



Resveratrol mitigates heat stress-induced testicular injury in rats: enhancing male fertility via antioxidant, antiapoptotic, pro-proliferative, and anti-inflammatory mechanisms

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Abstract

This study investigates the protective effects of resveratrol (RSV) against heat stress (HS)-induced testicular injury in rats. Climate change has exacerbated heat stress, particularly affecting male fertility by impairing testicular function and sexual behavior. A total of 32 rats were allocated into four experimental groups: control, RSV control, HS control, and RSV + HS. The HS groups were subjected to a 43 °C water bath for 20 min to induce testicular hyperthermia, while the RSV + HS group received 20 mg/kg of RSV starting just before HS and continuing for eight weeks. Our findings reveal that HS significantly impairs male sexual behavior, evidenced by reduced mount and intromission numbers, and increased latencies. It also negatively affects the reproductive system, decreasing the weights of testes (Cohen's $d=1.8$), epididymis, and accessory sex glands, and deteriorating sperm profile parameters such as motility (Cohen's $d=2.1$), viability, and morphology. Furthermore, HS notably decreases reproductive performance in female rats, reducing litter size, live births, and conception rates. Biochemically, HS decreases activities of key antioxidant enzymes in the testes—glutathione peroxidase, superoxide dismutase, and catalase—while increasing lipid peroxidation, nitrite levels, and proinflammatory cytokines (IL-1 β and TNF- α). It also reduces serum levels of reproductive hormones like testosterone (Cohen's $d=2.0$) and 17 β -estradiol. These results were affirmed with the histopathological evaluation and the immunohistochemistry staining (Ki-67, PCNA, Bax 5, and caspase-3 protein expression). Remarkably, RSV treatment mitigated these adverse effects, restoring both physiological and biochemical parameters toward normal levels (e.g., testicular weight Cohen's $d=1.6$, sperm motility Cohen's $d=1.9$, and testosterone levels Cohen's $d=1.7$). This suggests that RSV's antioxidative, anti-inflammatory, antiapoptotic, and androgenic properties could effectively counteract the degenerative impacts of testicular hyperthermia. This highlights the potential of RSV as a therapeutic agent against climate change-induced fertility issues in males.

Keywords Apoptosis · Resveratrol · Sexual behavior · Scrotal hyperthermia · Reproductive performance · Oxidative stress

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Introduction

Temperature is one of the most changeable environmental elements that terrestrial creatures confront during their lives, and it is expected to have a significant impact on animal's physiology, behaviour, and health features (Angilletta 2009; Huey et al. 2012). The complicated and multifaceted effects of heat stress (HS) on reproduction are made worse by the mounting problems brought on by climate change. Fertility and animal welfare are two delicate metrics readily impacted by heat stress (Tattersall et al. 2012). HS causes a significant drop in testosterone levels, spermatozoa's ability to fertilize eggs, a decline in sexual behavior, and, ultimately, a decrease in the rate of reproduction. Finding workable solutions to mitigate the effects of climate change on animal welfare and fertility is imperative to protect the animals' productivity, profitability, and reproductivity (Khan et al. 2023). Numerous research studies have been conducted on the impact of environmental hazards on the reproductive system. Reproduction is one of the most crucial traits of living things that allows the survival of the species (Abdollahi et al. 2023). The testes are the primary male reproductive organs where sperm are produced. One of the most essential reproductive factors is normal spermatogenesis; a disruption in scrotal thermoregulation can result in both temporary or permanent male infertility and poor spermatogenesis (Zhang et al. 2020). When the testicles are 2–4 °C colder than the body's core temperature, normal spermatogenesis takes place. Several elements influence cellular temperature control, such as thin skin, sweat glands, the cremaster and dartos muscles, the pampiniform plexus, counter-current heat exchange between testicular veins, and arterial blood (Delkhosh et al. 2021). While climate change has exacerbated heat stress (HS) globally, rising infertility rates cannot be solely attributed to the approximately 1.5 °C increase over the past 150 years. Several medically realistic and occupational scenarios expose human testicles to heat stress, causing similar detrimental effects on male reproductive function: Occupational heat exposure: professions such as steel working, mining, agriculture, welding, and baking expose individuals to elevated environmental temperatures for prolonged periods. Combined with restrictive clothing and inadequate cooling, these occupational settings impair scrotal thermoregulation, leading to hyperthermia that adversely impacts spermatogenesis and testosterone production (Delkhosh et al. 2021). Lifestyle factors: modern habits such as prolonged laptop use on the lap, heated car seats, sedentary behavior, and tight-fitting clothing can elevate scrotal temperatures by 1–2 °C—enough to disrupt normal spermatogenesis and sperm quality (Robinson et al. 2023). Fever-induced hyperthermia: febrile illnesses, whether acute (e.g., infections) or chronic (e.g., inflammatory diseases), cause systemic

hyperthermia, which has been shown to impair testicular function and fertility (Shoorei et al. 2019). Regional heat waves: climate change has increased the frequency and intensity of heat waves in many regions. Prolonged exposure during extreme heat waves, particularly in warmer climates with limited access to cooling systems, poses a significant risk to male reproductive health (Khan et al. 2023). These scenarios emphasize that testicular heat stress is not limited to climate change but extends to occupational, lifestyle, and medical conditions. The use of scrotal heat stress in animal models, as in this study, provides a realistic approach for investigating mechanisms of heat-induced testicular injury and evaluating potential therapeutic interventions.

Some reports have reported that reactive oxygen species (ROS) have been related to scrotal HS. Although ROSs are produced in the testicles during normal oxygen metabolism and help in sperm capacitation, acrosome reaction, and other processes, an excess of ROSs induced by HS can lead to testicular germinal atrophy and spermatogenic arrest (Delkhosh et al. 2021). HS and Leydig cells are the somatic cells of the testes involved in spermatogenesis (Shoorei et al. 2019; Abdollahi et al. 2023).

Hydrogen peroxide levels in testicles have been shown to rise in response to HS exposure. Then, by reducing the activity of enzyme antioxidants such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), lipid peroxidation is increased (Houston et al. 2018; Khan et al. 2023). Conversely, germ cell death and cell cycle arrest may result from extrinsic (mitochondrial-dependent) and intrinsic (initiated in response to HS) apoptotic mechanisms (Fan et al. 2017). Additionally, nuclear factor- κ B (NF- κ B), linked to testicular inflammation and male infertility, may be activated by oxidative damage brought on by HS (Delkhosh et al. 2021).

Natural antioxidants were reported to bind to free radicals and reduce their damaging effects on cellular functioning (Hou et al. 2020). Thus, they may be employed to lessen the harmful effects of HS on the male reproductive system. Several plants, including red wine, peanuts, and grapes, are known to naturally contain resveratrol (trans-3,5,40-trihydroxystilbene, or RSV), a powerful antioxidant and polyphenolic phytoalexin (Aanyu 2016). By activating the hypothalamic-pituitary–gonadal axis, daily oral administration of RSV to healthy adult male rats boosted sperm production (Juan et al. 2005). It has been reported that RSV possesses antioxidant properties in cells that produce reactive oxygen species (ROS) and is an efficient scavenger of radicals produced by metals, hydroxyls, and superoxide.

Consequently, by serving as a metal chelator, enzyme regulator, and radical scavenger in testicular tubules, RSV may also be protective by preventing inherent oxidative stress (de Oliveira et al. 2021). It improves sperm motility,

viability, and in vivo production by blocking spermatocytes' lipid peroxidation (LPO) (Illiano et al. 2020). Additionally, it exhibits protective benefits against testicular toxicity caused by numerous anticancer medications and environmental pollutants, germ cell death in mice and rats (Lv et al. 2020). This study comprehensively examines the effects of RSV on male reproductive parameters in rats exposed to scrotal heat stress. Key parameters assessed include sexual behavior, sperm quality, reproductive hormone profiles, testicular oxidative and nitrosative stress markers, activities of steroidogenic enzymes, reproductive performance, levels of proinflammatory cytokines, histomorphometric alterations, and apoptosis markers using immunohistochemistry. To the best of our knowledge, this is the first detailed investigation of RSV's role in mitigating the effects of scrotal heat stress in male rats.

Materials and methods

Animals

In the current experiment, thirty-two male adult Wistar rats (weighing 200–220 g) at five months of age (rats were sexually mature) were housed in plastic cages (80 cm × 50 cm × 40 cm), (4 rats/cage)/2 cages per group under a controlled temperature (23 ± 2 °C), controlled humidity (50%), and a 12 h/12 h light/dark cycle with ad libitum access to food (standard laboratory pellet diet) and water throughout the experiments. Rats were obtained from the Medical Research Institute (animal breeding unit), acclimatized to laboratory conditions for two weeks. This experimental study was permitted by the Animal Care Review Committee at the Faculty of Veterinary Medicine (Ethical Committee Approval Number: 2023/013/206). The United States National Academy of Sciences' "Guide for the Care and Use of Laboratory Animals" was followed to provide the animals humane treatment, and every effort was made to lessen their suffering.

Work design

Four groups of eight rats each were formed via random assignment of the rats as follows:

- i. The rats in group 1 (normal control group) were not exposed to scrotal heat stress
- ii. The rats in group 2 (RSV group) were not exposed to scrotal heat stress and received RSV (R5010-500 mg, Sigma-Aldrich, St. Louis, MO, USA) at 20 mg/kg of bwt dose once /day by oral gavage for up to 8 weeks. Based on data from earlier research demonstrating

against testicular injury, the dose of RSV was chosen (Guo et al. 2019).

