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Original Article

Resveratrol improves asthenozoospermia by mitigating oxidative stress-induced mitochondrial dysfunction

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Abstract: Objectives: To investigate the protective effects and mechanisms of resveratrol against oxidative stress-induced sperm dysfunction in vitro and cyclophosphamide-induced asthenozoospermia in vivo. Methods: An H_2O_2-induced sperm oxidative stress model was established to evaluate the dose-dependent effects of resveratrol on viability, motility, reactive oxygen species (ROS), lipid peroxidation, antioxidant enzymes, mitochondrial function, and apoptosis. A cyclophosphamide-induced asthenozoospermic mouse model was used to assess in vivo efficacy on sperm quality and testicular antioxidant capacity. Results: Resveratrol significantly improved sperm viability, total motility, and progressive motility in a dose-dependent manner. It reduced ROS generation and malondialdehyde (MDA) levels while enhancing antioxidant enzyme activities. Mitochondrial function was enhanced, as evidenced by maintained membrane potential, increased Adenosine Triphosphate (ATP) production, elevated succinate dehydrogenase activity, and preserved mitochondrial ultrastructure. Furthermore, resveratrol suppressed mitochondrial-mediated apoptosis by modulating B-cell Lymphoma 2 (Bcl-2) family protein ratios. In vivo, resveratrol treatment markedly improved sperm parameters and testicular antioxidant capacity in asthenozoospermic mice. Conclusions: Resveratrol protects sperm against oxidative damage through multiple mechanisms, including antioxidative, mitochondria-preserving, and anti-apoptotic pathways. These findings support its potential as a therapeutic candidate for male infertility associated with oxidative stress.

Keywords: Asthenospermia, resveratrol, oxidative stress, mitochondrial function

Introduction

According to global epidemiological studies, about 15% of couples of reproductive age are unable to conceive, and male factors account for nearly half of these cases [1, 2]. The primary cause of male infertility is poor sperm quality, which includes reduced sperm motility, low sperm count, and increased morphological abnormalities. Among these reasons, asthenozoospermia is a relatively common cause of male infertility, characterized by reduced sperm motility (progressive motility < 32%) and poor viability in the fresh semen [3]. Recently, the incidence of male infertility has been increasing annually, making it a public health concern.

Spermatozoa are highly specialized, terminally differentiated cells that require a large energy supply from mitochondria to survival and function [4]. Due to their scant cytoplasm and weak antioxidant system, sperm cells are highly susceptible to oxidative damage to their membranes and DNA. Excessive ROS damage mitochondrial structure, reduce Adenosine Triphosphate (ATP) levels, impair motility, cause DNA damage, and even induce apoptosis, ultimately leading to male infertility [5, 6]. G3PP expression is elevated in both spermatocytes and spermatids during murine spermatogenesis. Conversely, G3PP-deficient mice exhibit reduced sperm motility, increased oxidative stress, and ultrastructural mitochondria damage [7]. Taurine has been shown to reduce oxidative stress in lead-exposed mouse gonads and sperm, and restore mitochondrial function and motility [8]. In addition, H_2S-releasing compounds have been shown to dose-dependently reverse H_2O_2-induced decline in sperm motility and viability, while also reducing lipid peroxidation and caspase-3 activity [9]. Glycine has

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been shown to prevent lead-induced reproductive damage in mice by reducing testicular histological damage, suppressing oxidative stress markers in both testes and spermatozoa, and enhancing sperm kinetic parameters, such as progressive motility rate and velocity indices [10]. Collectively, these studies indicate that sperm dysfunction is associated with mitochondrial oxidative damage. Thus, scavenging ROS to restore mitochondrial homeostasis represents a potential treatment method for asthenozoospermia.

Resveratrol is a natural polyphenol found in various plants, including grapes, peanuts, and Japanese knotweed (Polygonum cuspidatum). Resveratrol is a well-known antioxidant, anti-inflammatory, and anti-apoptotic agent. Therefore, this study employed resveratrol as a therapeutic agent for asthenozoospermia. Resveratrol has been shown to increase testosterone levels, sperm count, and sperm motility in rat models of testicular damage [11]. A previous report showed that although isoflurane exposure in mice caused damage to testicular weight, volume, sperm parameters, and histomorphology, along with increased in malondialdehyde (MDA) levels and decreased total antioxidant capacity, resveratrol treatment mitigated these adverse effects [12]. Additionally, a single-center clinical study found that a multivitamin supplement containing 150 mg of resveratrol (oral administration every 12 hours) improved sperm concentration and motility in men with idiopathic male infertility [13]. Polydatin, a resveratrol derivative, has been shown to reverse the downregulation of androgen receptors and testosterone-associated proteins, while reducing elevated MDA and reactive oxygen species (ROS) levels in asthenozoospermic mice [14]. Collectively, these findings suggest that resveratrol may be a promising agent for treating male infertility.

Since oxidative stress is a major cause of mitochondrial damage in sperm, this study explored the protective effects of resveratrol on mitochondrial structure and function. Two experimental models were used to evaluate the therapeutic effects on asthenozoospermia: H_2O_2-challenged sperm in vitro and cyclophosphamide-induced asthenozoospermia in mice in vivo. Resveratrol reduced mitochondrial ROS, inhibited sperm apoptosis, and improved sperm motility and viability. Collectively, these findings support the use of resveratrol in treating asthenozoospermia and provide further insights into the role of mitochondrial oxidative stress in this condition.

Materials and methods

Sperm preparation and treatment

Semen samples were collected from healthy donors with normal semen parameters according to World Health Organization (WHO) criteria. High-motility spermatozoa were isolated by density gradient centrifugation and resuspended in Human Tubal Fluid (HTF) medium supplemented with 0.3% bovine serum albumin (BSA). Oxidative damage was induced by exposure to 200 μM H_2O_2 for 2 hours at 37°C in a humidified 5% CO_2 incubator. For cytoprotection assays, resveratrol (RSV) was added to the sperm suspension at a low (10 μm) or a high (40 μm) concentration for 24 hours prior to H_2O_2 treatment. All procedures involving human samples were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, and written informed consent was obtained from all donors.

