Original Article

Effect of Electronic Cigarette Exposure on The Adrenal Cortex of Adult Male Albino Rats and the Possible Effect of its Withdrawal (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Electronic cigarettes, also known as "cigs," are gadgets created to provide nicotine through a vaping solution rather than smoke and without the burning of tobacco. E-cigarettes are aggressively promoted as consumables among people who want to live healthier lives and are seen as a safer alternative to cigarettes.

Aim of the Work: To assess structural changes in the adrenal cortex of adult male albino rats and explore effect of withdrawal of electronic cigarette.

Material and Methods: 30 adult male rats weighting 150-230 g which were purchased from the Animal house of the faculty of medicine, Zagazig University. The animals will be divided equally into 3groups. Each one contains 10 rats as follows: Group (1): (control group); which contain ten rats that will be exposed to fresh air for 28 days to measure normal basic parameters. Group (II): (E- cig treated group); which contain ten rats that will be exposed to 1ml/day of E- liquid containing 18 mg/ml Nicotine For consecutive 5 days/ week for 28 days. Group (III): (withdrawal group); which contain ten rats which will be exposed to 1ml/day of E- liquid containing 18 mg/ml Nicotine For 5 consecutive days/ week for 4 weeks and then will be left without exposure for another 4 weeks.

Results: E-cigarette has hazardous effects on adrenal gland cortex histological structure. Also, there were significant increase in adrenal gland weight and the three zones thickness with elevation of MDA level & reduction of SOD level and also increased the level of cortisol and aldosterone in the group treated with E-cig. Moreover, Caspase 3, COX II immune-expression percent area and the number of CD44 +ve cells were significantly increased in treated group.

Conclusion: Exposure of adult male albino rats to E-cigs caused histopathological alterations in the adrenal cortex structure with great improvement in its histological structure and biochemical activity after its withdrawal.

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Key Words: Albino rats, adrenal cortex, electronic cigarette.

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INTRODUCTION

Adrenal glands or suprarenal glands are two glands located on the top of each kidney. They secrete hormones which are responsible for regulation of metabolism, blood pressure, response to stress and immune system. It is made of two parts; outer largest part which is adrenal cortex and contain three parts: zona glomerulosa, fasciculate and reticularis, inner part is called adrenal medulla and it secretes stress hormones like adrenaline[1].

An electronic cigarette (E-cigarette), called as vaping, is an electronic device that is a handheld device and it imitates the cigarette smoking act. It acts by liquid heating for making a vapor that is indrawn by the smoker[2].

Cigarette smoking and products of tobacco have famous hazardous effects. The main component of tobacco is Nicotine and it is responsible for its addiction. In low doses, nicotine play a role of stimulant, but at a higher doses (> 50 mg) it can be dangerous. The nicotine stimulating effect causes it to be highly addictive[3]. Also, in cigarette smoking nicotine is the main active material. Tobacco products intake caused increased mortality rate in developed countries[4]. Cortisol is an endogenous hormone that is produced via adrenal gland and its endogenous level is increased after smoking of cigarette and decrease when people abandon smoking[5].

Moreover, some physiological and behavioral effects of nicotine were modified by cortisol. Also, in smoker males there was increase in the level of 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione and dehydroepiandrosterone sulfate[6,7].

Nicotine binds to the adrenal medulla receptors, causing elevated blood pressure, rapid respiratory rate , increased heart rate and high blood glucose levels due to increased secretion of adrenaline and noradrenaline[8].

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Many health problems as congenital anomalies, poisoning and cardiovascular diseases are associated with cigarette smoking[9]. Furthermore, it was found that the antioxidant defense mechanism was distracted by administration of nicotine in rats[10,11].

Nicotine was proved to cause histological architecture damage in the cells of zona fasciculata of adrenal gland by subcutaneous injection as cells swelling and cytoplasmic vacuolation[12,13].

The toxic effects of E-cig on the histological structure of adrenal gland is obscure. So, this work was done to indicate the changes in histological structure of adrenal cortex as a result of E –cig intake with ameliorative role of its withdrawal in rats.

MATERIAL AND METHODS

Experimental Animals

Sample size, sampling frame

Sample calculated to be (30rats) divided into 3 groups; (10) in each one according to (Canistro *et al*., 2017)[14].

Thirty albino rats weighting 150-230 gram, aging 2-3 months were utilized in this work. The animals were obtained from Faculty of Medicine Animal House, Zagazig University. They were stayed in separate cages which had good ventilation with free water ad libitum availability and standard diet. All the steps of experiment were done on animals according to IACUC instruction with approval number (ZU-IACUC/3/F/13/2021).

chemicals

E-liquid for electronic cigarettes: (Dollar Blends Company, Eg): Vegetable glycerin, propylene glycol (PG), natural and artificial flavors, and nicotine (18 mg/ml) are all ingredients in one milliliter of e-liquid.

Portable electric Incense Burner: was obtained from an Egyptian market (Home electric comp., China).

Formol saline, alcohol, xylene and paraffin wax for preparation for light microscopic examination, microtome for sectioning exposure was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Exposure guidelines for e-liquid smoke: Rats were exposed to E-liquid smoke vapor aerosol for one hour, five days per week, for a total of 28 days utilizing portable electric incense burner placed inside an inhaling chamber (36x 24 x 17 cm) with a hole for fresh air and another hole for E-liquid smoke vapor aerosol entry. The animals were then moved to a room with fresh air after exposure.2-3.

Experimental Design

After acclimatization for 1week the animals will be divided equally into 3groups. Each one contains 10 rats as follows:

Group (I): (control group); contains ten rats that will be exposed to air for 28 days to calculate normal basic parameters.

Group (II): (E- cig treated group); contains ten rats that will be exposed to 1ml/day of E-cig liquid which contain 18 mg/ml of Nicotine by inhalation of the E-cig aerosol by E- cig device for one hour for consecutive 5 days/ week for 28 days[14].

Group (III): (withdrawal group); contains ten rats that will be exposed to 1ml/day of E-cig liquid which contain 18 mg/ml Nicotine by inhalation of the E-cig aerosol by E- cig device for one hour for consecutive 5 days/ week for 28 days and then will be left without exposure for another 28 days.

When the experiment was ended, anaesthetization with inhalation of ether and then scarification of animals. The adrenal tissue were carefully dissected for histological, immunohistochemical and morphometric study.

Investigation with light microscope

The adrenal glands were divided longitudinally, preserved in 10% formalin saline, and prepared as 5-m-thick paraffin slices for H&E and silver staining[15].

Minute specimens ($1mm^3$) were postfixed in 1% osmium tetroxide in the same buffer for 1 h at 4 °C after being promptly fixed in 2.5% glutaraldehyde buffered with 0.1 mol/L phosphate buffer at pH 7.4 for 2 h, and then dehydrated and embedded in epoxy resin. Semithin sections were stained with toluidine blue and examined under a light microscope[16].

Study using immunohistochemistry

Immunohistochemical staining was done for:

- Caspase 3, marker for apoptosis.
- Cox II, a marker for inflammation.
- A marker for endogenous mesenchymal stem cells is CD44+ve.

