing the mean area % of Mallory trichrome among the studied groups using one-way ANOVA, followed by the LSD post hoc test. Values are represented as Mean ± SD. # Significant difference compared to the control group. *Significant difference compared to the exposed group [Mallory Trichrome x 400]

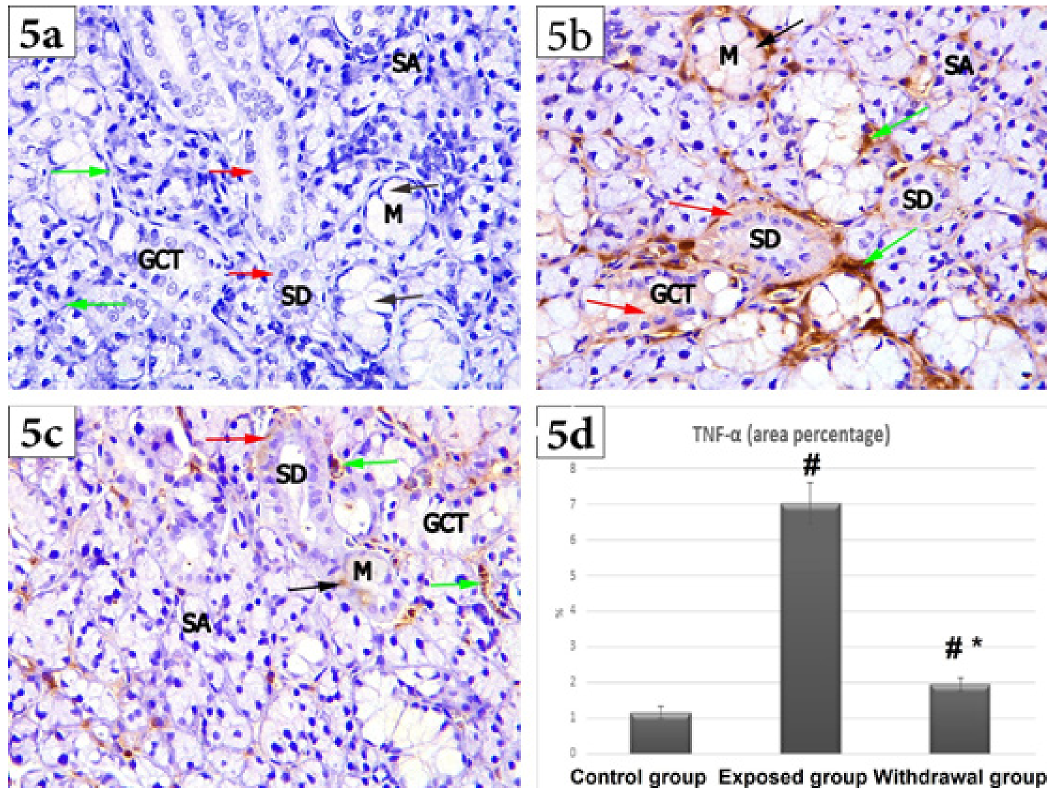
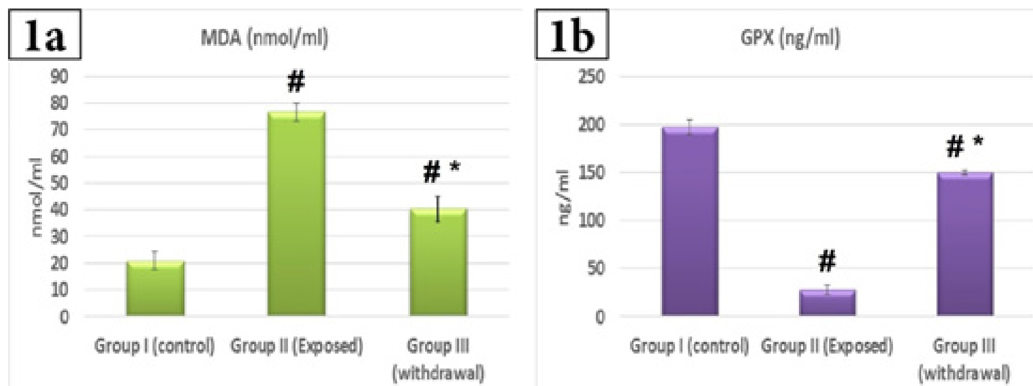


