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Multifaceted Impacts of Monosodium Glutamate on Testicular Health: Insights into Pyroptosis and Therapeutic Potential of Resveratrol

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ORIGINAL ARTICLE

Multifaceted impacts of monosodium glutamate on testicular morphology: insights into pyroptosis and therapeutic potential of resveratrol

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ABSTRACT

Background: Monosodium glutamate was considered one of the food additive and flavor enhancer in processed meat and soup that affects testicular histology, the aim of this research to investigates the impact of monosodium glutamate (MSG) on testicular structure in rats and explores the potential protective effects of resveratrol.

Materials and methods: Four experimental groups involved in our study 10 rats of each.: the first group as control group; the second group (Resveratrol group: control rats received 20 mg/kg of resveratrol, via oral gavage); the third group (MSG group: rats received monosodium glutamate (MSG) with a dose 60 mg/kg body weight daily, via gastric tube, and the fourth group (MSG + Resveratrol group). Serum level of testosterone, FSH, LH, were measured. Testicular specimens were prepared for measurement of oxidative stress markers, and gene expression of NLRP3, Caspase 3, and GSK-3β. Moreover, paraffin blocks contained testicular tissue used for histological and immunohistochemical examination. Additionally, seminal smear from epididymis were examined.

Results: MSG administration adversely affected testosterone production, hormonal levels, and sperm parameters, Histological examination revealed marked testicular degeneration, and oxidative stress assessments indicated elevated level of MDA a lipid peroxidation marker and decrease in SOD, CAT antioxidant enzymes. Moreover, MSG-induced apoptotic and pyroptotic markers and its gene expressions. Importantly. Administration of resveratrol reversed the detrimental effects of MSG, demonstrating its corrective influence on the hypothalamic–pituitary–gonadal axis disruption, improvement of sperm parameters, attenuation of oxidative stress, anti-apoptotic activity, and anti-pyroptotic effects. The expression of Ki-67 as a cell proliferation marker further supported the positive response to spermatogenesis dysfunction upon resveratrol treatment.

Conclusions: This comprehensive exploration sheds light on the protective effect of resveratrol against MSG-induced testicular damage with exploration of its mechanistic role.

Keywords: testicular damage, monosodium glutamate, resveratrol, pyroptosis, oxidative stress, apoptosis

INTRODUCTION

Monosodium glutamate (MSG), a sodium salt of L-glutamic acid, represents a prevalent food additive utilized for its preservative properties and enhancement of meal palatability [26]. The surge in processed food consumption due to evolving lifestyles has resulted in an escalated intake of MSG. According to reports, people in several European countries consume about 1 gram of MSG per day on average from processed foods, whereas people in Asia consume 4 grams, and people in Germany consume 10 grams [28, 44]. The detrimental and harmful effects of MSG on male fertility have been proven through numerous studies [4, 28, 47]. More specifically, elevated oxidative stress has been linked to MSG-induced testicular damage [26]. Reactive oxygen radical (ROS) and hydrogen peroxide overproduction can be triggered by oxidative stress, which can result in oxidative DNA damage, peroxidation of cell membranes, and ultimately, cell death [47].

Natural polyphenolic component resveratrol (3,4',5-trihydroxy-trans-stilbene) can be found in a variety of vegetables, such as berries, peanuts, and grapes. It has garnered increasing attention for its reported multifaceted benefits, encompassing anti-inflammatory, antidiabetic, anticancer properties, and cardiovascular protection. Additionally, resveratrol is associated with enhanced stress resistance, extended lifespan, and preventive effects against various diseases, such as cancer, ischemic injuries, and cardiovascular issues [11, 29, 50]. Notably, resveratrol's antioxidant attributes have demonstrated efficacy in shielding cells from hydrogen peroxide-induced oxidative stress and UV-irradiation-induced cell death when administered as a pretreatment [36, 37, 39]. In pharmaceutical applications, resveratrol exhibits the ability to delay lipid oxidation, reduce toxic oxidative byproducts, and extend shelf life while preserving nutritional quality [25, 43]. Due to its structural similarity to estradiol, resveratrol is postulated to potentially play a comparable role in the testicular function. Resveratrol application in vivo has demonstrated effectiveness in treating infertility, particularly in cases of dyszoospermia, where mitigated the impact induced by 2,5-hexanedione on spermatogenesis [31]. It has shown promise in improving sperm quality, likely facilitated by its ability to traverse the blood–testis barrier and confer protective effects to the

testicular structure in both humans and animals [3, 41]. Furthermore, oral administration of resveratrol in combination with coenzyme Q10 has been found to protect against radiation-induced spermatogenesis injuries, confirming a potential benefit in promoting male fertility [40]. Despite substantial research efforts, the precise role of resveratrol in male reproductive function remains unclear, warranting further investigation.

Hence, numerous initiatives have been undertaken to mitigate the impact of antibiotics on spermatogenesis [33, 46]. Resveratrol is recognized for its antioxidant properties, capable of scavenging ROS and thereby averting cellular damage in tissues. There is encouraging evidence supporting the effective preservation of spermatogenesis in animal models and the treatment of male factor infertility through the administration of antioxidants. Several studies have indicated that vancomycin can lead to testicular atrophy and compromised sperm quality in both animals and humans. This investigation is designed to explore whether resveratrol possesses a modulatory impact on the development of testicular injury and depression induced by monosodium glutamate in rats, along with an exploration of its underlying protective mechanisms.

MATERIALS AND METHODS

Experimental animals

Forty Wistar male rats weighing between 120 and 150 grams were bought from Zagazig University's Faculty of Veterinary Medicine. After a seven-day acclimatization period, during which they were housed in plastic cages, the rats were provided with a standard laboratory diet and had unrestricted access to water. After then, the animals were categorized into four groups, each consisting of eight rats.

Pharmaceutical interventions

Under license from Ajinomoto Co. Inc., Tokyo, Japan, monosodium glutamate (MSG), with a purity of 99% and the chemical formula C5H9NO4·Na, is commercially available in most open markets. Sixty grams of MSG crystals were dissolved in one thousand milliliters of distilled water to create a stock solution. To make sure that each animal received the same amount of MSG based on their weight, the dose regimen was adjusted and mixed with saline (0.9% NaCl) as suspension and given to animals immediately.

Resveratrol (purity ≥ 99%) and monosodium glutamate were obtained from Sigma Aldrich Co. (St. Louis, MO, USA).

Experimental design

The rats were allocated into four groups of ten each in random manner: Control group (n = 10), Resveratrol group (control rats receiving resveratrol at a dosage of 20 mg/kg daily via oral gavage for four weeks), and MSG group (rats administered MSG at a dose of 60 mg/kg body weight daily via gastric tube for four weeks). Additionally, there was a MSG + Resveratrol group where rats received both MSG (60 mg/kg b. wt) and resveratrol (20 mg/kg via oral gavage). The dose of resveratrol and monosodium glutamate according to the previous studies done by [2, 26] respectively.

Sample collection

After a 4-week treatment period, animals underwent a 24-hour fasting period before sacrifice. Anesthesia using ether was administered. Heparinized tubes were employed for blood samples that were drawn from the hepatic portal vein. To extract plasma, the samples were then centrifuged for 15 minutes at 1500 rpm. After that, the seminal vesicles, caudal epididymis, and testes were extracted, cleaned in saline solution, and dried. After severing the caudal epididymis from the testes, semen was collected using a microscope glass slide so that the characteristics of the sperm could be examined. Each rat was sacrificed, and its seminal vesicles and one testis were immediately preserved in a 10% formalin solution for microscopic inspection. The other testis was frozen at –80°C for future study. Testicular samples were homogenized at a 1:10 dilution in potassium phosphate buffer (0.1 M, pH 6.5). A portion of the homogenate was utilized for determining reduced oxidative stress markers.

