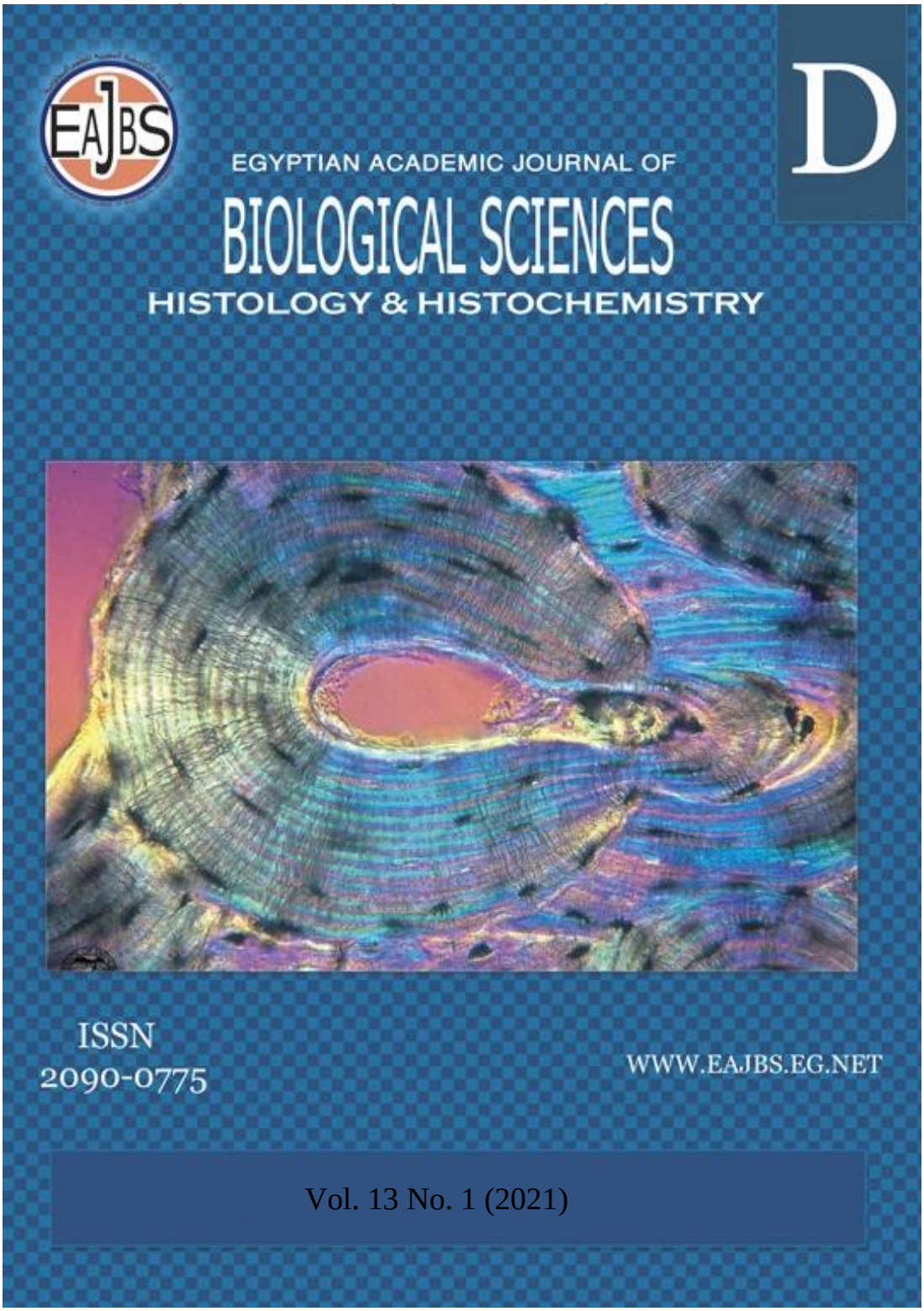


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.13(1) pp13-30(2020)*
DOI: 10.21608/EAJBSD.2021.154503

Egypt. Acad. J. Biolog. Sci., 13(1): 13- 30 (2021)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg

Testicular Ameliorative Effect of L- Carnitine on Monosodium Glutamate-Induced Testicular Structure Alterations in Male Mice

Abdelkarim M. Abdellateif and Wesam S. Twfeq
Zoology Department, Faculty of Science, Fayoum University
E.Mail : ama20@fayoum.edu.eg

ARTICLE INFO
Article History
Received:18/10/2020
Accepted:12/1/2021

***Keywords*:**
Monosodium glutamate, toxicity, testis, male mice and L-carintine

ABSTRACT

This study was carried out to investigate the ameliorative effect of L-carnitine on monosodium glutamate (MSG)-induced testicular toxicity in male mice. Sixty adult male mice were randomized into 6 groups (n = 10). In addition, to the control male mice group (Gp1) that orally administered distilled water, Gp2 mice received 150 mg/kg/day L-carnitine for 35 days. Monosodium glutamate (MSG) was orally administered to male mice at doses of 0.3 and 0.6 mg/g body weight individually (Gp3 & Gp4) and in combination with 150 mg/kg body weight of L-carnitine for 35 days (Gp5 & Gp6). The morphometric parameters, histopathological findings and immunohistochemical studies for PCNA, Ki-67 and Claudin-1 of the testis tissue demonstrated that L-carnitine attenuated and ameliorated the alterations in testicular tissues caused by MSG exposure.

Conclusions: The findings of the present study indicated that treatment of male mice with L-carnitine banned MSG-induced testicular toxicity by improving testicular structure status.

INTRODUCTION

Infertility is one of the worldwide and most important global medical concerns affecting millions of couples (Ross *et al*., 2010; Eslamian *et al*., 2012). Declines occurred in the semen quality maybe contribute to environmental pollutants, occupational exposures, or lifestyle (Homan *et al*., 2007). Moreover, Kaur *et al*. (2015) reported food additives as an important food component that affects semen quality. The food additives have some devastating effects on the consumer so, must be added in concentrations, regulated quantities, and should be within the acceptable daily intakes (Kunkel and Barbara, 2004).

Monosodium glutamate (MSG) is commonly used in processed food to preserve flavor and enhance taste or as flavouring agents or food additives (Rosa *et al.,* 2015). Many drug control and food agencies have certified MSG to be safe for human consumption without any specified dosage (Eweka and Om'Iniabohs, 2008). In developed countries, the average daily dietary of MSG consumption was reported to be from 0.3 to 1.0 g/day by Geha *et al*. (2000) or 0.6 g/day with extreme consumers having exposures of more than 2 g/day by Husarova and Ostatnikova (2013).

The LD_{50} of MSG in rats and mice was 15.000 – 18.000 mg/kg body weight, respectively (Walker and Lupien, 2000).

Various studies have shown that monosodium glutamate is gonadotoxic, neurotoxic, hepatotoxic and nephrotoxic. The mode of action of MSG through which it causes tissue damage might be related to the induction of oxidative stress (Vinodini *et al*, 2010).

Antioxidants are compounds that characteristically dispose of scavenging and halt the production of reactive oxygen species ROS or neutralize their actions. Antioxidants such as vitamin E and C, carotenoids and carnitine were found to be beneficial in restoring a balance between ROS generation and scavenging activities (Adewoyin *et al.*, 2017).

MATERIALS AND METHODS

Experimental Animals:

Sixty (ten weeks-old) male *Mus musculus* mice weighing 28±2 grams were purchased from VACSERA at Helwan, Egypt. Mice were housed and acclimatized to the animal house in accordance with standard laboratory conditions with access to food and water *ad libitum*, with 12 h light and dark cycles. This study has been approved by the scientific and ethical committee of Zoology Department, Faculty of Science in Fayoum University, Egypt 2009. Animals were randomly assigned to 6 groups of 10 mice per group orally and daily administered different doses of MSG and/or L-carintine for 35 days under the same conditions according to Kandeel *et al*. (2019). The control group (G1) administered distilled water for 35 days. L- carnitine group (G2) administered 150 mg/kg/day of L-carnitine for 35 days. MSG low dose group (G3) administered 0.3 mg MSG /g body weight as1/60 of LD_{50} for 35 days. MSG high dose group (G4) administered 0.6 mg MSG /g body weight as1/30 of LD_{50} for 35 days. Group 5: administered low dose 0.3 mg MSG /g body weight in combination with 150 mg/kg/day of L-carnitine for 35 days. Group 6: administered high dose 0.6 mg MSG /g body weight in combination with 150 mg/kg/day of L-carnitine for 35 days.

