RESEARCH ARTICLE

Effects of resveratrol on hydrogen peroxide-pyroptosis, apoptosis, and autophagy

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Oxidative stress is one of the main causes of testicular spermatogenesis disorders. Resveratrol (RES) can inhibit oxidative stress levels, reduce germ cell damage, and promote spermatogenesis. To investigate the molecular mechanism by which RES promotes spermatogenesis, the effects of hydrogen peroxide (H_2O_2) on the pathological damage of mouse testis tissue were assessed. The expression of key genes associated with pyroptosis, apoptosis, and autophagy, and the effects of RES on this process were determined. Experimental mice were divided into three groups with intraperitoneally injection of H_2O_2 (3 mmol/kg/d), RES (100 mg/kg/d) + H_2O_2 (3 mmol/kg/d), and normal saline, respectively. The testicular tissues were collected, and the pathological changes were observed by hematoxylin and eosin staining. The ultrastructure was observed using transmission electron microscopy (TEM). The transcriptional levels of pyroptosis, apoptosis, and autophagy-related genes were measured and analyzed by fluorescence quantitative polymerase chain reaction. The results showed that, in the H_2O_2 group, the basal membrane of spermatogenic tubules was damaged, atrophic, and thin. Interstitial cells were fragmented, and the gap of spermatogenic tubules increased. Compared with the H_2O_2 group, the pathological damage of testicular tissue structure in the H_2O_2 + RES group was significantly ameliorated. TEM results showed no evident damage to the cell structure in the control group, while apoptosis, autophagy, and pyroptosis were observed in the testicular tissue of H_2O_2 and H_2O_2 + RES groups, where cell damage was less, but apoptotic bodies, autophagosomes, and pyroptosomes were observed. The transcription levels of key pyroptosis genes increased in the H_2O_2 group but decreased significantly after RES treatment. The results indicated that H_2O_2 could induce pyroptosis, while RES inhibited pyroptosis and led to the release of inflammatory factors by regulating the expression of genes in signaling pathways. After H_2O_2 injection, the transcript levels of key apoptosis genes in testicular tissue were lower than those in the control group, while RES promoted the occurrence of H_2O_2-induced apoptosis, to achieve the purpose of protecting itself from oxidative damage. H_2O_2 could promote autophagy in mouse testicular tissue. RES enhanced the level of H_2O_2-induced autophagy, removed the damaged cells in the testicular tissue, and protected the development of the body. This study provided the experimental basis for the study of RES in oxidative damage, pyroptosis, apoptosis, and autophagy, and theoretical guidance and new ideas for drug development and treatment of male reproductive diseases in clinical practice.

Keywords: resveratrol; testes; pyroptosis; autophagy; apoptosis.

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Introduction

Hydrogen peroxide (H_2O_2) is a strong oxidant and an ideal inducer in the oxidative stress injury model. At a certain concentration, H_2O_2 can cause different degrees of oxidative damage to animal cells, and oxidative damage can cause serious spermatogenesis disorders in male animals [1]. This leads to pathological changes in animal testis tissue, a decline in the testicular index and sperm count, and an increase in sperm malformation rate, resulting in infertility of male animals [2]. Resveratrol (RES) is a very common natural polyphenolic antitoxin of plant origin. Since its discovery, RES has been detected in more than 700 kinds of substances with very high contents in peanuts, grapes, and polygonum cuspidatum [3]. RES has anti-cancer, anti-inflammatory, antibacterial, immune regulatory, and other beneficial biological effects [4, 5]. In terms of anti-oxidation, RES can effectively eliminate reactive oxygen species (ROS) from cells [6].

As a strong oxidant, H_2O_2 plays a significant role in destroying normal mitochondrial function and damaging DNA to induce oxidative stress [7]. It can lead to an increase in ROS content in the body, thereby initiating the caspase-mediated apoptotic pathway [8]. RES can promote the recovery of radiation-induced mice testicular spermatogenic function, and its mechanism may be related to the body's own recovery, the rebalancing of the testicular microenvironment, and the antioxidant effect of RES [9]. RES can effectively inhibit H_2O_2-induced cellular lipid peroxidation and increase testosterone content [10]. RES can also act on the development of male reproductive organs and improve sperm quality by inhibiting oxidative stress. Moreover, RES intervention can increase sperm motility and density, reduce sperm malformation rate, and decrease sperm DNA damage [11]. H_2O_2 has been shown to induce pyroptosis [12, 13], apoptosis [14, 15], and autophagy [8] in the *in vitro* oxidative stress model. After H_2O_2 treatment of placental trophoblast cells, the levels of ROS and malondialdehyde (MDA) increased, the activities of superoxide dismutase (SOD) and catalase (CAT) decreased, and the expression of caspase-1, interleukin (IL)-1β, light-chain-3 (LC3), and Beclin-1 proteins increased. However, RES (50 μmol/L) treatment for 8 h could ameliorate H_2O_2-induced injury, increase cell survival rate, reduce lactate dehydrogenase (LDH) release, decrease the level of MDA, increase SOD and CAT levels, and gown-regulate the expression of caspase-1, IL-1β, LC3, and Beclin-1 levels [16]. RES can inhibit autophagy and apoptosis of H_2O_2-induced cardiomyocytes [17]. As an autophagy specific substrate, p62 can interact with LC3 and infiltrate into autophagosomes, effectively degrading them through autophagosomes. Therefore, changes in the expression level of p62 can also be used to monitor changes in autophagy. Du *et al.* showed that RES could enhance autophagy by regulating the expression levels of retinoblastoma protein, LC3, and Sequestosome 1 (SQSTM1/p62), thereby protecting cells from H_2O_2-mediated damage [18]. Studies on the effects of RES on venous thrombosis showed that H_2O_2 could significantly reduce cell viability and increase apoptosis and ROS production, while RES alleviated this damage caused by H_2O_2 in a dose-dependent manner [19].