This experimental procedure was conducted in the Anatomy department of the Faculty of Medicine at Kafr Elsheikh University, Egypt. The experiment adhered to all guidelines for infection control, personal protective safety, and biosafety measures against zoonotic agents [38]. All procedures related to animal treatment, euthanization, and biological tissue sample management followed established biosafety protocols [5]. The disposal of deceased and euthanized rats was carried out using the alkaline hydrolysis method. The hygienic disposal of waste, hazardous materials, chemicals, and washed materials

followed recommended methods [13]. The experiment was initiated only after receiving approval from the Institutional Review Board (IRB).

Preparation of tissue homogenates and assessment of antioxidant capacity parameters

The dissected testicular specimens underwent a thorough wash with distilled water to eliminate blood, followed by the removal of adipose components. Subsequently, the tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) supplemented with 0.1 mM ethylenediaminetetraacetic acid (EDTA). Centrifugation was employed to separate the supernatant at 5000 rpm for 20 minutes at 4°C. This supernatant was utilized for the analysis of all biochemical parameters. Assessment of oxidative stress markers, including Superoxide Dismutase (SOD), Catalase (CAT), and Malondialdehyde (MDA), was performed on the testicular supernatant. Commercial test kits (Biodiagnostics Co., Cairo, Egypt) were employed. Using a spectrophotometer, the analyses were carried out in compliance with the manufacturer's methodology, which was described in the accompanying pamphlets.

Assessment of total sperm concentration

The right cauda epididymis was gently squeezed to discharge epididymal fluid onto a slide in order to determine the sperm count. Next, this fluid was suspended in phosphate-buffered saline (PBS) and pipetted up to the '0.5' mark using a white blood cell (WBC) pipette. One milliliter of 40% formaldehyde and five grams of sodium bicarbonate (NaHCO3) were dissolved in one hundred milliliters of normal saline to create a semen dilution solution. After drawing up the fluid to the pipette’s “11” mark, it was well combined. Neubauer's hemocytometer had one drop added to each side after the first one or two were discarded. After five minutes in a humid environment, the spermatozoa were allowed to settle in the hemocytometer. To count sperm, a high-power objective (40×) was used. The haemocytometer's five primary squares were used to count the total number of sperm, and the average was computed to determine the sperm count. To ascertain the sperm concentration in the original cauda epididymal semen sample, the dilution factor was considered, and the sperm count was calculated using the formula [18]: Sperm count/mL = (dilution factor) × (count in five squares) × (0.05×10^6).

For assessing the percentage of abnormal forms, seminal smears stained with hematoxylin and eosin were employed. A semen drop was applied to a glass slide after dilution and fixation with 95% ethyl alcohol. For every group, five air-dried smears were made on glass slides and stained with hematoxylin and eosin. Next, the percentage of aberrant sperm among the 200 spermatozoa on each slide was assessed [20].

Testicular histological staining

The Hematoxylin and Eosin (H&E) staining protocol for fresh frozen sections was done following the guidelines outlined by the Center of Musculoskeletal Research at the University of Rochester. The frozen tissue slides were subjected to fixation in a cold 10% neutral buffered formalin solution for a duration of 10 minutes. Subsequently, a triple rinsing step with 1X PBS, each lasting 3 minutes, was performed to eliminate residual optimal cutting temperature (OCT) or other tissue embedding compounds. Post-OCT removal, a gentle 1-minute tap water wash was executed. Hematoxylin staining ensued for a precise duration of 30 seconds, followed by a 20-second immersion in 1X PBS for nuclear counterstaining. Sequentially, the slides were consecutively immersed in 70% and 95% ethanol for 30 seconds each. Counterstaining with alcoholic-eosin transpired for 30 seconds. Dehydration procedures encompassed two iterations of 15-second exposures to 95% ethanol and three successive immersions in 100% ethanol for 15 seconds each. Tissue clearance was achieved through three changes of xylene, each lasting 1 minute. Subsequently, coverslips were applied. Microscopic examination of the slides revealed distinct features, including blue-stained nuclei, pink cytoplasm, and intensely red erythrocytes, alongside various other eosinophilic structures exhibiting hues of red, pink, or orange.

Immunohistological (IHC) assessment of the expression levels of Caspase-3, Bax, Bcl2, Ki67, IL-1beta, and Caspase-1

5 μm paraffin slices were rehydrated using descending ethanol strengths (100%, 95%, and 70%) and then washed for five minutes with distilled water in preparation for immunohistochemical examination. After that, the slices were cleaned with PBS (protein-buffered saline). Sections were treated with 0.1% hydrogen peroxide (H_2O_2) for 30

minutes to decrease endogenous peroxidase activity, and then they were washed with PBS. To avoid nonspecific binding, a blocking solution (10% normal goat serum) was administered and allowed to sit for one hour at room temperature. Primary antibodies targeting Caspase-3, Bax, Bcl2, Ki6, IL-1β, and Caspase-1 (Cat# MBS6005687, ab53154, MAB8272, 5F86, AAR15G, and MBS9700770) were then incubated with the sections for 60 minutes at room temperature. Following this, PBS rinsing was carried out. Secondary antibodies were applied for 20 minutes at room temperature, followed by another PBS rinse. Then, for ten minutes, streptavidin-horseradish peroxidase enzyme conjugates were administered. 3,3-diaminobenzoic acid (DAB) was used to visualize the conjugated secondary antibody sites, and PBS was then used to wash the samples. Hematoxylin counterstaining was carried out. A blinded professional pathologist conducted photography of slices from the prefrontal cortex and hippocampus using an Olympus digital camera at the Pathology Department of the Veterinary Medicine College, Mansoura University. Using ImageJ software, immunostained cell counts in fields extracted from at least three rats were measured and averaged per field for each animal.

Determination of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) by ELISA

On day 8, serum samples were obtained and individually processed for each animal. After centrifugation, the serum from each animal was subjected to separate analyses. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone serum levels have been evaluated using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The assays were carried out following the manufacturer's instructions (MyBioSource, Inc., San Diego, CA 92195-3308, USA).

Gene expression analysis of NLRP3, caspase 3, and GSK-3β through RT-PCR evaluation

Frozen testicular samples were treated with phenol and guanidinium isocyanate (Trizol reagent 15,596,026, Life Technologies, United States) to extract the entire RNA.

Using spectrophotometry, the concentration and purity of the RNA were measured at wavelengths between 260 and 280 nm. The Quali-Tect Reverse Transcription Kit (Qiagen) was implemented to convert 1 μg of RNA into single-stranded complementary DNA in accordance with the manufacturer's instructions. The primers employed for NLRP3, Caspase 3, and GSK-3β genes, the primers were used as follows: NLR Family Pyrin Domain Containing 3 **(**NLRP3) gene was forward 5′-AAAGGAAGTGGACTGCGAGA -3′ and reverse 5`-TTCAAACGACTCCCTGGAAC-3′; Glycogen synthase kinase- 3 beta (GSK-3β) gene was forward, 5′-GCTTCAACCCCTTCAAATGC-3′ and reverse, 5′-GACGCAGAAGCGGTGTTATTG-3; GAPDH housekeeping gene was forward 5'- CCTCGTCTCATAGACAAGATGGT -3' and reverse 5'- GGGTAGAGTCATACTGGAACATG -3'; caspase 3 gene was forward 5′-GGTATTGAGACAGACAGTGG-3′ and reverse 5′-CATGGGATCTGTTTCTTTGC-3′ . The primers were acquired from Vivantis Technologies, Malaysia, also known as Vivantis. The expression level of the genes was normalized to GAPDH and was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The results were presented as mean ± standard deviation (SD), and significant differences among groups were assessed using One-way Analysis of Variance (ANOVA) followed by a post hoc Bonferroni test. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS

Influence of resveratrol and MSG on testosterone, FSH, and LH levels

Our results revealed that oral ingestion of MSG produces a significant decrease in the hormonal levels of testosterone, FSH, and LH compared to the hormonal levels of control rats. Meanwhile, the MSG rats' group that received resveratrol showed a significant increase in the serum levels of hormones (Table 1). Regarding this finding, we demonstrated the upregulating effect of resveratrol on the hypothalamic-pituitary-gonadal axis in MSG-treated rats.