To verify whether the protective effect of RSV was mediated through mitochondrial oxidative stress, the mitochondria-targeted ROS scavenger Mito-TEMPO (Sigma-Aldrich, Cat# SML0737) was used. Spermatozoa were pre-treated with 50 μm Mito-TEMPO for 30 minutes at 37°C prior to H_2O_2 exposure or RSV intervention. For the combination group, spermatozoa were co-incubated with both RSV (40 μm) and Mito-TEMPO (50 μm) under the same conditions.

Sperm motility analysis

Sperm motility was assessed using a computer-assisted sperm analysis (CASA) system. A 5 μL aliquot of sperm suspension was added to a pre-warmed counting chamber and kept at 37°C on a phase-contrast microscope stage. To ensure the accuracy of the data, at least 200 spermatozoa per five randomly selected fields were counted. The system generated comprehensive parameters, including viability, total motility (progressive motility [PR] + non-progressive motility [NP]), progressive motility (PR), velocity indices (curvilinear velocity [VCL], straight-line velocity [VSL], average path veloci-

ty [VAP]), and trajectory traits (linearity [LIN], amplitude of lateral head displacement [ALH]).

Measurement of intracellular ROS levels

Intracellular ROS production was measured by monitoring DCFH-DA fluorescence. After 30 minutes of dark incubation at 37°C in serum-free medium, cells were washed three times with warm PBS to remove unbound probes, and strict light-protected conditions were employed to prevent signal decay. Fluorescence emission at 488/525 nm (Ex/Em) was obtained by a fluorescence microscope or a flow cytometer. The data are presented as mean fluorescence intensity, and the extent of intracellular DCFH-DA oxidation is shown.

Oxidative stress index detection

Lipid peroxidation was quantified as MDA content using the thiobarbituric acid reactive substances (TBARS) method. Additionally, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined using commercial kits. All procedures were performed according to the manufacturer's instructions.

Western blot analysis

Total protein was extracted using RIPA buffer containing 1% PMSF and a protease inhibitor cocktail, and protein concentration was quantified using a BCA protein assay kit. Equal amounts of protein (30 μg) were prepared by 10% or 12% SDS-PAGE and then transferred to PVDF membranes. Membranes were blocked in 5% non-fat milk in TBST for 1 h at room temperature, then incubated overnight at 4°C with primary antibodies against NRF2, HO-1, Bax, Bcl-2, and SOD2 (1:1,000), and GAPDH (1:5,000) as the internal control. After washing, the membranes were incubated with HRP-conjugated secondary antibodies for 1 hour. Signals were detected using an ECL kit, and band intensities were quantified by densitometry using ImageJ.

JC-1 staining and flow cytometry

To measure the mitochondrial membrane potential (ΔΨm), sperm cells were washed twice in PBS and adjusted to 1×10^6 cells/mL in pre-warmed JC-1 buffer. One milliliter of suspension was added to the JC-1 working solution for 20 minutes at 37°C in the dark, then washed twice with 1× buffer and resuspended in 500 μL for immediate acquisition on a 488 nm argon-ion laser flow cytometer. JC-1 aggregates (red, FL2: Ex/Em 585/590 nm) and monomers (green, FL1: Ex/Em 529/530 nm) were measured to assess mitochondrial status. FlowJo was used to process the data, and ΔΨm was taken as the ratio of red-to-green fluorescence.

Measurement of ATP content

Intracellular ATP levels were measured using a commercially available bioluminescence assay kit, and luminescence signals were recorded with a microplate reader. Succinate dehydrogenase activity was measured spectrophotometrically by monitoring the reduction of p-iodonitrotetrazolium violet at a wavelength of 490 nm. Phospholipid hydroperoxide glutathione peroxidase (GPX4) activity was determined using the 5,5'-dithiobis (2-nitrobenzoic acid)-coupled assay, with absorbance measured at 412 nm. All experiments were performed in triplicate, and then the data were normalized and expressed as units per milligram of protein.

Flow cytometry

To evaluate sperm apoptosis, cells were collected, washed twice with PBS, and suspended in 1× Annexin V binding buffer at a concentration of 1×10^6 cells/mL. One hundred microliters of the suspension were co-incubated with Annexin V-FITC (5 μL) and PI (5 μL) in the dark at room temperature for 15 minutes. Then, 400 μL of 1× binding buffer was added to stop the reaction, and immediately flow cytometry was carried out. FlowJo software was used to process all the data.

Animal model and grouping

SPF male C57BL/6 mice (6-8 weeks old, 20-25 g; Vital River, Beijing, China) were housed under standard conditions (22 ± 2°C, 50 ± 10% RH, 12-h L/D cycle) with ad libitum access to food and water. The animal study protocol was approved by the Animal Ethics Committee of The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine (SDUTCM20240613118), in accordance with NIH guidelines. The mice were randomly divided into four groups: blank control, asthenozoospermia

model, resveratrol treatment, and positive control. Asthenozoospermia was induced by intraperitoneal injection of cyclophosphamide (40 mg/kg) every 3 days for 35 consecutive days. Concurrently, oral administration was performed as follows: normal saline for the blank control and model groups, resveratrol (100 mg/kg/day) for the resveratrol group, and levocarnitine for the positive control group. To minimize animal suffering, all invasive procedures were performed under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). At the end of the experimental, mice were euthanized by cervical dislocation under deep anesthesia, followed by confirmation of death by absence of heartbeat and respiration. All experimental procedures were approved by the Institutional Animal Care and Use Committee of The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine.

