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The Effect of Aspartame on the Structure and Function of Prostate and Sperm Parameters in Adult Mice

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ABSTRACT

Background and Objective: Aspartame is a dipeptide composed of L-phenylalanine methyl ester and L-aspartic acid that is used as an artificial sweetener in diet sodas and chewing gum. It is metabolized in the body to produce phenylalanine, aspartic acid, and methanol. The aim of this study was to investigate the negative effects of aspartame on prostate structure and function and sperm parameters in adult mice.

Methods: 24 male mice weighing 20-25 grams and 8-10 weeks old were used in this experimental study, which were obtained from the university research institute. The mice were divided into 4 groups of 6. The four groups included control group, the sham group that received only the solvent aspartame, and the treatment groups that received aspartame at doses of 16 mg/kg and 32 mg/kg trough intraperitoneal injection. After 28 days, surgery was performed and prostate tissue was removed for histopathological changes and blood was removed from the heart for enzymatic studies. Furthermore, to determine sperm health factors, the epididymis was isolated and sperm count, motility, and structural health were examined.

Findings: Aspartame at doses of 16 mg/kg and 32 mg/kg caused a significant decrease in serum biomarkers of LH hormone (6.34±0.07 and 5.14±0.05) compared to the control group (9.27±0.07) ($p<0.001$). FSH hormone (4.34±0.04 and 3.21±0.07) significantly decreased compared to the control group (6.48±0.04) ($p<0.001$). Testosterone (4.36±0.05 and 3.22±0.06) also significantly decreased compared to the control group (6.08±0.04) ($p<0.001$). In addition, serum PSA levels (6.66±0.07 and 11.99±0.27) significantly increased compared to the control group (0.52±0.03) ($p<0.001$). Aspartame also negatively affected sperm parameters (count, motility, and structural integrity) and caused histopathological damage to prostate tissue.

Conclusion: The results of this study indicate the destructive and dose-dependent effects of aspartame on mouse prostate tissue.

Keywords: *Aspartame, Prostate Tissue, Histopathology, Serum Hormones, PSA Enzyme and Sperm Parameters.*

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Introduction

Aspartame is a dipeptide composed of L-phenylalanine methyl ester and L-aspartic acid and is used as an artificial sweetener in diet sodas and chewing gum and has an amino group around the alpha carbon of peptides (α-aspartame) (1). The main hydrolysis and breakdown products of aspartame are L-phenylalanine, aspartic acid, methanol, and diketopiperazine. Diketopiperazine is formed by the intramolecular reaction of the primary amine with the methyl ester group of aspartame, β-aspartame. Aspartame is a non-sweet isomer of alpha-aspartame. Aspartame is metabolized in the body to produce phenylalanine, aspartic acid, and methanol. Phenylalanine is an essential amino acid required for protein synthesis, while aspartic acid is a non-essential amino acid involved in energy production and other metabolic processes. Methanol is a toxic substance that can cause damage in high doses, but the small amount produced by aspartame is generally considered safe (2).

Methanol is widely distributed throughout the body, including the brain, muscle, fat, and nervous tissue. It is then metabolized to formaldehyde, which enters cells and binds to proteins and DNA. The cytogenetic effects of aspartame result from the contact of body cells with formaldehyde and the DNA damage from the formaldehyde that results from this contact. The nature of the damage generally involves the breaking and then cross-linking of genetic material, which alters the cells. Changes in genetic material have been linked to cancer in humans. There has been an increase in malignant brain tumors associated with aspartame consumption (3).

The vast majority of people can consume aspartame in moderate amounts. However, more research is needed to fully understand its effects on human health. The U.S. Food and Drug Administration (FDA) has set an acceptable daily intake of 50 mg per kilogram of body weight, which is equal to about 22 cans of diet soda for an average adult (4). On the other hand, some studies have reported adverse effects and carcinogenesis due to aspartame (5). The result of the study by Gezginci Oktayoglu et al. showed that long-term exposure to aspartame increases the population of cancer stem cells and tumor cell invasion through p21, NICD, GLI1 (6).

