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Evaluation of The Histological Effects of Aspartame on Testicular Tissue of Albino Mice

Nagla Zaky Ibrahim El -Alfy, Mahmoud Fathy Mahmoud
Mohamed Said Ebied Said, *Sally Ramadan Gabr Eid El-Ashry
Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, Cairo, Egypt
***Corresponding author:** Sally Ramadan Gabr Eid El-Ashry,
Email: sallyramadan@edu.asu.edu.eg, **Mobile:** +201099797341

ABSTRAT

Background: Aspartame, an artificial sweetener widely used in various food products, has raised concerns regarding its potential impact on male fertility. **Objective:** This research aimed to examine the histological effects of aspartame on testicular tissue in albino mice *Mus musculus*. **Materials and methods:** Twenty-five male albino mice (CD-1) of nearly the same age were individually weighed and randomly assigned to one of five groups: a control group received distilled water (the aspartame's solvent) orally and four treated groups received oral treatment with one of four doses of aspartame (250, 500, 750 and 1000 mg/kg body weight) once per day for one month. **Results:** Histological analysis displayed noticeable differences within the treated groups when compared to the control group. Mice treated with aspartame exhibited dose-dependent changes in seminiferous tubules and interstitial spaces. Alterations in seminiferous tubules were represented by atrophied seminiferous tubules, vacuolation, exfoliated germ cells, hypoplasia of the germinal epithelium and spermatogenic arrest at various stages of spermatogenesis. However, the intertubular changes included congested blood vessels and interstitial edema. These findings raise concerns about the potential impact of aspartame consumption on testicular histology. **Conclusion:** Current results bring attention to the necessity for additional research on the effects of aspartame on reproductive health. The observed alterations in testicular tissue of albino mice warrant further investigation into the implications for human health, especially for individuals regularly exposed to aspartame. Understanding the underlying mechanisms and long-term consequences of aspartame consumption on male reproductive health is crucial for informed decision-making regarding its use.

Keywords: Aspartame, Artificial sweetener, Testicular tissue, Testicular histology.

INTRODUCTION

With the widespread presence of aspartame in the modern diet, there is a growing interest in understanding the effects of it on various physiological systems, including its influence on testicular tissue fertility **(1)**. Aspartame is a food additive employed to enhance the sweetness of a wide range of items, including beverages, confections, cakes, chewing gum, yogurt, low-calorie and weight-management products, and even pharmaceuticals designed for oral consumption. Its inclusion in food products can be denoted either by its name or by its code E951 **(2)**.

Male fertility is a complex process that relies on the proper functioning of the male reproductive system, including the testes, where spermatogenesis takes place. Any disruption to this delicate process can have significant implications for fertility and reproduction **(3)**. Aspartame, chemically known as N-L-α-aspartyl-L-phenylalanine methyl ester, is a low-calorie artificial sweetener commonly used as a sugar substitute in various beverages, food products, and medications. Its prevalence in the modern diet and its incorporation into numerous consumables have led to concerns about its safety and potential health effects [(4)]. While aspartame is approved for consumption by regulatory authorities such as the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) [(5)], some studies have raised concerns about its safety and potential health effects [(6-8)]. However, the findings on aspartame's effects on testicular toxicity are not entirely conclusive, and further research is needed to establish a definitive link.

Some animal studies have suggested possible adverse effects on testicular function after aspartame treatment. For example, a study conducted on adult male Wistar rats exposed to aspartame showed alterations in the testicular morphology, decreased sperm count, and changes in hormone levels [(9)]. According to the study of **Hozayen *et al.***, aspartame's mode of action appears to involve the degradation and atrophy of Leydig cells in rats resulting in reduced testosterone synthesis and secretion due to the influence of formaldehyde produced from aspartame [(10)]. **Seif *et al.*** demonstrated that the treatment of rats with aspartame (1000 mg/kg body weight (b.wt.)) three times per week for 12 weeks induced severe testicular toxicity [(11)]. **Abu Tfaweel** that aspartame consumption led to decreases in red blood cells, white blood cells, testosterone levels, hemoglobin, platelet counts, and acetylcholinesterase enzyme activity [(12)].

In human studies, there is limited research on the direct impact of aspartame on testicular toxicity. However, a study in humans suggested a potential link between high consumption of beverages containing artificial sweeteners, including aspartame, and reduced sperm quality [(13)]. However, it is crucial to highlight that these studies have limitations, and more research is required to draw definitive conclusions about the effects of aspartame on testicular toxicity in humans.

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Overall, the existing literature points to the need for further investigation into the potential impact of aspartame on testicular health in animal models and humans. It is essential to conduct well-designed, controlled studies to better understand any potential risks associated with aspartame consumption and its effects on reproductive health.

In response to these concerns, current research intends to explore the histological consequences of aspartame on testicular tissue in albino mice (*Mus musculus*), providing valuable insights into its potential impact on male reproductive health.

