ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 15;2 http://apjmt.mums.ac.ir June 2026

ORIGINAL ARTICLE

The possible ameliorative effect of *Lepidium meyenii* (Maca roots) on monosodium glutamate-induced testicular toxicity in adult male albino rats

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Abstract

***Background*:** Monosodium glutamate (MSG) ranks among the most widely utilized food additives. Reports on its safety are conflicting, with some studies claiming safety and others noting deleterious health effects. The study objective was to evaluate whether MSG induces testicular toxicity and to determine if *Lepidium meyenii* has an ameliorative effect.
***Methods*:** A total of forty adult male albino rats were randomly assigned to four equal groups: Group I (control), Group II (maca), Group III (MSG), and Group IV (MSG plus maca). All treatments were administered orally for six weeks. Blood samples were collected for hormonal and biochemical analyses. Testicular tissues were prepared and stained with H&E, trichrome, and caspase-3. Monosodium glutamate produced deleterious effects on rats' testes as confirmed by a significant decrease in testosterone hormone ($P < 0.01$) and significantly disturbed oxidative stress indicators (TAC and MDA).
***Results*:** Histologically, in the MSG group, widening of the spaces between seminiferous tubules, with apparent disorganization, was evident. Excessive collagen deposition was observed in trichrome-stained sections. Additionally, few interstitial Leydig cells, destruction of most germinal epithelium, and elevated caspase-3 immunostaining. Maca administration evidently improved the degenerative changes.
***Conclusion*:** Monosodium glutamate induced significant degenerative changes, oxidative stress, and pronounced hormonal disturbance. *Lepidium meyenii* effectively attenuated hormonal, biochemical, and most histopathological alterations against MSG-induced testicular toxicity at the specific duration and dose investigated.

Keywords: *Lepidium meyenii*, Monosodium glutamate, Testicular toxicity, Rats

How to cite this article: Saleh EM, Boulos AN. The possible ameliorative effect of *Lepidium meyenii* (Maca roots) on monosodium glutamate-induced testicular toxicity in adult male albino rats. Asia Pac J Med Toxicol. 2026; 15(2):90-99.

INTRODUCTION

Poisoning-related morbidity and mortality remain a significant global concern, with WHO 2024-2025 reports estimating approximately 2 million deaths annually due to acute poisonings and chronic toxic exposures [1, 2]. Among these, plants account for about 5–10% of hazardous human exposures reported to Poison Control Centres in several Western countries, including the United States [3]. In India, a tropical nation with vast floral biodiversity, most plants are non-poisonous, yet a considerable number possess toxic potential. Within this spectrum, Yellow Oleander (*Thevetia peruviana*) is of particular importance because stands of its widespread distribution across India. It is a tropical and subtropical ornamental tree with milky sap-producing leaves and yellowish funnel-shaped blooms [4]. The diamond-shaped fruit contains multiple seeds that are highly toxic [4], with seeds carrying the highest concentration of Cardenolides (cardiac glycosides) among all plant parts [5, 6]. Owing to this, individuals aware of its potency often choose seeds for intentional consumption [6, 7].

The clinical profile of YOP is broad, ranging from asymptomatic cases to severe outcomes, including shock, syncope, and even death. Despite this spectrum, reliable data

substances that humans are exposed to, such as environmental chemicals, drugs, and food [8].

Many mechanisms are proposed for MSG induced toxicity, among which the oxidative stress mechanism,

where increased lipid peroxidation and decreased antioxidant activity were revealed in studies [9]. Despite the established role of synthetic antioxidants, such as vitamins C and E, against oxidation caused by free radicals [10], growing attention has been directed toward the use of alternative natural products for the prevention and treatment of human diseases. Natural medicinal herbs such as *Lepidium meyenii* are one of them [11, 12].

Lepidium meyenii is a medicinal plant rich in antioxidants. It is the scientific name of the Maca root plant, which contains large amounts of vitamins and flavonoids that protect cells from mutations and damage caused by free radicals [13]. Therefore, it has many medical uses, among them enhancing sexual potency and fertility through its impact on sex hormones and their receptors [14].

This study investigates the potential toxic effects of monosodium glutamate on testicular tissue in adult male albino rats, evaluates oxidative stress as a possible mechanism of toxicity, and assesses the potential ameliorative effects of Maca roots against MSG-induced testicular toxicity.

METHODS

In this experiment, forty mature male albino rats were used. The research ethics committee granted its approval to the study (IRB number 00012098, serial number 0307499). The Institutional Animal Research Committee of the Faculty of Medicine at Alexandria University in Egypt authorized the experimental protocol. The specified standards and regulations for the use and care of animals were followed.

