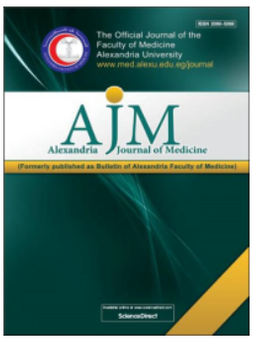

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The potential cytoprotective effect of Vitamin C and Vitamin E on monosodium glutamate-induced testicular toxicity in rats

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ABSTRACT

Background: Monosodium glutamate (MSG) has been recognized as flavor enhancer that adversely affects male reproductive systems.

Objective: The study was conducted to explore the conceivable protective effects of vitamin C and/or vitamin E on testicular toxicity induced by MSG in rats.

Materials and Methods: Thirty male Wistar albino rats were divided (six per group) into: control, MSG, MSG + Vitamin C, MSG + Vitamin E and MSG + Vitamin C + Vitamin E groups. The duration of the study was three weeks. Assessment of serum testosterone, leuteinizing hormone (LH), malondialdehyde (MDA), glutathione peroxidase (GPX), interleukin-10 (IL-10) ,and tumor necrosis factor (TNF-α) were done. Histopathological examination of the testes of the rats was performed using histological, histochemical (Periodic Acid Schiff reaction (PAS)), and immunohistochemical (Proliferating cell nuclear antigen (PCNA), androgen receptors (ARs), Caspase-3) techniques.

Results: MSG-group was associated with significant decrease in serum testosterone, LH, GPX, and IL-10 ($P < 0.05$) and significant increase in serum MDA and TNF-α ($P < 0.05$) when compared with control group. MSG-group revealed many histopathological changes in the testis including degeneration of the germinal epithelium, absence of sperms in the lumina of tubules, widened vacuolated interstitium, marked deposition of the collagen fibers, very strong PAS reaction and marked immunohistochemical changes. Administration of vitamin C or vitamin E significantly reduced these changes; however, the combination of vitamin C and vitamin E provided more potent protection against the toxic effect of MSG than using each vitamin alone. Also, there was insignificant difference ($P > 0.05$) between MSG +Vitamin C and MSG +Vitamin E groups.

Conclusion: Vitamin C and Vitamin E act synergistically in reducing MSG-induced testicular toxicity via antioxidant, anti-inflammatory and antiapoptotic effects of both vitamins.

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KEYWORDS
Monosodium glutamate; testosterone; oxidative stress; cytokines; caspase-3; Vitamin C and Vitamin E

1. Background

Male reproductive dysfunction describes a condition where one or more of the components of the male reproductive system is malfunctioning which may have a debilitating effect on the individual and may result in other secondary conditions [1]. The implicated factors for male reproductive dysfunction include hormonal disorders, reactive oxygen species (ROS), testicular inflammation, endocrinal disturbance, genital infection, chronic health problems, genetic defects, exposure to radiation, and diet [2].

A well-known food additive that may be present in the packed foods without appearing in label and has been found to be potent at initiating reproductive anomalies in males is Monosodium Glutamate (MSG) which is the sodium salt of glutamic acid [3]. The average daily intake of MSG is estimated to be 0.3–1.0 g, but can be higher, depending on the MSG content of individual food items and an individual's taste preferences [4]. Evidence indicates that MSG is safe in moderate amount; however, megadoses may causes harm [5].

Chinese restaurant syndrome and experimental findings have linked the intake of MSG with many structural and functional defects such as neurotoxic, hepatotoxic and reproductive-endocrine dysfunction. There is growing concern about the safety of ingesting MSG. In animals, high doses of MSG is neurotoxic as it destroys nerve cells in the hypothalamic nucleus through changes in the hypothalamic- pituitary (HP) axis [6].

The different mechanisms by which MSG may induce male reproductive dysfunctions include spermatogenic alteration resulting in a low sperm count, high sperm abnormality, reduced live sperm, oxidative damage, histological alteration, as well as gonadotropin imbalance which eventually culminate into reproductive abnormalities in the males [7]. MSG induces oxidative stress which leads to generation of free

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radicals, activation of proteases, phospholipases and endonucleases, transcriptional activation of apoptotic programs and genotoxicity. Proinflammatory cytokines might induce the formation of ROS which could trigger an inflammatory response through the activation of transcription factor NFκB forming an amplifying feed-forward loop and a vicious cycle leading eventually to cell death and tissue dysfunction [8].

To reduce the status of oxidative stress, antioxidants are needed. Enzymatic antioxidants consist of superoxide dismutase, catalase, glutathione peroxidase (GPX). While nonenzymatic antioxidants consist of vitamin C, vitamin E, and carotenoid, any substance that could alter their synthesis and secretion could also alter spermatogenesis [9].

Vitamin C (ascorbic acid) has a well-known antioxidant protective role, as it is considered the front line of defense against free radicals through; ROS scavenging, reduction of peroxides and repair of peroxidized biological membranes and sequestration of iron. Ascorbic acid also contributes to the redox mechanism by salvaging other antioxidants such as vitamin E, urate, and β-carotene from its oxidized form. It also quenches the free radicals present in the lipid membranes, preventing lipid peroxidation when combined with the tocopherols [10]. Vitamin C forms a major dietary component globally and its proposed influence on gonadotropin biosynthesis and secretion [11].

Vitamin E is an antioxidant that has a protective effect by either reducing or preventing oxidative damage. Vitamin E prevents lipid peroxidation chain reactions in cellular membranes by scavenging lipid peroxyl radicals [12].

Thus, this study aimed to investigate the possible toxic effect of MSG on the testicular tissue of young male rats, as a natural constituent of many food items. It also aimed at finding out whether or not administration of vitamin C and vitamin E either individually or in combination would proffer any form of ameliorative effect on MSG-induced testicular toxicity which may improve the male reproductive performance.

2. Materials and methods

2.1. Animals

The study was conducted on thirty male Wister albino rats weighing 200–250 g. After obtaining approval from "Research Ethical Committee," Faculty of Medicine, Menoufia University, Egypt. Experimental procedures followed the Guide for the Care and Use of Laboratory Animals, 8th edition (National Research Council 2011). The rats were housed in wire mesh cages (80×40×30 cm). Prior to experiment, all animals were conditioned for 2 weeks at constant environmental conditions and 12:12-h light/dark cycle. They were given free access to chow and water throughout the study period.

2.2. Experimental design

The animals were divided randomly (six rats per group) into five groups:

Group I (Control group): received 0.5 ml of distilled water by esophageal gavage, once daily and intraperitoneally (i.p.) injected with 0.5 ml olive oil twice weekly for three weeks.

Group II (MSG): received daily MSG (2 mg/g dissolved in 0.5 of distilled water by esophageal gavage for three weeks [13], Ajinomoto co.INC.Tokyo, Japan).

Group III (MSG+VC): received daily MSG (2 mg/g dissolved in 0.5 of distilled water) together with vitamin C (100 mg/kg dissolved in 0.5 ml of distilled water by esophageal gavage, once daily for three weeks [13], chemical industries development, Giza, Egypt).

Group IV (MSG+VE): received daily MSG (2 mg/g dissolved in 0.5 of distilled water) and vitamin E (600 mg/kg dissolved in 0.5 ml of olive oil via intraperitoneal injection twice weekly for three weeks [14], PHARCO Pharmaceuticals, Alexandria, Egypt).

Group V (MSG+VC+VE): received daily MSG together with VC and VE by the same doses in the previous groups for three weeks.

