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Protective Effects of Vitamin C and E Against Monosodium Glutamate Induced Histological Changes in The Epididymis of Adult Male Albino Rats



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THE purpose of the current study was to investigate the possibility of vitamin (Vit.) C and E in protecting rat epididymis from MSG-induced histological changes. Twenty-five adult male albino rats divided into five groups (n=5): control, MSG; MSG+Vit.C.; MSG+Vit.E. and MSG +Vit. C+Vit. E. This treatment lasted for forty days. The histological examinations include use of H&E stain and Masson trichrome and histochemical (Periodic Acid Schiff reaction (PAS)). The present results showed that, all groups' B.W. after treatment was significantly higher than that before treatment. However, after treatment, MSG, and MSG+Vit. E caused a high significant decrease in B.W., respectively. There were no appreciable variations in the epididymis weight between the groups MSG+Vit. E and MSG+Vit. C+Vit. E. Histological investigations, indicated none of the treated groups significantly altered the bulk of the epididymis tissues, except for some histological alteration; however, the MSG+Vit.C+Vit.E group showed few effects. These alterations include blood vessel congestion, inflammatory cell infiltration, giant cell formation, expansion of interstitial spaces, hyperplasia and vacuolation of epithelial tubule lining, and hydropic degeneration. MSG show marked deposition of collagen fibers in the capsule, epididymal tubules, interstitial space and basal lamina. While remaining groups indicated the presence of the moderate amount of collagen fibers. Basal lamina and interstitial cells in groups of the MSG; MSG+Vit.C. and MSG+Vit.E. ; exhibited a strong PAS response. Whereas, the MSG+ Vit.C+ Vit.E group had moderate PAS reaction. In conclusion, combining Vit. C and Vit. E with MSG may lessen its toxicity. It is imperative to reevaluate the use of MSG as a flavor enhancer.

Keywords: Monosodium glutamate, Epididymis, Vitamin C, Vitamin E, Histopathology.

Introduction

Monosodium glutamate (MSG) is a flavor enhancer that is often added to foods as an addition to enhancing flavor [1]. Its use has grown throughout time and can now be found it in a wide variety of processed goods and ingredients at any market or grocery shop. MSG imparts processed meals to a unique fragrance known as umami in Japanese. This flavor profile is often referred to as "Savoury"[2]. MSG is known as "China salt" in several nations [3].

Monosodium glutamate, a naturally occurring non-essential amino acid called glutamic acid, is the sodium salt of a substance known chemically as white crystalline powder [4]. According to Samuels, [5], MSG includes 78% glutamic acid, 22% salt, and water. It is generally known that glutamate is one of the most prevalent amino acids found in nature and serves as the main component of numerous proteins and peptides found in the majority of tissues. MSG is also created by the body, it is a crucial component of human metabolism and has been identified as a key excitatory amino neurotransmitter [6,7].

Glutamate occurs naturally in a wide variety of foods, including cheese, poultry, seafood

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(such as dried baby sardines, bonito flakes, tuna, yellow tail, tuna, mackerel, cod, shrimps, scallops and anchovies), and vegetables [8]. This flavor enhancer may be found in many different processed food products, including flavored potato chips, a variety of snacks, canned sauces and soups, marinated meats, frozen dishes, and prepared or stuffed poultry. It will be widely used in the restaurant and food industries [9].

Despite its ability to enhance flavor, taste, and hunger, MSG has been linked to a number of toxins and has been shown to cause oxidative stress. Chinese Restaurant Syndrome, obesity, metabolic abnormalities, neurotoxic effects, and negative effects on the reproductive system all have been related to MSG [1,3,10]. In addition to its neurotoxic effects [11], they discovered that animals exposed to high doses of MSC had reproductive-endocrine malfunction.

Monosodium glutamate is a well-known food ingredient that may be included in packaged foods without being listed on the label and has been demonstrated to be effective for starting male reproductive abnormalities [12], as well as negatively affecting the male reproductive system [10]. One of these toxic effects significantly enhances aberrant sperm morphology in male Wistar rats and causes substantial Oligozoospermia [13]. By producing testicular bleeding, degeneration, and modification of sperm cell population and shape, it has also been linked to male infertility [14,15]. A poor sperm count, damage, histological changes, and gonadotropin imbalance were all caused by MSG treatment, and these changes finally led to problems in male reproduction [16]. Moreover, MSG causes oxidative stress, which produces free radicals (ROS), activates proteases, phospholipases, and endonucleases, activates apoptotic transcriptional programs, and causes genotoxicity, all of which harm cells and tissues [17].

Enzymatic and nonenzymatic antioxidants are necessary to lower the level of oxidative stress. Superoxide dismutase, catalase, and glutathione peroxidase are among the enzymes (GPX). The vitamins (Vit) C and E, as well as carotenes, are nonenzymatic antioxidants, anything that may affect their synthesis and secretion could potentially affect spermatogenesis [18].

