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

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Background: *Monosodium glutamate is a food additive and flavour enhancer in processed foods and soups that is considered to affect testicular histology. The aim of this research was to investigate the impact of monosodium glutamate (MSG) on testicular structure in rats and explore the potentially protective effects of resveratrol.*

Materials and methods: *Four experimental groups involved in our study contained 10 rats in each. The first group was a control group; in the second group (the resveratrol group) control rats received 20 mg/kg of resveratrol via oral gavage; in the third group (the MSG group) rats received monosodium glutamate (MSG) at a dose of 60 mg/kg body weight daily, via a gastric tube. The fourth group we called the MSG + resveratrol group. Serum levels of testosterone, FSH, and 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 smears 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 an elevated level of MDA, a lipid peroxidation marker, and decreases in SOD and CAT, two antioxidant enzymes. Moreover, MSG induced apoptotic and pyroptotic markers and its gene expressions. Importantly, the administration of resveratrol reversed*

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these detrimental effects of MSG, demonstrating its corrective influence on hypothalamic–pituitary–gonadal axis disruption, the improvement of sperm parameters, the attenuation of oxidative stress, and 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 article sheds light on the protective effect of resveratrol against MSG-induced testicular damage, with an exploration of its mechanistic role.* (Folia Morphol 2025; 84, 1: 151–166)

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

INTRODUCTION

Monosodium glutamate (MSG), a sodium salt of L-glutamic acid, is a widely prevalent food additive utilised for its preservative properties and enhancement of meal palatability [26]. A 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].

A natural polyphenolic component, resveratrol (3,4',5-trihydroxy-trans-stilbene), can be found in a variety of fruit and vegetables, such as berries, peanuts, and grapes. It has garnered increasing attention for its reported multifaceted benefits, encompassing anti-inflammatory, antidiabetic, and anticancer properties, and cardiovascular protection. Additionally, resveratrol is associated with enhanced stress resistance, extended lifespan, and preventive effects against various diseases such as cancer, ischaemic 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 oestradiol, resveratrol is postulated to potentially play a comparable role in testicular function. Resveratrol application *in vivo* has demonstrated effectiveness in treating infertility, particularly in cases of dyszoospermia, where it has mitigated the impact induced by 2,5-hexanedione on spermatogenesis [31]. It has shown promise in improving sperm quality, probably facilitated by its ability to traverse the blood–testis barrier and to confer protective effects on 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 recognised 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.

Our investigation was 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 male Wistar rats weighing between 120 and 150 grams were bought from Zagazig University's Faculty of Veterinary Medicine. After a seven-day acclimatisation 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 that, the animals were categorised into four groups, each consisting of ten rats.

Pharmaceutical interventions

Under licence 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 1,000 mL 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 a suspension, and given to the animals immediately. Resveratrol (purity ≥ 99%) and monosodium glutamate were obtained from Sigma Aldrich (St. Louis, MO, USA).

Experimental design

The rats were allocated into four groups of 10 each in a random manner: Group 1 was the control group; Group 2 was the resveratrol group (control rats receiving resveratrol at a dosage of 20 mg/kg daily via oral gavage for four weeks); and Group 3 was the MSG group (rats administered MSG at a dose of 60 mg/kg body weight daily via gastric tube for four weeks). Additionally, there was an MSG + resveratrol group (Group 4) where rats received both MSG (60 mg/kg body weight) and resveratrol (20 mg/kg via oral gavage). The doses of resveratrol and monosodium glutamate were according to previous studies [2, 26].

Sample collection

After a 4-week treatment period, animals underwent a 24-hour fasting period before sacrifice. Anaesthesia using ether was administered. Heparinised 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 1,500 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 homogenised at a 1:10 dilution in potassium phosphate buffer (0.1 M, pH 6.5). A portion of the homogenate was utilised 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, euthanisation, and biological tissue sample management followed established biosafety protocols [5]. The disposal of deceased and euthanised 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 homogenised 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 5,000 rpm for 20 minutes at 4°C. This supernatant was used 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, analyses were carried out in compliance with the manufacturer's methodology as 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 millilitre of 40% formaldehyde and 5 g of sodium bicarbonate ($NaHCO_3$) were dissolved in 100 ml 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 haemocytometer 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 haemocytometer. 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 haematoxylin 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 haematoxylin and eosin. Next, the percentages of aberrant sperm among the 200 spermatozoa on each slide were assessed [20].