At the end of the experiment (three weeks), all rats were undergoing an overnight fasting. Morning blood samples were withdrawn from all rats through the retro-orbital route using heparinized capillary tubes and the blood samples were allowed to clot for 30 minutes at room temperature. Then the blood samples were centrifuged at 10,000 rpm for 20 minutes. The serum was separated and stored at −20°C till the biochemical analysis. Also, dissection of the testes of the rats was done for histological and immunohistochemical studies.

2.3. Biochemical analysis

Serum samples were used for estimation of serum testosterone and luteinizing hormone (LH) using ELISA kits (Labor diagnostika Nord GmbH & Co. KG) malondialdehyde (MDA) and glutathione peroxidase activity (GPx) using the conventional colorimetric (QuantiChrom™, BioAssay Systems, USA), serum Tumor Necrosis Factor-α (TNF-α), and Interleukin-10 (IL-10) using ELISA kit (Quantikine, Abcam company, Cambridge, UK) . All of the above assays were carried out according to the manufacturer's instructions.

2.4. Histopathological examination

At the end of the experiment, the rats were sacrificed by a high dose of ether. Their right sided testes were gently dissected, then fixed in Bouin's solution and

processed for paraffin blocks. Sections of 4 µm thick were cut and subjected to the following studies.

2.4.1. Histological study

Using Hematoxylin & eosin (H&E) stain for routine histological examination, Masson trichrome for detection of collagen and histochemical study by Periodic Acid Schiff reaction (PAS) for neutral mucopolysaccharides [15].

2.4.2. Immunohistochemical study

Used for detection of Proliferating Cell Nuclear Antigen (PCNA), Androgen Receptors (ARs), and caspase-3 [16].

2.5. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Analysis of Variances (ANOVA) was used for statistical analysis of the different groups, using Origin® software and the probability of chance (P values). P values < 0.05 were considered significant.

3. Results

3.1. Serum Testosterone and LH hormones

The mean value of serum testosterone level in MSG-group was significantly lower when compared to the control group (2.46 ± 0.40 vs. 4.69 ± 0.32 ng/ml respectively, P < 0.05). Serum testosterone levels in MSG+VC, MSG+ VE, and MSG+ VC+VE treated-groups were significantly higher when compared to MSG-group (3.18 ± 0.35, 3.53 ± 0.37, 4.13 ± 0.27 ng/ml respectively, P < 0.05) but still significantly lower in MSG+VC and MSG+VE groups when compared to the control group (P < 0.05). However, there was insignificant diffrence (P > 0.05) between MSG+VC +VE and control group. Serum testosterone level of MSG+ VC+VE treated group was significantly higher when compared to the corresponding values of VC-treated and VE-treated groups (P < 0.05). However, there was insignificant difference (P > 0.05) in serum testosterone level between VC-treated and VE-treated groups (Figure 1).

The mean value of serum LH level in MSG-group was significantly lower when compared to the control group (1.13 ± 0.33 vs. 4.11 ± 0.69 mLU/ml respectively, P < 0.05). Serum LH levels in MSG+VC, MSG+ VE, and MSG+ VC+VE treated-groups were significantly higher when compared to MSG-group (2.14 ± 0.37, 2.39 ± 0.32, 3.13 ± 0.47 mLU/ml respectively, P < 0.05) but still significantly lower when compared to the control group (P < 0.05). Serum LH level of MSG+ VC+VE treated group was significantly higher when compared to the corresponding values of VC-treated and VE-treated groups (P < 0.05). However, there was insignificant difference (P > 0.05) in serum LH level between VC-treated and VE-treated groups (Figure 2).

3.2. Serum inflammatory markers (IL-10 and TNF- α)

The mean value of serum IL-10 level in MSG-group was significantly lower when compared to the control group (11.15 ± 0.79 vs. 18.59 ± 1.32 Pg/ml respectively, P < 0.05). Serum IL-10 levels in MSG+VC, MSG+ VE

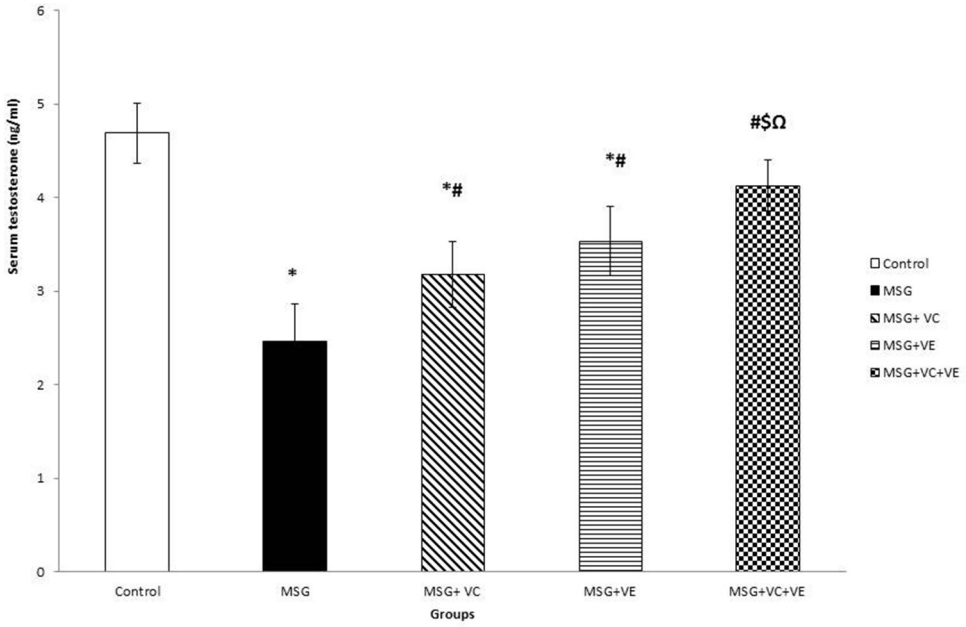

Figure 1. Serum Testosterone (ng/ml) in all studied groups.
(Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG+VE group. Number of rats = six per group).

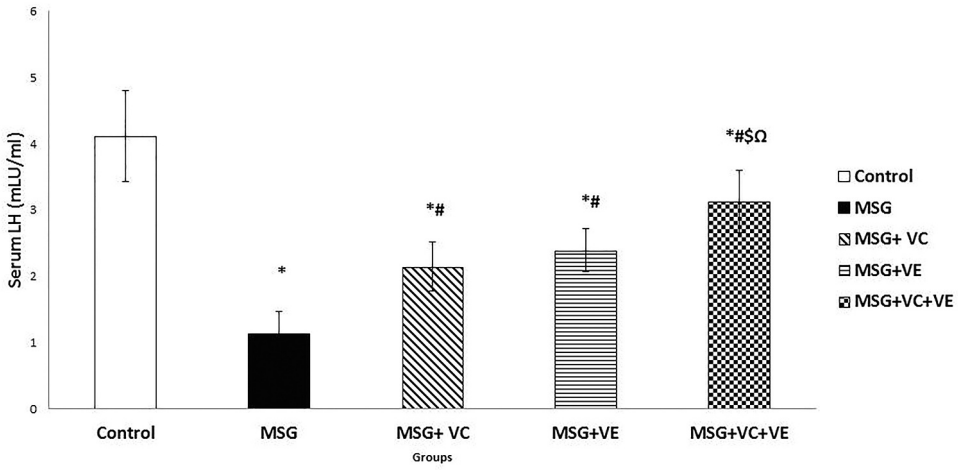

Figure 2. Serum LH (mLU/ml) in all studied groups.
(Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG+VE group. Number of rats = six per group).

