Zagazig University Medical Journal
www.zumj.journals.ekb.eg
Volume 29, Issue 1, January 2023, Page (266-276) Supplement Issue

Manuscript ID ZUMJ-2207-2607 (R3)

DOI **10.21608/zumj.2022.152238.2607**

ORIGINAL ARTICLE

Protective Effect of Resveratrol against Testicular Dysfunction Induced by Forced Swimming Exercise in Adult Rats

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Submit Date 2022-07-24
Revise Date 2022-10-11
Accept Date 2022-11-08

ABSTRACT

Background: The effect of vigorous exercise on testicular function is poorly understood. This work aims to identify the resveratrol influence on the altered reproductive function induced by forced exercise and to screen the relevant possible mechanisms.

Methods: The study comprised control, forced exercise (FE), and resveratrol treatment groups carried out on a total of 24 adult male rats. After 8 weeks of the exercise, the pituitary and testicular hormones, corticosterone, tumor necrosis factor (TNF-α), IL-6, epididymal sperm parameters, testicular oxidant–antioxidant markers, testicular ornithine decarboxylase (ODC) activity, polyamines, testicular histopathology, and immunohistochemistry for apoptotic reactions were all measured.

Results: The FE group showed significant reductions in the pituitary and testicular hormones, sperm count, and motility, while corticosterone, TNFα, IL-6 serum levels, and aberrant forms of sperms were significantly increased. Testicular tissue displayed abnormal architecture, apoptotic changes with a significantly increased malondialdehyde level, ODC activity and polyamines, and a reduction in superoxide dismutase activity. In comparison to the FE group, administration of resveratrol significantly improved the gonadal hormones, corticosterone, TNF-α, IL-6 levels, sperm parameters, oxidant–antioxidant system, ODC activity, and polyamines with an enhancement of testicular architecture and apoptosis.

Conclusion: Resveratrol was effective in attenuating testicular dysfunction caused by forced swimming exercise in rats.

Keywords: resveratrol, testicular dysfunction, forced exercise.

INTRODUCTION

The intensity of exercise among athletes has increased in recent years, along with the level of competitive exercise. Adaptive changes, such as an increase in the number of mitochondria in muscle cells and capillaries in the muscles, are also permitted by moderate exercise, which enhances cardiopulmonary performance. On the other hand, prolonged high-intensity exercise may cause athletes to have clinical or subclinical health issues [1]. According to previous researches, exercise-hypogonadal male disease and reduced spermatogenic function are the results of long-term high-intensity exercise [2]. Several studies have hypothesized that high-intensity exercise-induced reproductive failure is related to the hypothalamic-pituitary-gonadal regulatory axis, even if the precise physiological mechanism behind this association is unknown [3].

Additionally, the testis is one of the most susceptible organs to oxidative stress, which raises the formation of reactive oxygen species (ROS), during periods of high exercise. The male reproductive system may then become dysfunctional, which has a major impact on testicular steroidogenesis and spermatogenesis [4]. Putrescine, spermidine, and spermine are polyamines, organic cations that are essential for cell proliferation, survival, and apoptosis. Polyamines regulate a variety of biological processes, including nucleic acid synthesis, protein synthesis, and cell signaling [5]. Ornithine decarboxylase (ODC) is the enzyme that catalyzes the important rate-limiting step in the polyamine production pathway. Various stress stimuli, such as

exercise, heat stress, radiation, and ischemia, can cause rapid activation of ODC and a rise in polyamine levels [6].Ornithine decarboxylase is highly expressed in human reproductive organs such as the testes, ovaries, and prostate. Polyamines are essential for spermatogenesis and germinal epithelium proliferation [7]. Transgenic mice that overexpressed ODC developed infertility due to excessive amounts of putrescine, resulting in smaller testis and impaired spermatogenesis [7]. Consequently, utilizing antioxidant therapy to reduce the rise in high-intensity exercise-induced oxidative stress is now being argued as being counterproductive and even avoiding the health-promoting effects of exercise **[8].** Resveratrol (RSV), a polyphenolic phytoalexin derived from grapes, has been discovered to be the primary active ingredient of stilbene phytoalexins. It is formed by a variety of plants in response to harm [9].Resveratrol has a long list of benefits, including antioxidant, cardioprotective, and anti-aging properties as well as anti-inflammatory properties **[10]** due to its anti-ROS scavenging abilities, in particular. Trans-resveratrol, the most prevalent isomer, protects against DNA damage and lipid peroxidation brought by ROS [11]**,** as well as modulation of enzymes and pathways that yield inflammatory mediators **[10].**

Resveratrol has been studied in rats and other animal species for its impact on spermatological and oxidative stress parameters. RSV reduces the histological and apoptotic changes caused by diabetes in the testes **[12]**, as well as RSV improves the diminished Leydig cell steroidogenesis in obese mice **[13]**. RSV, on the other hand, has been shown to have negative effects on sperm parameters and testicular morphology **[14].** However, there is a paucity of data on the link between forced exercise/RSV and spermatological markers.

