Original Article

The Possible Effect of β -carotene on Nicotine Withdrawal in Testicular Tissue of Adult Male Albino Rats. Histological and Immunohistochemical Study

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ABSTRACT

Background and Objectives: Nicotine in cigarette smoking possesses damaging effect on testis. This study was designed to evaluate and compare the prophylactic and therapeutic effects of β carotene on nicotine induced testicular damage during nicotine withdrawal.

Materials and Methods: Thirty-nine adult male albino rats were used, 9 rats were control (GI), the remaining rats divided equally into 5 groups: GII (β carotene), GIII (Nicotine), GIV (Nicotine withdrawal), GV (Nicotine withdrawal therapeutic), GVI (Nicotine withdrawal prophylactic group). β carotene (10mg/kg/day) was injected intraperitoneally for 4 weeks. Nicotine (4 mg/kg/day) was injected intraperitoneally for 4 weeks. Before sacrifaction, blood samples were drawn to assess serum levels of malondialdehyde (MDA) and testosterone. After sacrifaction, testicular sections were stained with H&E, Masson trichrome (to reveal collagen fiber deposition and distribution) and immunohistochemically for Vimentin (Sertoli cell intermediate filament(. Morphometric measurements were done by computerized image analysis.

Results: Groups I and II showed similar results with normal testicular histological architecture, biochemical and morphometrical results. Deterioration of histological architecture, biochemical and morphometric parameters were recorded in groups III, IV and V (mostly GIII) with significant decrease in diameter of seminiferous tubules, height of spermatogenic epithelium and area percent of vimentin reaction, significant increase in area percent of collagen fibres deposition compared to GI. GVI showed better results than GIII, GIV and GV with restoration of normal testicular histological structure.

Conclusion: β carotene could help in improving spermatogenesis. Administration of β carotene prophylactically during nicotine exposure and withdrawal gives better histological, biochemical and morphometrical results than therapeutic administration during nicotine withdrawal period only.

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Key Words: β carotene, nicotine, smoking, testis, vimentin.

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INTRODUCTION

Among teenager and young adults, cigarette smoking is a highly addictive personal habit. Tobacco smoke is formed of more than 4000 components as oxidants, aldehydes and carcinogens causing inflammation and injury to the cells[1]. Nicotine is one of the most abundant organic particles in cigarette smoke[2]. The addictiveness of nicotine is mediated by acetylcholine receptors in the central nervous system[3]. The rise in drug abuse and smoking had made negative effects on the reproductive system and glands beside its known detrimental effects on the vital organs such as the heart and brain[4]. Increased reactive oxygen species production had linked to deleterious toxic effects of nicotine[5]. Testes are the most male reproductive system vital organs with sensitive cellular composition and high rate of mitotic activity[6]. Long-term exposure to cigarette smoke suppresses spermatogenesis, thus leading to male infertility[7]. Carotenoids are naturally occurring lipid-soluble compounds with strong antioxidant effects[8]. β Carotene is the most prominent carotenoid can improve male fertility through capturing and neutralization of reactive oxygen radicals[9].

MATERIAL AND METHODS

A- Material

Drugs

Nicotine (S)-3-(1-Methyl-2pyrroli-dinyl) pyridine powder, diluted in saline to be injected intraperitoneally.

β carotene (Provitamin A) Synthetic powder >93 %, diluted in saline and injected intraperitoneally. Both were purchased from Sigma-Aldrich chemical company (Cairo, Egypt)

Animals

Thirty-nine male adult rats, mean body weight of 150-200 gm, were bred in Kasr Al-Ainy Animal House, according to guidelines of Cairo University animal ethical committee and institutional animal care and use committee (IACUC). They were kept in sanitary cages under constant day/night cycle, feeding on standard rat diet and water.

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The rats were divided into the following groups:

Group I (Control): (n=9) divided to

- Subgroup Ia: 3 rats injected with saline intraperitoneally daily for 4 weeks then sacrificed after last day of injection.
- Subgroup Ib:3 rats injected with saline intraperitoneally daily for 4 weeks then left for another 8 weeks then sacrificed.
- Subgroup Ic: 3 rats injected with saline intraperitoneally for 8 weeks then sacrificed after last day of injection.

Group II (β carotene): 6 rats were injected intraperitoneally with β carotene (10mg/kg/day) for 4 weeks[10] and sacrificed after the last dose.

Group III (Nicotine): 6 rats were injected intraperitoneally with nicotine (4 mg/kg/day) for 4 weeks[11] and sacrificed after the last dose.

Group IV (Nicotine withdrawal): 6 rats were injected intraperitoneally with nicotine (4 mg/kg/day) for 4 weeks as group III, then left for another 8 weeks then sacrificed.

Group V (Nicotine withdrawal therapeutic group): 6 rats were injected intraperitoneally with nicotine as group III for 4 weeks then given β carotene as group II for next 4 weeks then were sacrificed.

Group VI (Nicotine withdrawal prophylactic group): 6 rats were injected intraperitoneally with β carotene (10 mg /kg/day) and nicotine (4 mg/kg/day) simultaneously from the beginning then stopped nicotine after 4 weeks and continued β carotene for next 4 weeks then were sacrificed.

B- Methods

I-Laboratory investigations

At the end of the study, blood samples were collected from tail veins of the rats to assess serum level of malondialdehyde (MDA) and testosterone. Both were measured in Biochemistry Department, Faculty of Medicine, Cairo University.

II- Histological Study

The rats were sacrificed after intraperitoneal injection of 60 mg/Kg phenobarbital[12], a midline incision was done with careful dissection and weighing of the left testis from each rat. Testis to body weight ratio was calculated for each rat. Specimens from the testis were fixed in Bouin's fixative, dehydrated in ascending grades of ethanol and embedded in paraffin blocks then processed for paraffin sections of 5 micrometers thickness. (Sections mounted on Canada balsam coated slides in case of ordinary stains and charged poly-L-lysine coated slides in case of immunostaining). Sections were subjected to:

- Hematoxylin and Eosin (H&E)[13].
- Masson's Trichrome stain[14].
- Immunohistochemical staining for Anti Vimentin antibody[15]: Sections were deparaffinized, rehydrated, incubated with 3% hydrogen peroxide for 10 minutes to inhibit endogenous peroxidase activity, then incubated at room temperature for one hour with 2 drops (=100 µl) of the primary antibody (mouse monoclonal antivimentin Ab, IgG1 isotype) (J144), cat#MA3-745., Thermoscientific (USA). Histostain SP kit detection system (LAB-SA system, Zymed Laboratories Inc, San Francisco, CA 94080, USA, cat no 95-9643) was used to complete Immunostaining. For counter staining, Mayer's hematoxylin (cat no TA- 060- MH) was used.

Morphometric study

Ten non-overlapping fields of each group were used to measure

- Height of spermatogenic epithelium (x100).
- Diameter of the seminiferous tubules (x100).
- Area percent of collagen fibers (x 200).
- Area percent of vimentin immunopositive cells (x200).

Measurements were carried out at Histology Department, Faculty of Medicine, Cairo University using Leica Qwin-500 LTD-software image analysis computer system Ltd. (Cambridge, England).

Statistical analysis

Data were statistically analyzed using "IBM SPSS statistics v.21" through One-way analysis of variance (ANOVA) followed by "Tuckey" post-hoc test (*P value*<0.05 was considered significant). Data were presented as mean ±standard deviation (SD)[16].

RESULTS

There was no reported mortality.

Testis to body weight ratio results (Table 1)

There was non-significant difference in the mean value of testis to body weight ratio in GII and GVI when compared to (GI). This value showed a significant decrease in groups GIII, GIV and GV in comparison with all other studied groups.

Biochemical results (Table 2)

There was non-significant difference in mean serum MDA level in GII and GVI in comparison with GI, while this value showed significant increase in groups GIII, IV and V in comparison with all other studied groups with significant decrease in GV when compared to Groups III and IV. Concerning mean value of serum testosterone level, there was non- significant difference in GII, GV and GVI in comparison with GI while this value showed significant decrease in GIII and GIV in comparison with all other groups. There was non-significant difference between groups GIII and GIV.

