Histological Study to the Effect of Resveratrol Nanoparticles in the Testis in White Male Rats Treated with Cisplatin

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Abstract

The current study was designed with the aim of determining some of the histopathological effects caused by the drug cisplatin, as well as identifying the role played by the regular and nano-extract of resveratrol in reducing the toxicity caused by the chemical drug. -12) weeks, and the animals were divided into six groups (ten animals per group) the first group represented (G1) negative control dosed with distilled water and diet for two months and the second group (G2) positive control was dosed with the drug (Cisplatin) at a dose of 2 mg / kg Only of body weight for two months and the third (G3) orally dosed with regular extract resveratrol at a dose of (20) mg/kg of body weight for two months. As for the fourth (G4), it was dosed with nano-extract of resveratrol (20) mg / kg of body weight for two months. And the fifth group (G5) was dosed with the normal extract of resveratrol (20 (mg/kg of body weight) simultaneously with the drug for two months, and the sixth group (G6) was dosed with the nano-extract of resveratrol (20 (mg/kg of body weight) simultaneously with the drug for two months and after the expiry of a period of time. In the experiment, the animals were sacrificed and blood was drawn from them for the purpose of examining the pathological effects of the studied criteria, and the results of the statistical analysis recorded a significant decrease (<0.05) in the level of the studied criteria, and it included (testosterone, LH and FSH, as well as the number of sperm cells and the diameters of the seminiferous tubules) in the group. Treatment with cisplatin drug at a concentration of 2 mg/kg body weight (G2) when compared with the control group (G1) and with the rest of the experimental groups, while the results indicated a significant increase (<0.05) for the groups of normal and nano extract and groups concurrent with the drug when compared with Positive control (G2), while the histological study of rat testes showed clear pathological changes for the drug group compared with the groups of normal and nano extract and others that were dosed concurrently with the drug. It showed a clear improvement in the tissue of the testes due to the role of the effective extract in reducing the damage and toxic effects caused by the drug.

KeyWords: Cisplatin, Regular and Nano-Resveratrol Extract.

DOI Number: 10.14704/nq.2022.20.8.NQ44380 **NeuroQuantology2022; 20(8): 3540-3550**

Introduction

Cisplatin is an anticancer and antineoplastic chemotherapy drug classified as an alkylating agent. It is used in the treatment of advanced bladder, ovarian, testicular, bladder, head, neck, esophageal, lung, small and non-small cell, breast, cervix, stomach and prostate cancers. Lymphoma, neuroblastoma, sarcoma, multiple myeloma, melanoma, mesothelioma [1]. Cisplatin is given intravenously, a chemical that can cause inflammation in the vein it is given through, if cisplatin leaks out. From a vein it can cause tissue damage [2].

Common side effects of the drug include bone marrow suppression, hearing problems, kidney damage, and vomiting [3]. Other serious side effects include numbness, difficulty walking, allergic reactions, deformity and heart disease. Use during pregnancy can also harm the baby [4].

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Cisplatin belongs to the family of platinum-based antitumor drugs and works in part by binding to

DNA and preventing its replication. Cisplatin interferes with DNA replication, killing the fastest spreading cells, which are theoretically considered cancerous. After administration, one chloride ion is slowly displaced by water to give the aqueous compound in a process called aquation. Intracellular chloride dissociation is preferred because the intracellular chloride concentration is only 3–20% of the 100 mM chloride concentration in the extracellular fluid [5].

Cisplatin binds to DNA in several different ways, interferes with cell division by mitosis and damaged DNA causes DNA repair mechanisms to appear, which in turn activate apoptosis when repair proves impossible [6].