Mitigation of MSG-induced testicular oxidative stress by resveratrol: an antioxidant perspective

Oral intake of MSG showed severe testicular oxidative stress, manifested as a significant increase in the lipid peroxidation marker MAD, along with a decrease in testicular SOD and CAT antioxidant enzymes. Fortunately, intake of resveratrol with MSG significantly decreased testicular MDA and increased SOD and CAT (Table 1). From the above, we can conclude that resveratrol revealed potent antioxidant capacity against MSG-induced oxidative insult.

Investigating the impact of resveratrol and MSG on sperm histology, sperm counts, and abnormal sperm morphology

The semen smear of the control rats' group revealed a normal histological structure of sperms, with a few rats in the resveratrol group exhibiting sperms with detached heads (Fig. 1A, B). In contrast, the MSG groups showed a large number of sperms with detached heads, bent middle pieces and heads, dwarf headless sperms, and other sperms with twisted heads (Fig. 1C). Treatment of the MSG rats' group with resveratrol clearly improved the seminal smear, with only a few sperms showing detached heads and dwarfs headless (Fig. 1D). This result was confirmed by statistical analysis of the percentage of abnormal sperm morphology, which showed a significant increase by 472% compared to the control rats'. Conversely, the MSG + resveratrol group exhibited a significant decrease by 57.39% compared to the MSG group (Fig 2F). Additionally, the MSG + resveratrol group showed a significant increase in sperm count compared to the MSG group's (Fig 1E).

Resveratrol's ameliorative impact on MSG-induced testicular damage

H&E examination of testicular sections in control and resveratrol rats' groups revealed normal morphology, characterized by apparent seminiferous tubules lined with normal spermatogonia, primary (1ry), and secondary (2ry) spermatocytes. The process of spermatogenesis appeared normal, as evidenced by a full lumen with spermatozoa attached to Sertoli cells, and the interstitial tissue containing Leydig cells (Fig. 2A–D). In

contrast, the testicular sections of the MSG group exhibited marked degeneration, with vacuolation and necrosis of the lining epithelial cells and numerous vacuolated and necrotic shrunken seminiferous tubules (Fig. 2E–H). Fortunately, the MSG-treated group with resveratrol showed a clear improvement in their testicular sections, with only a small number of vacuolated and necrotic epithelial linings in shrunken seminiferous tubules (Fig. 2I, J).

Resveratrol's suppressing influence on MSG-induced testicular pyroptosis

Oral intake of MSG by rats significantly increased testicular pyroptosis markers, as evidenced by elevated gene expression of GSK-3β and NLRP3 (Fig. 3N, O) and increased immune expression of caspase-1 and IL-1β (Fig. 3E–J, 4E, F) compared to control rats. In contrast, the MSG + resveratrol group showed a significant decrease ($p < 0.001$) in mRNA expression of GSK-3β and NLRP3, and testicular sections immunoassaying of caspase-1 and IL-1β (Fig. 3K, L, 4G, H). These findings demonstrate the anti-pyroptotic effect of resveratrol against MSG-induced pyroptosis.

Resveratrol's protective role against MSG-induced testicular apoptosis

Oral intake of MSG for 4 weeks resulted in a significant increase in immune staining of apoptotic markers caspase-3 and Bax, as well as gene expression of caspase-3 (Fig. 5E–J, 6E, F, 5N), with a decrease in immunoassaying of the antiapoptotic protein Bcl2 (Fig 7E–H) compared to normal rats. Conversely, treatment of MSG rats with resveratrol significantly decreased the expression of positively immunostained caspase-3 and Bax apoptotic cells, as well as mRNA expression of caspase-3 (Fig. 5K, L, 6G, H, 5N). Additionally, there was an increase in immunoexpression of the antiapoptotic Bcl2 (Fig. 7I–L) in relation to the MSG group. Resveratrol exhibited a strong antiapoptotic effect against MSG-induced testicular damage.

Resveratrol's safeguarding influence against MSG-induced alterations in spermatogenic Ki-67 expression

The MSG group rats showed a significant decrease in Ki-67 immunoexpression of spermatogenic cells in relation to the control group (Fig. 8E, F). Conversely, MSG rats that received resveratrol exhibited a marked increase in the proliferation of spermatogenic cells, as indicated by a significant elevation in Ki-67 immunoexpression (Fig. 8G, H). This finding confirms the strong proliferative effect of resveratrol on spermatogonia.

DISCUSSION

The testes perform dual primary functions, namely, [26] the synthesis of the male sex hormone testosterone and [44] the generation of spermatozoa, the male gametes [34]. In this investigation, the potential toxic impact of monosodium glutamate (MSG) was assessed and explore the potential protective influence of resveratrol on the structural and functional aspects of the testes. Monosodium glutamate (MSG), commonly employed as a flavor enhancer in culinary applications, is recognized as a hazardous substance with documented effects on the morphological and physiological aspects of the male reproductive system in both human and animal models [1]. The present study scrutinizes the biochemical, histological, and ultrastructural alterations manifested in the testes of adult rats following MSG-induced toxicity. Additionally, the mitigating effects of resveratrol against MSG-induced testicular injury in rats are investigated. Substantial evidence strongly implicates oxidative stress, cellular antioxidant insufficiency, and mitochondrial disruption as pivotal pathogenic pathways associated with MSG exposure.

Resveratrol, a naturally occurring polyphenolic compound present in various vegetables such as grapes, berries, and peanuts, is renowned for its antioxidant attributes [16]. Extensive research has focused on the antiaging characters of resveratrol and its potential in preventing age-related illness, like diabetes and Alzheimer’s disease [6]. Moreover, resveratrol exhibits potential in enhancing cognitive function [30], mitigating the risk of heart disease and stroke [19], and displaying anti-inflammatory and anti-cancer properties according to accumulating data [12]. Further investigations suggest that resveratrol may contribute to increased human longevity, improved stress tolerance, and inhibition of the progression of certain diseases [9]. Previous studies have demonstrated the efficacy of resveratrol treatment in vivo in reducing oxidative stress in the testes of

rats subjected to chemotherapy and hyperthyroid conditions [56]. In the context of the present experiment, the inquiry extends to the examination of resveratrol's capacity to shield rat spermatozoa from damage induced by MSG.

Firstly, in this experiment, the impact of MSG on testosterone production by the testes and hormones of the Hypothalamic-Pituitary-Gonadal Axis was assessed in rats. The results revealed a decrease in serum testosterone levels, contradicting the findings of [27], which reported a notable reduction in plasma testosterone levels and partial infertility in male rats exposed to high amounts of MSG. Additionally, MSG administration adversely affected the serum levels of FSH and LH, crucial hormones in reproductive organ maturation and male gamete production, consistent with [22, 24]. Conversely, a different study indicated that oral gavage with MSG increased anterior pituitary LH and FSH secretion, along with heightened FSHRH and LHRH secretion by paraventricular and supraoptic hypothalamic nuclei, negatively impacting the reproductive system. The inhibitory impact of MSG is attributed to its ability to suppress the secretion of various reproductive neuropeptides, including Neurokinin B, Proopiomelanocortin (POMC), Neuropeptide Y (NPY), Agouti-related protein (AgRP), all pivotal in reproductive function regulation. The inhibitory effects of MSG were counteracted by the administration of Resveratrol, in line with [10, 31], affirming Resveratrol’s capacity to rectify disruptions in the hypothalamic–pituitary–gonadal axis induced by MSG.

Secondly, the impact of MSG on sperm production by the testes was evaluated, revealing a decrease in epididymal sperm count and an increase in the percentage of sperm with abnormal morphology, contrary to [16, 54]. Conversely, treatment with resveratrol improved sperm parameters, aligning with [32]. The effects of MSG and resveratrol on sperm parameters were consistent with the histological examination of testicular sections using H&E staining. MSG induced marked testicular degeneration with necrotic shrunken seminiferous tubules, supporting [4], who explained the inhibitory effect of MSG on testosterone production. Resveratrol administration successfully alleviated testicular degeneration, as reported in [8].