Currently, the mechanism of RES on H_2O_2-induced testicular injury and spermatogenesis has not yet been elucidated. In this study, mice were intraperitoneally injected with H_2O_2, and then RES was used for reparative treatment. Hematoxylin and eosin staining, fluorescence quantitative polymerase chain reaction (PCR), and transmission electron microscopy (TEM) were used to detect the pathological damage, the transcription levels of key genes, and ultrastructural changes in testicular tissue. The effects of H_2O_2 and RES on testicular histology, pyroptosis, apoptosis, and autophagy were explored in this study, and the relationship between morphological changes and three cell death modes were analyzed with an aim to further understand the effects of RES on different cell death modes, providing a theoretical basis for research on male reproductive development,

and laying a scientific foundation for drug development and application.

Materials and methods

Feeding and handling of animals

Thirty Sprague Dawley (SD), specific pathogen-free, male mice aged 23-25 days were obtained from Huaxing Laboratory Animal Farm (Zhengzhou, Henan, China), and were randomly categorized into three groups including H_2O_2, RES + H_2O_2, and control groups with 10 mice in each group. The mice in corresponding group were intraperitoneally injected with H_2O_2 (3 mmol/kg/d), RES (100 mg/kg/d) + H_2O_2 (3 mmol/kg/d), and normal saline, respectively, once every 3 days. After seven injections, the mice were sacrificed by neck dislocation, and the testicles were removed and weighed. One of the testicles was quickly preserved in liquid nitrogen, while the other one was fixed in formalin. All procedures were approved by the Institutional Animal Care and Use Committee (Huanghuai University, Zhumadian, Henan, China).

Histopathological observation of the testis

The tissue samples were paraffin-embedded and sectioned. Specifically, the testis was removed from the fixing solution, dehydrated in different concentrations of ethanol at room temperature, treated with the clarifier for transparent processing, and then dipped in soft wax at 62℃ for 30 mins followed by hard wax at 62℃ for 30 mins. The wax-dipped testis was embedded in hard wax at 62 - 65℃. After cooling down appropriately, the wax block was sliced into sections of 5 µm thickness using HistoCore BIOCUT microtome (Leica Biosystem, Deer Park, IL, USA). The sections were unfolded in a water bath at 40-46℃, then baked at 37℃ for 2 h before stored in a refrigerator at 4°C. After conventional HE staining, the sections were dehydrated with gradient alcohol, sealed with neutral gum, and observed and photographed under a microscope.

Observation of testicular tissue ultrastructure

A small piece of testicular tissue was dissected and placed on clean cardboard with a drop of cooled 2.5% glutaraldehyde fixing solution. The sample was sliced into small pieces about 1 mm wide and 2-3 mm long using a new, grease-free sharp blade. The sample was processed as quickly and accurately as possible. These small sample pieces were placed into glutaraldehyde fixation solution at 4℃ one-at-a-time, and then sent to Wuhan Servicebio Biotechnology Co., Ltd. (Wuhan, Hubei, China) for ultrastructural analysis.

Primer designing

The sequences of key genes for necroptosis including NOD-like receptor protein (NLRP3), gasdermin D, (GSDMD), caspase-1, IL-18, IL-1β, for apoptosis including caspase-3, caspase-8, Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), for autophagy including Beclin-1, autophagy-related 16-like 1 (ATG16L1), unc-51-like kinase 1 (ULK1), LC3, and the reference gene (β-actin) were downloaded from National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). The primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and sent to Sangon Bioengineering (Shanghai) Co., LTD. (Shanghai, China) for synthesis. The primer sequences were listed in Table 1.