& MSG+ VC+VE treated-groups were significantly higher when compared to MSG-group (14.36 ± 0.61, 15.06 ± 0.61, 16.69 ± 0.93 Pg/ml respectively, P < 0.05) but still significantly lower when compared to the control group (P < 0.05). Serum IL-10 level of MSG+ VC+VE group was significantly higher when compared to the corresponding values in VC-treated and VE-treated groups (P < 0.05).However, there was insignificant difference (P > 0.05) in serum IL-10 level between VC-treated and VE-treated groups (Figure 3).

The mean value of serum TNF-α level in MSG-group was significantly higher when compared to the control group (50.58 ± 4.31 vs. 24.21 ± 1.66 Pg/ml, respectively, P < 0.05). Serum TNF-α levels in MSG+VC, MSG+ VE, and MSG+ VC+VE treated-groups were significantly lower when compared to MSG-group (35.91 ± 2.72, 33.16 ± 1.75, 29.66 ± 1.47 Pg/ml respectively, P < 0.05) but still significantly higher when compared to the control group (P < 0.05). Serum TNF-α level of MSG+ VC+VE treated group was significantly lower when compared to the corresponding values of VC-treated and VE-treated groups (P < 0.05).However, there was insignificant difference (P > 0.05) in serum TNF-α level between VC-treated and VE-treated groups (Figure 4).

3.3. Serum oxidative stress markers (MDA and GPx)

The mean value of serum MDA level in MSG-group was significantly higher when compared to the control group (16.18 ± 1.87 vs. 5.05 ± 0.75 mmol/ml

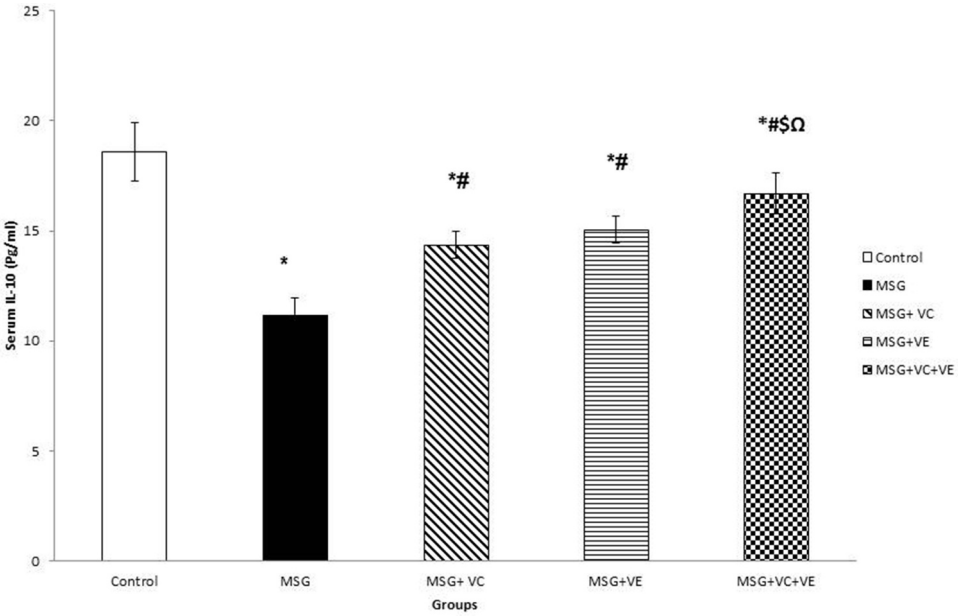

Figure 3. Serum IL-10 (pg/ml) in all studied groups.
(Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG+VE group. Number of rats = six per group).

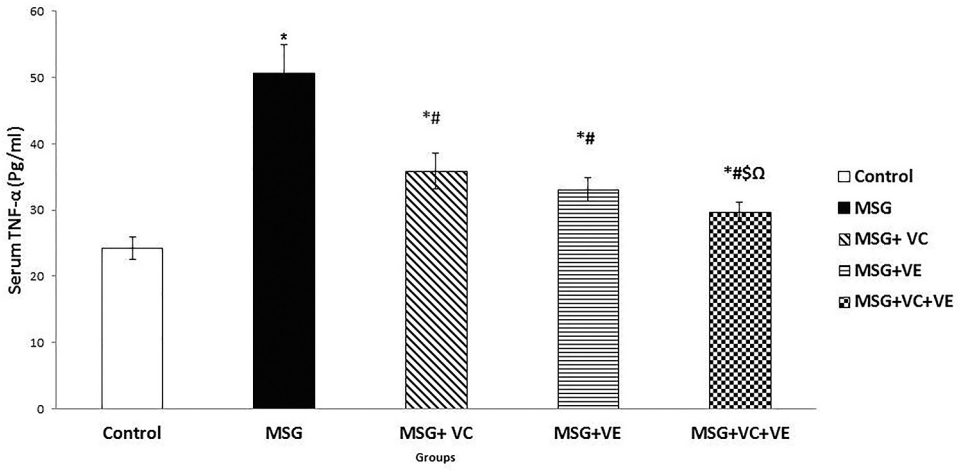

Figure 4. Serum TNF-α (pg/ml) in all studied groups.
(Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG+VE group. Number of rats = six per group).

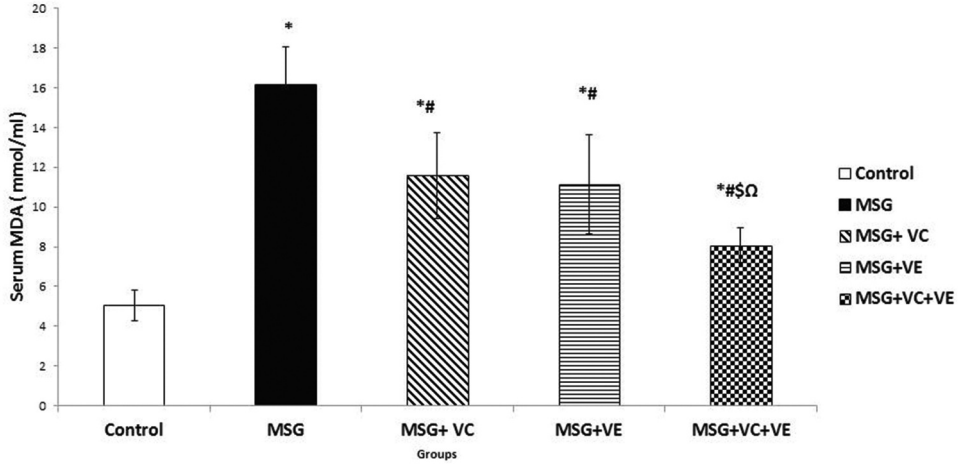

Figure 5. Serum MDA (mmol/ml) in all studied groups.
(Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG+VE group. Number of rats = six per group).

respectively, P < 0.05). Serum MDA levels in MSG +VC, MSG+ VE, and MSG+ VC+VE were significantly lower when compared to MSG-group (11.59 ± 2.16, 11.13 ± 2.49, and 8.08 ± 0.87 mmol/ml respectively, P < 0.05) but still significantly higher when compared to the control group (P < 0.05). Serum MDA level of MSG+VC+VE group was significantly lower when compared to the corresponding values of VC-treated and VE-treated groups (P < 0.05).However, there was insignificant difference (P > 0.05) in serum MDA level between VC-treated and VE-treated groups (Figure 5).

