Synergistic Protective Effects of L-Carnitine and Sodium Thiosulfate Against Monosodium Glutamate-Induced Testicular Toxicity in Male Rats

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Abstract

Monosodium glutamate (MSG) is a common food additive to improve taste with the potential of health-damaging effects, specifically on reproductive capacity. MSG is known to produce oxidative stress, which causes the decrease of steroid hormone synthesis, ultimately interfering with male reproductive organs function. This study aims to investigate the protective effects of carnitine and sodium thiosulfate as antioxidant monotherapy and their combination in protecting from MSG-induced testicular injury in male rats. A total of forty male Wistar rats were assigned to five groups of 8 [8/Group]. Group 1 (Control): received daily oral gavage of 1 mL distilled water; Group 2 (Positive control): monosodium glutamate at a dose of 1000 mg/kg bw, diluted in normal saline, delivered from orogastric tube once daily; Group 3: administered L-carnitine (100 mg/kg bw/day orally one hour prior to administration of MSG (1000 mg/kg bw/day); and group 4: received an intraperitoneal injection with sodium thiosulfate (400 mg/kg bw/day) one hour before the administration of MSG. Group 5: Monosodium Glutamate (1000 mg/kg bw/day) + L-carnitine (100 mg per kg bw each day) orally and intraperitoneally, sodium thiosulfate at a dose of 400 mg/kg/body mass /day. The experiment was conducted for 60 days. MSG exposure caused significant male reproductive toxicity, as evidenced by a decreased sperm count, motility, and morphology; this effect was accompanied with increased levels of malondialdehyde (MDA) but decreased superoxide dismutase (SOD). Individual administration of L-Carnitine (LC) and Sodium Thiosulfate (STS) alleviated these effects; however, the combinatory treatment manifested greater outcomes, leading to more significant amelioration in sperm quality and antioxidant capacity.

Conclusion: The present study shows that MSG administration leads to the development of a dose-response relationship for the impairment of testicular hormone regulation, sperm count, pathohistological changes, and biomarkers of oxidative stress in adult male Wistar rats. The results indicate that the combination treatment with antioxidants works as a synergistic protector against MSG-induced reproductive toxicity.

KEY WORD:

Monosodium glutamate, oxidative stress, testicular damage, L-carnitine, sodium thiosulfate, spermatogenesis

1. Introduction

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Monosodium glutamate (MSG) is the sodium salt of L-glutamic acid and is the most widely used food additive for preservation or be added to meals as a flavor booster. (Hamza and AL-Harbi, 2014). Several studies have reported detrimental toxic effects of MSG on the male reproductive system (Rahimi Anbarkeh *et al*., 2019; Alalwani, 2014). MSG-induced testicular toxicity has been reported to be associated with increased oxidative stress (Hamza and AL-Harbi, 2014). Oxidative stress is the overproduction of reactive oxygen species and hydrogen peroxide, which leads to oxidative DNA damage and lipid peroxidation of cell membranes, resulting in cell death (Rahimi Anbarkeh *et al*., 2019). The reproductive system is a common target for injury induced by glutamate because of the large number of glutamate receptors present in the reproductive organs and the sperm itself, rendering the reproductive system vulnerable to excitotoxic damage by excess glutamate in the body. Furthermore, glutamate toxicity is known to invoke horrible direct effects on the hypothalamic-pituitary-gonadal axis that can cause reproductive homeostasis disruption (Igwebuike UM, Nwankwo IA, Ochiogu IS. 2010).

L-Carnitine is a biologically active amino acid present in meat and milk; these foods are considered as the main sources of exogenous carnitine for human nutrition (NG *et al*., 2004). LC has antioxidant as well as free radical scavenging activities, reporting anti-inflammatory, anti-apoptotic, neuroprotective, cardioprotective and gastroprotective properties. (Dokmeci *et al*., 2007). Moreover, LC also protects the body from the harmful effects of ROS, owing to its act of being an antioxidant. Reactive oxygen species (ROS) damage the unsaturated fatty acids, decreasing the fusogenicity of sperm plasma membranes and long-chain fatty acids (LC), which protect spermatozoa from oxidative stress by removing harmful excess intracellular acetyl coenzyme A, as well as substitute fatty acids in membrane phospholipids (Micic *et al*., 2019). It is a major player in sperm energy metabolism and also a potential fuel of sperm motility during the epididymal transit, at extracellular and intracellular levels.

Sodium thiosulfate (STS) is an inorganic, odorless, and water-soluble compound with a chemical formula of Na□S□O□ and a molecular weight of 158.11g/mol. It is one of the principal oxidation products of hydrogen sulfide (H□S). It is usually encountered as a white crystalline or powdered pentahydrate (Na□S□O□·5H□O) (Ma *et al*., 2019). There is growing evidence to suggest that STS has antioxidant and anti-inflammatory properties and, therefore, can be a potential protective candidate molecule targeting multiple molecular pathways in a variety of diseases and drug-induced toxicities. STS can protect endocrine organs and regulate hormone secretion by its antioxidative effect and ion channel regulation. H□S acts in the endocrine system to modulate pituitary hormone secretion and testicular function (Zhang *et al*., 2021). Given the increasing exposure to MSG in the modern diet and its reported detrimental effects on male reproduction, it is necessary to investigate effective protective interventions. Thus, the present study was designed to evaluate the possible protective effects of sodium thiosulfate and L-carnitine separately or in combination against MSG-induced testicular damage in rats. The investigation is focused on the study of biochemical, hormonal, and histopathological parameters to understand the underlying mechanisms of protection.

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2. Materials and Methods

2.1 Chemicals and reagents

MSG L-Glutamic acid monosodium salt hydrate, BioReagent, suitable for cell culture (≥99 % purity), LC (L-Carnitine) of reagent grade purity ≥98%, STS Sodium thiosulfate. All were bought from a well-respected company (Al-Bashir Scientific Office, Baghdad, Iraq), were used in this study. Normal saline: 0.9% w/v sodium chloride from (Al-Mustaqbal Pharmaceutical Co. Ltd., Baghdad, Iraq). was used as a solevant. Biochemical analyses. The Enzyme-Linked Immunosorbent Assay (ELISA) kits from (East Lake Hi-TECH (ELK Biotechnology), Wuhan, China), were applied to determine hormonal levels, including LH, FSH, and testosterone. The SOD assay kit potentially exhibited by ELK Biotechnology Co. (Wuhan, China), and MDA, which is from (Solarbio Wuhan, China), utilized in conjunction with TBA spectrophotometry were run on the oxidative stress biomarkers. All reagents and kits were used according to the instructions of the manufacturer.

A stock solution was prepared by dissolving 1000 mg of MSG in normal saline, 100 mg of LC in normal saline, and 400 mg of STS in normal saline.

2.2 Animals

Methods: 40 male Wistar rats, aged 10 to 12 weeks and weighing between 200 and 250 grams, were chosen for this study. All animals were sourced from a facility in Najaf, which focuses on the breeding and care of exotic animals, including rats. Iraq. Animals were kept in ventilated cages in a well-ventilated room with temperatures between 21 and 23 °C at standardized conditions without stress. They were kept in a 12/12 light-dark cycle, with free access to humidity and drinking water (tap water), as well as standard rodent chow continuously.