Fluorescent quantitation polymerase chain reaction (PCR)

Total RNAs were extracted from testicular tissue using Trizol (Takara, Beijing, China) method and reverse transcribed into cDNA according to the instructions provided in the kit for reverse transcription. Briefly, 10 µL of TB Green Premix Ex Taq (Tli RNaseH Plus) (2X) (Takara, Beijing, China) was mixed with 0.4 µL of each 10 µM forward and reverse primers and 1,000 ng cDNA template. Sterilized water was used to make the final volume of 20 µL. The PCR was performed using LightCycler 480 System (Bio-Rad Laboratories, Hercules, California, USA) with the reaction program as pre-denaturation 95℃ for 30 s at 20℃/s, followed by 40 cycles of 95℃ for 5 s at

Table1. Primer sequences.

Gene	GenBank ID	Primer Sequences (5'→3')
β-actin	V01217.1	F: 5'-AGAAGCTGTGCTATGTTGGCATA-3' R: 5'- AGACAGCACTGTGTTGGCATA-3'
Beclin-1	XM_048197769.1	F: 5'- TTGTTGCTCCATGCTTTGGC-3' R: 5'-AGGGACTCCAGATACGAGTGA-3'
ATG16L1	XM_032901570.1	F: 5'-CATTGACCTGCGGACAAACG-3' R: 5'-GTGCTGCTTGGAGAGAACCT-3'
ULK1	XM_034488884.1	F: 5'-ATGCGCACACTGAGTGAAGA-3' R: 5'-CATAGGAGAGCCACAGAGCG-3'
LC3	XM_021193888.2	F: 5'-CGACCAGCACCCCAGTAAGA-3' R: 5'-TCTGGGACCAGAAACTTGGTCT-3'
Caspase-3	XM_030243266.1	F: 5'-TGGCTTGCCAGAAGATACCG-3' R: 5'-TCGAATTCCGTTGCCACCTT-3'
Bax	NM_017059.2	F: 5'-TGGCGATGAACTGGACAACA-3' R: 5'-CACGGAAGAAGACCTCTCGG-3'
Bcl-2	NM_016993.2	F: 5'-GGGGCTACGAGTGGGATACT-3' R: 5'-GACGGTAGCGACGAGAGAAG-3'
Caspase-8	AH006519.2	F: 5'-TGAGCCTCAAAATGGCGGAA-3' R: 5'-TTATGTCTTCCCGGGCCTTG-3'
NLRP3	XM_006246457.4	F: 5'-GGTCAGCTGCTGTCTCACAT-3' R: 5'-GGTCAGCTGCTGTCTCACAT-3'
IL-1β	NM_008361.4	F: 5'-ATCTCGCAGCAGCACATCAA-3' R: 5'-ACGGGAAAGACACAGGTAGC-3'
IL-18	NM_008360.2	F: 5'-TCAGACAACTTTGGCCGACT-3' R: 5'-TCAGTCTGGTCTGGGGTTCA-3'
Caspase-1	NM_009807.2	F: 5'-CGAGGGTTGGAGCTCAAGTT-3' R: 5'-TCCTTGTTTCTCTCCACGGC-3'
GSDMD	AB103383.1	F: 5'-GCGTGTGACTCAGAAGACCT-3' R: 5'-ACCTCGGTCACCACAAACAG-3'

20℃/s, 60 ℃ for 20 s at 20℃/s, 95℃ for 0 s at 20℃/s, Additional 65℃ for 15 s at 20℃/s and 95℃ for 0 s at 0.1℃/s was performed at the end of the program. The $2^{-\Delta\Delta CT}$ formula was used to calculate the relative expression of genes. The exogenous gene, β-actin, was used to normalize the qPCR data.

Statistics analysis

SPSS software (version 21.0) (IBM, Armonk, New York, USA) was used for statistical analysis. The one-way analysis of variance-least significant difference (ANOVA-LSD) method was used for multiple comparisons between groups. The results were expressed as mean ± standard deviation (SD). GraphPad Prism 6.0 software (GraphPad Software, Boston, MA, USA) was used for graphing. $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated a very significant difference.

Results

Histopathological observation of testis tissues

The results demonstrated that the early spermatozoa and mature spermatozoa were observed in the lumen of spermatogenic tubules in the control group with normal spermatogenesis, intact myoid cells, and normal interstitial cells without obvious pathological changes (Figure 1, A and a). In the H_2O_2 group, the testicular tissue morphology was evidently abnormal. The basement membrane of spermatogenic tubules was damaged, atrophic, and thin, and evident vacuoles were observed.