Chemicals and biochemical kits

- *Lepidium meyenii* (maca) was purchased from NOW® Foods (Bloomingdale, IL, USA) (Product code: NOW-0420, code number 4762C v7). This is row maca roots, traditionally grown in the Andes. Main Ingredients: MACA, Other Ingredients: Hypromellose (cellulose capsule) and Stearic Acid (vegetable source). Preparation and administration: 500mg containing capsule was freshly dissolved in distilled water before administration and given orally through a nasogastric tube.
- Monosodium glutamate (MSG) was purchased from Wei Zhi Huang, China, and dissolved in distilled water.
- Testosterone concentrations were determined using a competitive ELISA kit (Calbiotech Inc., El Cajon, CA, USA; Catalog No. TE373S).
- Total Antioxidant Capacity, Biodiagnostic, Giza, Egypt; Catalog No. TA 25 13
- Lipid Peroxide [Malondialdehyde], Biodiagnostic, Giza, Egypt; Catalog No. MD 25 29

Experimental Animals

Forty sexually mature male albino rats, weighing 150–200 g, were used in this study. The animals were obtained from Alexandria University's animal house and were in good health. For the length of the experimental period, the rats were kept in hygienic, well-ventilated metal cages with ten rats each, and they had unrestricted access to nutrition and water. The animals were kept in regular laboratory settings with a 12-hour light/12-hour dark, regulated humidity, and a constant temperature of 24 ± 2°C.

Study design and treatment protocols

Animals were randomly allocated to groups using a computer-generated randomization sequence to minimize selection bias. After allocation, animals were assigned coded group identifiers. The choice of doses of maca roots and MSG was based on the results of a previous study, as follows: 15

- Group I (Control Group): represented a negative control group in which animals were given 2 ml distilled water orally.
- Group II (Maca Group): represented the positive control group in which animals were given 500 mg/kg/day maca root orally through a nasogastric tube.
- Group III (MSG Group): represented the treated group in which animals received 900 mg/ kg/day MSG orally through a nasogastric tube.
- Group IV (MSG + Maca roots Group): represented the protected group in which 500 mg/kg/day maca was added to the administered MSG (900mg/kg/day).

At the beginning and at the end of the study, the weight of animals was measured and recorded. Solutions of these chemicals were freshly prepared before administration and were formed by dissolving the amount of substance needed to reach the reference dose in distilled water. The chemical concentrations were adjusted per animal's respective weight so that each animal received 2 mL solution from the needed chemical once daily for 6 weeks [15].

The male albino rats were sacrificed under ether anesthesia at the endpoint of the experiment. Serum was conserved for laboratory analysis after blood samples were collected and centrifuged.

Biochemical and Hormonal analysis

- Hormonal assay: Immediately following scarification, two milliliters of blood were obtained from the inferior vena cava for the hormonal assay; heparin was then added as an anticoagulant and centrifuged. Before being used for the testosterone assay, serum was frozen at -20 °C.
- Biochemical assay: Following the manufacturer's instructions, a colorimetric assay kit was used to measure the levels of total antioxidant capacity (TAC) and malondialdehyde (MDA).

Histological preparation

Testicular tissues were dissected, cleaned, weighed, grossly examined, and fixed in Bouin's fluid for 48 hours. The samples were then dehydrated, cleared, embedded in paraffin, sectioned at 5 µm, and stained with Hematoxylin

and Eosin, Masson's trichrome, and caspase-3 immunohistochemistry. For caspase-3 staining, sections underwent antigen retrieval with EDTA (pH 8), endogenous peroxidase blocking with 3% H_2O_2, and blocking with 5% BSA. The sections were incubated with primary monoclonal anti-caspase-3 antibody, followed by secondary anti-mouse IgG antibody. Finally, 3,3-diaminobenzidine tetrahydrochloride DAB chromogen was applied to visualize positive staining as a brown precipitate [16].

Histopathological evaluation

Histological specimens were then processed and evaluated using the codes only, so that the observers were unaware of the treatment allocation during histopathological assessment. The histological evaluation was performed independently by two blinded observers, and any disagreement was resolved by joint review to reach a final consensus.

Semiquantitative histological injury score (0-3) adapted from standard testicular histopathology grading schemes e.g., modified Johnsen and interstitial injury scores commonly used in reproductive toxicology, was applied. The score investigated six parameters (as shown in the supplementary materials) and ranged from 0-18 [17].

For morphometric analysis, ImageJ software (National Institutes of Health, Bethesda, MD, USA) was utilized. Five randomly chosen rats from each group had five non-overlapping high-power fields (×200 magnification) investigated. The average percentage of fibers of collagen in testes slices stained with Masson's trichrome were measured using the intensity and distribution of immunohistochemical staining. Similarly, H-score analysis of caspase-3 immuno-expression was evaluated [18].

Statistical analysis

IBM SPSS software package version 20.0 was used to analyze the data. (IBM Corp., Armonk, NY, published 2011). To confirm that the data were normally distributed, the Kolmogorov-Smirnov test was employed. Quantitative data were described using minimum, maximum, mean, standard deviation and 95% confidence interval. The results were evaluated for significance at the 5% level. F-test (ANOVA): When comparing more than two groups of normally distributed quantitative variables, the Post Hoc test (Tukey) is used for pairwise comparisons. The magnitude of the group effect was reported using fixed-effect omega squared (ω^2) with 95% confidence interval. A post hoc power analysis was done based on the observed ANOVA effect sizes. The achieved statistical power exceeded 99.9% for all the measured parameters [19, 20].