Design of experiments

Animals were separately classified into five groups (n = 8 for every group):

• Group 1 (Control): Dietary Gavage — Critically, every day fed 1 mL of distilled water by way of oral gavage.

• Group 2: 1000 mg/kg body weight of MSG diluted in distilled water orally once daily via gavage.

• Group 3: Rats were given L-Carnitine (100 mg / kg / day) orally one hour prior to MSG (1000 mg/kg/day).

• Group 4: Rats were treated with STS (400 mg/kg/day) by intraperitoneal route one hour before to MSG (1000 mg/kg/day).

• Group 5: L-Carnitine + STS (400 mg/kg/day, i.p) and MSG (1000 mg/kg/day).

All groups were administered for 60 days. Dose Selection of MSG, LC, and STS. The doses in MSG, LC, and STS were decided based on previous studies evaluating their effects in rodents (Ogunmokunwa and Ibitoye, 2025: Ashraf Kassem, 2022; Aboul-Naga *et al*., 2020).

2.3 Sample collection

Meanwhile, after 60 days of the last treatment period, male rats were anaesthetized by an intraperitoneal injection of ketamine (0.3 mL/kg body weight) and xylazine (0.1 mL/kg bw), dissected, and blood samples were collected from abdomen vein into non-heparinized tubes. Samples of blood serum were isolated by centrifugation at 3000 rpm for 5 minutes and the extracted serums were stored in ⁻20 C until the hormones concentrations were determined. The testes and seminiferous tubules were carefully separated without adjacent tissue

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and weighed. It was then stored at −80°C for biochemical analysis.

2.4 Hormonal assays

Serum concentrations of LH, FSH, and testosterone were measured with enzyme-linked immunosorbent assay (ELISA) kits (ELK Biotechnology Co., Wuhan, China), following the manufacturer's instructions. Absorbance was measured with a pre-coated microplate reader at 450 nm ± 10 nm, and hormone concentrations were determined by comparing the optical density of samples to the corresponding standard curves.

Malondialdehyde and Superoxide dismutase levels were measured in the Homogenates from testicular tissue that were used as biochemical indicators of oxidative stress.

The levels of malondialdehyde (MDA) were determined by the thiobarbituric acid (TBA) method described by Buege and Aust for serum, in which MDA reacts with TBA to form a pyronic chromophore that can be detected at 535 nm. (Buege and Aust, 1978).

SOD activity was measured using an ELISA assay kit (ELK Biotechnology co., Wuhan, China).

2.5 Histological examination

Tissue samples were fixed using 10% Neutral Buffered Formalin, rendering them amenable for staining, with an optimal fixation duration of 24-28 hours (Luna, 1968).

2.6 Statistical analysis

Data were shown as mean ± standard error of the mean (SEM) and analysed using one-way ANOVA. The assumptions of ANOVA were met: random allocation of subjects to groups ensured independence; normality was satisfied according to the Shapiro-Wilk test ($p>0.05$); and homogeneity of variances was demonstrated using Levene's test ($p>0.05$). Tukey's post hoc test was performed to assess significant differences ($p<0.05$) between groups. Data analysis was performed using GraphPad Prism version 8 (GraphPad Software, Inc., USA).

3. Results and discussion

3.1 Sperm parameter

The administration of MSG (G2) led to statistically significant and notable impairment of sperm characteristics, as shown in Table 1 and Figure 1, such as sperm count (51.98 ± 5.31 x 10□/mL against 97.20 ± 3.16 x 10□/mL), motility (40.52 ± 6.13% against 80.15 ± 2.82%), and normal morphology (41.67 ± 1.95% against 82.38 ± 3.19%). These results showed serious damage to spermatogenesis processes ($P<0.001$). Treatment with L-Carnitine (G3) and Sodium thiosulfate (G4) was equally effective in restoring all mentioned parameters, which significantly exceeded those of G2 ($P<0.001$). Namely, the sperm count reached 80.80 ± 3.56 and 77.28 ± 5.55 × 10□/mL, the motility increased to 65.54 ± 3.71% and 66.45 ± 3.96%, while the normal morphology achieved 66.48 ± 2.63% and 64.55 ± 4.33%. There was no statistical difference in improvement between G3 and G4 groups ($P>0.05$), which means that the two treatments had similar efficiency. Of interest is the fact that combined treatment (G5) yielded the best outcomes in terms of sperm count (91.43 ± 4.41 x 10□/mL), motility (74.56 ± 4.73)

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Table 1: Sperm Count, Motility, and Normal Morphology across experimental groups

Group	Normal Morphology (%)	Motility (%)	Count (×10^6 /mL)
Control	82.38 ± 3.19 A	80.15 ± 2.82 A	97.20 ± 3.16 A
MSG	41.67 ± 1.95 D	40.52 ± 6.13 D	51.98 ± 5.31 D
MSG + L-Carnitine	66.48 ± 2.63 C	65.54 ± 3.71 C	80.80 ± 3.56 C
MSG + STS	64.55 ± 4.33 C	66.45 ± 3.96 C	77.28 ± 5.55 C
MSG + LC + STS	73.08 ± 3.96 B	74.56 ± 4.73 B	91.43 ± 4.41 B
LSD (P<0.05)	3.376	4.478	4.565

Note: Values are Mean ± SE (n = 8 per group). Different letters within each column indicate significant differences (P<0.05, Fisher's LSD). LC = L-Carnitine; STS = Sodium Thiosulfate.

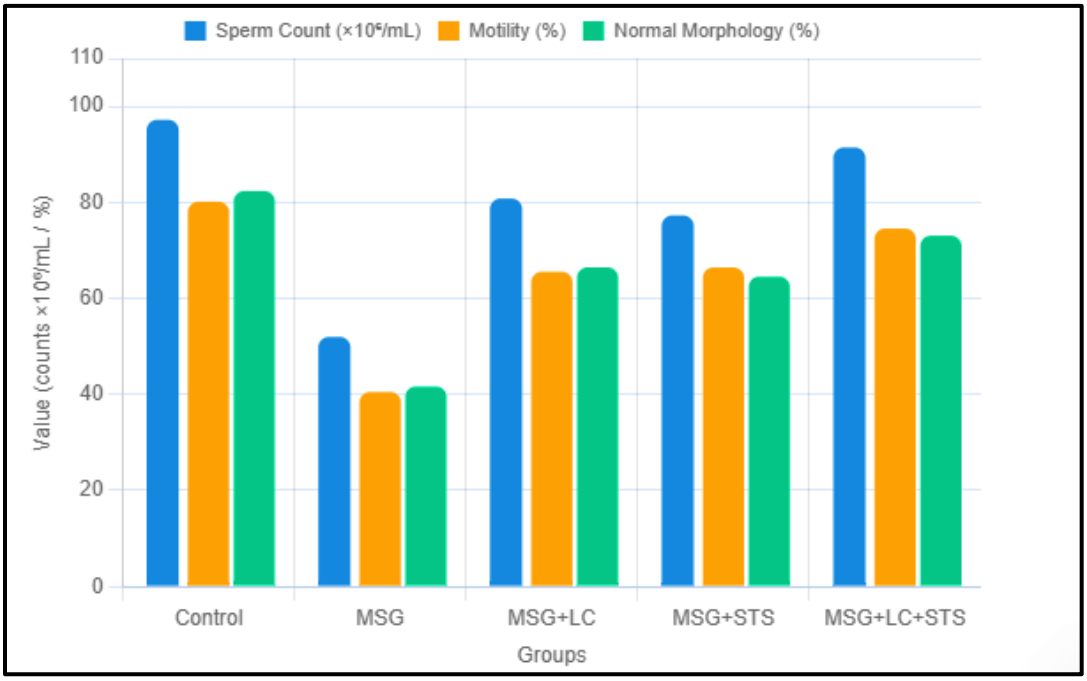


Figure 1: Sperm Count, Motility, and Normal Morphology across experimental groups