- iii. The rats in group 3 (HS control group) were exposed to scrotal heat stress
- iv. The rats in group 4 (RSV + HS group) were exposed to scrotal heat stress and given RSV as described with group 2.

Resveratrol was dissolved in normal saline. Each rat in the RSV-treated group was administered 0.5 ml of the solution daily via oral gavage, corresponding to a dose of 20 mg/kg body weight. Rats in the control and HS control groups received an equivalent volume of normal saline using the oral gavage for up to 8 weeks.

A simple randomization method was used to allocate the animals into groups. Each rat was assigned a unique identifier (1–32), and a computer-generated random sequence was produced using Microsoft Excel's random number function. This sequence was used to allocate the rats into the respective groups. The randomization process was conducted by an independent researcher who was not involved in the experimental procedures to minimize selection bias. To reduce potential bias during treatment administration and outcome assessments, personnel responsible for animal care, sample collection, and data analysis were blinded to the group allocation throughout the study (Suresh 2011).

Scrotal heat induction

The procedure used to induce scrotal hyperthermia was based on a prior study. Briefly, rats in groups 3 and 4 were first given ketamine/xylazine (10 mg/kg)/ IP injection. Then, the scrotum was palpated to check for the presence of testicles, and every other day, the scrotum and the rats' testicles were submerged in a water bath at 43 °C for 20 min for 8 weeks (Kheradmand et al. 2011; Delkhosh et al. 2021). RSV treatment began before heat exposure and continued every day for eight weeks (rat's spermatogenic cycle varied from 48 to 56 days, which is why this treatment period was selected (Guo et al. 2019)).

Sexual behavior

Sexual behavior as estimated by fertility test as follows:

Dinoprost tromethamine (LUTALYSE®) 0.1 mg/100g bwt was intraperitoneally injected into 32 female rats (Tso and Tam 1977) two times per day (in the morning and late afternoon) for synchronization of estrous. Next, a vaginal smear was performed to confirm the presence of estrous cells (irregular-shaped cornified cells). Each male present (at the beginning of the 7th week of the heat induction and the treatments) was presented to the estrous females in their plastic cage with 3–4 cm of wood shavings for bedding. The

test was video recorded for 15 min using a video camera. A vaginal smear was performed again on the following day to detect sperm, and that day was rendered to be day zero of gestation (Luna 1968). The following measurements were determined (Yakubu et al. 2007):

Mount frequency	mounts number till ejaculation
Intromission frequency	intromissions number till ejaculation
Mount latency	time from the female entrance till the first mount
Intromission latency	time from the female entrance till the first intromission
Ejaculatory latency	time from intromission till ejaculation

Sample collection and preparation

Following the course of the treatments (at the end of the 8 weeks), the rats were euthanized under a ketamine/xylazine anesthetic (7.5, 1.0 mg/kg IP). Blood samples were collected from the medial canthus of the eye via a heparinized microhematocrit tube. Testosterone and 17 β -estradiol values were determined using serum samples collected after blood centrifugation at 5000 rpm for 10 min. The testes were separated, the epididymis and accessory sex gland samples were weighed, and the right testes were homogenized to produce a 10% homogenate (w/v) in 0.1 M Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4. This was done using an ice-chilled glass homogenizing vessel in a homogenizer equipped with a Teflon pestle (Glass-Col, USA). Subsequently, the homogenate underwent a 10-min cooling centrifugation at 3000 $\times$ g and 4 °C to exclude debris and nuclei. The testicular antioxidant system (catalase, glutathione peroxidase, and superoxide dismutase) activities, oxidative/nitrosative stress markers (lipid peroxidation indicator and malondialdehyde), the activity of steroidogenic enzymes (3 β hydroxysteroid dehydrogenase, or 3 β HSD) and 17 β hydroxysteroid dehydrogenase, or 17 β HSD), and proinflammatory cytokines (interleukin 1 beta, or IL1 β) and tumor necrosis factor-alpha, or TNF α) were then measured in the supernatant, which was maintained at –80 °C. Testicular tissue from the left side (fixed in 10% neutral-buffered formalin) was utilized for histopathological and immunohistochemical analysis.

Reproductive organ index weights

The reproductive organs' index weight (IW) was established previously (Matousek 1969), including the prostate, seminal vesicles, and epididymis: $IW = 100 \times [\text{organ weight (g)}/\text{body weight (g)}]$.

Sperm characteristics

Sperm characteristics were assessed as previously described (Soliman et al. 2021). The cauda epididymis was carefully dissected, sliced into small pieces, and incised to release sperm onto a clean, wet glass slide. A total of 2 μ l of sperm was mixed with 20 μ l of sodium citrate (2.9%) to prepare the sample. Sperm motility was independently evaluated in at least four microscopic fields at a magnification of 400 $\times$, using 1 drop per slide repeated five times per rat. Sperm concentration was determined using a Neubauer hemocytometer and examined at 40 $\times$ magnification. Seminal smears were prepared on slides, stained with eosin-nigrosin, and analyzed for abnormalities in sperm morphology, including head, midpiece, and tail defects. Sperm viability was assessed using eosin-nigrosin staining, where unstained sperm were viable, and stained sperm were non-viable. Sperm motility was evaluated by placing a drop of the suspension on a pre-warmed slide and observing under a light microscope at 400 $\times$ magnification. All assessments were conducted with equipment and slides maintained at physiological conditions (37 °C) to ensure accuracy.

Reproductive performance of female rats

Reproductive performance was estimated according to Ibeh and Saxena (Ibeh and Saxena 1997), and the measurements recorded were as follows:

Total litter at birth:	the overall number of born pups.
Live litter at birth:	alive born pups number.
Dead litter at birth:	dead pups number at birth.
Birth weight (g):	each pup's weight at birth.
Conception rate:	% of pregnant females/group.

Estimation of serum reproductive hormonal levels

Testosterone and 17 β -estradiol (E2) levels were measured using ELISA kits (Catalog Nos. TE373S and ES380S, respectively) in accordance with the manufacturer's instructions (Calbiotech Inc., El Cajon, USA).

Testicular steroidogenic marker enzyme activities

The activities of 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD) in testicular tissue were determined using the method described by (Bergmeyer and Bernt 1974). After initial standardization, the enzyme experiments were carried out under the parameters of zero-order kinetics. The expression of enzyme activities was given as follows: nmol of NADPH transformed to NADP per mg protein per minute (17 β -HSD),

or nmol of NAD converted to NADH per mg protein per minute (3β -HSD).

Estimation of testicular antioxidant system and oxidative/nitrosative stress markers level

The activities of antioxidant enzymes and oxidative/nitrosative stress markers were assessed using spectrophotometric methods and commercial assay kits. Superoxide dismutase (SOD) activity was determined following the method of (Nishikimi et al. 1972), glutathione peroxidase (GPx) activity was measured as described by (Glatzle et al. 1974), and catalase (CAT) activity was evaluated using the protocol of (Bergmeyer 1974). Oxidative stress markers, including malondialdehyde (MDA), a lipid peroxidation marker, and nitric oxide (NO), were analyzed in testicular tissue homogenates. MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) assay described by (Buege 1978). Nitric oxide (NO) levels were indirectly estimated by determining nitrite concentrations using the Griess diazotization reaction (Buege 1978). All parameters, including SOD, GPx, CAT, MDA, and NO, were measured using commercial kits provided by Biodiagnostic Co. (SOD: catalog # SD25; GPx: catalog # GP10; CAT: catalog # CA25; MDA: catalog # MD25; NO: catalog # NO25), following the manufacturer's protocols. The protein content in testicular tissues was quantified to normalize enzyme activities and oxidative/nitrosative stress marker levels using following (Lowry et al. 1951).

Analysis of testicular proinflammatory cytokines

Tumor necrosis factor-alpha (TNF- α ; catalog # MBS824824) and interleukin-1 β (IL-1 β ; catalog # MBS825017) levels were measured in testicular tissue homogenates using enzyme-linked immunosorbent assay (ELISA) kits purchased from BioSource Inc. (San Diego, CA, USA). The assays were performed in accordance with the manufacturer's instructions and the protocol described by (Seriolo et al. 2006). Optical density readings were obtained using an ELISA plate reader (Bio-Rad, Hercules, CA, USA) to facilitate accurate quantification.

Histopathological examination

Specimens' preparation

At the end of the experiment, the left testis was collected and immediately fixed in 10% neutral-buffered formalin for a minimum of 24 h. The samples were then processed using a conventional paraffin-embedding technique. Tissue sections, 5- μ m thick, were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination under a light

microscope. The histopathological analysis was performed following the standard technique described by (Bancroft and Gamble 2008). To minimize investigator bias, the individual responsible for the histological and immunohistochemical assessments was blinded to group allocation throughout the analysis process. The slides were coded by a separate researcher not involved in the assessments, ensuring that the evaluator was unaware of the experimental groups during the examination.