Assessment of oxidative stress was carried out to elucidate the mechanisms influencing the impact of MSG and resveratrol on testicular structure and function.

Oxidative stress occurs when the production of ROS exceeds the capacity of cellular antioxidants, such as Catalase and Superoxide dismutase [10]. Elevated ROS levels can induce lipid peroxidation and disrupt the functionality of proteins, DNA, and RNA in spermatozoa and other testicular cells, thereby impairing male reproductive processes and potentially causing infertility. This interference may occur directly or indirectly by disrupting the hypothalamus-pituitary-gonadal (HPG) axis or influencing unfavorable interactions with other hormonal axes [54]. The study results revealed that MSG administration increased tissue levels of malondialdehyde (MDA), a final product of lipid peroxidation, and decreased the levels of Superoxide dismutase (SOD) and Catalase, indicating an association between MSG administration and oxidative stress. These findings align with previous studies [14, 15] that reported MSG's ability to induce oxidative stress. Resveratrol, known for its antioxidant properties, demonstrated an increase in catalase and superoxide dismutase levels and a decrease in MDA levels, consistent with [6,35]. These effects can be attributed to resveratrol's activity in suppressing NADPH oxidase expression and activity, thereby preventing the generation of ROS. Furthermore, this polyphenolic substance reduces mitochondrial superoxide production, inhibits superoxide formation from uncoupled endothelial nitric oxide synthase by upregulating GTP cyclohydrolase I, and promotes the expression of several antioxidant enzymes [26].

Tissue homeostasis relies on apoptosis, a programmed cell death process [45]. Controlling apoptosis is crucial for the growth, differentiation, and function of germ cells, as evidenced by higher levels of apoptotic cells in the seminal fluid of infertile men compared to fertile men [49]. In this study, oral intake of MSG increased the immunoexpression of apoptotic markers, caspase-3 and Bax, and upregulated gene expression of caspase-3, while concurrently reducing the immunoassaying of the antiapoptotic protein Bcl2. These findings substantiate the testicular apoptotic effect of MSG, aligning with [2, 47]. This supports the hypothesis that MSG-induced testicular damage involves mitochondrial-mediated apoptosis. As a remedial measure, resveratrol was administered, resulting in the reversal of the apoptotic effect of MSG in damaged testis tissue. This aligns with studies by [7, 57], which demonstrated the anti-apoptotic effect of resveratrol in alleviating diabetes-induced testicular dysfunction. This study

further reveals that resveratrol modulates the expressions of mitochondrial Bcl-2, Bax, and caspase-3 proteins, suppressing the mitochondrial death pathway, consistent with findings reported by [42].

This study presents the inaugural investigation into the impact of MSG and resveratrol on the testicular pyroptosis mechanism. Pyroptosis, a form of inflammatory apoptosis, serves the primary purpose of inducing robust inflammatory responses that aid the immune system in defending against microbial infections [21]. Given the involvement of pyroptosis pathways in various illnesses, including gout, Alzheimer's disease, sepsis, HIV infection, and Cadmium-induced testicular damage, these pathways emerge as crucial targets for therapeutic intervention [17, 23, 51, 53, 60]. In the classical pyroptosis pathway, the N-terminal fragment of Gasdermin D (GSDMD) anchors in the cell membranes, forming pores post-cleavage by pro-inflammatory Caspase-1 activated by the NLRP3 inflammasome. Activated Caspase-1 can further stimulate interleukin-18 (IL-18) and IL-1β [59]. The study observed a significant increase ($p < 0.001$) in testicular pyroptosis markers in rats following MSG oral intake, evident through elevated gene expression of GSK-3β and NLRP3, as well as increased immune expression of caspase-1 and IL-1β. These findings confirm MSG's ability to activate pyroptosis, contributing to testicular damage and fibrosis [59], consistent with [55] which associated MSG with depression in rats via the activation of the pyroptosis pathway. The anti-inflammatory properties of resveratrol were explored, revealing its anti-pyroptotic activity by inhibiting the expression of GSK-3β, NLRP3, caspase-1, and IL-1β. Previous studies have corroborated this activity in microglia cells [52] and intestinal cancer cells [48].

Finally, the study assessed the expression of Ki-67 as a cell proliferation marker in spermatogenic cells following the administration of MSG alone and with resveratrol. While the MSG groups exhibited mild positive expression, the MSG + resveratrol group displayed strong positive expression. The heightened positive expression levels of PCNA and Ki-67 indicated a favorable response to spermatogenesis dysfunction [58]. However, in this work we did not perform electron microscope examination for semen to discover the ultrastructural abnormalities in the sperm's heads, acrosomal and plasma membranes, middle piece, and sperms tail, also electron microscopic examination of testicular

specimen to explore the ultrastructural changes in the different lining cells of seminiferous tubules such as spermatogenic cells, primary spermatocyte and spermatids which is considered a limitation of this study.

CONCLUSIONS

In conclusion, this study provides a comprehensive examination of the intricate effects of monosodium glutamate (MSG) on testicular structure and function in rats, with a particular focus on the novel exploration of the pyroptosis mechanism. The observed adverse impacts of MSG on the Hypothalamic-Pituitary-Gonadal Axis, testosterone production, sperm parameters, oxidative stress, apoptosis, and pyroptosis underscore the complex nature of its influence on male reproductive health. The findings indicate that MSG administration leads to disruptions in hormonal balance, compromised sperm quality, testicular oxidative stress, and activation of testicular apoptotic and pyroptotic pathways. These outcomes align with some existing literature but also present novel insights into the involvement of the pyroptosis mechanism, highlighting its potential role in testicular damage induced by MSG. Crucially, the administration of resveratrol emerges as a promising therapeutic intervention, effectively mitigating the deleterious effects of MSG. Resveratrol demonstrates its protective prowess by correcting disruptions in the hypothalamic–pituitary–gonadal axis, improving sperm parameters, attenuating oxidative stress, exhibiting anti-apoptotic activity, and suppressing the pyroptosis pathway. This study not only contributes to the expanding body of knowledge on the reproductive consequences of MSG but also introduces a novel perspective by unraveling the involvement of pyroptosis in testicular damage. The potential therapeutic implications of resveratrol in alleviating MSG-induced reproductive impairments underscore its significance as a candidate for further exploration and consideration in the context of male reproductive health. Ultimately, these findings encourage continued research to elucidate the intricate mechanisms underlying MSG-induced reproductive toxicity and to explore additional avenues for therapeutic intervention. From all of this finding, we encouraged to spread our study to human subject to establish our outcomes and to assess the remedy of resveratrol in patients suffering from the complication of infertility

associated with monosodium glutamate administration as a food additive with a large dose.

Article information and declarations

Data availability statement

The data that support this research will be shared upon reasonable request to the corresponding authors.

Ethics statement

The study was conducted in accordance with the Canadian Council on Animal Care Guidelines and approved by the committee of Research Ethics, Kafrelsheikh University (KFS-IACUC/183/2024).

Author contributions

Medhat Taha: conceptualization, methodology, writing & original draft. Lashin Saad Ali: methodology, writing — review & editing. Mohammad El-Nablaway: supervision, visualization. Mohie Mahmoud Ibrahim: writing — review & editing. Alaa. M. Badawy: conceptualization. Amira E. Farage: investigation. Hany Sabry A. Ibrahim: methodology. Randa A. Zaghloul: methodology. Emadeldeen Hussin: investigation.