3.2 Reproductive Hormones (Testosterone, LH, and FSH)

MSG exposure (G2), as shown in Table 2 and Figure 2, resulted in a marked and statistically significant suppression of serum testosterone (7.27 ± 1.78 ng/mL) compared to the control (28.34 ± 7.24 ng/mL; P<0.001). Both L-Carnitine (G3: 18.09 ± 2.91 ng/mL) and Sodium thiosulfate (G4: 13.55 ± 2.11 ng/mL) significantly elevated testosterone relative to the MSG group (P<0.001), with G3 demonstrating significantly greater recovery than G4 (P = 0.003). Notably, the combined treatment (G5: 35.06 ± 3.18 ng/mL) exceeded control levels (P = 0.031) and both monotherapies

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(P<0.001), indicating a supra-physiological androgenic restoration. A similar pattern was observed for gonadotropins. Serum LH was significantly reduced in the MSG group (2.98 ± 0.92 mIU/mL) versus control (17.91 ± 1.68 mIU/mL; P<0.001). L-Carnitine (G3: 15.28 ± 1.08 mIU/mL) restored LH to near-normal levels, while Sodium thiosulfate (G4: 10.39 ± 0.73 mIU/mL) showed partial recovery, remaining significantly lower than both control and G3 (P<0.001). The combination group (G5: 22.61 ± 1.98 mIU/mL) significantly exceeded all groups, including control (P<0.001), indicating synergistic stimulation of LH secretion. FSH levels followed a comparable trend, with significant suppression in G2 (3.97 ± 1.78 mIU/mL) compared to control (21.62 ± 3.68 mIU/mL; P<0.001). L-Carnitine (G3: 18.30 ± 2.56 mIU/mL) achieved near-complete restoration (P = 0.055 vs control), whereas Sodium thiosulfate (G4: 10.58 ± 2.21 mIU/mL) showed moderate improvement but remained significantly lower than control and G3 (P<0.001). The combination therapy (G5: 28.01 ± 3.63 mIU/mL) significantly surpassed control (P = 0.004) and both individual treatments (P<0.001), confirming a synergistic gonadotropin-enhancing effect.

Table 2: Serum Testosterone, LH, and FSH concentrations across experimental groups

Group	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
Control	21.62 ± 3.68 B	17.91 ± 1.68 B	28.34 ± 7.24 B
MSG	3.97 ± 1.78 D	2.98 ± 0.92 D	7.27 ± 1.78 D
MSG + L-Carnitine	18.30 ± 2.56 B	15.28 ± 1.08 B	18.09 ± 2.91 C
MSG + STS	10.58 ± 2.21 C	10.39 ± 0.73 C	13.55 ± 2.11 C*
MSG + LC + STS	28.01 ± 3.63 A	22.61 ± 1.98 A	35.06 ± 3.18 A
LSD (P<0.05)	2.918	1.383	4.024

Note: Values are Mean ± SE (n = 8). Different letters within each column indicate significant differences (P<0.05, Fisher's LSD). * G3 vs G4 significantly different for testosterone (P = 0.003). LC = L-Carnitine; STS = Sodium Thiosulfate.

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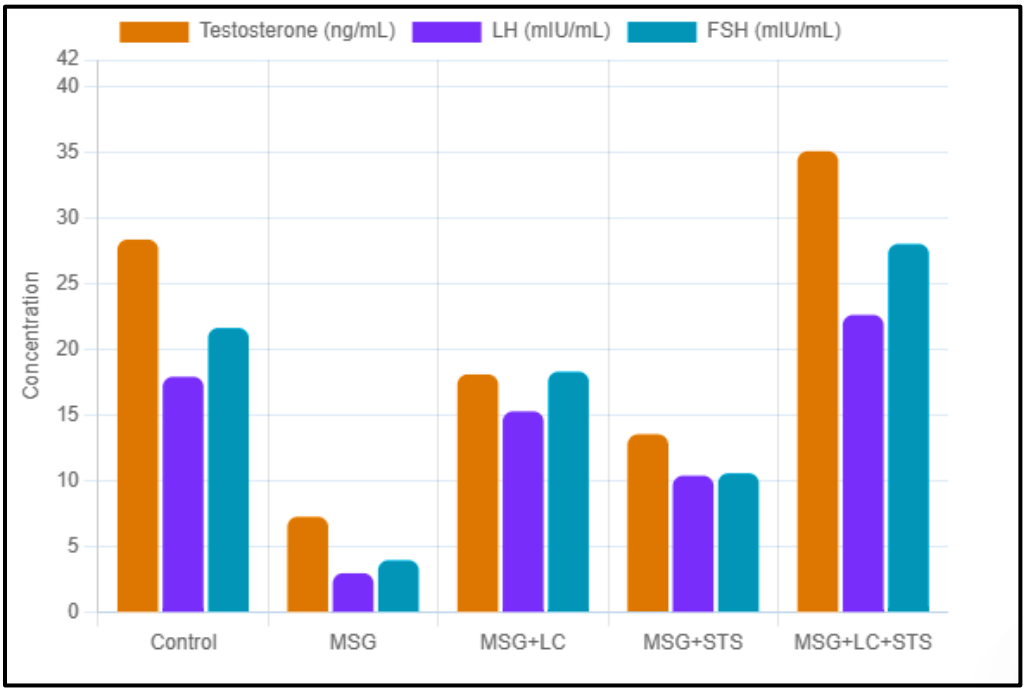


Figure 2: Serum Testosterone, LH, and FSH concentrations across experimental groups

3.3 Oxidative Stress Markers: MDA and SOD

The administration of MSG (Group 2), as shown in Table 3 and Figure 3, resulted in an increased state of oxidative stress as indicated by the higher level of MDA production (6.73 ± 0.49 nmol/mL) compared with the control (2.14 ± 0.12 nmol/mL; P<0.001) along with a depletion in SOD activity (0.606 ± 0.177 ng/mL vs. 2.838 ± 0.268 ng/mL; P<0.001). Both L-carnitine treatment (Group 3) and sodium thiosulfate (Group 4) were able to inhibit lipid peroxidation as well as improve the antioxidant system activity compared to MSG (P<0.001). Group 3 showed a higher antioxidant potential compared to Group 4 as reflected by a higher level of MDA production (3.50 ± 0.09 nmol/mL vs. 4.19 ± 0.38 nmol. Meanwhile, there was a significant increase in the level of SOD activity in G3 (3.777 ± 0.309 ng/mL), which exceeded that of controls (P<0.001), while G4 (2.040 ± 0.304 ng/mL) indicated partial recovery that was still statistically lower than controls and G3 (P<0.001). It is interesting to note that the combination therapy of both substances (G5) demonstrated the most significant effects on antioxidation, with minimal MDA concentrations (2.76 ± 0.08 nmol/mL) and maximal SOD activity (5.156 ± 0.563 ng/mL), significantly higher than either monotherapy alone (P<0.001) and almost reaching normal oxidative function, albeit with slightly increased MDA concentrations compared to controls (P<0.001).