Histological and immunohistochemical results

Hematoxylin and Eosin stained sections

Histological examination of testicular sections of both GI (all control subgroups) and GII rats revealed normal architecture of seminiferous tubules (STs), lined by several layers of spermatogenic cells and separated by interstitial tissue containing Leydig cells Each tubule was surrounded by thin basal lamina with flat myoid cells having flattened nuclei and was lined by seminiferous epithelium formed of Sertoli cells and spermatogenic cells demonstrating different stages of spermatogenesis, starting from the spermatogonia resting on regular basement membrane having small dark nuclei. Primary spermatocytes were inner to spermatogonia, with their relatively large size and large rounded characteristic nuclei with partially condensed chromosomes. Early spermatids appeared rounded with central rounded nuclei and prominent nucleoli. Late spermatids were seen near the lumen with dark blue heads and eosinophilic thread like flagella protruding into the lumen. Sperms were seen within the lumen of the tubules. Sertoli cells appeared with irregular poorly defined outlines among the spermatogenic cells. Their cytoplasm was pale acidophilic and contained triangular vesicular pale nuclei with prominent nucleoli. The interstitial tissue between the STs contained sparse connective tissue containing interstitial cells of Leydig that appeared as large polygonal cells with vesicular nuclei and prominent nucleoli and acidophilic cytoplasm (Figures 1.a,1.b). GIII showed loss of the normal architecture of the STs with thickened basement membranes and damaged spermatogenic epithelium. Degenerated spermatogenic cells possessed dark pyknotic nuclei and could not be distinguished from each other. The lumen was nearly devoid of sperms and the interstitial tissue showed clusters of interstitial Leydig cells with dark pyknotic nuclei, dilated congested blood vessels and acidophilic material (Figure 2.a). GIV showed distorted STs with disturbance of normal architecture lined with spermatogonia resting on the basement membrane and primary spermatocytes. The lumen of the tubules was nearly devoid of sperms. The interstitial tissue shows clusters of dark pyknotic interstitial cells of Leydig (Figure 2.b). GV revealed partial restoration of the normal architecture of STs. They were lined with multiple layers of spermatogenic cells. The lumen contained few sperms. The interstitial tissue contains interstitial Leydig cells with vesicular nuclei and prominent nucleoli and acidophilic cytoplasm (Figure 2.c). GVI illustrated normal appearance of the STs. They were surrounded by thin basal lamina with flat myoid cells and lined by seminiferous epithelium of Sertoli and several layers of all types of spermatogenic cells with their lumen full of sperms. STs were separated by interstitial tissue containing Leydig cells with their vesicular nuclei and prominent nucleoli and acidophilic cytoplasm (Figure 2.d).

Masson's Trichrome stain

Histological examination of trichrome stained rat testicular sections from GI and II showed minimal collagen deposition in between the tubules (Figures 3.a,3.b respectively). GIII and IV showed marked increase in collagen deposition (Figures 3.c,4.a respectively), GV showed moderate increase in collagen deposition (Figure 4.b), while GVI showed minimal collagen deposition in between the tubules (Figure 4.c).

Vimentin immunostained sections

Histological examination of vimentin immunostained rat testicular sections from GI and GII showed strong positive cytoplasmic immunoreaction in Sertoli cells extending to their apices (Figures 5.a ,5.b respectively) , GIII showed strong positive immunoreaction in the basal regions of Sertoli cells with no apical extensions (Figure 5.c). GIV showed strong positive cytoplasmic immunoreaction in the basal regions of Sertoli cells with weak positive immunoreaction in the apical extensions of few Sertoli cells (Figure 6.a), while GV & GVI showed strong positive cytoplasmic immunoreaction in the basal regions of many Sertoli cells as well as strong positive immunoreaction in few Sertoli cells apical extensions in GV (Figure 6.b) and many Sertoli cells apical extensions in GVI (Figure 6.c).

Morphometric Results (Figure 7)

There was a non-significant difference in the mean diameter of seminiferous tubules, mean height of spermatogenic epithelium, mean area percent of collagen fiber deposition and mean area percent of vimentin immunopositive reaction in GII and GVI in comparison with GI. All values showed a significant difference in groups III, IV and V in comparison with all other groups. However, the differences in the mean height of spermatogenic epithelium and mean area percent of collagen fiber deposition were significant in GV when compared with GIII and GIV. The mean area percent of vimentin immunopositive reaction was significant in GIV and GV when compared to GIII.

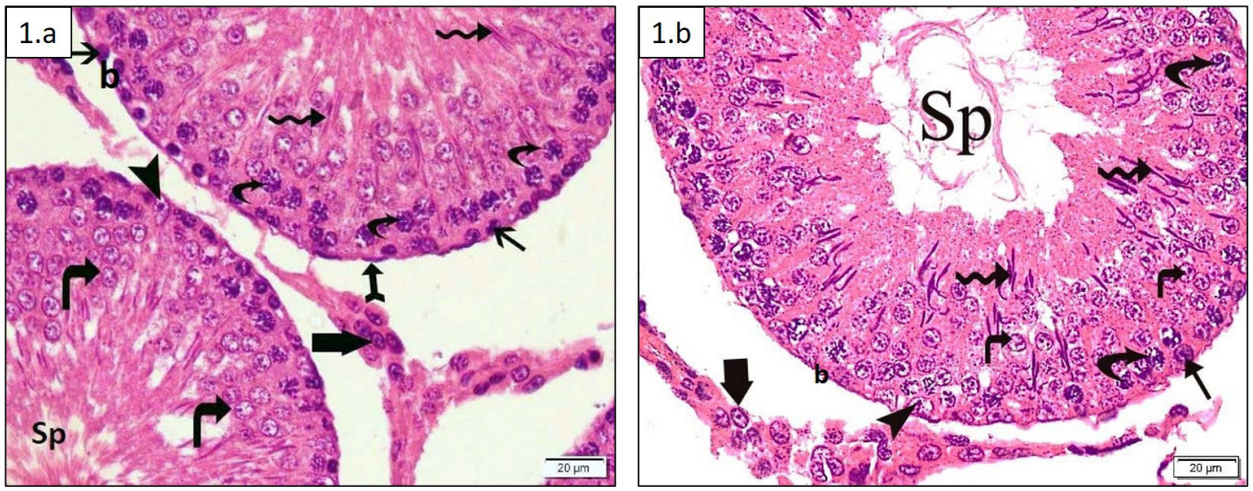

Fig. 1: photomicrograph of H&E stained testicular section: Fig1.a (GI x400) and Fig1.b (GII x400) showing seminiferous tubules with regular basement membrane (b), spermatogonia (thin arrows), primary spermatocytes (curved arrows) and early spermatids (right angled arrows). Note the presence of late spermatids (spiral arrows), Sperms (Sp) and Sertoli cell (arrowhead) with their triangular vesicular nucleus and ill- defined boundaries in between spermatogenic cells. Myoid (arrow with bisected tail) and interstitial cells of Leydig (Thick arrow) are also seen.