In another study, with the aim of investigating the potential role of aspartame in colorectal cancer, Maghiari et al. investigated two possible mechanisms of aspartame carcinogenesis, one was a cytotoxicity screening in human colorectal cancer HT-29 cells based on the assessment of cell viability, cell morphology, and cell migration, and the other was an evaluation in eggs for angiogenic and stimulatory potential using the chorioallantoic membrane assay. The results of this study showed a dose-dependent cytotoxic effect with a significant reduction in viable cells and morphological changes at the highest cell concentrations tested (7). Therefore, the present study was conducted to investigate the negative effects of aspartame on prostate structure and function and sperm parameters in adult mice.

Methods

After approval by the Ethics Committee of Mazandaran University of Medical Sciences with the ethics code IR.MAZUMS.AEC.1403.062, this experimental study was conducted on 24 adult male albino mice weighing approximately 20-25 grams and approximately 8-10 weeks old, which were obtained from Mazandaran Animal Research Institute. The animals were kept in special cages with easy access to water and food in the animal house of Mazandaran Animal Research Institute. The ambient lighting was set to 12 hours of light, 12 hours of darkness, and the temperature was set to 23±2.

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The mice were randomly divided into four groups (6 mice in each group), which included the control group, the sham group (carrier), and the groups receiving aspartame 16 mg/kg and 32 mg/kg. The study lasted for 28 days and aspartame was administered through intraperitoneal injection. 12 hours after the last injection, the mice were anesthetized with ketamine-xylose (K, 100 mg/kg; X, 10 mg/kg), and blood samples were taken for serum separation and evaluation of LH, FSH, testosterone, and PSA hormones. After blood collection, the testes and epididymis were immediately removed by forceps and scissors by making an incision on the scrotum, and after washing with normal saline, the left testis was weighed with an accurate scale. The gonadosomatic index for the testes was determined according to the initial weight of each animal. After separating the distal part of the epididymis and placing it in a dish containing 1 cc of Ham's F10 medium, the epididymis was cut into small pieces in order to completely release the sperm into the culture medium. The Petri dish was placed in a 37°C incubator for 10 minutes to completely release the sperm from the nephron tubules. Then, various sperm parameters were evaluated, including motility, viability, number, and morphology, and finally, the prostate tissue was placed in 10% formalin for pathological evaluation (8).

Measurement of sex hormone and PSA levels: To measure sex hormone levels, 1.5 ml of blood sample was taken from the animal's heart using a heparinized syringe. Then, the blood was centrifuged at 3500 rpm to separate the serum. The serum was stored at -80°C and LH, FSH, testosterone, and PSA were measured using a standard ELISA kit (8).

Evaluation of sperm parameters:

Sperm motility: Sperm motility was measured according to the World Health Organization (WHO) guidelines. Before taking the semen sample, we homogenized and mixed it thoroughly, then placed 10 µL of sperm suspension and culture medium on a slide and placed a slide on it. We immediately evaluated it with a magnification of 40 light microscope. At least 5 microscopic fields and 200 sperm were examined in each animal, and the percentage of motile sperm was calculated. The counting was performed 3 times for each sample and the mean value was reported. Then, the percentage of motile sperm compared to the total number of sperm counted in each repetition was calculated and finally the mean number of motile sperm in each animal was determined (8).

Sperm morphology: The number of normal sperm in a sample should be more than 50% and include sperm that are morphologically free in the head, neck and tail regions. A portion of the sperm suspension was mixed with 1% eosin in a ratio of 10:1 and then smears were prepared. They were allowed to air dry and then mounted. 200 sperm per suspension and per animal were evaluated using a light microscope at 100x magnification. Types of sperm morphology defects included narrow head, headless, tapered head, bent neck, double head, double tail, bent tail, coiled tail, stump tail and tail-less sperm (8).