Testicular histological staining

The haematoxylin and eosin (H&E) staining protocol for fresh frozen sections was done following the guidelines outlined by the Centre of Musculoskeletal Research at the University of Rochester, USA. The frozen tissue slides were subjected to fixation in a cold 10% neutral buffered formalin solution for 10 minutes. Subsequently, a triple rinsing step with 1X PBS, each lasting three 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. Haematoxylin staining ensued for precisely 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 one 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 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 then 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 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 10 minutes, streptavidin-horseradish peroxidase enzyme conjugates were administered. 3,3-diaminobenzoic acid (DAB) was used to visualise the conjugated secondary antibody sites, and PBS was then used to wash the samples. Haematoxylin 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.

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 is expressed as M ± SD. $^{aaa}p < 0.001$ vs. control; $^{bbb}p < 0.001$ vs. MSG; CAT — catalase; FSH — follicle-stimulating hormone; LH — luteinising hormone; MDA — malondialdehyde; MSG — monosodium glutamate; SOD — superoxide dismutase.

Determination of testosterone, follicle stimulating hormone (FSH), and luteinising 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), luteinising hormone (LH), and testosterone serum levels were evaluated using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The assays were carried out following the manufacturer's instructions (MyBioSource, San Diego, CA, 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, USA) 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 were 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 normalised 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 group that received resveratrol showed a significant increase in the serum levels of hormones (Tab. 1). Regarding this finding, we have 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 (Tab. 1). From the above, we can conclude that resveratrol revealed potent antioxidant capacity against MSG-induced oxidative insult.

