Egyptian Journal of Cell and Tissue Research
Print ISSN: 2812-5436 / Online ISSN: 2812-5444

Ameliorative Effect of Vitamin C against Aspartame Induced Histological Changes in Testis of Adult Male Albino Rats

Nehal Farid Tawfik El-Helbawy

Anatomy & Embryology Lecturer, Faculty of Medicine Tanta University, Zifta, Egypt.

Abstract

Background: Vitamin C is a widely used antioxidant. Aspartame has been used in diet and beverages to avoid obesity. Aspartame is associated by multiple disorders multiple disorders. **Aim of the work:** To clarify the ameliorative effect of vitamin C against aspartame induced histological changes in testis of adult male albino rats using light and electron microscopic examination. **Materials and methods:** Twenty four adult male albino rats were divided into three groups (8 rats each): Group I (control): were given 2 ml distilled water daily orally. Group II (Aspartme group): were given 40 mg/kg body weight of ASP once daily dissolved in distilled water by gavage needle. Group III (Aspartame and vit. C group): were given 40 mg/kg body weight ASP plus 150 mg/kg body weight of vit C once daily dissolved in distilled water by gavage needle. All rats were given treatment for 90 consecutive days then sacrificed. **Results:** Group II revealed a highly significant decrease in body weight compared with group I. Group III showed a significant decrease in body weight compared with group I. Group II and group III showed a non significant difference in body weight. Histopathological changes of testes were noticed in group II. Group III revealed improvement in the structure of the testes. **Conclusion:** This study highlights that Vitamin C is able to improve the histological changes induced by aspartame on the testes and also ameliorate spermatogenesis.

Key words:

Aspartame, Vitamin C

1. Introduction

Overweight and obesity may lead to many disorders. Diabetes mellitus, cardiovascular diseases, Alzheimer's disease, musculoskeletal diseases, depression and cancer may follow obesity [1].

One of the most common causes of overweight and obesity is high sugar intake. So, several people replace dietary sugar with low or non-caloric sweeteners [2].

Two types of sweeteners are present either nutritive or non-nutritive. The nutritive sweeteners are known as natural sweeteners like sucrose, fructose, and Stevia. Five non-nutritive sweeteners (aspartame, neotame , saccharin, acesulfame potassium and sucralose) are used [3].

Aspartame (ASP) has different trade names such as diet sweet, Nutra Sweet and canderel. Only a small amount of aspartame can give the same level of sweetness as large amount of sucrose as it is sweeter 200 times more than sucrose. Therefore it lowers the caloric intake. It is also used in different products such as soft drinks, multi-vitamins, desserts, breakfast cereals, and tabletop sweeteners [4,5].

Aspartame is a methyl ester of L-phenylalanine and L-aspartic acid. It is hydrolyzed and absorbed in the gastrointestinal tract. Its digestion leads to formation of phenylalanine, aspartate, and methanol which are toxic components affecting organs of the human body such as spleen and lymph nodes. These organs are injured due to increased oxidative stress consequently, low immunity. Formaldehyde is the metabolite of methanol in the liver and then transformed into formic acid. These metabolites are harmful to liver cells [6,7].

Dietary antioxidants, including vitamins showed their effectiveness against free radicals and oxidative stress. Therefore, they can protect the different body organs. Vitamin C is one of the natural antioxidants that protects organs against tissue damage results from oxidative stress [8]. So, the present research was made in order to clarify the ameliorative effect of vitamin C against aspartame induced histological changes in testis of adult male albino rats.

2. Materials And Methods:

Animals:

Twenty four (24) adult male albino rats weighed (180-220 grams each) were involved in this experiment. They were collected from animal house of faculty of medicine,Tanta University, Egypt.

They were kept under the same environmental conditions. Standard laboratory diet & water were available ad libitum. This animal experiment was approved by the local ethical committee of the Faculty of Medicine, Tanta University, Egypt (Approval number: (36264PR402/10/23)

Experimental Design:

Rats were divided into three groups (8 rats each):

Group I (control):

Animals of this group received 2 ml distilled water daily orally by gavage needle for 90 consecutive days until the end of the study then sacrificed.

Group II (ASP treated group):

Animals of this group received 40 mg/kg body weight of ASP once daily dissolved in distilled water by gavage needle for 90 consecutive days then sacrificed [9].

Group III (ASP and vit. C treated group):

Animals of this group received 40 mg/kg body weight ASP concomitantly with 150 mg/kg body weight of vit C once daily dissolved in distilled water by gavage needle for 90 consecutive days then sacrificed [9].

Chemicals:

Aspartame was purchased from Sigma Chemical Product Co. (Quesna, Menoufia, Egypt).

Vitamin C was purchased from Hikma Pharma SAE company (6th October City, Egypt).

Measurement of body weight

Rats were weighed 2 times; at the onset of the experiment and after 90 days before scarification. After that, animals from all groups were anaesthetized with chloral hydrate then sacrificed.

Tissue preparation and examination:

Specimens from testes were extracted and processed for light and ultrastructural study.

Sample Preparation and Examination

The specimens of testes of different subgroups were extracted. The right one was fixed in 10% formol saline for light microscopic examination. The left one was fixed in 3% glutaraldehyde in 0.1 phosphate buffer solution for transmission electron microscopic examination [10]. Hematoxylin and Eosin staining was used for light microscopic examination of testicular tissue [11].

Regarding light microscopic examination, all specimens were examined and photographed using Olympus Light Microscopes with an attached camera in the Histology Department, Faculty of Medicine, Tanta University, Egypt.

Regarding electron microscopic

examination, the specimens were inspected and captured at Tanta University, Electron Microscopy Unit, using a JEOL-JEM-100 SX electron microscope.

Morphometric study

ImageJ software was used to measure the mean thickness of the germinal epithelium in Hx&E stained sections. For different subgroups, 10 non-overlapping randomly selected fields from images photographed from each slide of each rat of each group were obtained at 200 magnification (Fig. 1) [12,13].