Morphometric Analysis:

According to Batra *et al.* (2001), thirty round or nearly round seminiferous tubular profiles were chosen randomly and measured for each mice testis. The longitudinal and transverse tubular diameters were measured and expressed in µm at 100x magnification with the Imagej program associated with an Olympus BX-40 microscope. Also, the germinal epithelium height and tubular lumen were obtained with the same tubules used to determine tubular diameter. The germinal epithelium was assumed from the basement membrane to the latest stage of germinal cells, spermatids. The blood vessel diameters of the testes sections were also measured and expressed as the mean value.

Histological Examination:

Whole left testis and specimens of the right testis were fixed in 10% neutral buffer formalin and processed to get 4-6 µm thick paraffin sections with a rotary microtome, stained with hematoxylin and eosin, then examined by light microscope (Sheehan and Hrapchak, 1987). Other paraffin sections were subjected to other immunohistochemical procedures.

Immunohistochemical Technique:

PCNA Immunohistochemistry:

The 5µm paraffin tissue sections were dewaxed and rehydrated in xylene and distilled water. Afterward, antigen retrieval was conducted in a microwave oven. Endogenous peroxidase activity in sections was quenched by 3% hydrogen peroxide at room temperature for 30 min, and the sections were then blocked with 5% bovine serum for 15 min. The sections were subsequently incubated with

specific primary antibodies against PCNA (GB13030; Wuhan Saiweier Biological Technology, China) overnight at 4 °C in a humidified chamber. The sections were then washed with PB, incubated with secondary antibody, and then stained by adding 3, 3′-diaminobenzidine after being washed with PB. The sections were counterstained with hematoxylin and rinsed in tap water. IHC micrographs were observed under a microscope. PCNA is detected in the mitotically dividing spermatogonia as a brown colour.

Ki-67 Immunohistochemistry:

Ki-67 is commonly used as a marker to assess cell proliferation because of the special binds in actively growing and dividing cells. The expression of Ki-67 is strongly related to cell proliferation (Gerdes *et al.*, 1983). Sections were processed antigen retrieval, endogenous peroxidase blocking and non-specific proteins blocking respectively. Then, all sections were probed with rabbit anti-Ki-67 antibody (1:300 (v/v); Millipore, Co., USA) diluted in PBS and incubated. Each section was washed and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:300 (v/v); Invitrogen™, USA). All sections were washed and then were incubated with the Vector Nova RED peroxidase (HRP) substrate kit (Vector Laboratories, USA). The end product of this reaction is a reddish-brown colour. Continually, sections were dehydrated, cleared, and mounted with DPX, respectively. The sections were counterstained with hematoxylin and rinsed in tap water. Ki67-staine cells are mainly localized in the spermatogonial germ cells of the seminiferous tubules in a brown colour.

Claudin-1 Immunohistochemistry:

Concerning this procedure, testis sections from mice were processed according to the Streptavidin- Biotin-peroxidase Complex (SBC) protocol. After deparaffinization and dehydration, the sections were pretreated with 0.03% H_2O_2 for 30 minutes, at room temperature, to block endogenous peroxidase activity. Samples were then washed in phosphate-buffered saline pH 7.4 (PBS), two times for 5 minutes each, and immersed in a solution containing 5% fat-free dry milk (Molico, NestléW) in PBS for 15 minutes to block non-specific binding sites. Sections were incubated overnight at 4°C with antirabbit claudin-1 antiserum (MH25 Zymed/InvitrogenW, lot 50393527, cat. no. 71–7800) diluted (1:250) in 0.05 M Tris–HCl with 1% bovine serum albumin (BSA). They were washed in PBS three times for 5 minutes and incubated with the diluted biotinylated anti-rabbit IgG for 30 minutes, then washed in PBS three times for 5 minutes and incubated for 30 minutes with Streptavidin-biotin peroxidase complex. Immunoreactive sites were revealed by using a buffered solution of 3, 3’-diaminobenzidine–tetrahydrochloride (DAB) (Dako cytomationW, USA). The sections were dehydrated, mounted and analyzed in a Zeiss Axioskop 2 photomicroscope and the images were captured by Pixera (Pixera Corporation, USA). As a negative control, normal rabbit IgG (Vector Laboratories) was used instead of the first antibody in every experiment. Hematoxylin was used for counterstaining. The localization of immunoreactive claudin-1 in the seminiferous epithelium appeared as a reddish-brown precipitate in the basal compartment of normal mouse seminiferous tubules. Moreover, immunoreactive specificity could be observed in the nucleus of premeiotic germ cells, but not in pachytene spermatocytes, secondary spermatocytes, or rounds spermatids.

RESULTS

Clinical Symptoms:

On the second day of the experiment, mice treated with both MSG doses (0.3 and 0.6 mg/g) showed an increase in the water and food consumption with general weakness, decrease in activity, diarrhea, disgusting smell of urine, and palpitation. All these symptoms disappeared by the time. These symptoms disappeared among mice treated with MSG and co-administered with L-carnitine. Also, an increase in fats in the belly area and around the testes was noticed during the dissection of these animals. Also, scrotal sacs appeared reddish and swollen due to dilatation of blood vessels and accumulation of fluids (Fig. 1).

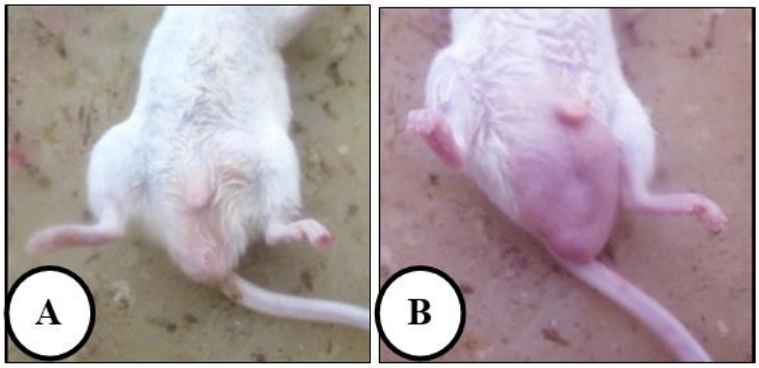


Fig. 1: Photomicrograph showing normal scrotal sacs in control group (A) and reddish swollen ones in MSG treated groups (B).

Morphometric Analysis:

Data concerning the morphometric parameters were shown in Table 1 and Figures 2&3. A significant reduction in the longitudinal diameter of the seminiferous tubules in low and high doses of MSG treated groups (110.03±6.25 and 91.69±6.1µm) in comparison with the control (177.74±5.89µm) and L-carnitine (182.90±3.19µm) groups. A significant improvement has been shown in these parameters (176.6±2.53µm and 173.31±1.99 µm), after treatment with L-carnitine concurrently with low and high doses of MSG. In the same manner, the transverse tubular diameter of the seminiferous tubules in case of low and high doses of MSG treated groups showed a significant reduction with 70.57±4.46 and 54.32±4.36µm in comparison with control (82.24±4.68µm) and L-carnitine (86±1.48µm) groups but treatment with L-carnitine concurrently with low and high doses MSG was showed significant improvement (82.21±4.15 and 84.54.31±2.16 µm).

The height of the spermatogenic cells lining the seminiferous tubules showed marked reduction in low and high doses of MSG treated groups (22±2.25µm and 15.6±2.09µm) relative to control and L-carnitine groups (37.29±2.13µm and 38.62±1.47µm) whereas treatment with L-carnitine concurrently with low and high doses MSG showed definite significant improvement (36.23±1.62µm and37.4±1.6µm). So, the lumen of the seminiferous tubules was dilated in MSG treated groups with low and high MSG doses (26.57±3.93µm and 23.12±4.14µm), in comparison with control (7.67±3.91µm) and L-carnitine (8.76±3.23µm) treated groups and returned back within normal levels in low and high doses of MSG concurrently treated with L-carnitine groups to 9.75±3.35µm and 9.75±3.84µm for both doses, respectively. In addition, the thickness of the tunica albugina was decreased in

low and high doses of MSG treated groups (23.66±5.46µm and 17.35±5.56µm) in comparison with the control (29.79±5.26µm) and L-carnitine (30.76±1.21µm) groups. In groups treated with low and high doses of MSG concurrently treated with L-carintine, the thickness of the tunica albgina was found within the

the normal level of the control for both treatments (30.27±0.73µm and 28.46±2.51µm) (Table 1 and Figs. 2 & 3). In addition, the diameter of blood vessels of the testicular tissue was increased in MSG low and high doses treated groups (78.63±2.96µm and 80.94±3.33µm) in comparison with the control (64.08±3.23µm) and L-carnitine (67.14±2.23µm) groups. After treatment with L-carnitine it became within the normal diameter values in both treatments (64.96±3.32µm and 69.09±3.31µm).

