Consequence of Aging on Male Albino Rats' Testes and Possible Protective Role of Resveratrol

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ABSTRACT

Background: Aging is a normal physiological process that causes several changes in all organs, and changes in testicular histology are one consequence of aging that leads to decline in male fertility.

The aim of the study: is to examine the impact of aging on the testes and the possible protective role of resveratrol.

Method: Twenty-four male of Wistar albino rats were used in the present study, which divided into three groups of eight animals each. A 6-month-old control group A. Group B the 24 month old group. Group C the old age group treated with resveratrol received 25 mg/kg/day dissolved in distilled water given orally by gastric tube. Treatment were performed for 3 months. At end of experiment, blood was collected for hormonal assessment, the animals were scarified, and the testes were taken and prepared for histological examination.

Results: revealed that the testes had regressive age-related structural alterations include atrophy and sclerosed seminiferous tubules, arrest of spermatogenesis, decrease in the spermatogenic cells and Sertoli cells, thickening of the tunica albuginea, increase in Leydig cells associated with decrease testosterone and increase in FSH (follicular stimulating hormone) and LH (luteinizing hormone), increase oxidative marker MDA (Malonaldehyde), in addition to deterioration in sperm analysis. The use of RES (Resveratrol) improve the testosterone, FSH and LH with decrease the MDA and improve the histological changes of aged testes.

Conclusion: aging has deleterious effect on testes cause histological changes, reduction in spermatozoa's quality and quantity with aging and increase oxidative stress of aged testes and the use of RES as a preventative and/or therapeutic drug for aging-related testicular alterations is possible.

Keywords: aging, testosterone, rats, Resveratrol.
INTRODUCTION

Aging is a physiological process characterized by major structural changes in all body organs as a result of both internal and external influences. Testicular morphological changes are major consequences of ageing on the male reproductive system. These alterations may reduce spermatogenic quality and quantity.

Recently many studies showed that increased age is related to the high incidence of fetal abnormalities such as Down's syndrome, malignancies such as leukemia, tumors of the nervous system, and numerous neurological and psychiatric diseases in the progeny.

Age-associated alteration in the hormones production, morphology of testes, and spermatogenesis have a detrimental effect on the morphology and quantity of spermatozoa, hence impairing the functions of the male reproductive system. Atrophy associated with ageing has been observed in both humans and animals, resulting in a decline in spermatozoan quantity, motility and viability.

The onset of testicular atrophy that comes with ageing is unknown. In rats and humans, the ageing process of tubular atrophy starts in the focal area in seminiferous tubules, where atrophic seminiferous tubules are frequently detected close to tubules with normal spermatogenesis.

Due to the testicular tissue's rapid rates of cell division and metabolism, oxidative stress can be particularly harmful, making the tissue's antioxidant capacity crucial. The state of Oxidative stress cause alteration in the DNA of testicular cells, which can result in infertility and testicular cancer. Therefore, maintaining a healthy antioxidant balance is important for the overall health of the testes.

Resveratrol (RES)

The bioactive substances known as polyphenols are widely present in plant-based foods and have a variety of health-promoting benefits through several pathways, including anti-oxidation and anti-inflammatory. Natural phenolic compounds can be found in a wide range of foods including grape, peanut, and blueberry. RES has been demonstrated to have several biological impacts including antioxidant, anti-carcinogenic, anti-inflammatory, immunomodulatory, hypotensive, and hypolipidemic properties. Many studies have stressed its importance in ageing treatment by lowering oxidative stress, and inflammation, increasing mitochondrial function, and managing apoptosis.

Resveratrol was identified in the dried root of Japanese knotweed (Polygonum cuspidatum), commonly termed Kojokon in Japan, in 1963. For thousands of years, this plant has been used in traditional Chinese and Japanese medical treatments for heart disease, hyperlipidemia, gonorhea, athlete's foot, and vascular inflammation. Large range of plant species and fruits have been found to contain RES such as purple grapes, blueberries, mulberries, cranberries, peanuts, groundnuts, pines Coconut and cocoa, and RES is nearly entirely produced in the skin of grapes especially those that have been infected by Botrytis cinerea, and its concentration peaks shortly before the grapes are ready to harvest. So the skin and seeds of grapes have the highest concentration of RES. Many investigations have indicated that RES can be utilized successfully as a testicular antioxidant.