Prior treatment was required for immunostaining[17]. To do this, sections were boiled for 10 minutes in a 10Mm pH 6 citrate buffer for antigen retrieval, and then allowed to cool at ambient temperature for 20 minutes. The primary antibodies were then treated with the sections for an hour. Polyclonal rabbit anti-active caspase 3 antibody (ab13847) was diluted between 1/50 and 1/00 (Abcam, Cambridge, MA, USA)[18], polyclonal rabbit anti-COX-II antibodies were diluted between 1:300 (Dako, Glostrup, Denmark) [19], and anti-CD44 antibody (IW-PA1021) was diluted between 1:200 (IHC World, Ellicott City, USA) overnight at 4°C[20]. Mayer's hematoxylin was used to counterstain after the immunostaining was finished using the Ultravision detecting system. Malondialdehyde (MDA) which is a marker for lipid peroxidation, antioxidant enzyme (SOD) and levels of cortisol and aldosterone were measured from rat blood collected from left ventricle[21].

Morphometric study

For each group, five immune-stained sections were used for evaluation of immunoreaction percent area by using image analysis. Olympus microscope with well fitted Olympus digital camera (E24-10 mega pixel; Olympus, China) through a photo adaptor which is 0.5×, utilizing a 40× objective lens. Video Test Morphology software of an Intel Core 13 computer was used for evaluation of resulting images[22].

Statistical analysis

By using the Student t-test; adrenal weights, thickness of the three zones, biochemical and morphometric data were estimated and calculated as mean value ± standard deviation. *P* was significant when it ($p < 0.05$) and highly significant when ($p < 0.01$)[23].

RESULTS

Light microscopic results

The adrenal cortex capsule with underlying rounded or oval clusters of zona glomerulosa and parallel cell cords of zona fasciculata were seen in the stained H&E sections of the control group's adrenal cortex. Between the cells, there were sinusoidal blood capillaries with flat endothelial cells (Figure 1a). It was possible to see the zona reticularis, which has sinusoidal blood capillaries between anastomosing cords of cells. ZF and a portion of the medulla were seen (Figure 1b). E-cigarette treatment group displayed a corrugated capsule and loss of zona glomerulosa and zona fasciculata normal architecture. While some ZG cells displayed ballooning and hazy cell borders, other ZG cells displayed vacuolated cytoplasm and deeply discolored nuclei. The majority of the zona fasciculata cells had darkly colored nuclei and vacuolated cytoplasm (Figure 1c). In-between cells of zona fasciculata, a deformed capsule and a condensation of fine, wavy collagen fibers were visible (Figure 1d). Dark compacted nuclei with many dilated congested blood sinusoids were visible in zona reticularis cells. The medulla was visible in part (Figure 1e). Zona glomerulosa cells with pale, spherical nuclei and few vacuoles were visible in the withdrawal group. The zona fasciculata resembled that of the control quite closely. There were a few dilated sinusoidal blood capillaries found in the spaces between the cells (Figure 1f). While some cells of the zona reticularis had vesicular nuclei, others displayed dark condensed nuclei. There was a mitotic activity found. Between cell cords, a small number of dilated sinusoidal blood capillaries were visible. The medulla was visible in part (Figure 1g)

The control group's stained semithin slices of adrenal cortex revealed sinusoidal capillaries, big cells with vacuolated cytoplasm, rounded nuclei with conspicuous nucleoli of zona fasciculata, and zona glomerulosa with polyhedral cells and vesicular nuclei (Figure 2a). There were sinusoidal capillaries separating the polygonal cells in the zona reticularis from the vesicular nuclei (Figure 2b). On top of the large corrugated capsule, the treated group displayed many oval cells with rich granular cytoplasm. In contrast to the others, one of them displayed a central nucleus. Cells from the zona glomerulosa had pyknotic nuclei and vacuolated cytoplasm. Increasing cytoplasmic vacuolation and black pyknotic nuclei were visible in zona fasciculata cells (Figure 2c). Polygonal cells with noticeable sinusoidal capillary dilatation were visible in the zona Reticulata. The medulla (M) was visible (Figure 2d). Zona glomerulosa cells with vacuolated cytoplasm and vesicular nuclei were visible in the withdrawal group. Zona fasciculata cells with dark nuclei and vacuolated cytoplasm. Between cells, there were sinusoidal capillaries. (Figure 2e). Polygonal cells with vesicular nuclei and dilated sinusoidal capillaries were visible in the zona reticularis (Figure 2f).

Fine reticular fiber strands were seen in the capsule, zona glomerulosa, zona fasciculata, and zona reticularis of the adrenal cortex in control group slices after being stained with silver (Figure 3a). Zona glomerulosa, zona fasciculata, and zona reticularis in the E-cigarette treatment group displayed thick capsule and dense reticular fibers (Figure 3b). The reticular fibers distribution in the capsule, zona glomerulosa, zona fasciculata, and zona reticularis in the withdrawal group was almost normal (Figure 3c).

In the nuclei of all adrenocorticocytes of all zones, the control group's caspase-3 immune-stained slices revealed a negative reaction for caspase-3 (Figure 4a). E cigarette treated group showed marked reaction for caspase-3 antibodies in the nuclei of most adrenocorticocytes of all zones (Figure 4b). Withdrawal group indicated that the nuclear reaction was mild positive for caspase-3 in nuclei of few adrenocorticocytes (Figure 4c).

All of the adrenocorticocytes in the control group's cox-II immune-stained sections displayed a negative reaction for cox-II (Figure 5a). Most adrenocorticocytes in the group that had been exposed to e-cigarettes had a conspicuously positive reactivity for cox-II antibodies (Figure 5b). Few adrenocorticocytes from the withdrawal group had a mildly favorable reaction to cox-II antibodies. (Figure 5c).

Sections from the control group stained with CD44 revealed no immune- expression in the cortex (Figure 6a). Those who have smoked electronic cigarettes had few spindle cells at the capsule (Figure 6b). Multiple spindle cells were visible in the cortex and at the capsule in the withdrawal group (Figure 6c).

Effect of E-cig on the weight of adrenal glands

In comparison to the control group, the weight of the adrenal gland increased significantly in the treatment group ($P < 0.05$) but not significantly in the withdrawal group (Table 1).

Effect of e-cigarettes on the three zones of the adrenal cortex's thickness

In comparison to the control group, the thickness of the three zones increased significantly in the e-cigarette

treated group but not significantly in the withdrawal group (Table 2)

Biochemical results

Effect of E-cig on the level of MDA and SOD in different studied groups

In comparison to the control group, the treatment group's MDA level increased significantly, but the withdrawal group's level did not increase much. While there was no significant difference in the amount of SOD in the withdrawal group in comparison to the control group, there was a highly significant decrease in the serum level in the treatment group. (Table 3)

Hormone levels of cortisol and aldosterone as a result of e-cigarette use

Cortisol and aldosterone levels highly significantly increased in the treatment group and significantly increased in the withdrawal group in comparison to the control group. (Table 4)

Morphometric and Statistical results

Comparing the treatment group to the control group, there was a highly significant rise in the area percent of Caspase-3 immune-reaction and a non-significant increase in the area percent of Caspase-3 immune-reaction in the withdrawal group.