MATERIALS AND METHODS

1-Animals:

Twenty-five mature male albino mice (CD1) of six to eight weeks old with an average body weight (25 ± 2 g) were used in this investigation. They were obtained from the closed colony of Theodor Bilharz Research Institute, Cairo. Throughout the entire duration of the experiment, the mice were maintained in animal housing facilities with appropriate conditions. They were provided with a standard rodent pellet diet and had access to water.

Ethical considerations:

Animal care and use and all the experimental procedures were approved by the Experimental Animal Care and Research Ethics Committee of Aims Shams University (approval No. sci1332307009).

2-Chemicals:

Aspartame was purchased from Sigma-Aldrich (St Louis, MO, USA, CAS No. 22839-47-0). It was dissolved in distilled water to facilitate oral administration to mice. The structural formula of aspartame is shown in **figure 1** [(14)]. The rest of the chemicals utilized were of analytical grade and obtained from commercially available sources.

All doses of aspartame examined in the study were in accordance with the human acceptable daily intake, as outlined by the World Health Organization, which ranges from 40 to 50 mg per kilogram of body weight per day. Dose conversion between human and mice were made according to allometric scaling approach described by **Nair and Jacob** [(14)].

1

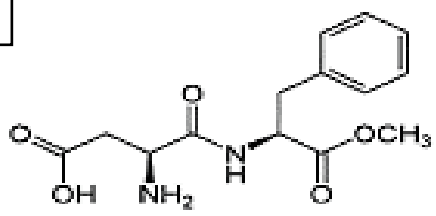

Figure 1: Structural formula of aspartame

After the mice were labeled, they were randomly distributed into five groups, each comprised of five mice. The groups receiving treatment were given aspartame through gavage for a period of 30 days, as follows:

The first group, designated as the control group, was given 1 ml of distilled water. The second group received a daily dosage of 250 mg/kg body weight of aspartame. The third group was administered 500 mg/kg body weight of aspartame per day. The fourth group received 750 mg/kg body weight of aspartame daily, and the fifth group was given 1000 mg/kg body weight of aspartame each day.

4-Histological preparation of testis:

In this study, the experiment was concluded by sacrificing the animals, then their testes were fixed in Bouin's fixation solution for a period of 24 hours. To remove excess picric acid, the testis underwent a wash in 70% alcohol mixed with a few drops of saturated lithium carbonate. Afterward, the specimens were dehydrated using a series of increasing concentrations of ethyl alcohol and then cleared in xylene. The testis specimens were subsequently embedded in pure paraffin wax (58-60º C) in an oven, undergoing three changes of wax, each lasting 30 minutes. Transverse sections of 4-6 microns were cut and mounted on clean slides, left to dry for 24 hours. The slides were then rehydrated using descending grades of ethanol and stained with hematoxylin and eosin before being mounted in DPX. Finally, the histology images were observed using a digital camera-fitted light microscope with a magnification range of 280x to 660x.

RESULTS

In the hematoxylin and eosin-stained (H&E) transverse sections of testes obtained from control group of albino mice (Fig. 2), the histological examination revealed the presence of typical and healthy features within the seminiferous tubules. These tubules were notably densely packed, signifying an abundance of spermatogonial cells and Sertoli cells, indicative of an actively occurring spermatogenesis process. Furthermore, numerous spermatids were prominently visible, which is a clear indication of the ongoing and well-established maturation of sperm within these tubules.

Beyond the seminiferous tubules, the interstitial tissue was observed to be intact and displayed normal characteristics. This included the presence of normal and functional blood vessels, which contribute to the proper transport of nutrients, hormones, and other essential components necessary for the normal functioning of the testes. Overall, the histological findings in the control group depicted a state of reproductive health, with active sperm production and an unremarkable interstitial tissue structure.

