Biochemical and Histopathological Effects of Electronic Cigarette Vapor on Testicular and Cardiac Tissues in Adult Male Albino Rats

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ABSTRACT

Over the past decade, the popularity of e-cigarettes has surged steeply. Instead of burning tobacco, these devices supply nicotine through an inhaled aerosol, a trait that has led many people to view them as an apparently safer substitute for ordinary cigarettes. Even so, published work cautions that, despite a lower toxin load than conventional cigarettes, e-cigarettes can still harm health. The aim here was to judge how e-cigarette exposure might harm the heart and testis of mature albino male rats, assessed through a hormonal panel (FSH, LH, Testosterone), the oxidative-stress marker MDA, troponin measurement and histopathology. Thirty adult albino male rats were enrolled and split at random into 3 matched sets of 10 animals each; the first (group I) acted as control, the second (Group II, low-dose arm) inhaled 1 ml of E-liquid smoke vapor carrying 6mg nicotine, and the third (Group III, high-dose arm) inhaled 1ml of E-liquid smoke vapor carrying 18mg nicotine. The protocol received clearance from Benha University's Faculty of Medicine (Research Ethics Committee). **Results:** a stepwise fall in FSH, LH, Testosterone appeared as exposure rose from control through the low dose and then to the high dose. By contrast, MDA, which flags oxidative stress, climbed sharply in testicular and cardiac tissue of both treated groups versus controls. Likewise, cardiac troponin I climbed significantly across both treated arms relative to the control. Consistent histopathological alterations in both organs. **Conclusion:** e-cigarettes lead to oxidative stress together with disturbance of the hormonal panel and troponin, alongside histopathological change.

Keywords: E-cigarette, nicotine, oxidative stress, heart, testis

Introduction

Over the past decade, e-cigarette and vaping use has expanded dramatically, especially among youth and young-adult populations. By supplying nicotine in an aerosolized form, such products are framed as an apparently safer option than ordinary cigarettes ***(Rose et al., 2023).*** A common marketing claim from producers is that breathing in e-cigarette vapor carries less harm than inhaling tobacco smoke ***(Marques et al., 2021).***

Although the chemical toxin count in e-cigarettes is smaller than in conventional cigarettes, these products still pose dangers. Research points to possible health threats arising from contact with damaging agents, among them nicotine, volatile organic compounds and the chemicals used for flavoring. Health authorities at the CDC similarly stress that e-cigarette use can adversely affect health ***(CDC, 2022).***

A vape is built around a tank holding E-liquid, a mixture made up of vegetable glycerin, propylene glycol (PG) and both natural and artificial flavorings. Within E-liquids the nicotine content ranges from nicotine-free formulations up to 36 mg/ml. Concentrations of 6, 12, 18, or 24 mg/ml are the ones most commonly met in E-liquids ***(Kar et al., 2019).***

Through vaping, users come into contact with an intricate blend of damaging chemicals capable of producing toxicity. Among the documented harmful actions of e-cigs is a rise in pro-inflammatory and pro-fibrotic markers. Such products also raise free-radical levels, a change that can in turn drive oxidative stress ***(Fajariyah et al., 2021 & Zong et al., 2024).*** On top of this, warming e-liquids liberates toxic aldehydes able to injure DNA, while nicotine on its own exerts toxic actions that add to the systemic burden ***(Gotts et al., 2019).***

Habitual electronic-cigarette use can damage male reproductive health. Beyond this, the agents added for flavor may injure testicular cells, since they have been tied to inflammatory reactions, gonadotoxicity and carcinogenesis ***(Montjean et al., 2023).***

Because the cardiovascular system reacts strongly to contact with toxic compounds, even modest amounts of toxicants may bring about harm. The damage tobacco-cigarette smoking does to the heart and vessels is firmly established, yet how toxic e-cigarettes are by comparison stays unclear, given that only a handful of investigations have described harmful cardiovascular outcomes from e-cigarette vaping ***(Middlekauf, 2020 & Rose et al., 2023).***

Aim of the study

This study sets out to gauge the probable adverse effects of one popular electronic cigarette (e-cigarette) on the heart and testis of adult albino male rats, through biochemical work (hormonal assay, oxidative stress, and troponin level) together with histopathological study.

Materials and Methods

Animals: A total of 30 rats, each weighing 200-250 gm, took part in the experiment. For one week beforehand the animals were acclimatized on a fixed diet of wheat, bread and milk, free of any medication and overseen by a trained technician (housed in the animal unit of Benha University's Veterinary Faculty of Medicine), so that their mental and physical wellbeing could be tracked.

Chemicals and Devices:

1. The vaping liquid (E-Liquid) employed in this study was supplied by an Egyptian vendor (Dollar blends company, Egypt).

2. The portable electric incense burner used as the vapor source came from the same Egyptian vendor (home electric company, China).

3. Testosterone enzyme-linked immunosorbent assay (ELISA) kits were obtained from BOSTER BIO Company, USA.

4. Follicle-stimulating hormone (FSH) enzyme-linked immunosorbent assay (ELISA) kits were obtained from CUSABIO Company, USA.