RESULTS

Observational results

A noticeable increase in food intake and, subsequently rats' weight was found after MSG administration. While the body weight partially decreased after adding Maca roots.

Gross examination of the testes revealed that there were no marked differences in the shape and surface features among the studied groups of rats in comparison to the control groups.

Weight, Hormonal and Biochemical analysis (Table 1)

The mean serum testosterone level was 1.10 ± 0.08 ng/ml in the control group. Maca administration significantly increased testosterone to 1.36 ± 0.10 ng/ml ($p < 0.001$ as compared to the control group). Conversely, MSG markedly decreased testosterone levels to 0.11 ± 0.09 ng/ml ($p < 0.001$ vs control). Co-administration of maca partially restored the hormone level (0.62 ± 0.10 ng/ml, $p < 0.001$ vs MSG; $p < 0.001$ vs control).

There was no significant difference between the mean body weight in the control group and the Maca group ($p= 0.937$). In contrast, MSG administration significantly increased body weight (357.50 ± 9.95 g, $p < 0.001$). Co-administration of maca partially attenuated this weight gain (296.9 ± 15.97 g), showing significantly lower body weights compared to the MSG group ($p < 0.001$).

The control group exhibited a mean TAC of 1.02 ± 0.03mM/L, while the Maca treatment significantly increased TAC (1.19 ± 0.02mM/L, $p < 0.001$). On the contrary, MSG administration caused a marked reduction in TAC (0.52 ± 0.04mM/L, $p < 0.001$) in comparison to the control group. Co-administration of Maca partially restored the mean TAC level (0.81 ± 0.04), which was significantly higher than the MSG group ($p < 0.001$).

Serum MDA levels in the control group recorded a mean value of 8.95 ± 0.41 nmol/L, while the Maca-treated group exhibited a significant reduction (6.97 ± 0.40 nmol/L; $p < 0.001$ vs. control). In contrast, MSG administration markedly elevated MDA (14.05 ± 0.40 nmol/L; $p < 0.001$ vs. control). Co-administration of Maca significantly lowered MDA compared with MSG alone (9.98 ± 0.21 nmol/L; $p < 0.001$), even though the values remained higher than the control group's ($p < 0.001$).

The difference between the studied groups showed extremely large effect sizes for testosterone ($\omega2 = 0.964$), body weight ($\omega2 = 0.919$), TAC ($\omega2 = 0.983$), and MDA ($\omega2 = 0.980$).

Histological Results

H&E Stain:

Groups I & II (negative & positive control groups) revealed the testes' typical histological architecture. Numerous seminiferous tubules bordered by Sertoli and germinal epithelial cells made up the testicular tissue. Spermatogonia, spermatocytes, spermatids, and sperm are included in the layers of spermatogenic cells that make up the germinal epithelium. The long tails of the sperm were visible in the tubule lumen. A distinct basement membrane enclosed each tubule. Groups of Leydig cells with vesicular nuclei and acidophilic cytoplasm inhabited the interstitial spaces between the tubules (Figure 1a,b).

Group III (MSG group) revealed evident testicular tissue

Table 1. Comparison between the different studied groups according to testosterone level, body weight, total antioxidant capacity (TAC) and serum malondialdehyde (MDA)

	Control (n = 10)	Maca (n = 10)	MSG (n = 10)	MSG + Maca (n = 10)	F ω² (95% CI)	p
Testosterone level (ng/ml)						
Min - Max	1.0 - 1.20	1.20 - 1.50	0.0 - 0.20	0.50 - 0.80		
Mean ± SD	1.10 ± 0.08	1.36 ± 0.10	0.11 ± 0.09	0.62 ± 0.10		
95% CI	1.04 - 1.16	1.29 - 1.43	0.05 - 0.17	0.55 - 0.69	354.204*	< 0.001*
p_1		< 0.001*	< 0.001*	< 0.001*	0.964 (0.932 – 0.973)	
p_2			< 0.001*	< 0.001*		
p_3				< 0.001*		
Weight (g)						
Min - Max	225.0 - 270.0	239.0 - 260.0	340.0 - 374.0	278.0 -321.0		
Mean ± SD	251.7 ± 16.70	248.3 ± 7.21	357.50 ± 9.95	296.9 ± 15.97		
95% CI	239.8 - 263.6	243.1 - 253.5	350.4 - 364.6	285.5 - 308.3	151.894*	< 0.001*
p_1		0.937	< 0.001*	< 0.001*	0.919 (0.850 – 0.941)	
p_2			< 0.001*	< 0.001*		
p_3				< 0.001*		
TAC (mM/L)						
Min - Max	0.95 - 1.06	1.16 - 1.23	0.47 - 0.57	0.75 - 0.86		
Mean ± SD	1.02 ± 0.03	1.19 ± 0.02	0.52 ± 0.04	0.81 ± 0.04		
95% CI	1.00 - 1.04	1.18 - 1.20	0.49 - 0.55	0.78 - 0.84	751.190*	< 0.001*
p_1		< 0.001*	< 0.001*	< 0.001*	0.983 (0.967 – 0.987)	
p_2			< 0.001*	< 0.001*		
p_3				< 0.001*		
MDA (nmol/L)						
Min - Max	8.30 - 9.60	6.40 - 7.70	13.50-14.60	9.60 - 10.30		
Mean ± SD	8.95 ± 0.41	6.97 ± 0.40	14.05±0.40	9.98 ± 0.21		
95% CI	8.66 - 9.24	6.68 - 7.26	13.76-14.34	9.83 - 10.13	663.410*	< 0.001*
p_1		< 0.001*	<.001*	< 0.001*	0.980 (0.963 – 0.986)	
p_2			<.001*	< 0.001*		
p_3				< 0.001*		

