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Effect of Astaxanthin Treatment on the Sperm Quality of the Mice Treated with Nicotine

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ABSTRACT

Introduction: Today, smoking has become a common habit, and researchers have largely been concerned with the adverse health effects of smoking. Some approaches have been proposed to minimize these effects. Nicotine is an alkaloid, which is considered to be a detrimental agent in smokers. The present study aimed to investigate the protective effects of astaxanthin against the adverse effects of nicotine.
Methods: In this study, 42 BALB/c male mice were purchased from Mashhad University in Mashhad, Iran and randomly divided into six groups. Group one received one milliliter of normal saline daily, group two received nicotine (1.5 mg/kg), group three was administered with astaxanthin (25 mg/kg), group four also received astaxanthin (50 mg/kg), group five was administered with astaxanthin (25 mg/kg) and nicotine (1.5 mg/kg), and group 6 was administered with astaxanthin (50 mg/kg) and nicotine (1.5 mg/kg). After the experiments, the epididymis was collected, and the motility, viability, and count of the sperms were evaluated.
Results: Nicotine at the dose of 1.5 mg/kg decreased the count, viability, and motility of sperm. In contrast, astaxanthin at the doses of 25 and 50 mg/kg was observed to diminish the destructive effects of nicotine.
Conclusion: According to the results, astaxanthin is a potent antioxidant for the protection of the reproductive system against nicotine-induced toxicity.

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Introduction

In recent decades, the outbreak of infertility has increased partly due to increased exposure to harmful agents (1-4). Infertility is a major health concern, affecting 10-15% of couples worldwide. It is defined as the inability to conceive after 12 months of unprotected intercourse (5,6). Evidence suggests that the rate of male infertility is higher compared to female infertility (7). Smoking is considered to be a significant contributing factor to infertility. Cigarettes contain numerous toxic agents, including nicotine, tar, and carbon monoxide (8).

Nicotine is a toxic agent found in cigarettes and exerts various detrimental effects on human health (9). Nicotine is an alkaloid with a high concentration in Nicotiana tabacum, where it acts as an insecticide (10). This active pharmacological agent has detrimental effects on the reproductive system and fertility in men (11,12).

There are red or yellow pigment carotenoids in

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animals and plants, which have antioxidant, anti-inflammatory, antitumor, and immunomodulatory properties (13,14). Among the carotenoids found in microalgae and seafood (e.g., salmon, trout, and shrimp), astaxanthin has been reported to have the highest antioxidant activity (15). A study in this regard investigated the antioxidant activity of astaxanthin and vitamin C, reporting the antioxidant activity of astaxanthin to be approximately 600-fold higher than vitamin C (16). Therefore, astaxanthin is extensively applied in the production of cosmetics and dietary supplements.

The effects of astaxanthin are exerted through several mechanisms, such as the scavenging of singlet oxygen via conjugated double bonds, inhibition of lipid peroxidation, and suppression of nuclear factor-kappa B (NF-kB) (17-19). Moreover, astaxanthin could be transferred to the brain tissue and protect the brain vessels against cerebrovascular diseases, such as cerebral ischemia and subarachnoid hemorrhage (20,21).

Oxidative stress is involved in the deterioration of various diseases due to the overgeneration of reactive oxygen species (ROS), superoxide radical, hydroxyl radical, and hydrogen peroxide (H_2O_2). According to reports, astaxanthin could ameliorate oxidative stress (22). Among various carotenoids (e.g., β-carotenoid), astaxanthin has been reported to be comparatively more beneficial, especially in the elimination of singlet oxygen (23).

The structure of astaxanthin consists of conjugated double bonds and terminal ring sections, which are associated with the enmeshing of radicals on membrane surfaces and internal membranes (24) (Figure 1).

The present study aimed to investigate the protective effects of astaxanthin against the adverse effects of nicotine on the motility, morphology, and viability of sperm.