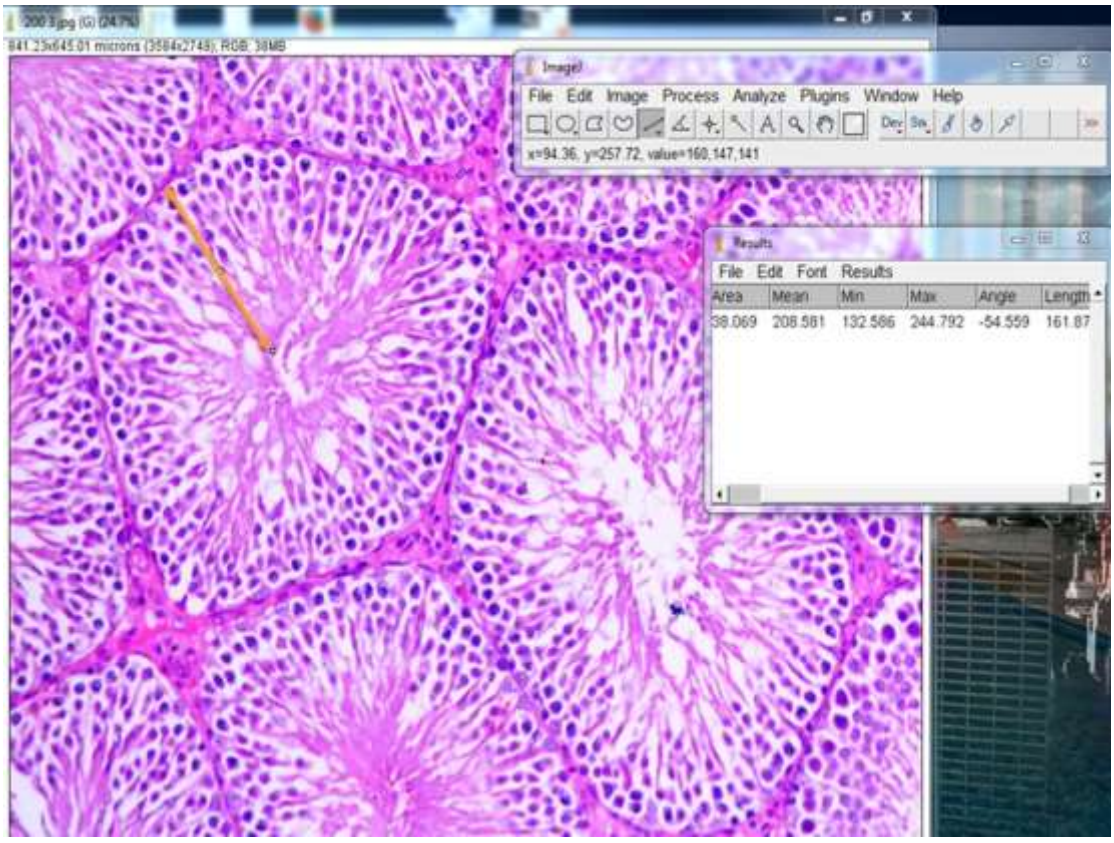

Fig. 1: A photomicrograph showing the mean thickness of the germinal epithelium measurement using Image J software. For each group, values were obtained from the image (Hx & E) at 200 magnifications.

Statistical Analysis:

Data collected of the mean body weight and the germinal epithelium thickness were statistically analyzed using ANOVA single factor test. This was followed by T test two samples assuming equal variances for comparison between the groups. The mean, the standard deviation (S.D) and the (P) value were calculated using Statistical Package for the Social Sciences (SPSS, version 20). Differences were regarded as significant if P-value <0.05 * &** Highly significant if P value ≤ 0.001).

3. Results:

Body weight measurements :

The mean initial body weight of rats from all groups ranged from 180 to 220 gm. Statistical analysis of the final body weight at time of scarification revealed a highly significant decrease in the mean body weight of rats from group II (ASP treated group) as compared with group I (control group). On the other hand, group III (ASP &vit. C treated) displayed a significant decrease in the means of their body weight when compared with control group. A significant decrease was noticed in the mean body weight of rats from group III (ASP & vit. C treated) as compared with group I (control group). Rats from group II (ASP treated group) showed non significant difference in the mean body weight in comparison with group III (ASP and vit. C treated).

Table 1 The final body weight (gm) of rats from all groups at scarification time.

Groups	**Final body weight (g)**		**ANOVA**	
	Range	**Mean±SD**	**F**	**P Value**
Group I (control)	**265-310**	**284±16.142**	**11.6068**	**<0.001**
Group II (ASP treated)	**239-270**	**251.375±12.960**		
Group III (ASP and vit. C treated)	**243-280**	**258.125±13.590**		
T test:				
P Value	Groups I and II	Groups II and III		
	Groups I and III			
	<0.001**	0.326		
	<0.05*			

ANOVA, Analysis of variance. P>0.05, no significant difference.

* P≤0.05, significant difference. **P≤0.001, highly significant difference.

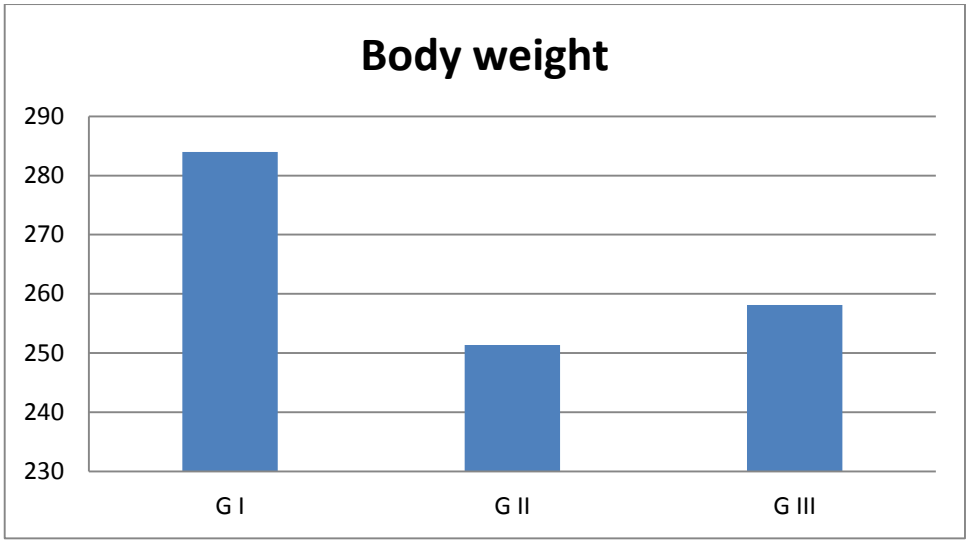

Fig. (2): A histogram showing the final body weight of rats from all groups at scarification time.

Morphometric study:

1- Mean of Germinal Epithelium Thickness:

The current study revealed revealed a highly significant decrease in the mean germinal epithelium thickness of rats from group II (ASP treated group) as compared with group I (control group). On the other hand, group III (ASP &vit. C treated) displayed a significant decrease in the mean germinal epithelium thickness when compared with (group I) control group. Non significant difference in the mean germinal epithelium thickness was noticed in the mean body weight of rats from group III (ASP & vit. C treated) as compared with group I (control group).

Table 2: Mean germinal epithelium thickness in all groups

Groups	**Mean thickness of the germinal epithelium (µm)**		**ANOVA**	
	Range	**Mean±SD**	**F**	**P Value**
Group I (control)	**109.848-183.945**	**139.9465±22.135**	**10.020**	**<0.001**
Group II (ASP treated)	**78.222-108.229**	**99.4077±14.288**		
Group III (ASP and vit. C treated)	**78.928-162.109**	**125.5605±23.884**		
T test:				
P Value	Groups I and II <0.001**	Groups II and III <0.05*	Groups I and III 0.179	

ANOVA, Analysis of variance. P>0.05, no significant difference.
* P≤0.05, significant difference. **P≤0.001, highly significant difference.

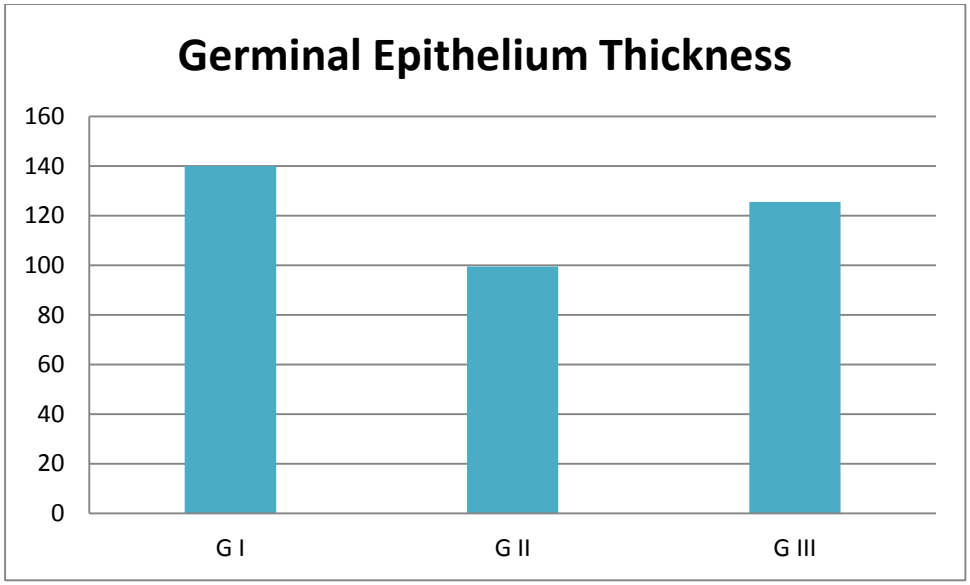

Fig. (3): A histogram showing mean germinal epithelium thickness in all groups.