5. Luteinizing hormone (LH) enzyme-linked immunosorbent assay (ELISA) kits were obtained from CUSABIO Company, USA.

6. Cardiac troponin enzyme-linked immunosorbent assay (ELISA) kits were obtained from SimpleStep ELISA kit , Abcam, UK.

7. Malondialdehyde (MDA) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biodiagnostic; Dokki, Giza, Egypt

Study design:

When the experiment commenced, the rats were allocated at random into 3 groups of 10 animals each, organized as follows:

a-Group I (control group) 10 rats: inhaled only fresh air throughout the four-week period.

b-Group II (low dose of e-cigs exposed group) 10 rats: inhaled 1 ml of E-liquid smoke vapor (6mg), a quantity matching the light-smoker dose, over 1 hour on 5 consecutive days each week for four weeks ***(Morgan & Jones, 2020).***

c-Group III (high dose of e-cigs exposed group) 10 rats: inhaled 1ml of E-liquid smoke vapor (18mg), a quantity matching the heavy-smoker dose, over 1 hour on 5 consecutive days each week for four weeks ***(Abdel-Kareem et al., 2022).***

Animals from groups II and III were placed inside an inhalation chamber formed by a propylene box (38 × 26.5 × 19 cm) holding 19 liters. With a portable electric incense burner, e-cigs vapor was delivered for one hour/day, the rats being treated in whole-body fashion at two rats/ chamber. Afterwards each was transferred to a fully sterile room ***(Canistro et al., 2017 & Saygın et al.,2023).***

Ethical considerations:

Approval for this work (code: MS-36-2-2025) was granted by the Scientific Research Ethics Committee of Benha University's Faculty of Medicine.

Studied parameters

Biochemical study:

Once the experimental period closed, each rat received an intraperitoneal injection of thiopental sodium (50 mg/kg) for anesthesia before specimens were taken ***(Wawryk-Gawda et al., 2019).*** From the retro-orbital plexus, venous blood (5 ml) was drawn through capillary glass tubes. After the blood had clotted, it was spun at 2000 revolution per minute (rpm) for 15 minutes so that serum could be separated for biochemical analysis. This serum was then gathered and held at −80°C until assayed, following the procedure of ***Kumar (2017).***

With rat ELISA (enzyme-linked immunosorbent assay) kits, serum was assayed for cardiac troponin I along with the hormones luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone.

Oxidative stress parameters:

Following sacrifice of the animals, testicular and cardiac tissues were excised and chopped into small fragments, which were rinsed with phosphate buffered saline (PBS) at PH 7.4 carrying 0.16 mg/ml heparin so that red blood cells and clots were washed away; the tissue was next ground in a glass homogenizer using 5 to 10 ml of cold buffer (namely 50 mM potassium phosphate, pH 7.5) for every gram. These homogenates underwent centrifugation at 4000 r.p.m. for 15 minutes at 4 ºC, after which the supernatant was drawn off, kept on ice and applied to measure Malondialdehyde (MDA) in the testicular and cardiac tissues with diagnostic kits as the manufacturer directed ***(Hussein et al., 2018).***

2-Histopathological examination of testis and heart:

After the rats' testicular and cardiac tissues had been dissected, they were gathered, trimmed and preserved in 10% buffered neutral formalin, taken through ascending grades of ethyl alcohol (70-100%), cleared in xylene and set in paraffin wax. Sections about 4-5 µm thick were then rinsed in tap water, dehydrated through graded alcohol concentrations, cleared in xylol and again embedded in paraffin wax. With a rotating microtome, serial sections of 3-5-micron thickness were cut and stained using Mayer's haematoxylin and Eosin for histopathological study ***(Suvarna et al., 2019).*** A light microscope (Olympus, Japan) was used to inspect the stained sections at the Pathology Department, Animal Health Research Institute, Zagazig Laboratory.

Data analysis:

Data were entered, coded and analysed in IBM SPSS Statistics for Windows (Version 28.0; IBM Corp, Armonk, NY; released 2021).

Prior to running the statistics, every quantitative variable was checked for normal distribution with the Shapiro-Wilk test, the option favoured when sample sizes are small (n = 10 per group) ***(Razali and Wah, 2011).***

The quantitative findings of this work are reported as means ± Standard deviation along with median, minimum and maximum.

For parametrically distributed data across the three groups, the F-based ANOVA test (analysis of variance) was applied, whereas the Kruskal-Wallis (KW) test handled non-parametric data. To compare any two groups afterwards, post hoc LSD served the parametric data and the Mann Whitney test (U) the non-parametric data.

Depending on how the data were distributed, correlations were examined with either the Spearman's or the Pearson's test. Spearman's correlation gauged the strength and direction of association between two non-parametric variables, while Pearson's was reserved for parametric ones; the resulting coefficients always lie between -1 (strong negative relationship) and +1 (strong positive relationship).

Significance for the data was fixed at 95%; accordingly, a p value >0.05 was read as a non-statistically significant

difference. In contrast, a p value < 0.05 denoted a statistically significant difference, and a p value <0.01 marked a highly statistically significant difference.

Results

Biochemical Results and Oxidative stress parameters (MDA)

Effect of e-cigarette exposure on hormonal levels and MDA in testis across the studied groups:

A highly significant progressive decline in FSH, LH, Testosterone levels was observed across the three groups ($p < 0.001$). The low and high-dose groups showed significantly lower FSH, LH, Testosterone levels compared to the control group, Additionally, the FSH, LH, Testosterone levels of the high-dose group was significantly lower than the low-dose group ($p < 0.001$), indicating a dose-dependent suppressive effect of e-cigarette exposure on FSH, LH, Testosterone secretion as shown in **Table 1**

A highly significant progressive increase in MDA levels in testicular tissue was observed across the three groups ($p < 0.001$). The low- and high-dose groups showed significantly higher MDA levels compared to the control group. Additionally, the MDA level of the high-dose group was significantly higher than the low-dose group ($p < 0.001$), indicating a dose-dependent elevation in oxidative stress within testicular tissue following e-cigarette exposure as shown in **Table 1**

Effect of e-cigarette exposure on Cardiac Troponin I levels and MDA in heart across the studied groups:

A highly significant progressive increase in cardiac troponin I levels was observed across the three groups ($p < 0.001$). The low- and high-dose groups showed significantly higher CTnI levels compared to the control group. Additionally, the CTnI level of the high-dose group was significantly higher than the low-dose group ($p < 0.001$), suggesting a dose-dependent myocardial injury effect of e-cigarette exposure, as shown in **Table 2**

A highly significant progressive increase in MDA levels in cardiac tissue was observed across the three groups ($p < 0.001$). The low- and high-dose groups showed significantly higher MDA levels compared to the control group. Additionally, the MDA level of the high-dose group was significantly higher than the low-dose group ($p < 0.001$), indicating a dose-dependent elevation in cardiac oxidative stress following e-cigarette exposure as shown in **Table 2**

Correlation between MDA in testis and reproductive hormones across the studied groups:

In low-dose group, a highly significant strong negative correlation was found between MDA and both FSH and LH in addition to a significant negative correlation with testosterone In the high-dose group, highly significant strong negative correlations were demonstrated between MDA and FSH, LH, testosterone suggesting that increasing oxidative stress in testicular tissue is strongly associated with suppression of the hypothalamic-pituitary-gonadal axis in a dose-dependent manner, **as shown in Table 3 and Figs. 1-2-3-4-5-6.**

Correlation between MDA in heart and Cardiac Troponin I across the studied groups:

In the low-dose group, a highly significant strong positive correlation was found between MDA in heart and CTnI. In the high-dose group, this association became even stronger, with a highly significant positive correlation, suggesting that e-cigarette-induced cardiac oxidative stress is closely linked to myocardial injury in a dose-dependent manner, **as shown in Table 4 and Figs. 7-8.**

Table (1): Comparison of hormonal levels in blood and oxidative stress markers (MDA) in testis among control, low dose, and high dose groups of rats

variable	Groups			Test of significance	P value	Pair-wise comparison
	Control group (n=10)	Low dose group (n=10)	High dose group (n=10)			
FSH (U/ml) Mean ± SD Median (min-max)	8.23±0.63 8.20(7.25-9.06)	4.27±0.57 4.01(3.69-5.22)	2.46±0.35 2.46(2.09-3.04)	**K=**25.89	<0.001**	p<0.001**1 p<0.001**2 p<0.001**3
LH (U/ml) Mean ± SD Median(min-max)	30.81±1.03 31.04(29.39-32.23)	24.5± 1.65 24.49(22.24-26.63	14.17±1.51 14.14(12.56-16.39	**F=**348.19	<0.001**	p<0.001**1 p<0.001**2 p<0.001**3
Testosterone: (ng/ml) Mean ± SD Median(min-max)	6.11±0.16 6.19(5.84-6.24)	3.6±0.29 3.54(3.29-4.11)	2.07±0.15 2.04(1.88-2.26)	**K**=25.94	<0.001**	p<0.001**1 p<0.001**2 p<0.001**3
MDA in **testis**(**nmol-mg**) Mean ± SD Median (min-max)	0.81±0.03 0.81(0.77-0.86)	1.93±0.31 1.92(1.46-2.37)	3.77±0.39 3.92(3.19-4.24)	**F**=264.73	<0.001**	p<0.001**1 p<0.001**2 p<0.001**3

SD: standard deviation, Min: Minimum, Max: Maximum, K: Kruskal-wallis, F: ANOVA (analysis of variance) test, **: Highly significant at P<0.01 , MDA: Malondialdehyde, FSH: follicle-stimulating hormone, LH: luteinizing hormone

Pair-wise comparison: P value of Post hoc LSD test, P_1: comparison between control group versus low-dose group, p_2: control group versus high dose group, p_3: low dose group versus high dose group *: significant at P<0.05, **: Highly significant at P<0.01

Table (2): Comparison of oxidative stress markers (MDA) in heart and cardiac troponin I in blood among control, low dose, and high dose groups of rats

variable	Groups			Test of significance	P value	Pair-wise comparison
	Control group (n=10)	Low dose group (n=10)	High dose group (n=10)			
MDA in heart(nmol-mg) Mean ± SD Median(min-max)	0.64±0.03 0.66(0.59-0.68)	1.23±0.16 1.28(0.96-1.40)	2.13±0.38 2.19(1.69 -2.59)	**K**=25.13	<0.001**	p<0.001**1 p<0.001**2 p<0.001**3
CTN (Pg-ml) Mean ± SD Median(min-max)	103.48±1.78 102.89(101.45-105.52)	260.38±25.74 256.65(229.81-301.10)	530.93±94.19 555(420.87-659.25)	**K**=27.41	<0.001**	P<0.001**1 p<0.001**2 p<0.001**3

SD: standard deviation, Min: Minimum, Max: Maximum, K: Kruskal-wallis, **: Highly significant at P<0.01, MDA: Malondialdehyde, CTN I: cardiac troponin I

Pair-wise comparison: P value of Post hoc LSD test, P_1: comparison between control group versus low-dose group, p_2: control group versus high dose group, p_3: low dose group versus high dose group *: significant at P<0.05, **: Highly significant at P<0.01.