The mean value of serum GPx level in MSG-group was significantly lower when compared to the control group (1.56 ± 0.36 vs. 4.68 ± 0.25 Mg/ml respectively, P < 0.05). Serum GPx levels in MSG+VC, MSG+ VE, and MSG+ VC+VE groups were significantly higher when compared to MSG-group (2.41 ± 0.39, 2.64 ± 0.37, and 3.53 ± 0.30 Mg/ml respectively, P < 0.05) but still significantly lower when compared to the control group (P < 0.05). Serum GPx level of MSG+VC+VE group was significantly higher when compared to VC-treated and VE-treated groups (P < 0.05).However, there was insignificant difference (P > 0.05) in serum Gpx level between VC-treated and VE-treated groups (Figure 6).

3.4. Histological and histochemical results

3.4.1. Hematoxylin and eosin staining

Light microscope examination of the H&E stained sections of testis of the control group showed rounded to oval seminiferous tubules separated by narrow interstitial spaces. Each tubule was bounded by basement membrane and flat peritubular myoid cells. The seminiferous tubules were lined by stratified epithelium composed of two populations of cells; the spermatogenic cells (germinal epithelium) and supporting Sertoli cells. The spermatogenic cells represented

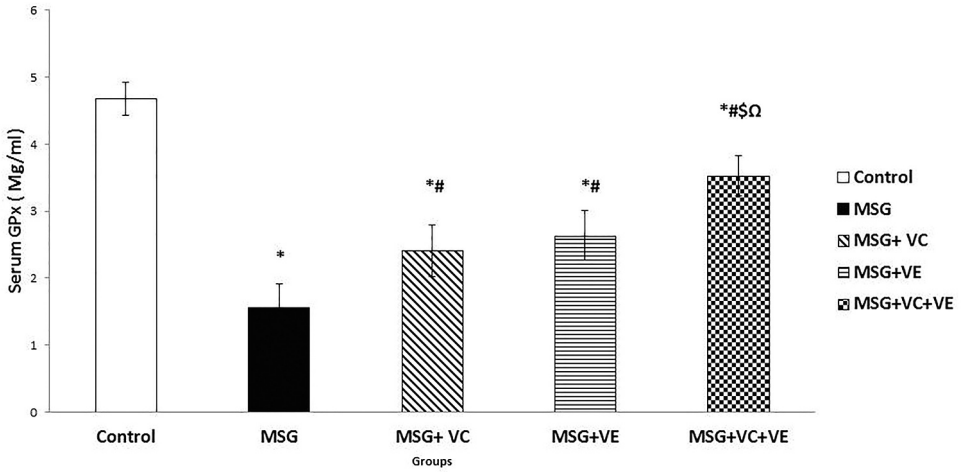

Figure 6. Serum GPx (Mg/ml) in all studied groups. (Significance = P < 0.05, *: Significant when compared to control group, #: Significant when compared to MSG group,$: Significant when compared to MSG+VC group, Ω: Significant when compared to MSG +VE group. Number of rats = six per group).

different stages of spermatogenesis and arranged in several layers. The basal layer was spermatogonia with rounded to oval nuclei. Primary spermatocytes were seen above the spermatogonia with large rounded nuclei. The spermatids were seen in several rows lying close to seminiferous tubule lumen. Spermatozoa were seen in lumen of the tubules. Sertoli cells were detected on basement membrane at intervals in between spermatogenic cells. Sertoli cells were elongated cells with basal, triangular and vesicular nuclei. The narrow interstitial spaces in-between tubules were occupied by loose C.T. containing blood vessels and clusters of Leydig cells with acidophilic cytoplasm and vesicular nuclei (Figure 7(a–c)). On the other hand, sections from MSG group revealed many histological changes as compared to control group. The seminiferous tubules appeared irregular with disorganized germinal epithelium. There was focal loss of spermatogenic cells leaving empty spaces with a noticeable decrease in their number. Some spermatogenic cells appeared shrunken with pyknotic nuclei. There was absence of sperms in the lumina of tubules which were filled with acidophilic hyaline streaks. The detachment of spermatogenic cells from basal lamina was observed in some tubules. The interstitium appeared widened, vacuolated and was occupied with acidophilic hyaline material, numerous interstital cells and congested blood vessels (Figure 7 (d–f)). Sections from MSG+VC group or from MSG +VE group revealed less degenerative changes as compared to the MSG group. Some tubules exhibited normal structure with mature spermatozoa in the lumen. However, other tubules appeared degenerated with few germ cells and empty spaces. Slightly widened interstitium with acidophilic hyaline material was observed (Figure 7(g–h)). Interestingly, sections from MSG+VC+VE group revealed a picture nearly similar to control group (Figure 7(i–j)).

3.4.2. Masson trichrome staining

Masson trichrome-stained sections of testes of control group revealed C.T. capsule (tunica albuginea) formed of collagen fibers and seminiferous tubules outlined by fine collagen fibers (Figure 8(a)). MSG group revealed marked deposition of collagen fibers in capsule, interstitial spaces, and basal lamina. Congested blood vessels were observed in capsule which appeared thickened (Figure 8(b–c)). Sections from MSG+VC group or from MSG+VE group revealed moderate deposition of collagen fibers in capsule, interstitial spaces and basal lamina (Figure 8(d–e)). Sections from MSG+VC+VE group revealed the capsule nearly similar to control and seminiferous tubules outlined by minimal collagen fibers (Figure 8(f)).

3.4.3. Periodic Acid Schiff reaction (PAS)

PAS-stained sections of testes of control group revealed moderate PAS reaction in the basal lamina of tubules and interstitial spaces (Figure 9(a)). MSG group revealed very strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (Figure 9(b)), while sections from MSG+VC group or from MSG+ VE group revealed strong PAS reaction in basal lamina, interstitial spaces, and spermatogenic cells (Figure 9(c–d)). Sections from MSG+VC+VE group revealed a reaction similar to that of control group (Figure 9(e)).

3.5. Immunohistochemical results

3.5.1. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA)

The control group revealed positive PCNA immunoreactivity (deep brown nuclear reaction) in all nuclei of the germ cells (Figure 10(a)). Sections from MSG group revealed few positive PCNA immunoreactive germ cells (spermatogonia). Some karyolitic cells with marked