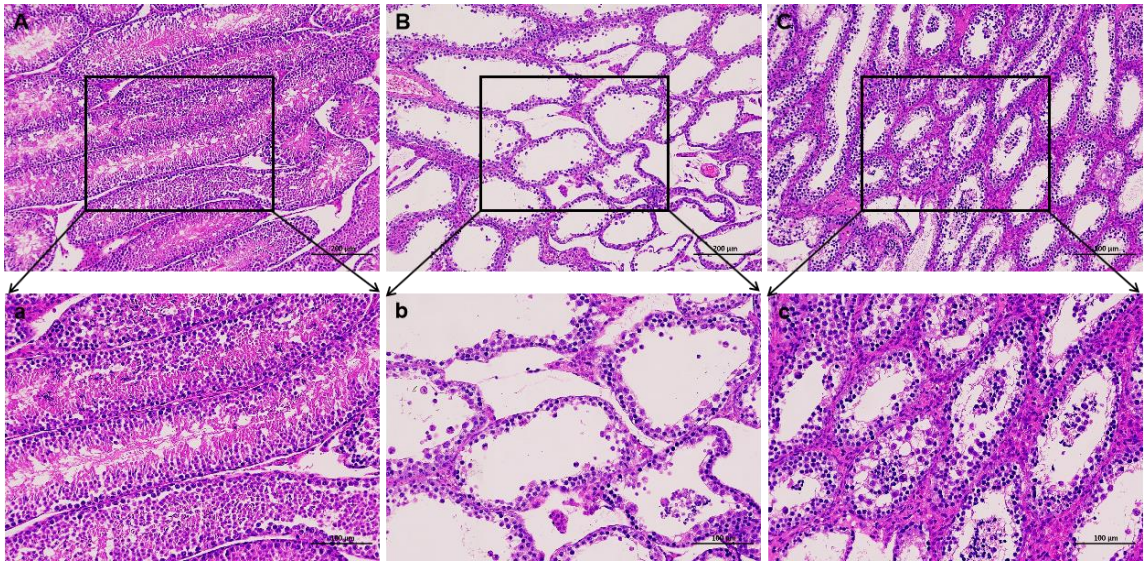

Figure 1. Observation of testicular morphological damage by hematoxylin-eosin (HE) staining. **A** (20×) and **a** (40×): normal saline. **B** (20×) and **b** (40×): H_2O_2 (3 mmol/kg/d). **C** (20×) and **c** (40×): RES (100 mg/kg/d) + H_2O_2 (3 mmol/kg/d).