In compared to the control group, there was a highly significant rise in the area percent of Cox-II immunological reactions in the treatment group and a considerable increase in the withdrawal group.

In comparison to the control group, there was a non-significant rise in the mean count of CD44 positive cells in the withdrawal group but a significant increase in the treatment group. (Table 5).

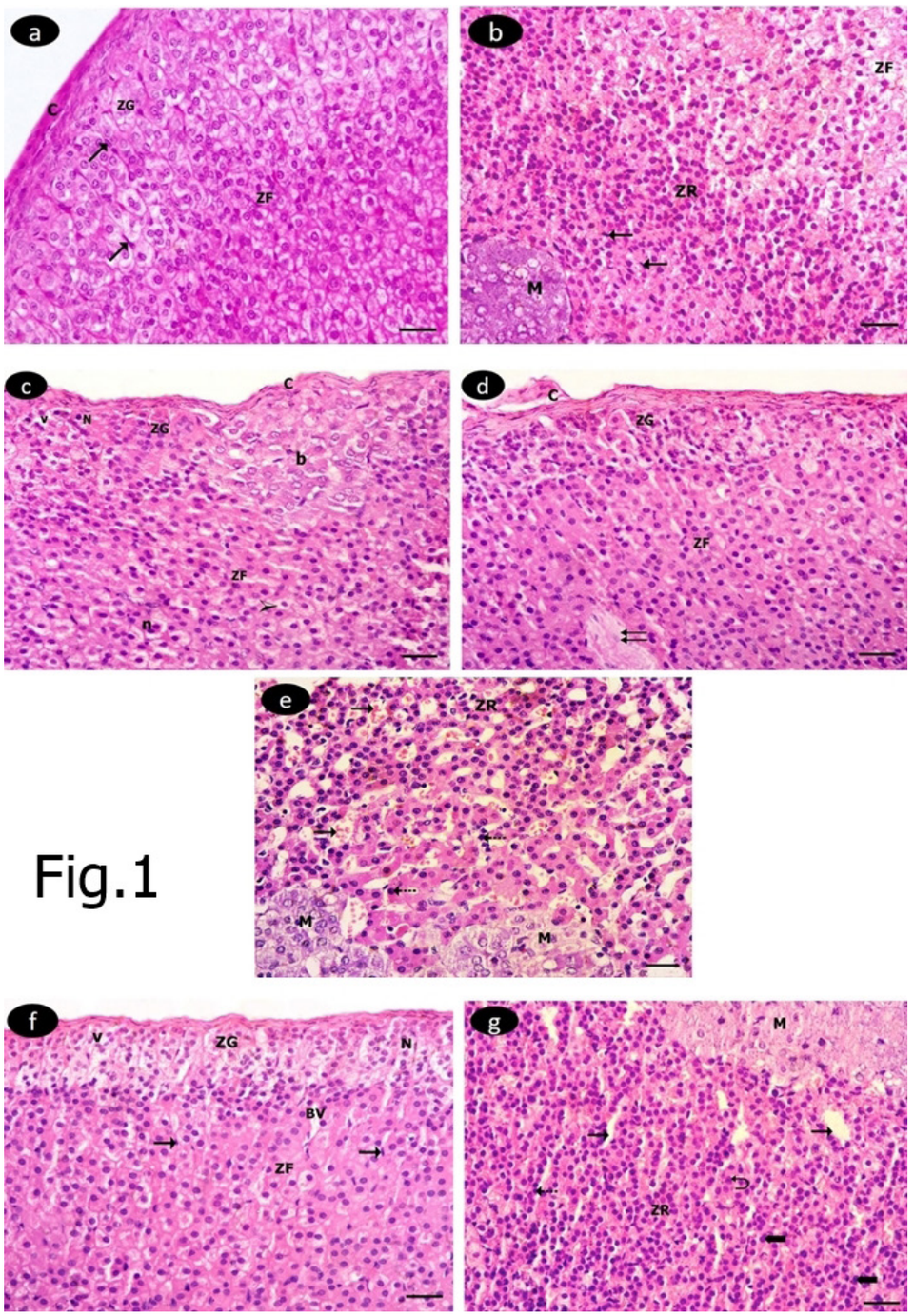

Fig.1

Fig. 1: Photomicrographs of sections of the adrenal cortex of control group showing (a) the capsule (C) with underlying rounded or oval clusters of zona glomerulosa (ZG) and parallel cell cords of zona fasciculata (ZF). Sinusoidal blood capillaries lined by flat endothelial cells (arrow) are present in-between cells. (b) Zona reticularis (ZR) with anastomosing cords of cells and sinusoidal blood capillaries (arrow) in-between are seen. Notice a part of the medulla (M) and a part of zona fasciculata (ZF).

(c) Treated group showing a corrugated capsule (C) and loss of normal architecture of zona glomerulosa (ZG) and zona fasciculata (ZF). Some ZG cells show ballooning with indistinct cell boundaries (b). Other cells show vacuolated cytoplasm (v) and darkly stained nuclei (N). Most of zona fasciculata cells show vacuolated cytoplasm (arrow head) with darkly stained nuclei (n). (d) A distorted capsule (C) and zona glomerulosa (ZG) are observed. Condensation of thin wavy collagen fibers (double arrow) is seen in-between cells of zona fasciculata (ZF). (e) Zona reticularis cells (ZR) show dark condensed nuclei (knotted arrow) with many dilated congested blood sinusoids (arrow) in-between cells. A part of medulla (M) is seen.

(f) Withdrawal group showing zona glomerulosa cells (ZG) with pale rounded nuclei (N) and few vacuoles (v). The zona fasciculata (ZF) appear nearly similar to that of control. Sinusoidal blood capillaries (arrow) are present in-between cells with few dilated ones (BV). (g) Some zona reticularis cells (ZR) show dark condensed nuclei (knotted arrow) while others show vesicular nuclei (curved arrow). Mitotic activity is noticed (thick arrow). Few dilated sinusoidal blood capillaries (arrow) are seen in-between cell cords. A part of medulla (M) is seen. H&E x400, scale bar 20um