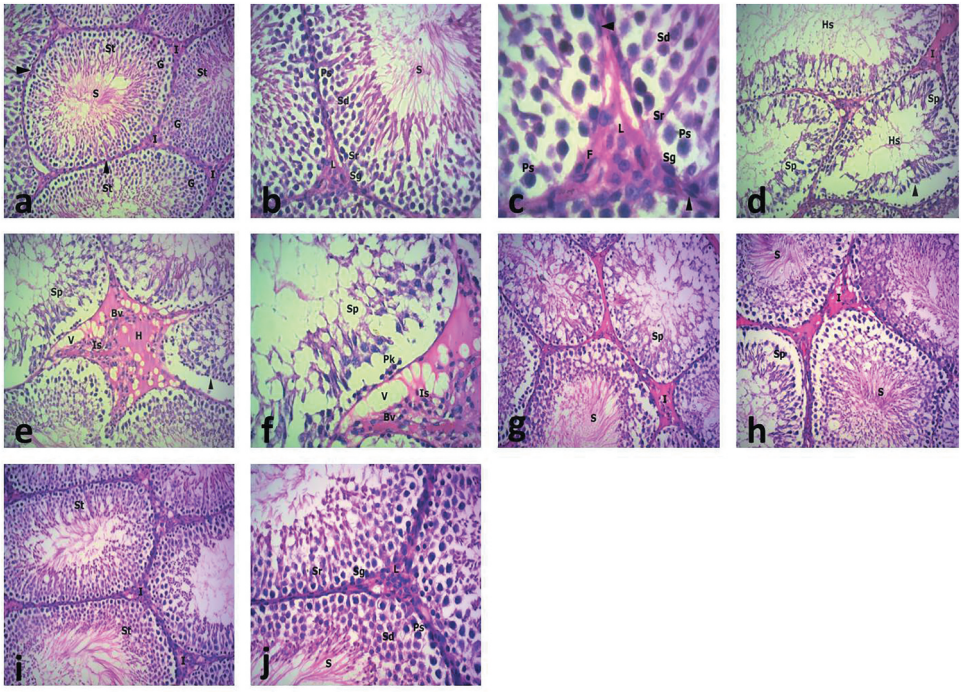

Figure 7. A photomicrograph of H&E-stained sections of testes of the different groups.
(a) control group showing seminiferous tubules (St) bounded by basal lamina (arrow heads), germinal epithelium (G), mature spermatozoa (S) within lumen and interstitial spaces (I). (b) control group showing spermatogenic cells: spermatogonia (Sg), primary spermatocytes (Ps), spermatids (Sd), spermatozoa (S) and Sertoli cells (Sr) in between them. The interstitial space contains Leydig cells (L). (c) Higher magnification of previous section showing Leydig cells (L) with vesicular nuclei and fibroblast (F) in interstitial space. Myoid cells (arrow head) surround the tubules. Notice: spermatogonia (Sg), primary spermatocytes (Ps), spermatids (Sd) and Sertoli cells (Sr) with vesicular nuclei in nearby tubules. (d) MSG group showing irregular seminiferous tubules, focal loss of spermatogenic cells leaving empty spaces (Sp) and absent sperms from lumina of tubules which are filled with acidophilic hyaline streaks (Hs). Notice: spermatogenic cells detachment from basal lamina (arrow head) and widened interstitium with acidophilic material (I). (e) MSG group showing widened interstitium with acidophilic hyaline material (H), numerous interstitial cells (Is), congested blood vessels (Bv) and many vacuoles (V). Notice: empty spaces (Sp) and spermatogenic cells detachment (arrow head). (f) MSG group showing few spermatogenic cells which appear shrunken with pyknotic nuclei (Pk). Notice: empty spaces (Sp), widened interstitium with many vacuoles (V), numerous interstitial cells (Is) and congested vessels (Bv). (g) MSG+vit C group showing some normal seminiferous tubules with mature spermatozoa (S) in lumen and other degenerated tubules with few germ cells and empty spaces (Sp). Slightly widened interstitium (I) with acidophilic hyaline material is observed. (h) MSG+vit E group showing some normal tubules with mature spermatozoa in lumen (S) and some degenerated tubules with few germ cells and empty spaces (Sp). Slightly widened interstitium (I) with acidophilic hyaline material is observed. (i) MSG+vit C + vit E showing normal seminiferous tubules (St) and interstitial space (I). (j) Higher magnification of previous section showing nearly normal seminiferous tubules with spermatogonia (Sg), primary spermatocyte (Ps), spermatids (Sd), spermatozoa (S), and Sertoli cells (Sr). The interstitium shows normal Leydig cells (L). H&E, (a, d, e, g, h, i) × 200, (b, f, j) × 400, (c) × 1000.

decrease in PCNA reactivity were detected (Figure 10 (b)). Sections from MSG+VC group or from MSG+VE group revealed some positive PCNA immunoreactive germ cells (Figure 10(c–d)). Sections from the MSG +VC+VE group revealed positive PCNA immunoreactivity in most nuclei of germ cells (Figure 10(e)).

3.5.2. Immunohistochemical staining for androgen receptor (AR)

The control group revealed very strong positive nuclear androgen receptor (AR) immunoreactivity (brown nuclear staining) in Sertoli and Leydig cells (Figure 11(a)). Sections from MSG group revealed very weak nuclear AR immunoreactivity in these cells (Figure 11(b)). Sections from the MSG+VC group or from MSG+VE group showed weak to moderate AR immunoreactivity (Figure 11(c–d)). Sections from MSG+V C+ VE group revealed a picture nearly similar to the control (Figure 11e).

3.5.3. Immunohistochemical staining for caspase-3

The control group revealed negative caspase-3 immunoreactivity in the cells of seminiferous tubules and interstitial spaces (Figure 12(a)). Sections from MSG group revealed strong positive caspase-3 cytoplasmic immunoreactivity in the cells of seminiferous tubules and interstitial spaces (Figure 12(b)). Sections from MSG+VC group or from MSG+VE group showed decrease in caspase-3 immunoreactivity (moderate or weak immunoreactivity) as compared to the MSG group (Figure 12(c–d)). Sections from MSG+VC+ VE group revealed a picture similar to control except for a weak caspase-3 immunoreactivity in interstitial spaces (Figure 12(e)).

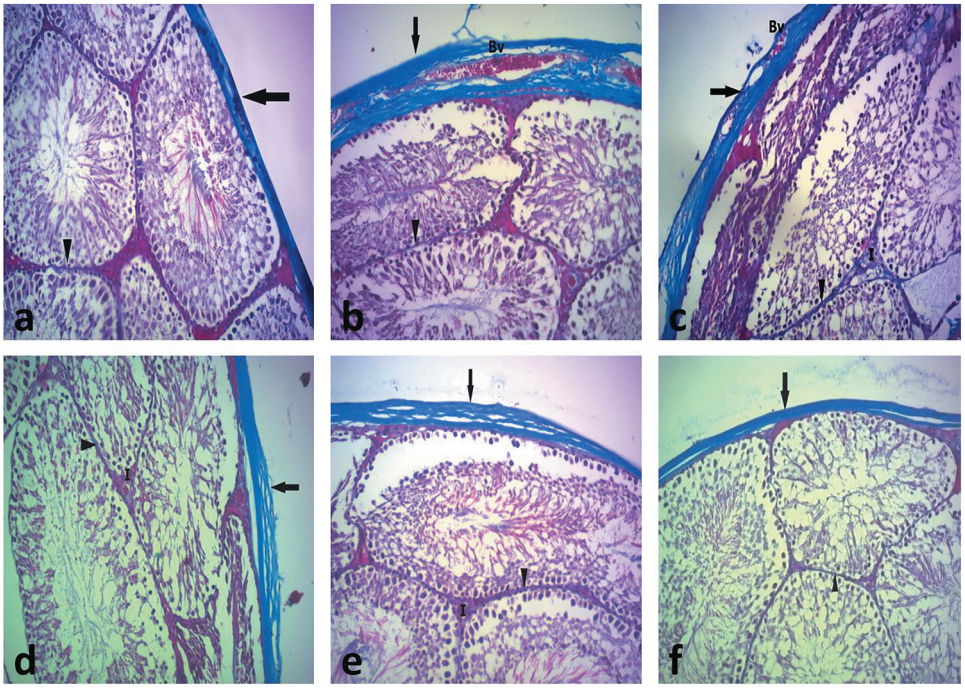

Figure 8. A photomicrograph of Masson Trichrome-stained sections of testes of the different groups.
(a) control group showing C.T. capsule (tunica albuginea) formed of collagen fibers (blue) (arrow). Seminiferous tubules are outlined by fine collagen fibers (arrow head). (b) MSG group showing marked deposition of collagen fibers in the capsule (arrow) which appears thickened with a congested blood vessel (BV) in it. Notice: the collagen fibers show corrugation. Seminiferous tubules are outlined by minimal collagen fibers (arrow head). (c) MSG group showing marked deposition of collagen fibers in the capsule (arrow), intersitial spaces (I) and basal lamina (arrow head). Notice congested blood vessels (BV) in the capsule. (d) MSG+ vit C group showing moderate deposition of collagen fibers in capsule (arrow), intersitial spaces (I) and basal lamina (arrow head). (e) MSG+vit E group showing moderate deposition of collagen fibers in capsule (arrow), intersitial spaces (I) and basal lamina (arrow head). (f) MSG+vit C+ vit E showing the capsule (arrow) nearly similar to control and seminiferous tubules outlined by minimal collagen fibers (arrow head). Masson's trichrome × 200.