Ascorbic acid, a water-soluble form of Vit. C, is a potent scavenger of aqueous radicals that degrade membrane lipids. As it is thought to be

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the first line of defense against ROS by peroxide reduction, ROS scavenging, repair of peroxidized cellular membranes, and iron sequestration, it has a well-known antioxidant protective role in the majority of the toxicities [19]. Globally, Vit. C is a key dietary component with potential effects on the production and release of gonadotropins [20].

Vitamin E is another antioxidant that has a protective impact by lowering or avoiding oxidative damage and by scavenging lipid peroxyl radicals from cellular membranes, it inhibits the chain processes that lead to lipid peroxidation [21].

The goal of the current study was to determine the effects of MSG on adult male albino rats' epididymis organs and examine the protective effects of Vits. C and E, either individually or together, against these effects through the investigation of body and epididymal weight, histological examination and Masson trichrome stain for detection of collagen and histochemical study by Periodic Acid Schiff reaction (PAS) for neutral mucopolysaccharides.

Material and Methods

Animals

Twenty-five mature male albino rats with initial body weights of (164-168) grams were used in the current investigation. They were acquired from the Animal Breeding House, Faculty of Science, Zakho University. These animals were housed under typical environmental settings all day long, including enough lighting, temperature (22–25 °C), ventilation, and free access to food and drink. The Animal Ethics Committee of the Faculty of Science, University of Zakho, approved this work (Code: AEC-016). The laboratory for Zoology in the Department of Biology, Faculty of Science, Zakho University, served as the site of this study's experimental work.

Monosodium glutamate (MSG)

Monosodium glutamate $(C_5H_8NNaO_4)$ ((Negin Tejarat Payam Co., Iran under the license of Huifenghe, China), Vit. C Vials (Osve Co., Iran), and Vit. E Capsules 400 IU (Pharmachem Biotech India PLC).

Experimental design

Following the procedures of El-Kotb et al.[10], with few modifications, the animals of the present investigation were weighted with an electrical balance before treatment and randomly divided into five groups (five male rats per group), as follows: - Group I (Control group): Animals in this group daily received 0.5 ml of distilled water via esophageal gavage once per day and 0.5 ml of olive oil via intraperitoneal (i.p.) injection twice per week.

- Group II (MSG): animals in this group were daily received (2 mg MSG /g B.W. dissolved in 0.5 ml of distilled water via esophageal gavage.

- Group III (MSG+Vit.C): Animals in this group were daily received (2 mg MSG/g B.W. dissolved in 0.5 ml of distilled water) plus Vit. C (100 mg/kg which dissolved in 0.5 ml of distilled water) via esophageal gavage.

-Group IV (MSG+Vit.E): Animals in this group were daily received (2 mg MSG/g B.W. dissolved in 0.5 ml of distilled water) and Vit. E (600 mg/ kg which dissolved in 0.5 ml of olive oil via intraperitoneal injection (i.p.) twice weekly.

-Group V (MSG+Vit.C+Vit.E): Animals in this group were daily received (2 mg MSG /g B.W. dissolve in 0.5 ml of distilled water) and together with Vit. C (100 mg/kg which dissolved in 0.5 ml of distilled water) and Vit. E (600 mg/kg which dissolve in 0.5 ml of olive oil via i.p. twice weekly)

The duration of this treatment lasted for forty days. The first day, when the animals were treated was considered experimental day 0. As mentioned above all animals underwent a forty-day course of treatment, followed by a weight check and an overnight fast.

Then, the next day the animals were sacrificed by anesthetized with xylazine hydrochloride (10 mg/kg i.p.) and ketamine hydrochloride (100 mg/kg i.p.) [22], then dissected, the epididymis organ was immediately removed and separated from the surrounding tissues and lipid, weighed using an electrical sensitive balance (the two epididymis (left and right) of each male rat were weighted and the average value was considered as one measurement). After that, they were fixed in Bouin's solution for 48 hours in order to undergo additional histological analysis [10].

Histopathological examination

The fixed epididymis was washed several times with 70% alcohol to remove the yellow color of the fixative, then; these samples were dehydrated, cleared, and embedded in paraffin wax. Then by using a rotary microtome (KEDEE: Korea), 4 μ m thick sections were trimmed from the paraffin block, and cut in the transverse section.

Histological study

Hematoxylin and eosin (H&E) stains were used for routine histological examination, Masson trichrome for detection of collagen and histochemical study by Periodic Acid Schiff reaction (PAS) for neutral mucopolysaccharides [23].

Statistical analysis

The collected data was submitted to SPSS program [24], in order to analyze statistically. However, the means within ANOVA (both one and two way) were separated using Duncan's multiple range test [25].