Table 1: Morphometric analysis of the testes of male albino mice of control, and different studied groups.

Parameters / Groups	Tubular diameter		Epithelial height	Tubular lumen	Thickness of tunica albugina	Diameter of blood vessels
	L.S	T .S				
Control	177.74 ±5.89A	82.24 ±4.68 A	37.29 ±2.13 A	7.67 ±3.91 C	29.7914 ±5.26 A	64.08 ±3.23 C
L-carnitine 150mg/kg/day	182.90 ±3.19 A	86.00 ±1.48 A	38.62 ±1.47 A	8.76 ±3.23 C	30.7646 ±1.21 A	67.14 ±2.23 C
MSG Low dose (0.3 mg/g/day)	110.03 ±6.25 B	70.57 ±4.46 B	22.00 ±2.25 B	26.57 ±3.93 A	23.6664 ±5.46 B	78.63 ±2.96 B
MSG High dose (0.6 mg/g/day)	91.69 ±6.10 C	54.32 ±4.36 C	15.60 ±2.09 C	23.12 ±4.14 B	17.3543 ±5.56 C	80.94 ±3.33 A
L-carnitine +MSG low dose	176.60 ±2.53 A	82.21 ±4.15 A	36.23 ±1.62 A	9.75 ±3.35 C	30.2777 ±0.73 A	64.96 ±3.32 C
L-carnitine +MSG high dose	173.31 ±1.99 A	84.54 ±2.16 A	37.40 ±1.60 A	9.75 ±3.84 C	28.4628 ±2.51 A	69.09 ±3.31 C
ANOVA	**	**	**	**	**	**

Data are represented as the mean of 10 samples ±SE (µm). Means with the same letter for each parameter in the same column are not significantly different, otherwise, they do (Duncan multiple range tests). **P<0.01 Highly Significant.

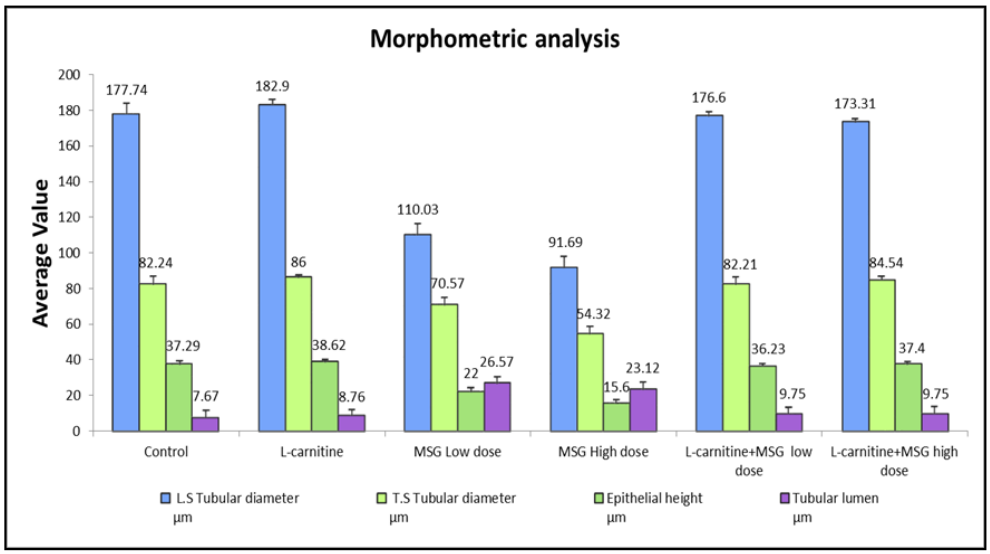


Fig. 2: Morphometric analysis of testes tubular diameter, epithelial height and tubular lumen (µm) in control male albino mice and those treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

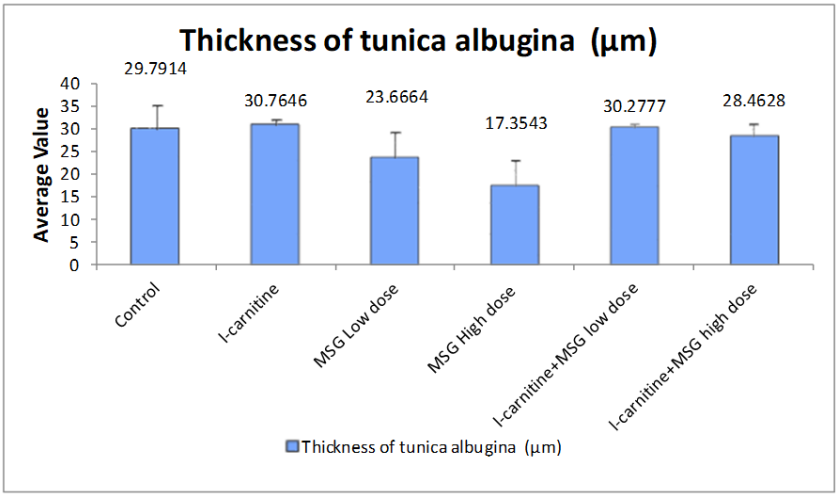


Fig 3: Morphometric analysis of testes thickness of tunica albugina (µm) in male albino mice treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

Histological Examination of The Testis:

Control and L- Carnitine Groups:

Histological examination of transverse sections in testes of control male mice and that treated with L-carnitine for 35 days showed similar histological architecture as seen in figure 4. The testis consists of tunica albuginea that consists of dense connective tissue containing blood vessels, numerous seminiferous tubules each of which is round, oval, or elongated compartment containing several layers of spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The intertubular connective tissue contains interstitial cells (leydig cells) which are a darkly stained group of small polygonal cells that represent the endocrine portion of the testis. Spermatogonia are small round cells with spherical nuclei forming the outermost layer of cells of the seminiferous tubules. Between these cells, Sertoli cells are found to have a slender or pyramidal shape and contain oval nuclei. The primary spermatocytes are large cells with prominent nuclei that contain distinct chromatin threads, however, the secondary spermatocyte is smaller cells lying nearer to the tubule lumen. The spermatids are small round cells with darkly stained nuclei found within the tubule lumen and the spermatozoa aggregate toward the sertoli cells.

MSG and/or L-Carnitine Treated Groups:

Microscopic investigation of transverse sections of testes of mice treated with low and high doses of MSG (0.3 mg/g and 0.6 mg/g) showed disruption of the germinal epithelium which was irregularly placed on the basement membrane and in some places detached from the basement membrane towards the lumen of the seminiferous tubules. Many vacuoles were observed in the spermatogenic tissue and hyaline material was observed in the tubules lumen. Within several tubules, in many sections of the testes, spermatogenic arrest phenomena were found. Also, many seminiferous tubules were seen filled with primary spermatogonia only without sperm formation due to the sloughing of spermatogenic cells with pyknotic nuclei. Accumulation of sloughed cells was detected in the center of the seminiferous tubules. Giant multinuclear spermatocytes were shown in the lumina of many seminiferous tubules. In addition, the interstitial tissue showed acidophilic hyaline material, edematous stroma containing small groups of leydig cells in comparison with the normal control mice. Furthermore, dilated and congested blood vessels and extensive

bleeding were observed in the interstitial tissue.

All these lesions are dose-dependent and recorded in tabulated figure 1 according to their incidence. Also, the testes of mice treated with both doses of MSG concurrently treated with L-carnitine (150 mg/kg) showed more or less normal architecture with an improvement in most of the previously mentioned lesions including the germinal epithelium of the seminiferous tubules and the interstitial tissue. Most of the seminiferous tubules appeared nearly normal and lined with the normal arrangement of spermatogenic cells and between Sertoli cells. Also, their lumina filled with mature spermatozoa.