Immunohistochemical assessment

The proliferating cell nuclear antigen (PCNA) serves as a marker for assessing germ cell proliferation and evaluating germ-cell kinetics; Ki-67 is used to detect the expression of the Bax and caspase-3 proteins, and the proliferative indexes are all detected (Seriolo et al. 2006). Positively charged slides of paraffin tissue slices from testicles were subjected to the conventional horseradish-peroxidase immunohistochemistry protocol. Testicular tissue sections, 4- μ m thick, were deparaffinized using xylene, rehydrated through a series of graded alcohol solutions, and pre-treated with 3% hydrogen peroxide (H_2O_2) to block endogenous peroxidase activity. Antigen retrieval was performed by placing the slides in 10 mM sodium citrate buffer (pH 6.0) and microwaving them for 10 min. The sections were incubated with biotin-conjugated goat anti-rat IgG antiserum for 60 min, after which they were rinsed with PBS and incubated for 30 min with streptavidin-peroxidase conjugate. Slides were then incubated with the specific primary antibody for Ki-67 (ab279653), PCNA (ab264494), Bax (ab216494), and caspase-3 (ab184787), diluted in 1% BSA/PBS pH 7.4 at a ratio of 1:100. The sections were stained microscopically with Mayer's hematoxylin after being detected with 3,3-diaminobenzidine tetra-hydrochloride substrate chromogen solution. The ocular detection of brown hue indicates positive staining.

Statistical analysis

SAS software was used to perform the statistical analysis (Sneller 1994). Testicular oxidative/nitrosative stress markers, sperm parameters, fertility performance, sexual behavior, testosterone hormone, and reproductive organ index weights were all examined using one-way ANOVA followed by Duncan's test as post-hoc analyses. It was decided to set the overall significance at $P < 0.05$. Data were displayed as the mean $\pm$ SD.

Results

Sexual behavior

Heat stress adversely affected male fertility during the fertility tests. It caused a significant reduction ($P < 0.05$) in sexual behavioral patterns compared to the control and RSV-treated rats, as summarized in Table 1. Heat stress significantly disrupted sexual behavior ($P < 0.001$), with the patterns in heat-stressed rats differing markedly from other groups. However, RSV administration significantly improved all sexual behavioral parameters, restoring them to normal levels.

Reproductive organ index weights

Scrotal heat stress caused a significant reduction ($P < 0.05$) in body weight and relative weights of reproductive organs compared to the control and RSV-treated groups. Conversely, RSV administration substantially improved these parameters, restoring them to normal physiological values, as shown in Table 2.

Sperm characteristics

Scrotal heat stress induced reproductive dysfunction, characterized by a significant reduction ($P < 0.001$) in epididymal

sperm mass, individual motility, count, and the percentage of live sperm, along with an increase in sperm abnormalities compared to the control group, as presented in Table 3. RSV treatment markedly improved sperm count, sperm mass motility, and the percentage of live sperm while reducing sperm anomalies compared to the heat-stressed group. However, these improvements were comparable to the control group values.

Reproductive performance of female rats

Scrotal heat stress led to reproductive failure, as shown in Table 4. It significantly reduced ($P < 0.05$) the conception rate, number of litters, number of live litters, and birth weight, while increasing the number of dead litters. Treatment with RSV significantly mitigated these adverse effects, improving all reproductive performance parameters.

Serum reproductive hormonal levels and testicular steroidogenic enzyme activities

As shown in Fig. 1, heat stress caused a significant reduction ($P < 0.05$) in serum testosterone and 17β -estradiol levels and the activity of testicular steroidogenic enzymes (17β -HSD and 3β -HSD) compared to the control group. RSV treatment significantly improved these hormonal levels and enzymatic

Table 1 Effect of RSV on sexual behavior during fertility test of scrotal heat-stressed male rats

Groups	Mount number	Intromission number	Mount latency (sec)	Intromission latency (sec)	Ejaculatory latency (sec)
NC	16.85 ± 2.56^a	16.17 ± 2.97^a	7.00 ± 1.29^a	7.67 ± 1.26^a	645.7 ± 71.6^a
RSV	16.87 ± 2.93^a	17.67 ± 1.48^a	8.50 ± 2.68^a	9.50 ± 2.68^a	619.2 ± 70.8^a
HS	8.00 ± 2.83^b	10.33 ± 2.23^b	22.00 ± 7.41^c	23.67 ± 7.31^c	367.5 ± 46.2^c
RSV + HS	14.33 ± 0.70^{ab}	15.00 ± 2.45^{ab}	10.67 ± 1.67^b	12.17 ± 3.28^b	560.8 ± 63.1^b
<i>P</i> value	< 0.0005	< 0.005	< 0.0001	< 0.0001	< 0.0001

NC normal control, RSV resveratrol, HS heat stress, RSV + HS resveratrol + heat stress

Values showed as mean $\pm$ standard deviation (SD)

^{a,b,c}Means in the same column without a common superscript letter differ significantly ($P < 0.05$)

Table 2 Effect of RSV on body weight and different reproductive organ index weights of scrotal heat-stressed male rats

Groups	Body weight (g)	Testes IW	Epididymis IW	Accessory IW
NC	178.75 ± 6.35^a	1.905 ± 0.17^a	0.859 ± 0.083^a	0.992 ± 0.038^a
RSV	175.50 ± 5.02^a	1.809 ± 0.09^a	0.829 ± 0.074^a	0.932 ± 0.015^a
HS	142.63 ± 4.23^c	1.217 ± 0.08^c	0.425 ± 0.023^c	0.602 ± 0.068^c
RSV + HS	158.00 ± 5.63^b	1.524 ± 0.12^b	0.635 ± 0.038^b	0.839 ± 0.049^b
<i>P</i> value	< 0.0001	< 0.005	< 0.005	< 0.005

NC normal control, RSV resveratrol, HS heat stress, RSV + HS resveratrol + heat stress, IW index weight

Values mean $\pm$ standard deviation (SD)

^{a,b}Means in the same column without a common superscript letter differ significantly ($P < 0.05$)

Table 3 Effect of RSV on sperm characteristics (sperm count, mass sperm motility %, individual sperm motility %, sperm abnormalities, and live sperm %) of scrotal heat-stressed male rats

Groups	Sperm count (million/ml)	Mass sperm motility (%)	Individual sperm motility (%)	Sperm abnormalities	Alive sperm (%)
NC	332.00 ± 13.71 ^a	93.95 ± 6.17 ^a	75.24 ± 5.12 ^a	6.54 ± 1.82 ^a	90.88 ± 3.65 ^a
RSV	333.63 ± 28.63 ^a	95.09 ± 7.48 ^a	78.16 ± 6.22 ^a	6.81 ± 1.91 ^a	90.85 ± 4.18 ^a
HS	182.25 ± 20.60 ^c	40.17 ± 3.04 ^c	30.41 ± 2.08 ^c	21.52 ± 2.14 ^c	62.75 ± 3.22 ^c
RSV + HS	210.38 ± 22.35 ^b	65.54 ± 5.12 ^b	55.51 ± 4.22 ^b	11.29 ± 1.56 ^b	75.75 ± 2.83 ^b
<i>P</i> value	< 0.0001	< 0.001	< 0.001	< 0.0001	< 0.001

NC normal control, RSV resveratrol, HS heat stress, RSV + HS resveratrol + heat stress

Values are mean ± standard deviation (SD)

^{a,b,c}Means in the same column without a common superscript letter differ significantly ($P < 0.001$)

Table 4 Effect of RSV on female rats' reproductive performance after mating with scrotal heat-stressed male rats

Groups	Total litter at birth	Live litter at birth	Dead litter at birth	Birth weight (g)	Conception rate (%)
NC	9.33 ± 1.17 ^a	9.33 ± 1.17 ^a	0.00 ± 0.00 ^a	6.76 ± 0.51 ^a	100.00 ± 00.00 ^a
RSV	9.00 ± 1.95 ^a	9.00 ± 1.95 ^a	0.00 ± 0.00 ^a	7.01 ± 0.34 ^a	98.93 ± 16.67 ^a
HS	3.50 ± 1.18 ^a	1.17 ± 0.14 ^a	2.33 ± 1.03 ^b	3.80 ± 0.16 ^c	19.67 ± 10.08 ^c
RSV + HS	7.17 ± 2.43 ^c	7.17 ± 2.43 ^c	0.00 ± 0.00 ^a	6.15 ± 0.28 ^b	88.67 ± 21.08 ^b
<i>P</i> value	< 0.0001	< 0.001	< 0.005	< 0.001	< 0.0001

NC normal control, RSV resveratrol, HS heat stress, RSV + HS resveratrol + heat stress

Values showed as mean ± standard deviation (SD)

^{a,b,c}Means in the same column without a common superscript letter differ significantly ($P < 0.05$)

activities, ameliorating the reproductive dysfunction caused by heat stress.

Testicular oxidative/nitrosative status

Heat stress significantly increased ($P < 0.05$) the levels of malondialdehyde (MDA) and nitrite, while significantly decreasing the activities of SOD, GPx, and catalase compared to the control group. RSV treatment reduced oxidative and nitrosative markers, partially restoring the antioxidant defense mechanisms in heat-stressed rats Fig. 2.

Testicular proinflammatory cytokines status

Heat stress significantly increased ($P < 0.05$) testicular proinflammatory cytokines (IL-1 β and TNF- α) compared to the control group. RSV administration effectively reduced these cytokine levels, mitigating the inflammatory response in heat-stressed rats Fig. 2.