Sharma et al., stated that RES enhanced the count of sperm production, mobility, and the number of viable sperm, as well as, it increases blood level of Testosterone hormone, FSH (follicular stimulating hormone), LH (luteinizing hormone), GSH (glutathione), CAT (catalase), SOD (superoxide dismutase), and it lowered lipid peroxidation (LPO). RES treatment alone can improve sperm parameters and the testicular antioxidative defense system.

El bana et al., mentioned that 4 weeks of RES treatment at a level of 20 mg/kg in male rat help in healing testicular damage induced by tramadol as RES protects against oxidative stress-induced lipid peroxidation and DNA damage. RES has also been shown to increase sperm maturation, and viability, it also reduces the number of apoptotic cells caused by tramadol.
Resveratrol appears to improve sperm quality in humans and animals through its ability to cross the blood-testicular barrier, allowing it to exert its role in testicular protection. RES has been demonstrated to reduce germ cell apoptosis, increase blood testosterone levels and enhance sperm quality and count of epididymal sperm 18.

MATERIAL AND METHOD

Animals and Housing
Twenty-four male Wistar albino rats were obtained from the University of Mosul's animal house. Polypropylene cages with stainless steel top grill housing the rats in groups. The ethical approval was given by the Mosul Medical College's medical research ethics committee. All animals were assessed for general health, fed commercially prepared ad libitum foods, and observed for three months.

Experimental design: distribution of rats into three separate groups (8 animals in each group) was as follows:-

Group A (adult-aged group): the rats in this group were aged 6 months and received 1 ml of distilled water.

Group B (old age group): the rats in this group aged 24 months received 1 ml of distilled water.

Group C (RES treated old age group): the rats in this group aged 24 months received 25 mg/kg/day of RES orally for 3 months duration.

Resveratrol: -the drug was obtained from the local pharmacy in Iraq. Trans – RES is an antiaging drug manufactured by NOW FOODS company/ USA. As 50 mg/capsule. Using gastric gavage, it was administered orally to animals at a rate of 25 mg/kg b. w. each day after being dissolved in distilled water 18.

The relative weight of testes: The weight of the body and testes were taken at the end of the experiment by using an electronic scale, and calculate the testicular weight by using the ratio of testicular weight to final body weight, testicular weight / body weight × 100.

Collection of the Blood: To evaluate the serum level of testosterone, follicular stimulating hormones (FSH), and luteinizing hormones (LH), a blood sample was taken from the retro-orbital vein.

Necropsy: After 3 months experimental period the animals were euthanized via inhalation of diethyl ether in a glass desiccator, and then a longitudinal midline in the abdomen region was made with a scissor, and both testicles were removed.

Handling and fixation of tissue: To remove the blood the testis was rinsed with normal saline, and then dried on filter paper. The testes were preserved in Bouin's solution for 48 hours. After that change to neutral buffered formalin at 10%.

and to determine the MDA concentration, one piece of testis should weigh at least 1gm and stored in aluminum foil in the refrigerator.

Tissue processing: Dehydration by using increased alcohol concentrations: 70%, 90%, and absolute alcohol. Three changes of xylene impregnation used for clearing and three changes of paraffin wax immersion result in a solid block containing the tissues that become ready for sectioning of 4-5 mm thick sections with a Reichert rotary microtome, then put the section on cleaned glass slides. Deparaffinization was done with two xylene changes utilizing increasing grades of alcohol and water. Staining the slides with (Hematoxylin and Eosin) H&E stain, (Masson's Trichrome) MT stain, and (Periodic Acid-Schiff stain) PAS 20-22.

Testosterone, FSH, and LH were detected by using the rat testosterone, follicular stimulating hormone (FSH), and luteinizing hormone (LH) ELIZA Kit ( ELK 8314, ELK1315, ELK 2367) respectively from ELK Biotechnology. The control group's value was regarded as standard and compared to the results of the other groups.

MDA (malonaldehyde): Elabsscience Biotechnology's MDA (malonaldehyde) ELISA Kit was used to detect MDA levels in the tissue of the testes (E-EL-0060) after tissue homogenization. The homogenate fluid is then centrifuged for 5-10 minutes at 5000 round to obtain the supernatant fluid for the ELIZA assay. The control group's value was regarded as standard and was utilized to compare with the results of the treated group.