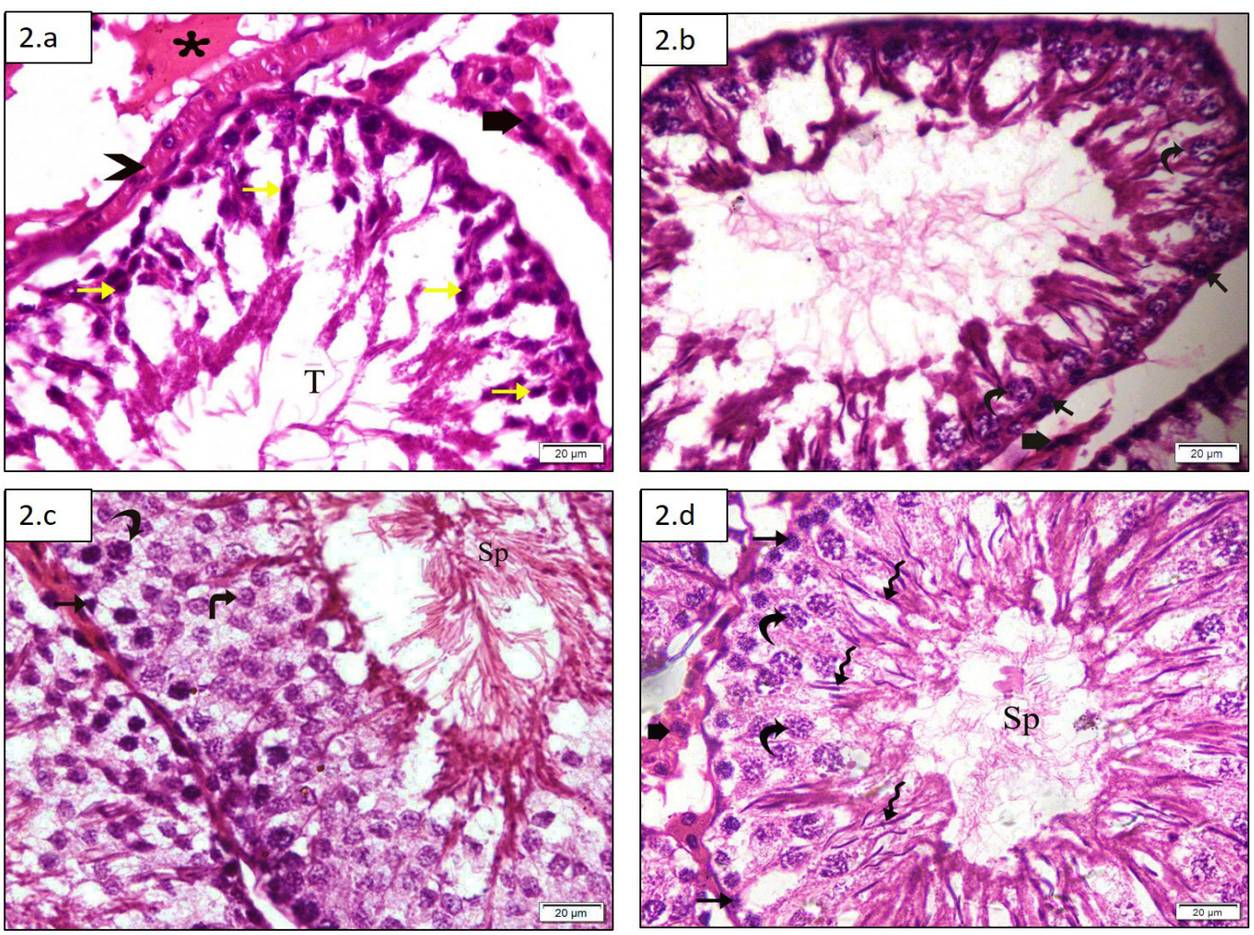

Fig. 2: photomicrograph of H&E stained testicular section :Fig 2.a (GIII X 400) showing part of a distorted seminiferous tubule (T), nearly devoid of sperms, lining spermatogenic epithelium with dark pyknotic nuclei (yellow arrows), clusters of dark pyknotic interstitial cells of Leydig (thick arrow), acidophilic material (star) and a congested blood vessel (arrowhead) in the interstitial tissue., Fig 2.b (GIV X 400) showing seminiferous tubule lined with spermatogonia (arrows) and primary spermatocytes (curved arrows). Dark pyknotic interstitial cells of Leydig (thick arrow) are seen in the interstitial tissue. Fig 2.c (GV X 400) showing adjacent parts of two seminiferous tubules lined with spermatogonia (arrow), primary Spermatocyte (curved arrow), early spermatids (right angle arrows) and sperms (Sp). Fig 2.d (GVI X 400) showing part of seminiferous tubule with normal architecture, lined with spermatogonia (arrows), primary spermatocytes (curved arrows), late spermatid (spiral arrow) and sperms (Sp). Note the presence of interstitial cells of Leydig (Thick arrow).

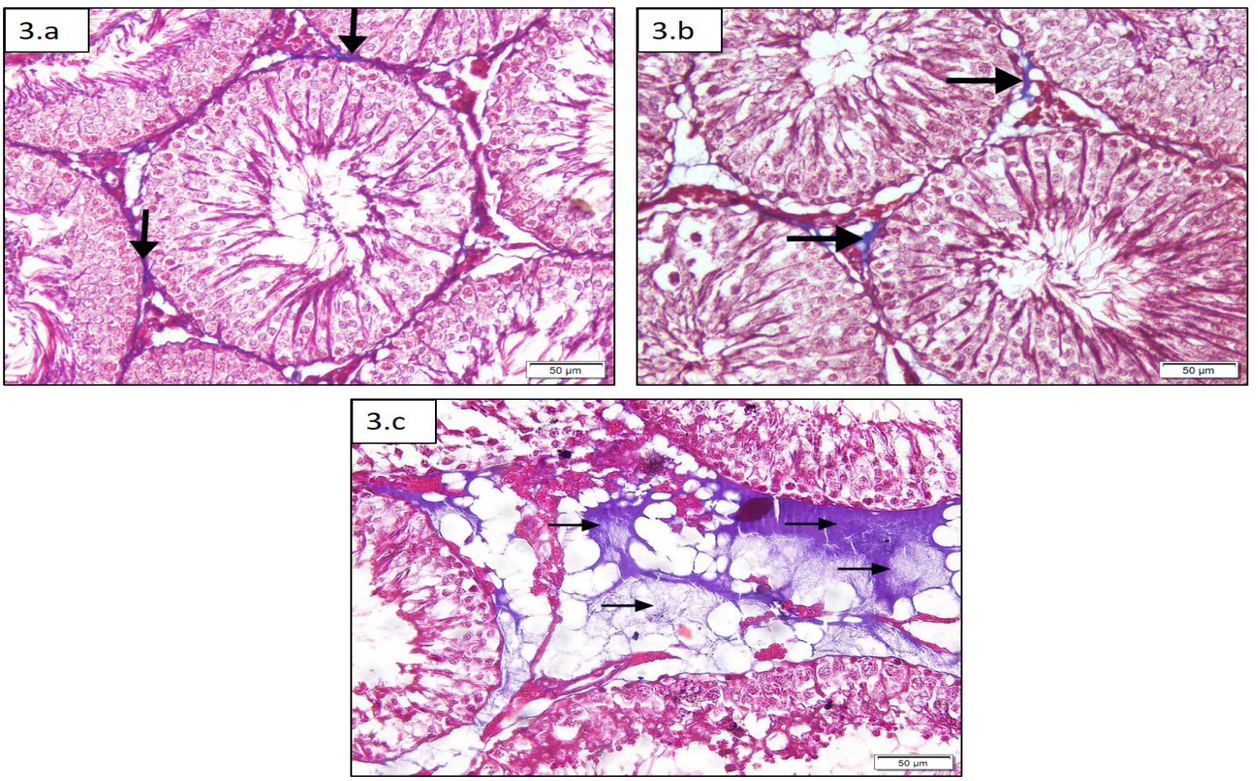

Fig. 3: photomicrograph of Masson's Trichrome stained testicular section: Fig 3.a (GI X200) & Fig 3.b (GII X200) showing seminiferous tubules with minimal collagen deposition in between the tubules (arrows)., Fig 3.c (GIII X200) showing marked increase in collagen deposition in between the tubules (arrows).