From these results, the repairing up of L-carintine against reproductive toxicity of MSG was more pronounced in case of a low dose of MSG (0.3mg/g) than the higher dose (0.6mg/g) where some cells stay vacuolated and detached from the basement membrane (Fig5 A&B).

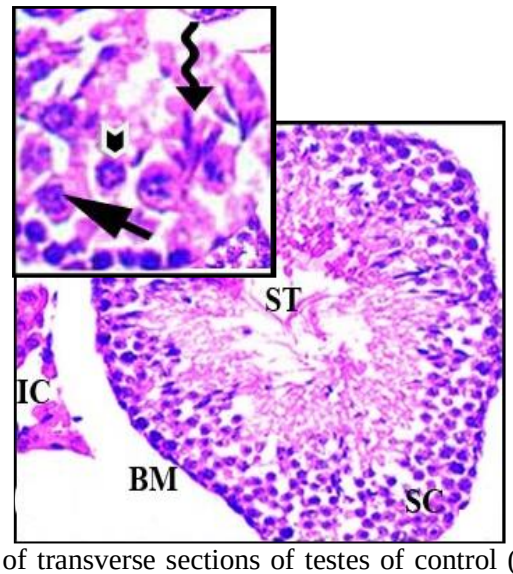


Fig. 4: Photomicrographs of transverse sections of testes of control (A) and L-carnitine (B) treated mice showing normal histological structure of the testes. Note: complete seminiferous tubule (ST), spermatogonia (SG), primary spermatocytes (arrow), secondary spermatocytes (arrowhead), spermatozoa (bent arrow in A and Z in B) and Sertoli cell inbetween (SC). Interstitial connective tissue (IC) with Leydig cells (Lc) (HE, X 400- the insertion part X1000).

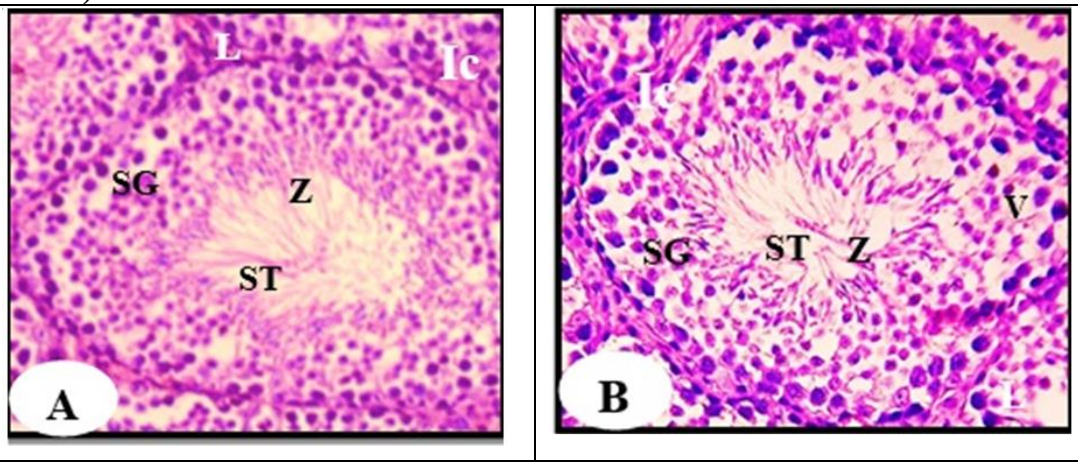
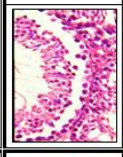
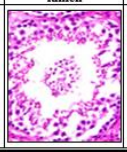
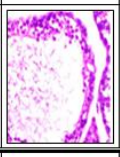
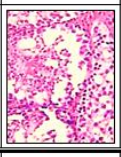
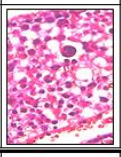
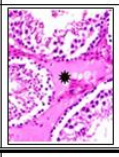
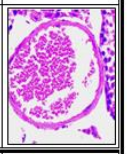


Fig. 5: Photomicrographs of transverse sections in the testes of mice co-administered L-carintine with low dose of MSG (A) and with high dose of MSG (B).
Note: The seminiferous tubules (ST) showed nearly with normal histological architecture and lined with different spermatogenic cells (SG). Lumina of tubules filled with mature spermatozoa (Z). Also, normal interstitial connective tissue (Ic) with Leydig cells (L). Note: some cells stay vacuolated (V) and detached from the basement membrane (HE, X400).

Tabulated figure 1: Showed the histopathological lesions in the testis of the male albino mice treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days. HE, X400.

Treatment and doses	Types of histopathological lesions							
	Detached spermatogenic cells and Pyknosis	Sloughing necrotic spermatocytes with pyknotic nuclei in tubular lumen	Spermatogenic arrest.	Vacuolation	Giant multinuclear spermatocyte	Hyaline material in lumina of the seminiferous tubules	Acidophilic hyaline material in interstitial tissue containing small groups of Leydig cells	Dilatation and congestion of blood vessels
								
Group 1: Control	-	-	-	-	-	-	-	-
Group 2: L-carnitine (150mg/kg/day)	-	-	-	-	-	-	-	-
Group 3: MSG Low dose (0.3 mg/kg/day)	++	++	+	++	-	+	+	+
Group 4: MSG High dose (0.6 mg/kg/day)	+++	+++	++	+++	++	++	++	++
Group 5: L-carnitine +MSG Low dose	+	-	-	-	-	-	-	-
Group 6: L-carnitine +MSG High dose	+	-	-	+	-	-	-	-

Immunohistochemical Studies:

Proliferating Cell Nuclear Antigen (PCNA) Expression:

PCNA is a marker for deoxyribonucleic protein which is necessary for deoxyribonucleic acid (DNA) synthesis in a mammalian cell. The positive PCNA reaction appeared as brown granules present in the nuclei of spermatogonia and part of spermatocytes. In the control and L-carnitine treated mice, the spermatogonic cells and primary spermatocytes showed strong immunopositive PCNA reaction. The seminiferous tubule epithelium exhibited a positive immune response reaction (brown colour) for PCNA in the nuclei of the spermatogonic cells (Fig. 6 A, B). Other sections of testes of mice treated with low and high doses of MSG showed decrease PCNA positive expression in the nuclei of the seminiferous epithelial cells as violet colour (Fig. 6 C & D) comparing to the control and L-carnitine treated groups. On the contrary, sections of testes of mice treated with low and high doses of MSG concurrently treated with L-carnitine showed an elevation in PCNA immune expression in spermatogenic cells compared to other groups treated with MSG (Fig. 6 E, F). Sertoli cells and leydig cells exhibited negative PCNA reactions in all groups examined.

Ki-67 Expression:

Ki-67 is commonly used as a marker to assess cell proliferation. The positive reaction appeared as brown granules present in the nuclei of spermatogonia as strong and moderate immunopositive reaction in the seminiferous tubules cells and Sertoli cells of both control and L-carnitine treated mice groups, respectively (Fig. 7 A & B). Figure 7 (C & D) showed a significant decrease in Ki-67 positive cells in the groups treated with low and high doses of MSG alone when compared to the control and L-carnitine groups. The nuclei of spermatogonic cells in case of low and high doses of MSG groups showed violet colour indicating weak reaction in Ki-67 immunostaining expression. The positive reaction was improved after treatment with 150 mg/kg of L-carnitine concurrently with low and high doses of MSG (Figs. 6 E, F).

Claudin-1 Expression:

The localization of claudin-1 in the epithelium of seminiferous tubules was examined to assess the possible changes in the blood testes barrier during the MSG induced spermatogenesis changes. The

localization of immunoreactive claudin-1 in the seminiferous epithelium of the normal control mice and L-carnitine treated mice appeared as a reddish-brown precipitate in the basal compartment of the seminiferous tubules (Fig. 8 A & B). This reaction was observed also in the adluminal compartments of each tubule in every stage of the germinal epithelium cycle at the junction between the borders of Sertoli cell with spermatids and sperms. Furthermore, immune-reactive specificity could be observed in the nuclei of premeiotic germ cells, but not in pachytene stage of spermatocytes, secondary spermatocyte, or around spermatids. The distribution of claudin-1 following treatment with low and high doses of MSG (Figs. 8 C & D) dwindled only as few expressions in the adluminal compartment. Whereas mice co-administered MSG with L-carnitine showed retention of the claudin-1 reaction to its normal reactivity as that observed in the control and L-carnitine treated groups (Figs. 8 E & F).