Micro-morphometric measurement: Numerous parameters were measured including the diameter of the seminiferous tubule (µm), the germinal epithelium thickness (µm), the number of Leydig and Sertoli cells/40X field, and the thickness of the tunica albugenia (µm), using a 40x microscope. Use a specialized digital camera (OMAX 18 MP, China) with USB 3.0 for morphometric estimation.

Sperm analysis: The sperm sample was obtained by excision of cauda epididymis, then the pieces that excised were put in a petri dish containing 2 ml normal saline (0.9% sodium chloride) at 37°C, the sperms were released by piercing the cauda epididymis in normal saline and homogenized. Then motility, viability, deformity and count of the sperms were estimated by a computer-aided sperm analyzer (cobas e 411) 23.

Statistical analysis: The values were analyzed by using IBM SPSS statistical analysis version 21. ANOVA was used to examine the mean differences between the experimental groups, followed by a post-hoc Duncan test. The statistical findings were presented as a mean ± SE, the mean Differences were considered statistically significant at ≤ P 0.05.
RESULTS

**Testicular weight**: testicular weight / 100gm of body weight was significantly decreased at (0.05) in old age rats (B) (0.275± 0.007) compared to the control group (A) (0.361±0.013), while in the treated group with RES (C) showed significant improvement in testicular weight (0.322± 0.014) compared to old age group. As in table (1)

![Table (1): the mean of relative weight of testis (gm./100gm body weight)](image)

- Repeated litters in a row indicate no statistically significant difference (p>0.05).
- A significant difference at the p≤0.05 level is shown by the presence of Different litters in a row.

**Hormonal Analysis**

**Testosterone hormone**: the aging process led to significant reduction in serum testosterone level in old age group (B) (2374.83±613.77) compared to control group (A) (8939.20±822.79) at P ≤0.05. However, the administration of old age group with RES (C) led to a significant elevation of testosterone level (8558.41±864.21) compared to old age group table (2).

**FSH and LH hormones**: Old age group (B) had significantly higher hormone levels than control group (A) at p ≤0.05. Control group (A) FSH and LH hormone levels were 21.15± 0.95 and 4800.46± 646.10, respectively, with maximum elevation of FSH and LH hormones were observed in old age group (B) (60.55± 2.82 , 7836.14± 111.80 ). The FSH and LH levels in treated group with RES (C) significantly reduced (21.78± 1.33, 5030.96± 458.96) compared to old age group (B) at p ≤0.05. Table (2).

![Table (2): serum concentration of hormones in different age groups of male rats, the data expressed as Mean ± SE.](image)

- Repeated litters in a column indicate no statistically significant difference (p>0.05).
- A significant difference at the p≤0.05 level is shown by the presence of Different litters in a column.

**Oxidative stress biomarker measurement in testis**: There was significant difference P(≤0.05) between old age group (B) (54.38± 6.00 ) and control group (A) (33.43±0 .78 ) , between old age group and RES treated groups(C) (45.24± 1.98 ) . The highest MDA value was detected in old age group when compared to control group, followed by reduced MDA level in group C compared to old age group B. table (3).

![Table (3): MDA levels in testes of various age group. Data is given as Mean ± SE.](image)

- Repeated litters in a row indicate no statistically significant difference (p>0.05).
- A statistical significant difference at the p≤0.05 level is shown by the presence of Different litters in a row.

**Micro-Morphometric Measurement**

1. **Diameter of seminiferous tubule (ST- DM)**: the morphometric measurement of ST diameter in different groups showed significant reduction in (old age group B) comparing to (adult group A), ST diameter increase significantly in old age group treated with RES (C) compared to (old age group A) at (P≤0.05).
2. **Epithelial thickness (EP-TH):** there were significant reduction in the height of germinal epithelium in old age group (B) when compared to control group (A). However in treated group with RES(C) there was improvement of the epithelial thickness compared to old age group at (P≤0.05).

3. **Leydig cells number (LEY-CELL):** the morphometric measurement showed significant raise in Leydig cells number in (old age group B) compared to (control group A) with significant decrease observed in RES treated groups (C) compared to old age group.