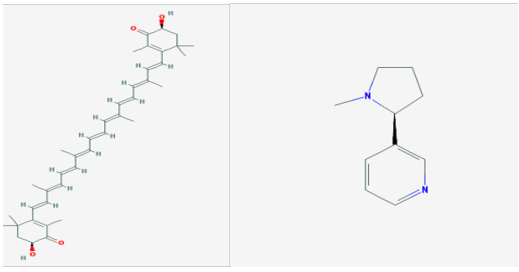

Figure 1. Structures of Astaxanthin (3S,3′S-Dihydroxy-β,β-carotene-4,4′-dione; left) and Nicotine (C10H14N2; right)

Methods

Experimental Animals

In this study, 42 BALB/c male mice weighing 25-30 grams were purchased from the School of Animal Sciences at Mashhad University in Mashhad, Iran. The animals were housed in a room with the temperature of 22±2°C under controlled environmental conditions within a 12-hour light/dark cycle and had free access to water and food. The ethical and humane principles were observed during the experiments (4, 25).

Experimental Design

The animals were randomly divided into six groups of seven. Group one received one milliliter of normal saline daily, group two received nicotine (1.5 mg/kg), group three was administered with astaxanthin (25 mg/kg), group four was also administered with astaxanthin (50 mg/kg), group five received astaxanthin (25 mg/kg) and nicotine (1.5 mg/kg), and group six received astaxanthin (50 mg/kg) and nicotine (1.5 mg/kg). Astaxanthin was administered via intraperitoneal injection twice per week, and nicotine was injected intraperitoneally once a day. The treatment period was four weeks.

Sample Collection for Sperm Analysis

After the separation of cauda epididymis from the testes, it was divided into small pieces on one milliliter of Ham's F10 culture medium. Hemocytometer with light microscopy with the magnification of 400* was used to evaluate the epididymal sperm count. In addition, phase contrast microscopy with the magnification of 400* was applied to assess sperm motility, showing 10 microscopic fields. The average counted sperms was expressed as sperm motility in each rat. In order to evaluate sperm viability, 20 milliliters of sperm suspension was combined with 20 milliliters of 0.05 Eosin-Y, and the slides were observed via bright-field microscopy with the magnification of 400*.

Statistical Analysis

Data analysis was performed using one-way analysis of variance (ANOVA) and Dunnett's test, and the data were expressed as mean and standard error of the mean (SEM) at the significance level of $P<0.05$.

Results

The sperm count decreased more significantly in group two compared to group one. On the other hand, supplementation with astaxanthin significantly increased the sperm count, and the maximum sperm count was observed in group four (Figure 2).

Sperm motility reduced more significantly in group two compared to group one. However, astaxanthin administration significantly increased sperm motility, and the maximum sperm motility

was observed in group four (Figure 3).

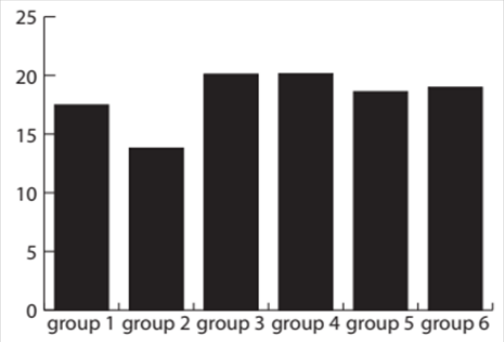

Figure 2. Decreased Sperm Count in Group Two Compared to Group One and Increased Sperm Count in Groups Five and Six Compared to Group Two.

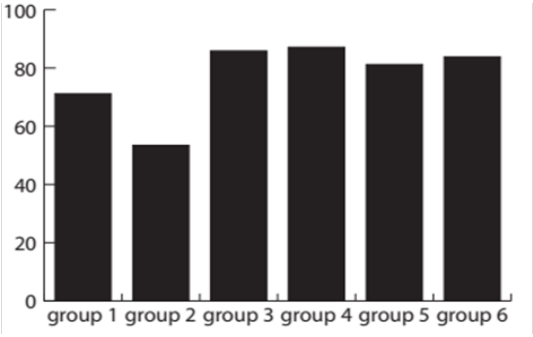

Figure 3. Decreased Sperm Motility in Group Two Compared to Group One and Increased Sperm Motility in Groups Five and Six Compared to Group Two.

