Original Article

Histopathological Analysis of Testis: Effects of Astaxanthin Treatment against Nicotine Toxicity

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ABSTRACT

Background: Nicotine is a toxic compound in the cigarette smoke and has destructive effects on various human organs. Astaxanthin is a carotenoid with high antioxidant property. In this study, we investigated the protective effects of astaxanthin against nicotine-induced toxicity in mice testes.

Methods: Forty-two inbred balb/c male mice were divided into six groups:*Group 1*, received 1 ml normal saline daily; *Group 2*, received nicotine (1.5mg/kg); *Group 3*, received astaxanthin (25mg/kg); *Group 4*, received astaxanthin (50mg/kg); *Group 5*, received astaxanthin (25mg/kg) plus nicotine (1.5mg/kg); and *group 6*, received astaxanthin (50mg/kg) plus nicotine (1.5mg/kg). After collecting testes samples, microscopic slides were prepared at the School of Veterinary Medicine, University of Mashhad, and the prepared slides were examined under light microscopy.

Results: The histological structures of the testes were normal in the control group and those receiving astaxanthin, regardless of nicotine (groups 3, 4, 5 & 6). However, group 2 that received only nicotine, showed transformed testicular histology with severe hemorrhage.

Conclusion: Based on the results, nicotine caused harmful effects on the mice testes and astaxanthin appeared to protect the organs against the toxic effects of nicotine.

Keyword: Astaxanthin, Balb/c mice, Nicotine toxicity, Testicular Hemorrhage.

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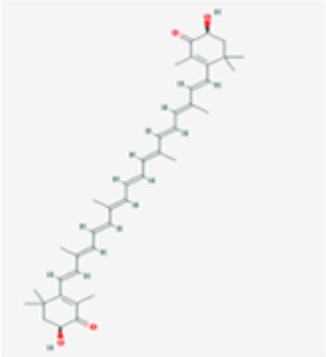
INTRODUCTION

Infertility is defined as the absence of conception after at least 12 months of regular unprotected intercourse in couples (1). Infertile men are associated with 30-50% of all cases of infertility. Various physiological conditions, systemic pathologies, genetic abnormalities, environmental pollution and even oxidative stressors of the reproductive organs can cause infertility (1). Cigarette smoking has adverse impacts on human health and fertility.

Nicotine, as an active alkaloid component of tobacco, results in testicular toxicity by affecting testosterone synthesis. Also, it impairs sperm function through oxidative stress, DNA damage and cell apoptosis (2). Furthermore, nicotine increases the production of cholesterol, triglycerides, phospholipids and free fatty acids in testes, and increases peroxidative damages with harmful impacts on spermatogenesis (2). Reduced semen quality and adverse effects on pituitary and gonadal hormones are other results of nicotine toxicity (3). Using compounds with antioxidant properties can reduce or even prevent the pathologic effects of nicotine. Astaxanthin is a lipophilic, pinkish-orange compound that is synthesized by seafood, various plants, and particularly by the green algae known as, *Haematococcuspluvialis or Chlorophyta* (4). The algae are mobile, mono-cellular and capable of synthesizing astaxanthin in response to environmental conditions (5).

Astaxanthin is used as a dietary additive in USA, Japan, South Korea and Sweden, which has significant protective antioxidant and anticancer properties. Also, it decreases oxidative stress and inflammatory response in cells and is beneficial for ischemic-reperfusion of clogged arteries, hypertension and dyslipidemia. Also, it has been shown that astaxanthin is the most powerful natural carotenoid antioxidant. The distinct chemical structure, polar ends interacting with phospholipid groups or water in the aqueous environment, results in the antioxidant property of astaxanthin (6). In this study, we investigated the protective effects of astaxanthin against the destructive impacts of nicotine on the histological structures of mice testes.



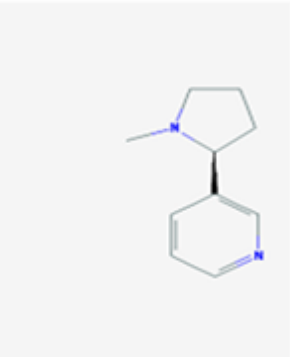


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Figure 1. The molecular structures of astaxanthin, 3*S*,3′*S*-Dihydroxy-β,β-carotene-4,4′-dione (*left*) and nicotine, C10H14N2 (*right*).

MATERIALS AND METHODS

Chemicals

Nicotine (C10H14N2) and astaxanthin ($C_{40}H_{52}O_4$) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Nicotine was diluted with normal saline (0.9%). Also, astaxanthin powder was dissolved in olive oil, as a solvent and diluted in normal saline (0.9%).

Animals

Forty-two inbred balb/c male mice with the weight of 25-30 g were obtained from Faculty of Animal Sciences of Mashhad University. These mice are also well known for the production of monoclonal antibodies. The mice were housed in a room at 22 ± 2°C under controlled light/dark cycle (12/12/hr.) with free access to water and food. The ethical and humane principles were observed for the animals during the conduction of the experiments (7-9).