Fig. 5: Photomicrographs of TNF-α imunnohistochemical staining of rat SMG sections of different groups: 5a) Control group showing serous acini (SA), mucinous acini (M), striated duct (SD) and granular convoluted tubule (GCT) with negative reaction in the interstitium (green arrows), in the acinar (black arrows) and ductal cells (red arrows). 5b) Exposed group showing serous acini (SA), mucinous acini (M), striated duct (SD) and granular convoluted tubule (GCT) with extensive positive cytoplasmic reaction to TNF-α in the acinar (black arrow), ductal cells (red arrows) and in the interstitium (green arrows). 5c) Withdrawal group showing serous acini (SA), mucinous acini (M), striated duct (SD) and granular convoluted tubule (GCT) with positive cytoplasmic reaction in a few acinar (black arrow), ductal cells (red arrow) and in limited areas of the interstitium (green arrows). 5d) Bar charts showing mean area % of TNF-α among the studied groups using one way ANOVA, followed by LSD post hoc test. Values are represented as Mean ± SD. # Significant difference compared to the control group.*Significant difference compared to the exposed group [TNF-α x 400]



Histogram 1: Bar charts showing mean oxidant/antioxidant among different studied groups using the ANOVA test, followed by the LSD post hoc test. Values are represented as Mean ± SD. 1a) In MDA, # significant difference compared to the control group. *Significant difference compared to the exposed group. 1b) In GPx1, # significant difference compared to the control group. * Significant difference compared to the exposed group.

DISCUSSION

In the present study, there was a highly statistical increase in MDA and a decrease in GPX-1 levels in the E-cigs vaping exposed group compared with the control group. These results were in accordance with El Golli *et al*.[16] who reported an elevation in MDA content and a reduction in antioxidant enzyme activities including SOD, catalase, and glutathione-S-transferase following an intra-peritoneal injection of E-liquid containing 0.5 mg of nicotine/kg of body weight/day diluted in physiological saline for 28 days for adult rats, highlighting the promotion of lipid peroxidation and oxidative stress. Also, our results were in line with Suryadinata *et al*.[17] who documented that E-cigs (5 min. per day for 3 weeks) increase free radicals and MDA in the blood and decrease the level of SOD just like tobacco cigarettes do. This is because exposure to E-cigs smoke contains many hazardous chemicals that increase the free radicals in the airways. Hasnis *et al*.[18] stated that the cause of the oxidant/antioxidant imbalance (increased MDA and decreased GPX-1) upon E-cig vaping exposure is most probably due to the E-liquid nicotine level. Nicotine is known to cross the cell membrane and produces hydroxyl free radicals and reactive oxygen species (ROS) like hydrogen and superoxide peroxide. Hemnani and Parihar[19] and Sudheer *et al*.[20] reported that these ROS induce cytotoxic and nucleic acid damage and DNA band breakage.

Del Rio *et al*.[21] and Mahapatra *et al*.[22] stated that MDA is a recognized biomarker of oxidative stress. It is one of the main oxidation products of peroxidized polyunsaturated fatty acids. Lipid peroxidation was hypothesized as an important mechanism of nicotine-induced toxicity. Esterbauer *et al*.[23] and Eder *et al*.[24] reported that many products derived from lipid peroxidation can cause DNA damage, especially MDA, which is the most mutagenic product that causes alteration of gene expression.

On the other hand, Abdollahi *et al*.[25] stated that GPx is an important antioxidant which helps to protect cells from free radical oxidative damage. Also, Sharma and Sangha[26] reported that antioxidant enzymes protect cells from damage caused by oxidative stress, so they are considered the first line of defense. The highly reactive superoxide anion is neutralized by SOD by converting it to hydrogen peroxide (H_2O_2), which is then degraded to water by GPx.

Interesting, our study revealed that MDA levels were highly statistically decreased and anti-oxidant enzyme (GPx-1) was highly statistically increased in the withdrawal group in comparison to the E-cigs vaping exposed group. These promising findings were somewhat aligned with Oyeyipo *et al*.[27] who supplemented the rats with 1.0 mg/kg BW of nicotine orally for 30 days followed by withdrawal for another 30 days and reported a significant decrease in MDA levels and an increase in SOD and GPx levels in the homogenate of testicular tissue in the withdrawal group in comparison to the nicotine treated group.

In the present study, examination of H & E-stained sections of the submandibular salivary gland of the control rats showed the normal histological architecture. The gland was divided into numerous lobules separated by thin connective tissue septa which conveyed interlobular excretory ducts. Each lobule contained closely packed mixed acini and intralobular ducts. These normal findings were similar to those described by Kamath[28] and by Kassab & Tawfik[29].

In the present study, exposure to E-cigs vapor induced histopathological changes in the submandibular salivary gland of rats upon H & E-staining that revealed widely spaced acini and ducts with vacuolation & thickened connective tissue septa. Dilated excretory ducts with degenerated epithelial lining and retained secretion with dilated congested blood vessels and mild mononuclear cellular infiltration were also observed. These findings were in agreement with Fujinami *et al*.[30] whose study was done on the parotid & submandibular glands of rats exposed to cigarette smoke containing 1.4 mg of nicotine for 30 days. They have observed dilated blood vessels, hyperemia, inflammatory cells, and vacuolar degeneration. Also, our results agreed with Arslan *et al*.[31] who found that degeneration in serous cells and striated duct cells, dilation and congestion of blood vessels in the stroma of the submandibular gland of rats exposed to nicotine sulphate (2 mg per kg) for 28 days. Also, they found enhanced expression of vascular endothelial growth factor (VEGF) in vascular endothelial cells together with inflammatory cells around the excretory ducts in the stromal area, an increase in fibrous tissue and edema of the gland.