Sperm count: Sperm counting was performed using a hemocytometer and the guidelines provided by the World Health Organization. After preparing the appropriate dilution of the sperm suspension, approximately 10 µL of it was transferred to each chamber of Neubauer Counting Chamber and a coverslip was placed on it. After 5 minutes, we counted 200 sperm in the RBC Neubauer Chamber and calculated the number of sperm per animal based on the formula provided. All observations were made using a light microscope at 40x magnification (8).

Formula for counting sperm in RBC ⟶ $C= (N/n) \times (1/20) \times X$ sperm per nl

N= Total number of sperm counted in 3 replicates

n= Total number of rows in 3 replicates

X= Based on dilution used

Histopathological examination: Abdominal prostate samples were collected from five animals in each group. The samples were fixed by immersion in 10% formalin solution and placed in Paraplast. The embedded samples were cut into 5 cm thick sections and the sections were stained with hematoxylin-eosin,

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Gomori's silver stain and Masson's trichrome. Light microscope images of the stained slides were photographed using a digital camera and the size of the nucleus and cytoplasm and the percentage of space occupied by the nucleus and cytosol in the cell were calculated and compared in each group (9).

All statistical calculations were performed using Prism Ver. 8 using nonlinear regression. Data were compared using one-way analysis of variance (ANOVA) and the corresponding post-test (Tukey-Kramer multiple comprehension test). Graphs were drawn using the same graphics program, and $p<0.05$ was considered significant.

Results

Biochemical results of the mouse serum: The results related to the serum biochemical factors of the studied mice, which include LH, FSH, testosterone and PSA factors, were evaluated (Figure 1). The results of the study showed that the levels of LH, FSH, testosterone and PSA in the prostate tissue of the experimental groups and the sham group did not show a significant difference compared to the control group. Furthermore, aspartame at doses of 16 mg/kg and 32 mg/kg caused a significant decrease in serum biomarkers of LH hormone (6.34±0.07 and 5.14±0.05) compared to the control group (9.27±0.07) ($p<0.001$). FSH hormone (4.34±0.04 and 3.21±0.07) significantly decreased compared to the control group (6.48±0.04) ($p<0.001$). Testosterone (4.36±0.05 and 3.22±0.06) significantly decreased compared to the control group (6.08±0.04) ($p<0.001$). In addition, serum PSA levels (6.66±0.07 and 11.99±0.27) significantly increased compared to the control group (0.52±0.03) ($p<0.001$).

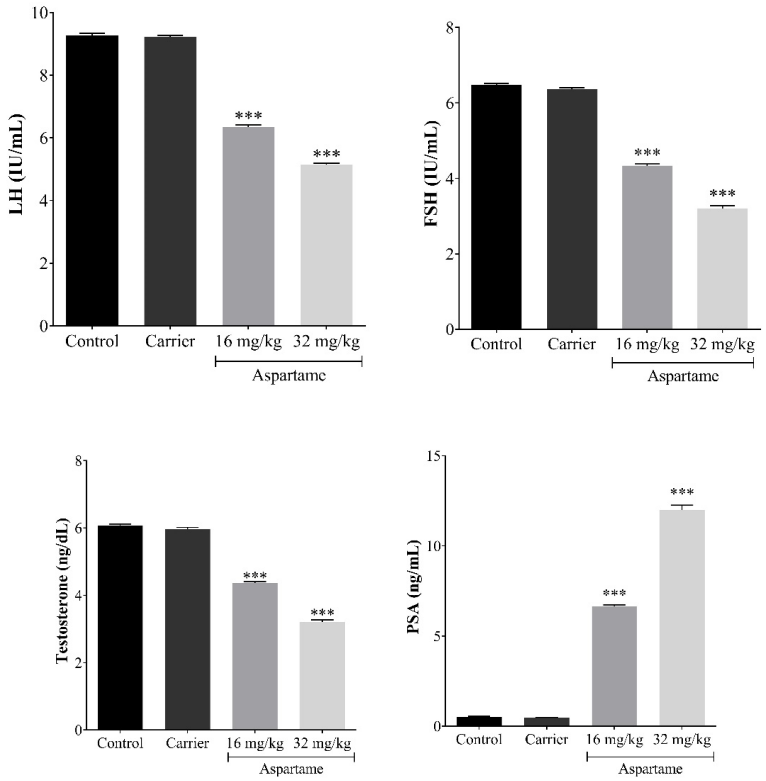

Figure 1. Effect of aspartame at concentrations of 16 and 32 mg/kg on serum LH, FSH, Testosterone, and PSA levels in mice after 28 days of intraperitoneal injection. Data are based on Mean±SD in each group (n=6). * (p<0.001): Significant compared to the control group.**