Results

Body and epididymis weights

Table (1) and Fig. (1) indicated that when comparing final body weight (after 40 days of treatment) with the initial body weights in each group revealed a highly significant increase (p<0.01) in the body weight in all experimental groups. But if compared the mean body weight after treatment in all treated groups either with control or within each other, the result indicated that there were no significant differences (P>0.05) in the increasing of the weights between group of (MSG +Vit.C +Vit.E) and groups of control, MSG and MSG +Vit.C. Whereas groups of MSG and MSG +Vit.C, showed significant increasing (P<0.05) in the body weight in comparison with control. Except group of MSG+Vit.E, revealed a highly significant decrease (P<0.01) in the mean body weight if compared with all other groups (Table 1 and Fig. 1). The same table also showed the percentage of body weight changes in all groups, the result indicated that group of MSG + Vit. E caused 39.61% in decreasing mean body weight changes in comparison with control (49.54%), MSG (55.07%), MSG +Vit.C (53.67%) and MSG +Vit.C+Vit.E (50. 46%).

Figure (2) and Table (1) also showed the effects of treatment with MSG (with or without Vit.C and E) on the mean epididymis weight. The statistical analysis showed that treated with MSG +Vit.E and MSG +Vit.C +Vit.E caused no significant differences (P>0.05) in the weight of the epididymis compared with their weight in the control group. While treated with MSG individually resulted in a highly significant decrease (P<0.01) and MSG +Vit.C caused significant decrease (p<0.05) in this weight in comparison with control and with other groups.



Fig. 1. Effects of treated with MSG (with or without VC and VE) on the mean body weight.



Fig. 2. Effects of treated with MSG (with or without Vit.C and E) on the epididymis weight.

TABLE 1. Body and epididymis weight (gram) of control and MSG treated groups Mean \pm S.E.

Group	Ν	Body weight before treatment (Mean+SE)	Body weight after treatment (Mean+SE)	Weight changes (%)	Epididymis weight after treatment	Sig. (p)
Control	5	164.677 ± 1.135	326.40 ±2.249 ^b	49.54	0.453 ± 0.014	
MSG	5	164.150 ± 1.439	365.40 ±2.159 ª	55.07	0.302 ± 0.017	0.0001
MSG +VC	5	167.113 ± 1.476	360.75 ±3.250 ª	53.67	0.357 ± 0.018	**
MSG +VE	5	166.170 ± 1.905	275.20 ± 20.272 °	39.61	0.409 ± 0.023	
MSG +VC+ VE	5	167.712 ± 1.138	338.60 ± 2.542 ab	50.46	0.421 ± 0.014	

Similar letters within each column refer to the (NS) non-significant difference (P>0.05), whereas varying letters in the same column refer

**= Highly significant (p<0.01); *= Significant (p<0.05).

Microscopic examination

Microscopic examination of hematoxylin and eosin (H&E) section of epididymis in all groups:

The microscopic examination of transverse sections of rat's epididymis (head and cauda) in control groups, revealed normal histological appearance of epididymal tubules lined by pseudostratified epithelium with stereocilia. The height of these linings was different according to the site of epididymis (head and cauda) (Fig. 3 A, B, C & D). These figures also indicated the presence of packed mass of spermatozoa in the lumen of the epididymal tubules in addition to the normal interstitial spaces and their elements.



Fig. 3. Transverse section of the head and cauda epididymis of the rats in the control group (A, B (high magnification of A), C and D) showing normal histological structure of tubules and intratubular elements. Note: Lumen contains densely packed spermatozoa (blue star); and black arrow indicate epididymal tubules lining epithelium with stereocilia (black arrow). H&E stain. A: 100x, B, C, and D: 400x).

The microscopic examination of the epididymis sections in all treated groups (MSG, MSG+Vit.C, MSG+Vit. E, and MSG+Vit. C+Vit.E), generally showed normal architecture in some epididymal tubules and interstitial elements. In addition, the clearly packet mass of spermatozoa was seen in most of the lumen of these tubules. But the histological examination indicated that few of these epididymal tubules revealed some histopathological changes were recorded and observed in all MSG treated groups whether individually or with Vit.C, Vit.E and Vit. C+ Vit.E. However, the least of these changes

were observed in group of (MSG+Vit.C+Vit.E), and as follows:

MSG treated group

As mentioned before some of epididymal tubules of the MSG treated group showed normal histological patterns as indicated in Figs.(4 A, B and C), these figures indicate the normal structure of the epididymal tubules lined by pseudostratified columnar epithelium with stereocilia, in their lumen a packed mass of mature spermatozoa has been noticed. Figure (4 C) showed the normal cells of the epididymal lining, the principal cells (columnar cells) which were the most abundant

cells in the epididymal tubule lining, and the basal cells which appeared as flat elongated cells, located at the base of epithelium lining. Both principal cells and basal cells form the majority of the epithelium.