In this study, it was also looked at whether RSV would help with testicular dysfunction brought on by forced exercise in rats.

METHODS

Animal and Experimental Design: 24 healthy adult male albino rats of the local strain were used for this research, weighing (180±15 gm) in the animal house of the Faculty of Medicine (Zagazig University). Rats were evenly and randomly distributed into six cages (four /cage) for the course of the study, all of which were maintained in a hygienic manner with free access to food and water and an ambient temperature of (22±2ºC). According to the Institute of Laboratory Animal Resources [15], all animals received care that was compliant with animal care and ethical standards. The experimental protocol was approved by the institutional animal care and use committee of Zagazig University (approval number: ZU-IACUC/3/F/162/2022).

Exercise protocol: The rats were randomly assigned into three equal groups (n=8) following one-week of acclimatization, and they underwent six-days training program to become accustomed to swimming before beginning of the experiment. In plastic tanks with dimensions x 50x100x50 cm filled with water that was between 32°C and 35°C, the rats were first trained to swim for 10 minutes. After then, the swimming period was increased by 10 minutes daily until it reached 60 minutes [16]. **Group 1: (Control).** The rats were retained in normal physiological state. **Group II: Forced swimming exercise (FE group).** The rats were made to swim for 60 minutes each day while having 6% of their body weight suspended from the base of their tails. The previous studies were used to calculate the workout load. Five days a week were used to carry out the protocol [16]. **Group III: Resveratrol treated (FE +RSV group).** The same workout regimen as for the FE group was used. The most commercially available active and stable form of RSV is Trans-Resveratol, which is suspended in 0.2 mL of normal saline and administered by gavage. The rats were given RSV (50 mg/kg/day) until the completion of the experiment [17]. RSV was supplied by Sigma-Aldreich Co (3050 Spruce Street, Saint Louis, MO 63103, USA. CAS No.: 501-36-0). The rats were given an ip injection of 60 mg/kg sodium thiopental 24 hours following the last day of exercise. Blood samples were collected from the retro-orbital venous plexus and centrifuged at 3000 rpm for 20 minutes to separate the serum. The serum was stored at -20°C until use. The testes and epididymides from the various groups were immediately excised and weighed after the rats had been decapitated. For the tissue biochemical analysis and semen analysis, the left testes and epididymides were used, respectively. Histopathological and immunohistochemical examinations were performed on the right testes.

Biochemical analysis: Using corresponding rat ELISA kits, follicle-stimulating hormone (FSH) [18], luteinizing hormone (LH), and testosterone (T) [19] concentrations in serum were estimated (Biocompare.Co, Cat. No. LS-F6305-1, LS-F27508-1, and LS-F10018-1 respectively). According to Henning [20], a rat ELISA kit (Sigma-Aldrich Co, Cat.no. AR E-8100) was used to measure the serum corticosterone level. According to Fernando et al. findings [21], TNF-α and IL-6 serum levels were also determined using rat ELISA kits from Sigma-Aldrich Co, Cat. Nos. RAB0480 and RAB0311.

Epididymal sperm analysis: The standard Neubauer's chamber method was used to count the

sperm in a suspension made from the left epididymis of each rat, which was carefully removed and minced in 1.0 ml of phosphate buffered saline (pH 7.2). A Leukocyte hemocytometer was used to measure a portion of the suspension (up to 0.5), which was then diluted up to the mark of 11 with phosphate buffered saline. Before the suspension was added to Neubauer's counting chamber, it was thoroughly mixed. The entire sperm count was obtained using a light microscope at 400X, in 8 squares of 1 mm^2 each, with the exception of the center of erythrocyte region, which was determined and multiplied by 5×10^4 to indicate the number of spermatozoa/ml [22]. By dividing the number of motile sperm cells over the total number of sperm cells (both motile and non-motile), the sperm motility percentage was determined following the method outlined by Murthy et al. [23]. The approach described by Oyeyemi et al. [24] was also used to find the abnormal sperm types.

Evaluation of the testicular oxidant-antioxidant markers, ornithine decarboxylase (ODC) activity, and polyamine content: To make a 10% homogenate (w/v) that was centrifuged for 10 minutes at 1000 rpm, the left testes were cut into thin slices and homogenized in cold 50 mM phosphate buffer (pH 7.0) with 0.1 mM EDTA. Using spectrophotometric kits (Biodiagnostic, Giza, Egypt) and following the procedures outlined by Ohkawa et al. [25] and Kakkar et al. [26], the resulting supernatant was used to estimate each of these: the malondialdehyde (MDA) level and superoxide dismutase (SOD) activity. ODC activity was measured in the testicular homogenate as described by [27]. Enzyme activity was expressed as pmol of released C02 /h/mg protein. Polyamine concentration was detected in testicular homogenate supernatant via high-performance liquid chromatography with the use of fluorescence detection. Polyamines have been separated on a reversed-phase column Hypersil ODS column (3 × 150 mm ID, particle size 5 mm, Analytic, Germany) as defined previously [28] Polyamine content was determined as nmol/mg protein.