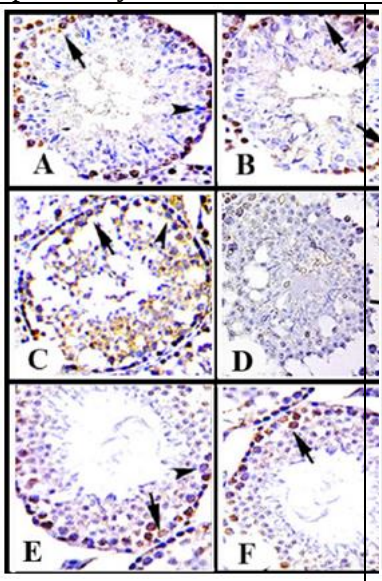


Fig.6: Photomicrographs showing immunohistochemical demonstration of PCNA in transverse sections of mice testes of different studied groups. **(A)** Control group **(B)** L-carintine (150 mg/kg L- carintine). **(C)** MSG low dose (0.3 mg/g) **D)** MSG high dose (0.6 mg/g). **E)** MSG low dose (0.3 mg/g) co-administered 150 mg/kg L- carintine. **F)** MSG high dose (0.6 mg/g) co-administered 150 mg/kg L-carintine. Note: nuclei of spermatogonic cell exhibited brown colour indicate positive reactivity (arrow) others give violet colour indicating weak or negative reaction (arrowhead). X400.

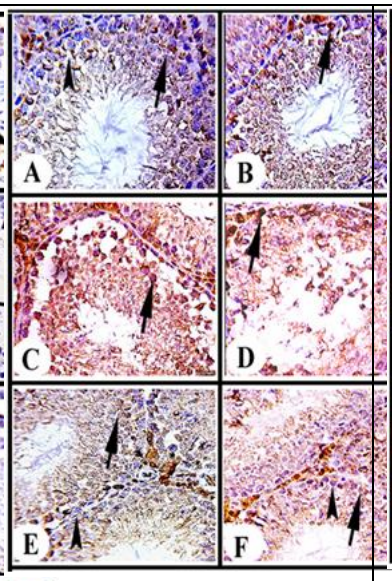


Fig. 7: Photomicrographs showing immunohistochemical demonstration of Ki-67 in transverse sections of mice testes of different studied groups. **(A)** Control group **(B)** L-carintine (150 mg/kg L- carintine). **(C)** MSG low dose (0.3 mg/g) **D)** MSG high dose (0.6 mg/g). **E)** MSG low **dose** (0.3 mg/g) co-administered 150 mg/kg L-carintine. **F)** MSG high dose (0.6 mg/g) co-administered 150 mg/kg L- carintine. Note: nuclei of spermatogonic cell and Sertoli cells exhibited brown colour granules indicate positive reactivity (arrow) others give violet colour indicating weak or negative reaction (arrowhead). X400.

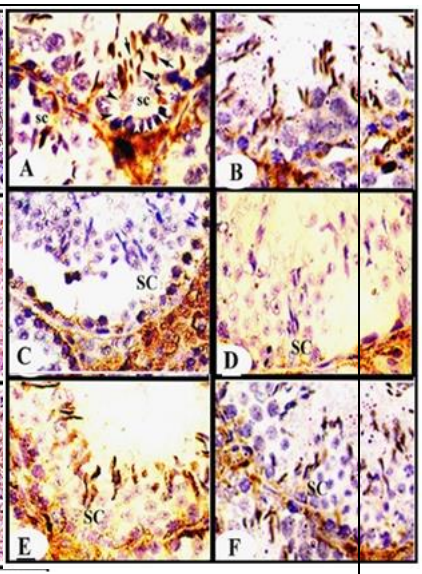


Fig. 8: Photomicrographs showing immunohistochemical demonstration of claudin-1 in transverse sections of mice testes of different studied groups. **(A)** Control group **(B)** L-carintine (150 mg/kg L- carintine). **(C)** MSG low dose (0.3 mg/g) **D)** MSG high dose (0.6 mg/g). **E)** MSG low dose (0.3 mg/g) co-administered 150 mg/kg L- carintine. **F)** MSG high dose (0.6 mg/g) co-administered 150 mg/kg L- carintine. Note: basement membrane of spermatogonic cell and borders of Sertoli cells exhibited brown colour granules indicate positive reactivity (arrow) others give violet colour indicating weak or negative reaction (arrowhead).X 1000.

DISCUSSION

Testicular Morphometric Change:

In the present study, mice treated with MSG showed marked changes in the measured morphometric parameters of the testicular tissues compared with the control and L-carintine treated mice. These changes included a decrease in tubular diameter, germinal epithelium height, tunica albuginea and an increase in the blood vessel diameter and lumen of the seminiferous tubules. In this respect, Nosseir *et al*. (2012) recorded that rats daily received *IP* 4 ml/kg body weight of MSG (1 gm of MSG in 1 ml of distilled water) for 14 days, showed a decrease in tubular diameter, reduction in germinal and epithelium height. In addition, Franca *et al.* (2006) reported a significant reduction (P<0.05 or less) in the seminiferous tubule diameter, seminiferous tubule volume, total length of seminiferous tubules, percentage of seminiferous tubule lumen and seminiferous tubule lumen volume, Leydig cell nuclear volume, and the total number of Sertoli and Leydig cells per testis in male rat pups at age of 1 or 4 months, that pups of 2 days after birth, were injected *ip* with 4 mg/g body weight MSG every 2 days up to 10 days of age and weaned at 21 days of age. Similarly, the diameter of seminiferous tubules and their epithelial height were significantly decreased in rats who received MSG at a dose level of 4mg/kg b.wt, daily for 4 weeks compared with controls (Sakr and Badawy, 2013). Sarhan (2018) demonstrated significant marked damage of seminiferous tubules with a significant reduction in diameter and height of the lining epithelium in testes of male rats orally received 6 mg/g/day of MSG for 45 days.

The germ cell height was markedly reduced in compare with control and L-carnitine groups. These results were in accordance with Franca *et al* (2006) who reported similar histopathological changes in the testis after administration of MSG. Whereas Sazegar *et al*. (2014) found that L-carnitine administration to diabetic rats in doses of 40 mg/kg for 16 days was effective on the diameter and thickness of seminiferous tubules.

Histological Studies:

The microscopical examination of the testes obtained from MSG treated mice showed that treatment with the MSG caused testicular damage in the form of atrophied seminiferous tubules with focal disorganization of seminiferous tubules with marked depletion of the spermatogenic cell populations. Exfoliation of the damaged spermatocytes and spermatids were detected within the tubular lumina of many of the seminiferous tubules. Also, Sertoli cells were reduced in number and size and the intertubular connective tissue became hyalinized and showed a comparative reduction of interstitial cells (Leydig cells).

These results are in agreement with the results of Kianifard (2015) who reported that rats treated with monosodium glutamate (4 mg/kg b.w, i.p.) for 14 days, showed severe tubular atrophy and germinal epithelium depletion accompanied with derangement of spermatogenic cells in tissue samples of animals treated with MSG. Also, MSG led to an alteration in the population of spermatogenic cells and derangement of cellular junctions led to moderate deformity and atrophy of seminiferous tubules.

In addition, Onakewor *et al*. (1998) and Boodnard *et al*. (2001) mentioned that administration of MSG led to atrophic changes in the testis and destruction of sertoli cells and leydig cells. Kadir (2011) found in rats that received MSG (1.53, 3.07, 6.13, 12.27 g/kg b.w. daily) for 14 days that the testes tissue showed a reduction in spermatogenic cells, reduction in the interstitial tissue, reduction in leydig cells and irregular seminiferous tubules. Mohamed (2012) revealed that orally administered MSG (2 g/kg and 4 g/kg)

for two duration (10 and 30 days) caused a decrease in primary spermatocytes, necrotic spermatogenic cells, sertolli cell vacuolization and loss of late spermatids. These findings were in concur with Nosseir *et al*. (2012) who proved that histopathological changes detected in the testis of rats treated with MSG included; loss of spermatogenic cells, atrophy of the seminiferous tubules, intercellular vacuolation in the stroma and peritubular fibrosis.