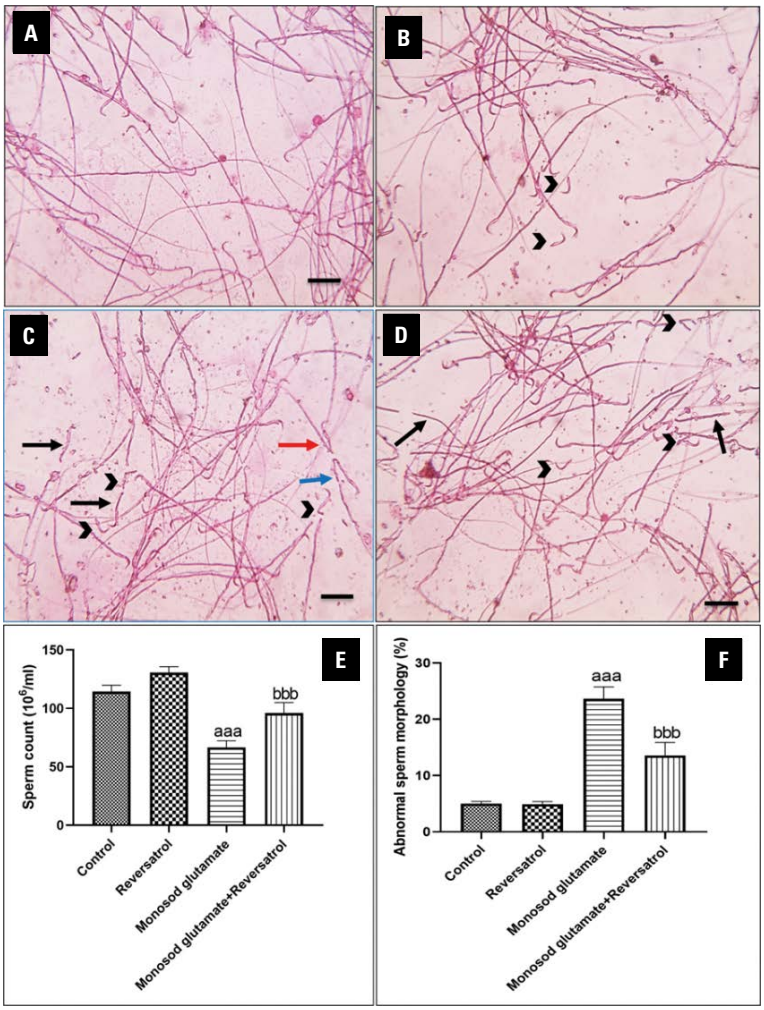

Figure 1. Microscopic images of epididymal smears in control group taken at 400× magnification using a light microscope and showing typical sperm morphology, including head, body, and tail. Smears from resveratrol group exhibit a few detached heads (black arrowheads). However, smear from MSG group reveals 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). Smear from 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. M — mean; MSG — monosodium glutamate; SD — standard deviation.

Investigating 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 dwarf headless sperms (Fig. 1D). This result was confirmed by statistical analysis of the percentage of abnormal sperm morphology, which showed a very 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 (Fig 1E).

Resveratrol's ameliorative impact on MSG-induced testicular damage

Haematoxylin and eosin examination of testicular sections in control and resveratrol rat groups revealed normal morphology, characterised by apparent

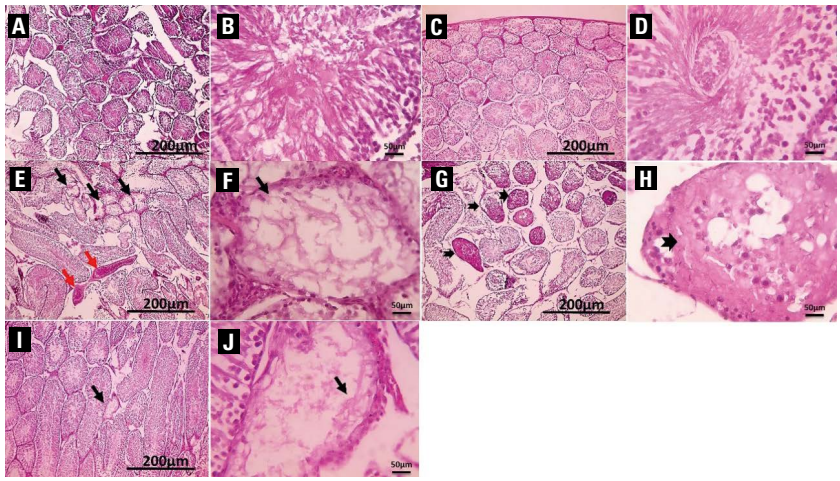

Figure 2. Microscopic images of H&E-stained testicular sections from control and resveratrol groups reveal normal features of seminiferous tubules, with several layers of spermatogonia, primary (1ry), and secondary (2ry) spermatocytes. Normal spermatogenesis is characterised by a full lumen with spermatozoa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

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 four 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, N, 6E, F), 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, N, 6G, H). 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