Tissue preparation for histopathological and immunohistochemical analyses: The testicular samples are placed in Bouin solution for 4 to 5 hours until they harden to a condition that allows them to be stored for the creation of paraffin blocks. 5 micrometer (um) sections were cut out and stained with Masson's trichrome to show collagen fibers and hematoxylin and eosin (H&E) to show the structural light microscopic modification [29]. Johnsen's testicular score system was used to assess the histological alterations in testicular tissue. According to Johnsen's standards [30], each tubule received a score ranging from 1 (very poor) to 10 (outstanding). For the immunohistochemistry test of caspase-3 (apoptotic protein) and α-SMA (smooth muscle actin) as peritubular myoid cell marker, sections of 4 µm thickness were employed [31]. **Image analysis:** The photos were taken using LEICA DM500 optical microscope at Zagazig University's department of anatomy and embryology. Quantitative measurements were made using Image J analysis software (Fiji Image J; 1.51 n, NIH, USA) to determine the mean area percentage of collagen fibers, the percentage of α-SMA-positive cells, the number of immunoreactive cells for caspase-3 in various stained sections, the number of Leydig cells/100 µm2, the number of Sertoli cells/tubule, and the thickness of peritubular myoid cells (µm) in 6 animals/group ,150 seminiferous tubules at 400× mag [32].

STATISTICAL ANALYSIS

The mean and standard deviation (SD) for all the animals in each group were calculated using the Statistical Package for Social Sciences (SPSS) version 22. Using the one-way analysis of variance (ANOVA test) and the LSD test, the statistical significance of the data was established. The significance threshold was established at $p < 0.05$.

RESULTS

The FE group in the present study showed a significant reduction in FSH, LH, and T serum levels ($P<0.05$) and a substantial increase in corticosterone levels ($P < 0.01$) as compared to the control group. In comparison to the FE group, the rats co-treated with RSV showed a substantial improvement ($P<0.01$) in the levels of FSH and corticosterone hormones. However, Serum levels of LH and T remained considerably lower ($P<0.05$) than in control rats **(Table 1)**.

The FE group's epididymal sperm motility and count were considerably lower ($P<0.01$ and $P<0.05$, respectively) in comparison to the control group, whereas the aberrant sperm shape was significantly more prevalent ($P<0.05$). Sperm motility, count, and aberrant forms significantly improved in the FE+RSV treated group as compared to the FE group ($P< 0.05$). **(Table 2).**

Additionally, the testicular homogenate MDA level significantly increased in the FE group, whereas testicular SOD activity significantly decreased ($P<0.01$), along with a considerable ($P<0.05$) increase in ODC activity compared to the control group. Furthermore, the FE group's polyamine testicular content was significantly ($p<0.05$) higher than that of the control group, whereas FE+RSV treated rats showed decreased levels of MDA ($p< 0.01$), ODC activity ($p<0.05$), putrescine, and spermidine in the tissues ($p< 0.01$),

and increased levels of SOD ($p<0.001$) in comparison to the FE group. RSV co-treatment significantly reduced both TNF-α and IL-6 serum levels compared to FE group, while TNF-α and IL-6 serum levels were significantly increased in the FE group compared to the control one ($P<0.01$ and $p<0.001$, respectively) **(Table 2).**

Histopathological and Immunohistochemical results:

The testes of control rats had seminiferous tubules that were normally separated by interstitial tissue. The seminiferous tubules had an oval or rounded shape, were encircled by a thin basal lamina, and were packed with Sertoli and germ cells. A sophisticated stratified germinal epithelium that was made up of layers of spermatogenic cells that lined each tubule were organized well and accumulated sperm bundles in their lumina **(Fig. 1A**). The seminiferous epithelium showed deterioration in the FE-group, and the seminiferous tubule lumen revealed an apparent decrease in the number of sperm (nearly empty from mature sperm). The germinal epithelium separated (shed), along with thickened myoid cells and a damaged basement membrane. The blood vessels seemed dilated, congested, and had thicker walls in the interstitial tissue, which also had vacuoles, fibrosis, and exudate (**Fig 1B**, **C).** The RSV-treated rats revealed that most of the seminiferous tubules appear nearly normal. While a few seminiferous tubules still showed mild degeneration with little exfoliated epithelium within their lumina (**Fig 1 D**). When forced swimming exercise was compared to control and FE+RSV-treated groups, the level of spermatogenesis significantly decreased in the FE group, according to inspection of Johnsen's scores in histopathological samples (**Fig 2A**). In the FE-group, there was a considerably lower mean number of Sertoli cells (**Fig 2B**) and Leydig cells (**Fig 2C**) ($P <0.05$). When compared to the control and FE+RSV-treated groups, the FE group's mean thickness of peritubular myoid cells dramatically increased (**Fig 2D**).