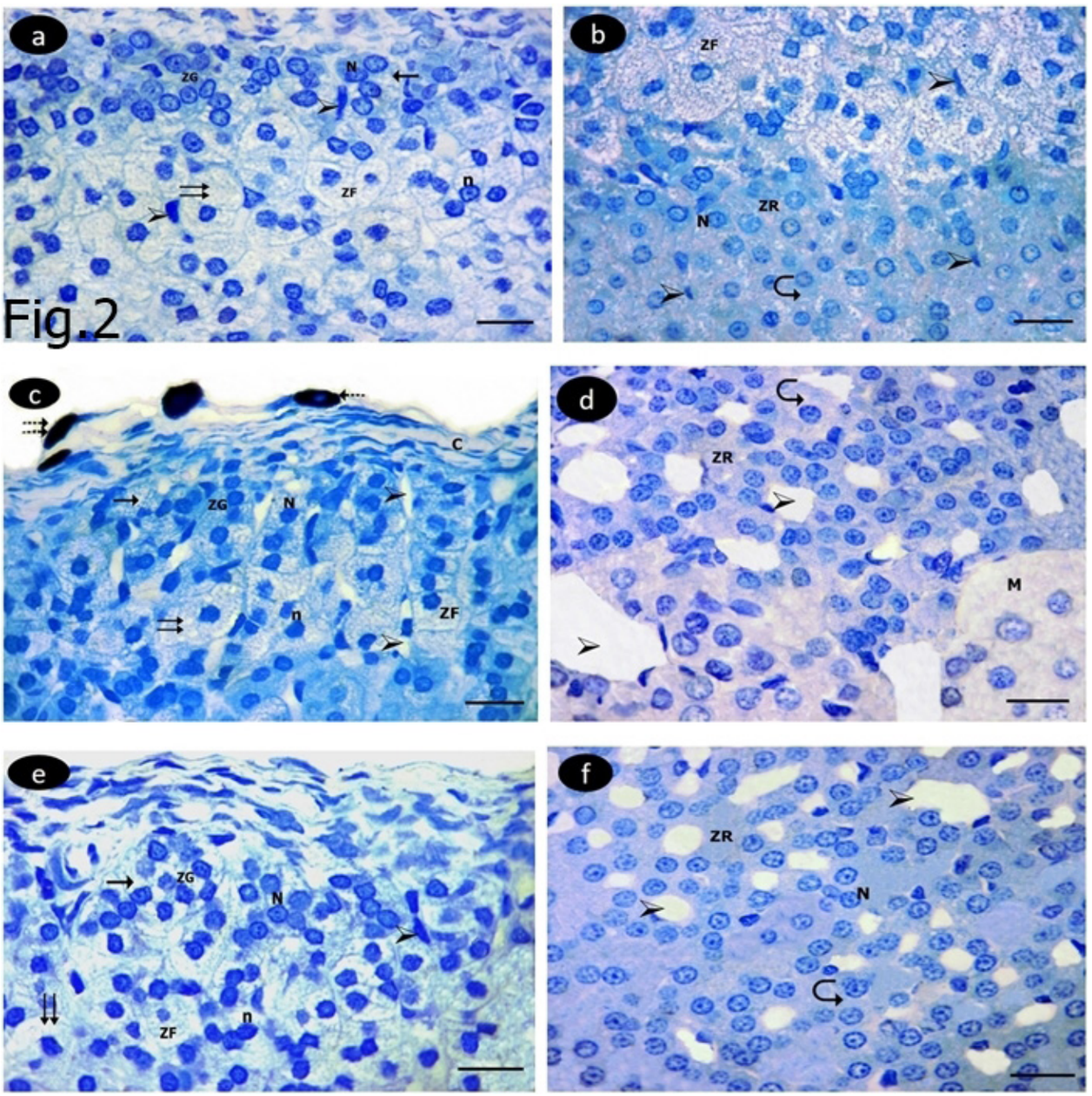

Fig.2

Fig. 2: Semithin sections of the adrenal cortex of control group showing (a) Zona glomerulosa (ZG) cells (arrows) and vesicular nuclei (N). Zona fasciculata (ZF) with large cells, vacuolated cytoplasm (double arrows) and rounded nuclei with prominent nucleoli (n). Note the sinusoidal capillaries (arrowheads). (b) Zona reticularis (ZR) with polygonal cells (curved arrow) and vesicular nuclei (N) separated by sinusoidal capillaries (arrow heads).

- Treated group showing (c) Multiple oval cells with dense granular cytoplasm are observed on the upper part of the thick corrugated capsule (C). One of them has central nucleus (knotted arrow) while the others show no nuclei (double knotted arrows). Zona glomerulosa (ZG) shows cells with vacuolated cytoplasm (arrow) and pyknotic nuclei (N). Zona fasciculata (ZF) shows cells with increase in the cytoplasmic vacuolation (double arrows) and dark pyknotic nuclei (n). (d) Zona reticularis (ZR) shows polygonal cells (curved arrow) with marked dilatation of sinusoidal capillaries (arrow head) between cells. A part of the medulla (M) is seen.

- Withdrawal group showing (e) Zona glomerulosa (ZG) shows cells with vacuolated cytoplasm (arrow) and vesicular nuclei (N). Zona fasciculata (ZF) shows cells with vacuolated cytoplasm (double arrows) and dark nuclei (n). There are sinusoidal capillaries between cells (arrow head). (f) Zona reticularis (ZR) shows polygonal cells (curved arrow) with vesicular nuclei (N). Notice dilated sinusoidal capillaries (arrow head) between cells. Toluidine blue x1000, scale bar 10um