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Table 1. Effect of resveratrol on hypothalamo-pituitary gonadal axis and oxidative stress markers

	Control	Resveratrol	MSG	MSG + Resveratrol
Testosterone	16.48 ± 0.66	15.92 ± 0.68	4.130 ± 0.54^{aaa}	9.320 ± 1.61^{bbb}
FSH	4.27 ± 0.53	4.09 ± 0.52	1.62 ± 0.27^{aaa}	2.63 ± 0.65^{bbb}
LH	8.63 ± 0.67	8.15 ± 0.55	4.93 ± 0.82^{aaa}	5.68 ± 0.62^{bbb}
MDA	10.33 ± 2.14	9.81 ± 1.96	40.52 ± 2.80^{aaa}	30.39 ± 3.87^{bbb}
SOD	198.6 ± 13.50	188.9 ± 13.25	91.30 ± 16.43^{aaa}	134.6 ± 16.46^{bbb}
CAT	5.17 ± 0.54	4.83 ± 0.56	1.30 ± 0.39^{aaa}	2.71 ± 0.39^{bbb}

All our data are expressed as M ± SD. $^{aaa}p < 0.001$ vs. control; $^{bbb}p < 0.001$ vs MSG.

CAT — Catalase; FSH — Follicle-Stimulating Hormone; LH — Luteinizing Hormone; MDA — malondialdehyde; SOD — Superoxide dismutase.

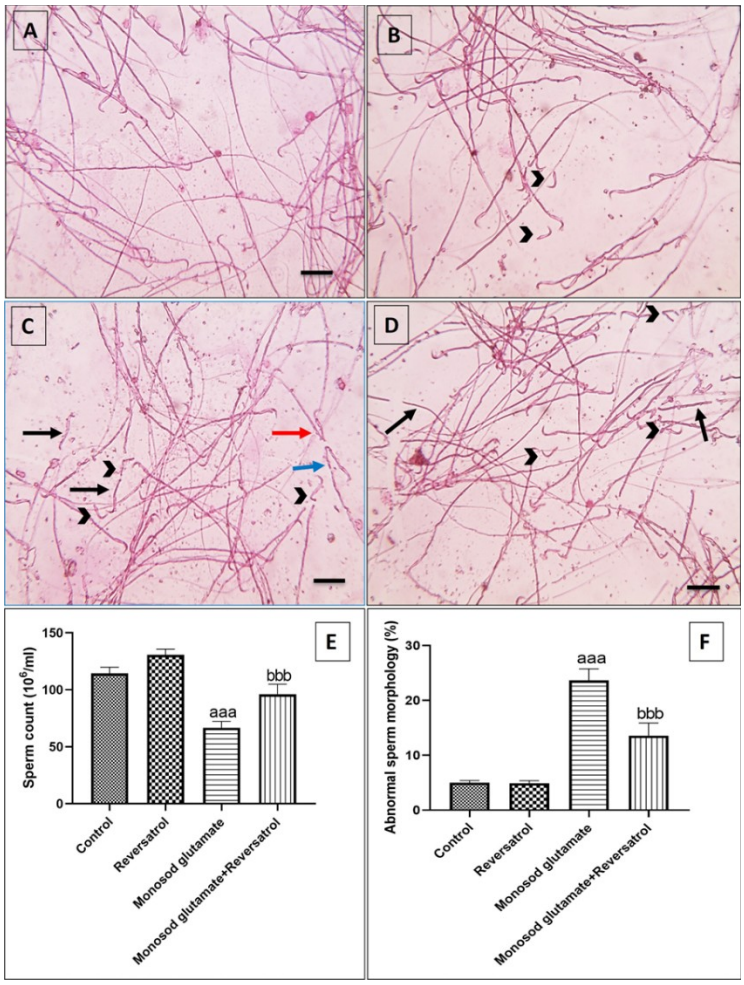

Figure 1. Microscopic images of epididymal smears in the control group taken at 400× magnification using a light microscope and showing typical sperm morphology, including the head, body, and tail. Smears from the resveratrol group exhibit a few detached heads (black arrowheads). However, the smear from the MSG group reveals several morphological abnormalities, such as detached heads (black arrowhead), bent middle pieces (blue arrow), bent heads (red arrows), dwarf headless sperms (black arrow), and twisted heads (closed arrowhead). The smear from the MSG + resveratrol group shows a few detached heads (black arrowheads) and a few dwarf headless sperms (black arrow). Staining was performed with a 0.05% aqueous solution of eosin-Y. Our findings are presented as M ± SD. $^{aaa}p < 0.001$ vs. control; $^{bbb}p < 0.001$ vs MSG

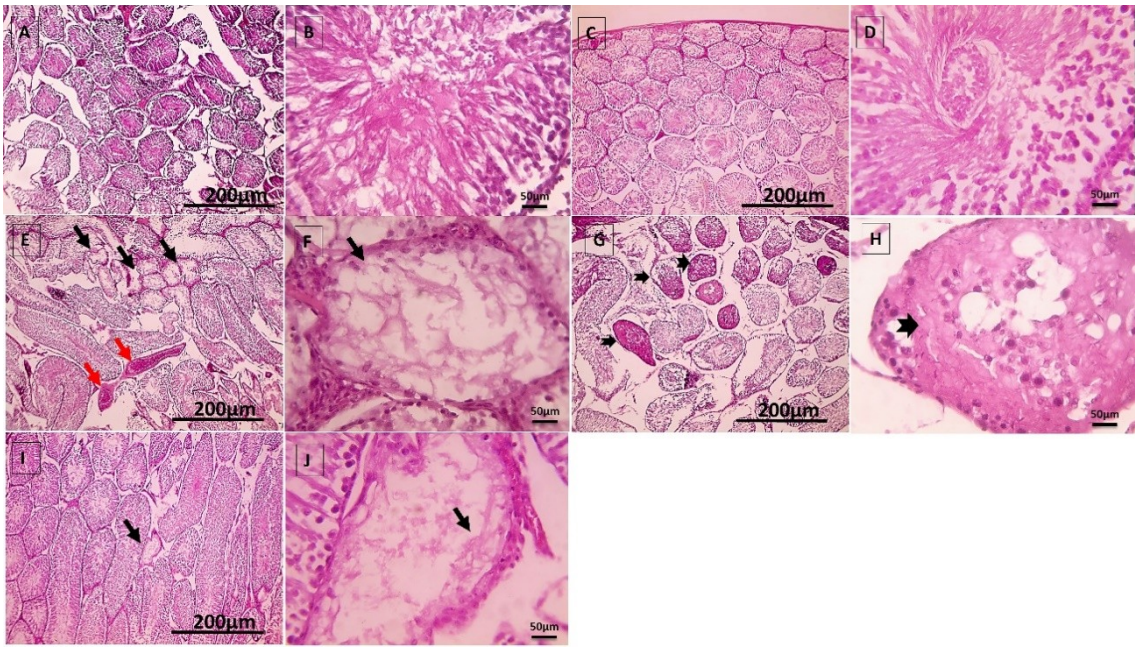

Figure 2. Microscopic images of H&E-stained testicular sections from the control and resveratrol groups reveal normal features of seminiferous tubules, with several layers of spermatogonia, primary (1ry), and secondary (2ry) spermatocytes. Normal spermatogenesis is characterized by a full lumen with spermatozoa attached to Sertoli cells, and interstitial tissue containing Leydig cells (A–D). Testicular sections from the MSG group display vacuolation and necrosis of the epithelial lining in many shrunken seminiferous tubules (thin black arrows) (E, F). Other testicular sections from the MSG group show vacuolation, necrosis, and hyalinization of the epithelial lining in many shrunken tubules (thick black arrows) (G, H). Testicular sections from the MSG + resveratrol group exhibit vacuolation and necrosis of the epithelial lining in individual shrunken seminiferous tubules (thin black arrows) (I, J). Low magnification ×: 40 bar 200, and high magnification ×: 400 bar 50.