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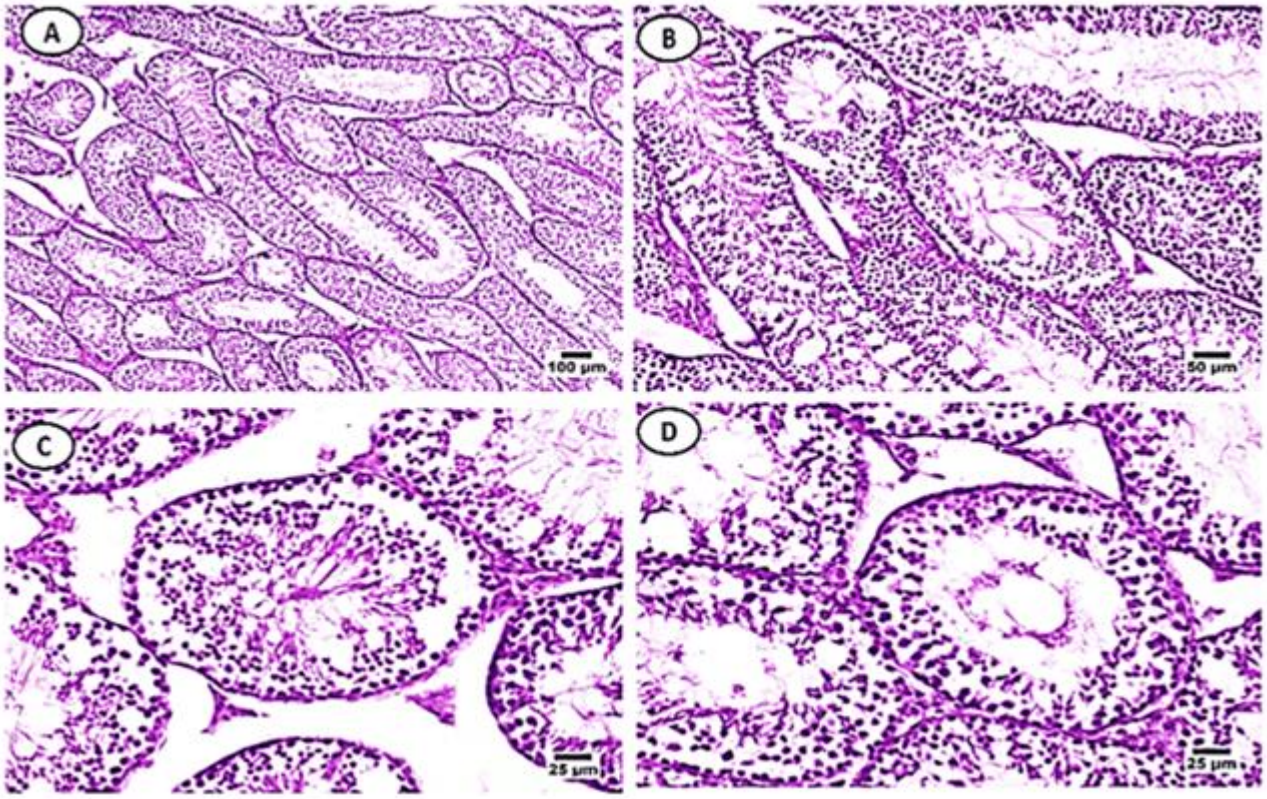

Figure 2: Cross-sections of the testes of first (control) group showing normal seminiferous tubules (H&E).

In the second group (Fig. 3), a range of histopathological alterations was discernible in the testicular tissue. Among these changes, there was a variable number of seminiferous tubules that appeared structurally normal. However, a notable feature was the presence of atrophied seminiferous tubules with irregular outlines. Vacuolation was observed in some seminiferous tubules, and a few germ cells were seen exfoliating into the tubule lumens. Additionally, interstitial edema, a condition characterized by fluid accumulation in the spaces between the tubules, was evident in certain examined sections.

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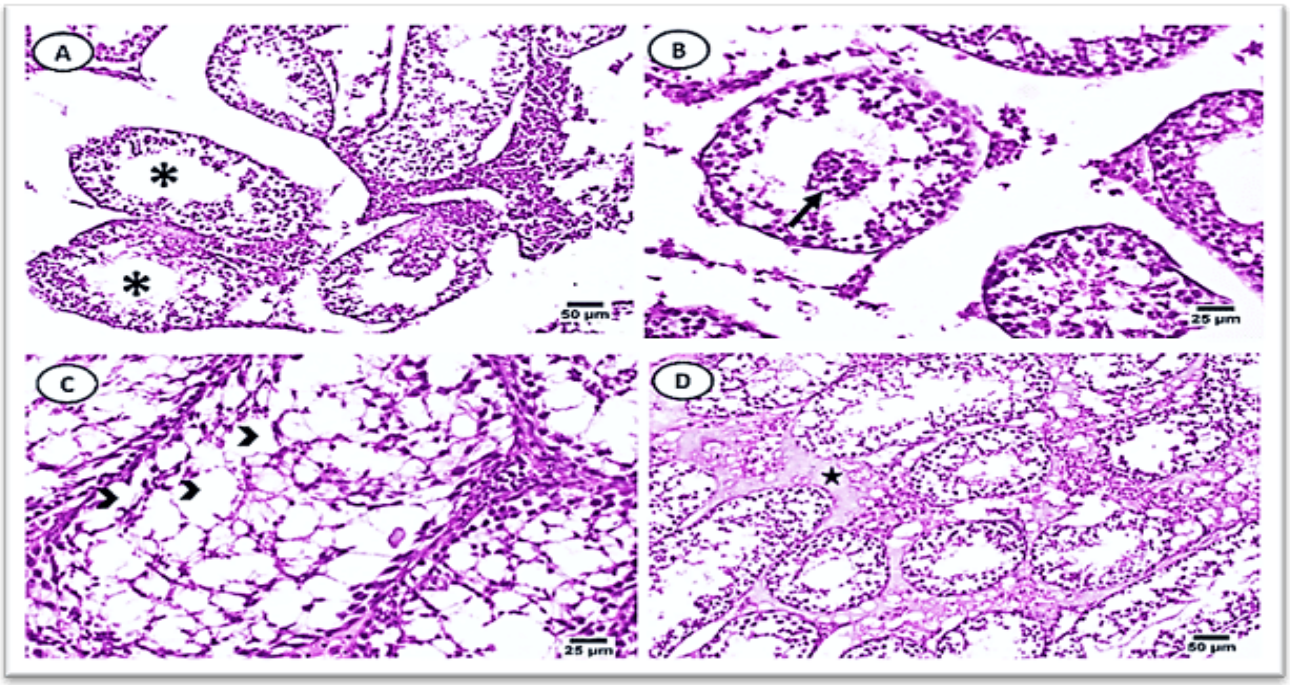