H&E staining

Testicular tissues were collected, fixed in 4% paraformaldehyde for 24 hours, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Sections of 5 μm thickness were cut and stained with haematoxylin and eosin (H&E). Morphological changes in seminiferous tubules and spermatogenic cells were observed under a light microscope.

Serum sex hormone levels

Blood was collected from the retro-orbital venous plexus one hour after the last dose, and serum was separated by centrifugation at 3,000 rpm for 10 minutes. Serum levels of testosterone (T), follicle-stimulating hormone (FSH), and luteinising hormone (LH) were determined using a commercial ELISA kit according to the manufacturer's instructions.

RT-qPCR

Total RNA was isolated from cells utilizing TRIzol reagent. Subsequently, first-strand cDNA was synthesized using the PrimeScript Reagent Kit, strictly adhering to the manufacturer's protocols. Quantitative real-time PCR amplification was performed using the SYBR Premix Ex Taq Kit. The primers were designed and synthesized by Sangon Biotech. Relative mRNA expression was quantified using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the endogenous reference control.

Statistical analysis

Data were analyzed using SPSS 21.0. Values are presented as mean ± standard deviation (SD) from three biologically independent replicates. The Shapiro-Wilk and Levene's tests were employed to assess normality and homogeneity of variance, respectively. ANOVA followed by post-hoc pairwise comparisons were performed among groups. Statistical significance was set at $P < 0.05$.

Results

Resveratrol increases the viability and motility of H_2O_2-damaged sperm cells

The H_2O_2-treated group showed significant reductions in sperm viability (**Figure 1A**), total motility (PR+NP%) (**Figure 1B**), and progressive motility (PR%) (**Figure 1C**) compared with the control group, thus, confirming successful establishment of the oxidative injury model. Resveratrol treatment produced dose-dependent recovery, with the high-dose treatment achieving greater improvements in both viability and PR% than the low-dose treatment. CASA kinematic analysis revealed that oxidative stress decreased VCL (**Figure 1D**), VSL (**Figure 1E**), VAP (**Figure 1F**), and LIN (**Figure 1G**), indicating impaired directional motility. Additionally, high-dose resveratrol significantly increased VSL, VAP, and LIN values, thereby enhancing progressive motility. Furthermore, high-dose resveratrol moderately increased the amplitude of lateral head displacement (ALH) (**Figure 1H**), which may indicate modulation of sperm capacitation and fertilization capacity.

Resveratrol inhibits H_2O_2-induced oxidative stress in sperm cells

Compared with the control group, the oxidative stress model group exhibited elevated intracellular ROS and MDA levels, and decreased activities of SOD, CAT, and GSH-Px. Resveratrol treatment reversed these changes, with both low- and high-dose groups showing reduced ROS (**Figure 2A**, **2B**) and MDA (**Figure 2C**) levels, and restored SOD (**Figure 2D**), CAT (**Figure 2E**), and GSH-Px (**Figure 2F**) activities, in a dose-dependent manner. Western blotting further showed that Nrf2 (**Figure 2G**, **2H**) and its downstream target HO-1 (**Figure 2G**, **2I**) were significantly downregulated in the model group, while resveratrol treatment upregulated both

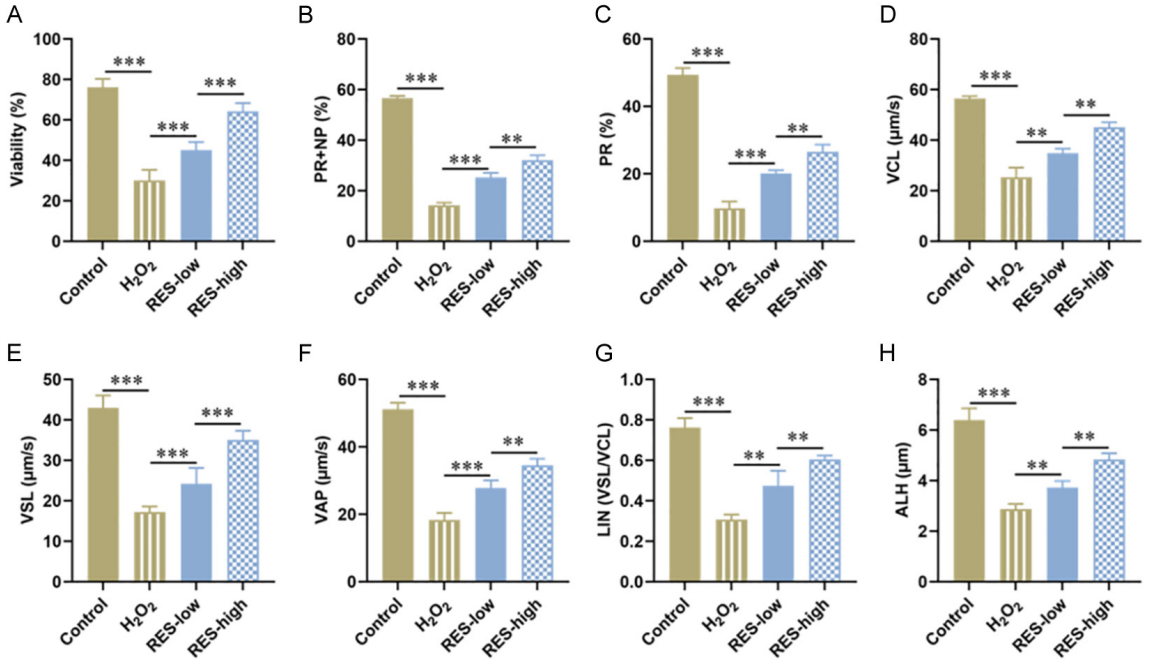


Figure 1. Effects of resveratrol on the motility of H_2O_2-stimulated sperm cells. A. Sperm viability. B. Total motility (PR+NP%). C. Progressive motility (PR%). D-F. CASA parameters: straight-line velocity (VSL), curvilinear velocity (VCL), and average path velocity (VAP); G, H. Linearity (LIN, VSL/VCL) and amplitude of lateral head displacement (ALH). N = 3. $^{**}P < 0.01$. $^{***}P < 0.001$. ns, not significant.