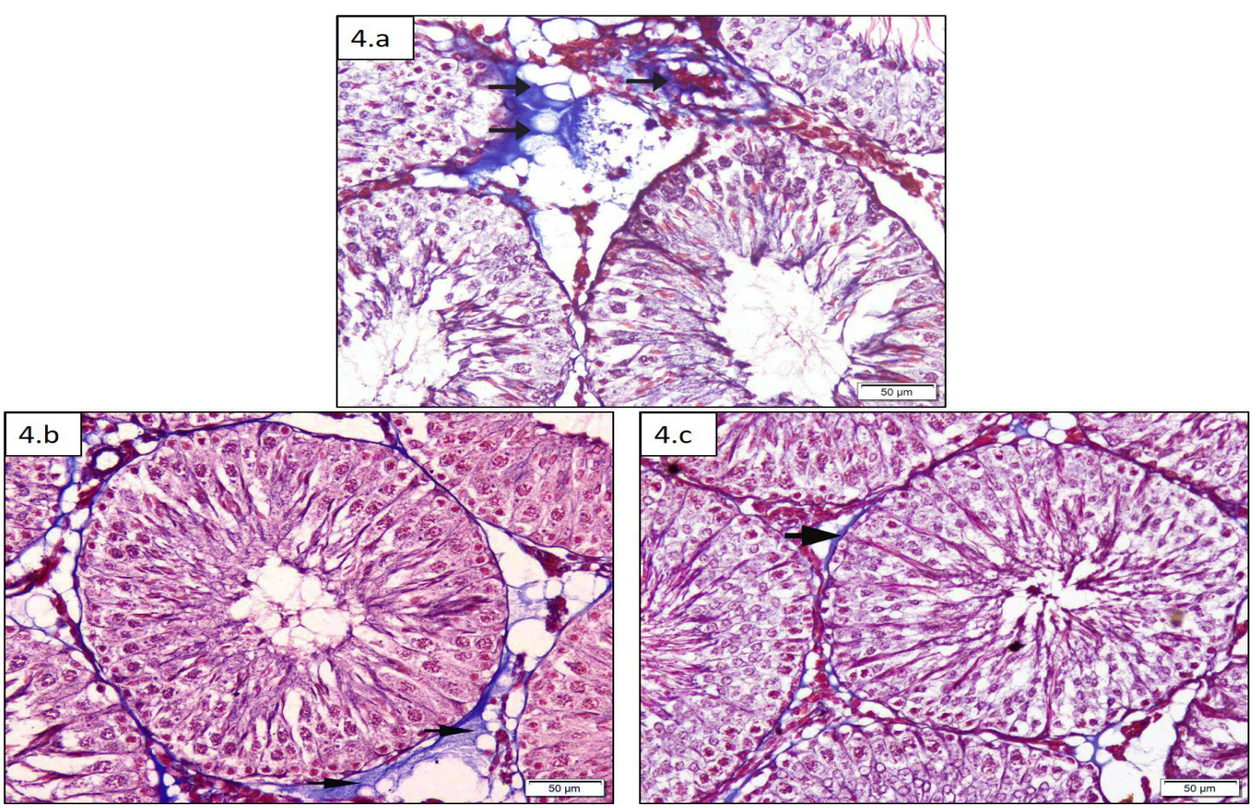

Fig. 4: photomicrograph of Masson's Trichrome stained testicular section: Fig 4.a (GIV X200) showing marked increase in collagen deposition in between the tubules (arrows)., Fig 4.b (GVX200) showing moderate increase in collagen deposition in between the tubules (arrows)., Fig 4.c (GVI X200) showing minimal collagen deposition in between the tubules (arrow).

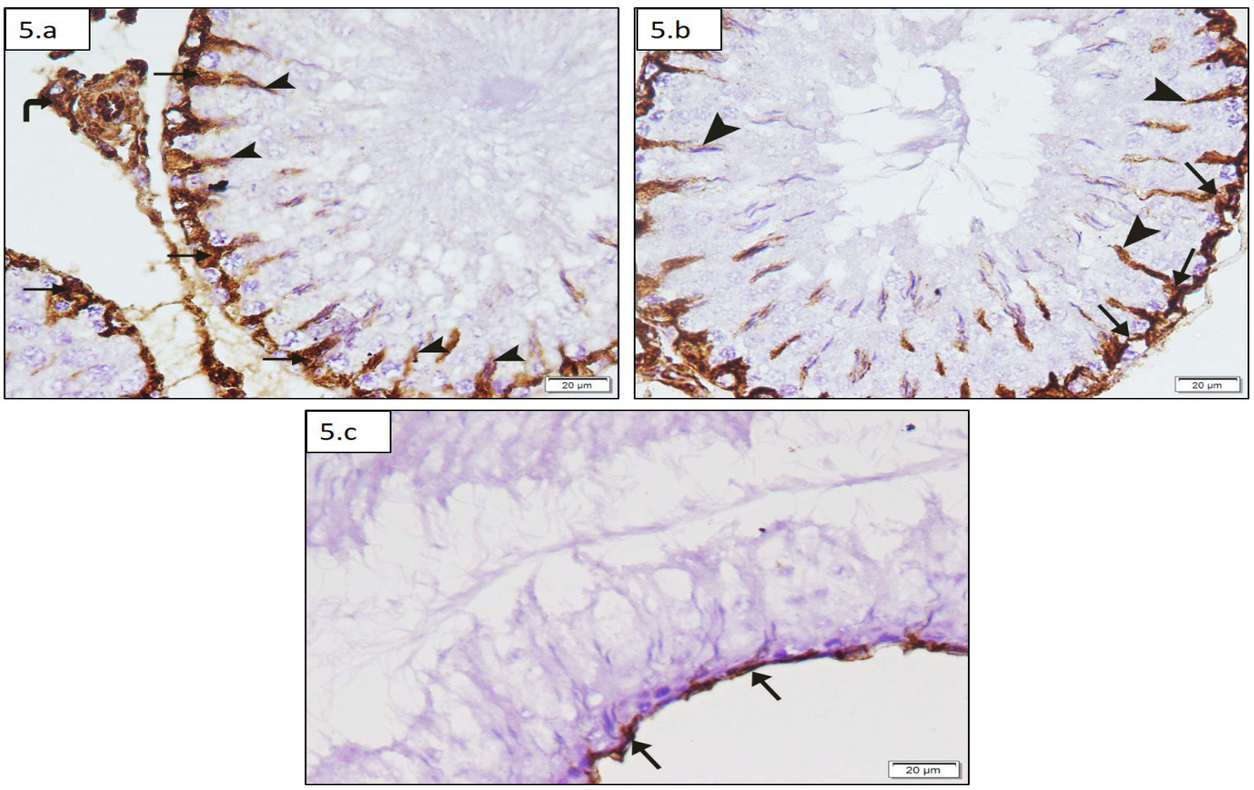

Fig. 5: photomicrograph of vimentin immunostained testicular section: Fig 5.a (GI X400) showing strong positive cytoplasmic immunoreaction in sertoli cells (arrows) with apical extensions (arrowheads) and positive immunoreaction in the interstitial tissue (right angle arrow)., Fig 5.b (GII X400) showing strong positive cytoplasmic immunoreaction in sertoli cells (arrows) extending to their apices (arrowheads).,Fig 5.c (GIII X400) showing strong positive immunoreaction in the basal region of sertoli cells (arrows) with no apical extensions.