As for the compound resveratrol derived from stilbene, it belongs to a group of plant compounds called polyphenols, as this polyphenolic stilbenoid is produced as a natural defense in response to damage to plants, bruises or attack of microbes such as bacteria or fungi, its chemical structure[7]. The compound resveratrol responsible for the pharmacological activity and which could eventually lead to a longer life expectancy was detected [8], and resveratrol was detected in a wide range of about 70 plant species, such as the purple grape Vitis vinifera, Blue berry, Cranberry, Peanut, Rhubarb, Cassia, Jackfruit and pine pines [9], primarily discovered in the Vitis vinifera vineyards in 1976 [10] And later in wine in 1992, the highest concentration of 50-100 µg/g of resveratrol was found, as was found in the casing and seeds of grapes. Unified to infect many types of pathological processes, it occurs when there is a serious disease that causes an imbalance between the production of ROS and antioxidants in the body, which is one of the defense systems [10] and these reactive oxygen species are among the highly reactive types that are formed enzymatically and non-enzymatically In mammalian cells, it causes cell damage either directly or indirectly by interfering with the mediators of diverse signaling pathways in cellular metabolism. [11]

Because of the instability of resveratrol, its poor solubility in water, its inefficiency in systemic delivery, and its low bioavailability, despite the successes it achieved (Lee et al, 2011; Signorelli and Ghidoni, 2005). In order to overcome the limitations of drug movement, it was loaded onto nanoparticles to give a more strategic force [12].

Materials and Methods

Resveratrol Dose ofResveratrol (98% purity) was obtained from Amazon in powder form, and the dose was prepared according to [13] at a concentration of 20 mg/kg body weight based on average weight and each animal was dosed daily orally orally using Stomach.

Cisplatin Dosage ofCisplatin was obtained from drug stores in the form of a liquid bottle with a concentration of 50 mg / 100 ml. The dose was prepared according to [14] at a concentration of 2 mg / kg of body weight by dissolving the required concentration depending on the average body weight of the animal. Each animal was injected weekly under the peritoneum for two months.

Preparation of the Nanomaterial

The nanoparticles were prepared for weekly loading of materials in the Graduate Studies Laboratory / Physiology Branch / College of Veterinary Medicine / Al-Qasim Green University.

Preparation of Nano-chitosan

Preparing of Chitosan Nanoparticles (CNP)

Chitosan nanoparticles were purchased from CAC Center and the produced solution was loaded from the Iranian Yekta Company according to the method of Pires et al.

Loading therapeutic materials on chitosan nanoparticles.

Loading of therapeutic materials on Chitosan Nanoparticles.

Therapeutic materials (resveratrol extract) were loaded onto nano-chitosan by ion gel method according to the method of Ibrahim et al. and Ali et al.

Characterization of Nanoparticles was Determined

By measuring the following checks to ensure that the material is loaded.

1. Particle size analysis
2. Diagnosis using FT-IR

FTIR (Fourier Transform Infrared Spectroscopy) Analysis.

3. Diagnosis using Electron Microscope FESEM

Diagnosis by Field-emission scanning electron microscopy (FESEM).

ExperimentalAnimals

The study was conducted in the animal house of the College of Veterinary Medicine / University of Al-Qadisiyah, under standard conditions of

temperature (22-25 degrees Celsius), ventilation and lighting duration (14 hours of light and 10 hours of darkness) and the animals were given free food and water for the duration of the experiment.

This experiment was conducted on 60 white male rats and divided randomly into six groups of (10) rats for each group, for a period of two months, as follows:

The first group, negative control (G1), represents the negative control group, only 1 ml of distilled water was dosed for two months.

The second group (G2): represents the positive control, injected subperitoneally weekly with cisplatin drug at a concentration of (2 mg/kg) of body weight.

The third group (G3): daily oral dose of resveratrol at a concentration of 20 mg / kg of body weight for two months.

The fourth group: (G4) daily dosed orally with nano-resveratrol at a concentration of 20 mg / kg of body weight for two months.

Fifth group: (G5) daily dosed orally with resveratrol at a concentration of 20 mg / kg of body weight and then injected with cisplatin drug at a concentration of 2 mg / kg of body weight per week simultaneously until the end of the experiment.

The sixth group: (G6) was given orally daily with nano-resveratrol at a concentration of 20 mg / kg of body weight, then injected with cisplatin drug at a concentration of 2 mg / kg of body weight per week simultaneously until the end of the experiment.

Study Criteria

1. Measurements of hormones

The concentrations of hormones (testicular lipid T, follicle-stimulating FSH and luteinizing hormone LH) were measured in the serum using the kits of analyzes for each of the aforementioned hormones produced by the Chinese company BT Lab, based on the examination by the well-known immunotechnology Enzyme-Linked Immunosorbent Assay (ELISA).