Figure 3: Cross-sections of the testes of second group treated with aspartame (250 mg /kg b.wt.) showing A) Empty seminiferous tubules wide lumen (∗). B) Vacuolation of seminiferous tubule with exfoliated germ cells. C) Vacuolation of seminiferous tubule (arrowheads). D) Interstitial edema (star). (H&E).

Within the third group (Fig. 4), the histopathological findings were more pronounced. Atrophied seminiferous tubules were frequently detected among the affected testicular tissue. These atrophied tubules exhibited structural abnormalities and compromised function. Congested blood vessels were also observed within the interstitial tissue, indicative of circulatory disturbances. Despite these changes, some sections still displayed seminiferous tubules that appeared to be structurally normal.

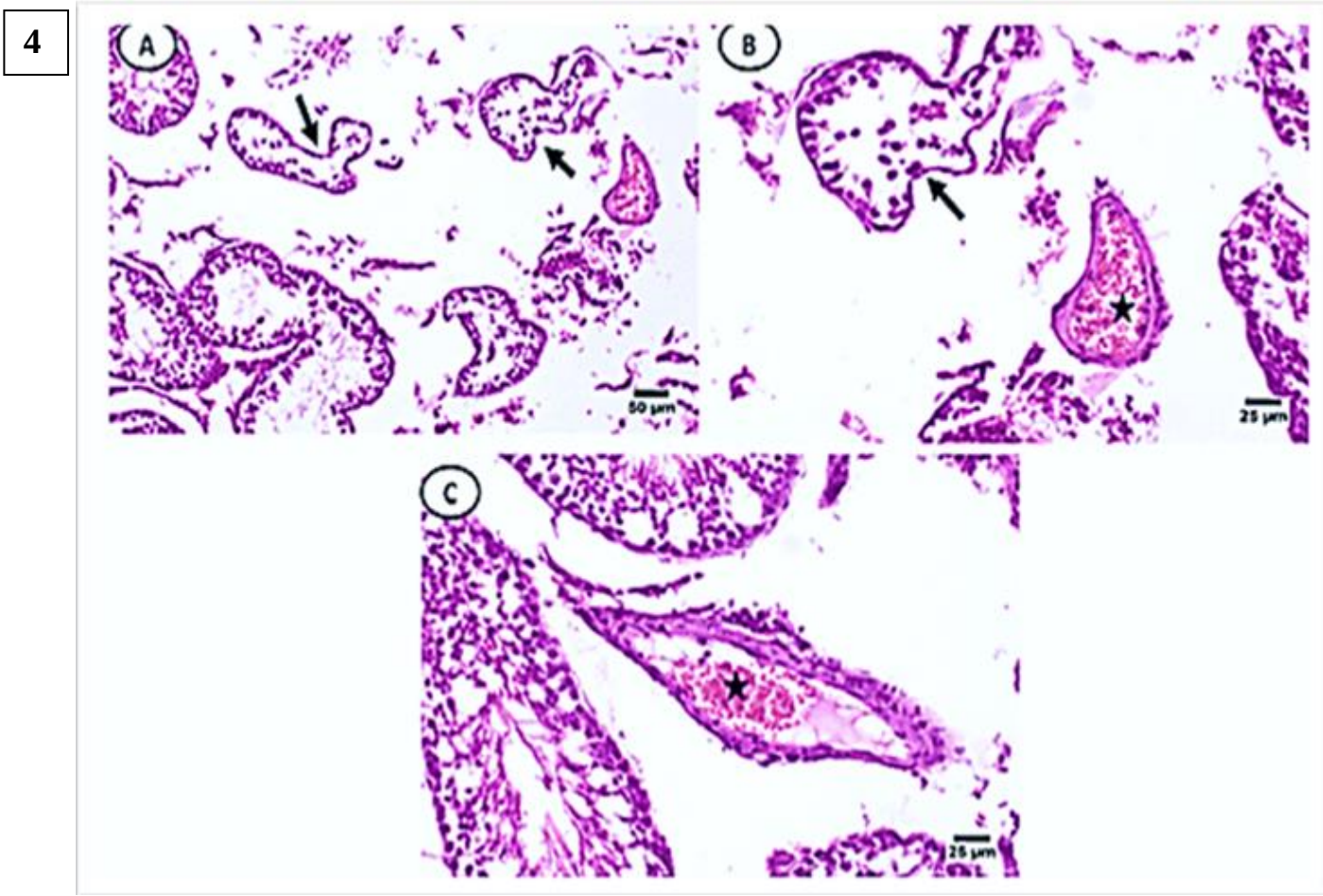

Figure 4: Cross-sections of the testes of third group treated with aspartame (500 mg /kg b.wt.) showing A) Marked atrophy of seminiferous tubules (arrows) with congested interstitial blood vessel. B) Marked atrophy of seminiferous tubule with wrinkled basement membrane (arrow) with congested interstitial blood vessel. C) Congested interstitial blood vessel (star) (H&E).