SD: Standard deviation; *: Statistically significant at $p \leq 0.05$. F: F statistic for one-way ANOVA, F (3, 36); pairwise comparisons were performed using Tukey post hoc test. ω²: Fixed-effect omega-squared; values are presented with 95% confidence intervals.
CI: Confidence interval. p: p-value for comparing between the studied groups. p_1: p-value for comparing Control with each other group.
p_2: p-value for comparing Maca with each other group. p_3: p-value for comparing MSG with MSG + Maca.

damage in the form of disorganization, and loss of spermatogenic cells. The nuclei of the cells were dark and pyknotic. In the lumen of the seminiferous tubules, spermatocytes, spermatids, and immature germ cells began to shed and exfoliate. Widening of interstitial spaces caused by oedema and hyaline material with few Leydig cells, and dilated, congested blood vessels were also noticed (Figure 2a-d).

Group IV (MSG + Maca group) demonstrated a partial return to the typical histological architecture. The majority of seminiferous tubules, were lined with spermatogenic and Sertoli cells and had a clearly defined basement membrane, seemed almost normal. Leydig cells were identified in the interstitial tissue, and spermatozoa were seen inside the seminiferous tubules' lumina. However, focal areas of spermatogenic cell loss and mild vascular congestion were still observed (Figure 3a–c).

Using semiquantitative injury score, MSG group exhibited the most severe injury (total score 17/18) representing 94.44%, while Maca administration improved tissue injury to reach 44.44% (table A1 and figure A1).

Masson's trichrome stain (Figure 4a-f)

The interstitial space and the seminiferous tubules' basal lamina in the control group had scant amounts of collagen fibers, as demonstrated by Masson's trichrome-stained sections (4a,b). In contrast, the MSG group showed increased deposition of collagen, as noticed in Figures (4c,d). Collagen deposition decreased and was nearly comparable to that of the control group after maca treatment (Figures 4e,f).

C*aspase-3 immunohistochemical staining (Figure 5a-e)*

Testicular tissue stained with anti-caspase-3 antibody showed diffuse negative immunoreaction in the control group (Figure 5a,b). In contrast, the MSG group demonstrated a diffuse strong positive cytoplasmic immunoreactivity in the germinal epithelial cells, (Figure 5c,d). In the MSG + Maca group, the immunoreactivity was weakly positive and diffusely distributed throughout the testicular tissue (Figure 5e).

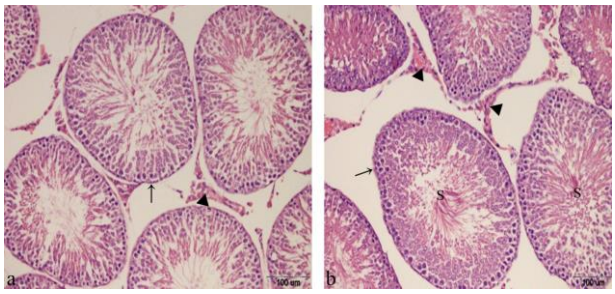

Figure 1 (a, b). H&E stain photomicrographs of the control rat testes a-group I, b-group II showing, seminiferous tubules resting on normal basal lamina (↑) and lined with Sertoli cells and spermatogenic cells, separated by interstitial spaces containing interstitial cell of Leydig (▲).
Note increase number of sperms (S) in the lumen of the tubules in group II. Mic Mag X 200