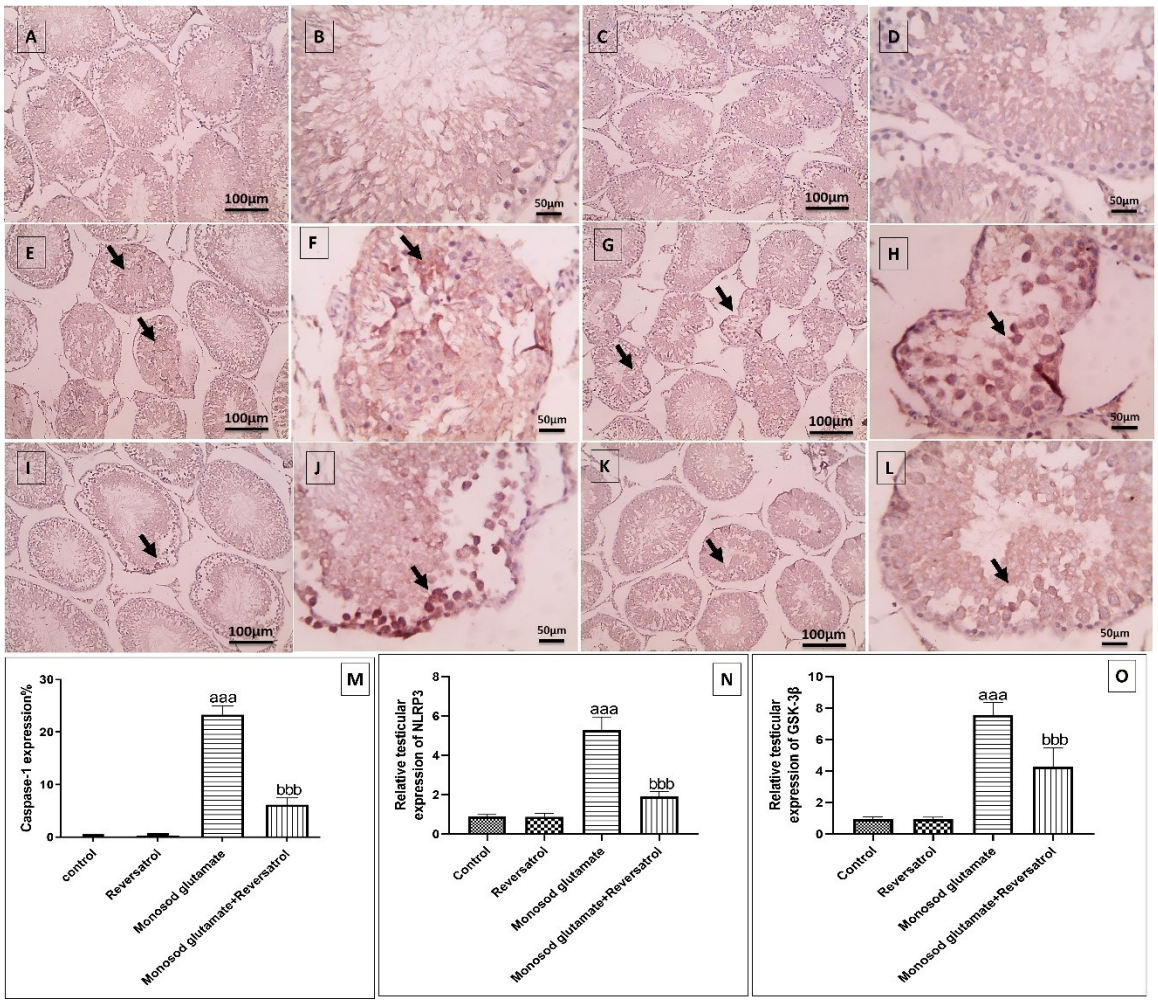

Figure 3. Microscopic images of immunostained testicular sections against caspase-1 show a negative tubular reaction in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit a prominent positive brown reaction against caspase-1, appearing in many cells lining numerous tubules (black arrows) (E–J). In contrast, testicular sections from the MSG + resveratrol group display a decreased positive brown reaction against caspase-1, observed in few cells lining very few tubules (black arrows) (K, L) IHC counterstained with Mayer's hematoxylin. Low magnification ×: 100 and high magnification ×: 400 bar 50. Bars represent means ± SE, demonstrating the percentage of caspase-1 expression in immunostained testicular sections, analyzed by one-way ANOVA test followed by Tukey's test (M) Relative testicular gene expression of NLRP3 and GSK-3β (N, O) Our data is presented as M ± SD. $^{aaa}p < 0.001$ vs control; $^{bbb}p < 0.001$ vs MSG.

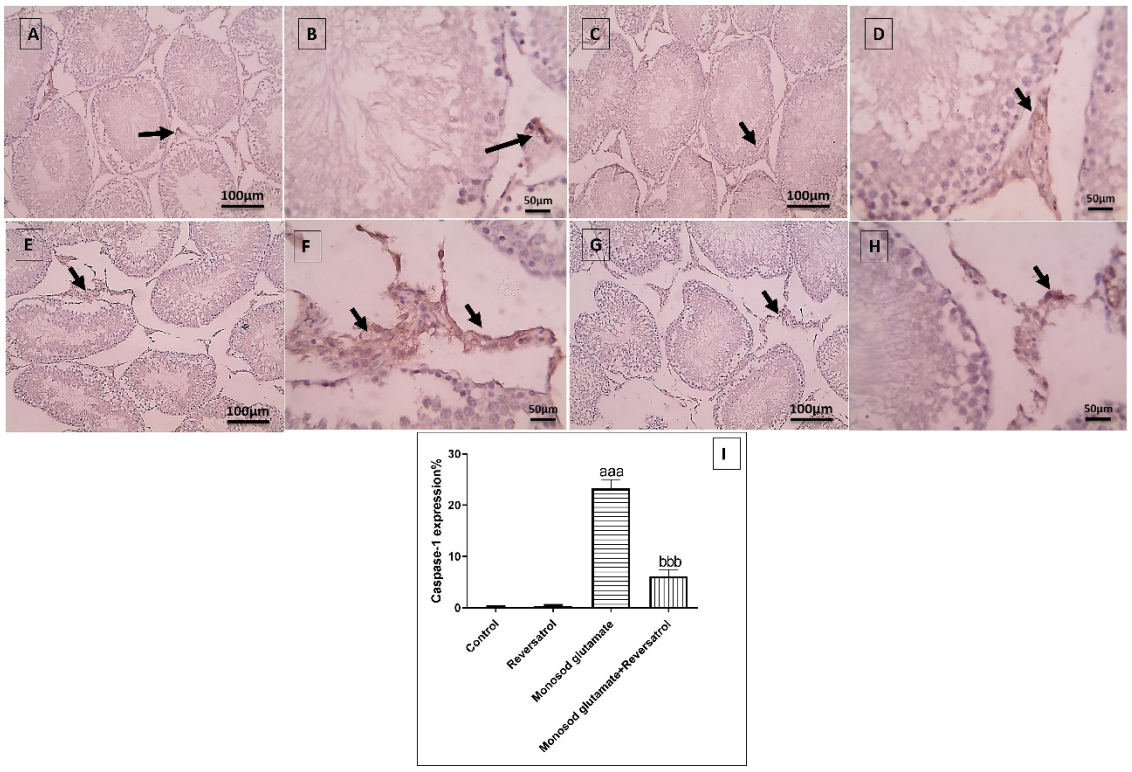

Figure 4. Microscopic images of immunostained testicular sections against IL-1β show a mild positive brown reaction appearing in the interstitial tissue (black arrows) in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit an increased positive brown reaction against IL-6 in the interstitial tissue (black arrows) (E, F). Conversely, testicular sections from the MSG + resveratrol group display a decreased positive brown reaction against IL-6 in the interstitial tissue (black arrows) (G, H). IHC counterstained with Mayer's hematoxylin. Percentage of IL-1β expression in immunostained testicular sections (I). All our data are presented as M ± SD. $^{aaa}p < 0.001$ vs control; $^{bbb}p < 0.001$ vs MSG. Low magnification ×: 100 and high magnification ×: 400 bar 50.