The fourth group (Fig. 5) displayed even more severe pathological alterations in the testicular tissue. Notably, there was a higher prevalence of seminiferous tubular degeneration, marked by empty lumens within the tubules. This degeneration indicated a significant disruption in the normal process of sperm production. Additionally, sloughing of germ cells into the lumens of seminiferous tubules was frequently observed, further suggesting impaired testicular function.

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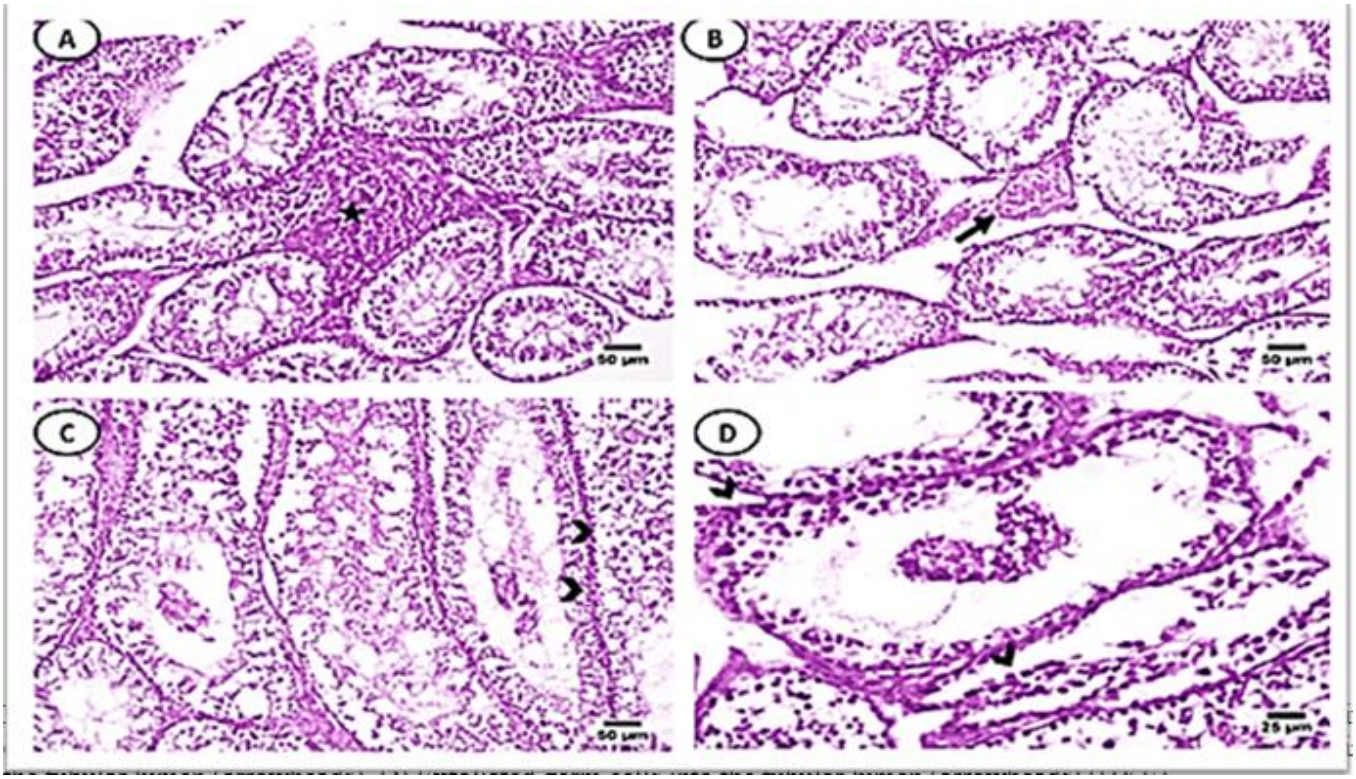

Figure 5: Cross-sections of the testes of fourth group treated with aspartame (750 mg /kg b.wt.) showing A) Interstitial orchitis (star). B) Testicular degeneration with congested interstitial blood vessel (arrow). C) Exfoliated germ cells into the tubular lumen (arrowheads). D) Exfoliated germ cells into the tubular lumen (arrowheads) (H&E).