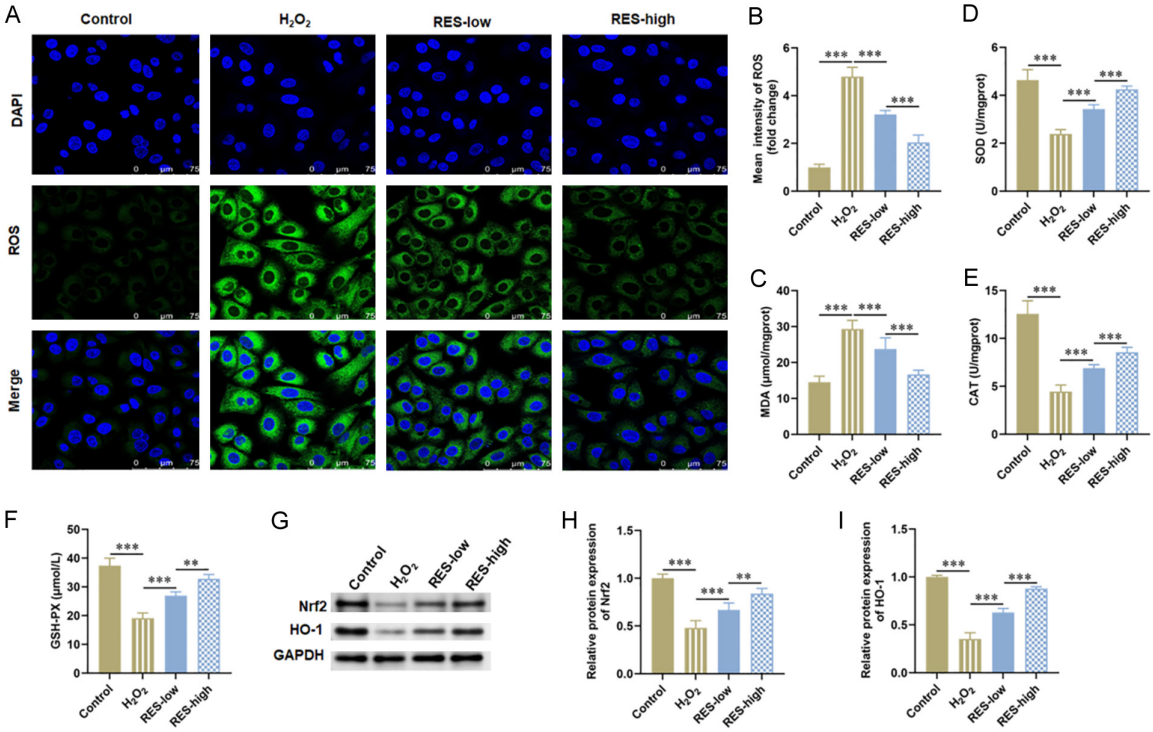


Figure 2. Effects of resveratrol on H_2O_2-induced oxidative stress in sperm cells. A, B. Reactive oxygen species (ROS) levels detected by the DCFH-DA fluorescent probe. Scale Bar = 75 μm. C. Malondialdehyde (MDA) content (TBA method). D-F. Activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). G-I. Protein expression levels of Nrf2 and HO-1. N = 3. $^{**}P < 0.01$. $^{***}P < 0.001$. ns, not significant.

proteins, with greater efficacy in the high-dose group.

Resveratrol reduces mitochondrial damage in sperm cells induced by H_2O_2

JC-1 staining showed that control sperm mitochondria exhibited predominantly aggregated JC-1 (red) with high mitochondrial membrane potential (ΔΨm), whereas the model group showed an increase in JC-1 monomers (green), indicating depolarization. Resveratrol administration effectively reversed this depolarization (**Figure 3A**, **3B**). ATP content was significantly lower in the model group than in the control group; however, resveratrol treatment increased ATP levels in a dose-dependent manner, with the high-dose group showing the best recovery (**Figure 3C**). Thus, resveratrol may regulate the energy metabolism by modulating mitochondrial organization. In addition, SDH activity was reduced in the model group, but restored by resveratrol in a dose-dependent manner (**Figure 3D**). OGG1, a key enzyme in mitochondrial DNA repair, showed reduced mRNA expression in the model group. Both low- and high-dose resveratrol treatments increased OGG1 expression (**Figure 3E**), suggesting that resveratrol protects mitochondrial genomic stability by enhancing oxidative damage repair. TEM showed that control sperm mitochondria were regularly oval with well-defined cristae, whereas the model group exhibited swollen mitochondria, disrupted cristae, and vacuolization. Resveratrol attenuated mitochondrial damage in a dose-dependent manner; at a low dose, only partial repair occurred, but at a high dose, it was the same as the control group and had undissolved cristae and a granular matrix. Resveratrol protected mitochondrial ultrastructure (**Figure 3F**).

Resveratrol inhibits H_2O_2-induced apoptosis of sperm cells

H_2O_2 exposure significantly increased both early and late apoptosis rates compared with the control. Resveratrol inhibited apoptosis in a dose-dependent manner, with greater protection in the high-dose group (**Figure 4A**, **4B**). The model group showed elevated caspase-3 activity, indicating ongoing apoptosis, while resveratrol effectively inhibited this process (**Figure 4C**). H_2O_2 upregulated the pro-apoptotic protein Bax (**Figure 4D**, **4E**) and downregulated the anti-apoptotic protein Bcl-2 (**Figure 4D**, **4F**). Resveratrol reversed these changes by increasing Bcl-2 and reducing Bax, especially at the high concentration. Therefore, the increased Bax/Bcl-2 ratio in the model group, indicative of mitochondrial apoptosis initiation, was significantly reduced by resveratrol, especially in the high-dose group (**Figure 4G**).