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Table 3: Serum MDA (nmol/mL) and SOD (ng/mL) concentrations across experimental groups

Group	SOD (ng/mL)	MDA (nmol/mL)
Control	2.838 ± 0.268 C	2.14 ± 0.12 D
MSG	0.606 ± 0.177 E	6.73 ± 0.49 A
MSG + L-Carnitine	3.777 ± 0.309 B	3.50 ± 0.09 C
MSG + STS	2.040 ± 0.304 D	4.19 ± 0.38 B
MSG + LC + STS	5.156 ± 0.563 A	2.76 ± 0.08 D*
LSD	0.354	0.291

Note: Values are Mean ± SE (n = 8). Different letters within each column indicate significant differences (P<0.05, Fisher's LSD). * G5 MDA remains significantly above control (P<0.001). LC = L-Carnitine; STS = Sodium Thiosulfate.

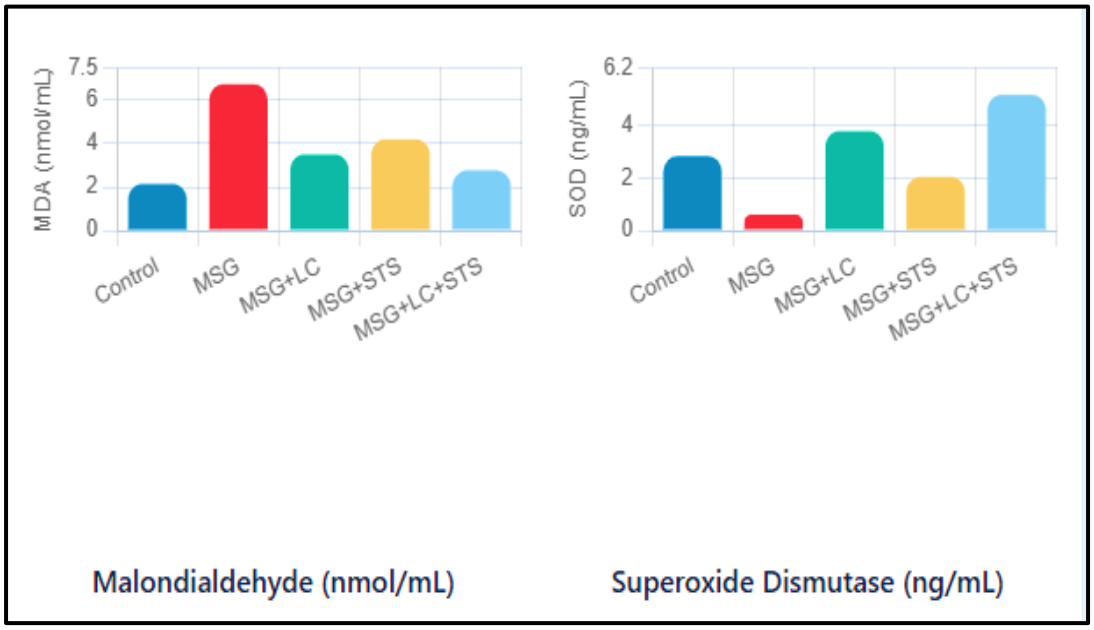


Figure 3: Serum MDA and SOD concentrations across experimental groups

3.4 Histopathological Examination

3.4.1 Group 1 (Negative Control Group)

The histopathological analysis of testis sections from negative control animals showed that the seminiferous tubules possessed normal anatomy. The seminiferous tubule wall was made up of well-differentiated layers of spermatogenic cells including spermatogonia, primary spermatocytes, secondary spermatocytes,

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spermatids, and spermatozoa (Figure 4.A). The lumen of seminiferous tubules had a dense population. The Sertoli cells were normal in appearance with intact nuclei and proper attachment to basement membranes. The interstitial tissue appeared normal, with healthy Leydig cells having nuclei centrally located. There had been no indications of vascular alterations, cellular necrosis, inflammatory cells, or cellular degeneration (Figure 4.B). This finding is consistent with that of (Yalçın *et al*., 2020), who reported similar effects, which states that germ cells are alive and well and seem active in the seminiferous tubules, where spermatogonia and the basement membrane are close together in a healthy state. Undetected alterations also included an accumulation in the tubular system and the breakdown of particular spermatogenic cells. Results are similar to those described for Leydig cells by (Tripepi *et al.,* 2000 ; Malhi *et al.,*2009). They had granular cytoplasm and round nuclei, and they were found in cords or groups of different sizes and shapes.