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Results related to sperm motility, morphology and number parameters: Based on the results obtained, the highest sperm count was in the control group and the lowest in the aspartame (32 mg/kg) group. Sperm motility in the group that received aspartame (32 mg/kg) was significantly reduced compared to the control group. Sperm morphology in the group that received aspartame (32 mg/kg) was also significantly increased compared to the control group, and the percentage of defective sperm increased (Table 1).

Results of histopathological studies of prostate tissue: In the group receiving aspartame mg/kg 16, the number of spermatogonia A and B cells decreased compared to the control group. Sertoli cells did not change. Spermatogenesis decreased and the prostate tubule and prostate thickness decreased. The number of prostate secretory cells decreased. Also, in the group receiving aspartame mg/kg 32, the number of spermatogonia and spermatocytes decreased significantly, and severe complications were seen in the prostate (tubule diameter and thickness). The number of prostate secretory unit cells decreased significantly (Image 1).

Table 1. Results related to sperm motility, morphology, and count parameters

Groups	Morphology	Sperm motility (%)		Sperm count × 10^6
	Percentage of abnormal sperm	Non-motile	Motile	Mean±SD
Control	14	29	71	47±5.11
Sham	15	31	69	46±4.82
Aspartame (16 mg/kg)	31	39	61	40±6.24
Aspartame (32 mg/kg)	42	43	57	36.33±4.51

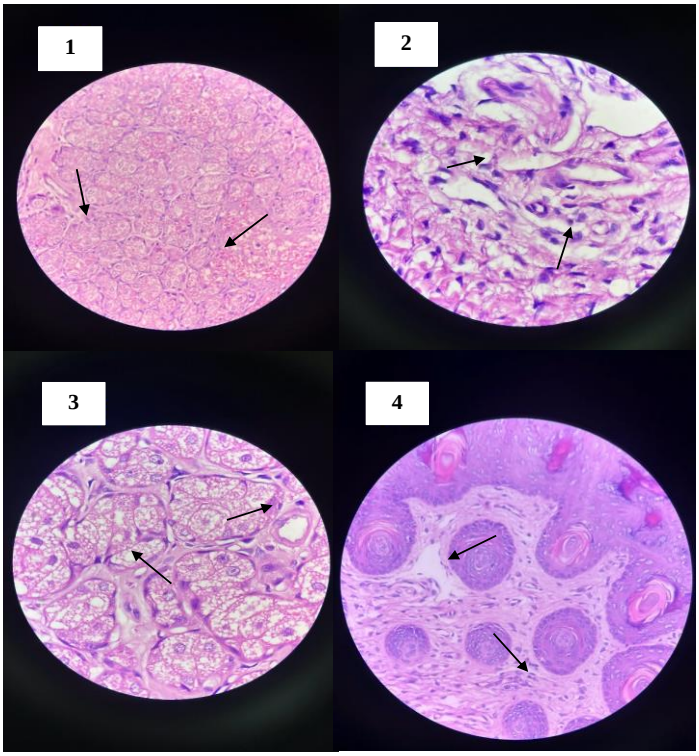

Image 1. Photomicrographs of prostate sections fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. Light microscopic examination of the tissues (objective lens 40x). 1. Tissue sections from the control group 2. Carrier or sham group 3. Aspartame 16 mg/kg, 4. Aspartame 32 mg/kg

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Discussion

The results of this study show that exposure to aspartame causes prostate tissue damage and reproductive toxicity in male rats, which is associated with a significant decrease in the concentration of pituitary-testicular axis hormones and a significant increase in serum PSA compared to the control group. Aspartame also caused histopathological damage to prostate tissue and a decrease in sperm quality, which is consistent with studies by other researchers (10, 11). The researchers attributed the toxicity of aspartame to the production of methanol and its metabolites following aspartame metabolism in the body. Methanol constitutes approximately 10% of the metabolized products of aspartame (12, 13).