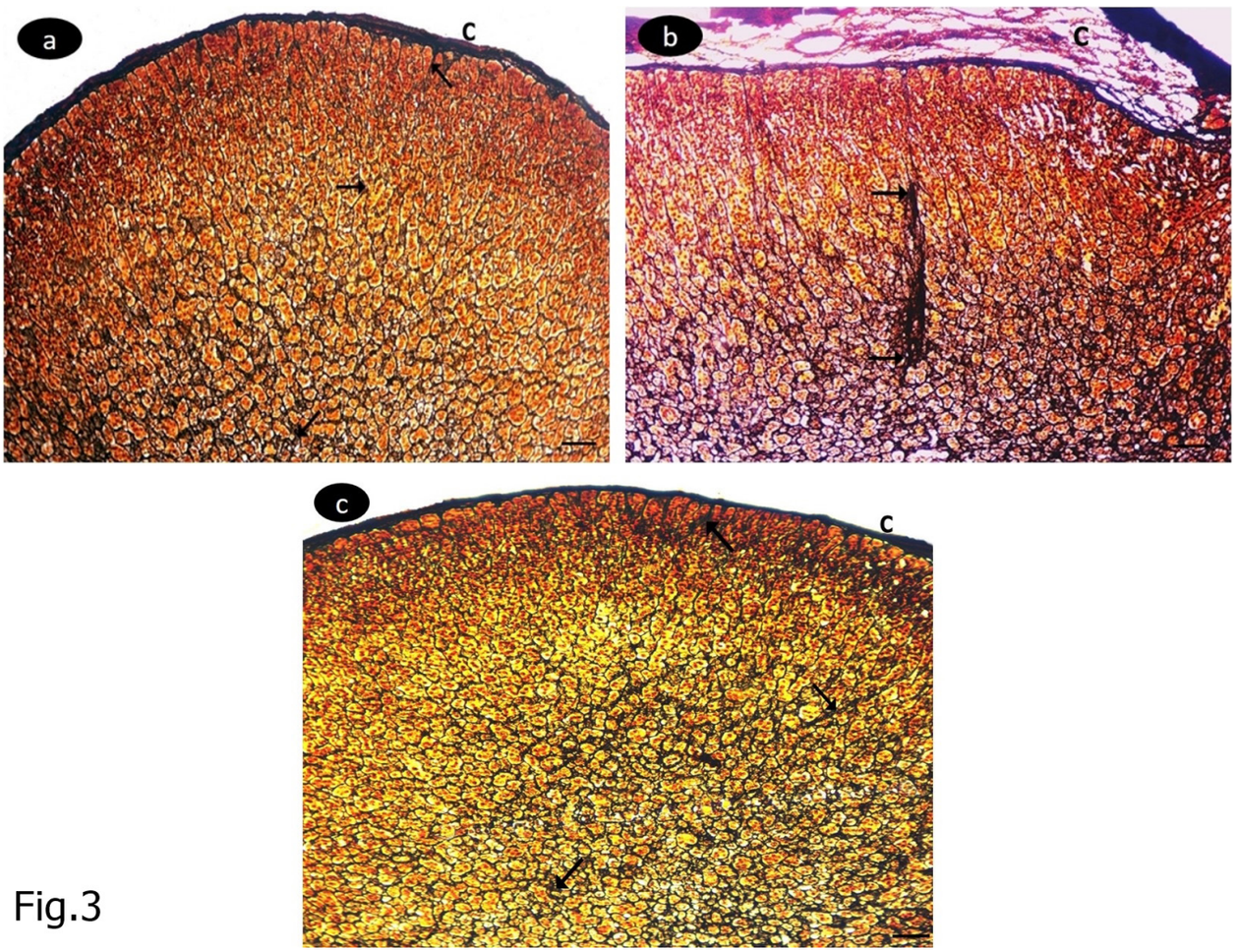

Fig.3

Fig. 3: Photomicrographs of sections of the adrenal cortex of (a) control group showing the capsule (C), zona glomerulosa, zona fasciculata and zona reticularis (arrow). (b) Treated group showing thick capsule (C) and dense reticular fibers (arrows) in zona glomerulosa, zona fasciculata and zona reticularis. (c) Withdrawal group showing nearly normal distribution of the reticular fibers in, zona glomerulosa, zona fasciculata and zona reticularis (arrow).
Silver stain x100, scale bar 50um

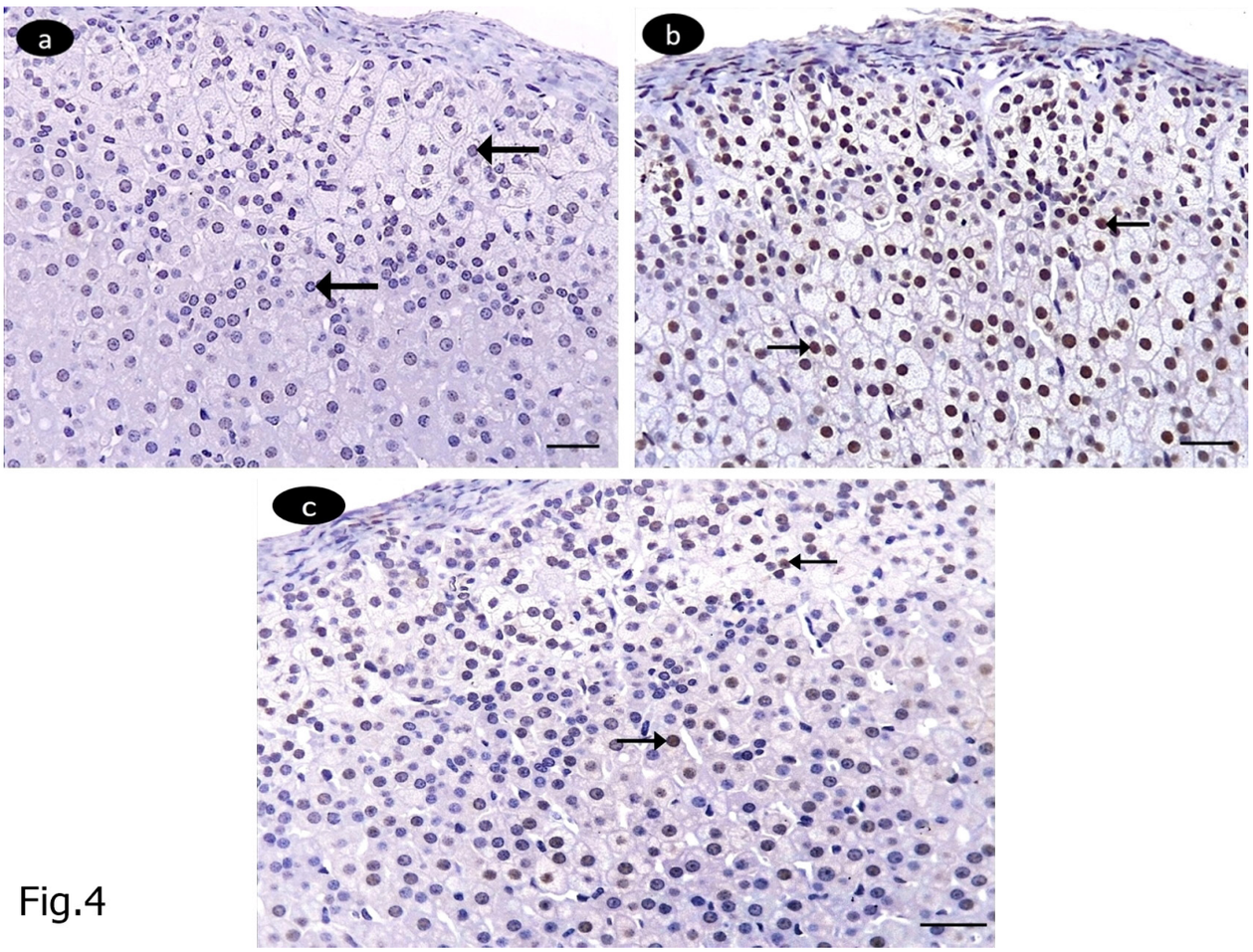

Fig.4

Fig. 4: Photomicrographs of immune-stained sections for caspase-3: (a) control group showing negative reaction for caspase 3 in the nuclei of all adrenocorticocytes of all zones (arrows). (b) e cigarette treated group showing marked reaction for caspase 3 antibodies in the nuclei of most adrenocorticocytes of all zones (arrows). (c) Withdrawal group showing mild positive nuclear reaction for caspase 3 in the nuclei of few adrenocorticocytes (arrows).
Immuno-peroxidase reaction for caspase 3 x400, scale bar 20um