Histopathological findings

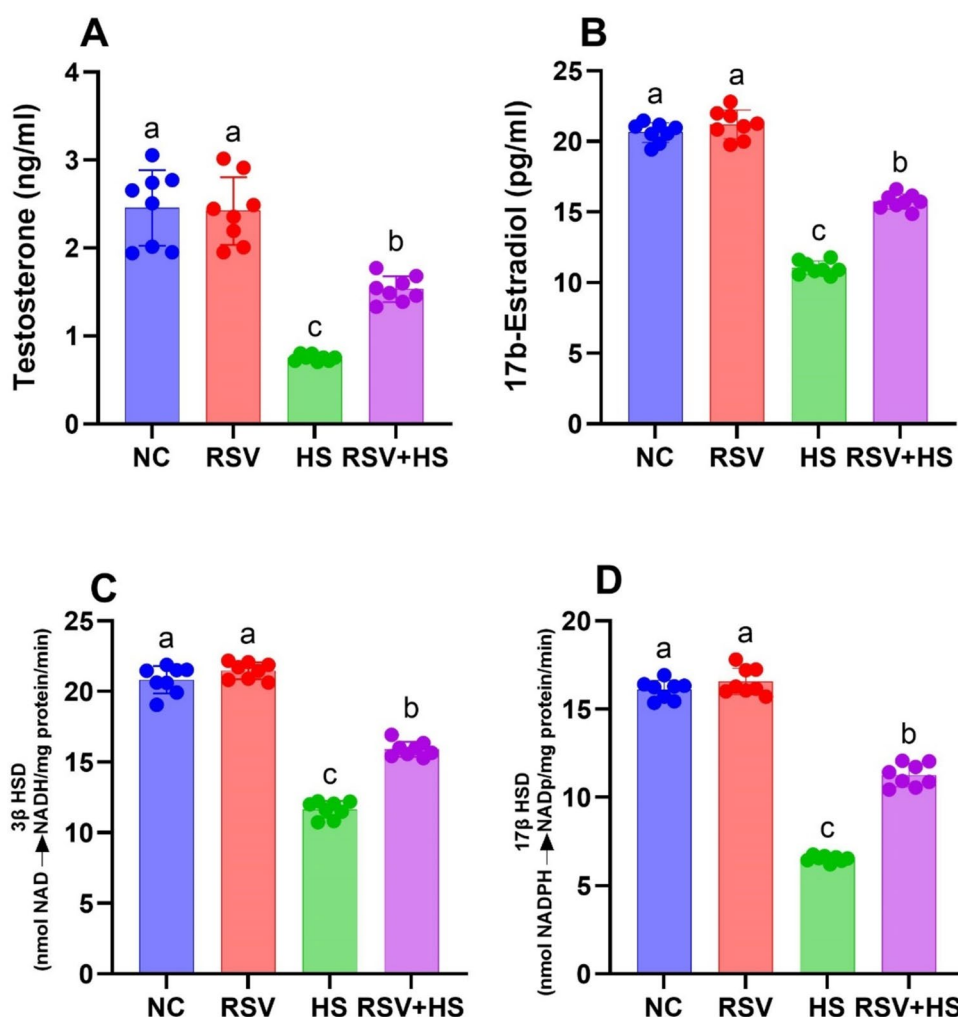
Histological examination of the testicular tissue in the control and RSV-treated groups revealed normal seminiferous

tubules and interstitium (Fig. 3a, b). In contrast, the heat-stressed group exhibited degenerative changes, including vacuolar degeneration of Sertoli cells and spermatogonia, sloughing of the germinal epithelium, necrosis, interstitial edema, and disorganized seminiferous tubules (Fig. 3c–g). Treatment with RSV significantly improved spermatogenesis and minimized histopathological lesions, although mild interstitial edema and minimal germinal epithelium degeneration were observed in some tubules (Fig. 3h, i).

Immunohistochemistry

Immunohistochemical analysis revealed strong positive Ki-67 and PCNA immunostaining in the spermatogonia and spermatocytes of the control and RSV-treated groups, with negative Bax and caspase-3 staining. Conversely, the heat-stressed group exhibited negative Ki-67 and PCNA immunostaining in germ cells and strong positive Bax and caspase-3 immunoreactivity in Leydig cells and germ cells. RSV treatment restored Ki-67 and PCNA immunoreactivity and reduced Bax and caspase-3 expression to weak levels (Fig. 4).

Fig. 1 Effect of RSV on the level of serum reproductive hormones: **A** testosterone (ng/ml) and **B** 17 β -estradiol (pg/ml). **C** Testicular activities of steroidogenic enzymes (3 β hydroxysteroid dehydrogenase (3 β HSD, nmol of NADH converted to NADH/mg protein/min) and **D** 17 β hydroxysteroid dehydrogenase (17 β HSD, nmol of NADPH converted to NADP/mg protein/min) (of scrotal-heat stressed male rats. Normal control (NC), resveratrol (RSV), heat stress (HS), resveratrol + heat stress (RSV + HS). Values are mean $\pm$ standard deviation (SD). ^{a,b,c}Means in the same column without a common superscript letter differ significantly ($P < 0.05$)



Clustering heatmap and the 3D PCA score plot

The hierarchical clustering heatmap (Fig. 5) visualized the biochemical and inflammatory profiles of samples under different conditions (NC, RSV, HS, and RSV + HS). Samples from NC and RSV groups clustered closely, indicating similar responses, while HS samples showed markedly elevated stress and inflammatory markers. RSV treatment moderated these effects, restoring the RSV + HS group closer to control levels. The 3D PCA score plot showed clear separation among groups, primarily along PCA 1, which explained 98.62% of the variance, highlighting significant biochemical differences between groups. PCA 2 and PCA 3 contributed minimally to the observed variability.

Discussion

Several environmental conditions, including heat, negatively impact spermatogenesis. Spermatogenesis works best at 2–4 °C temperature below the body's core (Robinson et al.

2023). Evidence from earlier research indicates that heat might cause a rise in DNA damage and germ cell apoptosis, which can cause oxidative stress and irreversible changes to sperm characteristics (Durairajanayagam et al. 2015). We reported disturbances in the activity of antioxidant biomarkers and sperm properties as evidenced by the reduction in the SOD, GPx, and CAT activities with increased levels of MDA and nitrite (Hou et al. 2020).

In our study, heat stress (HS) showed a significant reduction in the exhibition of sexual behavior and fertility of male rats. These may be attributed to several reports stating that heat stress causes a marked decrease in reproductive hormonal values of 17 β -estradiol and testosterone under the effect of scrotal heat stress, which was also reported by (Khan et al. 2023). This directly impacts the fertility of male rats and their libido and ability to mount other females and show normal sexual behavioral patterns. Moreover, (Houston et al. 2018) stated that a low concentration of circulating testosterone reduced sexual behavior, fertility, sperm motility, count, and weights of reproductive organs, which was also reported in our study. In those rats administered with

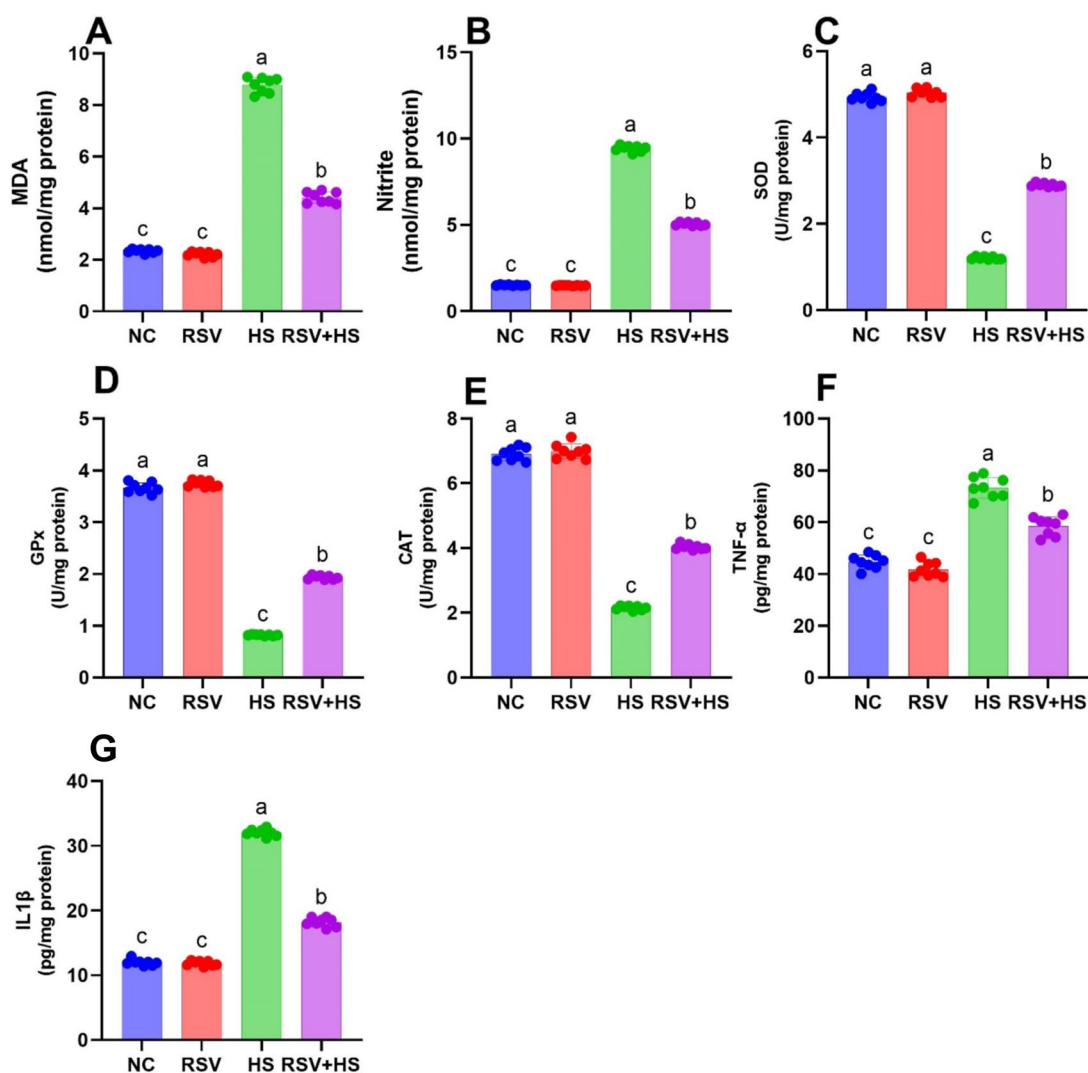


Fig. 2 Effect of RSV on testicular oxidative/nitrosative parameters: **A** malondialdehyde (MDA, nmol/mg protein), **B** nitrite (μ mol/mg protein), **C** superoxide dismutase (SOD, U/mg protein), **D** glutathione peroxidase (GPx, U/mg protein), and **E** catalase (CAT, U/mg protein). **F** Proinflammatory cytokines (tumor necrosis factor- α