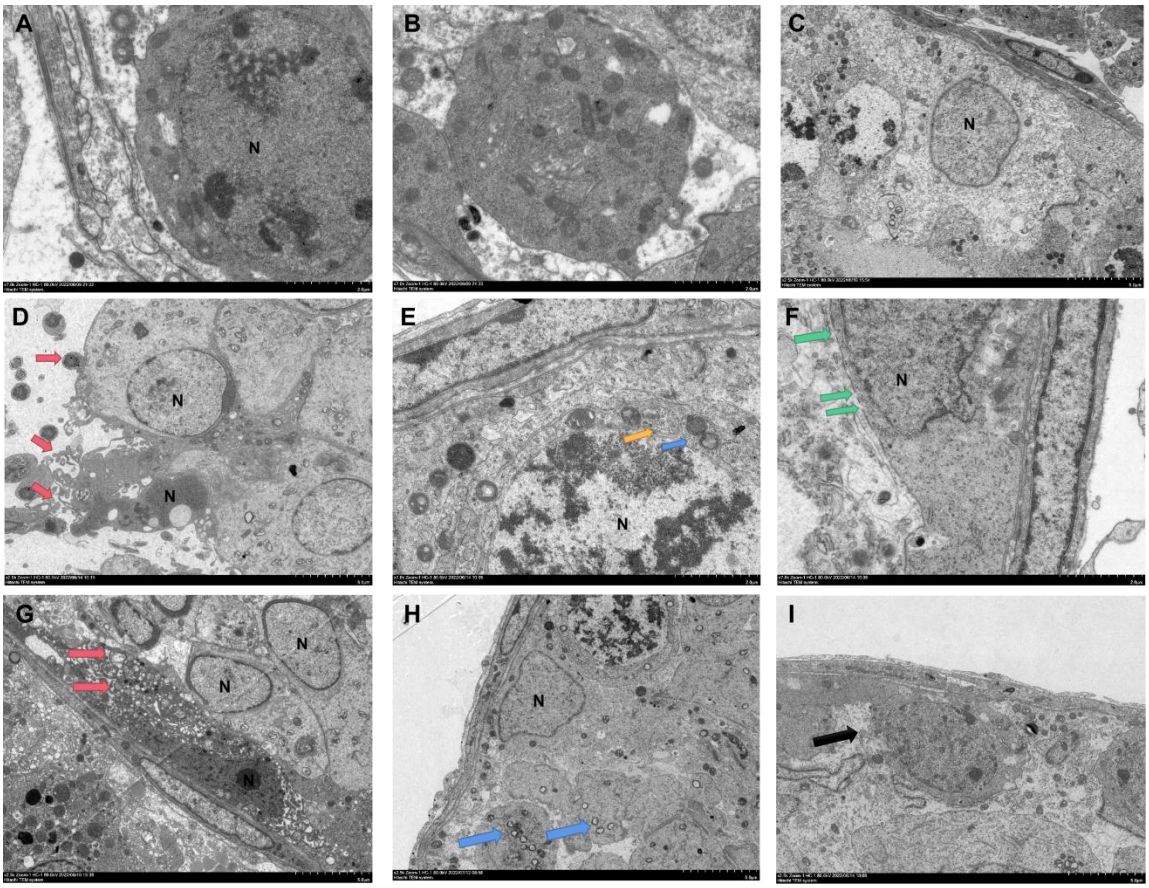

Figure 2. Transmission electron microscopy (TEM) results of testicular tissue in each group. **A-C**: the control group. **D-F**: the H_2O_2 group. **G-I**: the H_2O_2 + RES group. **Red arrow**: the apoptotic body. **Yellow arrow**: the autophagosome. **Blue arrow**: the mitophagosome. **Green arrow**: the membrane pore. **Black arrow**: the pyroptosome. **N**: the nucleus.

The Sertoli cells, primary spermatocytes, spermatocytes, and spermatozoa were fragmented and disappeared. Myoid cells disappeared, and interstitial cells were broken with enlarged space between spermatogenic tubules (Figure 1, B and b). However, in the testicular tissue of the H_2O_2 + RES group, compared with the control group, the secondary spermatocytes fell off, the number of spermatozoa decreased significantly or disappeared, the polarity of Sertoli cells disappeared, the number of cell layers decreased, and the arrangement became disordered. Compared with the H_2O_2 group, the pathological damage of testicular tissue structure was significantly ameliorated (Figure 1, C and c). Therefore, RES could reduce the degree of H_2O_2-induced pathological damage.

Observation of testicular tissue ultrastructure

The pathology of mice testis after different treatments was observed using TEM. The results showed no evident damage in the cell structure in the control group. The structures of mitochondria and nuclei appeared normal (Figure 2, A-C). In contrast, the testicular tissues in the H_2O_2 group showed apoptotic bodies (Figure 2D), autophagosomes (Figure 2E), and membrane pores (Figure 2F). In the testicular tissue of the H_2O_2 + RES group, the cell damage was reduced compared with the tissues of the H_2O_2 group, but apoptotic bodies (Figure 2G), autophagosomes (Figure 2H), and pyroptosomes (Figure 2I) were still observed. These results indicated that RES could alleviate the damage of H_2O_2 to testicular tissue to some extent.

Expression of key pyroptosis genes

The transcription results of the NLRP3 gene were shown in Figure 3A. The mRNA expression level of the NLRP3 gene in the testis tissue of the H_2O_2 group was significantly higher than that in the control group ($P < 0.01$), while that in the H_2O_2 + RES group was extremely lower than that in the H_2O_2 group ($P < 0.01$). The mRNA level of the Caspase-1 gene in the testis tissue of the H_2O_2 group was significantly higher than that of the control group ($P < 0.01$), while Caspase-1 mRNA of the H_2O_2 + RES group was significantly lower than that of the H_2O_2 group ($P < 0.01$) (Figure 3B). The mRNA level of the GSDMD gene in testicular tissue of the H_2O_2 group was significantly higher than that of the control group ($P < 0.01$), while the GSDMD mRNA of the H_2O_2 + RES group was significantly lower than that of the H_2O_2 group ($P < 0.01$) (Figure 3C). The transcription results of the IL-1β gene were shown in Figure 3D. The mRNA expression level of the IL-1β gene in testicular tissue of the H_2O_2 group was significantly higher than that of the control group ($P < 0.01$), while the IL-1β mRNA of the H_2O_2 + RES group was significantly lower than that of the H_2O_2 group ($P < 0.01$). The mRNA level of the IL-18 gene in testicular tissue of the H_2O_2 group was higher than that of the control group with no significant difference, and the IL-18 mRNA of the H_2O_2 + RES group was significantly lower than that of the H_2O_2 group ($P < 0.05$) (Figure 3E).

Expression of key apoptosis genes

The transcription level of the Caspase-3 gene in the H_2O_2 group was significantly reduced compared to that in the control group ($P < 0.05$), but it was significantly increased in the H_2O_2 + RES group compared with the control group ($P < 0.05$). The difference between the Caspase-3 mRNA of the H_2O_2 group and that of the H_2O_2 + RES group was very significant ($P < 0.01$) (Figure 4A). These results suggested that RES promoted caspase-3-mediated apoptosis. Compared with the control group, the addition of H_2O_2 significantly reduced the caspase-8 mRNA, and the difference between its transcript in the H_2O_2 group and the H_2O_2 + RES was very significant ($P < 0.01$) (Figure 4B). Compared with the H_2O_2 group, the addition of RES could increase the transcription level of this gene, suggesting that RES could promote apoptosis. There was neither a significant difference between the Bcl-2 transcript of the control group and that of the H_2O_2 group, nor between the H_2O_2 group and the H_2O_2 + RES group (Figure 4C). The transcript level of Bax mRNA in the H_2O_2 group was significantly decreased compared with that in the control group ($P < 0.05$), and there was a significant difference between the H_2O_2 group and the H_2O_2