Finally, the fifth group (Fig. 6) exhibited the most severe testicular degeneration among all groups. Widespread testicular degeneration was evident throughout the tissue samples. Abundant interstitial edema was observed in many examined sections, indicating extensive fluid accumulation in the interstitial spaces. This edema can disrupt normal testicular function. Furthermore, the sloughing of germinal epithelium into the seminiferous tubule lumens indicated a severe disruption in sperm production and overall testicular health.

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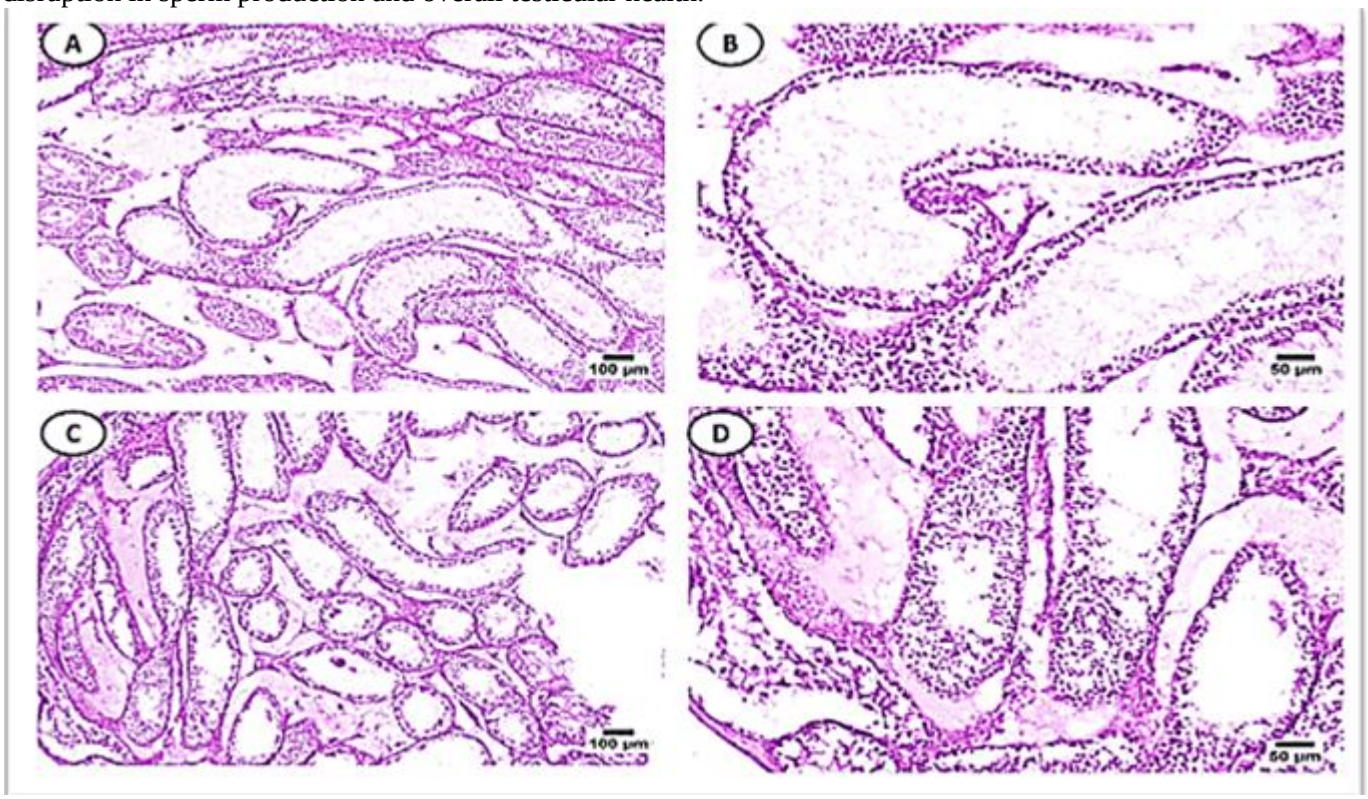

Figure 6: Cross-sections of the testes of fifth group treated with aspartame (1000 mg /kg b.wt.) showing A and B) Testicular degeneration. C and D) Diffuse testicular degeneration with excessive interstitial edema (H&E).

DISCUSSION

The current histological examination of testes from albino mice provided significant perspectives on the influence of aspartame on testicular tissue. Several studies showed that evaluating the histomorphometric parameters of testis is a suitable method for assessing the degree of injury or damage to this organ [15,16].