4. **Sertoli cells number (SER-CELL):** Sertoli cells were decreased in number in (old age group B) compared to (control group A), in addition to that the Sertoli cells increase in number in (RES treated group C) compared to (old age group B) at (P≤0.05).

5. **Tunica albugenia thickness (T-AL):** the result showed increase in the T-AL thickness in (old age group B) compared to (control group A), in (RES treated group C) there was a reduction in the T-AL thickness compared to (old age group B) at (P≤0.05). Table (4).

### Table (4): parameters of seminiferous tubules (µm), values of Leydig cells and Sertoli cells/40xfeild, data expressed as (mean ± SE).

<table>
<thead>
<tr>
<th>parameters groups</th>
<th>Seminiferous tubules diameter/ µm ST – DM</th>
<th>Epithelial thickness/µm EP-TH</th>
<th>Leydig cells number/40xfeild LEY-CELL</th>
<th>Sertoli cells number/40xfeild SER-CELL</th>
<th>Tunica albugenia thickness/µm T-AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult group</td>
<td>270.77 ± 1.76 a</td>
<td>86.42 ± 0.88 a</td>
<td>29.46 ± 0.93 a</td>
<td>38.08 ± 1.46 a</td>
<td>58.18 ± 2.01 a</td>
</tr>
<tr>
<td>Old age group</td>
<td>95.42 ± 0.57 b</td>
<td>46.71 ± 0.52 b</td>
<td>47.97 ± 0.88 b</td>
<td>25.38 ± 0.34 b</td>
<td>76.56 ± 0.80 b</td>
</tr>
<tr>
<td>Old age with RES</td>
<td>109.06 ± 3.24 c</td>
<td>50.90 ± 1.12 c</td>
<td>43.49 ± 1.35 c</td>
<td>32.46 ± 1.11 c</td>
<td>69.40 ± 2.75 c</td>
</tr>
</tbody>
</table>

- Repeated litters in a column indicate no statistically significant difference (p>0.05).
- A significant difference at the p≤0.05 level is shown by the presence of different litters in a column.

### Sperm analysis: There was a marked reduction in sperm count, viability, and motility, as well as a significant rise in sperm deformities in (old age group B) comparing to (control group A) (adult age group). However, sperm count, viability, motility were significantly improved and reduced sperm deformity in (RES treated group C) compared to (old age group B). At P ≤0.05. Table (5).

### Table (5): sperm parameters in seminal fluid analysis in different age group, the value expressed as mean ± standard error SE.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Sperms count</th>
<th>Sperm viability</th>
<th>Sperm deformity</th>
<th>Sperm motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult group</td>
<td>3006145.0 ±141829.60 a</td>
<td>85.43 ± 1.58 a</td>
<td>10.90 ± 1.22 a</td>
<td>90.46 ± 0.93 a</td>
</tr>
<tr>
<td>Old age group</td>
<td>855610.50 ±36633.660 b</td>
<td>54.4 ± 3.41 b</td>
<td>57.80 ± 2.17 b</td>
<td>64.20 ± 1.96 b</td>
</tr>
<tr>
<td>Old age with RES</td>
<td>2630263.3 ±70617.055 c</td>
<td>73.95 ± 2.39 c</td>
<td>22.31 ± 1.42 c</td>
<td>82.83 ± 1.57 c</td>
</tr>
</tbody>
</table>

- Repeated litters in a column indicate no statistically significant difference (p>0.05).
- A significant difference at the ps0.05 level is shown by the presence of Different litters in a column.
**Histological Findings**

a) Findings of H and E stain :-

**Group A (control group):** In the control group (A), the testis had typical architecture. It was composed of several spherical to oval seminiferous tubules STS separated by small connective tissue interstitial space, and it was encircled by a thin tunica albuginea. Every tubule is encircled by a continuous basal lamina, in which Sertoli and spermatogenic cells are arrested, in the interstitium there were blood vessels and groups of eosinophilic rounded Leydig cells. Figure 1 (A).