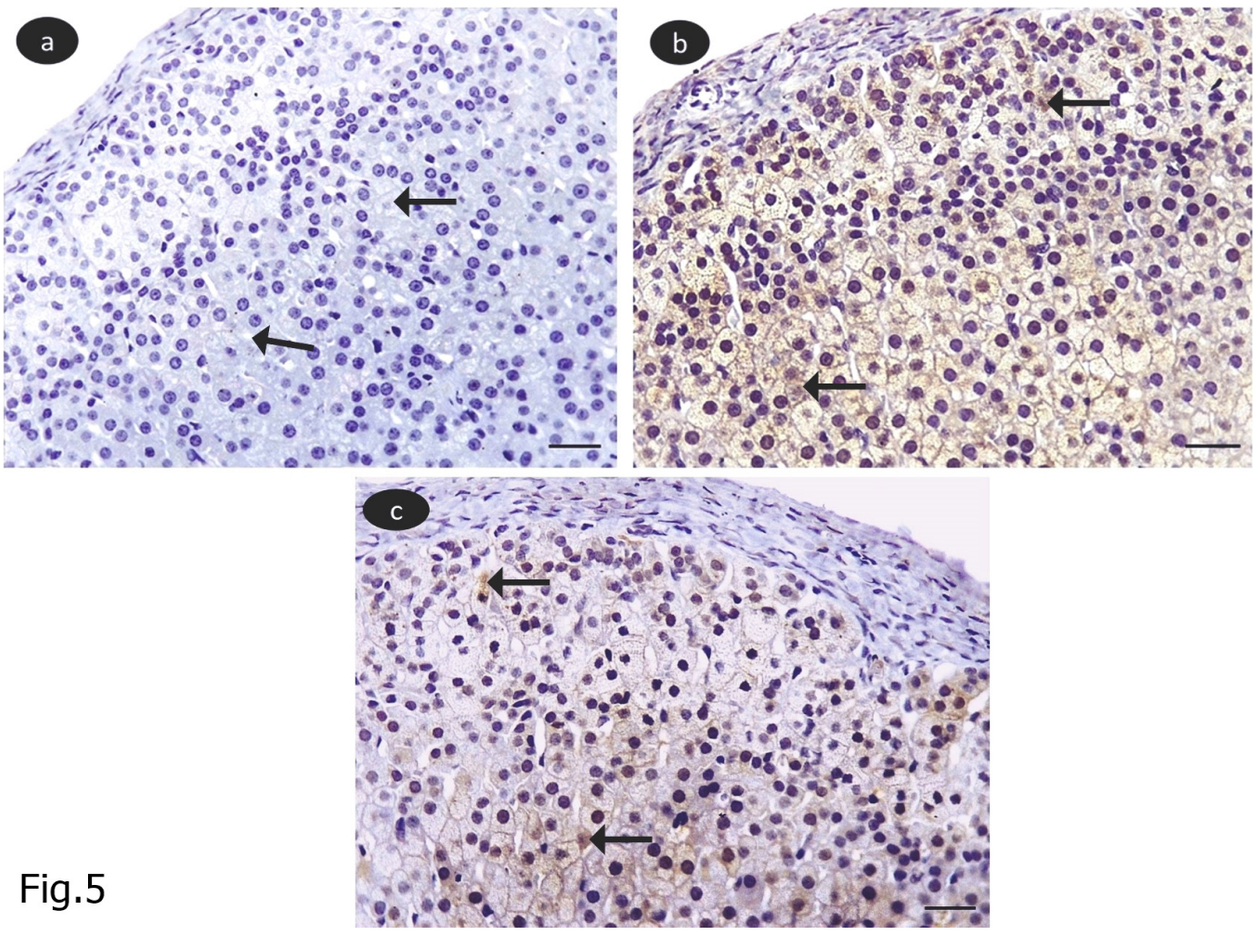

Fig.5

Fig. 5: Photomicrographs of immune-stained sections for cox-II: (a) control group showing negative reaction for cox-II in the cytoplasm of all adrenocorticocytes (arrows). (b) e cigarette treated group showing marked positive reaction for cox-II antibodies in the cytoplasm of most adrenocorticocytes (arrows). (c) withdrawal group showing mild positive reaction to cox-II antibodies in the cytoplasm of few adrenocorticocytes (arrows).

Immuno-peroxidase reaction for cox-II x400, scale bar 20um

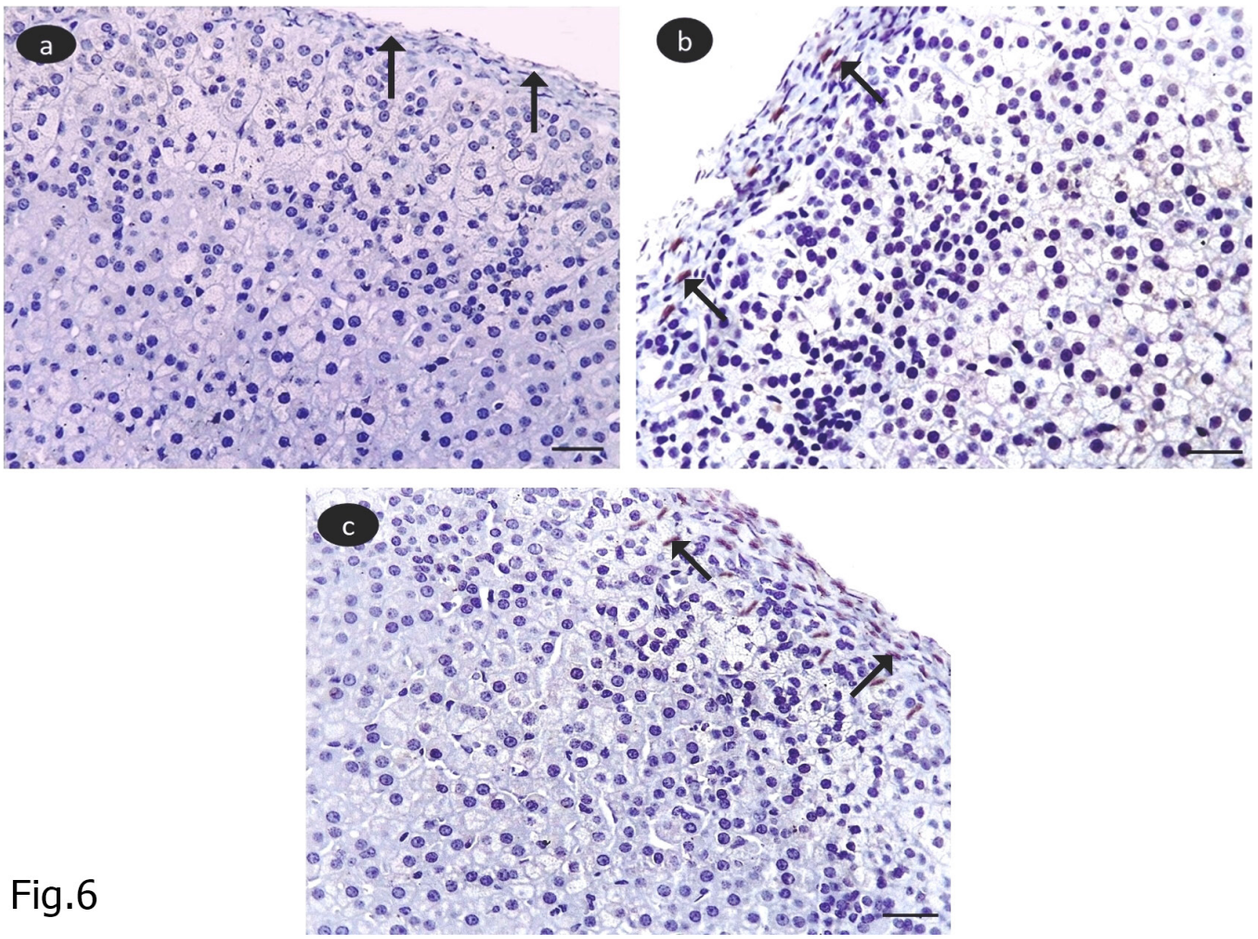

Fig.6

Fig. 6: Photomicrographs of immune-stained sections for CD44: (a) control group showing negative immune-expression in the cortex (arrows). (b) e cigarette treated group showing few spindle cells at the capsule. (c) withdrawal group showing multiple spindle cells at the capsule and in the cortex.
CD44 immunostaining x400, scale bar 20um