The decrease in hormone concentrations observed in the aspartame-treated groups compared to the control group may be attributed to neurodegenerative changes, particularly in the hypothalamus. These changes could lead to significant functional and structural changes in the hypothalamic-pituitary axis, which resulted in decreased levels of LH, FSH, and testosterone and a significant increase in serum PSA (14). In this study, aspartame injection at doses of 16 and 32 mg/kg resulted in a significant decrease in serum levels of LH, FSH, and testosterone and a significant increase in serum PSA compared to the control group. In a study by Anbara et al. on the mechanism of aspartame-induced toxicity in the male reproductive tract following long-term use in a mouse model, the results showed that long-term administration of aspartame at high doses significantly reduced the gonadosomatic index and serum concentrations of pituitary-testicular hormones (LH, FSH, and testosterone) (10). The decrease in LH, FSH, and testosterone concentrations and the increase in PSA levels in serum could be related to the direct or indirect negative effects of aspartame consumption and the subsequent induction of oxidative stress on the pituitary-gonadal axis. Ultrastructural studies in young mice and rabbits in the studies of Puica et al. showed that chronic administration of aspartame alters the structure of gonadotropic cells of the anterior pituitary gland (15, 16).

In this study, aspartame impaired sperm-related parameters, including sperm motility, sperm count, and sperm morphology. Given the role of LH, FSH, and testosterone in spermatogenesis, the decreased sperm count in our study could be a result of decreased production of these hormones (17). A second mechanism involves methanol toxicity, the primary metabolite of aspartame. It is thought to cause direct damage to the prostate and testes by damaging the structure and function of heat shock proteins (HSPs). These proteins are critical for regulating spermatogenesis, providing cellular protection, and increasing cellular resistance to environmental stress. This damage can also manifest as significant histopathological changes, which is consistent with the results of studies by Ali et al. and Azeez et al. (14, 18). In our study, aspartame caused histopathological damage to prostate tissue. In a study by El-Alfy et al. investigating the histological effects of aspartame on testicular tissue of albino mice, histological analysis revealed significant differences in the treated groups compared to the control group. Aspartame-treated mice showed dose-dependent changes in the seminiferous tubules and interstitial spaces. Changes in the seminiferous tubules were characterized by atrophied seminiferous tubules, vacuolation, exfoliated germ cells, hypoplasia of the germinal epithelium, and spermatogenic arrest at different stages of spermatogenesis. However, interstitial changes included vascular occlusion and interstitial edema. These findings raise concerns about the potential impact of aspartame consumption on testicular and prostate histology (19). Moreover, in a study conducted by Morovvati et al. on the effect of aspartame on the histological structure and histometry of the prostate gland in adult mice, histological and histometric results showed a significant decrease in the number of cells and epithelial height of secretory units in the ventral, anterior, and dorsal lobes of the prostate in the aspartame groups compared to the control group. The percentage of parenchyma to the ventral lobe of the prostate gland in the aspartame groups showed a significant decrease compared to the control group (20), which was consistent with the results of this study.

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Overall, the results of this study indicate that aspartame exposure in mice can induce prostate toxicity by inducing oxidative stress in sperm and prostate tissue, disrupting the pituitary-testicular axis at the level of germ cells. Moreover, the production of methanol and its metabolites following aspartame metabolism in the body ultimately causes a significant decrease in the biochemical factors LH, FSH, testosterone and an increase in serum PSA, as well as causing histopathological damage to prostate tissue and a decrease in sperm quality, which indicate the dose-dependent harmful effects of aspartame. However, more clinical studies on the possible effects of long-term aspartame consumption on prostate tissue seem necessary.

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