(TNF- α , pg/mg protein) and **G** interleukin-1 β (IL-1 β , pg/mg protein)) of scrotal-heat stressed male rats. Normal control (NC), resveratrol (RSV), heat stress (HS), resveratrol + heat stress (RSV + HS). Values mean $\pm$ standard deviation (SD). ^{a,b,c}Means in the same column without a common superscript letter differ significantly ($P < 0.05$)

RSV, the decrease in exhibition of sexual behavior and fertility was significantly recovered; these findings are due to the protective action of RSA on the body's sex hormones, which improve sperm count and adjust sperm production (de Oliveira et al. 2021) and its role as an antioxidant in contradiction of oxidative stress (Lv et al. 2020).

In the current investigation, various characteristics of reproductive dysfunctions in rats induced by HS have been validated, along with the administration of RSV intragastrically for 56 days during the spermatogenic cycle. The considerable reductions in body weight, testicular weight, epididymal weight, and accessory sex gland weight observed in the HS group in our data is strongly associated with

degenerative and necrotic changes in the reproductive organs (Delkhosh et al. 2021).

Rats with RSV treatment showed a considerable recovery in the weight loss of their reproductive organs and testicles. Notably, histopathological images in the HS group showed that HS caused various necrotic and degenerative changes. In contrast, the administration of RSV induced repaired morphology patterns. These findings are further validated by (Cheng et al. 2019), who reported that light microscopy of testicular tissue during scrotal hyperthermia revealed shrunken, disordered, and buckling seminiferous tubules with single or multiple cell layers. (Kopalli et al. 2017) Kopalli et al. (2017) suggested that the decreased testicular

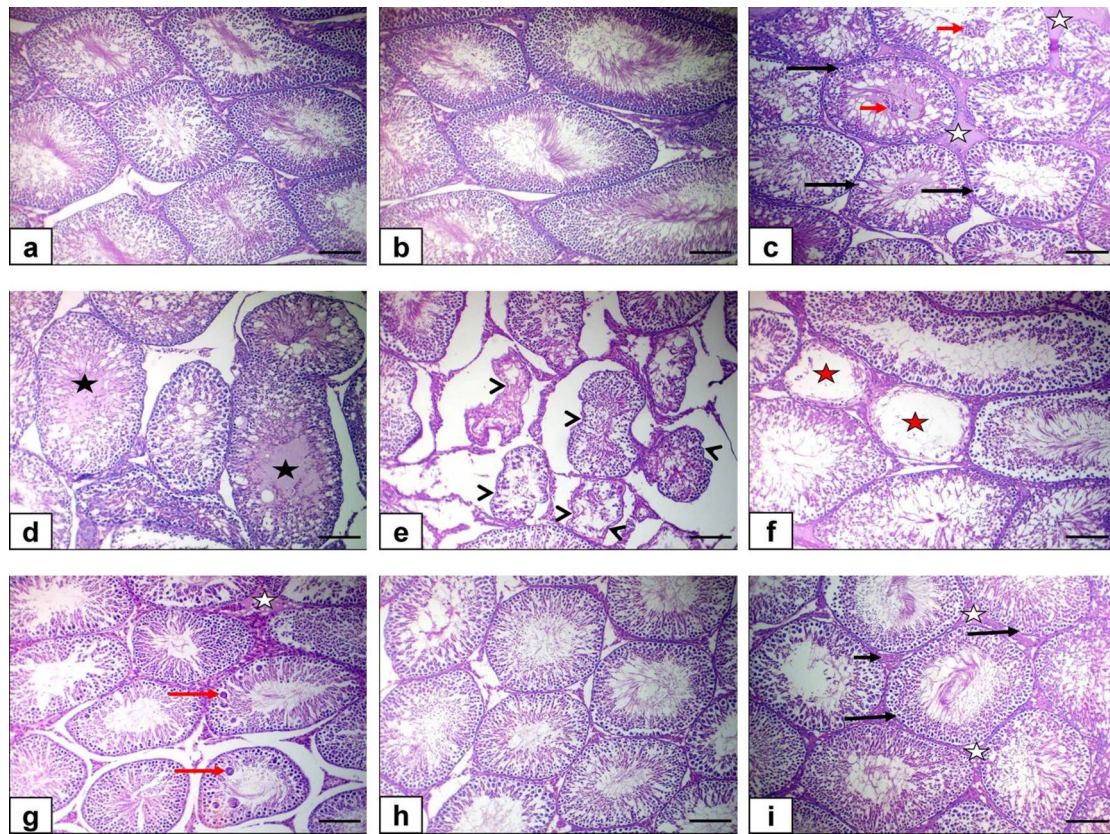


Fig. 3 Photomicrographs of rat testicular tissue sections stained with H&E: **a** control group showing normal testicular histo-architecture; **b** RSV-treated group showing normal testicular histo-architecture; **c–g** scrotal heat stress (HS) group showing vacuolar degeneration of the germinal epithelium and Sertoli cells (long black arrows), sloughing of the germinal epithelium into the lumen of seminiferous tubules (short red arrows), faint eosinophilic albuminous interstitial edema (white star), hyalinization of the luminal contents (black star),

shrunk, buckled, disorganized seminiferous tubules (arrowheads), and small-sized seminiferous tubules with single- or double-cell layers (red stars) containing giant cell formations (long red arrows) in the lumen; and **h, i** RSV + HS group showing marked improvement in spermatogenesis with only a few seminiferous tubules displaying vacuolar degeneration of the germinal epithelium (long black arrows), congestion of interstitial blood vessels (short black arrows), and mild interstitial edema (white star). Scale bar = 100 μ m

weight under heat stress conditions may result from degenerative testicular injury.

In contrast, the RSV-treated group experienced a reduction in these histological alterations. Remarkably, the RSV group had the usual histological characteristics, suggesting that RSV alone did not adversely affect the testicular tissue. Gao et al. (Gao et al. 2012) concluded that heat stress can significantly reduce testicular and epididymal weight over time, a finding that aligns with the testicular histological results observed in our investigation. Histological staining revealed a reduction in the number of cells and thickness of seminiferous tubules caused by heat stress, a decrease in the life span of primary spermatocytes, and a gradual degradation of round spermatids (Abdollahi et al. 2023). In contrast, the RSV-treated group experienced a reduction in these histological alterations. Remarkably, the RSV group had the usual histological characteristics, suggesting that RSV alone did not adversely affect the testicular tissue (Illiano et al. 2020).

Elevated temperatures may escalate the degree of oxidative stress in living organisms and induce modifications to the microenvironment, hormone levels, oxygen metabolism, and enzyme activity within the testis. These outcomes may culminate in feeble sperm, oligozoospermia, and more spermatogenic illnesses (Robinson et al. 2023). We revealed that hyperthermia markedly reduced spermatozoa count, percentage of live sperm, mass, and individual motility in rats while increasing the percentage of sperm abnormalities. These reproductive dysfunctions could be due to the reduced level of testosterone hormone, which was affected by the disruption of the oxidative/nitrosative balance induced by heat stress (Houston et al. 2018). RSV was found to help with spermatogenic dysfunction brought on by heat stress since it significantly improved these biomarkers.