The collagen fibers in the blood vessel walls were distributed normally in the control rat testis when examined with Masson's trichrome (**Fig 3A**). The substantial increase in collagen fibers is found in the blood vessel walls of the FE-group (**Fig 3B**). Compared to the control group, the FE+RSV-treated group showed that the collagen fiber distribution was almost normal (**Fig 3C**). By using Masson's trichrome staining, the mean area percentage of collagen fiber deposition was shown in (**Fig 3D**). The FE-mean group's area percentage of collagen fiber deposition was significantly higher than that of the control and FE+RSV-treated groups ($P< 0.05$).

The seminiferous tubules of the control group's showed any signs of the immune-positive reaction, which was confined in the nuclei of apoptotic cells (Fig **4A**). A large amount of caspase-3 immune-positive cells were discovered in the FE-group (**Fig. 4 B**). Compared to the control and FE+RSV-treated groups, the number of caspase-3 immune-positive cells per tubule was considerably higher in the FE-group. FE+RSV treatment markedly reduced the number of apoptotic cells (**Fig. 4C,D**). In the rats in the control group, the smooth muscle actin immunological reaction resulted in a positive response in the cytoplasm of the sole stretched layer of the myoid cells over the whole circle of the seminiferous tubules (**Fig. 5A**). In some of the FE-seminiferous group's tubules, dense peritubular myoid cells alternated with thin cells that showed negative α-SMA reactivity in the atrophic tubules (**Fig 5B**). Rats fed RSV had myoid cells in their testes that showed a positive α-SMA immune response (**Fig. 5C**). The percentage of the region with immunopositive expressions of seminiferous peritubular α-SMA was significantly lower in the FE-group as compared to the control group, according to statistics. Comparing the FE+RSV-treated group to the FE-group, the percentage of α-SMA expression increased considerably (**Fig. 5D**).

Table 1: Serum levels of testosterone (T), corticosterone (C), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and epididymal sperm parameters in the examined groups.

	Control group	FE group	FE+RSV group
FSH(μIU/ml)	37.69 ± 1.65	34.11 ± 0.12[a]	38.64 ± 2.42[b]
LH(μIU/ml)	21.51 ± 0.14	17.12 ± 0.31[a]	18.99 ± 0.5[a,b]
T(ng/ml)	23.78± 0.35)	18.40 ± 1.12[a]	21.11 ± 0.72[a,b]
Corticosterone (ng/ml)	62.22±11.34	133.85±20.83[a]	89.56±15.73[a,b]
Count (million/mm^3	110±2.1	79±5.1[a]	102±5.3[b]
Motility (%)	90±3.1	65±2.4[a]	76±1.3[a,b]
Abnormal forms (%)	3.1±1	17.74±5.35[a]	6.72±2.08[a,b]

Note: Data are presented as mean ± standard deviation (SD); [a] significance vs. control group; [b] significance vs. FE group (forced swimming exercise); RSV group (resveratrol treated).

Table 2: The testicular antioxidant and oxidant activity, ODC activity, polyamine concentration, and serum inflammatory markers for each group under investigation.

	Control group	FE group	FE+RSV group
SOD activity (U/mg ptn)	62.32 ± 1.65	52.31 ± 3.15[a]	84.46 ± 1.12[a,b]
MAD(nmol/g tissue)	4.02±0.5	6.01±0.21[a]	3.62±0.12[b]
ODC activity (pmol co2/h/mg ptn)	43.5 ± 6.5	120.2 ± 6.2[a]	86.2 ± 13.6[a,b]
Putrescine (nmol/mg ptn)	1.14 ± 0.16	2.34 ± 0.88[a]	1.21 ± 0.49[b]
Spermidine (nmol/mg ptn)	13.4± 3.9	22.73 ± 4.2[a]	16.95 ± 2.8[b]
Spermine (nmol/mg ptn)	9.8± 3.8	17.4± 3.8[a]	15.3± 3.9[a]
TNF-α(pg/ml)	20.53±1.8	55.27±1[a]	31.51±0.8[a,b]
IL-6(pg/ml)	31.65±2.6	60.32±0.9[a]	35.1±1.3[b]

Note: Data are presented as mean ± standard deviation (SD); [a] significance vs. control group; [b] significance vs. FE group (forced swimming exercise); RSV group (resveratrol treated).