Furthermore, the testes of rats received MSG at a dose level of 4mg/kg b.wt, daily for 4 weeks showed loss of the spermatogenic cells, deformed Sertoli cells and the interstitial tissue appeared with different vacuoles, blood hemorrhage and the leydig cells exhibited pyknotic nuclei (Sakr and Badawy, 2013). In the same respect, Hamza and El-Harbi (2014) observed seminiferous tubules that after MSG treatment filled by spermatogenic cells with few sperm formations and other seminiferous tubules filled by spermatogonia only. Also, Alalwani (2014) found atrophied seminiferous tubules, necrotic spermatogonia, vacuolization in germ cells, necrosis in leydig cells, and loss in the interstitial tissue, hyalinated interstitial tissue and dilated blood vessels in rats intraperitoneally injected with MSG in doses of 3, 6 g/kg b.w. daily for 56 days. Also, Hilwani *et al.* (2014) revealed damages to testes cytoarchitecture of male mice and degenerative structures in the seminiferous tubule of testes of *Mus musculus* after daily *IP* injection with different MSG concentrations at 250, 500 and 1000 mg/kg body weight, for 14 days. In addition, Iamsaard *et al.* (2014) reported a vacuolization in germ cells, loss in interstitial tissue and sloughing of spermatogenic cells in rats received orally MSG (0.25, 3, 6 g/kg b.w daily) for 28 days. In the same context, the semineferous tubules of male rats' testes showed disorganized spermatogenic cells, dark nuclei, reduction in number, maturation arrest, vacuolations of Leydig cells and interstitial tissue after orally receiving MSG at a dose of 6 mg/g/day for 45 days (Sarhan, 2018). Moreover, Al-Shahari and El-Kott (2019) reported that the examined testes of rats given 6 mg/g/day of MSG for two months showed marked degeneration and loss of spermatogenic cells that lining some seminiferous tubules with distorted Sertoli cells. Spermatogenic cells and cellular debris were seen in the lumen of seminal tubules. The blood hemorrhage, different vacuoles and Leydig cells with pyknotic nuclei were also shown. related with interstitial diffuse edema, necrosis and blood congestion.

In our study, animals treated with MSG and L-carnitine showed improvement in the histoarchitecture of the testes when compared with MSG treated groups. This finding was in agreement with Coşkun *et al*. (2013) who reported that administration of L-carnitine alleviated the reduction in germ cell numbers and morphological damage in testicular tissues and exhibited protective effects against the damage caused by radiotherapy. Experimental studies have reported that germ cell apoptosis is induced by treatment with MSG (Sarhan, 2018). The anti-apoptotic effect(s) of carnitine in the testes may also contribute to this apoptosis (Coşkun *et al.*, 2013). Mahmoud *et al*. (2018) reported that testes of male bats treated with α-chlorohydrin showed a thick tunica albuginea and slightly smaller tubules with a thick basement membrane. In addition, vacuolated Sertoli cells and vascular congestion, intact spermatogenic cells with significant hypoplasia, disrupted germinal epithelium, cytoplasmic swelling and nuclear pyknosis were also microscopically demonstrated. Cauda epididymis of the treated bats exhibited many abnormal spermatozoa and cellular debris in the epididymal duct. Sperm count was 1/10 of the control

and the observed abnormal forms of sperm were three times more frequent than that found in the control group.

Another antioxidant such as grape seed extract showed an improvement of the histopathological lesions as revealed when control animals contrasted, also, impaction of spermatids and sperms in the tubule lumen indicated that the normal arrangement of seminiferous tubule cells and complete spermatogenesis, however just seminiferous tubules dilatation was present (Al-Shahari and El-Kott, 2019).

Immunohistochemical Studies:

Spermatogenesis is a complex process in which the spermatogonia developed into mature spermatozoa by continuous mitosis, meiosis, and cell differentiation in the testis (Yao *et al.*, 2015; Griswold, 2016). Spermatogenic cells are frequently proliferating, continuously developed and differentiated with physiological significance during spermatogenesis. Several proliferation factors, including proliferating cell nuclear antigen (PCNA) and Ki-67, are involved in the proliferation and differentiation of spermatogonia in the testis (Wrobel *et al.*, 1996; Angelopoulou *et al.*, 2008). Thus, the positive protein expression levels of PCNA and Ki-67 in the testis are the standard markers of spermatogenic cell proliferation and evaluate the state of smooth spermatogenesis (Zhao *et al.*, 2018).

PCNA:

Although classical histopathology assessments are more sensitive measurements and conceded as the widely accepted standard for assessing potential testicular toxicity, generally they are neither quantitative nor sensitive enough to detect early toxicity. These methods are typically descriptive and unable to quantify the degree of toxicity. Immunolabeling cells with proliferating cell nuclear antigen (PCNA) has been used to identify proliferating spermatogonia; however, there have been no systematic attempts to quantify these changes (D'Andrea *et al*., 2008).

Proliferating cell nuclear antigen (PCNA) expression changes are used as a marker to evaluate cell proliferation. PCNA is an intranuclear polypeptide involved in DNA duplication and repair and it is a co-factor of DNA polymerase-d (Shivji *et al*., 1992; Georgescu *et al*., 2015). PCNA is an essential molecule for the initiation of DNA duplication, so it plays a critical role in the initiation of cell proliferation (Celis and Celis 1985; Robbins *et al*. 1987; Jaskulski *et al*. 1988; Xue *et al*., 2007). Furthermore, its staining intensity was used to evaluate the proliferation of cells and the spermatogenic function of testes in case of male infertility (Tousson *et al*., 2012).

The results of our study were to explore the capability of L-carintine in protecting or ameliorating the MSG induces reprotoxicity based on the evaluation of PCNA immunohistochemistry in the testes tissue. Many positive nuclear reactions were detected in spermatogonia and proliferating spermatocytes of the testes of both the control group and L-carintine treated group while the reactions were weak or negative in all MSG treated groups except those in group 5 and 6 where mice were co-administered with L-carintine during MSG treatment. Also, the number of PCNA-positive germinal cells and the signal density of positive cells were lower in the MSG-treated group. These results are coinciding with the lower values of sperm count in the MSG treated groups. The diminishing in PCNA in testicular germ cells indicates the decrease in proliferative activity and spermatogenesis (Kang *et al*., 1997; Sakr *et al*., 2017).

Free radicals may cause cell death directly or indirectly. Apoptosis is associated with oxidative stress in many cell types including spermatogenic cells. Apoptosis is the scheduled cell death that takes place in several

physiological and pathological events. Intracellular and extracellular death signals are effective in the beginning of the apoptotic process. When the cell receives these signals, the relevant genetic mechanism is triggered and apoptosis starts (Kus *et al*., 2008).

In agreement with our results, L-carnitine also reduce the late testicular and spermatic damage caused by cisplatin administration to prepubertal rats by inducing germ cell proliferation and preventing apoptosis where the number of PCNA positive cells in the cisplatin + carnitine group increased compared to the cisplatin group (Yaman and Topcu-Tarladacalisir, 2018). Parallel with these results, L-carnitine enhances the activity of DNA repairing enzyme (Boerrigter *et al.,* 1993). In addition, L-carnitine has been recently shown to act as an important anti-apoptotic mediator (Moretti *et al.,* 2002). The potent anti-apoptotic ability of acetyl L-carnitine to prevent oxidative stress-related mitochondrial damage and subsequent mitochondria-dependent apoptosis has been demonstrated in many cell types on experimental cisplatin nephrotoxicity (Tufekci *et al*., 2009).

Ki-67:

Ki-67 protein is a cell proliferative marker (Whitfield *et al.,* 2006; Angelopoulou *et al.,* 2008) expressed in the nuclear matrix of cells during the late G1, S, G2 and M phases of the cell cycle (Sasaki *et al.,* 1987). Also, it is a very important proliferation-associated nuclear antigen and its expression is of great significance for the regulation of the cell cycle (Schluter *et al*., 1993).

In the current study the Ki-67 expression is restricted to the nuclei of the basal compartment cells of the seminiferous tubules (spermatogonia and Sertoli cells) and its reactivity decreased in other cells towards the luminal compartment as previously reported by Steger *et al.* (1998) and Schwalm *et al.* (2007) who stated that, Ki-67 presents in the nuclei of spermatogonia and Sertoli cells of the testis. The migration of cells in the testis into the differentiated luminal compartment which contains germ cells in meiotic and post-meiotic phases is also associated with downregulation of Ki-67 as cells lose their ability to initiate DNA synthesis. This condition is reflected in the significant decrease in the Ki-67 labelling index from 24% for spermatogonia to 0.6% in spermatocytes in normal human testis (Eward *et al*., 2004).