While other tubules showed some histopathological changes, these changes include increase the interstitial spaces in addition to their degeneration (Fig. 4 D, E and G), these figures also revealed that being treated with MSG resulted in congestion of blood vessels which is associated with inflammatory cells infiltration and formation of fat cells. Multinucleated and binucleated giant cells (Fig. 4 F) and round bodies were observed in some lumens of epididymal tubules. MSG also resulted in vacuole formation and obvious cytoplasmic vacuolization of epithelial lining (Fig. 4 H), in addition to increasing the amount of collagen fibers that surround the epididymis stroma, destruction of some tubules and reduction in the spermatozoa numbers were also recorded (Figs. 4 H & I).

MSG+*Vit*.*C* treated group

Figures (5 A & B) showed normal histological patterns of some epididymal tubules with their lining and a great number of spermatozoa were seen in their lumen. While other tubules showed that this treatment resulted in congestion of blood vessels and increasing in interstitial spaces (Fig. 5 C). Treated with MSG +VC also caused the formation of giant cells (mononucleated) (Figs. 5 D & E) and formation of round bodies. This figure indicates the degeneration of some renal tubule lining and inflammatory cells infiltration. Other histopathological changes which were recorded in this group include: the detachment of the epididymal stroma from surrounding capsule, decreasing in the number of spermatozoa in epididymal tubule (Fig.5 F); cribriform changes in the epididymis tissues and vacuole formation in addition to the inflammatory cells and hyperplasia of tubule epithelial lining were recorded as indicted in figures (6 A, B, and C). While |Fig. (6 D & E) shows hydropic degeneration and presence of clear vacuoles within and between epithelial cells. Detachment of tubule epithelial lining from the basement membrane also was observed in few epididymal tubules (Fig. 6 F).

MSG+Vit.E treated group

The histological examination of epididymis *Egypt. J. Vet. Sci.* Vol. 54, No. 3 (2023)

transverse sections of rats treated with MSG+ Vit. E, showed normal histological structure of the epididymal tubules (Figs. 7 A & 8 A). However, the histopathological changes in this group were observed more than in other groups, these changes include inflammatory cells infiltration (Figs. 7A, F, and 8B); increasing in the amount of collagen fibers (Figs. 7A, E, F); some epididymal tubules showed reduction in spermatozoa mass within their lumen and other appeared without spermatozoa (Fig. 7B) ; blood vessels congestion (Figs.7, B, C & F); degeneration of tubules associated with detachment of the lining epithelial layer from the basement membrane (Fig.7D); vacuolation of the epithelial lining (vacuoles were observed between epithelial cells) (Figs.7E and 8E and F); mild hydropic degeneration (Fig.8B); in addition to the formation of round bodies (Fig.8C); and binucleated giant cell inside epididymal tubule lumen (Fig. 8D); (Fig. 8A) indicated the presence fat cells between collagen fiber which surround the epidydimal stroma and capsule.

MSG+Vit.C+Vit.E treated group

Figures (9 A & B), showed the normal histological appearance of some epididymal tubules and intertubular elements, but as in other MSG treated groups, the light microscopic examination of epididymis transverse sections in this group showed some histopathological changes were observed in other tubules, including mild hydropic degeneration which looked towards the tubule lumen (Fig. 9 C); cribriform changes were recorded in figures (9 D & E); vacuolation. of germinal epithelial lining tubules (Figs.9 E & F; 10 C), inflammatory cells infiltrations in interstitial tubules (between epididymal tubules) (Figs.9 F & 10 A); as indicated in figure (10 B), although there was a decrease in the thickness of the tubule lining epithelium, but these tubules contain densely packed spermatozoa, in addition, the lumen of some tubules observed empty from spermatozoa (Fig. 10 D), while others contained densely packed spermatozoa, (Fig.10 E); giant cell formation also was recorded in this group(Fig.10 F). Other change include degeneration of epithelial lining (Fig. 9 F); increasing in collagen fibers and fat cells (Fig.10B); RBC accumulation in blood vessel (Fig. 10 C) and increasing in the thicknesses of smooth muscle surrounding epididymal tubule (Fig.10 F).