Table 3: Spearman correlation between MDA in testis and hormones in blood in each group of the three studied groups

Variable	MDA in testis					
	Control group (n=10)		Low dose group (n=10)		High dose group (n=10)	
	R	P value	R	P value	R	P value
Follicular stimulating hormone (FSH)	- 0.100	0.783	**- 0.855**	**0.002****	**- 0.900**	**<0.001****
Luteinizing hormone (LH)	0.200#	0.580	**- 0.826#**	**0.003****	**-0.841**	**0.002****
Testosterone level	- 0.359	0.308	**- 0.700**	**0.024***	**0.850**	**0.002****

R: Spearman's rho correlation coefficient, #: Pearson`s correlation coefficient, *: Significant at p<0.05, **: highly significant at p<0.01, MDA: Malondialdehyde

Table 4: Spearman correlation between MDA in heart and cardiac troponin I in each group of the three studied groups

Variable	MDA in heart					
	Control group (n=10)		Low dose group (n=10)		High dose group (n=10)	
	R	P value	R	P value	R	P value
cardiac troponin I	0.300	0.400	**0.800**	**0.005****	**0.950**	**<0.001****

R: Spearman's rho correlation coefficient *: Significant at p<0.05, **: highly significant at p<0.01, MDA: Malondialdehyde

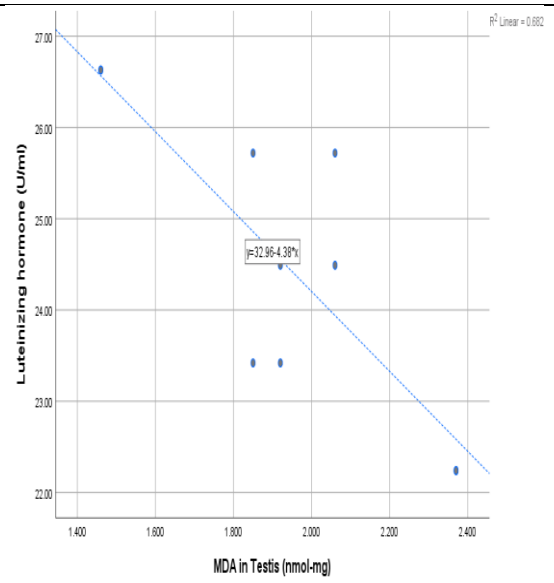

Figure (1): Scattered diagram showing correlation between MDA and LH hormone level in testis the low dose group.

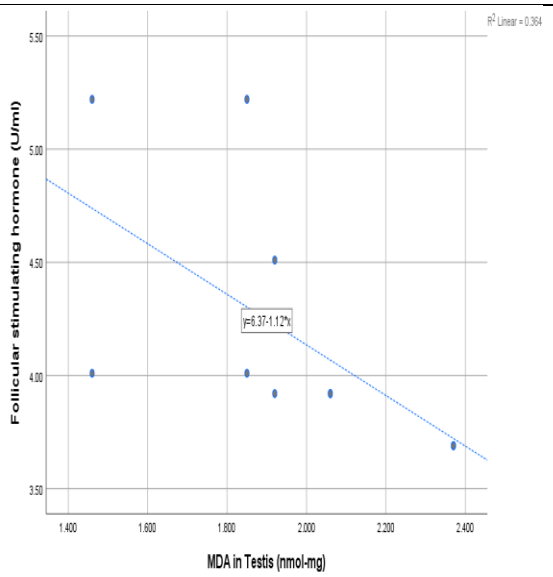

Figure (2): Scattered diagram showing correlation between MDA and FSH hormone level in testis the low dose group.

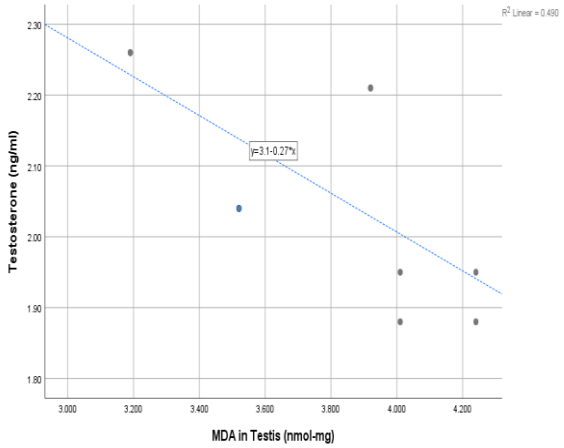

Figure (3): Scattered diagram showing correlation between MDA and LH level in testis the high dose group.

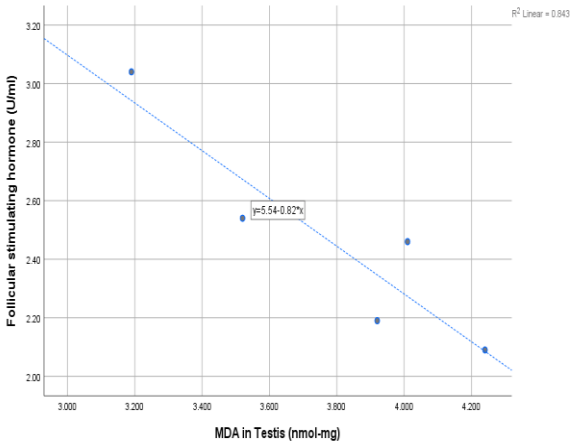

Figure (4): Scattered diagram showing correlation between MDA and FSH level in testis the high dose group.

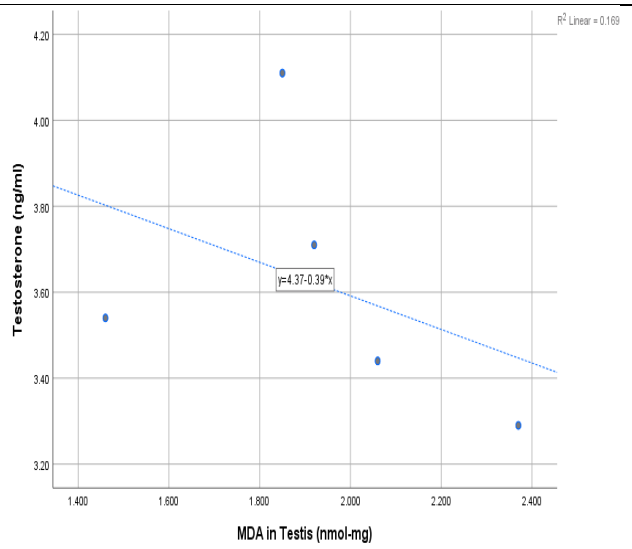

Figure (5): Scattered diagram showing correlation between MDA and testosterone hormone level in testis the low dose group.