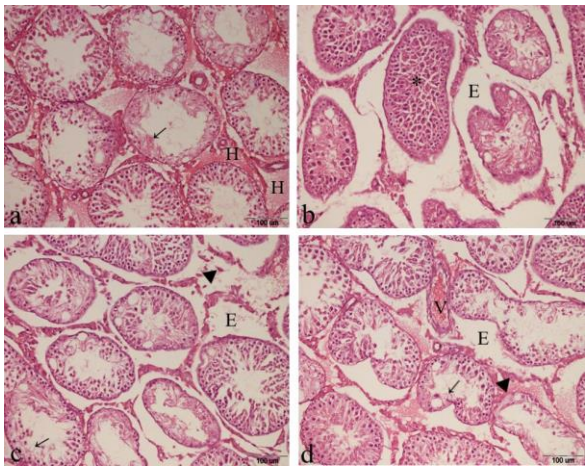

Figure 2 (a, b, c, d). H&E stain photomicrographs of monosodium treated rat testes showing, separation of the seminiferous tubules by edematous fluid (E), hyaline material (H) associated with congested blood vessels(V), pyknotic nuclei of degenerated spermatogenic cells, loss of cells (↑) and its exfoliation in the lumen of the tubules (*), decrease number of interstitial cell (▲). Mic Mag X 200

Morphometric analysis (table A2)

1. Collagen fiber mean area percentage

Morphometric analysis of the percentage area of fibrosis revealed a statistically significant difference among the studied groups ($F = 598.569$, $p < 0.001$). The mean percentage area of fibrosis was 10.00 ± 0.93% in the control group and 10.24 ± 0.86% in the Maca group, with no statistically significant difference between them ($p = 0.996$). In contrast, the MSG group showed a significantly higher mean percentage area of fibrosis (48.00 ± 2.41%) compared with both the control and Maca groups ($p < 0.001$ for each comparison). The MSG + Maca group showed a mean percentage area of fibrosis of 28.20 ± 1.88%, which was

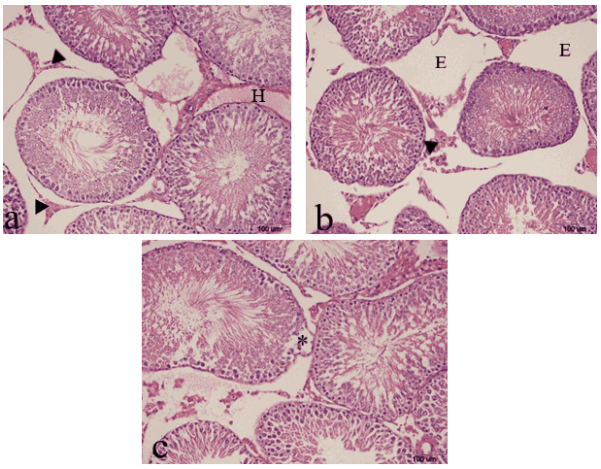

Figure 3 (a, b, c). H&E stain photomicrographs of the protected rat testes showing, more or less the same structure as the control group except mild increase of hyaline material(H), and edema (E).
Note focal area of degenerated spermatogenic cells (*) with more or less normal interstitial cell (▲). Mic Mag X 200

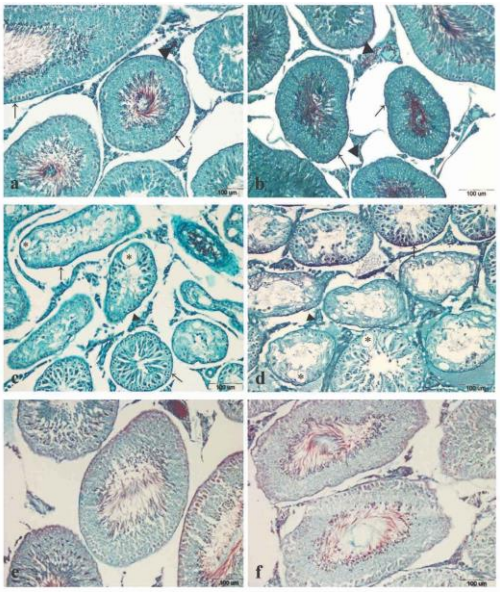

Figure 4 (a, b, c, d, e, f). Trichrome stain micrographs of the control rat testes
a: group I and b: group II showing, normal distribution of collagen fibers in the basal lamina (↑) and interstitial tissue between the seminiferous tubules (▲). Photomicrographs c and d of the MSG treated group: show, increase collagen fibers in the basal lamina (↑) and between the seminiferous tubules (▲) as compared with the control group. Note evident vacuolation (*) and loss of cells in the seminiferous tubules. Photomicrographs e and f of the Maca protected group show, more or less normal distribution of collagen fibers as compared with the control group. Mic Mag X 200