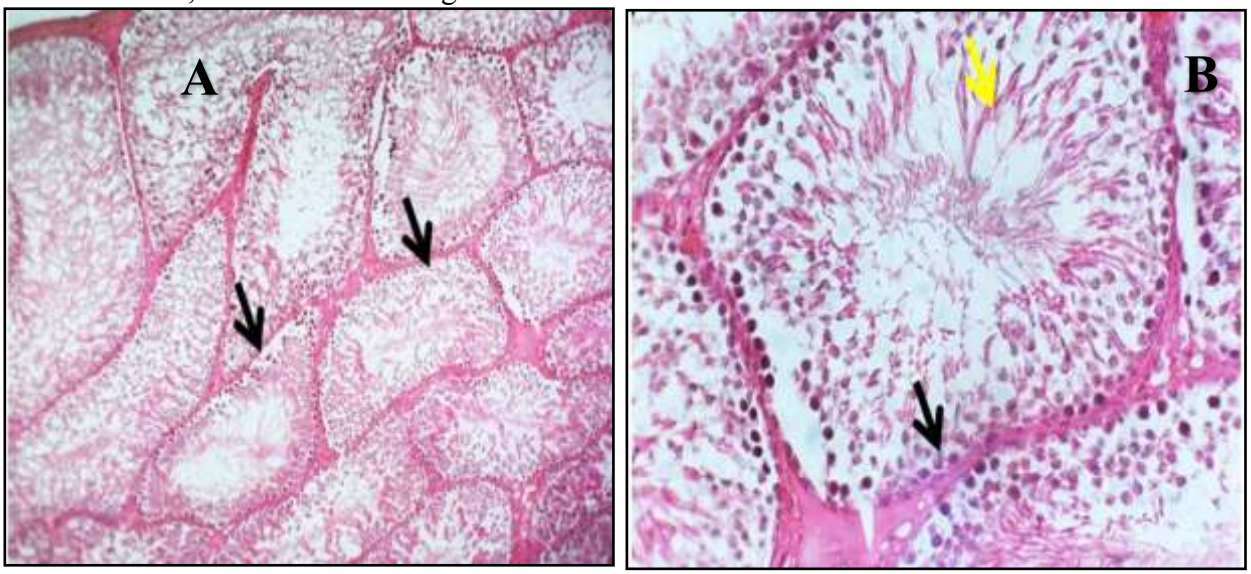


Figure 4: Histological section of testes (Group 1) **(A)** finding the typical architecture of seminiferous tubules with well-organized spermatogenetic cells (black arrows). 10X. H&E stain. Histological section of testes (Group 1) **(B)** finding the typical present of cellular stage with Sertoli cells appeared normal with intact nuclei (black arrow) and proper attachment to the basement membrane. The Tubular lumen was densely filled with mature spermatozoa (yellow arrow). 40X .H&E stain.

3.4.2 Group 2 (MSG Group)

In the testicular tissues of MSG-treated rats, significant pathological changes were observed. Seminiferous tubule epithelium seemed disordered and thinning with evidence of degeneration and necrosis of the spermatozoa cells. There was notable cell vacuolization and disintegration. The number of mature spermatozoa was decreased along with sloughing of the germ cells into the tubular lumen. Some of the seminiferous tubules were noted for atrophy and spermatogenic arrest. Basement membrane thickening and germ cell detachment were also seen. (Figure 5.A). There was notable degeneration of Leydig cells as evidenced by nuclear pyknosis and low cellular density which suggests decreased steroidogenic capacity. (Figure 5.B). This might be due to the oxidative

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stress caused by high production of ROS induced by MSG.

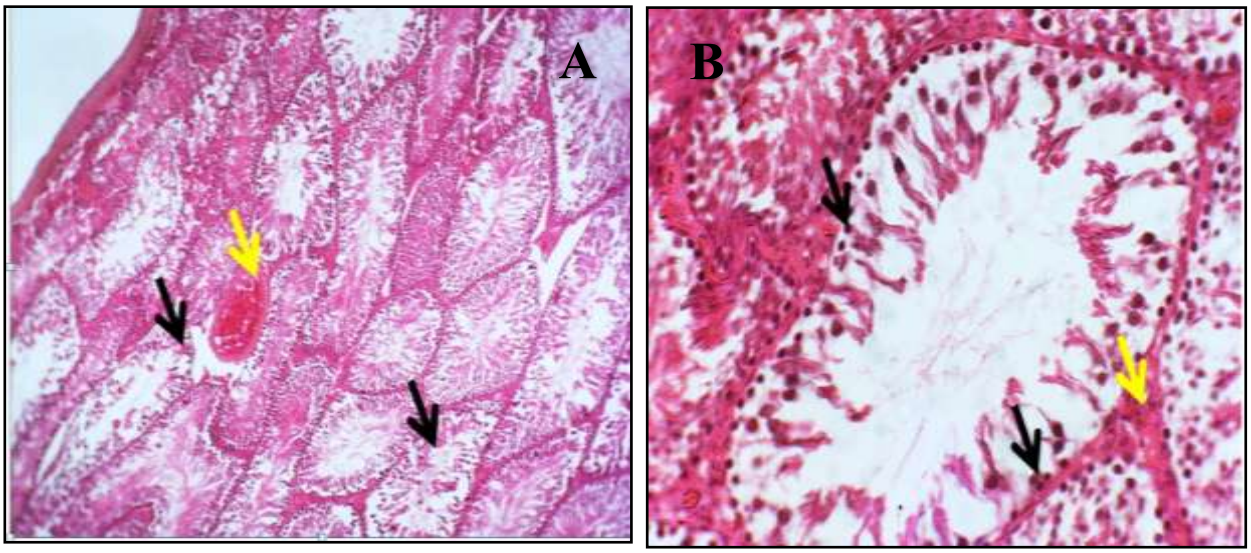


Figure 5: Histological section of testes (Group 2) **(A)** finding the damage in most of the seminiferous tubules and degeneration of germinal epithelial with reduced spermatogenetic cells density (black arrows). Presence of congestion in the interstitial space (yellow arrow). H&E stain. 10X. Histological section of testes (Group 2) **(B)** finding the sloughing of germ cells into the tubular lumen (black arrow), an observed thickness of the basement membrane, a decline in density of mature spermatozoa, and the Leydig cells appeared reduced in number with a restricted area (yellow arrow). Increased of inflammatory cells. H&E stain. 40X.

The current findings show that exposure to monosodium glutamate, or MSG, seriously alters testicular tissue structure, mainly in the seminiferous tubules and interstitial parts. The germinal epithelium degeneration—marked by thinning, disorganization, and loss of cell layering—shows a direct toxic effect on spermatogenic cells. This matches earlier research, which found that MSG disrupts seminiferous epithelium integrity and hinders spermatogenesis due to oxidative and metabolic stress (Ogunmokunwa and Ibitoye, 2025; Rahimi Anbarkeh *et al*., 2019).

The MSG-treated group showed significant histological signs of germ cell degeneration and necrosis, hitting primary spermatocytes and spermatids especially hard. This implies that rapidly dividing germ cells are really sensitive to MSG's toxic effects. Adding to that, we saw lots of cytoplasmic vacuolations and cellular disintegration, which shows some serious intracellular damage. It looks like this damage is probably from an overload of reactive oxygen species causing oxidative stress. This stress then leads to lipid peroxidation and mitochondrial dysfunction, ultimately triggering the apoptosis of those crucial germ cells (Ogunmokunwa and Ibitoye, 2025; Zhang *et al*., 2021). Additionally, the sloughing of germ cells into the tubular lumen and reduced density of mature spermatozoa observed in this study are indicative of impaired spermatogenic progression and defective Sertoli–germ cell adhesion. This detachment

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phenomenon suggests disruption of the blood-testis barrier and Sertoli cell function, which are essential for maintaining a controlled microenvironment for germ cell maturation. Similar observations have been reported in MSG-treated animal models, where germ cell exfoliation was associated with altered expression of junctional proteins and increased apoptotic signaling (Rahimi Anbarkeh *et al*., 2019). The thickening of the basement membrane and its partial separation from the germinal epithelium further reflect chronic degenerative changes. Basement membrane thickening is often associated with fibrosis and impaired nutrient exchange between the interstitium and seminiferous tubules, which can exacerbate spermatogenic arrest. This structural alteration has been previously linked to prolonged oxidative damage and inflammatory responses in testicular tissue exposed to toxic agents (Alalwani, 2014).