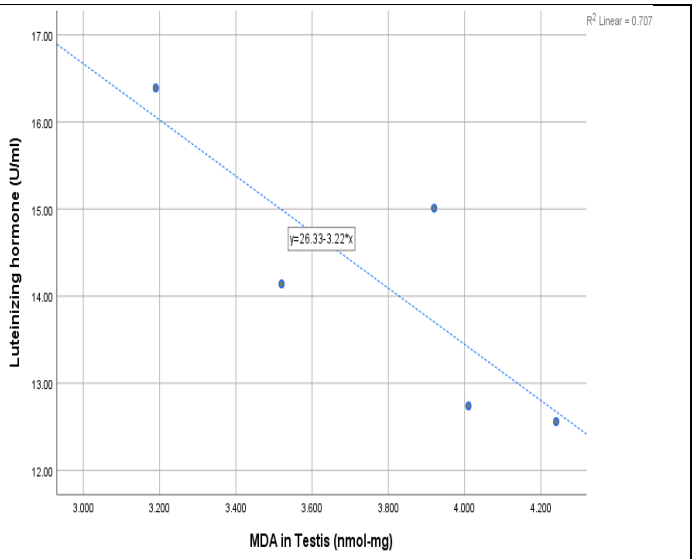

Figure (6): Scattered diagram showing correlation between MDA and testosterone hormone level in testis the high dose group.

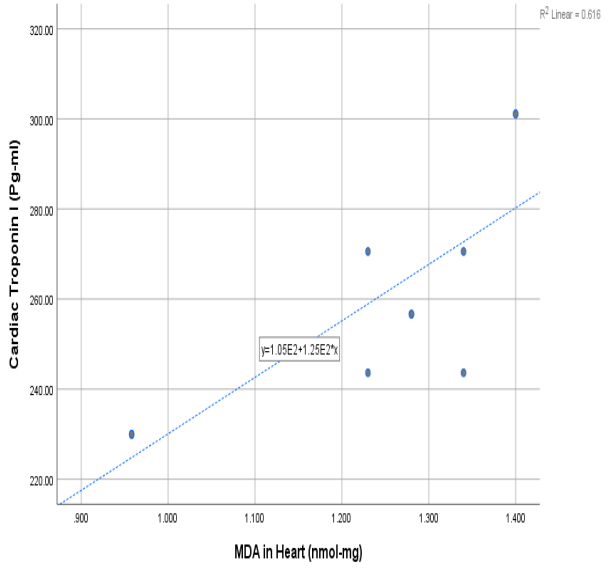

Figure (7): Scattered diagram showing correlation between MDA and Cardiac Troponin I level in heart in the low dose group.

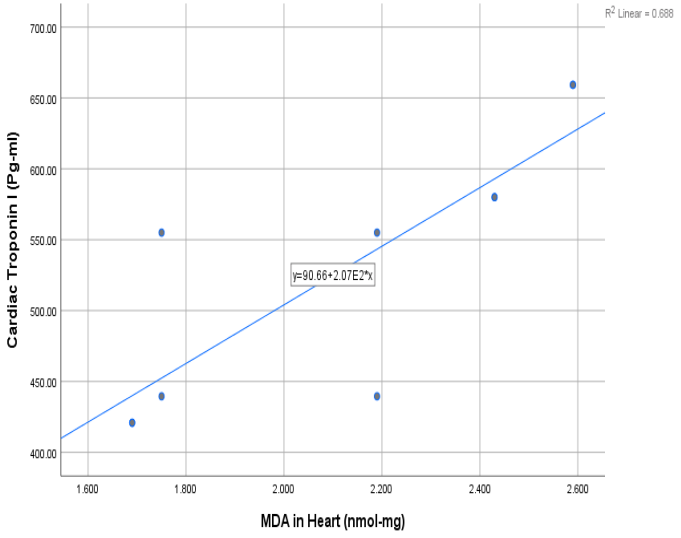

Figure (8): Scattered diagram showing correlation between MDA and Cardiac Troponin I level in heart in the high dose group.

Histopathological study (light microscopic examination):

1-Testis:

The examination of the testis by H&E-stained sections examined under the power of 100 showed that rats given the low e-cigarette dose, testicular tissue displayed localized atrophy in certain seminiferous tubules, with the inter-tubular parenchyma destroyed alongside it **(Fig. 9).** Additionally, diffuse congestion of testicular and scrotal blood vessels was observed in some sections, while the parenchyma and spermatogenesis stayed intact in other areas **(Fig. 10).**

In the high-dose e-cigarette group, the testicular changes were markedly more severe. Severe diffuse congestion of scrotal blood vessels was observed **(Fig. 11),** along with severe diffuse congestion of testicular blood vessels, destruction of the inter-tubular space by edema, and disruption of spermatogenesis **(Fig. 12).** Some sections revealed severe congestion with complete focal destruction of the testicular parenchyma **(Fig. 13),** while others showed hyperplasia of spermatogenesis cells, vascular congestion, and subepithelial edema **(Fig. 14).** in addition to hyperplasia of spermatogonia cells, vacuolation of epithelial cells, and inter-tubular destruction **(Fig. 15).** Furthermore, severe diffuse congestion of testicular blood vessels with focal partial destruction of the testicular parenchyma was also noted **(Fig. 16).**

2-Heart:

The examination of the testis by H&E-stained sections examined under the power of 100 showed that the low-dose e-cigarette group, the myocardial tissue showed inflammatory cell and fibroblast infiltration in addition to severe focal hyalinization of myocardial muscle fibers with slight abnormal myocardial arrangement **(Fig. 17).** while local marginal hyalinization was noted in other areas **(Fig. 18).**

In the high-dose e-cigarette group, the cardiac changes were considerably more pronounced. Focal myomalacia accompanied by severe diffuse congestion of myocardial blood vessels was observed **(Fig. 19).** Severe diffuse vascular congestion was also evident in sections that otherwise showed apparently preserved myocardial architecture **(Fig. 20).**

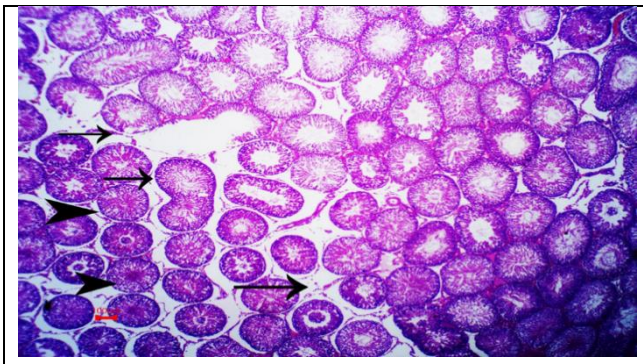

Fig.(9): Photomicrograph of H&E testis of rat treated with low dose of e-cig showing local atrophy of some seminiferous tubules **(arrows head)** with inter tubular parenchymal destruction **(arrows) (scale bar = 100 µm)**

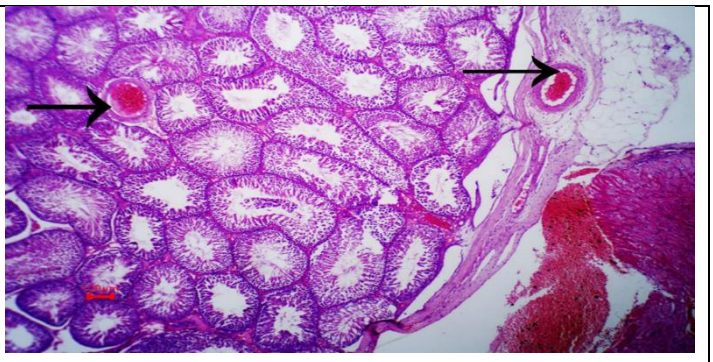

Fig.(10): Photomicrograph of H&E testis of rat treated with low dose of e-cig showing diffuse congestion of testicular and scrotal blood vessels **(arrows)** with normal parenchyma and full spermatogenesis **(scale bar = 100 µm)**

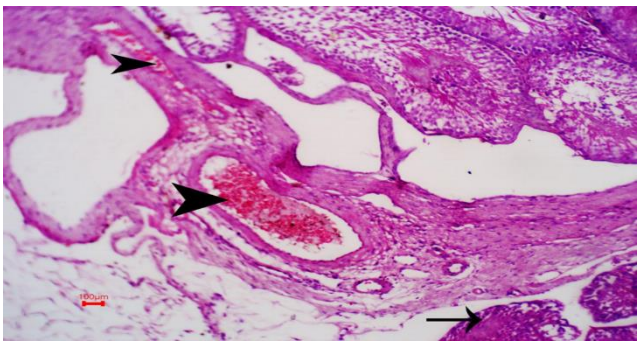

Fig.(11): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing severe diffuse congestion of scrotal blood vessels **(arrows head) (scale bar = 100 µm)**

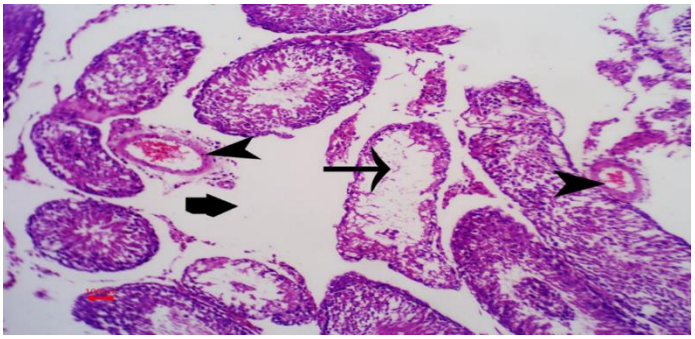

Fig.(12): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing severe diffuse congestion of testicular blood vessels **(arrows head)** with destruction of inter tubular space by edema **(thick arrow)** and destruction of spermatogenesis **(arrow) (scale bar = 100 µm)**

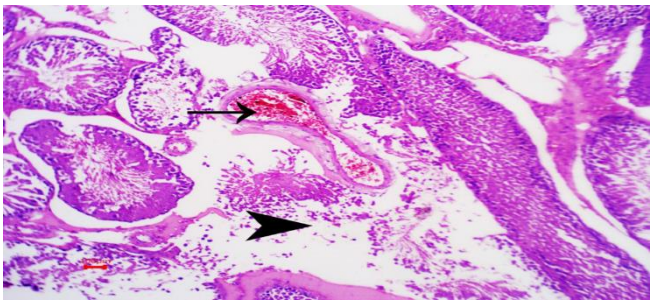

Fig.(13): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing severe diffuse congestion **(arrow)** with complete focal destruction of parenchyma **(arrow head) (scale bar = 100 µm)**