Light microscopic examination of the testis:

Hematoxylin and Eosin stained sections:

Examination of testis sections from the control group showed the normal shape and structure of seminiferous tubules with the interstitial Leydig cells. The lumen of the tubules was filled with spermatozoa. Normal spermatogenic cells; spermatogonia resting on the regular basement membrane, primary spermatocytes and spermatids were noticed. Also, Sertoli cells were seen Fig. 4 (A, B & C).

While, group II (ASP treated) showed distorted seminiferous tubule with wrinkled basement membrane and wide interstitial spaces. Congested interstitial blood vessel were also seen. Empty seminiferous tubules with wide lumen and germinal epithelium dislocation. Areas of focal loss of germinal epithelium and exfoliated spermatogenic cells were seen. Homogenous acidophilic material in the interstitial tissue and vacuolation were noticed. Spermatogonia containing pyknotic nuclei and vacuolated cytoplasm with wide separation between the spermatogenic cells were also seen Fig. 5 (A, B, C, D & E).

Regarding group III (ASP and vit. C treated), Hx & E stained sections of the testis showed improvement of the testicular tissue in comparison with group II and revealed restoration of the shape of the seminiferous tubules with normal different spermatogenic cells and spermatozoa were seen filling the lumen of the tubule. Spermatogonia were seen resting on the regular basement membrane. Focal widening of intercellular spaces and vacuolation of interstitium were noticed. Primary spermatocytes, spermatids and Sertoli cells were seen Fig. 6 (A & B).

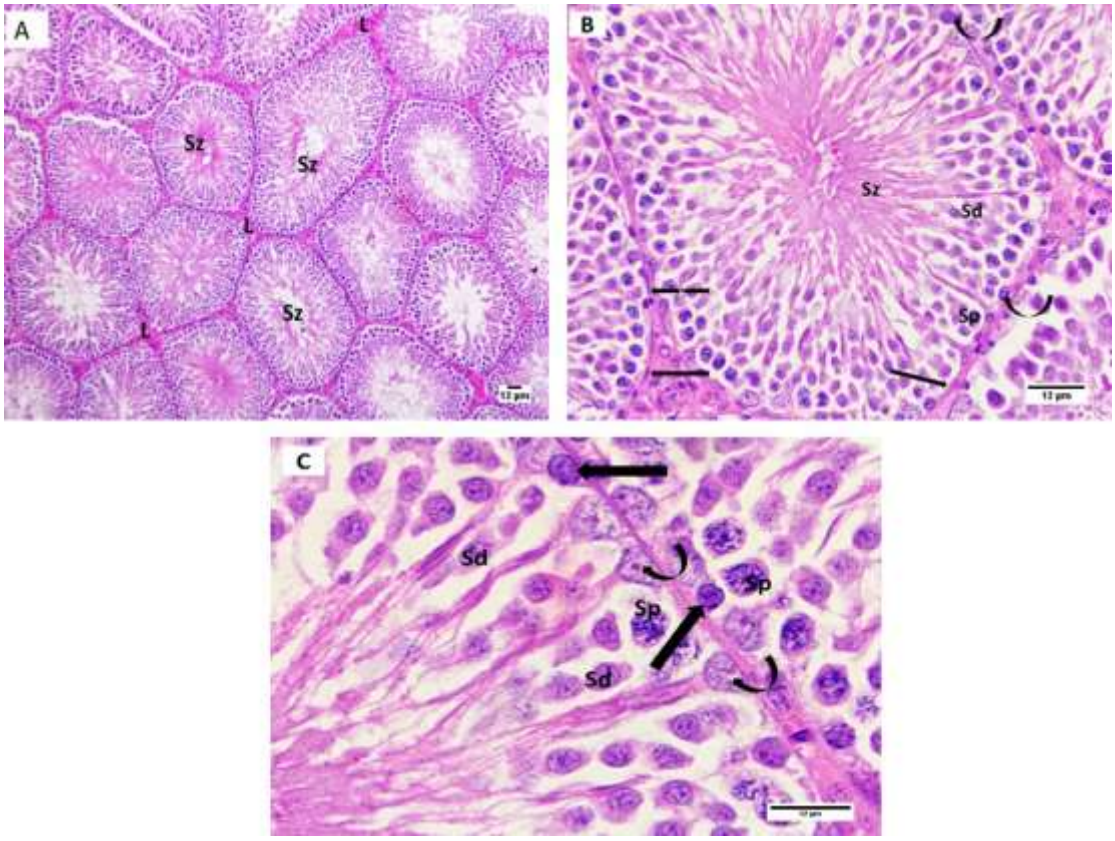

Fig. (4): Testis Hx. & E

Photomicrographs of cross sections of the testis of group I (control) ahowing: (A): The normal shape and structure of seminiferous tubules in cut section with the interstitial Leydig cells (L). The lumen of the tubules is filled with spermatozoa (Sz) (A: Hx&E x100). (B): Normal spermatogenic cells are seen. Spermatogonia (curved arrows) are resting on the regular basement membrane (arrows). Primary spermatocytes (Sp) and spermatids (Sd) are also present. Spermatozoa (Sz) are seen filling the lumen of the tubule (B: Hx&E x400). (C): Spermatogonia (arrows), primary spermatocytes (Sp), spermatids (Sd) and Sertoli cells (curved arrows) are noticed (C: Hx&E x1000).