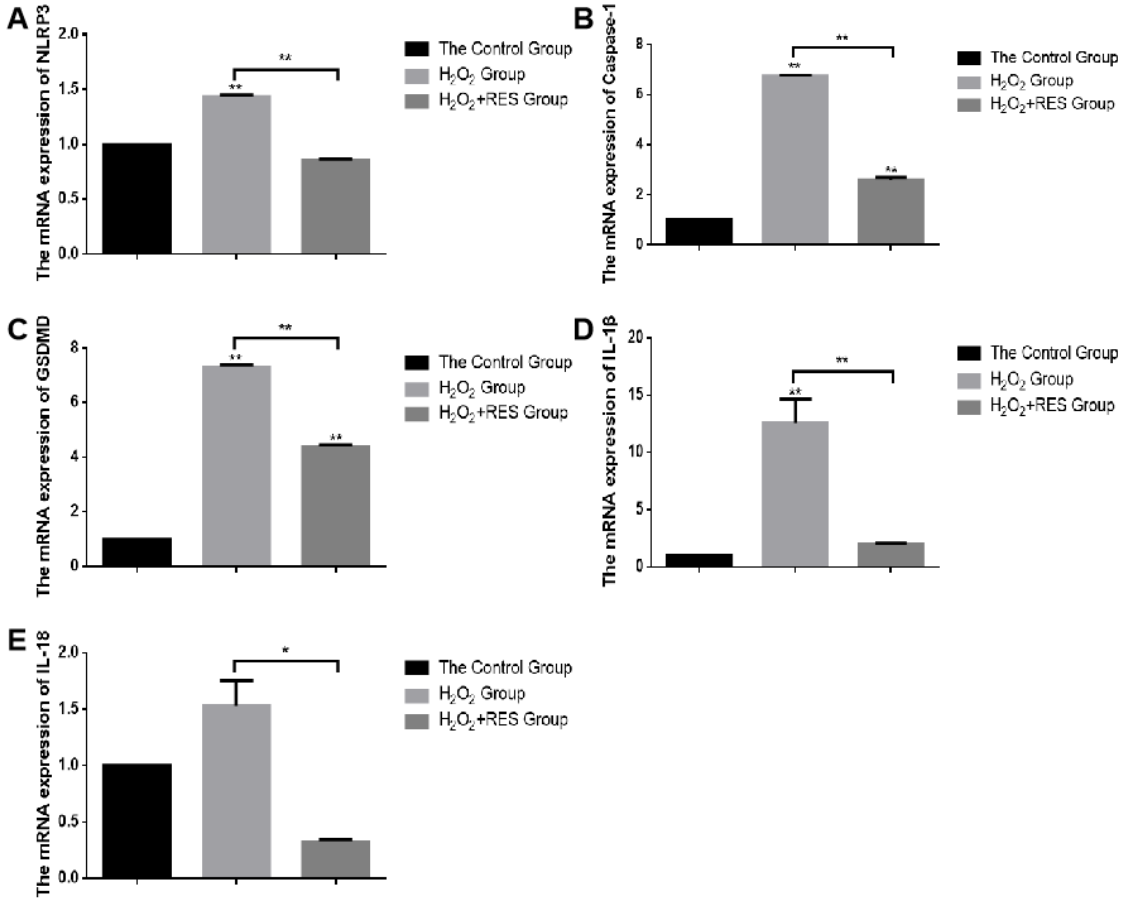

Figure 3. mRNA expression level of pyroptosis. **A**: NLRP3. **B**: Caspase-1. **C**: GSDMD. **D**: IL-1β. **E**: IL-18. *: very significant difference between groups ($P < 0.01$). **: significant difference between groups ($P < 0.05$).