**Group B (old age group):** The majority of the seminiferous tubules in this group have abnormal histological characteristics, including atrophy, involution, irregular tubule outline, reduction in germinal epithelium height, vacuolation of Sertoli cells and spermatagonia, wide interstitial tissues with vacuolated homogenous material (edema), absence of spermatozoa in the lumen, and presence of fibrin and hyaline production in the lumen. Figure 1 (B). Some tubules have a disordered germinal epithelium, spermatogenesis arrest, tubule hyalinization, vacuolation of Sertoli cells and spermatagonia, and the presence of giant cells in the tubule lumen. Figure 1 (C), some tubules show severe atrophy and the epithelium consists of a few numbers of Sertoli cells and spermatagonia. Vaculated homogenous acidophilic material infiltrates the interstitium, and widening the interstitial space. Figure 1 (D). Exfoliation of germ cells to the tubule lumen can be seen with germinal epithelium detachment from the basement membrane. Figure 1 (E).

**Group C (RES treated group):** The testicular section of this group showed an improvement, most of the tubules maintained normal histological appearance, normal cellular arrangement of germinal epithelial cells, the presence of sperm in the lumen of the tubule except for a small number of tubules that are still atrophied, disorganized, have reduced height of the germinal epithelium, and still have discrete small numbers of sperm. And few of them still showed separation of germinal epithelium from the basement membrane, discrete few spermatogenic cells were vacuolated. The interstitial tissue still infiltrated by homogenous material but less prominent than in the old age group. Figure 2 (A, B).

b) Periodic Acid Schiff (PAS) stain

**Control group:** The testicular section of this group shows normal +ve PAS reaction in the regular basement membrane and positive PAS particles inside the cytoplasm of spermatogenic cells close the basement membrane and in the late spermatid near the lumen of STs, the interstitial tissue revealed positive PAS reaction. Figure 3 (A, B).

**Old age group:** The section in this group revealed intense PAS +ve reaction of disrupted thick basement membrane and in the interstitial, mild PAS +ve reaction in the degenerated spermatogenic cells and spermatid, absence sperm in the lumen of STs compared with the control group. Figure 3(C).

**RES treated group:** When comparing the result of this group to the old age group, demonstrated a moderate PAS reaction of the normal regular basement membrane, as well as in the spermatogenic cells and the interstitial tissue. Figure 3 (D).

C- Masson's Trichrome (MT) stain

**Group A (control group):** Masson's trichrome (MT) staining of the testes reveals normal collagen fiber distribution, which is shown by the blue color of these fibers in the tunica albugenia, the basement membrane surrounding the seminiferous tubules, around blood vessels and the interstitial tissue. Figure 4 (A).

**Group B (old age group):** The section stained with Masson's trichrome (MT) from this group showed deposition of abundant collagen fibers in the tunica albugenia that seem thicker, as well as dense deposition of collagen in the basement membrane of seminiferous tubules, surrounding blood vessels, as well as in the interstitial tissue. Figure 4(B, C).

**Group C (RES Treated group):** Compared to the old age group, the sections in this group exhibit a marked reduction in collagen fibers deposition in the tunica albugenia, resulting in a decrease in thickness, as well as a reduction in collagen fibers in the basement membrane surrounding the tubules, blood vessels, and the interstitial tissue. Figure 4(D, E)
Figure 1 A: Microphotograph of testis of control group, showing rounded organized seminiferous tubules (ST), the lumen full by sperm. thin tunica albugения (arrow). Narrow interstitial tissues (IT). H&E, 100X. B: Microphotograph of testis of old age group, showing atrophied tubule with vacuolation of Sertoli cell and spermatogonia (black arrow). Decrease the height of germinal epithelium (blue arrow). Wide interstitial tissues with vacuolated homogenous material (edema) (star). H&E, 100X. C: Microphotograph of testis of old age group, showing tubule with arrest spermatogenesis and presence of giant cells in the lumen (arrow), wide interstitial tissues with vacuolated homogenous material (star). H&E, 100X.
Fig D: Microphotograph of testis of old age group, showing atrophy of the tubule with widening of the interstitial tissue (arrow), hyalinization of tubule (star). H&E, 100X. Fig E: Microphotograph of testis of old age group, showing exfoliation of the germinal epithelial cells in the tubule lumen (arrow head), separation of the basement membrane and germinal epithelium (arrow) H&E, 100X.