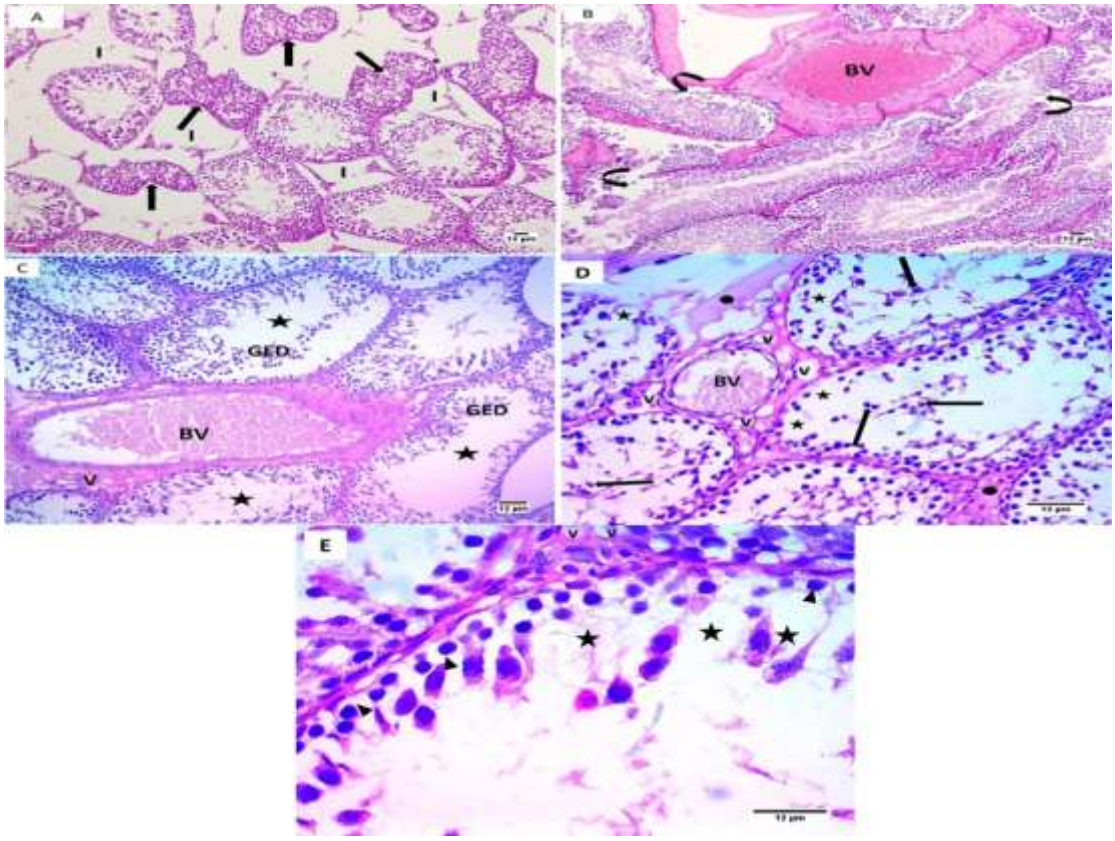

Fig. (5): Testis Hx. &E

Photomicrographs of cross sections of the testis of group II treated with aspartame showing (A): Distorted seminiferous tubule with wrinkled basement membrane (arrows) with wide interstitial spaces (I). (B): Distorted seminiferous tubule with disrupted basement membrane (curved arrows) and congested interstitial blood vessel (BV) are also seen (A,B: Hx&E x100). (C): Empty seminiferous tubules with wide lumen (stars), germinal epithelium dislocation (GED) and dilated congested interstitial blood vessels (BV) are seen (C: Hx&E x200). (D): Areas of focal loss of germinal epithelium (stars) and exfoliated spermatogenic cells (arrows) are seen. Homogenous acidophilic material (circle) in the interstitial tissue inbetween the seminiferous tubules with congested blood vessel (BV) are noticed (D: Hx&E x400). (E): Spermatogonia containing pyknotic nuclei and vacuolated cytoplasm (arrow heads) with wide separation between the spermatogenic cells are also seen (stars). The interstitium shows vacuolation (v) (E: Hx&E x1000).

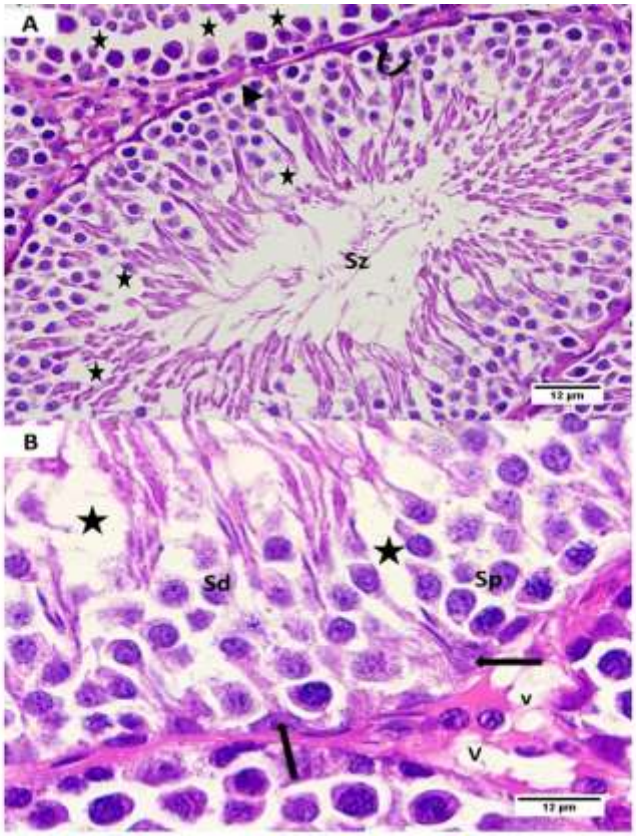

Fig. (6): Testis Hx. &E

Photomicrographs of cross sections of the testis of group III treated with aspartame and vit. C showing (A): Restoration of the shape of the seminiferous tubules with normal different spermatogenic cells. Spermatogonia (curved arrow) are seen resting on the regular basement membrane (arrow head). Focal widening of intercellular spaces (stars) is noticed. Spermatozoa (Sz) are seen filling the lumen of the tubule (A: Hx&E). (B): Primary spermatocytes (Sp), spermatids (Sd) and Sertoli cells (arrows) are seen. Focal widening of intercellular spaces (stars) is seen. Vacuolation of interstitium is noticed (B: Hx&E x1000).

Electron microscopic examination of the testis:

Examination of the ultrastructural sections of the testis of the control group showed spermatogonia with rounded nuclei resting on normal basement membrane. Also, primary spermatocytes and Sertoli cells were seen containing large euchromatic nuclei with prominent nucleolei and mitochondria. Spermatids containing peripheral mitochondria and euchromatic nuclei with the acrosomal cap extending over the anterior pole of the nuclei were noticed. Sections of middle pieces of spermatozoal tails showed an axoneme, nine outer dense fibers, circumferentially arranged mitochondria and flagellar membrane Fig. 7 (A, B & C).

While, group II (ASP treated) showed spermatogonia with disrupted cell membrane and lipid droplet separating it from the underlying irregular basement membrane. The cytoplasm of sertoli cell was rarified and contained mitochondria with destructed cristae. Primary spermatocyte contained rarified cytoplasm. Spermatids showed vaccuolated cytoplasm and abnormal acrosomal cap on the anterior pole of the nucleus. Primary spermatocytes showed vaccuoles in the cytoplasm and swollen destructed mitochondria. wide intercellualr spaces were noticed. The middle pieces of spermatozoal tails contained excess residual cytoplasm Fig. 8 (A, B & C).

Regarding group III **(**ASP and vit. C treated group), sections of the testis showed spermatogonia resting on the regular basement membrane. Primary spermatocytes were seen containg euchromatic nuclei and mitochondria. Some of them showed area of rarified cytoplasm. Intact junction between the cells was seen. Spermatids containing euchromatic nuclei with the acrosomal cap extending over the anterior pole of the nucleus and peripheral mitochondria were seen. Sections of middle pieces of spermatozoal tails contained axoneme, nine outer dense fibers, circumferentially arranged mitochondria and flagellar membrane Fig. 9 (A, B & C).

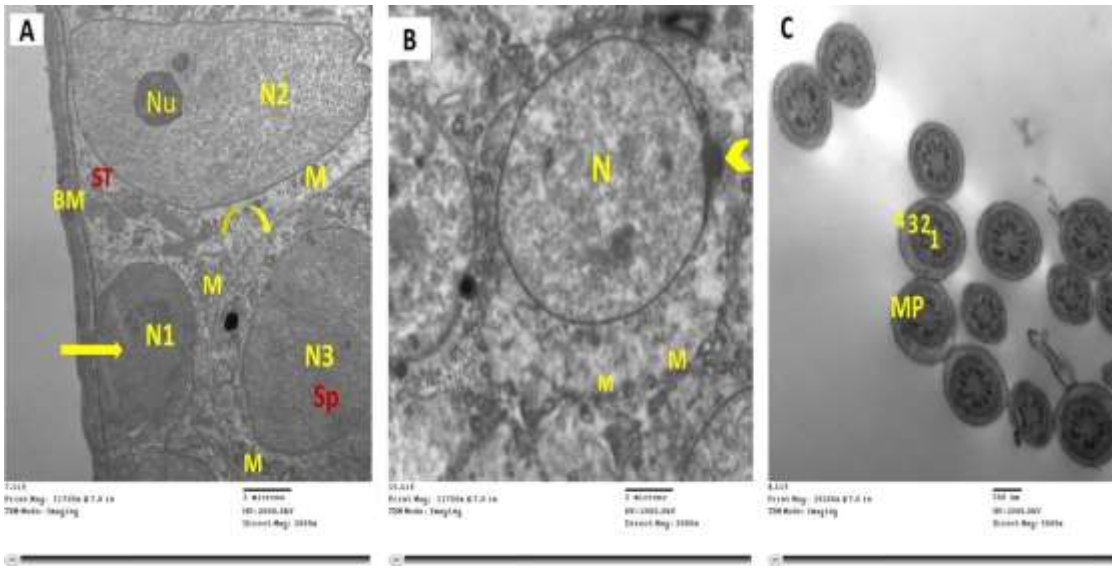

Fig.(7): An electromicrograph of Testis of control group. (A,B&C): (A): showing spermatogonia (arrow) with rounded nucleus (N1) resting on normal basement membrane (BM), Sertoli cell (ST) containing large euchromatic nucleus (N2) with prominent nucleolus (Nu)and mitochondria (M). Primary spermatocyte (Sp) is seen containg euchromatic nucleus (N3). The cytoplasm contains mitochondria (M). (B): shows a spermatid containing euchromatic nucleus (N) with the acrosomal cap (arrow)