Resveratrol reduces damage caused by H_2O_2-induced ROS in sperm mitochondria

The oxidative stress group exhibited elevated mitochondrial ROS (mtROS), reduced MMP, and increased cell apoptosis compared with the control group. Additionally, mitochondrial SOD2 (Mn-SOD) protein expression and GPX4 activity were decreased, confirming successful establishment of the mitochondrial oxidative stress injury model. Resveratrol treatment reduced mtROS levels (**Figure 5A**, **5B**), restored MMP (**Figure 5C**, **5D**), decreased cell apoptosis (**Figure 5E**, **5F**), increased SOD2 expression (**Figure 5G**, **5H**) and enhanced GPX4 activity (**Figure 5I**), demonstrating strong antioxidant and anti-apoptotic effects. The mitochondrial-specific ROS scavenger (Mito-TEMPO) group also attenuated these injury indicators. Notably, the combination of resveratrol and Mito-TEMPO did not provide additional benefit compared with Mito-TEMPO alone, suggesting a plateau effect. Therefore, the protective effect of resveratrol likely stems from mtROS-scavenging activity and modulation of the mitochondrial antioxidant system. The observed plateau effect further suggests that the therapeutic target of resveratrol is likely the mitochondrial oxidative stress pathway.

Resveratrol treatment reduces oxidative stress-induced injury in asthenospermia mice

Compared with the control group, the cyclophosphamide-induced asthenospermia model showed no significant changes in body weight (**Figure 6A**) or testicular weight (**Figure 6B**), but exhibited a substantial decrease in total sperm count (**Figure 6C**) and motility (**Figure 6D**), along with a high malformation rate (**Figure 6E**). Hormone analysis revealed decreased testosterone levels (**Figure 6F**) and elevated FSH (**Figure 6G**) and LH (**Figure 6H**) levels. Histopathological examination (**Figure 6I**) revealed disorganized seminiferous tubules, germ cell sloughing, and luminal vacuolization. Addi-

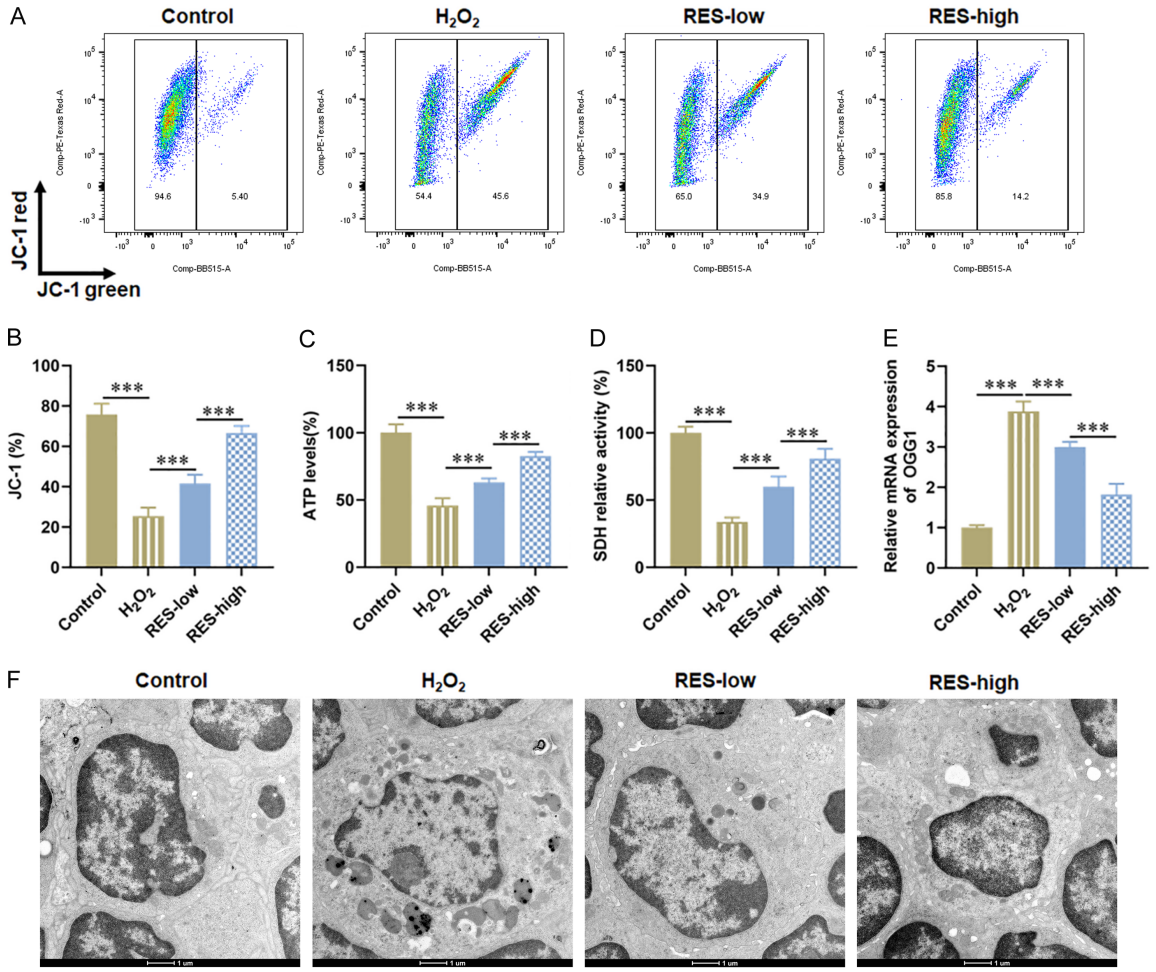


Figure 3. Effects of resveratrol on the mitochondrial function of H_2O_2-stimulated sperm cells. A, B. Mitochondrial membrane potential staining with JC-1. C. ATP content. D. Succinate dehydrogenase (SDH) activity. E. Relative mRNA expression of OGG1 was determined using the $2^{-\Delta\Delta Ct}$ method. F. Mitochondrial ultrastructure of spermatozoa observed by transmission electron microscopy. Scale bar = 1 μm. N = 3. ***P < 0.001. ns, not significant.