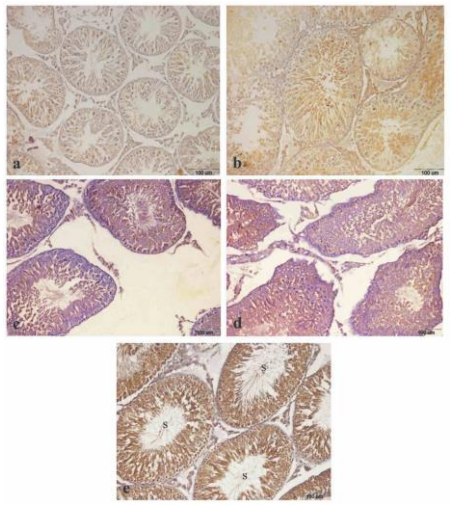

Figure 5 (a, b, c ,d, e). Caspase-3 immunohistochemical micrographs of the testes in negative (a) and positive (b) control revealed mild caspase-3 expression evidenced by faint cytoplasmic DAB.
In MSG treated group (c and d), strong extensive intracytoplasmic caspase-3 protein expression was evident. While in the protected group (e), a marked decline in caspase-3 expression in the spermatogonia and spermatocytes of seminiferous tubules was obvious. Additionally, increase number of sperms in the lumen of tubules (S). Mic Mag X 200

significantly lower than that of the MSG group ($p < 0.001$ for all comparisons).

2. *Mean Caspase-3 H-score*

Immunohistochemical assessment of Caspase-3 expression, quantified by H-score, revealed a statistically significant difference among the studied groups ($F = 687.930$, $p < 0.001$). The mean Caspase-3 H-score was 18.00 ± 2.55 in the control group and 16.60 ± 2.07 in the Maca group, with no statistically significant difference between them ($p = 0.993$). In contrast, the MSG group showed a significantly higher mean Caspase-3 H-score (217.60 ± 14.22) compared with both the control and Maca groups ($p < 0.001$ for each comparison). The MSG+ Maca group showed a mean Caspase-3 H-score of 111.80 ± 7.19, which was significantly higher than that of both the control and Maca groups and significantly lower than that of the MSG group ($p < 0.001$ for all comparisons).

DISCUSSION

A familiar flavor enhancer in a variety of goods is monosodium glutamate (E621). The Acceptable Daily Intake (ADI) of MSG is 30 mg/kg/day, and the No Observed Adverse Effect Level (NOAEL) is 3200 mg/kg/day, based on the official data from the European Food Safety Authority (EFSA). In the USA and Europe, the average daily consumption of MSG is between 0.3 and 1.0 g, but in Asian countries, it is between 1.2 and 1.7 g. Despite MSG's inclusion on the Generally Recognized As Safe list (GRAS) by the Food and Drug Administration (FDA), there is conflicting evidence regarding its safety in the literature [21].

There is usually debate between MSG doses in animal studies and human intake, as mentioned by Yoshida et al (2025). 22 The selected dose in the present study reflects an experimental model designed to reliably induce oxidative/testicular stress within 6 weeks, consistent with prior rodent toxicology literature. However, the actual daily intake of humans cannot be accurately established due to the wide range of products in which glutamates can be found. Additionally, hidden ingredient labels are listed under other trade names. 23 Although it is still generally regarded as safe for humans, studies have proven MSG to be toxic for experimental animals. Many calls are increasing for the revision of doses and assessment of their safety.

In light of the rising infertility rates, as mentioned by Miller et al. (2024), assessing the reproductive safety profiles of various substances linked to male infertility appears to be crucial [8]. As a result, the current study looks into the potential toxic effects of monosodium glutamate and how it can affect male infertility.

Studies have highlighted that excessive MSG exposure has been linked to adverse consequences such as headaches, numbness, flushing, generalized weakness, dizziness, and muscle aches [6, 7, 24]. These manifestations of neurotoxicity could be explained by the overstimulation of glutamate receptors and subsequently neurodegeneration and neuronal damage through a process called excitotoxicity [23].

In addition, oxidative stress and excessive production of reactive nitrogen and oxygen species are another proposed mechanism of toxicity of MSG. This mechanism explains the MSG linked numerous adverse consequences on different biological functions, including the male reproductive system, as emphasized by Kayode et al (2020). [25].

According to the current study, monosodium glutamate generates considerable oxidative stress; the MSG group showed a significant elevation of malondialdehyde and a significant decrease in total antioxidant capacity. These conclusions align with the outcomes of Al-Otaibi et al (2022) and Ogunmokunwa et al (2025) [26, 27].

Due to the established MSG-induced oxidative stress, the histological and caspase-3 immunohistochemical examinations of the testes revealed severe alterations in testicular tissue, which appeared as pyknotic nuclei, detached spermatogenic cells in degraded seminiferous tubules with few or no sperm. Those findings were also observed by Alalwani et al (2014), who concluded that the use of MSG can induce male infertility through its detrimental effects on the testis [28]. The study findings were also in agreement with Asadi et al. (2017), who explained that due to the high amount of unsaturated fatty acids, rapid cell division, and high mitochondrial oxygen demand, testicular tissues are vulnerable to oxidative stress [29].

Significant collagen fibres deposits in the seminiferous tubules' basal lamina, interstitial space, and capsules demonstrated the extent of the injury. Morphometric measures confirmed the findings of significant improvement in fibrosis after administration of Maca. Additionally, interstitial oedema and congestion of blood vessels were also noticed and could be explained by the inflammatory effect of MSG, as found by Banerjee et al (2020) [30].