Moreover, in our study, heat stress shows a significant adverse effect on the reproductive performance of female rats after mating with heat-stressed male rats, as it causes a substantial reduction in the conception rate of females and a

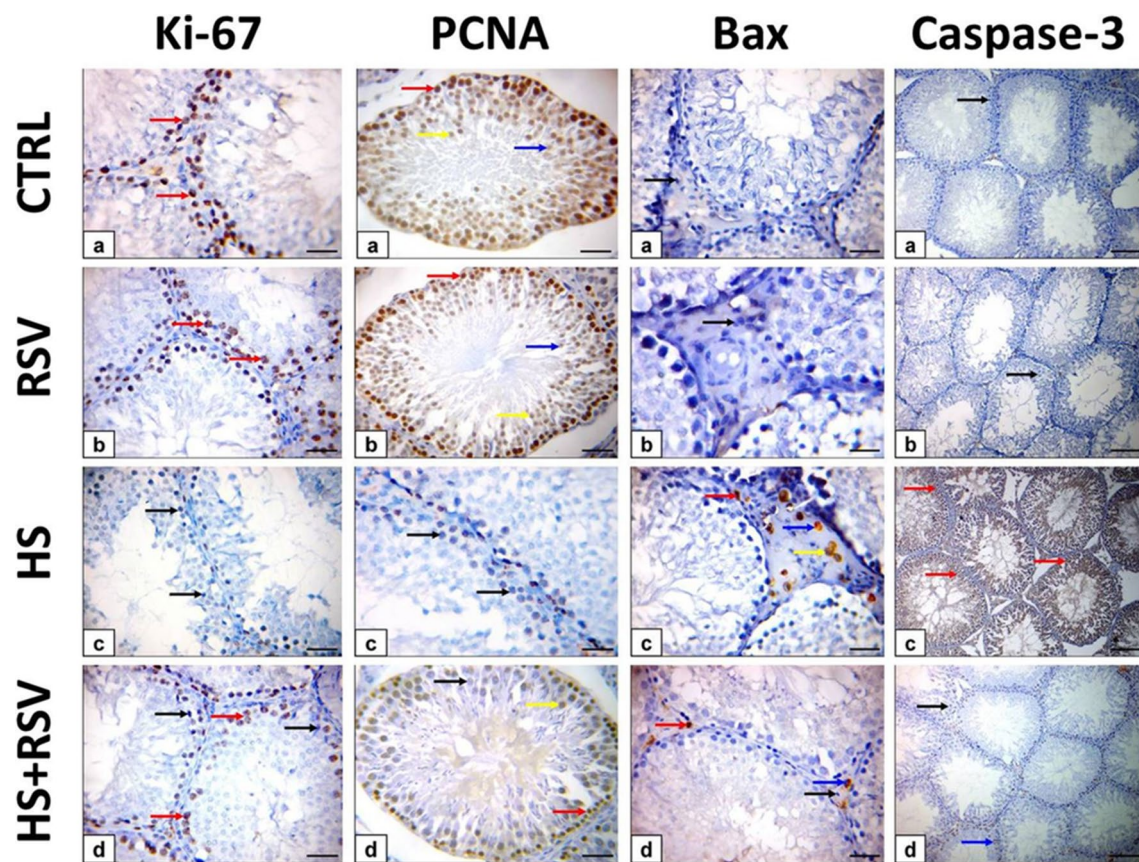


Fig. 4 Immunohistochemically stained rat testis sections showing protein expression of Ki-67, PCNA, Bax, and caspase-3. **a** Control (CTRL) group rats showed strong positive immunoreaction in the nuclei of spermatogonia and spermatocytes for Ki-67 and PCNA and negative immunoreaction for Bax and caspase-3. **b** The resveratrol (RSV)-treated group showed a similar pattern to the control group with strong positive immunoreaction for Ki-67 and PCNA and negative staining for Bax and caspase-3. **c** Heat stress (HS) group show-

ing negative immunoreaction (black arrows) for Ki-67 and PCNA and strong positive immunoreaction (red arrows) in the cytoplasm of Leydig cells for Bax and in germ cells for caspase-3. **d** HS + RSV-treated group showed weak immunoreaction (blue arrows) for Ki-67 and PCNA and reduced positive immunoreaction for Bax and caspase-3 compared to the HS group—scale bars: 20 μ m for all images except caspase-3, where the scale bar is 100 μ m

decrease in number of litters and their birth weight and the mortality rate of litters after birth. These results are caused by the significant reduction of spermatogenesis caused by heat stress (Abdollahi et al. 2023) and also causing oligozoospermia, weak sperm, further spermatozoa dysfunctions and abnormalities of sperm character (Houston et al. 2018), and low spermatoc count in semen samples (Hou et al. 2020), which led to a lower conception rate of females and adverse effect on the litter's number, weight, and mortality rate when they were born. RSV substantially enhanced all these adverse effects; consequently, it was established that RSV could enhance spermatogenic disorders and sperm characteristics triggered by hyperthermia due to its antioxidant role (Ullah et al. 2020). That's why RVS improves the conception rate of females and significantly increases the number of litters, live litters, and birth weight of litters, and there were no mortality rats with RVS-treated groups.

Numerous processes, including the heat shock response and the oxidative stress reaction, have been observed in the testes under heat stress conditions. According to (Illiano et al. 2020), one of the main causes of sperm oxidative damage is reactive oxygen species (ROS) produced by metabolism. Peroxides are delivered by the body's reaction to high temperatures. An infertile sperm can result from lipid peroxidation of the sperm membrane, which damages the spermatozoa's development, growth, morphology, and quantity; this happens when the body produces more ROS than it can remove (Shoorei et al. 2019). According to our findings, scrotal HS raised the levels of MDA and nitrite in the rats' testicles while lowering SOD, GPx, and CAT activities. An indicator of the state of oxidative stress and lipid peroxidation has been thought to be a high level of MDA. The nitrite level is measured to indicate the nitric oxide quantity that represents the nitrosative stress state (Hafeez et al. 2020).

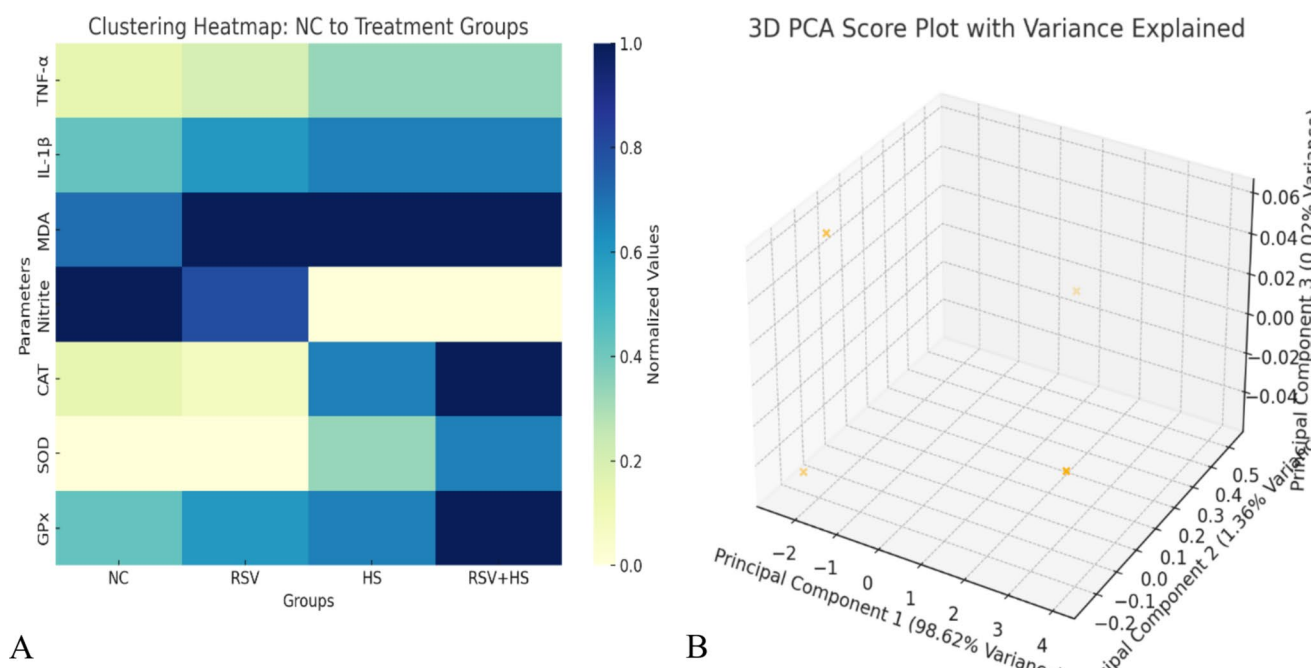


Fig. 5 **A** The clustering heatmap visualizes the data sets, with each color-coded cell indicating the concentration levels of the parameters (MDA, Nitrite, SOD, GPx, CAT, TNF- α , and IL-1 β). Columns represent the treatment groups (NC, RSV, HS, and RSV+HS), while rows correspond to the variable averages. On the gradient scale, darker colors indicate lower values, whereas lighter colors represent higher

values, allowing clear differentiation of the experimental groups. **B** The 3D principal component analysis (PCA) score plot illustrates the separation of experimental groups based on the first three principal components, effectively discerning the differences in biochemical profiles among the groups

Moreover, testing the MDA level could be used to assess the degree of testicular tissue injury. A reduction in endogenous antioxidant enzymes (such as CAT, GPx, and SOD) and the stimulation of testicular cell death are caused by increased lipid peroxidation, which also generates free radicals such as hydroxyl, superoxide, and hydroperoxyl radicals (Delkhosh et al. 2021). GSH-Px can restore the physiological state of unsaturated fatty acids on the cell membrane, and catalase helps SOD in the tissues to eliminate the hydrogen peroxide produced. SOD may also decrease the apoptosis of spermatogenic cells and contribute to male infertility (Shoori et al. 2019). In contrast, higher MDA, nitrite, and lower antioxidant enzyme levels were recovered to almost control levels in rats treated with HS and RSV. In a review, RSV was shown to lower oxidative/nitrosative stress indicators in the male reproductive system (Mongioi et al. 2021).

Resveratrol has a well-established protective effect against oxidative stress on the testes and epididymis (Lv et al. 2020), given that resveratrol has a role as an electron donor, free radical scavenger, and antioxidant (Ullah et al. 2020). It has been previously shown that resveratrol's antiapoptotic and peroxidative properties helped to lessen the testicular damage caused by doxorubicin in rats (Türedi et al. 2015). In humans and experimental animals, resveratrol administration enhanced the testicular antioxidant

defense system and epididymal sperm characteristics (Said et al. 2021). Furthermore, studies have demonstrated that resveratrol increases sperm production and protects sperm from apoptosis brought on by physiological injury (Mohammadzadeh et al. 2020). Thus, our study's findings showing the discrepancy between lipid peroxidation (MDA) and antioxidant enzymes (SOD, GPx, and CAT) suggest that RSV, via its antioxidant ability, is probably going to be essential for maintaining prooxidant-antioxidant equilibrium.