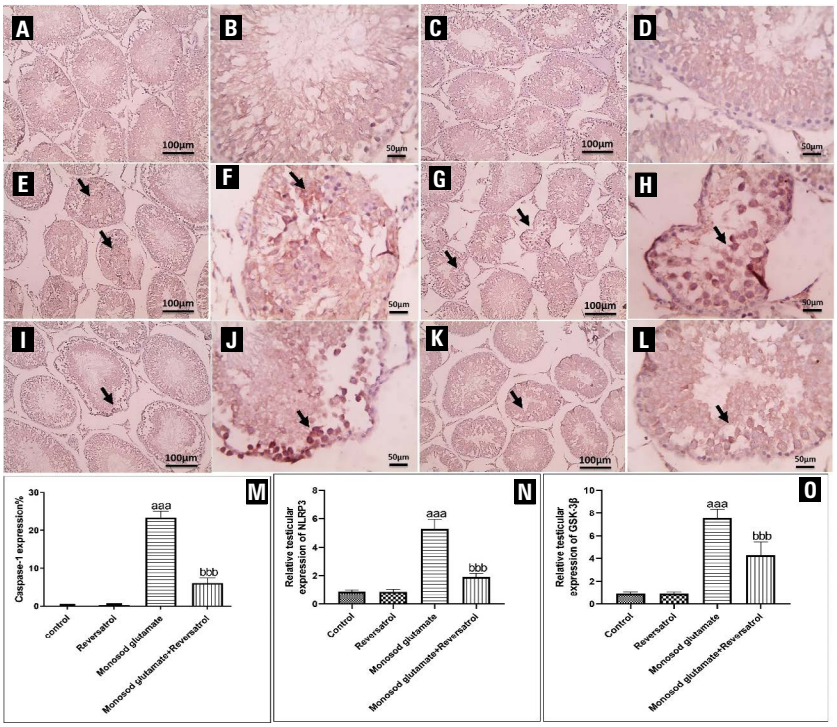

Figure 3. Microscopic images of immunostained testicular sections against caspase-1 show a negative tubular reaction in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit a prominent positive brown reaction against caspase-1, appearing in many cells lining numerous tubules (black arrows) (**E–J**). In contrast, testicular sections from 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 haematoxylin. Low magnification: 100× and high magnification: 400× bar 50. Bars represent means ± SE, demonstrating percentage of caspase-1 expression in immunostained testicular sections, analysed 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
IHC — immunohistochemistry; MSG — monosodium glutamate; SE — standard error.

the strong proliferative effect of resveratrol on spermatogonia.

DISCUSSION

The testes perform dual primary functions: [26] the synthesis of the male sex hormone testosterone and [44] the generation of spermatozoa, the male gametes [34]. In our investigation, the potentially toxic impact of monosodium glutamate (MSG) was assessed and we explored the potentially protective influence of resveratrol on the structural and functional aspects of the testes. Monosodium glutamate (MSG), commonly employed as a flavour enhancer, is recognised 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 scrutinises 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 fruits and vegetables such as grapes, berries, and peanuts, is renowned for its antioxidant attributes [16]. Extensive research has focused on the antiageing characteristics of resveratrol and its potential in preventing age-related illnesses such as 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

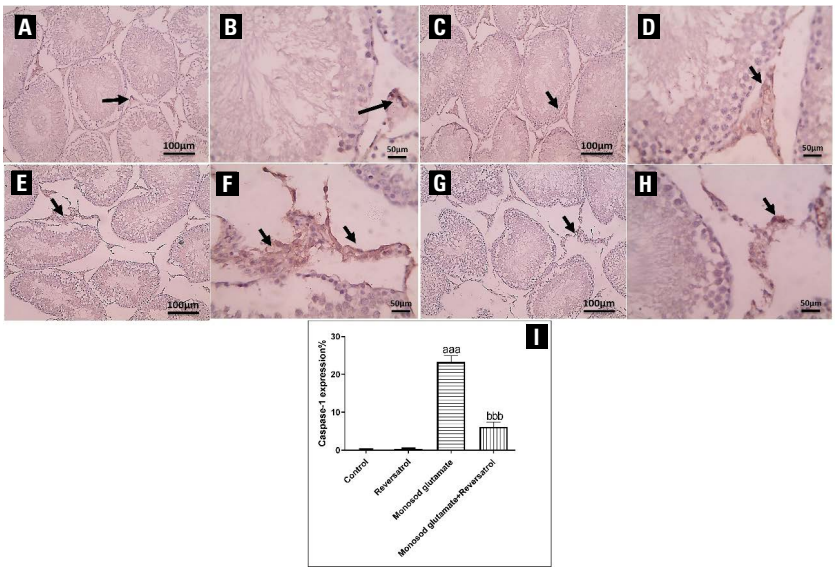

Figure 4. Microscopic images of immunostained testicular sections against IL-1β show a mild positive brown reaction appearing in interstitial tissue (black arrows) in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit an increased positive brown reaction against IL-6 in interstitial tissue (black arrows) (**E, F**). Conversely, testicular sections from MSG + resveratrol group display a decreased positive brown reaction against IL-6 in interstitial tissue (black arrows) (**G, H**). IHC counterstained with Mayer's haematoxylin. Percentage of IL-1β expression in immunostained testicular sections (**I**). All 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. IHC — immunohistochemistry; M — mean; MSG — monosodium glutamate; SD — standard deviation.