2. Histological Study

1. Histological measurements of the testicle

The study of the physiological changes of the testes sections included the following:

• Measurement of the diameters of the seminiferous tubules

The diameters of the seminiferous tubules were measured using an ocular micrometer after it was calibrated with a stage micrometer with a force of 40x. An average of 10 diameters of the seminiferous tubules of regular shape (circular or close to circular) in each section was calculated and then the general average was calculated to extract the average diameter of the seminiferous tubules (Ross et al. al., 2003).

• Calculating the numbers of sperm-forming cells

The numbers of sperm-forming cells represented by spermatids, sperm cells, and spermatids were calculated in 20 seminiferous tubules for each animal, and the method (Alwachi et al., 1986) was used to calculate the rate of the numbers of these cells.

2. The histological sections of the testes were prepared by following the method recommended by Humason, 1967).

Statistical Analysis

The results were subjected to a statistical analysis of the Statistical Package for the Social Sciences (SPSS) program to find out the differences between the averages of the studied criteria in the different groups, and accordingly, the significant differences were determined at the probability level of $P < 0.05$ using the One way analysis of variance (ANOVA) test. Significant differences between means were tested using the Least Significant Differences (LSD) Daniel and Cross (2018) test.

Results

1. Changes of Hormones

The results of the current study shown in the table below indicated a significant decrease ($p < 0.05$) in the level of testicular fat hormone, luteinizing hormone and follicle stimulating hormone for male rats treated with cisplatin at a concentration of 2 mg/kg compared with the negative control group (G1) and the rest of the other groups. The results of the statistical analysis also showed significant differences when treated with resveratrol extract at a concentration of (20) mg / kg of body weight, where a significant increase ($p < 0.05$) was recorded when compared to the positive control and groups (G1, G5, G6), while a significant decrease (p) appeared. < 0.05) when compared with the group of nano-extract (G4). While the current results indicate a significant increase ($p < 0.05$) for the group of nano-extract in the level of hormones compared to the positive control group and the other groups. When studying the results of the groups synchronized with the drug, the results

of the fifth group that were dosed with the normal extract with the drug indicated a significant and clear increase when compared with the positive control (G2) and a significant decrease (P<0.05) when compared with the rest of the other groups. The results of the last sixth group that were dosed with the nano-extract and the drug simultaneously showed a significant increase compared with the positive control group (the drug G2) at a significant level (p < 0.05), as well as with the group (G5) which showed a significant decrease with the other groups represented by (G1, G3)., G4).

Table 1. Shows the effect of cisplatin, resveratrol and nano-extract on the average concentrations of testicular lipotropic hormone, luteinizing hormone and follicle stimulating hormone in male white rats

Groups	FSH (mIu/ml)	LH (mIu/ml)	Test. (ng/ml)
G1	C 1.74±0.04	1.81±0.09C	0.90±0.06C
G2	F 0.75±0.1	0.83±0.09E	0.31±0.07F
G3	1.99±0.05B	2.07±0.16B	1.17±0.07B
G4	2.13±0.04A	2.25±0.29A	1.29±0.07A
G5	1.32±0.06E	1.47±0.04D	0.67±0.04E
G6	1.60±0.08D	1.64±0.03D	0.80±0.03D
Less significant	0.093	0.167	0.083

***Values are mean ± standard error**

*** Similar letters between any two groups indicate no significant differences, while different letters indicate significant differences at the level (p < 0.05).**

Calculating the number of sperm cells and the diameters of the tubules.

Spermatogonia, Spermatocyte, and Spermatid.

The results shown in the table below showed a significant decrease (P<0.05) in the numbers of spermatozoa, sperm cells, spermatogones and tubule diameters in male rats treated with cisplatin (G2) when compared with the negative control group (G1) and the rest of the groups (G3, G4 and G5). G6), and the results showed a significant decrease (P<0.05) in the number of cells for groups (G5 and G6) compared with the negative control group (G3 and G4), while it showed a significant increase (P<0.05 when compared with the group (G2)). When the rates of (G5 and G6) differed from each other significantly (P<0.05, while the rates of negative control and (G3) did not differ significantly (P<0.05), and the statistical analysis showed a significant difference (P<0.05) between the group averages) G4) where it was significantly higher than the rest of the other groups.