tionally, the testes showed compromised antioxidant status, with reduced SOD and GSH-Px activities and increased MDA levels (**Figure 6J-L**), confirming successful model establishment. Resveratrol administration significantly ameliorated these abnormalities, with effects comparable to those of the positive control. Collectively, these data suggest that resveratrol alleviates cyclophosphamide-induced asthenospermia in vivo by enhancing testicular antioxidant capacity, preserving tissue structure, and regulating hormone production.

Discussion

The present study systematically investigated the protective effect of resveratrol against H_2O_2-induced spermatozoal damage and elucidated the underlying molecular mechanisms. Resveratrol improved sperm vitality, reduced oxidative damage, protected mitochondrial structure, and inhibited apoptosis in both in vitro and in vivo models of asthenozoospermia. Mechanistically, these protective effects are likely mediated by reducing mitochondrial ROS, activating the Nrf2 antioxidant signaling pathway, and regulating mitochondrial bioenergetics.

Our results demonstrate that H_2O_2 damages sperm cells via ROS generation, leading to reduced viability and impaired motility. Resveratrol improved multiple motility parameters in a dose-dependent manner. CASA kinematic analysis further revealed that resveratrol enhanced VSL, VAP and LIN values, indicating

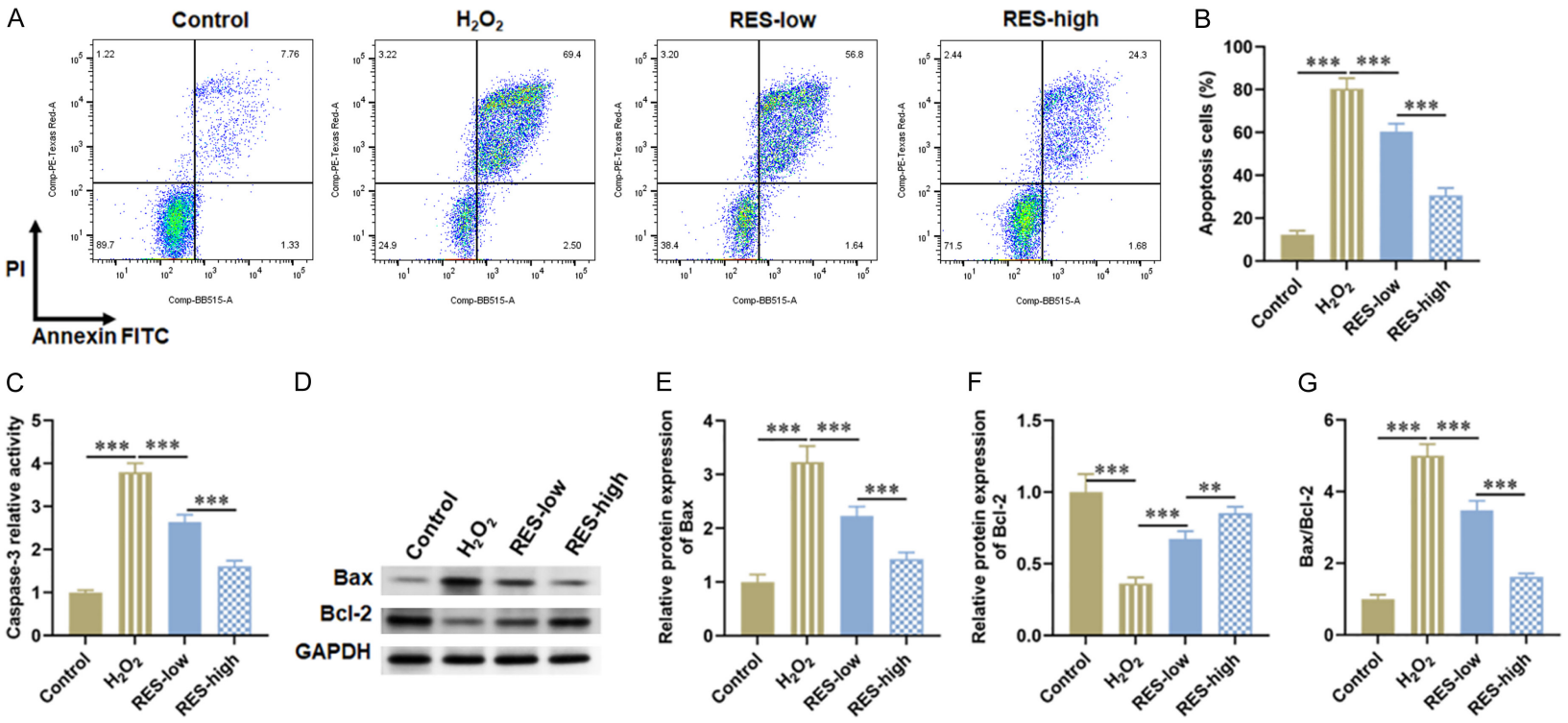


Figure 4. Effects of resveratrol on H_2O_2-induced apoptosis in sperm cells. A, B. Sperm cell apoptosis detected by Annexin V/PI double-staining flow cytometry. The upper right quadrant (Q2) and the lower right quadrant (Q3) are, respectively, late-apoptotic cells and early-apoptotic cells. C. Caspase-3 activity in sperm cells. D-F. Expression levels of apoptosis-related proteins Bax and Bcl-2. G. Bax/Bcl-2 protein ratio. N = 3. $^{**}P < 0.01$. $^{***}P < 0.001$. ns, not significant.