extending over the anterior pole of the nucleus. The cytoplasm shows peripheral mitochondria (M). (C): shows sections of middle pieces (MP) of spermatozoal tails containing an axoneme (1), nine outer dense fibers (2), circumferentially arranged mitochondria (3) and flagellar membrane (4).

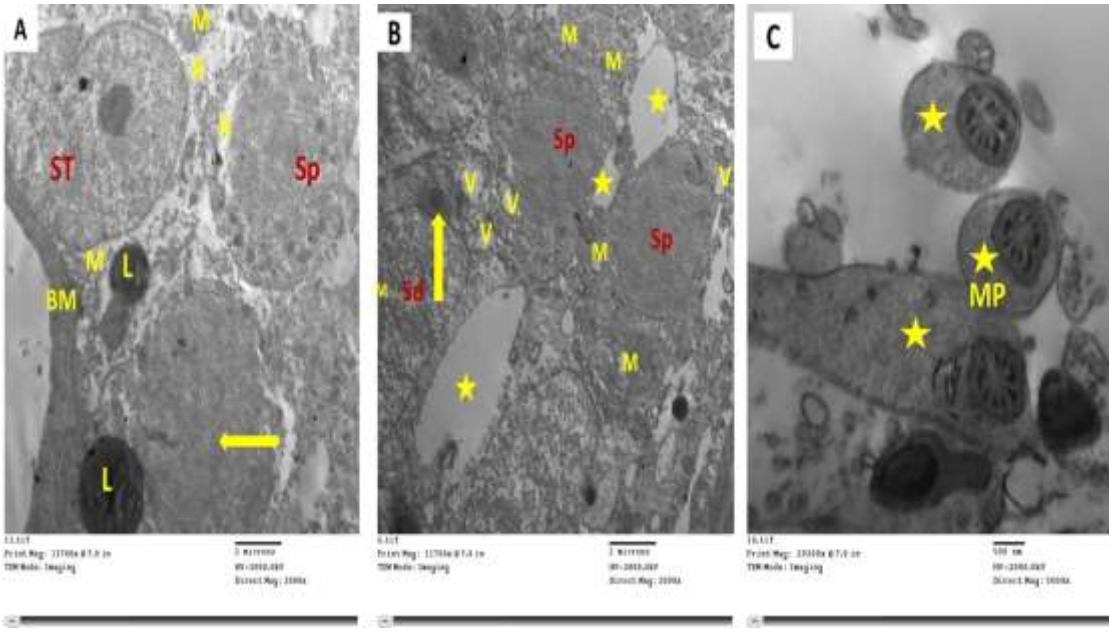

Fig.(8): An electromicrograph of testis of ASP group: A) shows spermatogonia (arrow) with disrupted cell membrane and lipid droplet (L) separating it from the underlying irregular basement membrane (BM). The cytoplasm of sertoli cell (ST) is rarified (R) and contains mitochondria with destructed cristae (M). Primary spermatocyte (Sp) containing rarified cytoplasm (R) is seen. B) shows a spermatid (Sd) with vaccuolated cytoplasm (V) and abnormal acrosomal cap (arrow) on the anterior pole of the nucleus. Two primary spermatocytes (Sp) showing vaccuoles in the cytoplasm (V) and swollen destructed mitochondria (M). wide intercellualr space is noticed (stars). C)shows the middle pieces of spermatozoal tails with excess residual cytoplasm (stars).

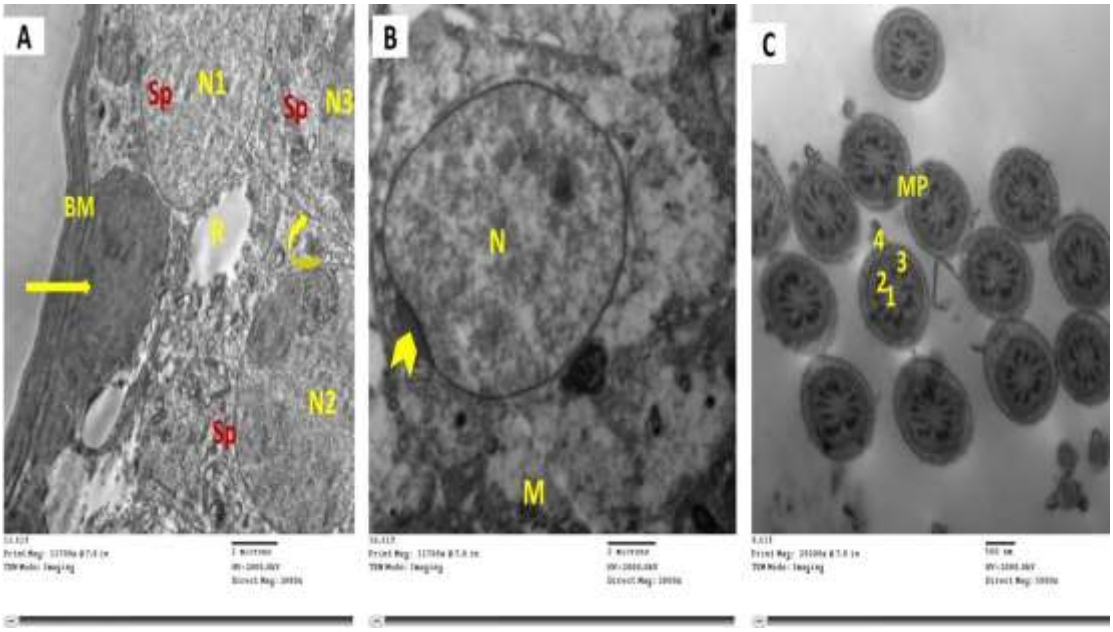

Fig.(9): An electromicrograph of testis of ASP and vit. C treated group; A) shows spermatogonia (arrow) resting on the regular basement membrane (BM), Three primary spermatocytes (Sp) are seen containg euchromatic nuclei (N1,N2&N3). The cytoplasm contains mitochondria (M). one of them shows area of rarified cytoplasm (R). intact junction between the cells is seen (curved arrow). B) shows a spermatid containing euchromatic nucleus (N) with the acrosomal cap (arrow head) extending over the anterior pole of the nucleus. The cytoplasm contains mitochondria (M) at the periphery. C) shows sections of middle pieces (MP) of spermatozoal tails containing an axoneme (1), nine outer dense fibers (2), circumferentially arranged mitochondria (3) and flagellar membrane (4).

4. Discussion

Aspartame is commonly used in diet and beverages to avoid blood glucose elevation and obesity [5]

Despite the widespread consumption of aspartame, it was found to be associated with multiple clinical disorders including neurological disorders, hepatotoxicity and nephrotoxicity [2,14].