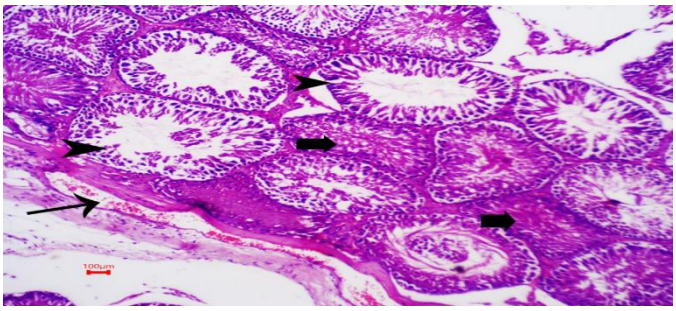

Fig.(14): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing hyperplasia of spermatogenesis cells (thick arrows) with vascular congestion **(arrow)** and subepithelial edema **(arrows head) (scale bar = 100 µm)**

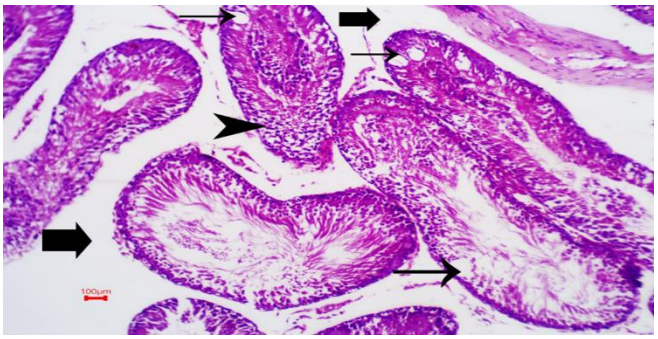

Fig.(15): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing hyperplasia of spermatogonia cells **(arrow head)** with vacuolation of few epithelial cells **(arrows)** andinter tubular destruction **(thick arrows) (scale bar = 100 µm)**

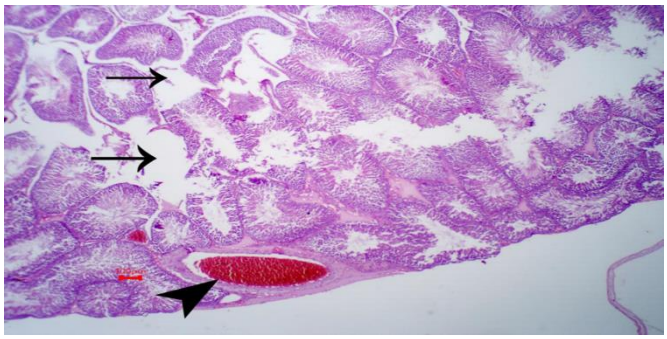

Fig.(16): Photomicrograph of H&E testis of rat treated with high dose of e-cig showing severe diffuse congestion of testicular blood vessels **(arrow head)** in addition to focal partial destruction of testicular parenchyma **(arrows) (scale bar = 100 µm)**

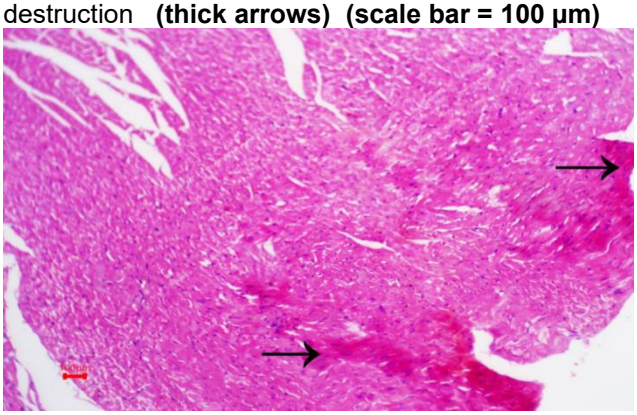

Fig.(17): Photomicrograph of H&E heart of rat treated with low dose of e-cig showing severe focal hyalinization of some myocardial muscle fibers **(arrows)** in addition to slight abnormal myocardial arrangement **(scale bar = 100 µm)**

Fig.(18): Photomicrograph of H&E heart of rat treated with low dose of e-cig showing local marginal hyalinization **(arrow) (scale bar = 100 µm)**

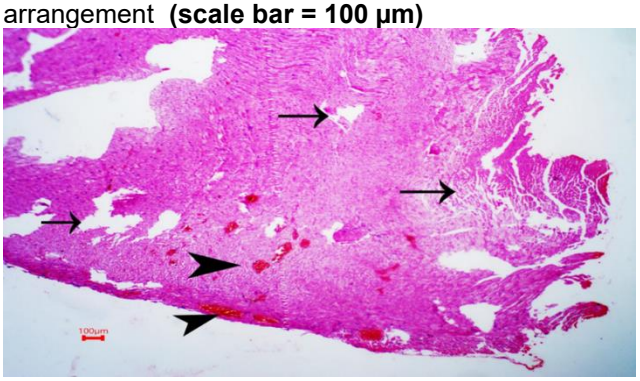

Fig.(19): Photomicrograph of H&E heart of rat treated with high dose of e-cig showing focal myomalacia **(arrows)** with severe diffuse congestion of myocardial blood vessels **(arrows head) (scale bar = 100 µm)**

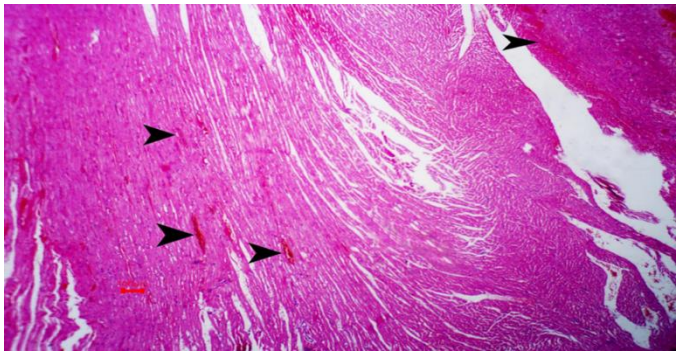

Fig.(20): Photomicrograph of H&E heart of rat treated with high dose of e-cig showing severe diffuse congestion of myocardial blood vessels **(arrows head)** with apparently normal myocardial architecture and arrangement **(scale bar = 100 µm)**

Discussion

This work examined how e-cigarette exposure affects testicular and cardiac tissue in adult albino male rats. Its findings showed a significant dose-dependent fall in the reproductive hormones (testosterone, LH and FSH), a steady climb in the oxidative stress marker (MDA) across both testicular and cardiac tissue, a pronounced climb of cardiac troponin I (CTnI) signalling myocardial injury, and matching histopathological changes in the two organs.