The hypothalamus-pituitary-gonadal axis can influence spermatogenic activity, and sperm generation and concentration are regulated by sex hormones in the body (de Oliveira et al. 2021). Testosterone can change into estradiol to bind to the estrogen receptor; testicular functions depend on estradiol, which is involved in the growth and differentiation of germ cells into mature spermatids (Kim et al. 2017). Testosterone controls sperm generation by binding to the AR in Sertoli cells and actively moving or diffusing into the vas deferens. Moreover, testosterone can support gonadal organ development, spermatozoa maturation in the epididymis, and preservation of normal physiological function (Hou et al. 2020). Testicular steroidogenic enzyme activities (3 β HSD and 17 β HSD) and blood levels of reproductive hormones (17 β -estradiol and testosterone) were considerably decreased by HS. In contrast,

the RSV-treated HS group had significantly greater levels of the abovementioned parameters. While the suprarenal glands in both sexes can produce modest amounts of testosterone, Leydig cells in the testicles of males secrete a substantial amount of it (Nishimura and L'Hernault 2017). On the other hand, in Leydig cells, steroidogenesis starts when cholesterol enters the mitochondrial inner membrane through the steroidogenic acute regulatory (StAR) protein. The cytochrome P450 cholesterol side-chain cleavage enzyme (P450_{scc}) in the mitochondria converts cholesterol to pregnenolone. Subsequently, 3 β -HSD, 17 α -hydroxylase/C17-20 lyase (P450_{c17}), and 17 β -hydroxysteroid dehydrogenase sequentially convert pregnenolone into progesterone, 17 α -hydroxyprogesterone, androstenedione, and testosterone, respectively (Payne and Hales 2004). Nishimura and L'Hernault (2017) further reported that the accurate synthesis of testosterone is critically dependent on the activities of the steroidogenic enzymes 17 β -HSD and 3 β -HSD.

Curcumin, an antioxidant, was shown in one study to regulate the 3 β -HSD gene and prevent HS-induced damage in Leydig cells, hence increasing androgen production, which is necessary for healthy spermatogenesis (Lin et al. 2015). Thus, these enzymes (17 β -HSD and 3 β -HSD) were employed in our investigation to examine a few of the pathways in testosterone synthesis. To discover how RSV stops the reduction in testosterone values under the HS condition, we also recommend that future research look at the other routes involved in testosterone secretion.

Regarding TNF- α and IL-1 β levels, we revealed that the HS group administered with RSV had lower levels than the untreated group. In contrast, the HS group substantially increased these proinflammatory markers compared to the control group. Testicular dysfunction may develop after increased proinflammatory cytokines despite these being essential for maintaining the testicles' equilibrium (de Oliveira et al. 2021)). In rats subjected to scrotal heat stress, elevated TNF- α and IL-1 β levels can reduce blood testosterone levels (Delkhosh et al. 2021). According to Abdollahi et al. (2023), this leads to oxidative imbalances, resulting in abnormalities in spermatogenesis, reduced sperm quality, and infertility.

It also enhances the migration of leukocytes into the reproductive system. An in vitro investigation showed that TNF- α or IL-1 β treatment inhibited steroidogenesis in Leydig cells by downregulating the expression of genes involved in testosterone production, StAR, 3 β -HSD, P450_{scc}, and P450_{c17} (Nishimura and L'Hernault 2017). These findings appear to have a possible role in our study's decline in testosterone levels. Conversely, we propose that RSV reduces TNF- α or IL-1 β levels to prevent inflammation. Nevertheless, more research is needed to fully assess the anti-inflammatory benefits of RSV (Mohammadzadeh et al. 2020).

Immunohistochemistry selected Ki-67 and germ cell proliferation (PCNA) expression as bio-indicators to assess cell proliferation in rat testes under heat stress in vivo. Heat stress inhibited intra-seminiferous tubule cell growth but increased interstitial cell proliferation, according to Ki-67 cell proliferation marker staining (Nguyen-Thanh et al. 2022). Proliferation-associated peptide PCNA, a marker of the G1 and S phases of the cell cycle, was found to be another essential element in the current investigation. Heat exposure may considerably lower the mean proportion of spermatocytes and immunostained spermatogonia against PCNA. PCNA was severely depleted in the spermatocytes of the HS group due to the simultaneous relative up-regulation of Bax in these cells (Kim et al. 2017). Similar findings were found in an increased testicular temperature model in humans, where the expression of PCNA was significantly downregulated in varicocele (up to 70%) compared to the control (Nguyen-Thanh et al. 2022). Significantly, RSV increased the levels of PCNA and Ki-67 in both spermatogonia and spermatogenic cells (Khan et al. 2023). Compared to HS rats, the RSV-treated heat stress animals exhibited a reduction in Bax substance level, which was closely correlated with increased PCNA expression. This led to faster regeneration of seminiferous tubule germ cells, which accelerated testicular recovery and the resuming of spermatogenesis (Guo et al. 2019).

It has long been understood that elevated testicular temperature causes apoptosis rather than necrosis and that this process can be triggered by reactive oxygen species (ROS), the translocation of the proapoptotic factors. The translocation of Bax and caspase-3 from the cytoplasm to the perinuclear region, or the release of cytochrome c from mitochondria (Hikim et al. 2003). (Fan et al. (2017) reported that heat-induced testicular damage in germ cells is primarily mediated by the apoptotic peptides Bax and caspase-3. Given that Bax and caspase-3 are crucial factors in determining cell fate, we thus postulated that RSV might be involved in their modulation (Houston et al. 2018). The current investigation suggests that RSV administration may considerably lower the expression of the proteins Bax or caspase-3 in the Leydig and germ cells. The data obtained further indicate that RSV can help reduce the adverse effects of heat exposure on the testicles, as it may mitigate heat-induced apoptotic events in spermatogenic cells, which are the most heat-sensitive cells (Cheng et al. 2019). The current study is likely the first among current publications. To our knowledge, no data compares the RSV influence on the heat-induced apoptotic effects in the testes.

Conclusion

Resveratrol effectively mitigates the detrimental effects of scrotal heat stress on various aspects of male reproductive health, including sexual behavior, sex hormone

regulation, inflammation, oxidative and nitrosative stress, apoptosis, and overall reproductive performance. The findings from our study demonstrate that RSV therapy not only suppresses the harmful impacts of prolonged heat exposure but also restores normal physiological functions. By reducing oxidative and nitrosative stress markers and enhancing the activity of antioxidant enzymes such as SOD, GPx, and CAT, RSV strengthens the testicular antioxidant defense system. Additionally, it decreases proinflammatory cytokines (IL-1 β and TNF- α), thereby attenuating inflammation-induced damage in the testis. Our histological and immunohistochemical analyses further confirm that RSV prevents heat-induced degeneration of testicular tissue by preserving the integrity of seminiferous tubules and reducing the expression of apoptotic markers like Bax and caspase-3. Moreover, RSV significantly improves serum testosterone and estradiol levels, as well as the activity of key steroidogenic enzymes (17 β -HSD and 3 β -HSD), which are critical for testosterone synthesis. Importantly, RSV treatment restores sexual behavior and sperm quality to near-normal levels, enhancing sperm motility, viability, and count while reducing abnormalities. In summary, our study verifies that RSV, as a potent antioxidant and anti-inflammatory agent, can effectively alleviate the adverse effects of thermal stress on the male reproductive system. These findings suggest that RSV could serve as a therapeutic intervention to protect against heat-induced testicular dysfunction and infertility. Further investigations are recommended to explore its potential clinical applications.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed MHH, MS, SEE, HGT and MSE. The first draft of the manuscript was written by MHH, HIG, SEE and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Data availability All source data for this work (or generated in this study) are available upon reasonable request.