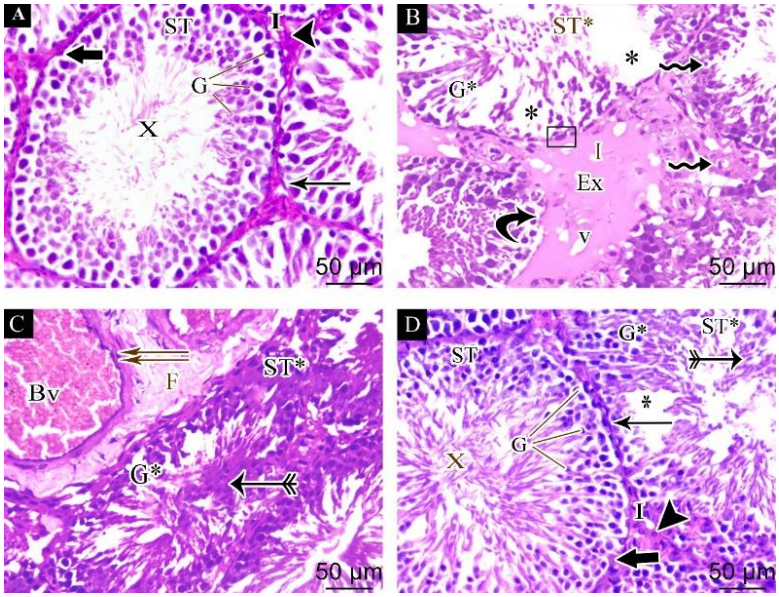

Fig.1 Illustrations of the testicular tissues of various groups **A**: The control group, the seminiferous tubules (ST) are lined with stratified germinal epithelium (G) and Sertoli cells are tightly impacted (Thick arrow). Their lumina are filled by spermatozoa (X). Groups of Leydig interstitial cells (Arrowhead) are visible in narrow interstitial tissue (I). Flat myoid cells (thin arrow) form a single layer around the seminiferous tubule. **B** and **C**: The FE-group has deformed seminiferous tubules (ST*), significantly reduced germinal lining (G*) with noticeably degraded cells (asterisk), and an asymmetrical basement membrane (curved arrows) with thickened myoid cells (boxed area). Apoptotic cells (zigzag arrows) are seen in the interstitial space and the tubules, as well as exfoliated germinal epithelium within the lumina (double-tailed arrow). The interstitial space shows exudate (Ex) and vacuolation (V). Other tubules show fibrosis (F) and dilated congested blood vessels (Bv) with thick walls (double arrow) in their interstitial spaces. In the **D**: FE+RSV-treated group, some nearly normal seminiferous tubules (ST) lined with stratified germinal epithelium (G) and Sertoli cells (Thick arrow), aggregated spermatozoa (X) are seen in their lumina. The disorganized seminiferous tubules (ST*) with disorganization of germinal epithelium (G*) show little areas of atrophied germinal epithelium (asterisks) and exfoliated cells (double-tailed arrows) within their lumina. Flat myoid cells (thin arrow) are also observed. Narrow interstitial tissue (**I**) exhibits groups of interstitial cells of Leydig cells (**arrowhead**).

50m scale bar, x400

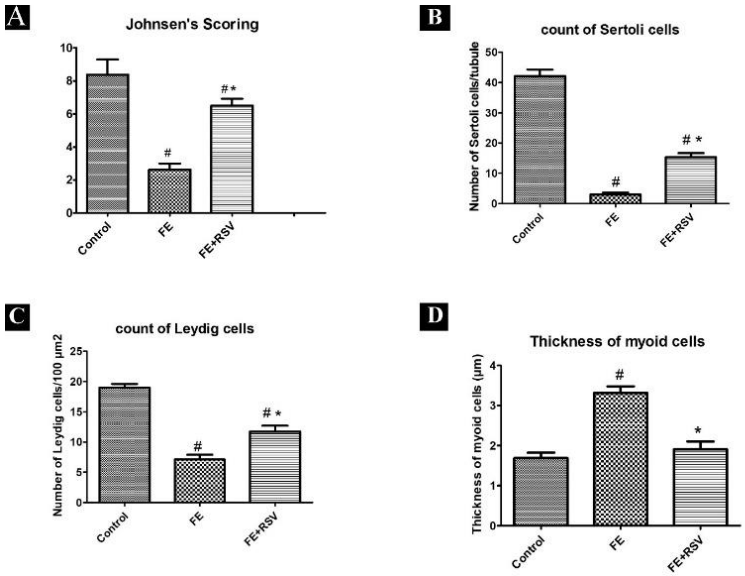

Fig.2. Bar charts show Johnsen's testicular score (**A**), count of Sertoli cells (**B**), count of Leydig cells (**C**) and the thickness of myoid cells (**D)** in control, FE, and FE+RSV-treated groups. # Significant difference compared to the control group, P < 0.05.*Significant difference compared to the FE-group, P < 0.05