Fig. 4. Transverse sections of rat's epididymis treated with MSG, showing: A, B & C (high magnification of part of B): Normal histological structure of epididymal tubules which lined by pseudostratified columnar epithelium with stereocilia (black arrow), in their lumen a packed mass of mature spermatozoa (blue star) and interstitial space (green star). In C, the yellow arrow indicates the principal cells while the red arrow indicates the basal cells. While Figs. (D, E, F, G, H and I) variable degree of epididymal degeneration. (D): Increasing interstitial space which contains adipose cells (red star), and congested blood vessels (black arrows). (E): Increasing interstatial space, congested blood vessels (red star), and inflammatory cell infiltration (orange star). (F): Binucleated giant cell (black arrow). (G): Degeneration and Increasing in the interstitial spaces (red star) and thickness of smooth muscle surrounded epididymal tubes (black arrows). (H): Vacuole formation (black arrow) and pronounced cytoplasmic vacuolization of the epithelial inning (red arrow). (I): Reduction in the population of mature spermatozoa in the tubule lumen (green star) and destruction of tubule (black arrow), increase in the collagen fiber surrounding the epididymis stroma (red arrow) and increase in the formation of adipose cells surround epididymis (red star). H & E stain. A: 40x; B, D, E, G, and I: 100x; C and H: 400x; F: 1000x



Fig. 5. Epididymis transverse sections of rats treated with MSG+Vit.C, showing: (A & B): normal histological structure of epididymal tubules which layered by pseudostratified epithelium with stereocilia (black arrow), A packed mass of spermatozoa were seen in the lumen of the tubules (blue star). (C): Increased interstitial spaces, collagen fibers (yellow star) and congested blood vessels (black arrow). (D): Degeneration of epithelial lining of tubule (black arrow), mononucleated giant cell (yellow arrow), inflammatory cells infiltration (yellow star). (E): High magnification of mononucleated giant. (F): Decreasing in the number of spermatozoa in epididymal tubule (green star), congested blood vessels (yellow arrow), detachment of the epididymis stroma from the capsule (black arrow). H&E stain. (A and D:40x; C and F:100x; B & E: 400x).



Fig. 6. Epididymis transverse sections of rats treated with MSG+Vit.C showing: (A & B): Cribriform changes in epididymal tubules (black arrow) associated with increasing connective tissues (yellow star), presence of inflammatory cells and hyperplasia of epithelial tubule lining (red line). (C): Hyperplasia of epithelial tubule lining (red line), hydropic degeneration (red star). (D & E high magnification of part of D): Hydropic degeneration (yellow arrow), increase the interstitial space (green star), presence of clear vacuoles within and between epithelial cells (black arrow). (F): Detachment of epithelial lining from the basement membrane (black arrow). H&E stain. (A, D and F: 100x; B, C and E: 400x).



Fig. 7. Epididymis transverse sections of rats treated with (MSG +Vit.E), showing: (A): Normal histological structure of epididymal tubules (red star). Notes: the presence of inflammatory cells (black arrow) and increase in collagen fiber (red arrow). (B): Some epididymal tubules showed reduced spermatozoa mass within the lumen (red arrow), and others appeared without spermatozoa (green star), in addition to the congested blood vessel (black arrow). (C): Increasing in both interstitial spaces (yellow star), and congested blood vessels (black arrow). (D): Degeneration of epididymal tubules associated with detachment of the lining epithelial layer form basement membrane (red star). (E): Increase in collagen fiber (yellow star), and presence of vacuoles within and between epithelial cells (black arrow). (F): Increase in smooth muscle surrounding the epididymal tubule (white arrow), congested blood vessels (black arrow), inflammatory cells (yellow arrow), collagen fiber (yellow star) and fat cells (red star). H&E. (A, B, C, and F:100x; D & E:400x).



Fig. 8. Epididymis transverse sections of rats treated with (MSG+Vit.E), showing: (A): Fairly normal epididymal tubules lined by pseudostratified epithelium with stereocilia and packed mass of spermatozoa were seen in the lumen of these tubules. Fat cell formation between collagen fiber (yellow star) surrounding epididymal stroma and capsule (red star). (B): Inflammatory cell infiltration (yellow star) and mild hydropic degeneration (red star). (C): Formation of round bodies in the lumen (black arrow). (D): Binucleated giant cell formation inside tubule (black arrow). (E and F: high magnification of part of E): Vacuolation of germinal epithelium (black arrow), some vacuole appeared within epithelial cell disrupt the epithelial alignment and displace the cytoplasm and nucleus to the periphery of the cell (red arrow). H&E stain. (A:40x; B, C, and E:100x, D&F: 400x)



Fig. 9. Epididymis transverse sections of rats treated with (MSG+Vit.C+Vit.E): showing: (A&B high magnification of part of (A)): Normal histological structure of the epididymal tubules lined by pseudostratified epithelium (black arrow) with stereocilia (yellow arrow) and packed mass of spermatozoa (blue star) were seen in the lumen of the tubule. (C): Mild hydropic degeneration towards the tubule lumen (red star). (D): Cribriform changes (yellow star), RBC accumulation (black arrow) and fat cells (red star). (E): Vacuolation of germinal epithelium (black arrow) and Cribriform changes (yellow star). (F): Degeneration of epithelial lining tubules. Note: presence of vacuoles between epithelial cells (black arrow) and some inflammatory cells (red arrow). H&E stain. (A, E, D: 100x; B, C, F: 400x).