Experimental Design

Animals were randomly divided into six groups (n = 7 per group). *Group 1*, received 1 ml normal saline daily; *Group 2*, received nicotine (1.5 mg/kg); *Group 3*, received astaxanthin (25 mg/kg); *Group 4*, received astaxanthin (50 mg/kg); *Group 5*, received astaxanthin (25 mg/kg) plus nicotine (1.5 mg/kg); and *group 6*, received astaxanthin (50 mg/kg) plus nicotine (1.5 mg/kg). Astaxanthin was injected intraperitoneally twice a week, while nicotine was administered intraperitoneally once a day. The treatment duration lasted for four weeks.

Histological Examination

Several laboratory phases were performed to prepare microscopic slides, as described in our previous study (10). The mice were anesthetized, and the left testes were removed. All testicular samples were fixed in 10% formaldehyde. Sample dehydration phase was performed by reducing the level of ethanol, clearing with xylene, loading in paraffin, followed by sectioning and preparing microscopic slides. The horizontal and perpendicular sections were stained with hematoxylin and eosin (H & E). Finally, the effects of nicotine and astaxanthin on the histological structures of the testes were evaluated under light microscopy. For histopathological evaluation, the grading system of Cosentino was used (11). Also, the grading system of Johnson (12) was used to assess the spermatogenesis maturation of the testes samples.

Table 1. Scoring system of Cosentino (11)

Score	Microscopic Characteristics
1	Normal testicular architecture with an orderly arrangement of germinal cells and without hemorrhage and necrosis.
2	Injury showed less orderly, non-cohesive germinal cells and closely packed seminiferous tubules with mild hemorrhage and necrosis.
3	Injury exhibited disordered, sloughed germinal cells, with reduced size of pyknotic nuclei and less distinct seminiferous tubule borders with moderate hemorrhage and necrosis.
4	Injury exhibited seminiferous tubules that were closely packed with coagulative necrosis of the germinal cells with severe hemorrhage and necrosis.

Table 2. Scoring system of Johnson (12)

Score	Microscopic Characteristics
1	No germ cells and no Sertoli cells present.
2	No germ cells, but only Sertoli cells present.
3	Only spermatogonia present.
4	Only a few spermatocytes present.
5	No spermatozoa or spermatids, but numerous spermatocytes present.
6	Only a few spermatids present.
7	No spermatozoa, but numerous spermatids present.
8	Only a few spermatozoa present in the section.
9	Numerous spermatozoa present, but the germinal epithelium is disorganized.
10	Complete spermatogenesis and normally organized tubules.

RESULTS

Histological changes of testicular tissue are presented in Figure 2. In *group 1*, which received normal saline (0.9%), the histological structures of testes, germinal cells and tubules were found to be normal. In *group 2*, which received nicotine, vacuolation, thickening of capsule, desquamation, irregular shape of seminiferous tubules, irregular distribution of Sertoli cells, hemorrhage and interstitial edema were observed (mean grade: 3.66; range: 3.5-4). In *group 5*, which received astaxanthin (25 mg/kg) plus nicotine (1.5 mg/kg), moderate hemorrhage and histological damages were observed in the testicular tissue samples (mean grade: 2.78; range: 2.25-4). In *group 6*, which received astaxanthin (50 mg/kg) plus nicotine (1.5 mg/kg), only

mild hemorrhage and histological damages were observed (mean grade: 1.66; range: 1.5-2). Besides, the spermatogenesis was assessed according to the scoring system of Johnson (12). Normal spermatogenesis was observed in *groups 1, 3 and 4* (mean score: 9.5). Severe histological damages were observed in *group 2* (mean grade: 2.62). Moderate and mild histological damages were observed in *groups 5* (mean grade: 5.28) and *6* (mean grade: 8.42), respectively.