Table 2. Shows the effect of cisplatin and normal and nano-resveratrol extract on the rate of spermatozoa, PSCs, spermatids and tubule diameters in male albino rats

Groups	spermatozoa	primary sperm cells	spermatids	tubules diameters
G1	76.82±4.25B	89.79±3.74B	109.7±6.09B	12.73±0.94C
G2	40.13±3.9E	39.27±3.14E	56.62±10.08E	5.27±0.41F
G3	79.72±3.37B	92.95±4.54B	111.53±4.85B	13.69±0.98B
G4	89.35±2.39A	A 99.10 ±1.15	118.26±5.09A	17.31±0.84A
G5	55.47±5.03D	59.19±3.28D	73.39±7.36D	8.24±0.42E
G6	66.97±4.57C	74.44±3.11C	90.76±6.32C	10.25±0.51D
Significant	5.24	4.34	6.66	0.95

***Values are mean ± standard error**

*** Similar letters between any two groups indicate no significant differences, while different letters indicate significant differences at the level (p < 0.05).**

2. Histological Changes in the Testis

Histological examination of the sections taken from the testicles in the negative control group (G1) showed the normal structure of the testicle, as it represented the appearance of dilated seminiferous tubules with a slightly wide lumen and filled with sperm, and the proliferation of spermatogonia was noted, and the process of spermatogenesis appeared integrated with the presence of primary spermatocytes Secondary and proliferation of Leydek cells in the interstitial tissue of the seminiferous tubules (Fig. 1 and 2).

In animals treated with cisplatin (G2) at a concentration of 2 mg/kg of body weight, rupture and disintegration of spermatozoa and inhibition of sperm formation were observed. The lumen of the seminal tubule appeared wide and filled with cellular debris with the presence of small numbers of sperm, and the emergence of clear changes represented in With necrosis and degeneration, as for Leydec's cells, few and non-proliferating cells appeared with a rupture of the basement membrane of the tubule, thickening of the fibrous capsule surrounding the testicular tissue and the presence of thrombus and congestion in the blood vessels in the interstitial tissue between the seminiferous tubules when compared with group

G1) (as in the figures) 3 and 4).

While the group treated with the normal extract at a concentration of 20 mg/kg body weight (G3) showed large, dilated seminiferous tubules filled with sperm with the completion of the process of sperm formation inside the tubule (Spermatogenesis), where the proliferation of spermatozoa was observed, with a large number of sperm cells and a clear proliferation in Ledic cells in the interstitial tissue, with large numbers of Sertoli cells. As in Figures (5 and 6), in the group treated with the nano-extract at a concentration of 20 mg / kg of body weight (G4), positive histological changes were observed, represented by the large expansion of the seminiferous tubules and their appearance full and with a very narrow lining, and the process of sperm formation inside the tubule was (Spermatogenesis). Complete with a clear proliferation of spermatozoa. A clear proliferation of Leydek cells was also observed in the interstitial tissue. Sertoli cells were very clear and large with many long and long sperms extending into the endothelium. As in Figures (7 and 8).

The histological sections of the group of the normal extract and the drug simultaneously (G5) indicated the presence of a very simple rupture in the spermatids in a few seminiferous tubules, which appeared a wide lining and the sperms gathered in them, and also showed a complete process of sperm formation with the proliferation of spermatozoa cells and Sertoli cells and an increase in the number of Lydec cells, (Fig. 9 and 10).

As for the group treated with the nano-extract and the drug simultaneously (G6), it was observed that there was an improvement in the tissue condition, as the seminiferous tubules appeared greatly enlarged and filled with sperm and with a very narrow lining, as well as the proliferation of primary and secondary sperm cells with an abundant number of Lydec cells in the interstitial tissue, and the proliferation of Sertoli cells and sperm are dense and extend towards the endothelium. As in Figure (11 and 12).

The above sections showed that the treatment with the nano-extract and the drug showed a more clear improvement in the testes tissue of the experimental animals. In Leydek cells between the seminiferous tubules, the number of primary and secondary spermatocytes increased, while the seminiferous tubules appeared enlarged with a large number of sperms.