Current results showed that the control group (Fig. 2) exhibited the normal histological features of seminiferous tubules. These tubules were densely packed, indicating an abundance of spermatogonial cells and Sertoli cells, which are crucial components of active spermatogenesis [17]. Numerous spermatids were also present, confirming the ongoing maturation of sperm within these tubules. Furthermore, the interstitial tissue displayed normal characteristics, including intact and functional blood vessels, essential for nutrient and hormone transport in support of normal testicular function. This is confirmed by the findings of recent studies, which showed that testis is composed of multiple seminiferous tubules, which have a rounded or oval appearance [18,19]. Each tubular structure is surrounded by a thin basement membrane, and on the outer side, it is further enclosed by fibrous connective tissue. The gaps between the seminiferous tubules contain an interstitial tissue stroma.

In contrast, the experimental groups exposed to varying doses of aspartame (250, 500, 750, and 1000 mg/kg b.wt.) once per day for one month demonstrated a range of histopathological alterations in their testicular tissue. These changes included atrophied seminiferous tubules, irregular outlines, vacuolation, sloughing of germ cells, and interstitial edema. Notably, the severity of these alterations appeared to correlate with the dose of exposure. These results are consistent with the histological observations of **Anbara and colleagues** [20] who revealed that aspartame has the potential to induce disarray and significant edema in the interstitial connective tissue in a dosage-dependent fashion. Also, **Anbara *et al.*** [21] suggested that prolonged aspartame consumption can lead to adverse effects on male reproductive health and a reduction in the levels of hormones related to the pituitary-testis axis, as well as the initiation of oxidative stress and apoptosis in the testes. Recent studies suggested that the toxic effects of aspartame on testes might be attributed to methanol intoxication, which leads to an elevation in lipid peroxidation [22,23].

In the second group (aspartame, 250 mg/kg b.wt. once per day for one month), while some seminiferous tubules remained structurally normal, the presence of atrophied tubules with irregular outlines and vacuolation indicated early pathological changes. These findings are consistent with recent studies reporting histopathological alterations in testicular tissue due to environmental toxins and radiation exposure [24,25]. On the other hand, the results of this study are in clear contrast to the safety and usage recommendations put forth by the European Union. These recommendations pertain to sweeteners such as acesulfame-K, aspartame, cyclamates, saccharin, sucralose, neohesperidin DC (NHDC), neotame, the salt of aspartame-acesulfame, and advantame, all of which have been officially approved as safe for consumption with established acceptable daily intake (ADI) levels [26].

The third group (aspartame, 500 mg/kg b.wt. once per day for one month) exhibited more pronounced histopathological findings, including a higher prevalence of atrophied seminiferous tubules. The presence of congested blood vessels in the interstitial tissue indicated circulatory disturbances, which can impact testicular function [27]. Despite these changes, some tubules still appeared structurally normal, suggesting a dose-dependent response to the experimental condition.

The fourth group (aspartame, 750 mg/kg b.wt. once per day for one month) displayed even more severe pathological alterations, with a higher prevalence of seminiferous tubular degeneration characterized by empty lumens, indicating disrupted sperm production. Frequent sloughing of germ cells into tubule lumens further underscored the impairment in testicular function [28]. These findings align with recent research demonstrating the vulnerability of testicular tissue to various stressors, leading to impaired spermatogenesis [29,30].

Finally, the fifth group (aspartame, 1000 mg/kg b.wt. once per day for one month) exhibited the most severe testicular degeneration, with widespread tissue damage and abundant interstitial edema. The prominent sloughing of germinal epithelium into tubule lumens indicated a severe disruption in sperm production and overall testicular health. These results align with the previous study of **Seif *et al.*** [11], which demonstrated that aspartame induces rat testicular toxicity at dose 1000 mg/kg when administered three times a week for a period of 12 weeks.

CONCLUSION

In conclusion, the histopathological findings in this study provide evidence of dose-dependent testicular damage in albino mice. These results underscore the vulnerability of testicular tissue to environmental stressors and toxic agents, which can lead to impaired spermatogenesis and testicular dysfunction. Understanding these histological changes is crucial for assessing the reproductive health risks linked to exposure to harmful agents and substances.

- **Sponsoring financially:** Nil.
- **Competing interests:** Nil.

REFERENCES

1-Magnuson B, Burdock G, Doull J *et al.* (2007): Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. Critical Reviews in Toxicology, 37(8): 629-727.

2-Shaher S, Mihailescu D, Amuzescu B (2023): Aspartame safety as a food sweetener and related health hazards. Nutrients, 15(16): 3627.

3-Sharpe R (2010): Environmental/lifestyle effects on spermatogenesis. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1546): 1697-1712.

4-Butchko H, Stargel W, Comer C *et al.* (2002): Aspartame: review of safety. Regulatory Toxicology and Pharmacology, 35(2): S1-S93.

5-Czarnecka K, Pilarz A, Rogut A *et al.* (2021): Aspartame—true or false? Narrative review of safety analysis of general use in products. Nutrients, 13(6):1957.

6-Otabe A, Ohta F, Takumi A *et al.* (2019): Mutagenicity and genotoxicity studies of aspartame. Regul. Toxicol. Pharmacol., 103:345-351.