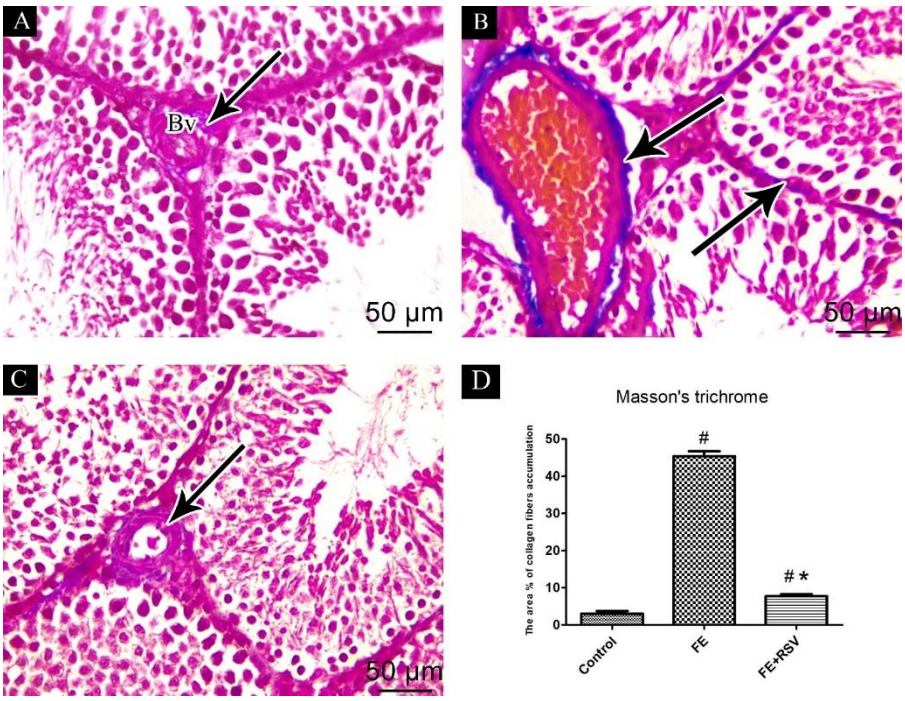

Fig.3. Representative Images showing Masson's trichrome stained testicular sections in different experimental groups: **(A)** control, **(B)** FE, and **(C)** FE+RSV-treated groups. Arrows indicate the blue staining of the collagen fibers around blood vessels in interstitial space and the walls of tubules. **(D)** The quantification analysis in the expressions of Masson's trichrome in seminiferous peritubular via area% in the testes sections (x400) of the three experimental groups is shown in the lower right panel. # Significant difference compared to the control group, P < 0.05.*Significant difference compared to the FE-group, P < 0.05.

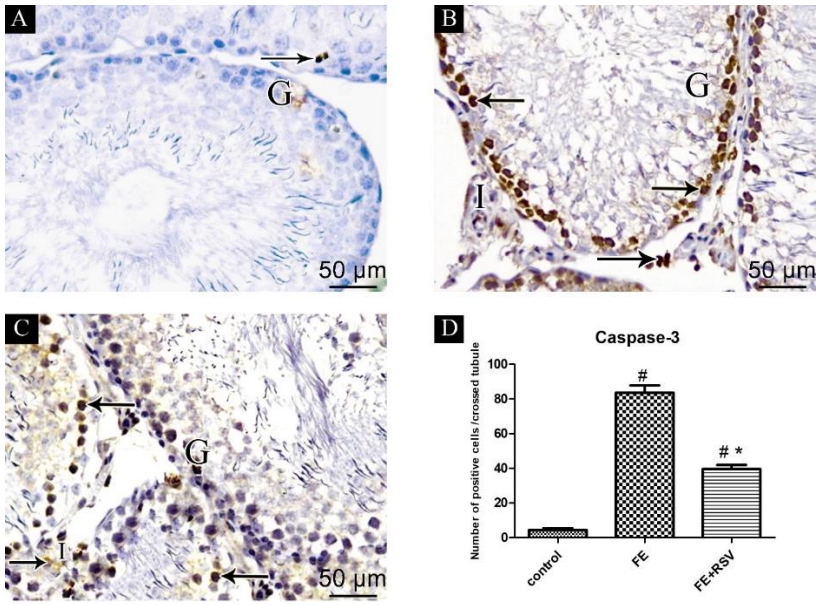

Fig.4. Representative Images showing caspase-3 immuno-positive cell expression, **(A)** control, **(B)** FE, and **(C)** FE+RSV-treated groups. Arrows demonstrate dark brown staining of immune-positive nuclei of apoptotic cells in germ cells (G) and interstitial cells (I). **(D)** The lower right panel shows a quantitative analysis of the number of caspase-3 immuno-positive nuclei in the three groups of testes. # Significant difference compared to the control group, P < 0.05.*Significant difference compared to the FE-group, P < 0.05.