The present work showed a highly significant decrease in the mean final body weight of rats from group II was observed in comparison with group I. A significant decrease was noticed in the mean body weight of rats from group III in comparison with group I. However, non significant difference in the mean body weight of rats was noticed between group II and group III. The results of the present study were in accordance to other researchers who

showed that aspartame reduces the body weight and this may be due to oxidative damage or induction of secretion of glucagon-like peptide by the digestive tract, that results in weight loss through reducing appetite and food intake. Some researchers noticed that significant weight loss occurred even with vit. C intake [15,16,17, 18 ,19,20].

These results were in agreement with other authors who found that ASP caused weight loss in human and was used to control obesity. Weight loss with aspartame could be due to reduction of the brain's neuropeptide Y which is important for metabolism [21,22].

On the other hand, other researchers reported that consumption of aspartame resulted in increase in the body weight [23]. Other reports revealed that aspartame induces obesity [24,25].

The current study revealed a highly significant decrease in the mean thickness of the germinal epithelium in group II (ASP treated group) in comparison with group I (control group). These results were coincided with **Anbara et al. 2020**[26] who found decreased germinal epithelial height in groups treated with ASP when in comparison with the control group.

The mean thickness of germinal epithelium in group III (ASP and vit. C treated) showed significant increase in comparison with group II (ASP treated). The mean thickness of the germinal epithelium showed non significant difference between group I and group III (ASP and vit. C treated). These changes were in accordance with **Ayoubi et al. 2015** [27] who found that vit. C improved the germinal epithelial thickness of the seminiferous tubules.

In this experiment, light microscopic sections of Hx & E stained sections of the testes from group II treated with ASP showed wide range of histopathological alterations in the testicular tissue. Distortion of seminiferous tubules with wrinkled basement membrane and wide interstitial spaces were observed. Areas of complete loss of basement membrane and its overlying germinal epithelium were also noticed. Exfoliated spermatogenic cells with pyknotic nuclei and vacuolation of interstitial Leydig cells were also seen. These results were coincided with other workers who revealed that prolonged use of aspartame affected function of testes and fertility through reduction in the levels of pituitary testicular axis hormones In addition ASP use causes oxidative stress and apoptosis in the

testes [28].

In this work, some empty seminiferous tubules with wide lumen and germinal epithelium dislocation were noticed in sections of the testis from ASP treated group. These findings were explained by some authors to be a sign of spermatogenesis failure [29]. Homogenous acidophilic material in the interstitium found in our study was explained by some authors to be due to impaired Sertoli cells phagocytosis leading to degenerated spermatogenic cells hyalinization from the damaged seminiferous tubules or due to lymphatic exudates and increased permeability of congested veins [16,30,31,32].

Congested interstitial blood vessels seen in the present study indicated circulatory disturbances that can affect the function of the testes. This was explained by some authors to be due to the spermicidal effect that was proved by reduction of spermatogenic cells and atrophy of seminiferous tubule. All these findings are indicators of spermatogenesis failure [29,33,34]. They added that aspartame had a negative impact on the reproductive system through its effect on the hypothalamic pituitary testicular axis.

Findings observed in this work showed vacuolation of interstitial Leydig cells. This was attributed by some researchers to oxidative stress [35]. Spermatogonia were seen containing pyknotic nuclei and vacuolated cytoplasm. Wide separation between the spermatogenic cells was seen. The interstitium shows vacuolation. Some researchers reported that these findings occurred due to inhibition of spermatogonial stem cells proliferation that would lead to infertility. In addition, apoptosis could occur due to oxidative stress that causes abnormal mitochondrial functions [36].

Ultrastructural features of the testes in this work from group II that was treated with ASP confirmed the light microscopic results and showed marked histopathological changes in the spermatogenic cells. Spermatogonia were seen with disrupted cell membarne and lipid droplet separating it from the irregular basement membrane. Sertoli cells contained rarified cytoplasm and mitochondria with destructed cristae. Primary spermatocytes contained rarified cytoplasm. Some of them showed vaccuolated cytoplasm and swollen destructed mitochondria. Spermatids containing vaccuolated cytoplasm and abnormal acrosomal cap on the anterior pole of the nucleus were found. Wide spaces between the cells was noticed. The middle pieces of spermatozoal tails contained excess

residual cytoplasm. This was explained by some authers who explained that ASP caused decrease in LH, testosterone and FSH blood levels. ASP breakdown gave aspartic acid, phenylalanine and methanol. Moreover, formaldehyde and formate are produced by methanol metabolism. These products strenghthen the oxidative stress and harm the reproductive systems of rats and sperm quality [9,37,38].

On the other hand, other researchers explained that the harmfulness of aspartame on the testes is dose dependent and some seminiferous tubules remained normal [39].

Vit C. is an important supplement for tissue protection due to its antioxidant effect with both radical scavenging and antioxidant properties. Therefore, it decreases oxidative stress and inflammatory biomarkers [40,41].

Light microscopic examination of testes sections in this study from group III treated with ASP and vit. c revealed improvement in the histological architecture of the testes compared to group II ASP treated but it did not return completely to normal structure. Spermatogonia resting on the regular basement membrane, restoration of seminiferous tubules shape with normal stages of spermatogenesis were noticed. Primary spermatocytes and spermatids were also noticed showing focal widening of intercellular spaces. Spermatozoa were filling the lumen of the tubule. In agreement with these results some researchers reported that vitamin C could reverse the effects of ASP on LH, and FSH concentration in blood. Therefore it protected the testes from the harmful effect of ASP [42].

In contrast with these results, some authors noticed that vit. C has no role in regeneration of seminiferous tubules. Moreover, they found that germinal epithelium remained destructed in rats treated with vit. C [43].

In our study, ultrastructural features of the testes from group III ASP and vit. C treated confirmed the light microscopic results and showed spermatogonia and sertoli cells containing large euchromatic nuclei on the basement membrane. Primary spermatocytes containing euchromatic nuclei and mitochondria were found. Some of them showed area of rarified cytoplasm. Intact junction between the cells was seen. Spermatids contained euchromatic nuclei with the acrosomal cap extending over the anterior pole of the nucleus and peripheral mitochondria. Middle pieces of spermatozoal tails contained an axoneme, nine outer dense fibers,

circumferentially arranged mitochondria and flagellar membrane were seen. These results were supported by some authors who showed the protective role of vit C on the testes and explained this by its antioxidant effect. Therefore, it reduced mRNA destruction, enhanced the sperm quality and improved the ultrastructure of the seminiferous tubule cells. Restoration of the intact junction between cells indicated improved spermatogenesis and this was explained by restoration of blood testis barrier and improvement of metabolism of testes[19,44,45].

5. Conclusion:

This study highlights that Vitamin C is able to improve the histological changes induced by aspartame on the testes and also ameliorate spermatogenesis.

Conflict of interest:

There is no conflict of interest.

6. References:

1. **Blüher M (2019)**: Obesity: Global epidemiology and pathogenesis. Nat Rev Endocrinol, 15(5), pp.288-298.
2. **Lizunkova, P., Enuwosa, E. and Chichger, H., (2019)**:. Activation of the sweet taste receptor T1R3 by sucralose attenuates VEGF-induced vasculogenesis in a cell model of the retinal microvascular endothelium. Graefe's Archive for Clinical and Experimental Ophthalmology, 257, pp.71-81.
3. **Erbaş, O., Erdoğan, M.A., Khalilnezhad, A., Solmaz, V., Gürkan, F.T., Yiğittürk, G., Eroglu, H.A. and Taskiran, D., (2018)**. Evaluation of long term effects of artificial sweeteners on rat brain: A biochemical, behavioral, and histological study. Journal of Biochemical and Molecular Toxicology, 32(6), p.e22053.
4. **Marinovich, M., Galli, C.L., Bosetti, C., Gallus, S. and La Vecchia, C., (2013)**:. Aspartame, low calorie sweeteners and disease: regulatory safety and epidemiological issues. Food and Chemical Toxicology, 60, pp.109-115.
5. **Maaruf, N.A.; Mahmood, Z.M. and Amen, P.J.M. (2017):**. Effect of Aspartame on the Rat's Thyroid Gland: A histological and Morphometrical Study. Diyala Journal of Medicine, 12(1), pp.63-69.
6. **Czarnecka, K., Pilarz, A., Rogut, A., Maj, P., Szymańska, J., Olejnik, Ł. and Szymański, P., (2021)**:. Aspartame—true or false? Narrative review of safety analysis of general use in products. Nutrients, 13(6), p.1957.
7. **Choudhary, A.K. and Devi, R.S., (2014):** Imbalance of the oxidant-

antioxidant status by aspartame in the organs of immune system of Wistar albino rats. Afr J Pharm Pharmacol, 8(8), pp.220-30.