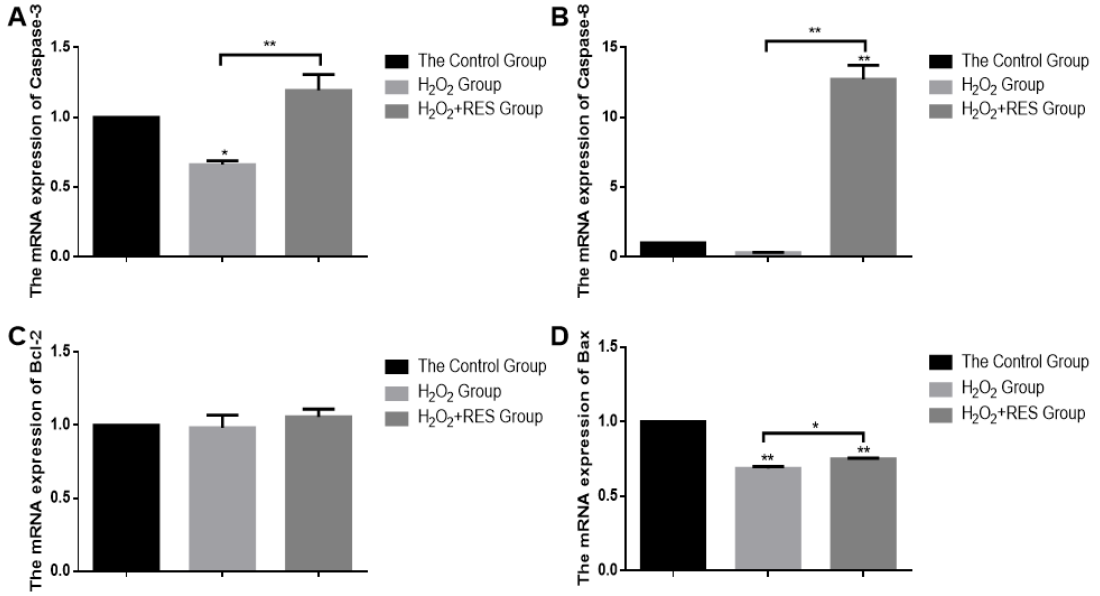

Figure 4. mRNA expression level of apoptosis. **A**: Caspase-3. **B**: Caspase-8. **C**: Bcl-2. **D**: Bax. *: very significant difference between groups ($P < 0.01$). **: significant difference between groups ($P < 0.05$).

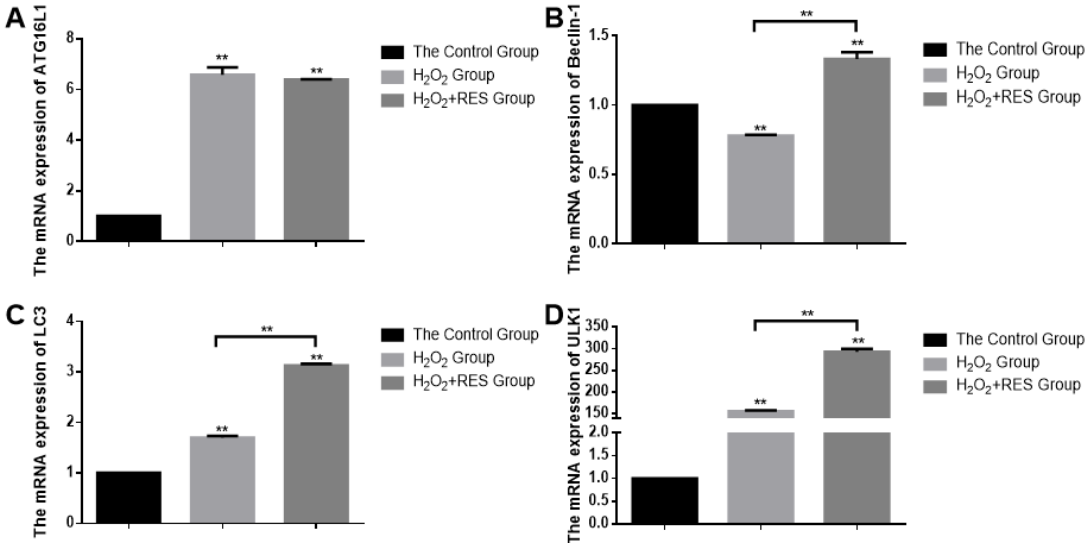

Figure 5. mRNA expression level of autophagy. **A**: ATG16L1. **B**: Beclin-1. **C**: LC3. **D**: ULK1. *: very significant difference between groups ($P < 0.01$). **: significant difference between groups ($P < 0.05$).

+ RES group ($P < 0.05$) (Figure 4D). These results indicated that the addition of H_2O_2 could reduce the transcript level of the Bax gene in mouse testicular tissue, and RES could enhance the expression of the Bax gene, thus promoting the apoptosis of mouse testicular cells.

Expression of key autophagy genes

The mRNA levels of the ATG16L1 gene in testicular tissues of both H_2O_2 and the H_2O_2 + RES groups were significantly higher than that of the control group with both *P* values < 0.01, but there was no significant difference between two experimental groups ($P > 0.05$) (Figure 5A). The Beclin-1 mRNA expression in testicular tissue of the H_2O_2 + RES group was significantly higher than that of the control group ($P < 0.01$) (Figure 5B). The mRNA level of LC3 in testicular tissue of both H_2O_2 and H_2O_2 + RES groups was significantly higher than that of the control group (both $P < 0.01$) (Figure 5C). The levels of ULK1 mRNA in testicular tissue of both the H_2O_2 group and H_2O_2 + RES group were significantly higher than that of the control group (both $P < 0.01$) (Figure 5D).