3.4.3 Group 3 (L-carnitine)

When compared to the MSG group, specimens from the treated group with L-carnitine showed an apparent enhancement in testicular histology. With germinal epithelial thickness restored, the majority of seminiferous tubules maintained comparatively normal architecture. With moderate to active spermatogenesis, spermatogenic cells were arranged in sequential layers (Figure 6.A). At certain tubules, particularly in dispersed spermatocytes, slight damage and vacuolation remained visible. There were occasionally removed germ cells in the lumen, but there were also a fair number of mature spermatozoa. The integrity of the structure of Sertoli cells was largely intact. Leydig cells showed mild regeneration. When contrasted to the MSG group, there was very little inflammatory invasion (Figure 6.B).

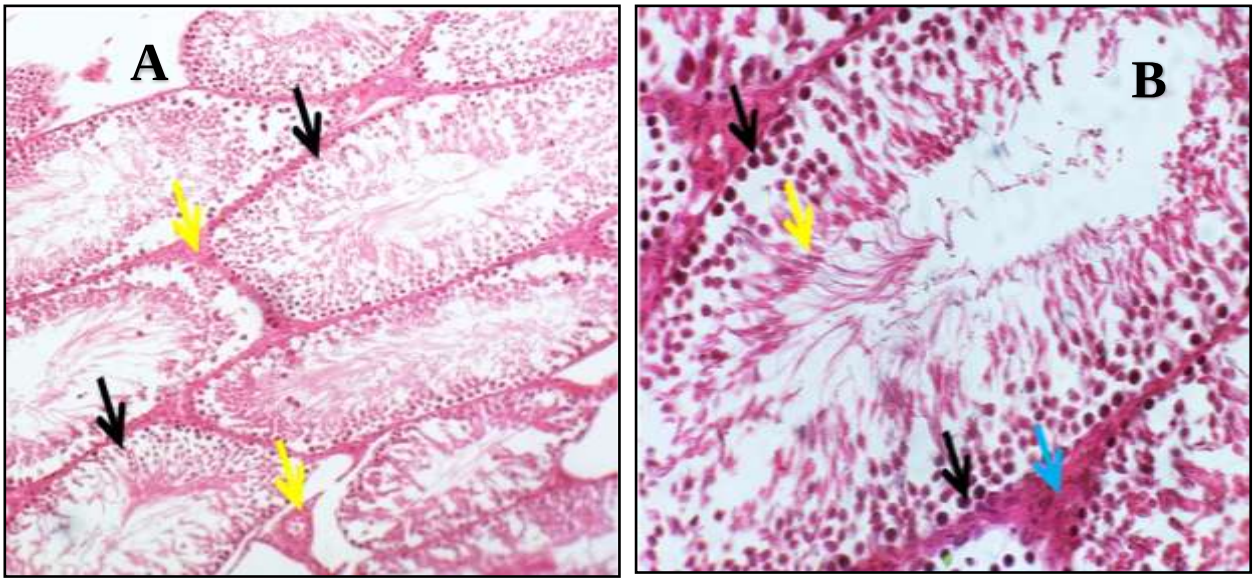


Figure 6: Histological section of testes (Group 3) **(A)** finding the testicular histology showed an apparent enhancement in contrast to the MSG group. Most of the seminiferous tubules maintained a comparatively regular architecture (black arrows). Some tubules still showed signs of slight degeneration. Well-identified spermatogenetic cord arrangement. Enhancement of Leydig cells (yellow

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arrow). H&E stain. 10X. Histological section of testes (Group 3) **(B)** finding the well-identified spermatogenetic cells with well-architectured germinal cells (black arrows). clear enhancement of spermatozoa maturity (yellow arrow). The Leydig cells appeared increased in number and spread over a wide area (blue arrow). H&E stain. 40X.

These findings match earlier studies showing that L-carnitine helps keep sperm alive and aids in their production through better mitochondrial function and energy use (Kooshesh *et al*., 2023; Yaman *et al*., 2018). While there was overall progress, some issues remained. Mild degeneration, like slight vacuolation and a few germ cells sloughing off into the lumen, were still present in some seminiferous tubules. This indicates that even though L-carnitine lessens MSG-induced toxicity, it might not fix all structural damage from severe or long-term exposure. Still, the fact that many mature sperm were in the tubule lumens shows significant recovery in sperm generation. Sertoli cells kept their structure too, with neat nuclei and a solid hold onto the basement membrane. This is crucial because they shape the microenvironment needed for germ cells to differentiate and offer lots of support to them. L-carnitine helps by reducing oxidative stress and stabilizing cell membranes, stopping the blood-testis barrier from getting messed up. Leydig cells in the interstitium showed some bounce back – their shape was better, and they spread out more like normal. This looks like they regained some steroid-making action, vital for churning out testosterone and guiding spermatogenesis. Plus, L-carnitine boosts how mitochondria burn fatty acids for energy, giving these cells what they need to keep up their hormone-making duties (Aboul-Naga *et al*., 2020; Ng *et al*., 2004). Another key point is the reduced inflammation in the areas studied, showing L-carnitine has anti-inflammatory benefits alongside its antioxidant actions. It combats oxidative damage by getting rid of reactive oxygen species and cutting down on lipid peroxidation, which keeps inflammatory responses in check. Earlier research has found that L-carnitine curbs the production of ROS, blocks apoptosis, and maintains cell health in reproductive tissues (Kooshesh *et al*., 2023). Mechanically, L-carnitine helps because it's a mitochondrial cofactor. It moves long-chain fatty acids into the mitochondrial matrix for beta oxidation, boosting ATP production. That's key for sperm health and movement. Besides, L-carnitine keeps the acetyl-CoA/CoA ratio in check, stops metabolic imbalance, and cuts down on oxidative stress. So, it prevents cell death and makes overall testicular function better. L-carnitine offers significant protection against monosodium glutamate-induced testicular harm. This is shown by restored seminiferous tubule structure, better spermatogenesis, and preserved Sertoli cell integrity, along with some Leydig cell recovery. While slight damage remains, the clear improvement suggests L-carnitine could be a useful tool in battling oxidative stress-related issues.

3.4.4 Group 4 (Sodium Thiosulfate Group)

In comparison to the MSG-treated group, seminiferous tubules displayed better cellular arrangement and higher spermatogenic cellular activity. Spermatogonia, spermatocytes, and spermatids were clearly visible in the somewhat broad germinal epithelium (Figure 7.A). Testes administered sodium thiosulfate displayed modest residual

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cellular degeneration, moderate spermatogenesis, and a partial enhancement in histological structure. There were small quantities of spermatozoa in the lumen, but some seminiferous tubules showed focal disarray of germinal cells. The basement membranes barely thickened and stayed largely intact. The interstitial spaces tissue showed minimal inflammatory cells. Leydig cells showed enhanced nuclear morphology and seemed to be essentially normalized, suggesting that sodium thiosulfate mitigated the oxidative and degenerative testicular destruction caused by MSG. in a notable decrease in the production of androgens, which is one of these cells' primary functions (Figure 7.B).