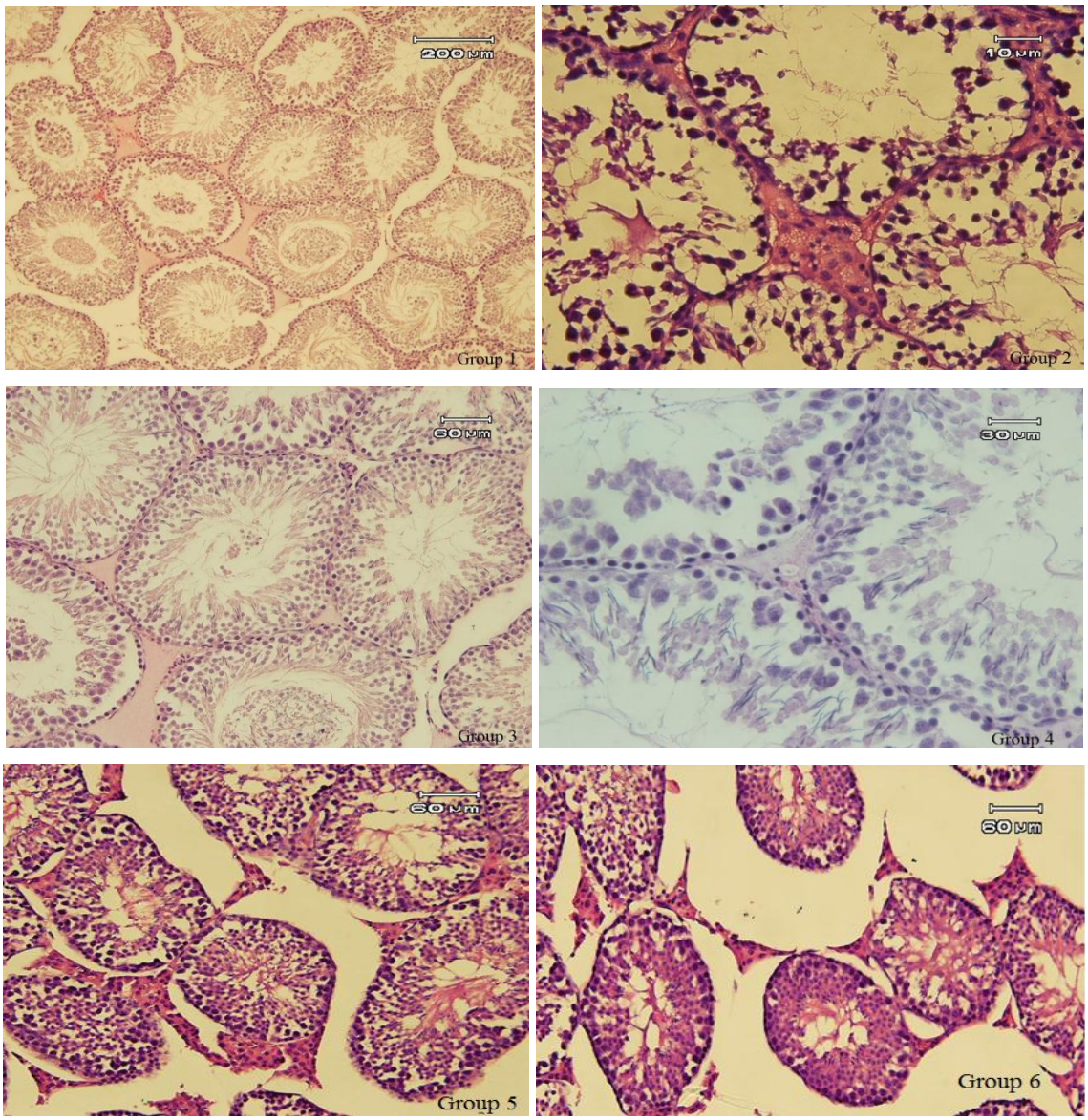

Figure 2. The histological features of the mice testicular tissues from the control and experimental groups as revealed under light microscopy (H & E staining).

DISCUSSION

The aim of this study was to investigate the toxic effects of nicotine on the reproductive system and the protective impacts of astaxanthin against nicotine. We observed coagulative necrosis, severe hemorrhage and disorganized testicular tissue features caused by nicotine. In contrast, the histological structures of the testicular tissue samples were found to be normal in the groups treated with astaxanthin, which were similar to those observed in the control group.

During recent years, much attention has been paid to nicotine toxicity (7-10,13,14). Spermatogenic cells have high levels of unsaturated fatty acids, several dual links in plasma membrane and low levels of cytoplasmic antioxidants, and are sensitive to oxidative stress (15). Oxidation of fatty acids in cell membranes leads to loss of membrane fluidity and diminishes the activity of enzymes and ion channels in sperms. Nicotine results in the generation of reactive oxygen species. It has been shown that an appropriate (i.e., low) level of reactive oxygen species are vital for the capacitation and acrosome reaction (16,17). Acrosome is a cap-like structure, derived from the Golgi apparatus that develops over the anterior half of the head in the sperm cells (18). High levels of reactive oxygen species result in decreased semen quality and volume, sperm count and motility, and forward regression (19). Nicotine, as a toxic compound, is absorbed via respiratory system, oral mucosa and skin (20). Nicotine is known to inhibit the release of follicular stimulating hormone, *FSH*, and

luteinizing hormone, *LH* (21). It has been demonstrated that nicotine decreases the levels of *androstenedione* and *testosterone* by producing defects in the testosterone biosynthesis pathway (22). It has also been reported that nicotine impairs spermatogenesis in a dose-dependent manner (22). These effects are reversible upon nicotine withdrawal. Furthermore, tobacco extract impairs the testicular spermatogenesis (22).

CONCLUSION

Nicotine, as a toxic substance in the cigarette smoke, causes adverse effects on the testis and consequently, can impair fertility. In this research, we demonstrated that astaxanthin can be considered as a potential candidate for supporting testicular tissue against the harmful effects of nicotine toxicity. Nicotine resulted in hemorrhage and irregular shape of seminiferous tubules, and astaxanthin reversed the negative effects of nicotine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in conducting this study.

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