Bao *et al*.[32] stated that the observed degenerative changes of the submandibular salivary gland may be contributed to the toxicity of nicotine or its related metabolites. Also, Williams *et al*.[33] stated that the degenerative changes may be due to silicate particles and metal elements in the E-cigs vapor that may have some cytotoxic effects.

In the current work, H & E stained sections of the submandibular salivary gland of the withdrawal group showed some degree of improvement compared with the exposed group. The gland showed some space between the acini and ducts. Some of them appeared normal and others were vacuolated. The excretory ducts appeared slightly dilated with some dilated congested blood vessels.

The previous findings of the withdrawal group were close to Lewis *et al*.[34] who reported an improvement in hepatocyte vacuolization caused by a high nicotine dosage after cessation of treatment. Also, Wong *et al*.[35] reported that after the 42-day recovery period, the incidence of nicotine-induced cytoplasmic vacuolation in liver cells returned to baseline.

In the present work, AB-PAS-stained sections of the submandibular salivary gland of the control group showed the normal appearance of the gland and its secretory granules containing salivary glycoprotein in the mucous

acini stained blue in color. These results were in agreement with Correia *et al*.[36]. However, AB-PAS-stained sections of the E-cigs exposed group revealed vacuoles with a decrease in mucous acinar secretory granules. This result was close to Phillips *et al*.[37] who noticed cytoplasmic vacuolation in AB-PAS staining of the liver in an inhalation study to E-cigs in rats for 90 days. On the other hand, AB-PAS stained sections of the withdrawal group revealed some normal mucous acini with their secretory granules that were stained blue in color and others revealed vacuoles with a decrease in their acinar secretory granules.

In the present work, submandibular salivary gland sections of the control group stained with Mallory's trichrome showed few collagen fibers in the connective tissue septa between the lobules of the gland. These results were in agreement with Kassab & Tawfik[29]. However, Mallory trichrome-stained sections of the E-cigs exposed group revealed deposition of abundant collagen fibers in the connective tissue septa between the lobules that extended to surround the intralobular secretory acini and ducts. These histopathological changes were confirmed statistically by increasing the area percentage of collagen distribution in Mallory-stained sections of this group compared with the control group. This result was in harmony with Otero *et al*.[38] who found flavored E-liquid, whether with or without nicotine, enhanced collagen type I protein expression substantially. Also, Ferragut *et al*.[39] showed a significant increase in the collagen distribution (Type I collagen production increased, followed by types II and III collagen production) in the stromal spaces of the submandibular gland exposed to chronic passive smoking for one hour per day, 7 days per week for 6 months.

In the present study, Mallory-stained sections of the submandibular salivary gland of the withdrawal group revealed a moderate amount of collagen fibers in the connective tissue septa among the lobules and around the acini and intralobular ducts. This histological improvement was certained statistically by decreasing the area percentage of collagen distribution of this group compared with the exposed group.

Kang *et al*.[40] stated that TNF- α is a key mediator of inflammation in response to infection. Increased TNF- α expression has been linked to inflammatory disorders. Limaye *et al*.[41] stated that TNF- α may serve as a marker of inflammation in a variety of salivary gland diseases, even if it isn't the only one.

In the present work, sections of the control group stained with TNF-α immunohistochemical staining revealed negative reaction in the interstitium and in the acinar and ductal cells. This result was in line with Fukuoka *et al*.[42].

In our present work, sections of the E-cigs vaping exposed group stained with TNF-α revealed extensive positive cytoplasmic reaction to TNF-α in the acinar, ductal cells and in the interstitium that were confirmed statistically by increasing the area percentage of TNF-α in the E-cigs exposed group in comparison to the control group. This result is in harmony with Hamza & El-Shenawy[43] who supplemented nicotine (intraperitoneally, 2.5 mg/kg for 4 weeks) and reported that nicotine increases the serum level of TNF-α in rats as compared to control animals.

Lerner *et al*.[44] and Canistro *et al*.[11] stated that E-cig aerosol up regulates the production of inflammatory cytokines, induces oxidative stress and depletes glutathione. Sussan *et al*.[45] reported that the vapor from E-cigs is high in free radicals. Airway inflammation, oxidative stress, decreased antibacterial and antiviral responses, reduced bacterial phagocytosis, and higher virus-induced morbidity and mortality were all linked to E-cig use.