4. Discussion

Monosodium glutamate is commonly marketed as a flavor enhancer. Many food and drug control agencies have certified MSG to be safe for human consumption without any specified dosage. However, inadvertent abuse of this food additive may occur because of its abundance, mostly without labeling, in many food ingredients [17].

Monosodium glutamate has been demonstrated to exert neuronal toxic effects in the CNS. This fact was clearly obvious in our results as the mean values of serum LH and testosterone hormones were significantly lower in MSG-group when compared with control group. This result was in agreement with previous studies [1]. The decrement in serum testosterone could be attributed to the disruption of the HP axis in the MSG-treated rats. Also, it is possible that the total number of Leydig cells responsible for testosterone production may decrease, as suggested by the marked shrinkage of the testicular interstitial tissues of the MSG-group compared to the control group [1]. The decrement of LH concentration could be attributed to the reported lesions MSG produces in the arcuate nucleus of the hypothalamus which secretes gonad-trophin-releasing hormone which controls the biosynthesis and secretion of LH by the anterior pituitary [18].

Sperm production can be affected by a number of factors, including vitamin deficiency with free radical generation in the testis which can reduce sperm concentrations which can result in male infertility [19]. It is suggested that toxic effects of MSG lead to alterations in the structural integrity of mitochondrial inner membrane, resulting in the depletion of mitochondrial GSH levels and increased formation of hydrogen peroxide [20]. The testis, epididymis, sperm, and seminal plasma contain high activities of antioxidant enzymes such as GPx, SOD, and CAT [21].The present investigation revealed that MSG caused significant decrease in serum GPx activities and these findings are greatly in accordance with other studies [22]. This could be attributed to its consumption during the breakdown of free radicals or the inhibition of these enzymes by these radicals. Thus, the changes in oxidative defense systems and increase in the level of oxidants in the testis are associated with MSG administration. This led to increased lipid peroxidation which was clearly evidenced in our study by significant high level of MDA in MSG-treated group when compared with control group. Our results were in harmony with Tezcan et al. who declared that MDA is one of the final decomposition of lipid peroxidation [23]. This assures our finding concluding the presence of

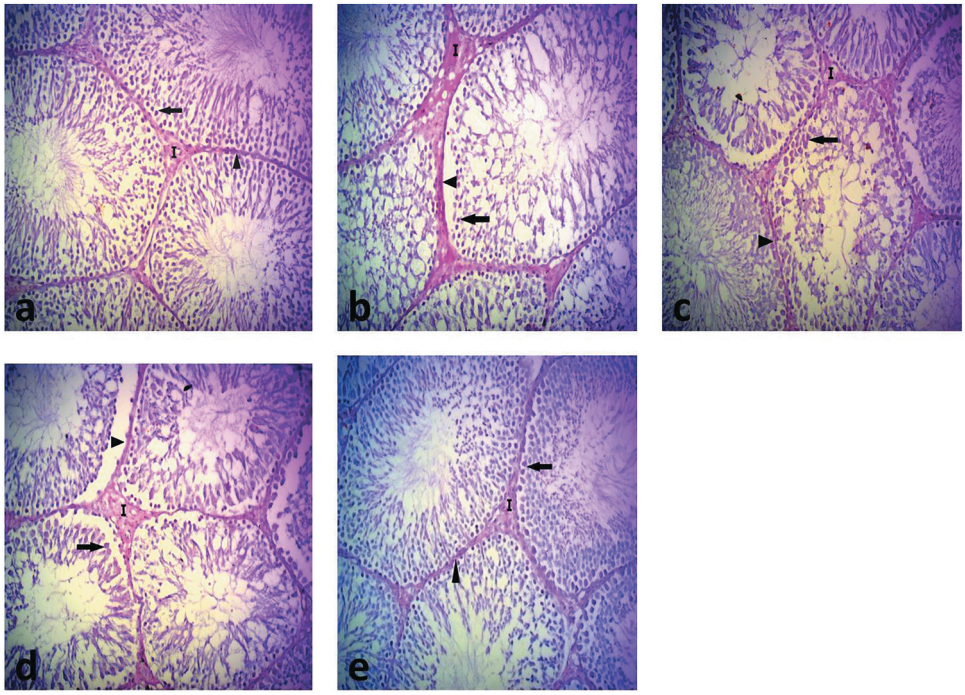

Figure 9. A photomicrograph of PAS-stained sections of testes of the different groups.
(a) control group showing moderate PAS reaction in basal lamina of seminiferous tubules (Arrow head), interstitial spaces (I) and spermatogenic cells (arrow). (b) MSG group showing very strong PAS reaction in basal lamina (arrow head), interstitial spaces (I), and spermatogenic cells (arrow). (c) MSG+vit C group showing strong PAS reaction in basal lamina (arrow head), interstitial spaces (I), and spermatogenic cells (arrow). (d) MSG+vit E group showing strong PAS reaction in basal lamina (arrow head), interstitial spaces (I), and spermatogenic cells (arrow). (e) MSG+vit C+ vit E group showing moderate PAS reaction in basal lamina (arrow head), interstitial spaces (I), and spermatogenic cells (arrow). (PAS ×200).

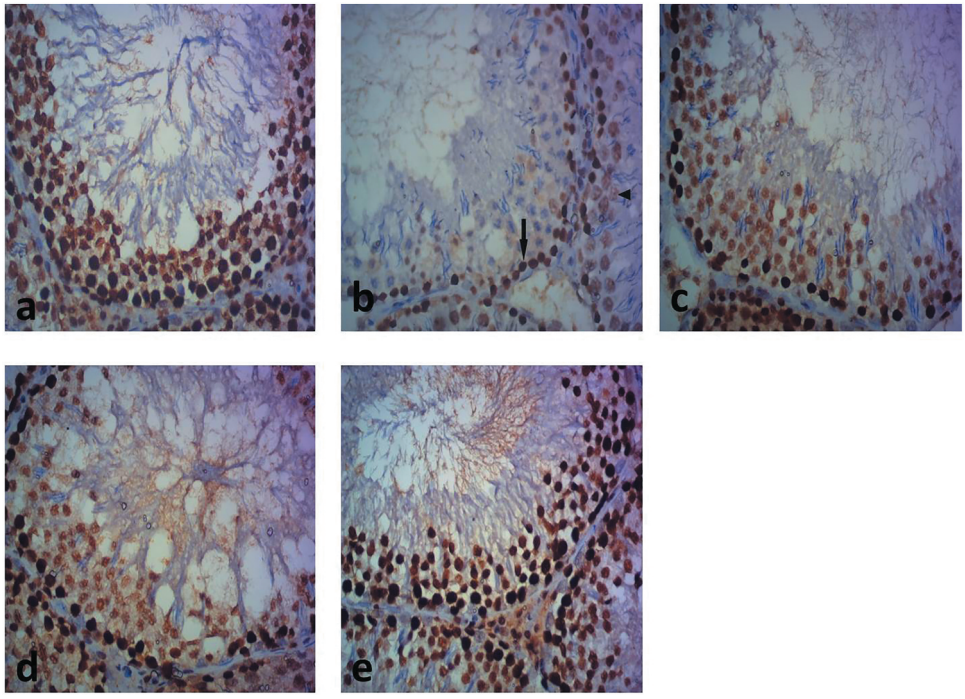

Figure 10. A photomicrograph of immunohistochemical stained sections for PCNA of testes of the different groups.
(a) control group showing positive proliferating cell nuclear antigen (PCNA) immunoreactivity (deep brown nuclear reaction) in all nuclei of germ cells. (b) MSG group showing few positive PCNA immunoreactive germ cells (spermatogonia) (arrow). Notice some karyolitic cells with marked decrease in PCNA reactivity (arrow head). (c) MSG+vit C group showing some positive PCNA immunoreactive germ cells. (d) MSG+vit E group showing some positive PCNA immunoreactive germ cells. (e) MSG+ vit C+ vit E group showing positive PCNA immunoreactivity in most nuclei of germ cells. (PCNA immunostaining, ×400).