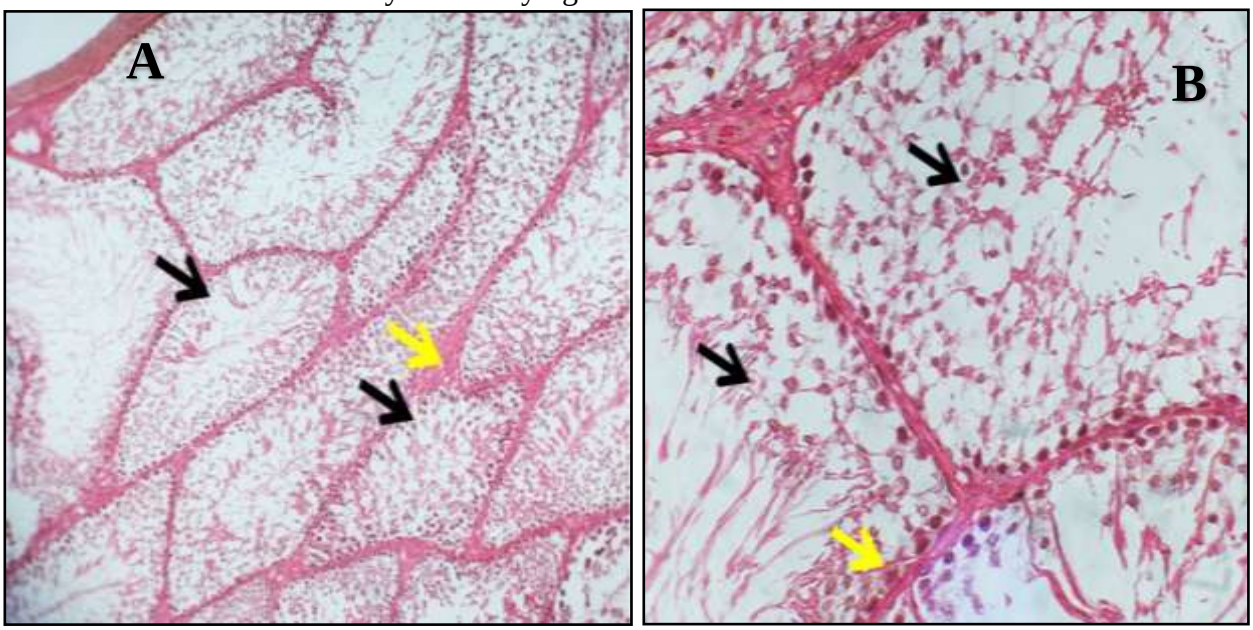


Figure 7: Histological section of testes (Group 4) **(A)** finding the revealed partial restoration of normal testicular architecture. Seminiferous tubules showed improved cellular organization and increased spermatogenic activity compared with the MSG-treated group (black arrows). well-organized Leydig cells (yellow arrow). H&E Stain.10X. Histological section of testes (group 4) **(B)** showing an intermediate abundance of spermatozoa in the tubular lumen. The basement membranes were mostly undamaged, though there were a few places where they were slightly thicker. Improved Sertoli cells intact with the basement membrane (yellow arrow). H&E stain. 40X.

This is corroborated by previous research suggesting that sodium thiosulfate provides a protective response to oxidative stress-related damage to cells due to its antioxidative and cytoprotective properties (Pateira, 2023; Zhang *et al*., 2021). A reduction in inflammatory cells and maintenance of basement membrane integrity in the present histological slides further confirm the involvement of sodium thiosulfate in preventing oxidative and inflammatory damage. It could be due to its role as an H□S donor, which has been known to modulate the cellular redox state, prevent apoptosis, and repair tissues (Zhang *et al*., 2021). Furthermore, the partial recovery of spermatogenesis and the presence of moderate numbers of spermatozoa in the lumen support the hypothesis that sodium thiosulfate could play an important role in the recovery of

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germ cell proliferation and differentiation. Such results were also obtained by Pateira (2023), who showed that sodium thiosulfate can alleviate toxic effects on Leydig cells and improve their testicular function. However, degenerative changes in seminiferous tubules still present in some testes could be explained by the insufficient protective properties of sodium thiosulfate since it provides incomplete protection. Thus, combination therapy resulted in better therapeutic effect than sodium thiosulfate therapy alone.

3.4.5 Group 5 Combination Group (L-Carnitine + Sodium Thiosulfate)

Testicular appearance was nearly normal and there was significant improvement in the combination therapy group's histological specimens. Regularly shaped seminiferous tubules with unaltered basement membranes seemed densely packed. According to spermatogonia to mature spermatozoa, the germinal epithelium showed a comprehensive and well-organized spermatogenic sequence (Figure 8.A). Activated spermatogenesis had been evident in the tubular lumen's densely packed sperm bundle population. Sertoli cells maintained adherence to the basement membrane and had clear nuclei, giving them a typical look. There were very few signs of degenerative alterations. The interstitial spaces showed no signs of inflammation or edema. Several architecturally sound Leydig cells had polygonal shapes, lots of eosinophilic cytoplasm, and centrally situated nuclei. When L-carnitine and sodium thiosulfate were administered together, they significantly reduced testicular damage caused by MSG and promoted healing (Figure 8.B). Because antioxidants have the ability to attenuate free radicals, reduce oxidative stress, and slow the apoptotic process, they can mitigate the deleterious effects of busulfan on the male reproductive system (Hakemi *et al.*, 2019). As a result, during busulfan treatment, antioxidants can lessen lipid peroxidation and DNA damage in testicular cells. With its antioxidant and antiapoptotic qualities, L-carnitine protects against infertility brought on by busulfan. Research has shown that L-carnitine can reduce or prevent the production of free radicals through its metal chelating properties and free radical scavenging activities (superoxide anion and hydrogen peroxide, respectively), which results in a reduction in the rate of DNA and protein oxidation as well as lipid peroxidation (Kooshesh *et al.*, 2023).

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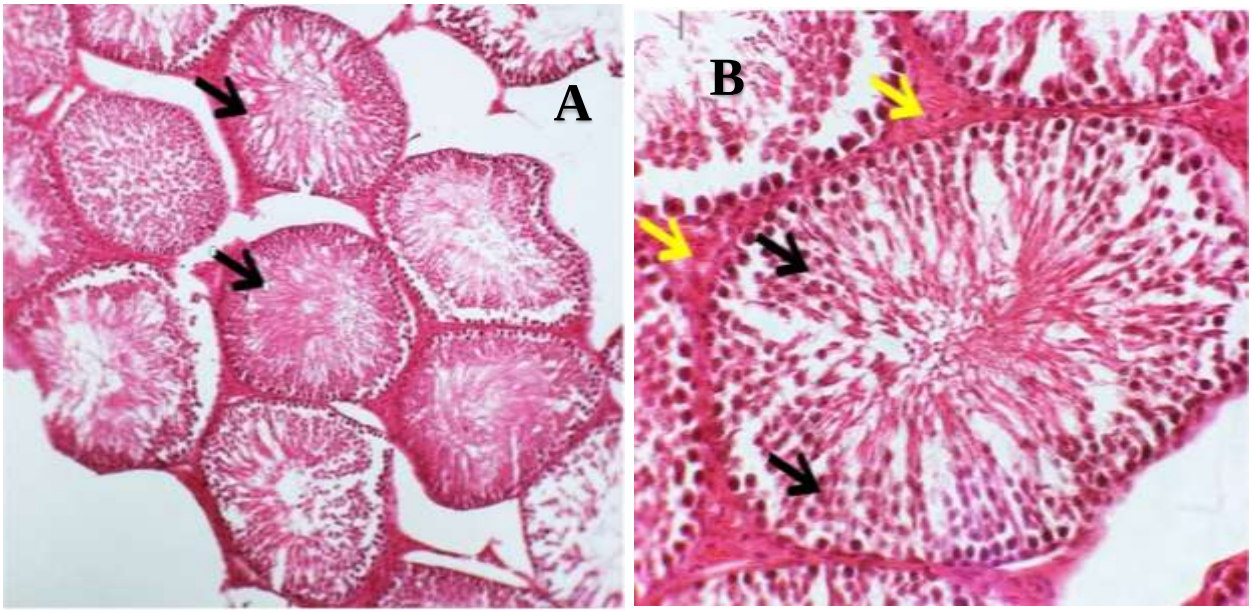