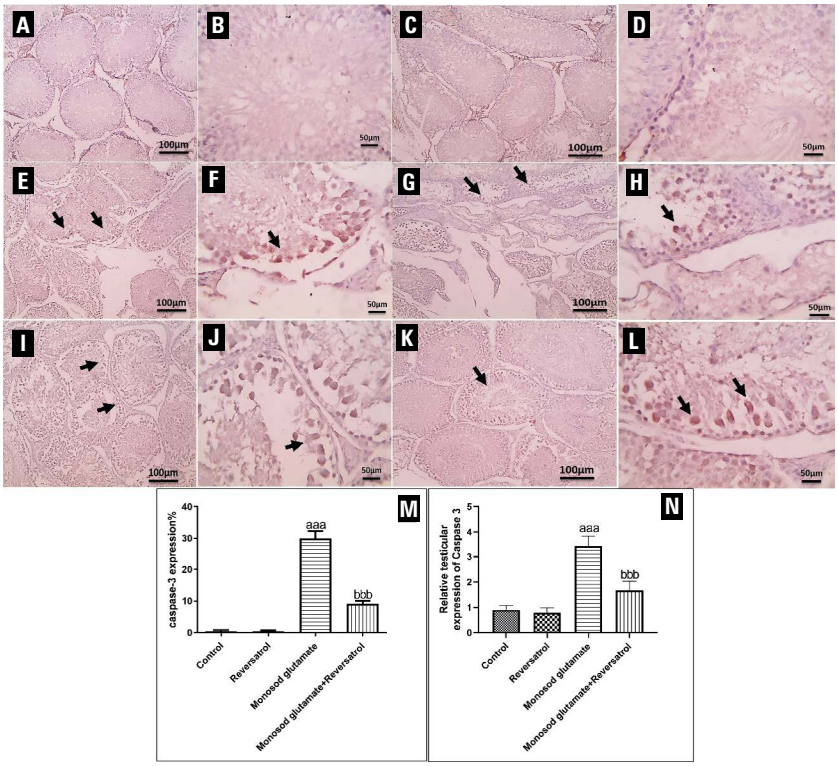

Figure 5. Microscopic images of immunostained testicular sections against caspase-3 show a negative tubular reaction in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit a prominent positive brown reaction against caspase-3, appearing in many cells lining numerous tubules (black arrows) (**E–J**). Conversely, testicular sections from 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 haematoxylin. Low magnification: 100× and high magnification: 400× bar 50. All our data is 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**). IHC — immunohistochemistry; M — mean; MSG — monosodium glutamate; SD — standard deviation.