7-Kirkland D, Gatehouse D (2015): Aspartame: A review of genotoxicity data. Food Chem. Toxicol., 84:161-168.

8-Desbonnet L, Clarke G, Shanahan F *et al.* (2014): Microbiota is essential for social development in the mouse. Mol. Psychiatry, 19(2):146-148.

9-Ashok I, Sheeladevi R (2012): Biochemical responses and mitochondrial mediated activation of apoptosis on long-term effect of aspartame in rat brain. Redox Biology, 1(1): 1-5.

10-Hozayen W, Soliman H, Desouky E (2014): Potential protective effects of rosemary extract, against aspartame toxicity in male rats. J. Inter. Acad. Res. Multidisc., 2(6): 111-125.

11- Seif H, Hozayen W, Solimana H (2014): Study of the chemopreventive effects of Zingiber Officinale roots against aspartame induced testicular toxicity in rat model. Journal of Physiology and Pharmacology Advances, 4(5):360.

12-Abu Tfaweel G (2016): Effect of monosodium glutamate and aspartame on the behavioral and biochemical parameters of albino mice. African J. Biotech., 15(15):601–612.

13-Xu X, Tian D, Hong X *et al.* (2018): Consumption of artificial sweeteners and its effect on male fertility: A case-control study. Medicine, 97(48): e13302.

14- Amchra F, Faiz C, Chaouqi S, *et al.* (2018): Effect of Stevia rebaudiana, sucrose and aspartame on human health: A comprehensive review. Journal of Medicinal Plants Studies, 6(1): 102-108.

15-Nair A, Jacob S (2016): simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm., 7(2):27-31.

16-Gong T, Wei Q, Mao D *et al.* (2016): Effects of daily exposure to saccharin and sucrose on testicular biologic functions in mice. Biology of Reproduction, 116: 1–13.

17-Asri-Rezaei S, Nourian A, Shalizar-Jalali A *et al.* (2018): Selenium supplementation in the form of selenium nanoparticles and selenite sodium improves mature male mice reproductive performances. Iran J. Basic Med. Sci., 21(6):577–585.

18-Hutson J, Li R, Southwell B *et al.* (2013): Germ cell development in the postnatal testis: the key to prevent malignancy in cryptorchidism? Front Endocrinol. (Lausanne), 3:176.

19-El-Alfy N, Isa M, Mahmoud M *et al.* (2013): The protective role of the royal jelly against histological effects of endoxan drug on the testis of the male albino mice. New York Sciences Journal, 6(1):96-101.

20-El -Alfy N, Mahmoud, M, Abdullah A (2016): Mitomycin-C induced genetoxic effect in lymphocytes and histological alterations in testes of male albino mice. The Egyptian Journal of Hospital Medicine, 62: 18 – 27.

21-Anbara H, Sheibani M, Razi M (2020): Long-Term Effect of Aspartame on Male Reproductive System: Evidence for Testicular Histomorphometrics, Hsp70-2 Protein Expression and Biochemical Status. Int J Fertil Steril., 14(2):91-101.

22-Anbara H, Sheibani M, Razi M *et al.* (2021): Insight into the mechanism of aspartame-induced toxicity in male reproductive system following long-term consumption in mice model. Environmental Toxicology, 36(2): 223-237.

23-Parthasarathy N, Kumar R, Manikandan S *et al.* (2006): Methanol-induced oxidative stress in rat lymphoid organs. J. Occup Health, 48(1):20–7.

24-Saoudi M, Ben Hsouna A, Trigui M *et al.* (2012): Differential oxidative stress responses to methanol in intraperitoneally exposed rats: ameliorative effects of Opuntia vulgaris fruit extract. Toxicol Ind. Health, 28(6):549–59.

25-Sharpe R, Skakkebaek N (1993): Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? The Lancet, 341(8857): 1392-1395.

26-Amann R, Veeramachaneni D (2007): Cryptorchidism in common eutherian mammals. Reproduction, 133(3):541-61.

27-Kearns M, MacAindriu F, Reynolds C (2022): The impact of non-caloric sweeteners on male fertility: A systematic review and narrative synthesis in rodent models. Frontiers in Nutrition, 9: 854074.

28-Auharek S, de França L, Hermo L (2007): Cellular events and molecules involved in the differentiation of the testicular spermatids. The Journal of Basic and Applied Zoology, 48(4): 191-200.

29-Saez J (1994): Leydig cells: Endocrine, paracrine, and autocrine regulation. Endocrine Reviews, 15(5): 574-626.

30-Lahousse S, Wallace D, Liu D *et al.* (2006): Testicular gene expression profiling following prepubertal rat mono-(2-ethylhexyl) phthalate exposure suggests a common initial genetic response at fetal and prepubertal ages. Toxicol Sci., 93(2): 369-381.

31-Telisman S, Cvitković P, Jurasović J *et al.* (2000): Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ Health Perspect., 108(1):45-53.