**Figure 2A:** Microphotograph of testis of old age group treated with RES, showing most of the tubules with normal histological appearance (arrow head), tubule shows stop of spermatogenesis (star), separation of the tubular epithelium from the basement membrane (arrow), H&E,100X. Fig B: Microphotograph of testis of old age group treated with RES, showing most tubules were normal architecture (star), atrophy of the tubule (arrow head), separation of germinal epithelium in tubule (arrow), few cells show vacuolation (blue arrow), less prominent homogenous material (red arrow) H&E,100X.
Figure 3: A Microphotograph of testicular section of control group revealed + ve PAS reaction of basement membrane (arrows) cytoplasm of spermatogenic cells (red arrows) and sperm in the lumen (star), mild PAS reaction in the interstitium (blue arrow), and in the blood vessels (green arrow). PAS, 100X. B: Microphotograph of testis of control group showing PAS +ve particles in the spermatogenic cells and in the enlongated spermatid (arrows), and in Leydig cells (curved arrow). PAS, 400X. C: Microphotograph of testis of old age group showing tense PAS +ve reaction in the thick disrupted basement membrane (black arrow), and in the interstitium (curved arrow), thickened wall of blood vessels (blue arrow), weak PAS reaction in the degenerated spermatogenic cells in STs (red arrows). Thining in the germinal epithelium with wide intercellular vacuoles (green arrow), no sperm in the lumen (star). PAS, 100X. D: Microphotograph of testis of old age RES treated group revealed moderate PAS +ve reaction in the basement membrane (arrow), spermatogenic cells (yellow arrow), and in the interstitium (curved arrow). No vacuoles in the germinal epithelium, presence of sperms in the lumen (red arrow). PAS, 100X.
Figure 4 A: Microphotograph of testis of control group revealed normal collagen fiber distribution in the Tunica albugenia (arrow), in the basement membrane of ST (head arrows) and in interstitium and around blood vessels (red arrows). MT, 200X.

B: Microphotograph of testis from old age group showing dense deposition of collagen fibers in the tunica albugenia increase its thickness (arrow), also increase collagen in the basement membrane of ST (head arrow) and in interstitium and around blood vessels (red arrows). MT, 200 X.

C: Microphotograph of testis from old age group showing increase deposition of collagen fibers in the basement membrane of ST (arrow) and in interstitium (red arrow). MT, 400 X.

D: Microphotograph of testis from old age treated with RES group showing mild increased collagen deposition in interstitium (arrow) and around blood vessels (red arrows). MT, 200 X.

E: Microphotograph of testis from old age treated with RES group showing normal collagen fibers distribution in the tunica albugenia (arrow), mild increased collagen deposition in interstitium (red arrows). MT, 200 X.
DISCUSSION

This research is aimed to assess the effect of ageing on the testes and the effectiveness of RES against age-related testicular changes. The testes of elderly rats revealed a decrease in testicular weight, according to Zhao and their colleague 24, during the ageing process, ROS degrade steroidogenesis in Leydig cells and inhibit testosterone biosynthesis, hence reducing testosterone levels. In this study, the application of antioxidants as RES will counteract the degrading impact of reactive oxygen species (ROS) on Leydig cells, boost testosterone production, preserve spermatogenic cells and germinal epithelium, and raise the testicular weight 25.

The level of testosterone in the current study decreased with ageing, starting gradually to decline in 12-month-old rats and continuing to decline in older rats. This decline in testosterone levels prompted feedback mechanisms to increase the level of LH and FSH hormones to stimulate the testes, in particular the Leydig cells to produce more testosterone hormones to cover this age-related decline in testosterone levels. Reduced sperm counts, germinal epithelial thinning, and other degenerative changes in the testis were all linked to lower testosterone levels in old rats and decreased testicular weight 26,27.