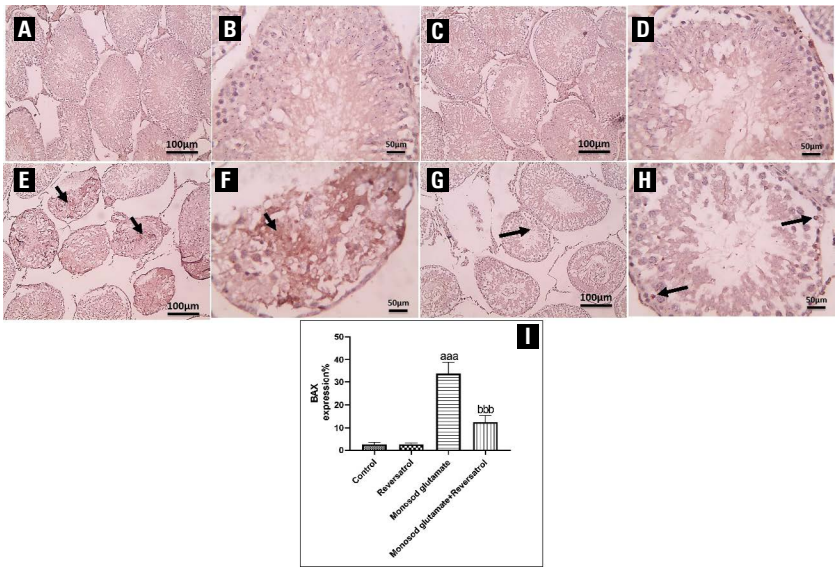

Figure 6. Microscopic images of immunostained testicular sections against Bax show a negative tubular reaction in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit a prominent positive brown reaction against Bax, observed in many cells lining numerous tubules (black arrows) (**E, F**). In contrast, testicular sections from MSG + resveratrol group display a decreased positive brown reaction against Bax, appearing in a 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. M — mean; MSG — monosodium glutamate; SD — standard deviation.

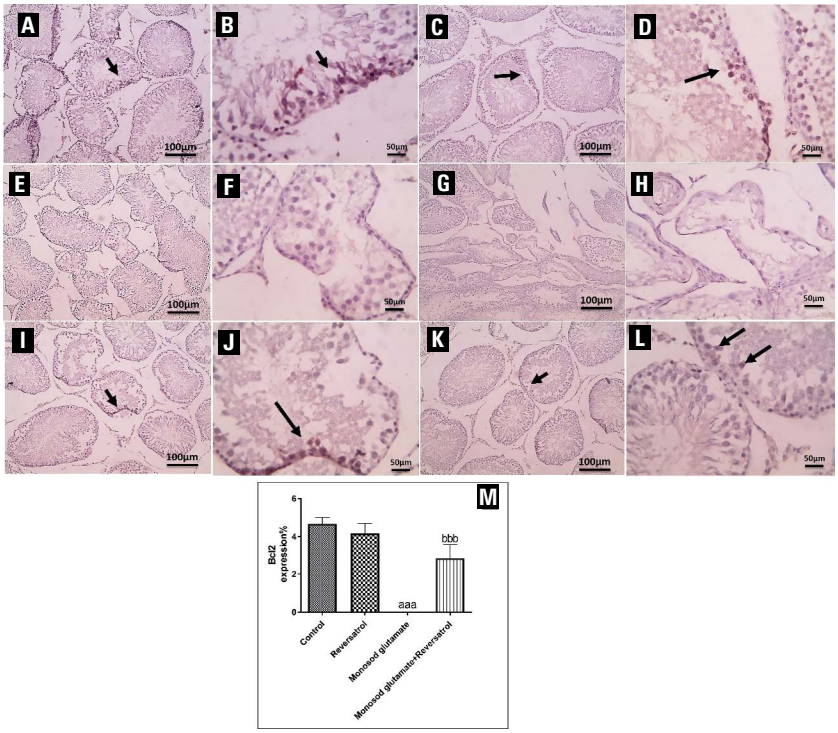

Figure 7. Microscopic images of immunostained testicular sections against Bcl2 show a mild positive brown reaction appearing in a few cells lining a few tubules (black arrows) in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit a negative tubular reaction against Bcl2 (**E–H**). Conversely, testicular sections from 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. M — mean; MSG — monosodium glutamate; SD — standard deviation.