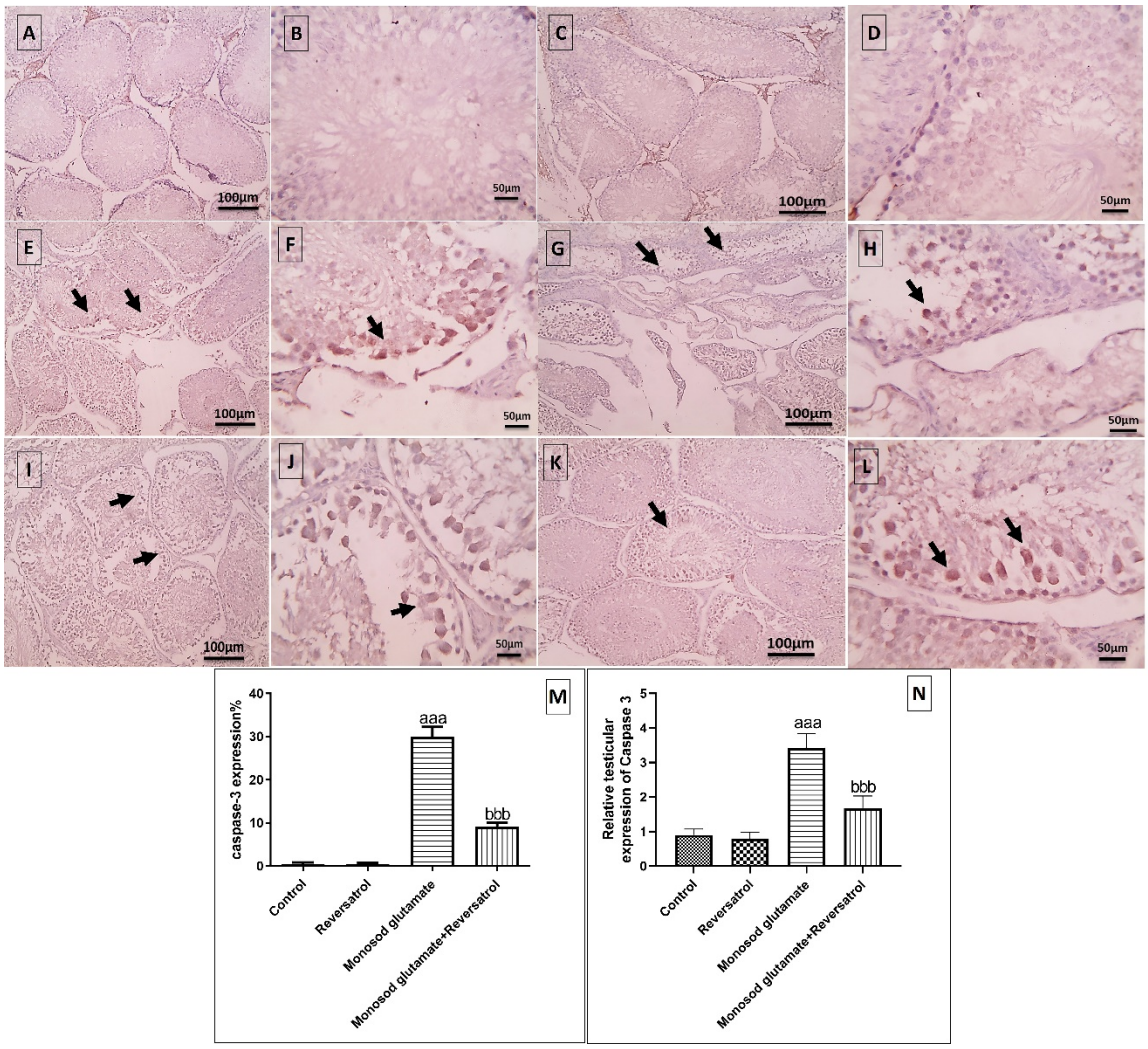

Figure 5. Microscopic images of immunostained testicular sections against caspase-3 show a negative tubular reaction in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit a prominent positive brown reaction against caspase-3, appearing in many cells lining numerous tubules (black arrows) (E–J). Conversely, testicular sections from the MSG + resveratrol group display a decreased positive brown reaction against caspase-3, observed in many cells lining very few tubules (black arrows) (K, L). IHC counterstained with Mayer's hematoxylin. Low magnification ×: 100 and high magnification ×: 400 bar 50. All our data are expressed as M ± SD. $^{aaa}p < 0.001$ vs. control; $^{bbb}p < 0.001$ vs MSG. Low magnification ×: 100 and high magnification ×: 400 bar 50. Relative gene expression of caspase-3 (N).

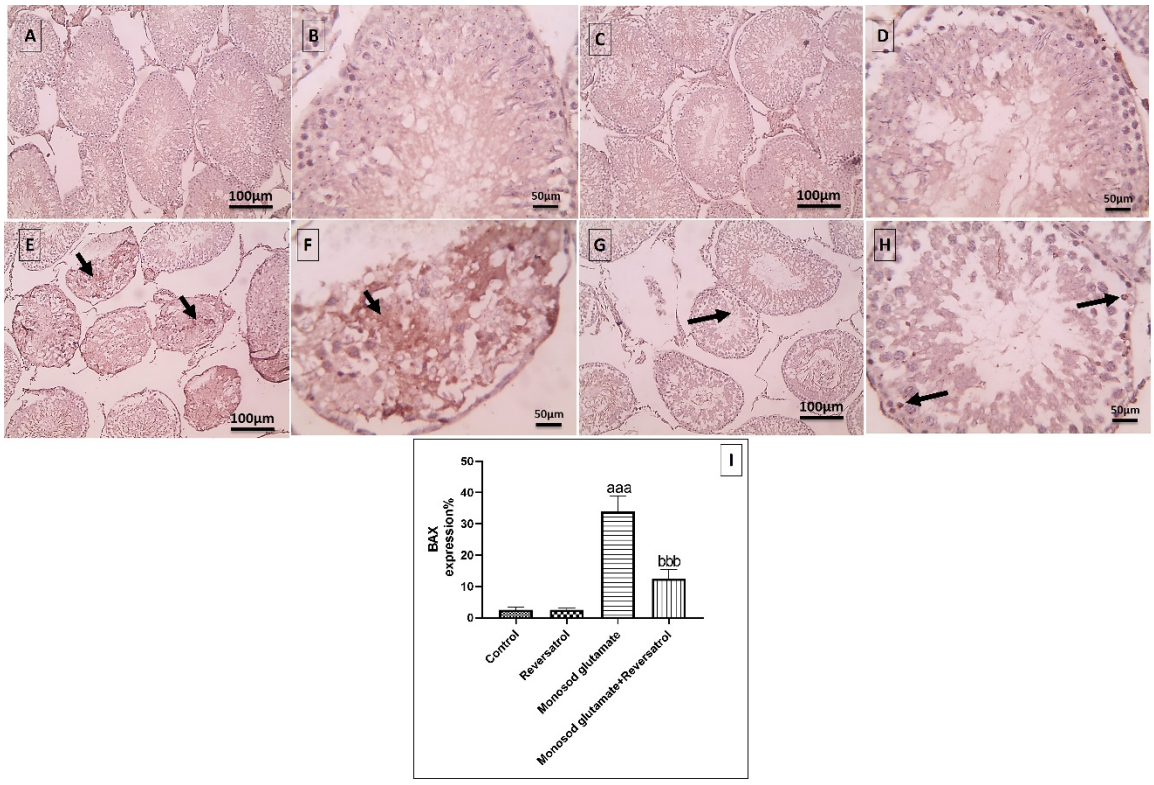

Figure 6. Microscopic images of immunostained testicular sections against Bax show a negative tubular reaction in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit a prominent positive brown reaction against Bax, observed in many cells lining numerous tubules (black arrows) (E, F). In contrast, testicular sections from the MSG + resveratrol group display a decreased positive brown reaction against Bax, appearing in few cells lining a few tubules (black arrows) (G, H). Our data is presented as M ± SD. $^{aaa}p < 0.001$ vs. control; $^{bbb}p < 0.001$ vs MSG. Low magnification ×: 100 and high magnification ×: 400 bar 50.