According to the findings, sperm viability decreased more significantly in group two compared to group one. On the other hand, supplementation with astaxanthin significantly increased sperm viability, and the maximum viability was observed in group four (Figure 4).

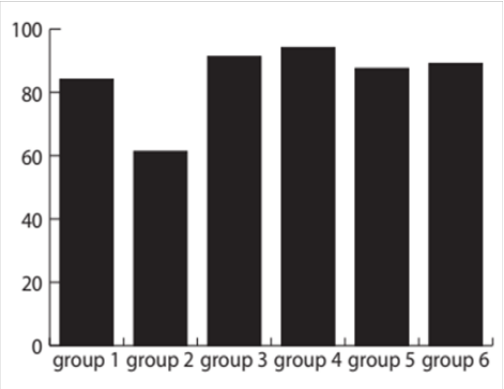

Figure 4. Decreased Sperm Viability in Group Two Compared to Group One and Increased Sperm Viability in Groups Five and Six Compared to Group Two.

Discussion

Infertility is a significant health issue, which adversely affects the personal, social, and economic aspects of life. Evidence suggests that male factors account for 40% of infertility cases (26). Reduced sperm count, sperm immaturity and abnormality, and lack of sperm motility are associated with sperm dysfunction (27). Over the past decades, special attention has been paid to the toxicity of various agents, and researchers have focused on finding compounds to decrease the adverse effects of these compounds (1-4,6,28-33).

According to reports, agents containing nicotine reduce sperm count and motility (34). Nicotine could readily cross the cell membrane and interact with the tubulin protein present in the cytoplasm of multiplying cells, thereby leading to cell division disorders (35). In a study in this regard, Racowsky and Kaufman (2008) demonstrated that nicotine leads to damages in the sperm membrane and DNA, stimulating apoptosis in the interstitial cells in the testes (36). The results of the present study confirmed the adverse effects of nicotine exposure on sperm count, motility, and viability. Accordingly, the intraperitoneal injection of astaxanthin increased sperm motility, count, and viability. Therefore, it could be concluded that the high antioxidant activity of astaxanthin could enhance the antioxidant defense of the body and decrease lipid peroxidation.

In another research, Aitken (1995) claimed that sperm motility may be considered a significant influential factor in fertility, and sperm motility is the main cause of various fertilities (37). Furthermore, nicotine has been reported to improve the activity of cannabinoids, resulting in decreased sperm motility via activating the CB1 receptors in mature sperm (38). According to the current research, nicotine could increase the motility of sperms, which could be due to the inhibition of cannabinoid activity by astaxanthin.

In general, there is a negative association between smoking and fertility (39), and nicotine has been shown to impair the reproductive system in men and women (40). The present study particularly investigated the effects of nicotine on the male reproductive system. Nicotine exposure may lead to decreased gametogenesis, reduced steroidogenesis, and inhibited secretion of gonadotropins (41-44). Several studies have indicated that nicotine use reduces testosterone release, estradiol, follicle-stimulating hormone, and luteinizing hormone. Furthermore, nicotine could adversely affect the count, motility, survival, and morphology of sperms. Nicotine has also been reported to reduce the weight of the testes and body, as well as the libido. In addition, nicotine exerts alterations in the pituitary-hypothalamic axis, thereby stimulating the release of cortisol,

vasopressin, and oxytocin and increasing oxidative stress. Nicotine exposure could also increase the risk of apoptosis in the reproductive system (45-53).

Oxidative stress is caused by the overproduction of oxygen radicals or dysfunction of the antioxidant enzymes, which are highly involved in the etiology of male infertility (54,55). Appropriate levels of ROS are vital to the human body, required for the sperm to undergo capacitation and acrosome reaction (56-58). The adverse effects of nicotine on sperm quality could be attributed to the increased levels of oxidative stress, and astaxanthin could suppress oxidative stress through its high antioxidant activity.

Conclusion

According to the results, astaxanthin could be a potential candidate for protection against the adverse effects of nicotine as it increased the indices of sperm count, motility, and viability.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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