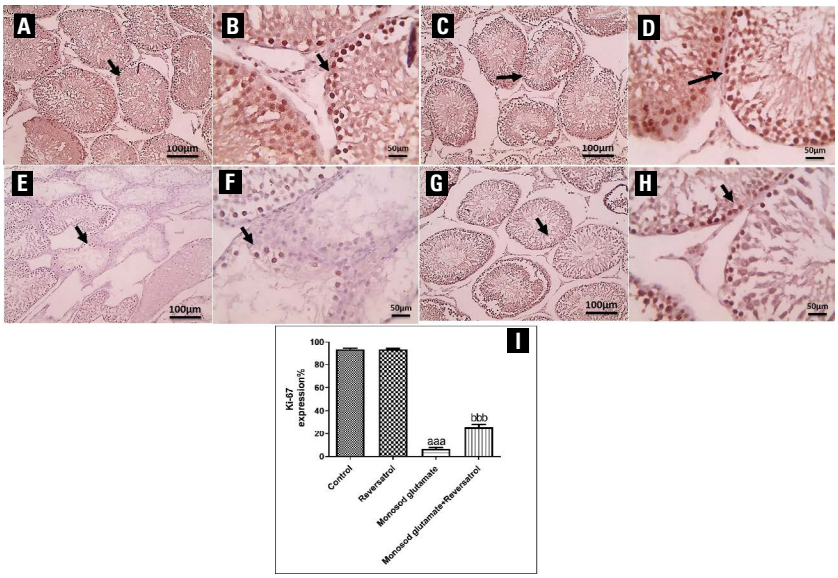

Figure 8. Microscopic images of immunostained testicular sections against Ki-67 show a strong positive brown reaction appearing in many cells lining all tubules (black arrows) in control and resveratrol groups (**A–D**). Testicular sections from MSG group exhibit a mild positive brown reaction against Ki-67, observed in few cells lining most tubules (black arrows) (**E, F**). Conversely, testicular sections from 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 + 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. M — mean; MSG — monosodium glutamate; SD — standard deviation.

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 upon 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), and 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, thereby supporting [4] a study which explained the inhibitory effect of MSG on testosterone production. Resveratrol administration successfully alleviated testicular degeneration, as reported in [8].

The 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 unfavourable interactions with other hormonal axes [54]. Our 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, thereby 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 our 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 [7, 57] which have demonstrated the anti-apoptotic effect of resveratrol in alleviating diabetes-induced testicular dysfunction. Our 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].

Our study presents an 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, and cadmium-induced testicular damage, these pathways are emerging 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]. Our 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, our 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 mildly positive expression, the MSG + resveratrol group displayed strongly positive expression. The heightened positive expression levels of PCNA and Ki-67 indicated a favourable response to spermatogenesis dysfunction [58]. However, in this work we did not perform electron microscopic examination of semen to discover the ultrastructural abnormalities in the sperm heads, acrosomal and plasma membranes, middle piece, and sperm tails, nor electron microscopic examination of testicular specimens to explore the ultrastructural changes in the different lining cells of seminiferous tubules such as spermatogenic cells, primary spermatocyte and spermatids. We acknowledge that this can be considered a limitation of our study.

CONCLUSIONS

Our 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 underline the complexity of its influence on male reproductive health. Our findings indicate that MSG administration leads to disruptions in hormonal balance, compromised sperm quality, testicular oxidative stress, and the activation of testicular apoptotic and pyroptotic pathways. These outcomes align with some existing literature and also 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.

Our study thus not only contributes to expanding the understanding of the reproductive consequences of MSG, but also introduces a novel perspective by unravelling the involvement of pyroptosis in testicular damage. The potentially therapeutic implications of resveratrol in alleviating MSG-induced reproductive impairments underline its significance as a candidate for further exploration and consideration in the context of male reproductive health.

Ultimately, our findings should encourage continued research to elucidate the intricate mechanisms underlying MSG-induced reproductive toxicity and to explore additional avenues for therapeutic intervention. We encourage the extension of our study to human subjects in order to establish our outcomes and to assess the remedial ability of resveratrol in patients suffering from the complication of infertility associated with monosodium glutamate's administration as a food additive delivered in large doses.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The data that supports 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 was approved by the Committee of Research Ethics, Kafr Elsheikh University (KFS-IACUC/183/2024).

Author contributions

MT: conceptualization, methodology, writing and original draft; LSA: methodology, writing — review and editing; MEI-N: supervision, visualization and methodolgy; MMI: writing — review and editing; AMB: conceptualization; AEF: investigation; HSAI: methodology; RAZ: methodology; EH: investigation.

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Conflict of interest

The authors declare no conflict of interest.

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