Figure 8: Histological section of testes (Group 5) **(A)** showing enhancement of structure of seminiferous tubules. Also appeared density of spermatogenetic cells and spermatozoa with decreased luminal space (black arrows). H&E stain. 10X. Histological section of testes (Group 5) (**B)** showing well-identified germinal epithelial cell and a great arrangement of primary and secondary stages of spermatocytes (black arrow). The spermatozoa have well-differentiation, and full density. The Leydig cells appeared in a great state, increased in number, and had full distribution (yellow arrows). H&E stain. 40x.

3.5 Conclusion

This study was designed to investigate the possible protective role of L-carnitine and sodium thiosulfate against MSG-induced oxidative and reproductive toxicity in male rats The results combined show that MSG treatment caused a marked impairment in sperm parameters, hormonal profiles, antioxidant enzyme activity and shape of the testes as observed by decreased sperm concentration, motility and morphology, reduction in testosterone, LH and FSH levels together with increased MDA and SOD activities along with severe histopathological damages. These findings support the hypothesis that oxidative stress induced by MSG is a major factor contributing to impaired male reproductive function. Each agent alone imparted measurable protective effect; however, their combination yielded significant effects that exceeded those of individual agents in restoring spermatogenic capacity to near normal levels, supra-physiological androgenic recovery and incrementally superior antioxidant potentiation. Histological evaluation supported these biochemical results with combination-treated testes showing almost normal architecture and active spermatogenesis. Thus, dosing regimens and mechanistic studies of this synergism should be pursued in future work. In conclusion, the concurrent administration of L-carnitine and sodium thiosulfate may become a future therapeutic approach in alleviating MSG-caused male reproductive toxicity. Overall, it can be

concluded that MSG produces considerable reproductive toxicity due to oxidative stress, L-carnitine as well as sodium thiosulfate showed a protective effect; however, the co-administration of both these antioxidants produced even better results regarding the improvement in reproductive characteristics.

Ethical approval

The work on the laboratory animals was conducted in accordance with the ethical standards and in compliance with the local and international guidelines. It was approved by the Ethical Committee of the Federal University of Al-Qassim Green, College of Veterinary Medicine, Department of Physiology, Pharmacology and Biochemistry (approval number: qgec/48/2025).

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التأثيرات الوقائية التآزرية للـ ال-كارنيتين ثيوسلفات الصوديوم ضد السُمية الخصَوية المُستحثة بغلوتامات أحادية الصوديوم في ذكور الجرذان.

تم إعداده من قبل **حسين محمد جليل.**

بأشراف أستاذ. دكتور. **أريج غازي حسن**

الملخص

أحد أكثر المواد (MSG)يُعدّ جلوتامات أحادي الصوديوم شيوعاً الذي يضاف ألى الأطعمة لتحسين النكهة و يمكن أن يؤدي إلى آثار صحية شديدة وضارة مع تأثير مُثبتَ على تثبيط ألقدرة الإنجابية. يُعدّ جلوتامات أحادي الصوديوم بالتأكيد مُسبِّبًا للإجهاد التأكسدي، وبالتالي يُقلّل تخليق الهرمونات الستيرويدية، مما يَتعطّل معه الأداء الفسيولوجي للأعضاء التناسلية الذكرية. كان الهدف من هذه الدراسة هو تقييم التأثيرات الوقائية لِلـ ال-كعلاجات (STS) وثيوسلفات الصوديوم (LC)كارنيتين مضادة للأكسدة منفردة وكمركّب وقائي ضد ضرر الخصية الناجمة عن جلوتامات أحادي الصوديوم في تم تقسيم أربعين جرذًا ذكرًا من سلالة .الجرذان الذكور ويستار إلى خمس مجموعات، بواقع ثمانية جرذان لكل مجموعة. المجموعة 1 (مجموعة السيطرة): 1 مل من الماء المقطر عن طريق التجريع الفموي يوميًا. المجموعة 2 (السيطرة الإيجابية): جلوتامات أحادي الصوديوم بجرعة 1000 ملغم/كغم من وزن الجسم، مخففة في محلول ملحي فيزيولوجي، عبر أنبوب معدي فموي مرة واحدة يوميًا. المجموعة 3: ل-كارنيتين (فموي، 100 ملغم/كغم من وزن الجسم/يوم) قبل إعطاء جلوتامات أحادي الصوديوم بساعة واحدة (1000 ملغم/كغم من وزن الجسم /يوم). المجموعة 4: ثيوسلفات الصوديوم (400 ملغم/كغم من وزن الجسم/يوم، حقن داخل الصفاق) قبل إعطاء جلوتامات أحادي الصوديوم بساعة واحدة. المجموعة 5: 1000 ملغم/كغم من وزن الجسم/يوم من جلوتامات أحادي الصوديوم + 100 ملغم/كغم من وزن الجسم/يوم من ل-كارنيتين فمويًا، وكذلك ثيوسلفات الصوديوم (400 ملغم/كغم من وزن الجسم/يوم) داخل استمرت التجربة لمدة إجمالية قدرها 60 يومًا. .الصفاق

أدى تجريع مادة جلوتامات أحادي الصوديوم إلى سمّية تناسلية ذكورية واضحة، ما أدى إلى انخفاض عدد الحيوانات المنوية وحركتها وسلامتها المورفولوجية. بالإضافة الى ذلك لوحظ انخفاض نشاط انزيم السوبر وهو ما ارتبط بزيادة تراكيز (SOD)اوكسايد دسميوتيز ووفقًا لذلك، فإن إعطاء ل-(MDA)ألمالونداي الدهايد منفردين (STS) وثيوسلفات الصوديوم(LC)كارنيتين خفف هذه التأثيرات الضارة، إلا أن العلاج المركّب أظهر استجابات أفضل، مما أدى إلى تعزيزٍ ملحوظ في جودة الحيوانات المنوية وقدرةً مضادات الأكسدة.

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