Declarations

Ethics approval This experimental study was permitted by the Animal Care Review Committee at the Faculty of Veterinary Medicine (Ethical Committee Approval Number: 2023/013/206). The United States National Academy of Sciences' "Guide for the Care and Use of Laboratory Animals" was followed to provide the animals humanely treatment, and every effort was made to lessen their suffering.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

References

- Aanyu M (2016) Effects of phytochemical compounds on growth and nutritional physiology of Nile tilapia (*Oreochromis niloticus*) [Ph. D thesis]. University of Stirling, p 110
- Abdollahi F, Amanpour S, Muhammadnajad A, Barzegar F, Dehghan SF (2023) Testicular histopathology in rats co-exposed to heat and psychological stressors. *Heliyon* 9:e14146
- Angilletta MJ (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford Univ. Press, Oxford
- Bancroft JD, Gamble M (2008) Theory and practice of histological techniques, 5th edn. Churchill Livingstone, New York
- Bergmeyer H (1974) Methods of enzymatic analysis, 2nd edn. Academic Press, Cambridge
- Bergmeyer HU, Bernt E (1974) UV-assay with pyruvate and NADH. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 2nd edn. Academic Press, Cambridge, pp 574–579
- Buege JA (1978) Microsomal lipid peroxidation. *Methods Enzymol* 52:302–310
- Cheng K, Yan E, Song Z, Li S, Zhang H, Zhang L, Wang C, Wang T (2019) Protective effect of resveratrol against hepatic damage induced by heat stress in a rat model is associated with the regulation of oxidative stress and inflammation. *J Therm Biol* 82:70–75
- Delkhosh A, Shoorei H, Niazi V, Delashoub M, Gharamaleki MN, Ahani-Nahayati M, Dehaghi YK, Raza S, Taheri MH, Mohaqiq M (2021) Coenzyme Q10 ameliorates inflammation, oxidative stress, and testicular histopathology in rats exposed to heat stress. *Hum Exp Toxicol* 40:3–15
- Durairajanayagam D, Agarwal A, Ong C (2015) Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online* 30:14–27
- Fan X, Xi H, Zhang Z, Liang Y, Li Q, He J (2017) Germ cell apoptosis and expression of Bcl-2 and Bax in porcine testis under normal and heat stress conditions. *Acta Histochem* 119:198–204
- Gao J, Zuo Y, So K-H, Yeung WS, Ng EH, Lee K-F (2012) Electroacupuncture enhances spermatogenesis in rats after scrotal heat treatment. *Spermatogenesis* 2:53–62
- Glatzle D, Vuilleumier J, Weber F, Decker K (1974) Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. *Experientia* 30:665–667
- Guo Y, Wang A, Liu X, Li E (2019) Effects of resveratrol on reducing spermatogenic dysfunction caused by high-intensity exercise. *Reprod Biol Endocrinol* 17:1–11
- Hafeez M, Shaheen R, Akram B, Haq S, Mahsud S, Ali S, Khan RT (2020) Green synthesis of cobalt oxide nanoparticles for potential biological applications. *Mater Res Express* 7:025019
- Hikim APS, Lue Y, Yamamoto CM, Vera Y, Rodriguez S, Yen PH, Soeng K, Wang C, Swerdloff RS (2003) Key apoptotic pathways for heat-induced programmed germ cell death in the testis. *Endocrinology* 144:3167–3175
- Hou Y, Yuan P, Fu Y, Zhang Q, Wei Y, Gao L, Liu L, Wang X, Zheng X, Feng W (2020) Duzhong butiansu prescription improves heat stress-induced spermatogenic dysfunction by regulating sperm formation and heat stress pathway. *Evid Based Complement Alternat Med* 2020:6723204
- Houston BJ, Nixon B, Martin JH, De Iuliis GN, Trigg NA, Bromfield EG, McEwan KE, Aitken RJ (2018) Heat exposure induces oxidative stress and DNA damage in the male germ line. *Biol Reprod* 98:593–606
- Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE (2012) Predicting organismal vulnerability to climate

- warming: roles of behaviour, physiology and adaptation. *Philos Trans R Soc B Biol Sci* 367:1665–1679
- Ibeh I, Saxena D (1997) Aflatoxin B1 and reproduction: I. Reproductive performance in female rats. *Afr J Reprod Health* 1:79–84
- Illiano E, Trama F, Zucchi A, Iannitti RG, Fioretti B, Costantini E (2020) Resveratrol-based multivitamin supplement increases sperm concentration and motility in idiopathic male infertility: a pilot clinical study. *J Clin Med* 9:4017
- Juan MEL, González-Pons E, Munuera T, Ballester J, Rodríguez-Gil JE, Planas JM (2005) trans-Resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J Nutr* 135:757–760
- Khan I, Mesalam A, Heo YS, Lee S-H, Nabi G, Kong I-K (2023) Heat stress as a barrier to successful reproduction and potential alleviation strategies in cattle. *Animals* 13:2359
- Kheradmand A, Dezfoulian O, Tarrahi MJ (2011) Ghrelin attenuates heat-induced degenerative effects in the rat testis. *Regul Pept* 167:97–104
- Kim MK, Cha K-M, Hwang S-Y, Park U-K, Seo SK, Lee S-H, Jeong M-S, Cho S, Kopalli SR, Kim S-K (2017) Pectinase-treated Panax ginseng protects heat stress-induced testicular damage in rats. *Reproduction* 153:737–747
- Kopalli SR, Cha K-M, Lee S-H, Ryu J-H, Hwang S-Y, Jeong M-S, Sung J-H, Kim S-K (2017) Pectinase-treated Panax ginseng protects against chronic intermittent heat stress-induced testicular damage by modulating hormonal and spermatogenesis-related molecular expression in rats. *J Ginseng Res* 41:578–588
- Lin C, Shin D-G, Park SG, Chu SB, Gwon LW, Lee J-G, Yon J-M, Baek I-J, Nam S-Y (2015) Curcumin dose-dependently improves spermatogenic disorders induced by scrotal heat stress in mice. *Food Funct* 6:3770–3777
- Lowry O, Rosebrough N, Farr AL, Randall R (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Luna LG (1968) Manual of histologic staining methods of the Armed Forces Institute of Pathology. Manual of histologic staining methods of the Armed Forces Institute of Pathology. pp xii, 258–xii, 258
- Lv Z-M, Ling M-Y, Chen C (2020) Comparative proteomics reveals protective effect of resveratrol on a high-fat diet-induced damage to mice testis. *Syst Biol Reprod Med* 66:37–49
- Matousek J (1969) Effects on spermatogenesis in guinea-pigs, rabbits and sheep after their immunization with sexual organ fluids of bulls. *Reproduction* 19:63–72
- Mohammadzadeh M, Ramezani V, Hamishehkar H, Marvast LD, Mangoli E, Rajabi M, Sales ZA, Talebi AR (2020) Does resveratrol affect prepared sperm parameters and chromatin quality in normozoospermic and asthenozoospermic patients before and after freezing? A lab trial study. *Int J Reprod BioMed* 18:755
- Mongioi LM, Perelli S, Condorelli RA, Barbagallo F, Crafa A, Cannarella R, La Vignera S, Calogero AE (2021) The role of resveratrol in human male fertility. *Molecules* 26:2495
- Nguyen-Thanh T, Dang-Van P, Dang-Ngoc P, Kim W, Le-Minh T, Nguyen-Vu Q-H (2022) Chronic scrotal heat stress causes testicular interstitial inflammation and fibrosis: an experimental study in mice. *Int J Reprod BioMed* 20:569
- Nishikimi M, Rao NA, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 46:849–854
- Nishimura H, L'Hernault SW (2017) Spermatogenesis. *Curr Biol* 27:R988–R994
- de Oliveira SA, Cerri PS, Sasso-Cerri E (2021) Impaired macrophages and failure of steroidogenesis and spermatogenesis in rat testes with cytokines deficiency induced by diacerein. *Histochem Cell Biol* 156:561–81. <https://doi.org/10.1007/s00418-021-02023-7>
- Payne AH, Hales DB (2004) Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev* 25:947–970
- Robinson BR, Netherton JK, Ogle RA, Baker MA (2023) Testicular heat stress, a historical perspective and two postulates for why male germ cells are heat sensitive. *Biol Rev* 98:603–622
- Said NI, Abd-Elrazek AM, El-dash HA (2021) The protective role of resveratrol against sulfoxafloer-induced toxicity in testis of adult male rats. *Environ Toxicol* 36:2105–2115
- Seriolo B, Paolino S, Sulli A, Ferretti V, Cutolo M (2006) Bone metabolism changes during anti-TNF- α therapy in patients with active rheumatoid arthritis. *Ann N Y Acad Sci* 1069:420–427
- Shoorei H, Khaki A, Khaki AA, Hemmati AA, Moghimian M, Shokoohi M (2019) The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. *Biomed Pharmacother* 111:568–578
- Sneller CH (1994) SAS programs for calculating coefficient of parentage. *Crop Sci* 34:1679–1680
- Soliman MM, Aldhahrani A, Gaber A, Alsanie WF, Shukry M, Mohamed WA, Metwally MM, Mohamed AA (2021) Impacts of n-acetyl cysteine on gibberellic acid-induced testicular dysfunction through regulation of inflammatory cytokines, steroid and antioxidant activity. *Andrologia* 53:e14036
- Suresh K (2011) An overview of randomization techniques: an unbiased assessment of outcome in clinical research. *J Hum Reprod Sci* 4:8–11
- Tattersall GJ, Sinclair BJ, Withers PC, Fields PA, Seebacher F, Cooper CE, Maloney SK (2012) Coping with thermal challenges: physiological adaptations to environmental temperatures. *Compr Physiol* 2:2151–2202
- Tso E-F, Tam W (1977) The effect of continuous treatment with prostaglandin F-2 α on oestrous cycle length and corpus luteum regression in hysterectomized guinea-pigs. *Reproduction* 50:335–336
- Türedi S, Yuluğ E, Alver A, Kutlu Ö, Kahraman C (2015) Effects of resveratrol on doxorubicin induced testicular damage in rats. *Exp Toxicol Pathol* 67:229–235
- Ullah S, Mustafa S, Ennab W, Muhammad J, Shafiq M, Kavita NM, Z-p LÜ, D-g MAO, F-x SHI (2020) A protective role of resveratrol against the effects of immobilization stress in corpora lutea of mice in early pregnancy. *J Integr Agric* 19:1857–1866
- Yakubu MT, Akanji MA, Oladiji AT (2007) Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *Pharmacogn Rev* 1:49–56
- Zhang P, Zheng Y, Lv Y, Li F, Su L, Qin Y, Zeng W (2020) Melatonin protects the mouse testis against heat-induced damage. *Mol Hum Reprod* 26:65–79

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