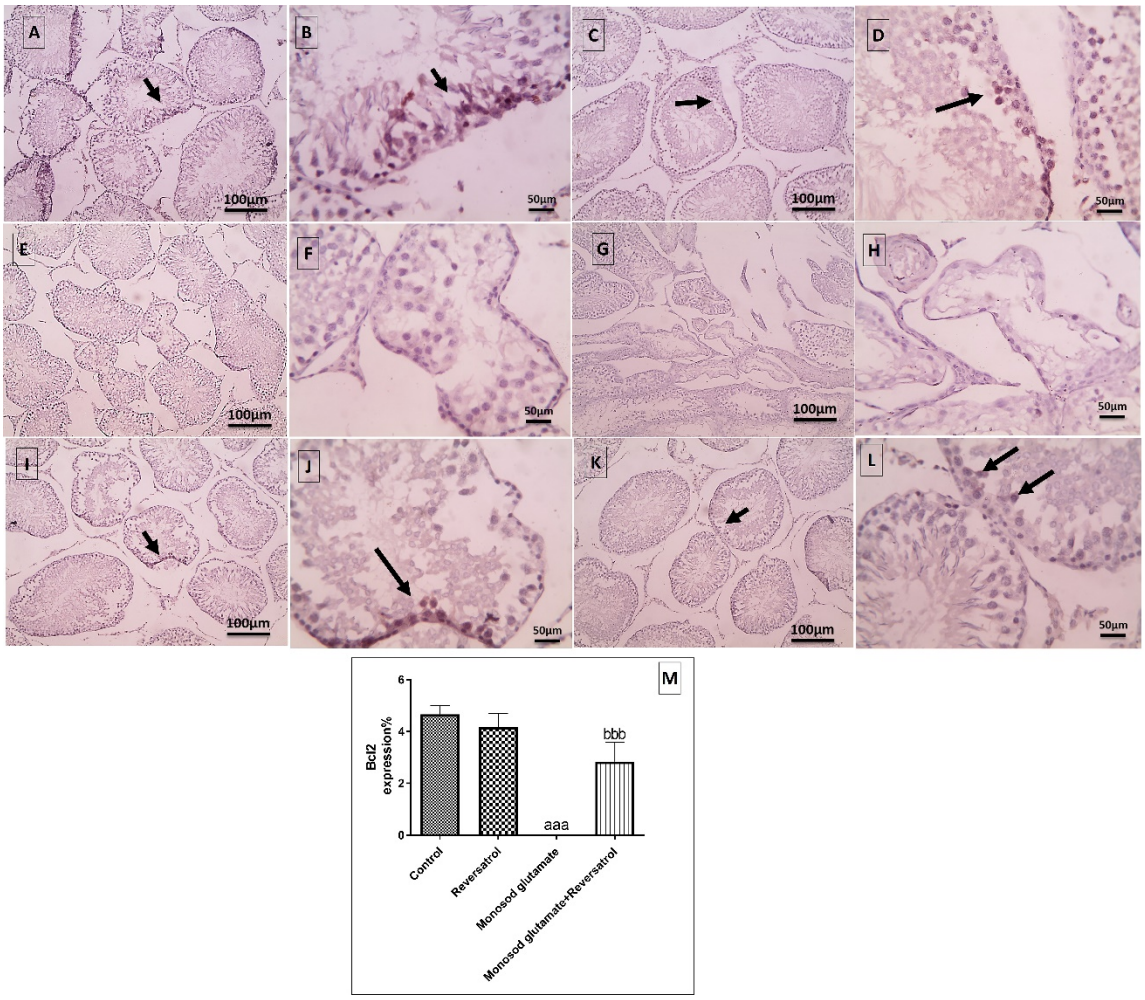

Figure 7. Microscopic images of immunostained testicular sections against Bcl2 show a mild positive brown reaction appearing in a few cells lining a few tubules (black arrows) in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit a negative tubular reaction against Bcl2 (E–H). Conversely, testicular sections from the MSG + resveratrol group display an increased positive brown reaction against Bcl2 (black arrows), observed in a few cells lining a few tubules (black arrows) (I–L). Our results are depicted as M ± SD. $^{aaa}p < 0.001$ vs control; $^{bbb}p < 0.001$ vs MSG. Low magnification ×: 100 and high magnification ×: 400 bar 50.

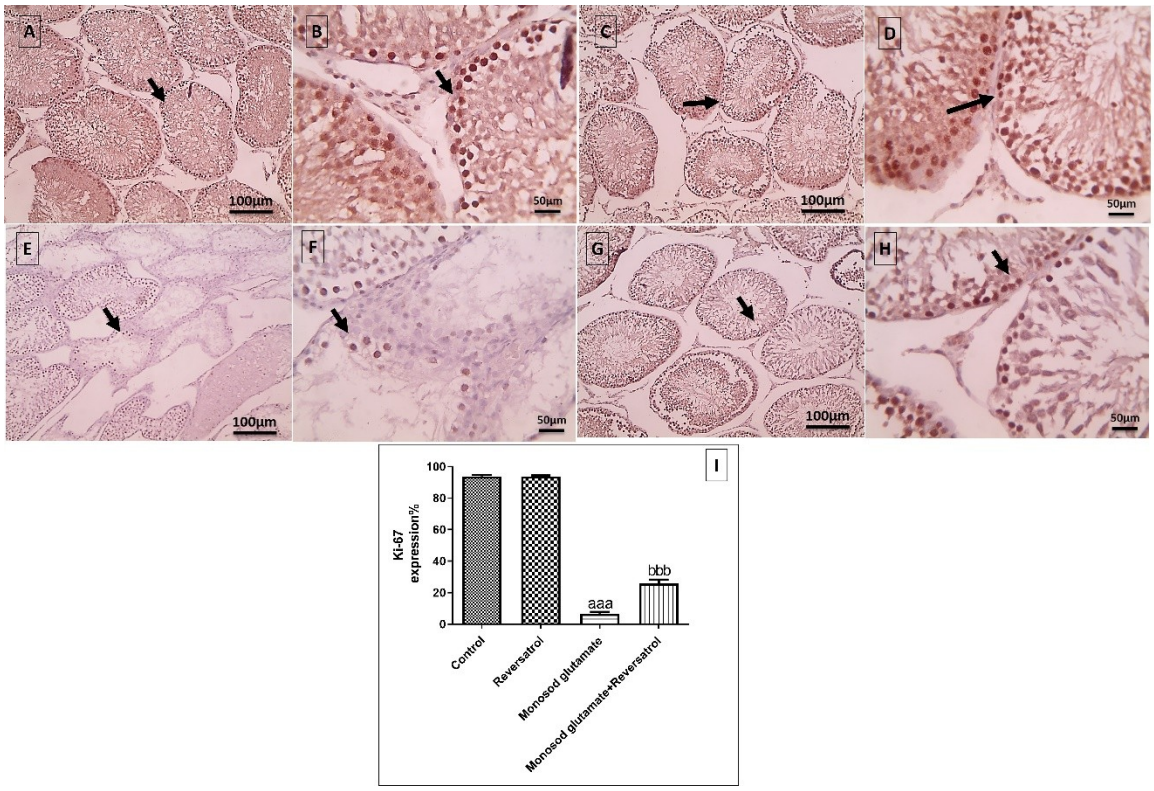

Figure 8. Microscopic images of immunostained testicular sections against Ki-67 show a strong positive brown reaction appearing in many cells lining all tubules (black arrows) in the control and resveratrol groups (A–D). Testicular sections from the MSG group exhibit a mild positive brown reaction against Ki-67, observed in few cells lining most tubules (black arrows) (E, F). Conversely, testicular sections from the MSG + resveratrol group display an increased positive brown reaction against Ki-67 (black arrows), observed in a higher number of cells lining most tubules than in the + ve group (black arrows) (G, H). Our data is presented as M ± SD. $^{aaa}p < 0.001$ vs control; $^{bbb}p < 0.001$ vs MSG. Low magnification ×: 100 and high magnification ×: 400 bar 50.