8. **Poli, V.; Aparna, Y.; Madduru, R; and Motireddy, S.R. (2022)**: Protective effect of Vitamin C and E on enzymatic and antioxidant system in liver and kidney toxicity of Cadmium in rats. Applied Food Research, 2(1), p.100098
9. **Azeez, O.H., (2021)**: Evaluation of Some Male and Female Rats' Reproductive Hormones Following Administration of Aspartame With or Without Vitamin C or E. Vet. Med, 45(2), pp.14-20.
10. **Kuo, J. (2007)**: Electron microscopy: Methods and protocols. (Editor:JohnKuo). Second edition, Publisher: Humana Press, Western Australia.2007;67.
11. **Bancroft J and Layton C. (2012)**: Connective and mesenchymal tissues with their stains (11) in Bancroft's theory and practice of histological techniques (editors: Suvarna S, Layton Ch and Bancroft J). Seventh ed: Churchill Livingstone, Elsevier. Philadelphia. P: 187-214.
12. **Lee, K.H., Park, J.H., Bunick, D., Lubahn, D.B. and Bahr, J.M., (2009)**: Morphological comparison of the testis and efferent ductules between wild type and estrogen receptor α knockout mice during postnatal development. Journal of Anatomy, 214(6), pp.916-925.
13. **Abd-El-Hafez M, El-Shafee M, Omar S, Aburahma A and Kamar S. (2020)**: The ameliorative effect of curcumin on cryptorchid and non-cryptorchid testes in induced unilateral cryptorchidism in albino rat: histological evaluation. Folia Morphologica. 2020; 1-18.
14. **Azez, O.H., Baker, S.A., Abdullah, M.A. and Nabi, R.K., (2023)**: Histopathological and the dose depended effects of aspartame toxicity on liver and kidney of rats. Journal of Applied Veterinary Sciences, 8(2), pp.16-22.
15. **Alkafafy, M.E.S., Ibrahim, Z.S., Ahmed, M.M. and El-Shazly, S.A., (2015):** Impact of aspartame and saccharin on the rat liver: Biochemical, molecular, and histological approach. International Journal of Immunopathology and Pharmacology, 28(2), pp.247-255.
16. **Abd Elfatah, A.A., Ghaly, I.S. and Hanafy, S.M., 2012.** Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. Pakistan Journal of Biological Sciences: PJBS, 15(19), pp.904-918.

17. **Kashif, S., Meghji, K.A., Memon, T.F., Channar, S.P., Khan, J. and Hanif, M.S., 2020**. Effects of Ascorbic Acid on Aspartame Induced Nephrotoxicity: An Experimental Rat Model. Journal of Islamic International Medical College (JIIMC), 15(2), pp.88-93.
18. **Olaniyi, O. S. Joseph, O. O.(2024):** Histomorphological and Neurobehavioural Effects of Aspartame on Weaned Wistar Rats**.** . American Journal of Biomedical Science & Research, 23(5)**,** pp**.**631-637.
19. **El Kotb, S.M.; El-ghazouly,D.E. and Ameen,O. (2020)**: The potential cytoprotective effect of Vitamin C and Vitamin E on monosodium glutamate induced testicular toxicity in rats, Alex-andria Journal of Medicine, 56:1, 134-147.
20. **Sabry, R. M., Ahmad, E. A., & Yousef, D. M. (2023)**: The Possible Protective Role of Vitamins C&E Supplemented Diet on the Testis of Adult Albino Rat with Induced–Diabetes, An Ultrastructural, Morphometrical and Biochemical Study. Egyptian Journal of Histology, 46(2), 973-992.
21. **Beck B, Burlet A, Max JP, Stricker-Krongrad A. (2002)**: Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, plasma leptin and body weight gain and composition. Physiol Behav.;75(1-2):41–47.
22. **De La Hunty A, Gibson S, Ashwell M. A (2006)**: review of the effectiveness of aspartame in helping with weight control. Nutr Bull. 31(2):115–128.
23. **Eiya, B.O. and Osunbor, J.O., (2023):** Aspartame and diet soda impact on blood sugar and insulin in Wistar rat. Scientia Africana, 22(3), pp.1-10.
24. **Fowler, S.P., (2016):.Low-calorie sweetener use and energy balance:** Results from experimental studies in animals, and large-scale prospective studies in humans. Physiology & behavior, 164, pp.517-523.
25. **Steffen, B.T., Jacobs, D.R., Yi, S.Y., Lees, S.J., Shikany, J.M., Terry, J.G., Lewis, C.E., Carr, J.J., Zhou, X. and Steffen, L.M., (2023):** Long-term aspartame and saccharin intakes are related to greater volumes of visceral, intermuscular, and subcutaneous adipose tissue: the CARDIA study.

International Journal of Obesity, 47(10), pp.939-947.

26. **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.
27. **Ayoubi, A.; Vaizadeh, R.; Omidi, A. and Abolfazli, M. (2015): Protective Effects of Vitamin C (Ascorbic Acid) in Lead Acetate Exposed Diabetic Male Rats:** Evaluation of Blood Biochemical Parameters and Testicular Histopathology, İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ., 41 (1), 84-91.
28. **Anbara, H., Sheibani, M.T., Razi, M. and Kian, M., (2021)**: Insight into the mechanism of aspartame- induced toxicity in male reproductive system following long- term consumption in mice model. Environmental Toxicology, 36(2), pp.223-237.
29. **Elkelany, M.M. and Kashef, S.M., (2023)**: Role of spirulina extract in ameliorating histological and immunohistochemical changes induced by sertraline in the testis of adult albino rat. Egyptian Journal of Histology, 46(1), pp.136-149.
30. **Yassien R and Ghoneim N. (2020)**: Comparative histological and immunohistochemical study on the effects of antidiabetic drugs (metformin versus sitagliptin) on the testes of adult male albino rat. Egyptian Journal of Histology. 43(1): 353-72.
31. **Ahmed F and Shaheen M. (2016)**: Histological study of effect of the food contaminant semicarbazide on the testis of albino rats and possibility of recovery. British Journal of Science.14 (1): 1-18.
32. **Salama N, Bergh A, Damber J-E. (2003)**: The changes in testicular vascular permeability during progression of the experimental varicocele. Eur Urol.; 43:84–91.
33. **Naik, A.Q., Zafar, T. and Shrivastava, V.K., (2023)**:**.** The impact of non-caloric artificial sweetener aspartame on female reproductive system in mice model. Reproductive Biology and Endocrinology, 21(1), p.73.
34. **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.