According to the current study’s morphometry results, the increasing Leydig cell number, with a decline in testosterone levels observed in the older age group were accompanied by increases in FSH and LH levels. This increased Leydig cell number may be due to dysfunction of the Leydig cells that limit their capacity for steroidogenesis or reduced the number of LH receptors on the Leydig cells 28. Or it may be due to an Age-related rise in arteriosclerotic lesions of testicular arterioles reducing testicular perfusion and subsequently, decrease oxygen level and level of LH, which are important for the activation of Leydig cells 29. Besides Reduced LH receptor coupling to G-protein results in decreased cholesterol translocation to the mitochondria and steroidogenic enzymes downstream of the smooth endoplasmic reticulum. It has been proven that reactive oxygen species have a negative impact on critical components of the steroidogenic pathway 28, so the use of RES in this study improves steroidogenesis by increasing ROS scavenging and antioxidant enzymes, it prevents ROS generation by inhibiting p38 MAPK activation, which improves STAR (the cholesterol transport proteins steroidogenic acute regulatory protein) gene transcription, which is required for cholesterol translocation to the mitochondria, and thus RES can protect steroidogenic pathway in Leydig cells and preservation of male reproductive health 25,30.

The rate of ROS formation is particularly high because of the significant oxygen consumption necessary for steroid and sperm synthesis in addition to that spermatozoa have little cytoplasm, they are deficient in antioxidant enzymes leads to oxidative stress, lipid peroxidation, DNA damage, and increased apoptotic rate, which eventually terminates with loss of sperm motility, vitality and function as observed in sperm analysis, and increased sperm deformity due to lipid peroxidation, so increased level of free radicals may be a significant contributor to idiopathic infertility 31,32. This is proven by the significant increase in MDA levels in old age rats in this study which is in accordance with the findings of other studies 2. Furthermore, the plasma membrane of sperm has a high level of unsaturated fatty acids, the primary target of oxidative damage. As a result, it is vulnerable to free radical attacks which might result in the creation of MDA, which have the potential to generate additional ROS, rendering the sperm functionally deficient due to plasma membrane damage and unable to unite with the egg for fertilization 31.

The most prevalent age-related testicular change is a mosaic of various lesions of the seminiferous tubule, extending from complete, decreased spermatogenesis to tubules that are fully sclerosed, with impaired spermatogenesis leading to germ and Sertoli cell loss. Thus the diameters of old seminiferous tubules and the height of the epithelium decline 32,33.

This study found that there was less germinal epithelium and the spermatogenesis was impaired, with tubules regressing and degenerated germ cells with wide space between them. This could be attributable to a disruption in the junction of the Sertoli-to-Sertoli cell, which has been linked to abnormal spermatogenesis and the breakdown of the immune barrier provided by the blood testicular barrier in old group rats. Large cells with more than one nucleus could be a sign of poor spermatogenesis or the failure of primary spermatocytes to finish meiotic division. It could be caused by the fusion of the cell membranes of nearby spermatocytes and spermatids, which kills the cells 2,29.

Vacuolation of the germinal epithelium was seen in aged testicular sections, which was referred to as germ cell loss induced by early germ cell exfoliation. Sertoli cells phagocytized the abnormal germ cells resulting in vacuolization 2,34.

Thickening of artery wall was seen in Masson's Trichrome (MT) stain in testicular sections of the elderly group, resulting in a decrease in blood flow to the testicular parenchyma associated with systemic arteriosclerosis and tubular involution of the testes, testicular fibrosis, causes the germinal
tubular epithelium to separate from the blood supply resulting in the involution of the tubule with age and testicular shrinkage. The increased glycosaminoglycans and proteoglycans may be linked to increasing basement membrane thickness collagen type IV accumulates in the basement membrane as a free radical defense mechanism. The interstitial edema (accumulation of homogenous material) found in this study was attributed to excess lymphatic exudate overflowing from deteriorated lymphatic arteries, as well as an increase in vascular permeability caused by free radical accumulation.

The sections from the older age group showed disrupted thickening +ve PAS reaction in the basement membrane and the absence of elongated spermatid and spermatooza in numerous STs, this indicates that spermatogenesis has been arrested. Because the lymph accumulated in the interstitial space contains protein derived from the extracellular matrix (ECM), including collagen, glycoprotein, and proteoglycans, the interstitial space showed an intense +ve PAS reaction. During stress or illness situations the amount and activity of these proteins in the lymph rise. Sertoli cells in senile rats lose the thin cytoplasmic extensions (pseudopodia) that surround germ cells and residual bodies causing the failure of spermatogenesis.