Allen *et al*.[46] and Gerloff *et al*.[47] stated that the flavoring chemicals of E-cigs vapor play a key role in E-liquids and E-liquid aerosol cytotoxicity. Tierney *et al*.[48] reported that attention has been raised regarding the toxicity of these favorable compounds when inhaled.

Farsalinos & Polosa[49] and Shin *et al*.[50] showed that heavy metals, chemicals, and glass particles, which are contained in the device's welding material and tubing, can be released by vaping pens. Also, Kar *et al*.[2] reported that metallic substances such as nickel, lead, chromium, and tin have been found in the E-liquid and smoke of E-cigs. Besides, phenolic chemicals, volatile organic compounds, and tobacco-specific nitrosamines are also present.

In our present work, sections of the withdrawal group stained with TNF-α revealed positive cytoplasmic reaction in a few acinar, ductal cells and in limited areas of the interstitium that confirmed statistically as the area percentage of TNF-α in the withdrawal group was highly statistically decreased than in the E-cigs vaping exposed group.

Finally, Sussan *et al*.[45] reported that the use of E-cigarettes among teens is quickly increasing, posing a risk to public health in terms of recurring bacterial or viral illnesses. Moreover, Korfei[51] stated that there is growing evidence to suggest that E-cigs may play active roles in the pathogenesis of some malignancies.

CONCLUSIONS

Adult male albino rats exposed to electronic cigarettes had degenerative alterations in the cytoarchitecture of the submandibular salivary gland. Stopping the exposure (withdrawal) resulted in some improvement. According to these findings, electronic cigarettes are hazardous to the public's health; they are not a safe substitute for smoking cigarettes and should not be used. So, further studies on the sublingual gland are recommended.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التغيرات الهستوباثولوجية التي يسببها دخان السجائر الالكترونية في الغدة تحت الفكية للجرذان

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الخلفية: السجائر الالترونية هي اجهزة لتوصيل النيكونين والتي تعمل عن طريق تبخير السائل الالكتروني بدلاً من حرق التبغ. كان يعتقد في البداية أن استخدامها امن. ولكن مع تزايد استخدام وشعبية السجائر الإلكترونية، اصبح من الضروري التفكير في مخاطرها المحتملة. وبسبب ندرة الدراسات المتعلقة بمخاطرها، كان من الضروري تقييم تأثيرها على الغدة اللعابية تحت الفكية.

الهدف من الدراسة: هو توضيح التغيرات التي قد تحدث في الغدة تحت الفكية للجرذان بعد التعرض للسجائر الإلكترونية وإمكانية التحسن بعد التوقف عن التعرض (الانسحاب).

الطرق المستخدمة: تم استخدام ثلاثين من ذكور الجرذان البيضاء وتم تقسيمها عشوائيًا إلى ثلاث مجموعات متساوية. المجموعة الضابطة (المجموعة ١). المجموعة المعرضة للسجائر الإلكترونية (المجموعة ٢): تعرضت لبخار دخان السائل الإلكتروني (١ مل / يوم) لمدة ساعة واحدة لمدة ٥ أيام متتالية في الأسبوع لمدة أربعة أسابيع. مجموعة الانسحاب (المجموعة ٣): تم تعريضها لنفس المدة والجرعة من بخار دخان السائل الإلكتروني مثل المجموعة ٢ ثم تركت دون التعرض لمدة أربعة أسابيع أخرى. بنهاية التجربة، تم استئصال الغدة تحت الفكية ودراسة الفحص النسيجي الهستوباثولوجي باستخدام صبغة الهيماتوكسيلين والايوسين، صبغة كاشف شيف حمض البيريوديك الازرق، صبغة مالوري الثلاثية والصبغة الهستوكيميائية المناعية عامل نخر الورم الفا. وكذلك تم قياس علامات الاكسدة البيوكيميائية (المالونداليديهيد وانزيم الجلوتاثيون بيروكسيداز).

النتائج: تسببت السجائرالإلكترونية في إصابة الغدة تحت الفكية للجرذان ، كما يتضح من التغيرات النسيجية في العناقيد والقنوات ، بما في ذلك وجود فجوات في السيتوبلازم. بالإضافة إلى زيادة نشاط عامل نخر الورم الفا ونسبته المئوية. علاوة على ذلك ، سببت السجائر الالكترونية ضررًا تأكسديًا عن طريق زيادة المالونداليديهيد وخفض مستويات إنزيم الجلوتاثيون بيروكسيداز. ومن المثير للاهتمام أن التوقف عن التعرض أدى إلى بعض التحسن في التغيرات النسيجية والكيميائية الحيوية

الخلاصة: تنتج السجائر الإلكترونية تغيرات مدمرة في التركيب النسيجي للغدة اللعابية تحت الفكية ويظهر الانسحاب درجة من التحسن.