Fig. 10. Epididymis transverse sections of rats treated with (MSG+Vit.C+Vit.E), showing: (A): Inflammatory cells infiltrate in interstitial spaces between the tubule (yellow star). (B): Decrease in the thickness of endothelial lining tubules (black arrow), but their lumen contained densely packed spermatozoa (blue star); increased in collagen fiber (yellow star) and fat cells (red star) in interstitial space. (C): Destruction of interstitial space, large vacuole formation (red arrow) and accumulation of RBS in blood vessels (black arrow). (D): Empty the lumen of some tubules from spermatozoa (black arrow). (E): Lumen contained densely packed spermatozoa (blue star), and increased the thickness of smooth muscle surround tubule (black arrow). (F): Giant cell formation in tubule (black arrow). H&E stain. (A, B, C:100x; D, E: 40x, F: 400x).

Microscopic examination of special stain section of epididymis in all groups: Periodic Acid Schiff reaction (PAS)

Periodic Acid Schiff-stained sections of epididymis of control group and group of MSG+ Vit.C + Vit.E, revealed moderate PAS reaction in basal lamina of epididymal tubules, interstitial spaces and spermatogenic cells (Figs.11 A&E). Strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells was recorded in groups (MSG, MSG+ Vit.C and MSG+Vit.E) (Fig.11B, C and D) respectively.

Masson trichrome staining:

Masson trichrome stained sections of epididymis in both control and (MSG +Vit.C

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+Vit.E) groups revealed that the tunica albuginea (capsules) was formed of collagen fibers and the epididymal tubules in control group outlined by fine collagen fibers and in group (MSG +Vit.C + Vit.E) outlined by minimal collagen fibers, that's mean the reaction of this group appeared nearly similar to control. While a group of the MSG showed marked deposition of collagen fibers in the capsule and the epididymal tubules, interstitial spaces but basal lamina were outlined by minimal collagen fibers (Fig.12 B). Moderate amount of collagen fibers were deposited in the capsule, interstitial spaces and basal lamina, in groups of (MSG +Vit.C and MSG +Vit.E), (Figs. 12 C&D) respectively.



Fig. 11. A photomicrograph of PAS-stained sections of epididymis of the different groups. (A): Control group showing moderate PAS reaction in basal lamina of epididymal tubules, interstitial spaces and spermatogenic cells (arrow head). (B): MSG group showing very strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (arrow head). (C): MSG+ Vit.C group showing strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (arrow head). (D): MSG+Vit. E group showing strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (arrow head). (D): MSG+Vit. E group showing strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (arrow head). (E): MSG +Vit. C+Vit.E group showing moderate PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (arrow head). (350x).



Fig. 12. A photomicrograph of Masson Trichrome-stained sections of epididymis of the different groups. (A): Control group showing a capsule (tunica albuginea) formed of collagen fibers (blue) (arrow). Epididymal tubules are outlined by fine collagen fibers (arrow head). (B): MSG group showing marked deposition of collagen fibers in the capsule (arrow) the collagen fibers show corrugation epididymal tubules, interstitial spaces (I) and basal lamina are outlined by minimal collagen fibers (arrow head). (C): MSG+ Vit. C group showing moderate deposition of collagen fibers in capsule (arrow), interstitial spaces (I) and basal lamina (arrow head). (D): MSG+Vit. E group showing moderate deposition of collagen fibers in capsule (arrow), interstitial spaces (I) and basal lamina (arrow head). (E): MSG+Vit.C+Vit.E showing the capsule (arrow) nearly similar to control and epididymal tubules outlined by minimal collagen fibers (arrow head). (350x).

Discussion

One of the primary flavor enhancers used in food goods all around the globe is monosodium glutamate, which is a glutamic acid salt derivative [4]. MSG has received certification from several food and medicine regulatory organizations stating that it is safe for ingestion by humans in any amount. Yet, because of its widespread usage, sometimes without labeling, in all food products, there may be unintentional misuse of this food additive. Nevertheless, MSG is known to influence both fertility and the anatomy and operation of the male reproductive system [16 & 26].