Table 1: Adrenal gland weight of the different studied group.

groups	weight	*P value*
control	0.028 ± 0.004	
treated group	0.058 ± 0.08**	*P*<0.001**
Withdrawal group	0.030 ± 0.005	

** Highly significant increase compared to control group

Table 2: Effect of E-cig on the thickness of zona glomurolusa, fasculata and reticularis of adrenal cortex of different studied groups

groups	ZG thickness\ mm	ZF thickness\ mm	ZR thickness\ mm
Control group	1.5 ± 0.26	6.8± 0.11	0.88 ± 0.04
Treated group	2.1 ± 0.25**	7.5 ± 0.22**	1.4 ± 0.03**
Withdrawal group	1.4 ± 0.42	6.7 ± 0.17	0.86 ± 0.03

** Highly significant increase compared to control group (*P*<0.001**)

Table 3: Levels of MDA and SOD in different studied groups

Groups	Level of MDA (nmol/g tissue)	*P value*	Level of SOD (U/g protein)	*P value*
Control	6.76 ± 0.55		78.04 ± 9.08	
treated group	14.98 ± 5.02**	*P*<0.001**	62.88 ± 8.03**	*P*<0.001**
Withdrawal group	6.78 ± 0.58		75.49 ± 8.66	

** Highly significant increase compared to control group

Table 4: Levels of cortisol and aldosterone hormones in different studied groups

Groups	Level of cortisol (µg/dl)	*P value*	Level of aldosterone (pg/ml)	*P value*
control	205.7 ± 14.1		498.3 ± 6.2	
treated group	397.3 ± 37.5 **	*P*<0.001**	683 ± 25.3**	
Withdrawal group	279.5 ± 41.7*	*P*<0.05*	520 ± 7.7*	*P*<0.001**

** Highly significant increase compared to control group

* Significant increase compared to control group

Table 5: Mean ± SD of area percent of the Caspase 3 immune-expression, Cox-II immune-expression and count of CD44 positive cells:

	Area % of the Caspase 3 immunoexpression	Area % of the Cox-II immunoexpression	Mean count of CD44+ve cells
Control group	0.25 ± 0.41	0.13 ± 0.21	0.18 ± 0.32
Treated group	30.82 ± 10.81**	38.44 ± 4.6**	4.32 ± 0.81
Withdrawal group	4.89 ± 0.90	14.61 ± 1.9*	8.25 ± 3.12*

*Significant increase compared to control group

** Highly significant increase compared to control group

DISCUSSION

The adrenal glands are called the life-saving glands as they possess a very important functions in the form of secreting many hormones that control essential body functions like glucocorticoids and mineralocorticoids[24].

Nicotine, which is the primary component in e-cigarettes, has a number of harmful effects on endocrine glands such the pituitary, thyroid, and adrenal glands. By reducing the quantities of antioxidants and increasing the levels of lipid peroxidation in the tissue, it disrupts the adrenal antioxidant function[25]. Therefore, this research was done to examine the negative consequences of e-cigarette use on the adrenal gland histological architecture as well as the impacts of stopping use.

The treated group's H&E sections demonstrate a loss of the zona glomerulosa and zona fasciculata's typical morphology. Some cells in the zona glomerulosa exhibit ballooning and blurry cell borders. Other cells have darkly pigmented nuclei and vacuolated cytoplasm. The majority of the cells in the zona fasciculata have vacuolated cytoplasm and darkly pigmented nuclei. These results were in agreement with[13] who explained that the lipid droplets aggregations and vacuolation of cytoplasm in zona fasciculata cells following the treatment with nicotine are caused by impairment of glucocorticoid synthesis. Zona reticularis cells exhibit dark condensed nuclei with many dilated congested blood sinusoids in between cells. Elshennawy *et al* explained that disruption of cytochrome P450 enzymes play a vital role in adrenal gland toxicity as it causes impairment of steroidogenesis process. So, inhibition of cholesterol synthesis occur which lead to lipid droplet aggregation and vacuolation of cytoplasm in zona fasciculata cells[26]. These results also were in harmony with[27] who said that administration of dexamethasone cause disruption of steroidogenesis which in turn cause lipid droplet aggregation in zona fasciculate and zona reticularis cells.

In semithin sections of treated group, mast cells which are oval cells with granular cytoplasm are seen above the capsule. The mast cells contain one nucleus (mononuclear) with a lot of secretory, dense granules which may hide the nucleus[28].

The hematopoietic stem cells of bone marrow give rise to mast cells which have progenitor cell type that circulate to different tissues and under the effect of local factors the mature mast cells arise from progenitor type and they are present in the connective tissue of all organs not in circulation[29]. The innate immune system utilizes the mast cells' inflammatory effects as its defense main line against foreign objects. In the event that a foreign body is detected, the inflammatory effects of mast cells lead to an increase in the number of immune cells in circulation[30,31].

Compared to the control and withdrawal groups, adrenal cortex slices stained with silver revealed thick capsules and dense reticular fibers in the zona glomerulosa, fasciculata, and reticulata of the E-cigarette treated group. These findings were consistent with[32] who indicated that silver stained sections of MSG treated group showed increased the amount of reticular fibers and these changes were due to damage of the tissue which is influenced by many humoral and cellular reactions that increase healing process and remove the damaged tissue. Ribeiro *et al* reported that all these reactions take place in connective tissue and depends upon collagen deposition[33].

In the nuclei of the majority of adrenocorticocytes in all zones, the immune stained sections for caspase-3 of the E cigarette treated group showed a marked reaction for caspase-3 antibodies. These results were consistent with[12], who also discovered increased immune-reactivity for caspase-3 in the cells of the adrenal gland following the exposure to nicotine. These results were also explained by[34] who said that caspase 3 immunoreaction was increased with apoptosis. These results also were in the same with[35] who said that increased immunoreaction to caspase 3 in the male piglets brain after exposure to nicotine. Also,

increased apoptosis and decreased integrity of the cell were the causes for increased the immune expression for caspase 3 in human gingival fibroblasts treated with nicotine[36].