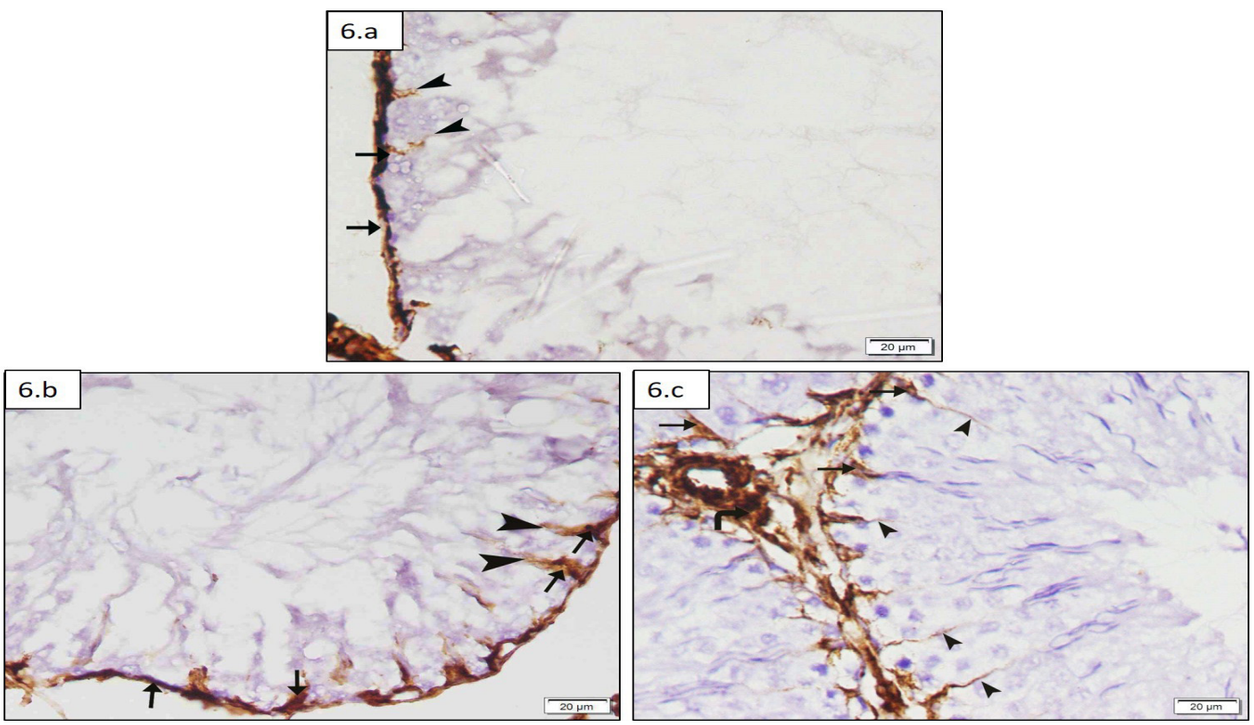

Fig. 6: photomicrograph of vimentin immunostained testicular section showing Fig 6.a (GIV X400) and Fig 6.b (GVX400) with strong positive immunoreaction in the basal region of sertoli cells (arrows) and weak positive immunoreaction in the apical extensions of few sertoli cells (arrowheads)., Fig 6.c (GVI X400) showing strong positive cytoplasmic immunoreaction in sertoli cells (arrows) with strong apical extensions in many sertoli cells (arrowheads). Note the presence of positive immunoreaction in the interstitial tissue (right angle arrow).

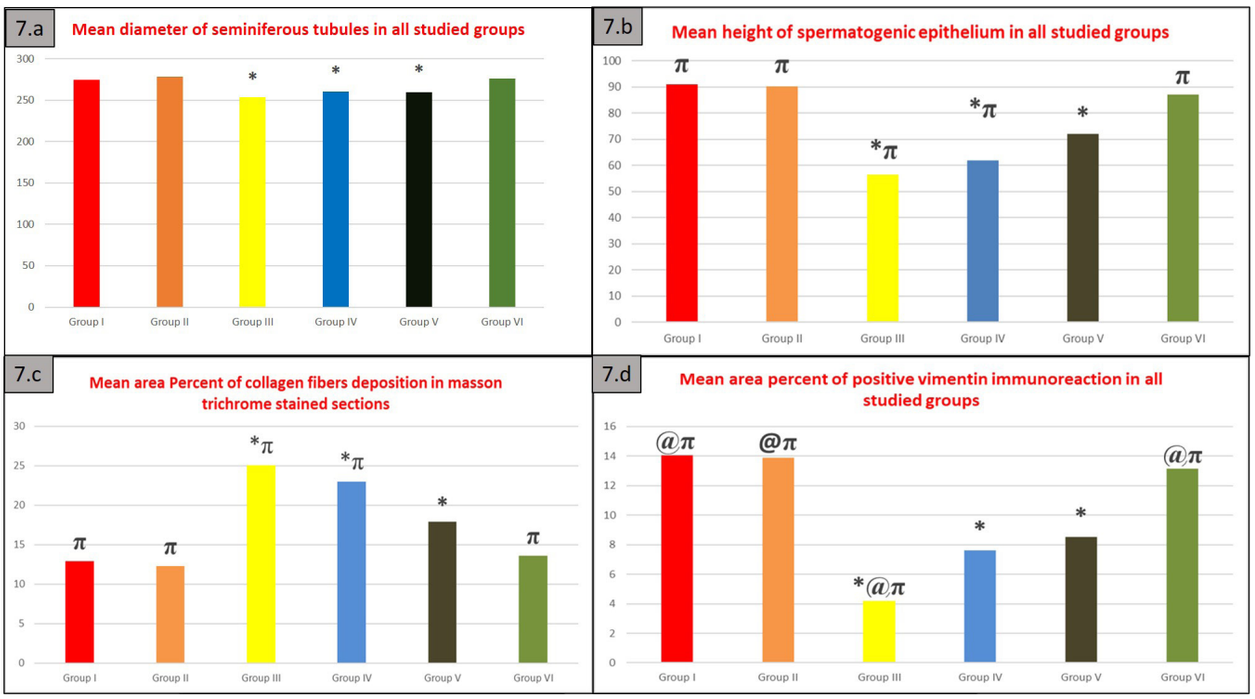

Fig. 7: a histogram showing mean values (± SD) of: Diameter of seminiferous tubules (Fig 7.a)., Height of spermatogenic epithelium (Fig 7.b)., Area percent of collagen fibers deposition (Fig 7.c) and mean area percent of vimentin immunopositive reaction (Fig 7.d) in all studied groups.
* Significant compared to group I, II and VI ($P < 0.05$).
@ Significant compared to IV ($P < 0.05$).
π Significant compared to V ($P < 0.05$).

Table 1: The Mean values of testis to body weight ratio (± SD) in all studied groups

Group	Mean testis to body weight ratio ± SD
GI	0.92 (±0.017)
GII	0.95 (±0.053)
GIII	0.47(±0.035)*
GIV	0.53(±0.13)*
GV	0.61(±0.087)*
GVI	0.89 (±0.074)

* Significant compared to group I, II and VI ($P < 0.05$).

Table 2: Mean value ± SD of biochemical parameters in all groups

Group	Mean Serum MDA level (nmol/ml) ± SD	Mean serum testosterone level (ng/ml) ± SD
GI	13.1(±0.12)•	1.3 (±0.18)
GII	12.9(±0.14)•	1.4 (±0.42)
GIII	38.7(±6.7)*•	0.53 (±0.25)*•
GIV	35.4(±3.6)*•	0.67 (±0.2)*•
GV	22.5(±4.9)*	1.08 (±0.13)
GVI	16.1(±0.38)•	1.12 (±0.33)

* Significant compared to group I, II and VI ($P < 0.05$).
● Significant compared to group V ($P < 0.05$).

DISCUSSION

About 6 million deaths and more than 600 000 negative smokers die each year from exposure to tobacco smoke[17,18]. Nicotine administration causes hypoxia that affects testis mainly owing to its high metabolic requirements for continuous spermatogenesis process[19]. Decrease in the spermatogenic cell layers of the seminiferous tubules as well as decrease in sperm count and motility was documented due to exposure to nicotine[20]. Carotenoids are naturally occurring lipid-soluble compounds, having strong antioxidant effects in humans and animals. One of these carotenoids is β carotene that has antioxidant properties through capturing and neutralizing reactive oxygen radicals, improving male fertility[8,21]. Based on the above-mentioned data, this study was designed to evaluate and compare the prophylactic and therapeutic effects of β carotene on nicotine induced testicular damage during nicotine withdrawal.