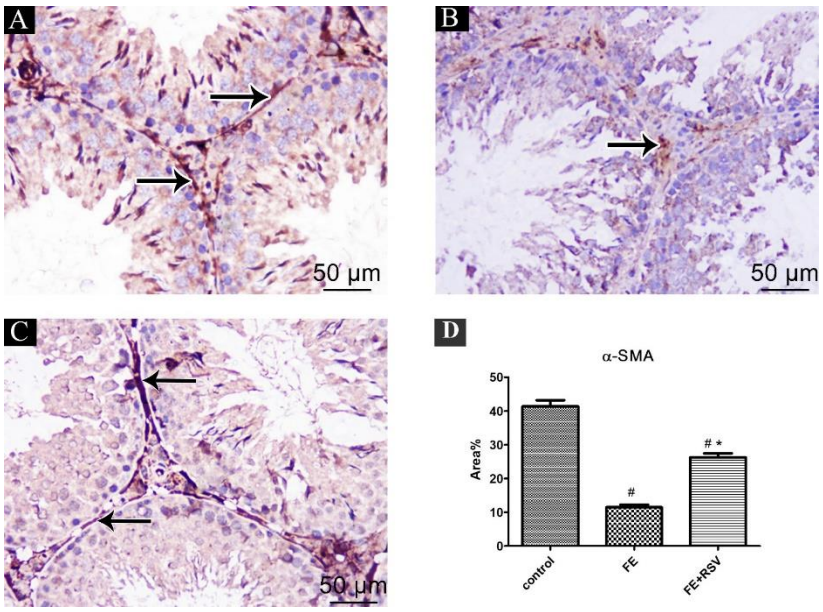

Fig.5. Representative images illustrating expressions of myoid cells in the peritubular area of seminiferous tubules through immunostaining of α-SMA in tissues of rat testes in all experimental groups, (**A**) control, (**B**) FE, and (**C**) **FE+** RSV-treated groups. Arrows signify dark brown staining of immune-positive myoid cells. (**D**) The quantification analysis of the expressions of α-SMA in seminiferous peritubular via area% positive cells in testes sections (x400) of the three experimental groups is shown in the lower right panel. # Significant difference compared to the control group, P < 0.05.*Significant difference compared to the FE-group, P < 0.05.

DISCUSSION

Forced swimming exercise decreased testosterone levels, other reproductive hormones, sperm count, motility, and viability in adult male rats, while increasing apoptotic germ cells and aberrant morphology [16, 33]. In a similar manner, our data revealed that serum corticosterone levels significantly increased while serum levels of testosterone, FSH, and LH decreased. Furthermore, in the FE group, sperm count and

motility were significantly reduced, while aberrant morphology was enhanced, which was associated with increased germ cell apoptosis. These hormonal and physiological changes point to the probability of testicular function impairment, which could be linked to a change in the hypothalamo-pituitary testicular axis (HPT).

After rigorous exercise in rats, **Yi et al. [34]** discovered an increased level of serum testosterone, sperm count, and improved sperm motility. Regardless of the type of exercise, the duration and frequency of exercise training programs varied between studies, which could explain the disparities in results.

Although research on the effects of forced exercise on testicular function is contradictory, numerous theories have been proposed to explain the harmful effects of high-intensity exercise on testicular function. Reducing testosterone secretion is one of them, while generating oxidative stress is another **[35]**. Exercise stimulates the hypothalamic-pituitary-adrenal (HPA) axis because it is a stress response to the body [**36**].

Athletes' testosterone secretion may be suppressed as a result of the repressed release of gonadotropins and the direct and indirect effects of elevated corticotropin-releasing hormone (CRH), corticotropin, and cortisol **[37].** Additionally, glucocorticoids reduce gonadal axis activity at the hypothalamic-pituitary level, and Leydig cells have been found to express CRH and its receptor, which inhibits T production **[36]**. The HPA axis has also been shown to have an inhibitory influence on the female reproductive system **[37].** These reports were partially concordant with our results for the increased serum corticosterone level in the FE group. Moreover, the decrease in testosterone output was related to a decrease in testicular blood flow during exercise, as exercise raises testicular temperature, which can reduce testosterone secretion and spermatogenesis **[4].**

However, the role of the HPT axis in these changes is debatable, with some studies reporting no significant changes in FSH and lower LH serum levels **[4]**, while others report higher LH serum levels **[38**]. Nevertheless, **Safarinejad et al [36]** have reported that high intensity exercise is associated with hyporesponsiveness of HPT axis as reflected by low serum levels of LH and FSH after the HPT axis activation. In this study, FE+RSV-treated rats had considerably higher serum testosterone, FSH, and LH levels, as well as sperm count and motility, but serum corticosterone and aberrant sperm forms were much lower than the FE group. Results from other research hypothesized that trans-resveratrol preserves the sex hormonal profile, testicular structure, spermatogenesis, and apoptosis after vigorous exercise in mice were consistent with our findings [**17].** According to Juan et al. [**39**], who attributed it to RSV's binding to the estrogen receptor (ER) as a mixed weak agonist/antagonist with no estrogenic qualities, RSV's effects on testicular function may be mediated by hypophisary stimulation. RSV may also increase NO production by stimulating LH and FSH release and acting centrally on the pituitary gland, which would then cause leydig cells to produce more testosterone [**40**]. However, Ranawat et al **[14]** reported that RSV analogues inhibited steroidogenesis in rat Leydig cells.