Body weight and Epididymis weight

The present findings showed that after 40 days of exposure, there were no significant changes in the effects of the MSG + Vit. C+ Vit. E on the main body weight compared to the control. This result indicates that the presence of vitamins C and E reduces the harmful effects of MSG. But the rat's body weight significantly increased when it was given MSG individually or in combination with Vit. C. This increase in body weight is due to the effect of MSG, as it causes the formation of large amounts of fat that accumulate inside the abdominal cavity and around the organs; this was clearly observed in this study when dissecting animals. This finding was in line with Rogers and Blundell, [27], who demonstrated that after three weeks of MSG treatment compared to control, the body weight growth was enhanced. Nevertheless, the findings of the present research did not agree with those of study Jubaidi et al., [28], which utilized MSG individually in two doses (60 and 120 mg/Kg BW), their results showed that there were no significant variations in body weight across all groups. The ability of MSG to stimulate the orosensory receptors, therefore, MSG did to induce weight gain or due to an improvement in the taste of foods by exerting a positive influence on the appetite center [29]. This may lead to the idea that MSG supplementation has a favorable impact on body weight. MSG has been linked to an increase in body weight and obesity in mice, according to some researchers [30&31].

The findings of the current research also showed that MSG alone generated a highly significant decrease in the weight of the epididymis, while MSG plus Vit. C caused a significant decrease in this weight. This result was consistent with Jubaidi et al., [28], who demonstrated that consuming MSG at a level of 120 mg per kg of body weight significantly decreased the weight of the male

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reproductive system organs and damaged the reproductive system (testis, epididymis, seminal vesicles and prostate). The male reproductive system is very vulnerable to various elements, including chemicals, environmental and industrial contaminants, as well as food, as indicated by Nordkap et al., [32], which is why the weight of the epididymis decreased. The most common food ingredient is MSG. as taste enhancers and preservative and it is affected by the secretion of the male androgen hormone. It is well known that the androgens play a significant role in maintaining the secretory function of accessory glands, the epididymis being one of them [33].

Nevertheless, the current study's findings also showed that when animals were given MSG + Vit. E or MSG + Vit. C +Vit. E, these vitamins were crucial in reducing the effects of MSG and did not significantly affect epididymis weight when compared to the control group. This result was consistent with the findings of some authors [3 &10], which revealed that the antioxidant and anti-inflammatory capabilities of vitamins A, C, D, and E may be used to reduce the toxicity of MSG.

Histological and histopathological studies:

The findings of the current study demonstrated that none of the treated groups (MSG, MSG+ Vit. C, MSG+ Vit. E, and MSG+ Vit. C+ Vit. E) caused histological changes in the majority of the tissues in the epididymal tubules, that's mean some of these tubules appeared normal in their architecture and clearly displayed a packet mass of spermatozoa in their lumen.

But generally, all MSG-treated groups, whether they were treated alone, with Vit. C, Vit. E, or Vit. C + Vit. E., had some histopathological alterations, but compared to other groups, the group that received MSG+ Vit. C+ Vit. E, had the fewest abnormalities., these changes include clogging of blood vessels, inflammatory cell infiltration, vacuolation of epithelial lining, giant cell formation, expansion of interstitial spaces with destruction of their components, hyperplasia of epithelial tubule lining, and hydropic degeneration. These findings concurred with previous findings [16 & 28]. They demonstrated that the injection of MSG altered many male reproductive system mechanisms, such as causing male reproductive dysfunction by altering the reproductive system's histology (testes, epididymis, seminal vesicle and prostate organs). Several additional research [17, 34 & 35], showed that MSG causes oxidative stress, which generates ROS in various tissues

of experimental animals. It was also noted by [27], that MSG produced histological damage to the reproductive system. This impact may have been brought on by ROS activity and a decline in antioxidant status, which led to oxidative damage. Antioxidants are required to lessen the states of oxidative stress. Enzymatic and non-enzymatic antioxidants are included in this group. The latter includes Vit. (C and E) and carotenoids, and any substance that could change their synthesis and secretion could also change spermatogenesis [18]. It has also been suggested that MSG toxicity can be reduced by using Vit. like A, C, D, and E because of their anti-inflammatory and antioxidant properties [3].

In (2003), Loo, et al. [36], showed that, Vit. C, was used as the first line of defense against ROS by scavenging ROS, reducing peroxides, repairing peroxidized cellular membranes, and sequestering iron. Hence, Vit. C is an important dietary component that has been linked to gonadotropin production and secretion [20]. Vit. E is a powerful antioxidant that protects cells by lowering or avoiding oxidative damage [21]. Moreover, this vitamin was thought to be the most powerful liposoluble antioxidant present in biological systems, particularly in the tissue of the testicles and their associated organs [37]. Kayode et al., in 2020 [16], showed the role of Vit. E in reducing or preventing oxidative damage, which has a beneficial antioxidant effect. According to the findings of the current study, MSG's effects are almost entirely reduced when it is combined with vitamins C and E.

As the epididymis is one of the auxiliary reproductive organs and also an androgenic dependent organ, MSG's effects on androgen (testosterone) hormone production are a further factor. Androgens play a crucial role in maintaining the secretory function of those organs [38], therefore any changes to the hormone's release harm those organs. The results of the current investigation (unpublished data) showed that the levels of the hormone testosterone were significantly lower in the MSG, MSG+Vit.C, and MSG+Vit.E groups than in the other groups. Nevertheless, in the MSG+ Vit. C+ Vit. E group, the amount of this hormone was much lower than in the control group, which may help to explain how MSG affects the tissue and weight of the epididymis organ. This outcome was consistent with Jubaidi et al., [28], who reported that the level of testosterone decreased following MSG treatment.