Following ingestion, MSG is converted to L-glutamate and sodium ions. Glutamate receptors are abundant in the reproductive organs, and when glutamate overstimulates these receptors, it increases the production of free radicals and lipid peroxidation. Sperm membrane malfunction, DNA damage, reduced sperm motility, and eventually male infertility will be the result [25].

It is well established that a significant drop in serum testosterone was detected in the MSG group in comparison to the control group and confirmed by the large effect size. These results could be explained by the direct toxic effect of glutamate on Leydig cells. Besides, the hypothalamic-pituitary-gonadal axis, a crucial system for regulating reproductive function, could be impacted by glutamate-induced toxicity and increased ROS levels, as mentioned by Damilare et al. (2024) [31]. These hormonal disruptions are linked to reduced spermatogenesis and hurt testicular tissue development and preservation, as explained by Asadi et al. (2017) [29].

Body weight was observationally and statistically considerably higher in the MSG group. That was in agreement with Hernández et al (2019), 4 who claimed that MSG could induce obesity and metabolic syndrome. The taste-enhancing effect of MSG, in addition to its effect on leptin, could explain the increased food intake and subsequently body weight of the studied animals and coincide with Kahe et al (2025) [32]. However, the study results were against those of Yoshida et al (2025), who stated that body weight gain and feed consumption were unchanged with MSG administration [22].

Due to the implication of oxidative stress as a mechanism for MSG induced testicular toxicity, antioxidants may be useful in correcting MSG-induced reproductive toxicity. 29 The study has proved that *Lepidium meyenii* powder significantly improved MSG induced disturbed levels of MDA and TAC. The ameliorative effects of *Lepidium meyenii* were also evidenced by noticeable improvement of many histopathological changes in testicular tissue.

The mechanism for maca's protective effects is its rich content of macamides, phytochemicals, flavonoids, and isothiocyanates. These compounds reduce oxidative stress, inhibit cellular damage, and thus promote the recovery of testicular health by their antiapoptotic and anti-inflammatory effects, as found by Ojeda et al (2024) [33].

In the MSG group, tissue damage caused by free radicals displayed overexpression of caspase-3, which causes DNA breakage and enters cells in stages of apoptosis. The histopathological adverse consequences were reduced by combined maca therapy. Maca reduced the expression of caspase-3 induced by monosodium glutamate. Therefore, *Lepidium meyenii* could be a potential adjuvant therapy for male infertility. Those findings coincide with Omolaoye et al (2025) and Shehab et al (2024) [15, 34].

CONCLUSION

The current study highlights how monosodium glutamate induced an increase in body weight and marked testicular damage resulting from oxidative stress and apoptosis. Treatment with Maca significantly improved most of the testicular tissue damage, enhanced total antioxidant capacity, and elevated testosterone levels at the specific duration and dose investigated. Multiple dose- response design could be more comprehensive in understanding dose-toxic relationship. Dose adjustments and assessment of toxic effects should be conducted for all products- containing MSG prior to their introduction for human consumption.

Ethical considerations:

The research ethics committee granted its approval to the study (IRB number 00012098, serial number 0307499). The Institutional Animal Research Committee of the Faculty of Medicine at Alexandria university authorized the experimental protocol. The specified standards and regulations for the use and care of animals were followed.

ACKNOWLEDGMENTS

Authors would like to express their gratitude to Professor Dr. Fardos Soror Karawya, Professor of Histology and Cell biology, Faculty of Medicine, Alexandria University, for her invaluable assistance, and insightful suggestions during the histological preparation and assessment of this study.

Conflict of interest: The authors declare no conflict of interest.

Funding and Support: None.

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Appendix

Semiquantitative Histopathology Injury Score

Scoring System

Sections were graded using a semiquantitative injury score adapted from standard testicular histopathology grading schemes (e.g., modified Johnsen-type and interstitial injury scores commonly used in reproductive toxicology). Six morphological parameters including; Seminiferous tubular atrophy/disorganization, Germinal epithelium depletion, Vacuolization, Interstitial edema, Interstitial haemorrhage, inflammatory cells infiltration were scored on a 0–3 severity scale: 0 = absent/normal, 1 = mild (focal, < 25% of field/tubules affected), 2 = moderate (multifocal, 25–50% affected), 3 = severe/marked (diffuse, >50% affected).