SOD activity was shown to be significantly reduced, while MDA levels were significantly higher in the FE group's testicular tissues, ODC activity and polyamine concentration were also significantly higher. This could explain the histopathological findings in this research. Because oxidative stress causes cytotoxic damage to cell membrane components, and could induce apoptosis, seminiferous tubule atrophy, and a reduction in the spermatogenetic cell cycle **[3].** Numerous studies [8] have shown that intracellular oxidative stress directly induces ODC activation and polyamine levels. Overexpression of polyamines, on the other hand, can result in the production of ROS during specific oxidase-mediated polyamine catabolism [**41**]

In our study, the hyperactive ODC was suppressed, the intracellular levels of biological polyamines were reduced, and testicular function was enhanced after RSV co-treatment. **Schneider et al. [42]** linked RSV's protective impact on human cancer colon cells to its inhibition of ODC activity and polyamine concentration, which is consistent with our findings. Overactivity of the ODC, as well as the resultant accumulation of putrescine, is supposed to contribute to stimulation of the apoptotic signaling cascades [**41**]**.** RSV-induced inhibition of ODC overactivity and polyamine testicular content can explain, to some extent, the reported antiapoptotic effect of RSV evidenced by fewer caspase-3 immunopositive cells in the RSV treated group. TNF- α and IL-6 serum levels were significantly higher in the FE group. In agreement with our findings, **Minuzzi et al. [43]** found that ROS can activate NF-κB dimers, which translocate into the nucleus and regulate the gene transcription of pro inflammatory cytokines. TNF-α has been shown to suppress the gene expression of the testosterone synthases StAR and 3-HSD via NF-kB activation, resulting in decreased testosterone production inside Leydig cells and sperm damage. [**43**]. On the contrary, **Morgado et al. [44]** found that after long-term high-intensity exercise, the number of blood monocytes and dendritic cells, as well as the generation of IL-6, TNF-α, and macrophages, were reduced in obese men. The

discrepancy in outcomes between this study and earlier studies could be due to variances in exercise programs and study species. It is noteworthy that in comparison with the FE group, RSV administration resulted in a considerable increase in SOD activity and a decline in MDA levels in testicular tissues, as well as a significant decline in serum IL-6 and TNF-α. RSV has protective effects against strenuous exercise-induced lipid peroxidation in rats, as evidenced by its ability to inhibit TNF-α, IL-6, and impair NF- κB activation, as well as increased levels and activity of the protein deacetylase enzyme-silent information regulator 2/sirtuin-1 (SIRT-1) **[45].**

Further findings in the present research showed that the testicular tissue exhibited dilated and congested blood vessels, expanded interstitial space with exudate, and damaged germinal epithelium. There was also an increase in the number of caspase-3 immune-positive cells in the FE-group as well as the presence of apoptotic cells in tubules and interstitial space, which agreed with the conclusions of some studies [4]. This extensive exercise-induced apoptosis was explained by the occurrence of testicular ischemia **[46]**.

Moreover, the current study assigned increased myoid cell thickness and significantly lower area percent expression in the FE-group. According to our findings, Upadhyay et al. [47] reported that myoid cell proliferation and immunophenotypic changes following experimental induction of testicular ischemia in adult rats increased the secretion of extracellular matrix components, causing thickening of the lamina propria, tubular fibrosis, and Sertoli cell lesions.

According to **Devkota et al. [48]**, myoid cells have androgen receptors and are involved in controlling spermatogenesis and testicular function. This revealed another function for RSV-linked improvement of myoid cell thickness, number of Sertoli cell, Leydig cell, and testosterone levels in our current work.

CONCLUSION

Therefore, by improving sex hormones, sperm parameters, and histological abnormalities, RSV treatment has the capacity to effectively attenuate and eradicate any testicular dysfunction brought on by forced swimming exercise in rats. Most likely, these benefits are caused by resveratrol's antioxidant, anti-ornithine decarboxylase, and anti-inflammatory capabilities, which are mediated by the HPT and/or peripheral stimulatory actions. However, additional research is needed to determine the precise mechanism of action.

Declaration of interest and **Funding information**: No conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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To Cite:

Khalil, S, Abdelghany, E., Abd El-Fatah, S., Elwany,N., Habib ,M. Protective Effect of Resveratrol against Testicular Dysfunction Induced by Forced Swimming Exercise in Adult Rats. *Zagazig University Medical Journal*, 2023; (266-276): -.doi: 10.21608/zumj.2022.152238.2607.