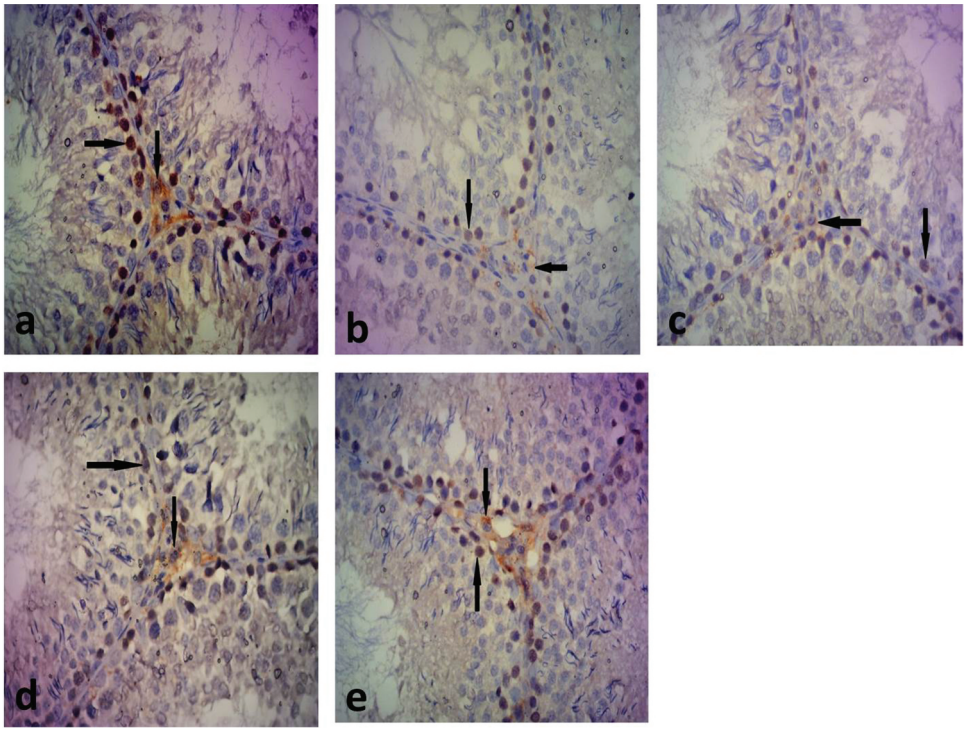

Figure 11. A photomicrograph of immunohistochemical stained sections for AR of testes of the different groups.
(a) control group showing very strong positive nuclear androgen receptor (AR) immunoreactivity in Sertoli and Leydig cells (arrows). (b) MSG group showing very weak positive nuclear AR immunoreactivity in Sertoli cells and Leydig cells (arrows). (c) MSG +vit C group showing weak positive nuclear AR immunoreactivity in Sertoli and Leydig cells (arrows). (d) MSG+vit E group showing moderate positive nuclear AR immunoreactivity in Sertoli and Leydig cell (arrows). (e) MSG+vit C+ vit E group showing strong positive nuclear AR immunoreactivity in Sertoli and Leydig cells (arrows). (AR immunostaining, × 400).

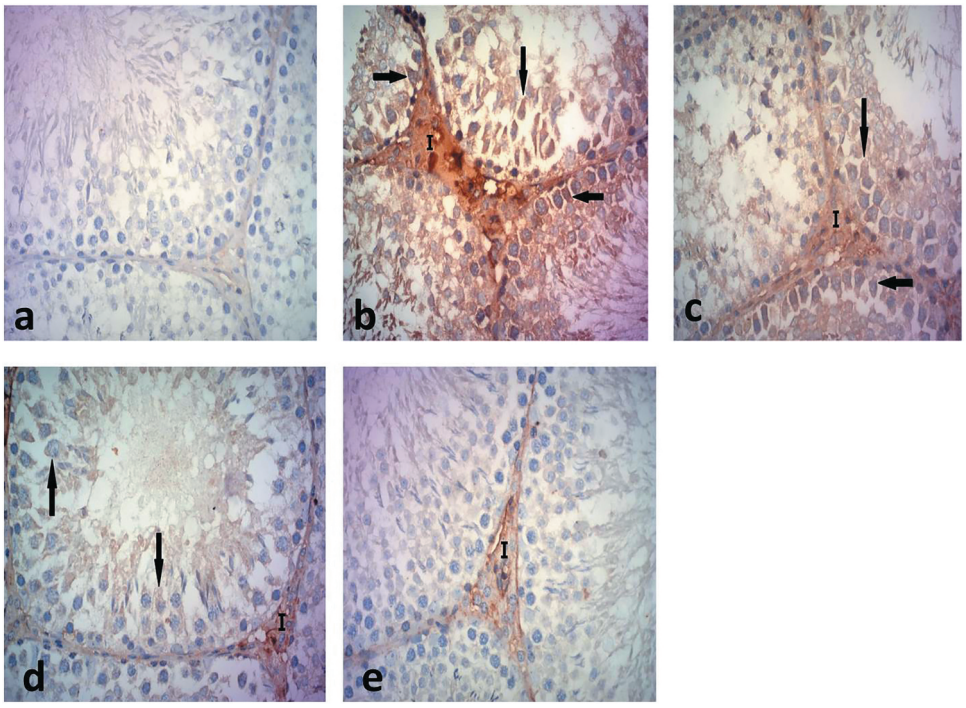

Figure 12. A photomicrograph of immunohistochemical stained sections for Caspase-3 of testes of the different groups.
(a) control group (I) showing negative cytoplasmic immunoreactivity for caspase-3 in cells of the seminiferous tubules and interstitial spaces. (b) MSG group showing strong positive caspase-3 cytoplasmic immunoreactivity in cells of seminiferous tubules (arrows) and intersitial spaces (I). (c) MSG+ vit C group showing moderate positive caspase-3 cytoplasmic immunoreactivity in cells of tubules (arrows) and interstitial spaces (I). (d) MSG+vit E group showing weak positive caspase-3 cytoplasmic immunoreactivity in cells of the tubules (arrows) and moderate immunoreactivity in cells of interstitial spaces (I). (e) MSG+ vit C+ vit E group showing negative cytoplasmic immunoreactivity for caspase-3 in cells of seminiferous tubules and weak immunoreactivity in cells of interstitial spaces (I). (Caspase-3 immunostaining ×400).

oxidative stress in rats treated with MSG. Moreover, MSG resulted in a decrease in the testicular ascorbic acid level that could lead to oxidative damage as reported by previous studies [8].

Membranes in testicular tissues and spermatozoa, in particular, are highly sensitive to ROS. ROS mediated damage to sperm is a significant contributing pathology in 30–80% of infertility cases. ROS can cause infertility by either damaging sperm membranes which reduces sperm motility and ability to fuse with the oocyte or directly damaging sperm DNA [24].