Here, FSH and LH dropped significantly among the vapor-exposed animals against the control group, the sharpest decline falling within the high-dose group. Such a result aligns with ***Omolaoye et al. (2022), and Sripratiwi (2019),*** who described nicotine as suppressing FSH and LH secretion by disrupting the pulsatile release of GnRH within the hypothalamus. This agreement may be mechanistically explained by the binding of nicotine to nicotinic acetylcholine receptors (nAChRs) in the hypothalamus, which alters GnRH secretion patterns and consequently reduces FSH output from the anterior pituitary, ultimately impairing spermatogenesis.

Testosterone in the present analysis also declined significantly, the most marked reduction arising within the high-dose group rather than the control group. ***El Golli et al. (2016)*** likewise recorded a comparable significant fall in serum testosterone in rats given e-cigarette refill liquid. These effects appear to result from a dual mechanism: first, the upstream suppression of LH secretion, which deprives Leydig cells of their principal stimulatory signal; and second, the direct testicular toxicity of nicotine, whereby it increases the expression of pro-apoptotic proteins p53 and caspase-3 while suppressing the anti-apoptotic bcl-2, leading to Leydig cell dysfunction and reduced steroidogenic capacity ***(Mosadegh et al., 2017).***

By contrast, ***George et al. (2019)*** detected no significant short-term harm to testosterone in subjects transitioning from traditional cigarettes to e-cigarettes. This discrepancy is most likely attributable to the acute nature of their human study versus the chronic animal exposure model employed in the present study, which allowed for sustained nicotine-induced hormonal disruption to become apparent.

Osadchuk et al., (2023) also found no significant differences in serum levels of FSH, LH, or testosterone between smokers and non-smokers in a large population-based study. This discrepancy may be attributed to the differences in study design, as their cross-sectional human study did not control for nicotine dose or exposure duration.

In the present study, MDA levels in testicular and cardiac tissues were significantly increased in the e-cigarette-exposed groups compared to the control group, with the greatest elevation observed in the high-dose group. These findings agree with***, Fajariyah et al. (2021), Saygın et al. (2023) and Shi et al. (2025).*** These effects may be attributed to nicotine's induction of reactive oxygen species (ROS) generation in vascular endothelial cells, cardiomyocytes and testis, leading to lipid peroxidation of cardiac membrane phospholipids, spermatozoa membrane and MDA accumulation.

In the present study, cardiac troponin I (CTnI) levels were significantly increased in the e-cigarette-exposed groups compared to the control group, with the greatest elevation observed in the high-dose group. These findings are consistent with ***Tsai et al. (2020***), who reported that chronic inhalation of e-cigarette aerosol chemicals significantly increases the risk of myocardial infarction. ***Siddiqi et al. (2023)*** also demonstrated that e-cigarette usage is associated with vascular impairment and cardiac injury markers in both humans and animals.

Under the microscope, testicular tissue in this study displayed focal atrophy of

seminiferous tubules, destruction of the inter-tubular parenchyma and vascular congestion throughout the low-dose group, whereas the high-dose group showed far more severe change. Such observations agree with ***Iring (2023),*** who reported similar dose-dependent testicular histopathological damage following e-cigarette exposure.

In the heart, the present study noted infiltration by inflammatory cells and fibroblasts, focal hyalinization of myocardial fibers and severe focal vascular congestion in the low-dose group, while the high-dose group revealed more marked features such as focal myomalacia, severe diffuse vascular congestion and pronounced interfibral edema. These observations are consistent with ***Tsai et al. (2020) and Shi et al. (2025),*** who described similar myocardial histopathological changes after chronic e-cigarette exposure in rats.

These structural changes may be explained by nicotine-induced elevation of ROS, which damages the blood-testis barrier and promotes apoptosis of spermatogenic cells through activation of p53 and caspase-3 pathways. Also promote inflammatory cell infiltration, collagen deposition by fibroblasts, and hyalinization of myocardial fibers.

On the other hand, ***Putra (2014)*** attributed testicular tubular changes primarily to decreased sperm production rather than direct nicotine cytotoxicity. This discrepancy may reflect a difference in perspective rather than a true contradiction, as decreased spermatogenesis in the present study is itself a downstream consequence of the oxidative and apoptotic damage to the germinal epithelium induced by nicotine exposure.

In contrast, ***George et al. (2019)*** found no significant immediate negative effects on ventricular musculature in subjects switching from traditional cigarettes to e-cigarettes. This discrepancy is likely explained by the acute duration of their human study, which was insufficient to produce the cumulative structural myocardial damage that develops progressively with chronic nicotine exposure as demonstrated in the present chronic animal model.

Conclusions:

Sustained breathing of e-cigarette vapor brings about significant, dose-dependent harm to the testicular and cardiac tissues of adult albino male rats.

Study limitations:

A four-week exposure window might not capture the full extent of the long-term harms that habitual vaping produces in people.

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