Discussion

Oxidative damage can cause severe disorders in spermatogenesis in animals, including pathological changes in testicular tissue, decreased testicular index and sperm count, and increased rate of sperm malformation, resulting in male infertility [20]. RES is a natural antioxidant that plays an important role in the treatment of oxidative damage, inflammation, allergies, and cardiovascular diseases. RES can exert its antioxidative effect by scavenging or inhibiting free radical generation, reducing lipid peroxides production, regulating the activity of antioxidation-related enzymes, and other mechanisms to achieve the purpose of alleviating or treating diseases [21]. RES exerts a protective effect against oxidative damage due to various factors. Furat Rencber *et al*. showed that RES could improve the antioxidant capacity of the body and increase the serum testosterone content [22]. RES also exerts a good anti-aging effect by improving the function of testicular endocrine cells, promoting the recovery of spermatogenic function, and delaying the decline in gonadal functions [23, 24]. RES can reduce mouse reproductive system damage and activate male germ cells autophagy [25]. The results of this study showed that RES could promote the expression of key genes of autophagy induced by

H_2O_2 in mouse testicular tissue, improve the pathological injury of the testicle, and benefit the ultrastructure of testicular tissue. This result is consistent with previous research results.

Considering the roles of pyroptosis, on the one hand, it can resist pathogen infection and maintain the normal operation of the body, and on the other hand, it can lead to inflammation of neighboring cells and tissues. The activation of NLRP3 inflammasome mediates the activation of caspase-1, which leads to the maturation and secretion of IL-1β and IL-18 and cleavage of GSDMD, thus inducing pyroptosis. There have been many studies on the effects of RES and H_2O_2 on pyroptosis. Chang *et al*. reported that RES could inhibit the activity of NLRP3 inflammasome and the secretion of IL-1β, thus inhibiting the occurrence of pyroptosis [26]. H_2O_2 can increase caspase-1 and IL-1β expression in placental trophoblast cells. RES treatment can relieve the damage caused by H_2O_2 and reduce the expression of caspase-1 and IL-1β protein [16]. Xie *et al*. reported that H_2O_2 could activate the inflammasome and promote the occurrence of pyroptosis [27]. Fan *et al*. and Yang *et al*. also reported that RES decreased NLRP3, caspase-1, IL-18, and IL-1β levels, as well as pyroptosis of macrophages and duck ileum cells [28, 29]. This study showed that H_2O_2 treatment led to a significant increase in the transcription levels of key genes associated with pyroptosis in tissues, while RES treatment led to a significant decrease in these indicators. H_2O_2 can form a large number of free radicals in mice, stimulating the activation of NLRP3 inflammasome and promoting the occurrence of pyroptosis, while RES can inhibit free radical production or eliminate them and reduce the transcription levels of key pyroptosis genes.

H_2O_2 produced under normal physiological conditions is necessary to maintain sperm production. However, excess H_2O_2 can initiate apoptosis by oxidizing lipids, proteins, and DNA or acting as a signaling molecule. H_2O_2 can significantly reduce cell viability, increase ROS, ·OH, and MDA contents as well as DNA damage, and significantly increase the total and early apoptosis rates [14]. RES can effectively reduce the expression of Bax/Bcl-2 (apoptosis-related genes), thus effectively inhibiting cell proliferation and promoting apoptosis [30]. In addition, RES protects against cisplatin-induced testicular injury and reproductive dysfunctions through the increase in testosterone levels, increase in sperm count, reducing testicular apoptosis through the antioxidant capacity, inhibiting endoplasmic reticulum stress, P53, extracellular signal-regulated protein kinase1/2, c-Jun N-terminal kinases, and activating protein kinase B [31]. Natural AhR antagonist RES is shown to reduce benzopyrene-induced spermatogenic tubule DNA adduct and apoptosis [32]. Previous studies, as well as the results of this study, confirmed the role of RES in the prevention and improvement of male reproductive diseases at the cellular and molecular levels, suggesting the potential of RES as a promising drug candidate for the treatment of reproductive diseases. However, current studies on RES have mainly focused on the cellular and animal levels, and more clinical studies are needed to ensure the safety of RES in the body. Further, the appropriate dose of RES in prevention and treatment of reproductive diseases also needs further investigation. Currently, only a few studies have been conducted on RES in the field of reproductive medicine. Nevertheless, based on the biological functions of RES and the current research results, RES can promote the removal of damaged and senescent male animal germ cells and may also be a promising candidate drug to treat reproductive problems. This study not only provided a theoretical basis for the study of male reproductive development, but also laid a scientific foundation for the development and application of related drugs.

Conclusion

To a certain extent, H_2O_2 can promote autophagy and the transcription of key pyroptosis genes and inhibit the transcription of apoptosis-related

genes in mice testis. In contrast, RES can increase the transcription level of autophagy-related genes in mice testis cells, promote the occurrence of autophagy, inhibit pyroptosis and release of inflammatory cytokines, and protect cells from oxidative stress by up-regulating the expression of genes associated with apoptosis.

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