Table A1. Semiquantitative Injury Scores (0–3 scale) for H&E- stained testicular tissue among the different studied groups

Group	Tubular atrophy	Germinal epith. depletion	Vacuoli-zation	Interstitial edema	Interstitial haemorrhage	Inflammatory cell infiltration	Total (0-18)	% of injury score
Group 1 (Control)	0	0	0	0	0	0	0	0%
Group 2 (MSG)	3	3	3	3	3	2	17	94.44%
Group 3 (MSG + Maca)	1	1	1	2	2	1	8	44.44%

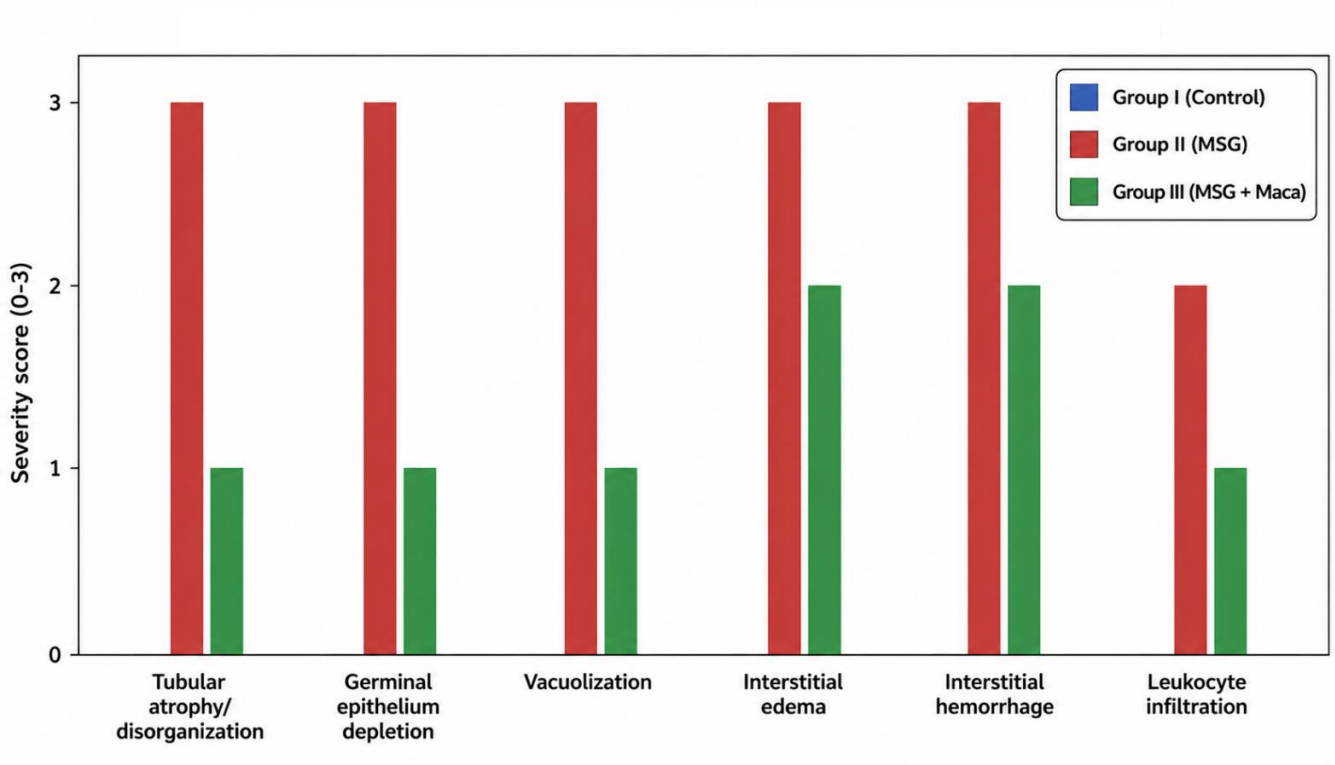

Figure A1. Semiquantitative histology injury score (0-3) for H&E-stained sections

Table A2. Comparison between the different studied groups according to Mean percentage area of fibrosis and Caspase-3 H-score

	Control	Maca	MSG	MSG AND Maca	F	p
Mean percentage area of fibrosis						
Min – Max	8.70 – 11.10	9.0 – 11.20	44.50 – 51.0	25.50 – 30.10	598.569*	< 0.001*
Mean ± SD	10.0 ± 0.93	10.24 ± 0.86	48.0 ± 2.41	28.20 ± 1.88		
p_1		0.996	< 0.001*	< 0.001*		
p_2			< 0.001*	< 0.001*		
p_3				< 0.001*		
Caspase-3 H-score						
Min – Max	15.0 – 21.0	14.0 – 19.0	198.0 – 236.0	102.0 – 121.0	687.930*	< 0.001*
Mean ± SD	18.0 ± 2.55	16.60 ± 2.07	217.6 ± 14.22	111.8 ± 7.19		
p_1		0.993	< 0.001*	< 0.001*		
p_2			< 0.001*	< 0.001*		
p_3				< 0.001*		

SD: Standard deviation; F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey); p: p value for comparing between the studied groups; p_1: p value for comparing between Control and each other group; p_2: p value for comparing between Maca and each other group; p_3: p value for comparing between MSG and MSG & Macca; *: Statistically significant at $p \leq 0.05$