In the present study, MSG-treated rats had significantly higher levels of serum TNF-α and lower level of IL-10 when compared with the controls. These results were in accordance with other studies [25]. TNF-α initiates the activation cascade of cytokines, chemokines, and growth factors involved in the inflammatory response and therefore considered as a proinflammatory cytokine [26].IL-10 can suppress pro-inflammatory cytokine production [27]. MSG triggers RNA expression of interleukin-6, TNF-α in visceral adipose tissue [28]. Proinflammatory cytokines might induce the formation of ROS which could trigger an inflammatory response through the activation of transcription factor NFκB. NF-κB then translocates into the nucleus where it activates a variety of inflammatory genes such as inducible nitric oxide synthase (iNOS), COX-2, IL-1β, IL-6, IL-8, and TNF-α [29]. IL-1β and TNF-α could activate NF-κB forming a vicious cycle leading eventually to cell death [30].

It has been suggested that toxicity of MSG can be overcome by the use of vitamins like A, C, D, and E. They have been recognized by their antioxidant and anti-inflammatory properties [28]. Our study demonstrated significant improvement in serum oxidative stress and inflammatory markers in both vitamin E and vitamin C treated groups. Vitamins C, E, and B protect and repair sperm DNA. They can strengthen the blood-testis barrier and can be effective in treating male infertility by reducing the damage caused by free radicals [31]. Also, vitamins can be effective in treating hormonal imbalance, and oligospermia leading to increased fertility [32].

Vitamin E is considered to be the most effective liposolouble antioxidant found in biological systems especially in the testicular tissue [24]. It has a protective antioxidant effect by either reducing or preventing oxidative damage. It functions as a chain-breaking antioxidant by preventing chain initiation and propagation of free radical reaction and lipid peroxidation in cellular membrane protecting them and lipid containing organelles from peroxidative damage by oxidative stress [17]. In addition, vitamin E supplementation influences the cellular response to oxidative stress through modulation of signal-transduction pathway [33]. Also, vitamin E functions as membrane stabilizer [34]. Furthermore, the protective effect of vitamin E may be due to its role in impairment of MSG absorption in the gastrointestinal tract [17]. Regarding the anti-inflammatory action of vitamin E, it directly inhibits the activation and translocation of NF-κB [35]. Moreover, Matsunaga et al. demonstrated that γ-tocotrienol prevented the degradation of inhibitory-κB and subsequently prevention of the activation of NF-κB [36].

Through the production of ROS, the inflammatory process may deplete stores of antioxidants, including vitamin C; so, its usage in treating inflammation associated with diseases has been considered. The literature indicates that the anti-inflammatory properties and antioxidant capacity of vitamin C can be attributed to their ability to modulate the DNA binding activity of nuclear factor kappa B. Vitamin C can reduce the plasma levels of the inflammatory mediators TNF-α and IL-6 via down regulation of hepatic mRNA expression [37]. Also, vitamin C restored the activity and the content of the antioxidants [38].

Vitamin C plays a role in the regeneration of vitamin E; it donates electron totocopheryl radical and reduces it to tocopherol. So, these two vitamins display a synergistic behavior with the regeneration of vitamin E through vitamin C, reinstating its antioxidant potential. This could explain the significant improvement in all measured biochemical parameters in combined vitamin C and E group when compared to vitamin C or vitamin E -treated groups alone [38].

The neuroprotective role of vitamin C may be due to its involvement in presynaptic reuptake of glutamate, which in turn inhibits binding of glutamate to NMDA receptor [39]. This could explain the significant increase in serum LH and consequently testosterone hormones in treated groups when compared with MSG-group, in addition to the antioxidant and anti-inflammatory effects of vitamin E and vitamin C.

In the present study, H&E-stained sections of MSG-group revealed many histological changes including disorganized germinal epithelium, a noticeable decrease in the number of spermatogenic cells and absence of sperms in lumina of tubules. Our findings came in harmony with the results of previous studies on MSG-induced testicular toxicity [17,40,41]. The maturation arrest observed in the present study, represented by few number of spermatogenic cells and absence of sperms, was explained by El-Wessemy who correlated this arrest to testosterone inhibition which caused stopping of spermatogenesis [42]. Also, MSG has toxic direct action on glutamate receptors and transporters that are expressed in epithelial cells of the seminiferous tubules [43]. Wide interstitial spaces filled with acidophilic hyaline material, congested

blood vessels and numerous interstitial cells were observed in our study which confirmed other findings [41]. Previous studies attributed wide interstitial spaces to shrinkage of seminiferous tubules and hyperplasia of Leydig cells as a result of endocrine homeostasis disturbance [44]. The observed interstitial hyperplasia was suggested to be a compensatory mechanism for the decreased serum testosterone level reported in our study or attributed to increased activity of Leydig cells as previously reported [45]. The acidophilic hyaline material could be a result of lymphatic exudates from degenerative lymphatic vessel or owing to increased vascular permeability of congested blood vessels [46].

Masson's trichrome-stained sections of MSG-group revealed marked deposition of collagen fibers in the capsule. Similar results were reported previously [41]. The oxidative stress occurring secondary to MSG may contribute to the observed testicular fibrosis as ROS can induce transformation of fibroblasts to more synthetic myofibroblasts [47].

Very strong PAS reaction was observed in MSG group. Such finding was confirmed by other studies [48] and attributed this to underutilization of glycogen by the few available sperms, or to inhibition of glycogen phosphorylase activity by MSG.

In our study, MSG group revealed few positive PCNA immunoreactive germ cells. Similar results were reported by other studies [49] on their study on pancreatic acinar cells of MSG group. They attributed these changes to decreased pancreatic DNA content caused by long-term ingestion of MSG. MSG group showed very weak positive AR immunoreactivity in Sertoli and Leydig cells. This could be explained by the fact that the actions of androgens such as testosterone are mediated via AR [50]. This was evidenced in our study by the decrement in testosterone level in MSGtreated rats. MSG group showed strong caspase-3 immunoreactivity in testis. Similar finding was reported previously by Sarhan [41] who attributed this to MSG-induced oxidative stress. Ryter et al. [51] stated that oxidative stress can cause cell death by triggering apoptotic pathways.

In our study, MSG+Vitamin C group showed improvement in the histological, histochemical and immunohistochemical results as compared to the MSG group. This finding was in consistence with other reported results [52]. Also, MSG+Vitamin E group gave results more or less similar to those of MSG+vit C group. These results were in harmony with others [17,53]. The protective effects of vitamin C and E could be attributed to their potent antioxidant and anti-inflammatory effects as discussed before in our results. The coadministration of both vitamin C and vitamin E to MSG treated rats exhibited more protection than groups supplemented with either of the vitamins alone. This group gave results nearly similar to control group. This was in harmony with previous study [54] who reported that vitamins C and E as antioxidants remove completely the toxic effects of abamectin on the histological structure of rat testes.

5. Conclusion

This study has demonstrated that MSG has toxic effects on the testicular tissue. Vitamin C or vitamin E may be a useful prophylactic agent against MSG toxicity. However, there was insignificant difference between the cytoprotective effects of both vitamins on MSG induced testicular toxicity. We concluded that, the combined supplementation with vitamin C and vitamin E has highly protective effect against MSG-induced testicular toxicity rather than using each vitamin alone. It appears that vitamins C and E display a synergistic behavior in reducing MSG toxicity via their antioxidant, anti-inflammatory, and antiapoptotic effects.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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