An experimental model of testicular damage due to nicotine exposure was developed in adult male albino rats. Rats were chosen since, rats have well-defined reproductive systems, the testis of rat more or less histologically similar to that of human and the compounds which can cause infertility in human males were also active in rats[22,23]. Testicular weight is considered an important parameter in the evaluation of sperms productivity due to high and positive correlation to sperm production[24]. Testis from each rat was weighted and the testis weight was divided by total body weight and multiplied by 100 to calculate testis to body weight ratio according to the formula described by Abd El-Aziz *et al*.,[25]. In our study, a significant decrease in this ratio was observed in groups in GIII, IV and V in comparison to all other groups. This could be explained by reduction in testosterone level due to nicotine effect on seminiferous tubules. Similar results were described by Budin *et al*., & Mosadeghet *et al*.,[7,26] who reported that promotion of spermatogenesis process needs physiologic interaction between testosterone and Sertoli cells. Therefore, decreased level of the testosterone after nicotine exposure leads to decreased tubular cellularity. Cellular depletion resulted in severe reduction in total testicular weight gain to total body weight. Although Fairuz *et al*.,[27] noticed no adverse effects of nicotine on testicular weight or testicular measurements in rats, this could be due to the small dosage of nicotine used in that study. Authors considered generation of reactive oxygen species (ROS) after nicotine one of the possible mechanisms that are negatively affecting the male reproductive functions[28].

Malondialdehyde (MDA) is considered as one of the most popular markers that determine oxidative stress[29]. This was proved in GIII, IV and V by the significant elevation in mean serum MDA level in comparison to all other studied groups (I and II and VI). The significant decrease in mean serum MDA value in combined β carotene and nicotine treated groups (GV and GVI) when compared to nicotine treated groups only (GIII and GIV) reflects the ameliorating effect of β carotene against the oxidative stress caused by nicotine exposure on testicular tissue especially when β carotene combined with withdrawal of nicotine. This was in agreement with Kini *et al*.,[30] who proved the protective role of β carotene against cadmium induced testicular damage and reported decrease in MDA level following β carotene administration for 30 days before cadmium exposure. Testosterone hormone is essential for male sexual differentiation, gamete production and maturation, spermatogenesis process and development of normal spermatogenic cells[31]. Regarding serum testosterone hormone level in our study control group, the values came in agreement with Orieke *et al*.,[32] where the normal serum testosterone level in adult male Wistar rats was (1.70±0.22 ng/ml) a result similar to that observed in our control group. On the other hand, there was a significant reduction in the mean testosterone hormone level in groups III and IV when compared to all other studied groups (I, II, V and VI). These results were consistent with many researchers who concluded that in nicotine exposed male albino rats, nicotine impaired testicular functions and suppressed testosterone hormone production[33-35]. The decrease in mean serum testosterone level observed in nicotine and withdrawal groups (GIII and GIV) could be explained by the disruption of testicular architecture affecting Leydig cells' number and function, as well as LH concentration. This came in agreement with many authors who stated that exposure to nicotine resulted in decreased reproductive hormones serum concentration through impairment of genes related to expression of steroidogenesis enzymes[25,36,37]. On the other hand, the mean serum testosterone level in withdrawal therapeutic and prophylactic groups (GV and GVI) demonstrated significant increase in comparison to (GIII and GIV) which reflects the protective effect of β carotene on nicotine induced testicular damage and Leydig cell functions, that agreed with a research reporting that β carotene pre-treatment leads to elevation of testosterone hormone level in titanium oxide nanoparticles-intoxicated mice[38]. In our study, normal architecture of the seminiferous tubules and intact stratified germinal epithelium were observed in β-carotene group (GII). Also there was no difference in the histological, biochemical and morphometric results among (β- carotene) and control groups. Histological examination of H&E stained testicular sections of rats from nicotine group (GIII) showed loss of the normal architecture of the seminiferous tubules with thickened basement membrane that might be due to active formation of fibrous tissue by the fibroblasts. This came in agreement with some authors who reported significant irregularity and thickening of the basal lamina of the seminiferous tubules in rat testis after daily tobacco smoke exposure[39,40]. Most spermatogenic cells possessed dark and pyknotic nuclei. The lumen was nearly devoid of sperms. The interstitial tissue showed clusters of interstitial Leydig cells with dark pyknotic nuclei, congested dilated blood vessels and acidophilic material that may be due to increase in the vascular permeability as postulated by Agarwal *et al*.,[41]. These findings were supported morphometrically in our study

by significant decrease in mean diameter of seminiferous tubules and mean height of the lining spermatogenic epithelium in GIII in comparison with control group. Similar findings were reported by some authors[7,42]. Our results were in agreement with authors who reported degenerative changes in seminiferous tubules in the form of disturbed architecture, detached seminiferous germinal epithelium from the basement membrane and sloughed degenerated cells in the lumens of seminiferous tubules after exposure to nicotine with different doses and duration[11]. Chronic cigarette smoke can induce apoptosis in rat testis with reduction in the number of germ cells, Leydig cells and Sertoli cells[43,44]. In our study, H&E stained testicular sections of rats from Nicotine withdrawal group (GIV) revealed distortion in the seminiferous tubules with disturbance of normal architecture, the tubules were lined mostly with spermatogonia and primary spermatocytes suggesting spermatogenic arrest at the primary spermatocyte stage. The lumen of the tubules was nearly devoid of sperms. The interstitial tissue showed clusters of dark pyknotic interstitial cells of Leydig. On the contrary, histological examination of testicular sections of withdrawal therapeutic group (GV) the tubules partially restored their normal architecture, the tubules were lined with multiple layers of spermatogenic cells starting from spermatogonia till late spermatids, Interstitial cells of Leydig were seen as polygonal acidophilic cells with vesicular nuclei. These results revealed the protective role of β-Carotene when given during the withdrawal period. On the other hand, the mean seminiferous tubule diameter and the mean spermatogenic epithelium height showed a significant decrease in the nicotine withdrawal and withdrawal therapeutic groups (GIV and GV) in comparison with control group. While histological examination of H&E stained testicular sections of withdrawal prophylactic group (GVI) revealed that the tubules restored their normal architecture lined with multiple layers of spermatogenic cells starting from spermatogonia resting on the basement membrane up to late spermatids, the lumen contained multiple sperms. These results were supported morphometrically by significant increase in the mean diameter of seminiferous tubules and the mean height of the lining spermatogenic epithelium in comparison to nicotine, nicotine withdrawal and withdrawal therapeutic groups (Groups III, IV and V) respectively. These results confirm the ameliorating effects of β-carotene on testicular tissue preserving spermatogenesis and maintaining the normal histological structure of seminiferous tubules along with preserved Leydig cell functions especially in GVI where β-carotene was administered during nicotine exposure and withdrawal and proved better histological, biochemical and morphometrical results when compared to GV where β-carotene was given only during nicotine withdrawal. Our results came in agreement with Lin *et al*.,[45] work that proved the protective role of β-carotene on testicular tissue of mice subjected to scrotal hyperthermia. This improvement in spermatogenic activity and functions in (GVI) could be explained by β carotene role in restoration of androgenesis and spermatogenesis[46].

Masson trichrome stained testicular sections revealed marked increase in collagen fibres deposition in the interstitial tissue and around the congested blood vessels in (GIII and GIV). This could be explained by the oxidative stress and associated decrease in collagen metabolism[47,48], with subsequent interstitial oedema[49]. On the other hand, the nicotine withdrawal therapeutic and prophylactic groups (GV and GVI) showed moderate and minimal collagen fibres deposition respectively in comparison to the control group. Morphometric results supported these histological findings where a significant increase in area percent of collagen fibres deposition in Groups (III, IV and V) respectively compared with all other studied groups. While GVI demonstrated significant decrease in collagen deposition compared to the previously mentioned groups reaching values approaching control values. According to a study, vimentin filaments present in Sertoli cells play an important role in maintaining spermatogenesis, where spermatogenesis damage and restoration are related to activity of those filaments[50]. The decrease in vimentin expression associated with testicular injury could be attributed to the collapse of Sertoli cell vimentin filaments away from the cell membrane which eventually might lead to detachment of spermatogenic cells and the detached cells might undergo apoptosis. It was documented by Paccola and Miraglia[51], that disruption of tight junctional complexes between the Sertoli and germ cells, sloughing of spermatids into the lumen, disorganization in seminiferous tubules and the morphological alteration of germ cells after nicotine exposure. Loss of vimentin filaments apical extensions and their collapse at the basal region was observed in Nicotine group (GIII) while in Nicotine Withdrawal group (GIV), vimentin filaments were collapsed and concentrated at the basal region with few Sertoli cells demonstrating weak positive reaction in their apical extensions. These results could be attributed to effect of oxidative stress associated with nicotine consumption on vimentin filaments. Similar findings were reported by Mohammed *et al*.,[52] who found that nicotine consumption releases oxidative radicals affecting negatively Sertoli cells vimentin filaments and in turn spermatogenesis. Vimentin immunostained GV sections revealed a strong positive cytoplasmic immunoreaction of Sertoli cells as well as strong positive immunoreaction in the apical extensions of few Sertoli cells while on the other hand GVI showed a strongly positive cytoplasmic immunoreaction in Sertoli cells with prominent apical extensions. Our morphometric results revealed a significant decrease in the mean area percent of vimentin immuno-positive reaction in groups III, IV and V compared to all other studied groups. While GII and GVI demonstrated a non-significant difference in this mean area percent when compared with control group. This could be explained by the protective effect of β carotene in ameliorating the toxic effect of nicotine on vimentin content and distribution in Sertoli cells.