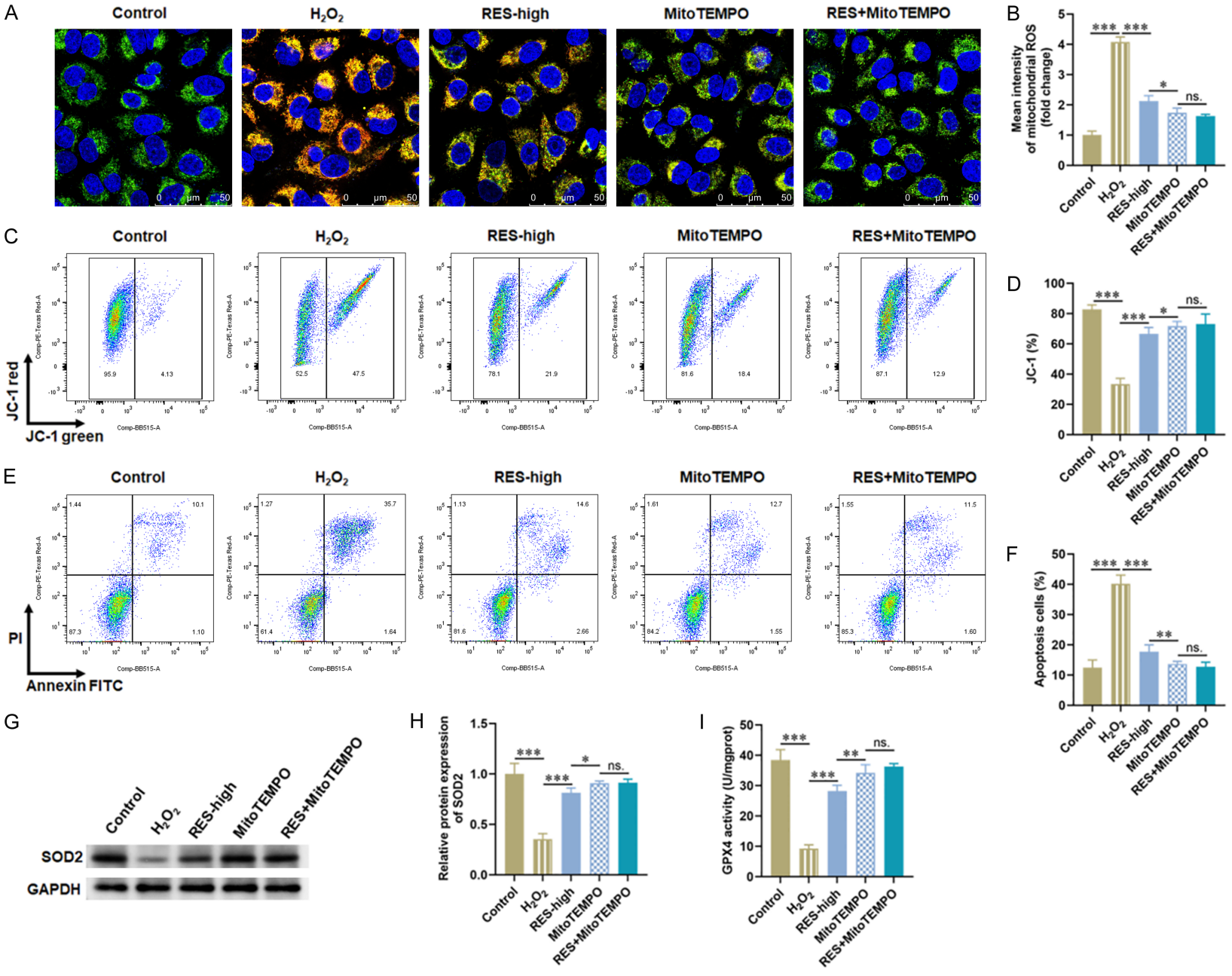

A
Control
H2O2
RES-high
MitoTEMPO
RES+MitoTEMPO
B
Mean intensity of mitochondrial ROS (fold change)
C
JC-1 red
JC-1 green
D
JC-1 (%)
E
PI
Annexin FITC
F
Apoptosis cells (%)
G
SOD2
GAPDH
H
Relative protein expression of SOD2
I
GPX4 activity (U/mgprot)

Figure 5. Effects of resveratrol on H_2O_2-induced mitochondrial oxidative stress in sperm cells. A, B. Mitochondrial ROS (mtROS) levels measured by Mito-Tracker and CellROX co-staining. Scale Bar = 50 μm. C, D. Mitochondrial membrane potential measured by JC-1 probe. E, F. Cell apoptosis. G, H. Protein expression of mitochondrial SOD2 (Mn-SOD). I. GPX4 activity measured by DTNB method. N = 3. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. ns, not significant.