Moreover, Ki-67 expression in testicular tissue showed great reduction in both MSG treated groups in comparing with control and L-carnitine groups where well-arranged spermatogenic germ cell series were present giving nuclei a violet colour indicating negative or week reaction. Whereas the co-administered MSG and L-carnitine groups showed more or less positive Ki-67 as normal level. Parallel with these results, Ki-67 expressions in both valproic acid and *Momordica cochinchinensis* treated groups were lower than the control group (Iamsaard *et al.,* 2017). This study suggested that low expression of this protein might associate with the decreasing of germ cell proliferation resulting in testicular damages, spermatogenic arrest, and damage of seminiferous tubule as previously reported by other authors (Steger *et al.* 1998; Schwalm *et al*., 2007). The positive expression of Ki-67 is conducive to smooth spermatogenesis (Angelopoulou *et al.,* 2008). As a cell proliferation-related nuclear antigen, Ki-67 exists in the cell nucleus and participates in cell mitosis at the prophase to prometaphase transition and DNA structure maintenance (Takagi *et al*., 2016).

Claudin-1:

It is an integral membrane protein at the blood testes barrier (BTB). The inter-sertoli tight junctions (TJ) constitute the BTB that protects the seminiferous epithelium from invasion by molecules or cells that may disturb the process of spermatogenesis. Gilio *et*

al. (2013) demonstrated that claudin-1 is found in the basal and adluminal compartments in the seminiferous epithelium of normal mice, suggesting that claudin-1 may have functions other than those involving TJ in the testis. The localization of claudin-1 in the epithelium of the seminiferous tubules was examined to assess the possible changes in the blood testes barrier (BTB) during the MSG induced spermatogenesis changes. In the present study, localization of immunoreactive claudin-1 in the seminiferous epithelium of the normal control mice and L-carnitine treated mice appeared as a reddish-brown precipitate in the basal compartment of the seminiferous tubules. This reaction was also observed in the adluminal compartments of each tubule at the junction between the borders of Sertoli cell with elongated spermatids and sperm.

In contrast, the immunohistochemical distribution of claudin-1 dwindled only as few expressions in the adluminal compartment after treatment with low and high doses of MSG. Weak immunoreactivity results may be due to the effect of MSG on claudin-1 protein in the BTB. Whereas the mice co-administered L-carnitine with MSG in ameliorated groups exhibited clear retention of the claudin-1 reaction to nearly normal reactivity as that observed in the control and L-carnitine treated groups.

The tight junction is a multimolecular membrane that comprises integral membrane proteins, including occludin and claudin family proteins (Morrow *et al.,* 2010). In testis, tight junctions (TJs) between adjacent Sertoli cells are important for the formation of the blood-testis barrier (BTB) and crucial for spermatogenesis. This barrier restricting the movement of water, solutes, and immune cells from circulation to seminiferous tubule, which creates a unique microenvironment for spermatogenesis. Moreover, BTB is crucial for the creation of a physiological milieu in a seminiferous tubule for spermatogenesis and for the protection of germ cells from the immune system (Russell and Peterson, 1985). The tight junctions between Sertoli cells have several unique features in comparison with the tight junctions of other polarized epithelial cells. In other polarized epithelia, tight junctions are located in a circumferential band towards the apical membrane; whereas in Sertoli cells, the tight junctions are located in a circumferential band toward the basement membrane (Wong *et al*., 2008).

According to Mruk and Cheng (2004), the blood-testis barrier divides the seminiferous epithelium into basal and adluminal compartments. Spermatogonia and pre-leptotene spermatocytes are below in the basal compartment, and leptotene and later spermatocytes and spermatids are above, in the ad luminal compartment. This physical separation establishes the distinct microenvironments needed for the different germ cell types (Onoda *et al.*, 1990; Mruk and Cheng, 2004). It also protects the primary spermatocytes and haploid spermatids from potentially harmful chemicals by limiting the movement of intercellular molecules from the interstitial space into the adluminal compartment (Mruk and Cheng 2004; Su *et al*., 2009). In association with other immunoprotective mechanisms, the BTB protects the haploid spermatids, which express ‘foreign’ proteins, from immunological attack and helps to establish testicular immune privilege (Fijak and Meinhardt, 2006). In addition, Claudin-1 and -11 may play an important role in the development of BTB and IN migration of early spermatocytes through BTB and the development of BTB, respectively. Besides, Claudin-1 and -11 may also associate with Sertoli cells–germ cells as well as Sertoli cell-ECM adhesion in the seminiferous epithelium of adult pheasant testis (Park *et al*., 2011). In

humans, alterations of BTB have been known to be related to diverse male fertility status (Cavicchia and Sacerdote, 1988).

In conclusion, the results of the current study reflect clearly that MSG-induced reproductive toxicity in male mice. The toxicity symptoms are decreased by L-carintine treatment suggesting that L-carnitine is a protective antioxidant against MSG induced male reproductive toxicity. Additionally, it is recommended to carry out further investigations using other parameters such as antioxidants measurements and other immunohistochemical markers to evaluate the protective or preventive effects of different antioxidants against the reproductive toxic effect of MSG. This study also spots light on the organization's human health, and responsible for food safety to use common salt and natural flavour instead of MSG.

REFERENCES

Acharya, U. R., Mishra, M., Patro, J., and Panda, M. K. (2008): Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reproductive Toxicology*, 25(1): 84-88.

Adewoyin, M., Ibrahim, M., Roszaman, R., Isa, M., Alewi, N., Rafa, A., and Anuar, M. (2017): Male infertility: the effect of natural antioxidants and phytocompounds on seminal oxidative stress. *Diseases*, 5(1):1- 26.

Alalwani, A. D. (2014): Monosodium glutamate induced testicular lesions in rats (histological study). *Middle East Fertility Society Journal*, 19(4): 274-280.

Angelopoulou R, Balla M, Lavranos G, Chalikias M, Kitsos C, Baka S. and Kittas C. (2008): Evaluation of immunohistochemical markers of germ cells' proliferation in the developing rat testis: a comparative study. *Tissue Cell*, 40(1):43–50.

Batra, N., Nehru, B., and Bansal, M. P. (2001): Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels. *Journal of Applied Toxicology: An International Journal*, 21(6): 507-512.

Boerrigter, M. E., Franceschi, C., Arrigoni-Martelli, E., Wei, J. Y., and Vijg, J. (1993): The effect of L-carnitine and acetyl-L-carnitine on the disappearance of DNA single-strand breaks in human peripheral blood lymphocytes. *Carcinogenesis*, 14(10): 2131-2136.

Boodnard, I., Gooz, P., Okamura, H., Toth, B.E., Halasz, B., and Nagy, G.M. (2001): Effect of neonatal treatment with monosodium glutamate on dopaminergic and DOPA neurons of the medial basal hypothalamus and on prolactin and MSH secretion of rats. *Brain Research Bulletin*, 55: 767- 774.

Coşkun, N., Hatipoğlu, M. T., Özoğul, C., Korkmaz, C., Akyol, S. N., Mıcılı, S. C. and Erdoğan, D. (2013): The protective effects of acetyl L-carnitine on testis gonadotoxicity induced by cisplatin in rats. *Balkan medical journal*, 30(2): 235.

Eslamian, G., Amirjannati, N., Rashidkhani, B., Sadeghi, M. R., and Hekmatdoost, A. (2012): Intake of food groups and idiopathic asthenozoospermia: a case–control study. *Human reproduction*, 27(11): 3328-3336.

Eweka, A., and Om'Iniabohs, F. A. E. (2008): Histological studies of the effects of monosodium glutamate on the testis of adult wistar rats. *The Internet Journal of Urology*, 5(2).

Franca, L. R., Suescun, M. O., Miranda, J. R., Giovambattista, A., Perello, M., Spinedi, E., and Calandra, R. S. (2006): Testis structure and function in a nongenetic hyperadipose rat model at prepubertal and adult ages. *Endocrinology,* 147(3):1556-1563.

Geha, R. S., Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J. and Saxon A. (2000): Review of alleged reaction to monosodium glutamate and outcome of a multicenter DoubleBlind Placebo-Controlled study. *Journal of Nutrition,* 130(4): 1058-1062.

Georgescu, R., Langston, L., and O'Donnell, M. (2015): A proposal: Evolution of PCNA's role as a marker of newly replicated DNA. *DNA repair,* 29: 4-15.