Most normal tissues lack COX-II, a prostaglandin synthase enzyme that triggers arachidonic acid conversion to prostaglandins. Nonetheless, it is turned on in inflammatory areas by cytokines, growth hormones, and tumor promoters[37]. The results of the current study's immunological stained sections for cox-II of the group that had been exposed to E cigarettes indicated a notable positive reaction for cox-II antibodies in the cytoplasm of most adrenocorticocytes and these results were in agreement with[38] who revealed increased the expression of COX-II immunohistochemical reaction in the BDR group.

In the present work, the statistical analysis and immunoreaction of the CD 44+ve cells number were increased in E-cigarette treated group as compared to other groups. CD44 + ve cells (cluster of differentiation) are cell adhesion and signaling molecules family cells special for mesenchymal stem cells which may reached the damaged area from the bone marrow through the circulation. Furthermore, many studies have revealed that in mammalian species many adrenocortical cells with stem-like properties may be present which possess the ability to migrate by membrane extensions[39,40,41,42].

In this study, the immune stained sections of CD 44+ve cells of E-cigarette treated group showed few spindle cells at the capsule and these results were in harmony with[43] who detected that increased the expression of CD44+ve cells in adrenal cortex of tributyltin treated rats.

This study's results were consistent with[44] who discovered that intra peritoneal administration of E-cigarette liquid containing nicotine significantly increased the adrenal gland weight. The adrenal gland weight was significantly higher in the E-cigarette treated group than in the control group and withdrawal group. The increased weight of the adrenal gland in the group that had been exposed to E cigarettes was due to enhanced cell swelling brought on by increased lipid deposition by nicotine[45].

According to[46] who claimed that nicotine administration at a dose of 0.5 mg/kg for 60 days caused an increase in the thickness of the adrenal gland zones and that this resulted from the tissue inflammation caused by nicotine, which led to an increase in cell size. In the current investigation, in comparison to the control and withdrawal groups, the treated groups' mean thickness of the adrenal cortex zones was noticeably increased.

Malondialdehyde (MDA) is a lipid peroxidation predictor utilized for indication of tissue damage which occur due to free radical liberation. As compared to the control and withdrawal groups, there was a rise in the amount of MDA and a decrease in SOD level in the E-cigarette treated group. These findings were consistent with[47,48] who said that nicotine possess a strong oxidant ability as it increases the free radical liberation which fuse with the cell membrane causing oxidative damage then cells death.

Cortisol and aldosterone levels in this work increased in a highly significant way, according to statistical analysis in E-cigarette treated group in relation to control and withdrawal group and these results were in agreement with[49] who said that the cortisol level was significantly increased after smoking cigarette and these results were also reported by[50] who said that exposure of rats to stressful stimulators lead to release of ACTH due to activation of hypothalamic pituitary axis.

All stained sections of the withdrawal group's adrenal gland tissues in the current study exhibited nearly the same structure as the control group, which was demonstrated by morphometric studies and statistical analysis for immunoreaction for caspase 3, CD44+, and COX-II as well as for levels of MDA, cortisol, and aldosterone levels. These findings were consistent with previous studies[51] who said that after nicotine withdrawal the seminiferous tubules, histological structure was greatly improved and also these data were in harmony with[52] who explained that there were partial improvement of histological structure of the uterus and ovary after stoppage of nicotine administration.

Finally, it can be said that using e-cigarettes has negative effects on the adrenal gland's histological structure as well as its chemical function. The histological and biochemical function of the adrenal gland significantly improved after e-cigarette usage was discontinued.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير التعرض للسجائر الالكترونية على قشرة الغدة الكظرية فى ذكور الجرذان البيضاء البالغة و الدور المحتمل لانسحابه (دراسة هستولوجية و مناعية)

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المقدمة: السجائر الالكترونية عبارة عن اجهزة صممت لامداد الجسم بالنيكوتين فى صورة سائلة بدلا من تدخين السجائر بدون استهلاك التبغ والتى تستخدم كبديل امن للتدخين العادى و لقد اصبح التسويق للسجائر الالكترونية على انها اختيار لنمط الحياة.

الهدف : لتقييم التغيرات النسيجية والكيميائية فى قشرة الغدة الكظرية فى ذكور الجرذان البيضاء البالغة نتيجة التعرض للسجائر الالكترونية و الدور المحتمل لانسحابة.

المواد والطرق المستخدمة: لقد استخدم فى هذه الدراسة ثلاثون من ذكور الجرذان البالغة و التى تزن ١٥٠-٢٠٠ جم وقد تم الحصول عليهم من بيت الحيوان كلية الطب البشرى جامعة الزقازيق ولقد تم تقسيمهم الى ثلاثة مجموعات تحتوى كل منها على عشرة جرذان المجموعة الاولى (الضابطة): والتى تحتوى على ١٠ جرذان لم يتم اعطاؤها شىء ويتم تقديم الطعام والشراب لها بصورة منتظمة من اجل قياس المعلومات الاساسية. المجموعة الثانية (االمعالجة بالسجائر الالكترونية):والتى تضم عشرة جرذان يتم تعرضها ل ١ مل يوميا من السائل الالكترونى والذى يحتوى على ١٨ مليجرام من النيكوتين لمدة خمسة ايام متتالية فى الاسبوع لمدة اربع اسابيع .المجموعة الثالثة (مجموعة الانسحاب): والتى تضم عشرة جرذان يتم تعرضها ل ١ مل يوميا من السائل الالكترونى والذى يحتوى على ١٨ مليجرام من النيكوتين لمدة خمسة ايام متتالية فى الاسبوع لمدة اربع اسابيع ثم تترك اربعة اسابيع اخرى بدون التعرض للسجائر الالكترونية

النتائج: ان تعرض ذكور الجرذان البيضاء للسجائر الالكترونية ادى الى تأثيرات ضارة بالنسيج التركيبى و كذلك الوظائف الكيميائية للغدة الكظرية فلقد فقد التركيب الهستولوجى الطبيعى للطبقتين المتكورة والحزيمية اما الطبق الشبكية فقد ظهرت خلاياها بنوايا سوداء مكدسة مع وجود العديد من الاوردة المحتقنة بين الخلايا .و لقد لوحظ ايضا زيادة وزن الغدة الكظرية و زيادة سمك الطبقات الثلاثة المتكورة والحزيمية والشبكية فى المجموعة المعالجة و هناك ايضا زيادة فى نسبة الميلانوالدهايد ونقص نسبة الانزيم المضاد للاكسدة سوبراكسيد ديسميوتاز وكذلك زيادة افراز هرمون الكورتيزول و الالديستيرون فى تلك المجموعة.واما بالدراسة المورفومترية فقد وجد زيادة فى النسبة المئوية للتمثيا الامنيوسى لمادة كاسبيز ٣ و كوكز ٢ وزيادة عدد خلايا السى دى ٤٤ وقد لوحظ تحسن ملحوظ فى جميع التغيرات السابقة فى المجموعة الثالثة (مجموعة الانسحاب).

الاستنتاج: يؤدى استخدام السجائر الالكترونية الى اضرار بالغة فى النسيج التركيبى لقشرة الغدة الكظرية وكذلك الوظائف الكيميائية ويؤدى انسحابه الى تحسن فى تلك التغيرات الضارة.