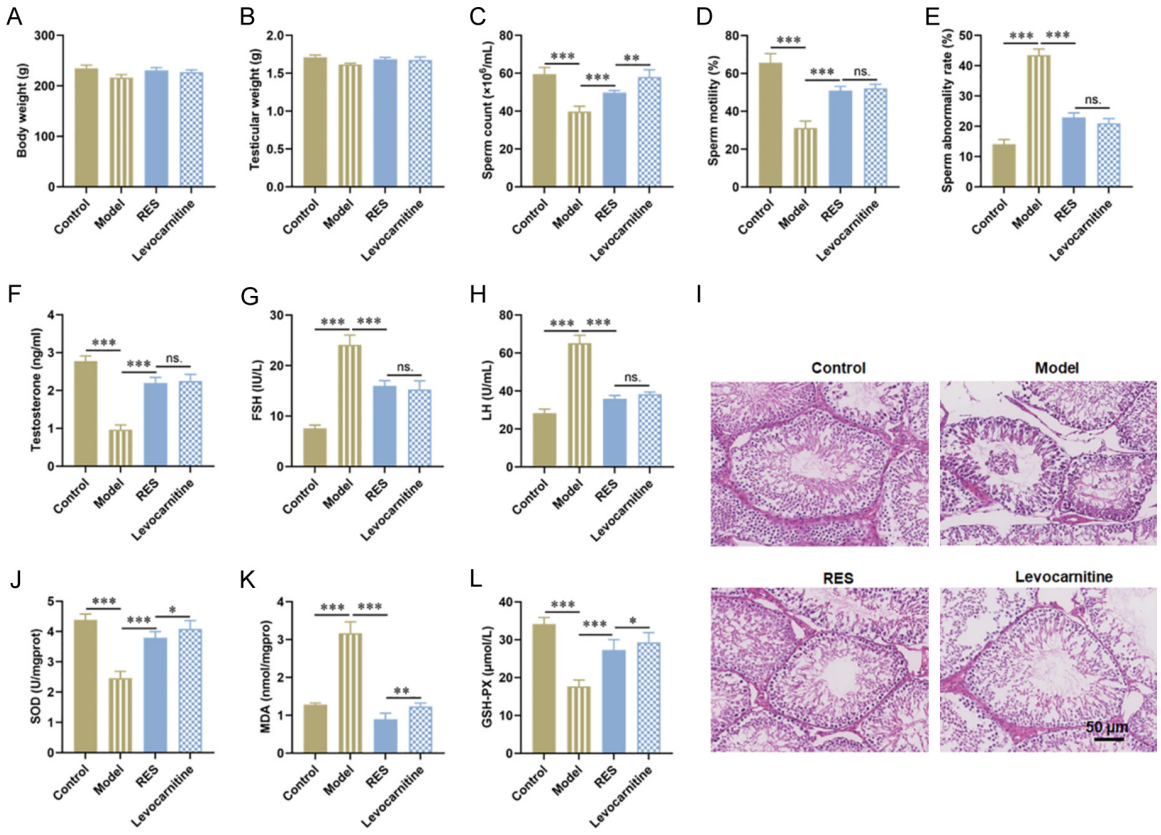


Figure 6. Therapeutic effects of resveratrol on asthenospermia in vivo. A, B. Body weight and testis weight. C, D. Sperm count and motility. E. Sperm deformity rate. F-H. Serum hormone levels: testosterone (T), follicle-stimulating hormone (FSH), and luteinising hormone (LH). I. Testis histopathology (H&E staining, scale bar = 50 μm). J-L. Antioxidant indices: SOD activity, GSH-Px activity and MDA content. N = 6. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. ns, not significant.

improved straight-line progressive motility. The moderate increase in ALH suggests that resveratrol may modulate sperm capacitation and fertilization ability. Resveratrol decreased intracellular ROS and MDA levels and activated antioxidant enzymes (SOD, CAT, GSH-Px) under oxidative stress conditions. Mechanistically, Western blotting revealed increased Nrf2 and HO-1 expression, suggesting that resveratrol likely enhances cellular antioxidant capacity via Nrf2 pathway activation. Nrf2 is an upstream regulator of the cellular antioxidant system, controlling the expression of multiple antioxidant enzymes. Resveratrol acts through multiple mechanisms, contributing to its potent antioxidant properties. Consistent with previous studies [15-17], resveratrol also acts as a direct free-radical scavenger that enhances endogenous antioxidant capacity through regulation of enzyme and gene expression.

Mitochondria in sperm cells are the energy powerhouses that drive motility and also play a central role in regulating oxidative stress. As sites of aerobic respiration, mitochondria inevitably generate electrons during ATP synthesis via the electron transport chain, leading to superoxide anion production and endogenous ROS generation [18, 19]. Spermatozoa are particularly vulnerable to oxidative damage due to their scant cytoplasm, low antioxidant enzyme levels compared with somatic cells, and plasma membrane rich in easily oxidized polyunsaturated fatty acids [20]. Excessive mitochon-

drial ROS production caused by external factors or metabolic abnormalities leads to lipid peroxidation, membrane damage, and impaired motility, while mitochondrial and nuclear DNA damage can trigger apoptosis and reduce fertilization capacity [21, 22]. Therefore, maintaining mitochondrial homeostasis and adequate energy supply without excessive oxidative damage is essential for sperm quality. In this study, resveratrol preserved ΔΨm, increased ATP production, stimulated SDH activity, and maintained mitochondrial morphology. Resveratrol also increased OGG1 mRNA expression, suggesting enhanced repair of oxidative damage to mitochondrial DNA and preservation of genomic integrity. Thus, these results indicate that mitochondria are key targets of the protective effects of resveratrol on sperm cells.

Furthermore, resveratrol demonstrated therapeutic efficacy in an in vivo mouse model of asthenozoospermia. Resveratrol improved all sperm parameters, corrected hormonal imbalances, restored testicular morphology, and showed efficacy comparable to that of the positive control. These in vivo findings further support the potential application of resveratrol in the improvement of male reproductive health. Although this study demonstrates protective effects, it also has certain limitations, including the use of only one oxidative stress model. Future studies should expand the experimental groups and employ various oxidative stress models to further validate these findings.

Resveratrol exerts protective effects through multiple targets and pathways, with mitochondrial ROS scavenging being a primary mechanism. Collectively, these findings provide strong theoretical and experimental support for the use of resveratrol as a functional food or therapeutic agent for male infertility.

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Disclosure of conflict of interest

None.

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