CONCLUSION

β carotene was proven to possess an ameliorating effect against nicotine induced testicular damage. Using β carotene during nicotine exposure and extending to the withdrawal period appeared to be more effective than consuming it during nicotine withdrawal only. This was evidenced by the biochemical, histological and morphometric results of this study.

CONFLICT OF INTERESTS

There are no conflics of interest.

REFERENCES

1. Autifi M, Elsagheir A and Al Mandouh M. Effect of nicotine on the testis of adult albino rats and the possible protective effect of vitamin E. Nat Sci. 2017;15(4):13-19.
2. Alberg AJ. Cigarette smoking: health effect and control strategies. Drugs Today (Barc). 2008; 44(12):895-90.
3. Gawish A, Ramadan S, Hassan A and Issa A. Morphometrical, histophathological and cytogenetical ameliorating effects of Green tea extract on nicotine toxicity of the testis of Rats. J cytol Histol. 2010; 1(2):101-105.
4. Mahmoud A and Shalaby N. Ameliorative Effect of Zinc Oxide Nanoparticles on Nicotine Induced Testicular Dysfunction; Biochemical and Histological Study. Toxicol. Environ. Health 2019; 11(2): 104-113.
5. Oyeyemi WA, Shittu ST, Kolawole TA. and Akinola AO. Protective Effect of Vitamin E on Nicotine Induced Reproductive Toxicity in Male Rats. Nigerian Journal of Basic and Applied Science 2015; 23(1): 7-13.
6. EL Shafai A, Zohdy N, EL Mulla K, Hassan M and Morad N. Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. Food and chemical toxicology 2011; 49:734-743.
7. Budin SB, Kho JH, Lee JH, Ramalingam A, Fatin FJ, Elda SL, Satirah Z, Izatus ST and Jamaludin M. Low-dose nicotine exposure induced the oxidative damage of reproductive organs and altered the sperm characteristics of adolescent male rats. Malays J Med Sci.2017; 24(6):50–57.
8. Bahadır A, Ceyhan A, Gergin Ö, Yalçın B, Ülger M, Özyazgan T M and Yay A. Protective effects of curcumin and beta-carotene on cisplatin-induced cardiotoxicity: An experimental rat model. Anatol J Cardiol.2018; 19: 213-221.
9. Ribeiro J, Verzeletti FB and Poletto RS. Male infertility: Reduction Of free radicals per action of Carotenoids. Global Journal of Reproductive health 2018; 4(1): 10-14.
10. Vardi N, Parlakpinar H, Ates B, Cetin A and Otlu A. Antiapoptotic and antioxidant effects of b-carotene against methotrexate-induced testicular injury. Fertil Steril.2009; 92(6):2028-2033.
11. Nesseim WH, Haroun HS, Mostafa E, Youakim MF and Mostafa TB. Effect of nicotine on spermatogenesis in adult albino rats. Andrologia 2011; 43:398-404.
12. Pourghasem M, Nasiri E and Shafi H. Early renal histological changes in alloxan-induced diabetic rats. Int J Mol Cell Med. 2014; 3: 11-15.
13. Keirnan JA. Histological and histochemical methods, theory and practice. 5th ed., Scion Publisher.UK.; 2015. 111-162.
14. Suvarna KS, Layton C and Bancroft JD. Immunohistochemical techniques. In: Bancroft's theory and practice of histological techniques, 7th edition, Elsevier Health Science, China; 2013. 381-426.
15. Bancroft JD and Gamble M. Immunohistochemical techniques in Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, London; 2008.433-473.
16. Emsley R, Dunn G and White I R. Statistical methods in medical research.19th Edition, Sage publications, USA.; 2010. 237-270.
17. Low WY and Binns C. Tobacco consumption. The Asia Pacific region's major public health problem. Asia Pac J Public Health 2013; 25: 7–9.
18. Dai JB, Wang ZX and Qiao ZD. The hazardous effects of tobacco smoking on male fertility. Asian Journal of Andrology 2015; 17: 954–960.
19. Reyes JG, Farias JG, Henríquez OS, Madrid E and Parraga M. The hypoxic testicle: physiology and pathophysiology. Oxid Med Cell Longev. 2012; 2012: 929285.
20. Gambo IM, Galam NZ, Adamu G, louis OA, Habeeb A, Bello M, Bello N, Rabiu A M and Odeh SO. The Effect of Aqueous Leave Extract of Nicotiana Tabacum (Tobacco) On Some Reproductive Parameters and Micro-Anatomical Architecture Of The Testis In Male Albino Wistar Rats. Journal of Natural Sciences Research 2013; 3:2224-3186.
21. Sandmann G. Antioxidant Protection from UV- and Light-Stress Related to Carotenoid Structures. Antioxidants (Basel).2019; 8(7):219.
22. Cho Ping N, Hashim NH and Hasan AD. Effects of nigella sativa (Habbatus sauda) oil and nicotine chronic treatments on sperm parameters and testis histological features of rats. Evidence-Based Complementary and Alternative Medicine 2014; 2014: 218293

23. Treuting PM, Dintzis SM and Montine KS. Male reproductive system. In: Comparative anatomy and histology: a mouse, rat, and human atlas, 2nd ed., Academic Press Elsevier, USA.;2017.335-339.

24. Pandey G and Jain GC. Aluminium Chloride-Induced Testicular Effects in Rats: A Histomorphometrical Study. AJAST.2017; 1(9):46-52.

25. Abd El-Aziz G, El-Fark M and Hamdy R. Protective effect of Eruca sativa seed oil against oral nicotine induced testicular damage in rats. Tissue and Cell 2016; 48(4): 340-348.

26. Mosadegh M, Hasanzadeh S and Razi M. Nicotine-induced damages in testicular tissue of rats; evidences for bcl-2, p53 and caspase-3 expression. Iran J Basic Med Sci.2017; 20:199-208.

27. Fairuz AM, Hashida NH and Mahanem MN. Effect of nicotine and goat milk coadminstration on rat testis and sperm parameters. Aust.J.Basic Appl. Sci.2011;5:2738-2741.

28. Adewoyin M, Ibrahim M, Roszaman R, Md Isa ML, Alewi N, Abdul Rafa AA and Anuar MN. Male Infertility:The Effect of Natural Antioxidants and Phytocompounds on Seminal Oxidative Stress. Diseases 2017;5(1): 9.

29. Bas H and Kalender S. Antioxidant status, lipid peroxidation and testis-histoarchitecture induced by lead nitrate and mercury chloride in male rats. Brazilian Archives of Biology and Technology 2016; 59: e16160151.

30. Kini RD, Kumar NA, Noojibail A, Bhagyalakhshmi K, Bhoja S and Chatterjee PK. Protection against Cadmium Induced Testicular Toxicity. Pharmacog J. 2018; 10(6):66-70.

31. Gevrek F, Biçer Ç, Kara M and Erdemir F. The ameliorative effects of Ginkgo biloba on apoptosis, LH-R expression and sperm morphology anomaly in testicular torsion and detorsion. Andrologia 2018; 50(4): e12969.