35. **Goluža, T., Boscanin, A., Cvetko, J., Kozina, V., Kosović, M., Bernat, M. M., & Ježek, D. (2014)**: Macrophages and Leydig cells in testicular biopsies of azoospermic men. BioMed research international, 2014(1), 828697.
36. **Fotouh, G. I. A., Abdel-Dayem, M. M., Ismail, D. I., & Mohamed, H. H. (2018)**: Histological study on the protective effect of endogenous stem cell mobilization in busulfan-induced testicular injury in albino rats. Journal of Microscopy and Ultrastructure, 6(4), 197-204.
37. **Ashok I, Poornima P, Wankhar D, Ravindran R, Sheeladevi R. (2017)**: Oxidative stress evoked damages on rat sperm and attenuated antioxidant status on consumption of aspartame. Int J Impot Res. 29(4): 164-170.
38. **Hozayen WG, Soliman HAE, Desouky EM. (2014)**: Potential protective effects of rosemary extract, against aspartame toxicity in male rats. J Inter Acad Res Multidisc. 2(6):111–125.
39. **El-Alfy, N. Z. I., Mahmoud, M. F., Said, M. S. E., & El-Ashry, S. R. G. E. (2023)**: Evaluation of The Histological Effects of Aspartame on Testicular Tissue of Albino Mice. The Egyptian Journal of Hospital Medicine. 93, 7349-7355.
40. **May, J.M.; Harrison, F.E. (2013)**: Role of vitamin C in the function of the vascular endothelium. Antioxid. Redox Signal. 19, 2068–2083.
41. **Tan,B.L.; Norhaizan, M.E.; Liew, W.P.& Sulaiman Rahman, H. (2018)**: Antioxidant and OxidativeStress: AMutual Interplay in Age-Related Diseases. Front. Pharmacol. 9, 1162.
42. **Ali W, Mohamed S, Abdallah E, El-Deen E. (2019)**: Protective effect of vitamin C against aspartame toxicity to the testes: A review article. Sohag Med J. 23(2): 52-55.
43. **Ziamajidi, N., Khajvand-Abedini, M., Daei, S., Abbasalipourkabir, R., & Nourian, A. (2023)**: Ameliorative Effects of Vitamins A, C, and E on Sperm Parameters, Testis Histopathology, and Oxidative Stress Status in Zinc Oxide Nanoparticle- Treated Rats. BioMed Research International, 2023(1), 4371611.
44. **Anbara H, Shahrooz R, Razi M, Malekinejad H, Najafi G. (2018)**: The effects of vitamin C on mice hemolytic anemia induced by phenylhydrazine: An animal model

study using histological changes in testis, pre-implantation embryo development, and biochemical changes. Iran J Basic Med Sci. 21(7): 668-677.

45. **Maresch, C.C.; Stute, D.C.; Alves, M.G.; Oliveira, P.F.; de Kret-ser, D.M. and Linn, T. (2018)**: Diabetes induced hyperglycemia impairs male reproductive function: a systematic review, Human Reproduction Update; 24(1): 86–105.

Figure Legends:

Fig. (1): A photomicrograph showing the measurement of the mean thickness of the germinal epithelium using Image J software. For each group, 10 values were obtained from the slide at 200 magnification.

Fig. (2): A histogram showing the final body weight of rats from all groups at scarification time.

Fig. (3): A histogram showing the mean value of thickness of the germinal epithelium in all groups.

Fig. (4): Testis Hx. & E

Photomicrographs of cross sections of the testes of group I (control) ahowing: (A): The normal shape and structure of seminiferous tubules in cut section with the interstitial Leydig cells (L). The lumen of the tubules is filled with spermatozoa (Sz) (A: Hx&E x100). (B): Normal spermatogenic cells are seen. Spermatogonia (curved arrows) are resting on the regular basement membrane (arrows). Primary spermatocytes (Sp) and spermatids (Sd) are also present. Spermatozoa (Sz) are seen filling the lumen of the tubule (B: Hx&E x400). (C): Spermatogonia (arrows), primary spermatocytes (Sp), spermatids (Sd) and Sertoli cells (curved arrows) are noticed (C: Hx&E x1000).

Fig. (5): Testis Hx. &E

Photomicrographs of cross sections of the testis of group II treated with aspartame showing (A): Distorted seminiferous tubule with wrinkled basement membrane (arrows) with wide interstitial spaces (I). (B): Distorted seminiferous tubule with disrupted basement membrane (curved arrows) and congested interstitial blood vessel (BV) are also seen (A,B: Hx&E x100). (C): Empty seminiferous tubules with wide lumen (stars), germinal epithelium dislocation (GED) and dilated congested interstitial blood vessels (BV) are seen (C: Hx&E x200). (D): Areas of focal loss of germinal epithelium (stars) and exfoliated spermatogenic cells (arrows) are seen. Homogenous acidophilic material (circle) in the interstitial tissue inbetween the seminiferous tubules with congested blood vessel (BV) are noticed (D: Hx&E x400). (E): Spermatogonia containing pyknotic nuclei and vacuolated cytoplasm (arrow heads) with wide separation between the spermatogenic cells are also seen (stars). The interstitium shows vacuolation (v) (E: Hx&E x1000).

Fig. (6): Testis Hx. &E

Photomicrographs of cross sections of the testis of group III treated with aspartame and vit. C showing (A): Restoration of the shape of the seminiferous tubules with normal different spermatogenic cells. Spermatogonia (curved arrow) are seen resting on the regular basement membrane (arrow head). Focal widening of intercellular spaces (stars) is noticed. Spermatozoa (Sz) are seen filling the lumen of the tubule (A: Hx&E). (B): Primary spermatocytes (Sp), spermatids (Sd) and Sertoli cells (arrows) are seen. Focal widening of intercellular spaces (stars) is seen. Vacuolation of interstitium is noticed (B: Hx&E x1000).

Fig.(7): An electromicrograph of Testis of control group. (A,B&C): (A): showing spermatogonia (arrow) with rounded nucleus (N1) resting on normal basement membrane (BM), Sertoli cell (ST) containing large euchromatic nucleus (N2) with prominent nucleolus (Nu)and mitochondria (M). Primary spermatocyte (Sp) is seen

containg euchromatic nucleus (N3). The cytoplasm contains mitochondria (M). (B): shows a spermatid containing euchromatic nucleus (N) with the acrosomal cap (arrow) extending over the anterior pole of the nucleus. The cytoplasm shows peripheral mitochondria (M). (C): shows sections of middle pieces (MP) of spermatozoal tails containing an axoneme (1), nine outer dense fibers (2), circumferentially arranged mitochondria (3) and flagellar membrane (4).

Fig.(8): An electromicrograph of testis of ASP group: A) shows spermatogonia (arrow) with disrupted cell membrane and lipid droplet (L) separating it from the underlying irregular basement membrane (BM). The cytoplasm of sertoli cell (ST) is rarified (R) and contains mitochondria with destructed cristae (M). Primary spermatocyte (Sp) containing rarified cytoplasm (R) is seen. B) shows a spermatid (Sd) with vaccuolated cytoplasm (V) and abnormal acrosomal cap (arrow) on the anterior pole of the nucleus. Two primary spermatocytes (Sp) showing vaccuoles in the cytoplasm (V) and swollen destructed mitochondria (M). wide intercellualr space is noticed (stars). C)shows the middle pieces of spermatozoal tails with excess residual cytoplasm (stars).

Fig.(9): An electromicrograph of testis of ASP and vit. C treated group; A) shows spermatogonia (arrow) resting on the regular basement membrane (BM), Three primary spermatocytes (Sp) are seen containg euchromatic nuclei (N1,N2&N3). The cytoplasm contains mitochondria (M). one of them shows area of rarified cytoplasm (R). intact junction between the cells is seen (curved arrow). B) shows a spermatid containing euchromatic nucleus (N) with the acrosomal cap (arrow head) extending over the anterior pole of the nucleus. The cytoplasm contains mitochondria (M) at the periphery. C) shows sections of middle pieces (MP) of spermatozoal tails containing an axoneme (1), nine outer dense fibers (2), circumferentially arranged mitochondria (3) and flagellar membrane (4).