The basal lamina and interstitial cells of groups of MSG, MSG +Vit.C, and MSG +Vit.E showed a high PAS reaction. While the MSG +Vit.C +Vit.E group displayed a minimal PAS reaction. This result was in agreement with some investigators [10 & 13], which showed that the outcome was brought about by MSG's decreased glycogen phosphorylase activity.

Masson trichrome stanning section, of epididymis in the group of the MSG showing marked deposition of collagen fibers in the capsule (tunica albuginea), epididymal tubules, interstitial space and basal lamina. Same results were obtained by [10,39], they showed that the oxidative stress occurring secondary to MSG may contribute to the observed testicular fibrosis as ROS which can induce transformation of fibroblasts to more synthetic myofibroblast. While other groups (MSG +Vit.C, MSG +Vit.E and MSG +Vit.C +Vit.E) indicated the presence of a moderate amount of collagen fibers which were deposited in the capsule, interstitial spaces and basal lamina.

In conclusion, combining Vit. C and Vit. E with MSG may lessen its toxicity. It is imperative to reevaluate the use of MSG as a flavor enhancer.

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Conflict of Interest

The authors declare no competing interests.

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التأثيرات الوقائية لفيتامين C وفيتامين E ضد التغيرات النسجية التي يسببها الغلوتامات أحادية الصوديوم في بربخ الجرذان البيضاء البالغة

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الهدف من الدر اسة الحالية هو التحقق من إمكانية فيتامين (فيتامين C و E) بحماية بربخ الجرذان من التغير ات النسجية التي يسببها الغلوتامات أحادية الصوديوم. ٢٥ جردًا بالغًا من ذكور الجرذان البيضاء قسمت إلى خمس مجاميع (ن = ٥): مجموعة التحكم، MSG+Vit.C + Vit.E ؛ MSG +Vit.C و MSG+Vit.C و MSG+Vit.C استمرت المعاملة لمدة أربعين يومًا. لغرض الدراسات النسجية تم استخدام ملون الهيماتوكسالين والأيوسن و ملون ماسون ثلاثي الكروم بينما للدر اسة الكيمايئية النسجيةتم استخدام كاشف شيف- حامض البريوديك. بعد أربعين يوما من المعاملة. أظهرت النتائج بأنه هنالك زيادة معنوية عالية في اوزان الحيوانات ولكافة المجاميع فيما لو تم مقارنتها ما قبل المعاملة. ولكن ، تسببت المعاملة مع MSG و MSG +Vit.E في انخفاض معنوي. كبير وواضح في وزن الجسم على التوال. لم تكن هناك اختلافات ملحوظة في وزن البربخ بين المجموعتين MSG+Vit.E و MSG+Vit.C+ Vit.E. أشارت نتائج الدراسات النسجية إلى أن أيا" من المجموعات المعاملة لم تسبب تغيرات نسجية كبيرة في أنسجة البربخ ، باستثناء بعض التغيرات النسجية.ومع ذلك ، سجلت مجموعة MSG + Vit.C + Vit.E تأثيرات قليلة. شملت هذه التغيرات احتقان الأوعية الدموية ، وتسلل الخلايا الالتهابية ، وتكوين الخلايا العملاقة ، وتوسع المسافات البينية ، وتضخم وتفجي في بطانة الأنابيب الظهارية، والتنكس المائي. سببت المعاملة بالغلوتامات أحادية الصوديوم ترسبًا ملحوظًا لألياف الكولاجين في الكبسولة والأنابيب البربخية والمسافات البينية والصفيحة القاعدية. بينما سييت المعاملات الاخرى إلى ترسب كمية معتدلة من ألياف الكو لاجين. الصفيحة القاعدية والخلايا الخلالية في مجموعات من MSG + Vit.C ؛ MSG. ؛ Vit. E+ MSG. + Vit.C + أظهر استجابة قوية لكاشف شيف- حامض البريوديك . في حين أن مجموعة + E+ MSG. Vit.E كان لها تفاعل معتدل لهذا الكاشف الاستنتاج: قد يؤدي المعاملة بفيتامين C و E مع MSG إلى تقليل سميت. نتيجة لذلك من الضروري إعادة النظر في استخدام مادة الغلوتامات أحادية الصوديوم كمُحسِّن للنكهة.

الكلمات المفتاحية : غلوتامات أحادية الصوديوم ، البربخ ، فيتامين C , فيتامين E , علم امر اض الانسجة

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