32. Orieke D, Ohaeri OC, Irene Ijeh I, Ijioma SN. Semen quality, hormone profile and histological changes in male albino rats treated with Corchorus olitorius leaf extract. Avicenna J Phytomed. 2019; 9(6):551-562.

33. Mosbah R, Yousef MI and Mantovani A. Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: The protective effects of green tea extract. Experimental and Toxicologic Pathology 2015; 67(3): 253–259.

34. Nair SV and Rajamohan T. The role of coconut water on nicotine- induced reproductive dysfunction in experimental male rat model. Food and Nutrition Sciences 2014; 5(12): 1121–1130.

35. Bisong S, Ukoh I, Nna V and Ebong P. Vitamin E attenuates nicotine- and noise-induced reproductive impairment in male albino Wistar rats. Andrologia 2018; 50(7): e13050.

36. Kolawole TA, Oyeyemi WA, Adigwe C, Leko B, Udeh C, and Dapper DV. Honey Attenuates the Detrimental Effects of Nicotine on Testicular Functions in Nicotine Treated Wistar Rats. Niger. J. Physiol. Sci. 2015; 30: 11-16.

37. Guo X, Wang H, Wu X, Chen X, Chen Y, Guo J, Li X, Lian Q and Ge R. Nicotine affects rat Leydig cell function in *vivo* and *vitro* via down-regulating some key steroidogenic enzyme expressions. Food and Chemical Toxicology 2017; 110: 13-24.

38. Orazizadeh M, Khorsandi L, Absalan F, Hashemitabar M and Daneshi E. Effect of beta-carotene on titanium oxide nanoparticles-induced testicular toxicity in mice. J Assist Reprod Genet. 2014; 31:561-568.

39. Hanadi BA, Kelany AH, ElQudsi FM, Ameen HA and El Karium SA. the possible protective role of antioxidants (selenium,Vitamin E) in reducing smoke effects on testes of albino rats. Ass. Univ. Bull. Environ. Res.2011; 14(1):61-76.

40. Abdul Ghani R, Qazzaz M, Dabdoub N, Muhammad R and Abdul Ghani AS. Studies on cigarette smoke induced oxidative DNA damage and reduced spermatogenesis in rats. J Environ Biol. 2014; 35: 943–947.

41. Agarwal A, Virk G, Ong C and Du Plessis S. Effect of oxidative stress in male reproduction.World J Mens Health 2014; 32(1):1-17.

42. Elshaari FA, Elfagih RI, Sherif DS and Barassi IF. Testicular Torsion-Detorsion- Histological and Biochemical Changes in Rat Testis.J CytolHistol. 2012; 3: 99-115.

43. Ahmadnia H, Ghanbari M, Moradi MR and Khaje Dalouee M. Effect of cigarette smoke on spermatogenesis in rats. Urol J. 2007; 4: 159–163.

44. La Maestra S, De Flora S and Micale RT. Effect of cigarette smoke on DNA damage, oxidative stress, and morphological alterations in mouse testis and spermatozoa. Int J Hyg Environ Health 2015; 218: 117–122.

45. Lin C, Choi YS, Park SG, Gwon LW, Lee JG, Yon JM, Baek IJ, Lee BJ, Yun Y Wand Nam SY. Enhanced Protective Effects of Combined Treatment with β-Carotene and Curcumin against Hyperthermic Spermatogenic Disorders in Mice. BioMed Research International 2016; (5):1-8.

46. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D and Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. The Journal of Steroid Biochemistry and Molecular Biology 2008; 109(3):323-330.

47. Altindag O, Erel O, Aksoy N, Selek S, Celik H and Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatology International 2007; 27(4):339-344.

48. Salem MM, Altayeb ZM and El-Mahalaway AM. Histological and Immunohistochemical Study of Titanium dioxide Nanoparticle Effect on the Rat Renal Cortex and the Possible Protective Role of Lycopene. Egyptian Journal of Histology 2017; 40(1):80-93.

49. Kim SH, Lee IC, Baek HS, Shin IS, Moon C, Kim SH and Kim JC. Melatonin prevents gentamicin-induced testicular toxicity and oxidative stress in rats. Andrologia 2014; 46(9):1032-1040.

50. ElGhamrawy TA, Helmy D and Elall HF. Cadherin and vimentin immunoexpression in the testis of normal and induced infertility models of albino rats. Folia Morphol. 2014; 73(3): 339-346.

51. Paccola C and Miraglia S. Prenatal and lactation nicotine exposure affects Sertoli cell and gonadotropin levels in rats. Reproduction 2016; 151(2):117-133.

52. Mohammed M, Abd elfadeel K, Abdel Aziz H, Fawzy A and Samy W. The effect of chronic stress on the testis of adult albino Rats and the possible protective effect of astaxanthin supplementation (Histological, immunohistochemical and biochemical studies. J Med Histo. 2019; 2(2): 115-130.

الملخص العربى

التأثير المحتمل لكاروتين ب على إنسحاب النيكوتين في نسيج خصية ذكور الجرذان البيضاء البالغة. دراسة هستولوجية وهستوكيميائية مناعية

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الخلفية والأهدف: يمتلك النيكوتين الموجود داخل أدخنة السجائر تأثيرا مدمرا على خلايا الخصية، وقد تم تصميم هذه الدراسة لتقييم ومقارنة التأثيرالوقائى والعلاجى المحتمل لكاروتين ب علي تلف الخصية الناجم عن النيكوتين في نموذج الجرذ اثناء انسحاب النيكوتين .

المواد وطرق البحث: تم إدراج تسعة و ثلاثين من ذكور الجرذان البيضاء البالغة في هذه الدراسة وتم تقسيمهم إلى ٦ مجموعات : المجموعة الأولى (الضابطة)، المجموعة الثانية (مجموعة كاروتين ب)، المجموعة الثالثة (مجموعة النيكوتين)، المجموعة الرابعة (مجموعة إنسحاب النيكوتين)، المجموعة الخامسة (المجموعة العلاجية لإنسحاب النيكوتين)، المجموعة السادسة (المجموعة الوقائية لإنسحاب النيكوتين (تم حقن ال كاروتين بـ١٠ مجم / كجم /يوم) داخل الغشاء البريتوني يوميا لمدة أربعة أسابيع . تم حقن النيكوتين (٤ مجم / كجم /يوم) داخل الغشاء البريتوني يوميا لمدة أربعة أسابيع. وقبل الذبح: تم سحب عينات الدم لقياس مستوى المالوندالدهيدو هرمون التستوستيرون، وبعد الذبح: تم تشريح الخصية وصبغتها بصبغة الهيماتوكسيلين والإيوسين وصبغة ماسون ثلاثي الألوان وصبغة هستوكيميائية مناعية (فايمنتين). وتم إجراء القياسات المترية الشكلية

النتائج: أظهرت المجموعة الأولى والثانية نتائج متشابهه مع سلامة البنية النسيجية والقياسات الكميائية والمترية للخصية. حدث تدهور في كل من البنية النسيجية والقياسات الكميائية والمترية للخصية في المجموعات الثالثة والرابعة والخامسة (خاصة المجموعة الثالثة) مع انخفاض ذى دلالة إحصائية في متوسط قطر الأنابيب المنوية ومتوسط ارتفاع النسيج الطلائي ومتوسط النسبة المئوية لمساحة الخلايا المتفاعلة إيجابيًا مع الفايمنتن وارتفاع ذى دلالة إحصائية في متوسط النسبة المئوية لمساحة ألياف الكولاجين مقارنة بالمجموعة الضابطة. أظهرت المجموعة السادسة نتائج أفضل من المجموعات الثالثة والرابعة والخامسة مع استعادة التركيب النسيجى الطبيعى للخصية.

الاستنتاج: يستطيع كاروتين ب تحسين عملية انتاج الحيوانات المنوية. يعتبرالتأثيرالوقائى لكاروتين ب اثناء تعاطى وانسحاب النيكوتين أفضل من التأثيرالعلاجى لكاروتين ب اثناء انسحاب النيكوتين فقط وذلك بواسطة الدراسات الكيميائية والهستولوجية والهستوكيميائية المناعية والقياسات المترية.