Sertoli cells in the present study revealed degenerative structural changes and reduction in number when compared to the control group in the morphometric study. This has an impact on spermatogenesis because Sertoli cells are essential for the proper development of spermatogonia into spermatooza. The quantity of Sertoli cells, as well as whether these cells were functional or not, influenced spermatogenesis. Sertoli cells in senile rats lose the thin cytoplasmic extensions (pseudopodia) that surround germ cells and residual bodies causing the failure of spermatogenesis.

Antioxidant therapies have a great deal of promise as effective treatment options to lessen the damage that ageing does to the system of male reproduction. So the use of RES in this group reduce the oxidative stress in the testes and ameliorate the histological changes observed in the old age group, most of the seminiferous tubules showed normal histology, basement membrane and there was an improvement in the spermatogenesis and presence of sperm in the lumen, normal interstitial space and decrease in the edema, improvement in MDA level and testosterone level, these observations were confirmed by MT stain which revealed improvement in the tunica albuginea, tubular basement membrane and normal interstitial tissue, regarding the morphometric study the parameter of the ST, value of spermatogenic cells, Sertoli cells and Leydig cells were improved comparing the old age rats. This result is in agreement with Hamza and their colleagues who mentioned that supplementation of RES (20 mg/kg) to aged rats improves various histological damage, biochemical parameters, the sections of testicular tissues demonstrating normal spermatogonia, normal seminiferous tubules, and normal Leydig cells, a significant increase in antioxidant enzyme, and reduction in MDA level, in addition to that RES improves the level of testosterone, increases the sperm viability, and motility. Moreover, compared to other known antioxidants, RES is more effective at minimizing DNA damage.

Furthermore, Hamdy and Basma confirmed the significance of RES in protecting testicular tissue from cisplatin oxidative damage, restoring the tubular basement membrane thickness, the weight of testes, complete spermatogenesis, presence of spermatogenic cells, Sertoli cells, and Leydig cells function are restored, together with the normal histological structure of the STs and interstitial tissue. Hence it is possible to think about RES as a unique therapeutic target for any pathological disorders affecting the testis. RES also reduced aberrant sperm morphology and inhibited the deteriorating effects of ROS on sperm viability, motility, and count.

Resveratrol is the most effective natural substance that activates SIRT-1 (silent information regulator 1), the most conserved mammalian NAD+-dependent protein and a member of the SIRT family. SIRT1 promotes mitochondrial biogenesis and increases mitochondrial mass. Several scientists have investigated how RSV affects male fertility and have shown that spermatogenesis including sperm differentiation and quantity has improved. RSV alters anti- and pro-apoptotic proteins to prevent DNA degradation and cell death.

Additionally, The AMPK (Adenosine monophosphate-activated protein kinase) pathway is activated by RES, which is associated with better mitochondrial performance and greater concentrations of the antioxidant enzymes catalase, glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), to manage the cellular redox status by altering the metabolic pathway while under stress, by minimizing oxidative damage, these defense mechanisms raise sperm quality and spermatooza fertility. The capacity of RES to improve membrane fluidity which aids in a more efficient interaction with radicals, may be the cause of its antioxidant effects against lipid peroxidation. Moreover, RES is linked to the activation of the genes for mitochondrial biogenesis and oxidative
phosphorylation. Moreover, it could stimulate the energy metabolism of spermatozoa, increasing their vitality.

**Highlights**

1. The study aims to see the effect of aging on the testes.
2. The aging process cause progressive histological changes in the testes.
3. These age-related histological changes are associated with a decline in male fertility.
4. There was an elevation in the oxidative stress marker MDA which proves the relation of oxidative stress state with aging.
5. The use of resveratrol improve the histology of testes, and the testosterone hormone level.
6. Resveratrol decrease the oxidative stress marker MDA, this indicate that resveratrol has protective and therapeutic role in aging and age-related changes in the testes.

**Conclusion**

Ageing has a deleterious effect on the testes leading to changes in the histology of the testes affecting sperm and steroid production, causing atrophy of seminiferous tubules decrease in the sperm viability and motility and increasing oxidative stress of aged testes and the RES improves these changes and decreases the oxidative status so it can be used as for protection or treatment of testicular changes during the ageing process.

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**Conflict of Interest**

According to the writers there is no possible conflict of interest.

**REFERENCES**