Gerdes, J., Schwab, U., Lemke, H., and Stein, H. (1983): Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *International journal of cancer,* 31(1): 13-20.

Gilio, J. M., Portaro, F. C., Borella, M. I., Lameu, C., Camargo, A. C., and Alberto-Silva, C. (2013): A bradykinin-potentiating peptide (BPP-10c) from Bothrops jararaca induces changes in seminiferous tubules. *Journal of Venomous Animals and Toxins including Tropical Diseases,* 19(1): 28.

Hamzaa, R. Z. and AL- Harbib, M. S. (2014): Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. *Toxicology Reports,* 1037-1045.

Hilwani, I. N., Nasibah, R., Nurdiana, S., and Norashirene, M. J. (2014). Gonadotoxic and Cytotoxic Effect of Induced obesity via Monosodium Glutamate on *Mus musculus* Testis Cytoarchitecture and Sperm Parameter. World Academy of Science, Engineering and Technology, *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering,* 8(9): 1000-1003.

Homan, G.F., Davies, M. and Norman, R. (2007): The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Human Reprod Update,* 13: 209-23.

Husarova, V., and Ostatnikova, D. (2013): Monosodium glutamate toxic effects and their implications for human intake: a review. *JMED Research*, 1-12.

Iamsaard, S., Sukhorum, W., Samrid, R., Yimdee, J., Kanla, P., Chaisiwamongkol, K., and Kondo, H. (2014): The sensitivity of male rat reproductive organs to monosodium glutamate. *Acta medica academica,* 43(1).

Kadir, R. E. (2011): Effects of Monosodium Glutamate on Semen Quality and the Cytoarchitecture of the Testis of Adult Wistar Rats. *International Journal of Biochemical and Health Sciences,* 7(1).39-46.

Kaur, R. P., Gupta, V., Christopher, A. F., and Bansal, P. (2015): Potential pathways of pesticide action on erectile function–a contributory factor in male infertility. *Asian Pacific Journal of Reproduction,* 4(4): 322-330.

Kianifard, D. (2015): Protective Effects of *Morus Alba (M. alba*) Extract on the Alteration of Testicular Tissue and Spermatogenesis in Adult Rats Treated with

Monosodium Glutamate. *Medicine Science*, 4(1):1947-58.

Kubota T., Nishimura K., Kanemaki M.T. and Donaldson A.D. (2013): The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Molecular Cell*, 50(2):273–280.

Kunkel, E. M., and Barbara, H. D. (2004): Nutrition and Well-being A to Z. The Gale Group Inc., *Macmillan Reference USA*, New York, Gale.

Mohamed, I. K. (2012): The effects of oral dosage of monosodium glutamate applied for short-and long-terms on the histology and ultrastructure of testes of the adult rats. *Journal Animal Veterinary Advanced*, 11(1): 124-133.

Moretti, S., Famularo, G., Marcellini, S., Boschini, A., Santini, G., Trinchieri, V., and De Simone, C. (2002) L-carnitine reduces mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *International Journal of Cancer*, 31(1):13–20

Morrow, C. M., Mruk, D., Cheng, C. Y., and Hess, R. A. (2010): Claudin and occludin expression and function in the seminiferous epithelium. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1546): 1679-1696.

Nosseir, N. S., Ali, M. H. M. and Ebaid, H. M. (2012): A histological and morphometric study of monosodium glutamate toxic effect on testicular structure and potentiality of recovery in adult albino rats. *Research Journal of Biology*, 2(2): 66-78.

Rosa, S. G., Quines, C. B., da Rocha, J. T., Bortolatto, C. F., Duarte, T., and Nogueira, C. W. (2015): Antinociceptive action of diphenyl diselenide in the nociception induced by neonatal administration of monosodium glutamate in rats. *European journal of pharmacology*, 758: 64-71.

Ross, C., Morriss, A., Khairy, M., Khalaf, Y., Braude, P., Coomarasamy, A., and El-Toukhy, T. (2010): A systematic review of the effect of oral antioxidants on male infertility. *Reproductive biomedicine online*, 20(6): 711-723.

Sakr, S. A., and Badawy, G. M. (2013): Protective effect of curcumin on monosodium glutamate-induced reproductive toxicity in male albino rats. *Global J ournal Pharmacology*, 7: 416-422.

Sarhan, N. R. (2018): The ameliorating effect of sodium selenite on the histological changes and expression of caspase-3 in the testis of monosodium glutamate-treated rats: Light and electron microscopic study. *Journal of microscopy and ultrastructure*, 6(2): 105-115.

Sasaki, K., Murakami, T., Kawasaki, M., and Takahashi, M. (1987): The cell cycle associated change of the Ki-67 reactive nuclear antigen expression. *Journal of cellular physiology*, 133(3): 579-584.

Sazegar, G., Ebrahimi, V., Saeedi Boroujeni, M. J., Mohammadi, S., and Salimnezhad, R. (2014): Morphometric study of testis tissue and spermatogenesis following carnitine administration in diabetic rat induced with stereptozotocin. *Iranian Journal of Diabetes and Metabolism*, 14(1): 9-14.

Schluter, C., Duchrow, M., Wohlenberg, C., Becker, M. H. G., Key, G., Flad, H. D. and Gerdes, J. (1993): The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements,

representing a new kind of cell cycle-maintaining proteins. *Journal Cell Biololgy*, 123, 513-522.

Sheehan, D. C. and Hrapchak, B. B. (1987): Theory and practice of Histotechnology. 2nd Edition. Columbus: Battelle Memorial Institute, pp 39-40.

Shivji, K. K., Kenny M. K. and Wood, R. D. (1992): Proliferating cell nuclear antigen is required for DNA excision repair. *Cell*, 69(2): 367-374.

Vinodini, N. A., Nayanatara, A. K., Ramaswamy, C., Ranade, A. V., Kini, R. D., Gowda, K. M., ... and Bhat, R. (2010): Study on evaluation of monosodium glutamate induced oxidative damage on renal tissue on adult Wistar rats. *Journal of Chinese clinical medicine*, 5(3) 144-147.

Walker, R., and Lupien, J. R. (2000): The safety evaluation of monosodium glutamate. *The Journal of nutrition*, 130(4): 1049-1052.

ARABIC SUMMARY

التأثير الوقائي لـ إل-كارنتين على تغيرات بنية الخصية التي يسببها الغلوتامات أحادية الصوديوم في الفئران الذكور

عبد الكريم محمد عبد اللطيف - وسام س توفيق
قسم علم الحيوان، كلية العلوم، جامعة الفيوم

أجريت هذه الدراسة لمعرفة التأثيرالوقائي ل ال - كارنتين ضد سمية الغلوتامات أحادية الصوديوم في خصية ذكور الفئران. وقد تم اختيار 60 من الفئران الذكور البالغين وتقسيمهم إلى 6 مجموعات بصورة عشوائية (ن=10) كما يلي: المجموعة الأولي (الضابطة) وهي مجموعة ذكور الفئران والتي تناولت الماء المقطرعن طريق الفم لمدة 35 يومًا والمجموعة الثانية أعطيت 150 مجم / كجم / يوم ال – كارنتين لمدة 35 يومًا. بينما تم إعطاء الجلوتامات أحادية الصوديوم عن طريق الفم إلى ذكور الفئران بجرعات 0.3 و0.6 مجم / جرام من وزن الجسم بشكل فردي في المجموعتين الثالثة والرابعة لمدة 35 يومًا. اما فئران المجموعتين الخامسة والسادسة فقد اعطيت جرعات 0.3 و0.6 مجم / جرام من وزن الجسم مصحوبا ب 150مجم / كجم من وزن الجسم من ال - كارنتين لمدة 35 يومًا. أظهرت القياسات المورفومترية والنتائج الهستوباثولوجية والدراسات الكيميائية المناعية لـ PCNA وKi-67 وClaudin-1 لأنسجة الخصية أن ال - كارنتين يخفف ويحسن التغيرات الضارة في أنسجة الخصية والناتجة عن التعرض لـ MSG. الاستنتاجات: علاج الفئران الذكور بـ ال - كارنتين يقلل من تسمم الخصية الناجم عن MSG عن طريق تحسين حالة بنية الخصية.

الكلمات الدالة: الغلوتامات أحادية الصوديوم، السمية، الخصية، ذكور الفئران ، ال كارنتين