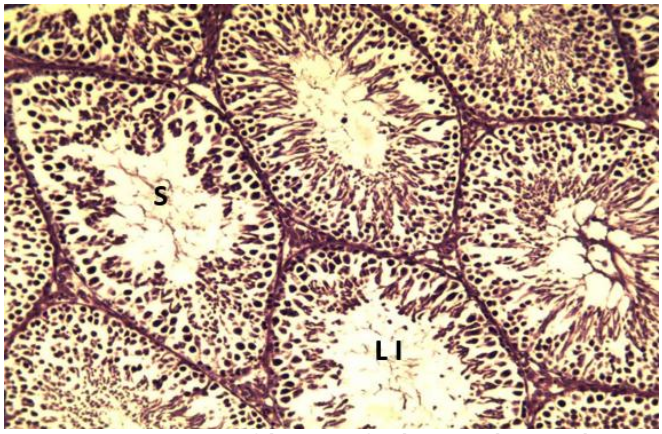

Figure 1. A cross section of the testis of male rats in the control group, where the normal structure of the testis (LI) is observed, in which normal and dilated seminiferous tubules are observed with a slightly wide lumen and contains sperm, (S) with the proliferation of sperm cells and progenitors. 10X H&E

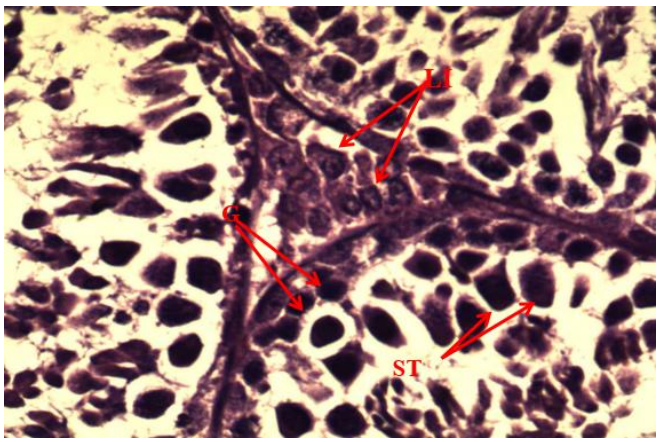

Figure 2. A cross section of the testis of male rats in the control group showing the presence of Leydig cells in the interstitial tissue (LI) with a slight proliferation of spermatozoa, (G), primary and secondary spermatocytes with a number of Sertoli cells (ST). 40X H&E

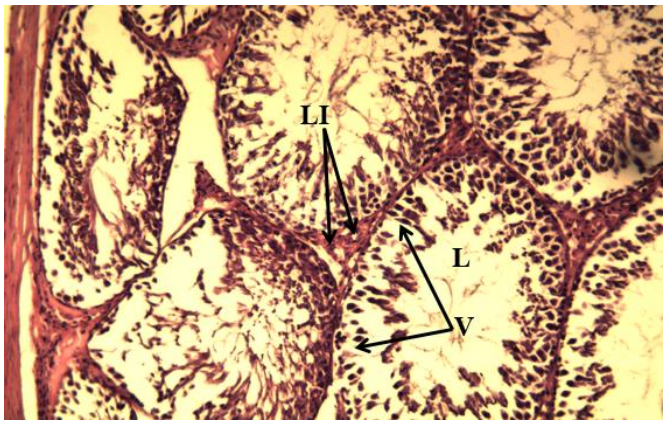

Figure 3. A cross-section of the testis of male rats in the drug group cisplatin shows clear bursting of the spermatids (V) with the presence of a small number of sperm cells, the lumen of the tubules appears enlarged (L). Degeneration and very small numbers of Leydig cells in the interstitial tissue (LI). 10X H&E

Figure 4. A cross section of the testis of male rats in the cisplatin group shows a clear atrophy of the seminiferous tubules (A) with an expansion of the lining of the tubules, which appear devoid of sperm (L). The fibrous capsule surrounding the testicular tissue (CA) thickens, with pronounced hyperemia (C). 40X H&E

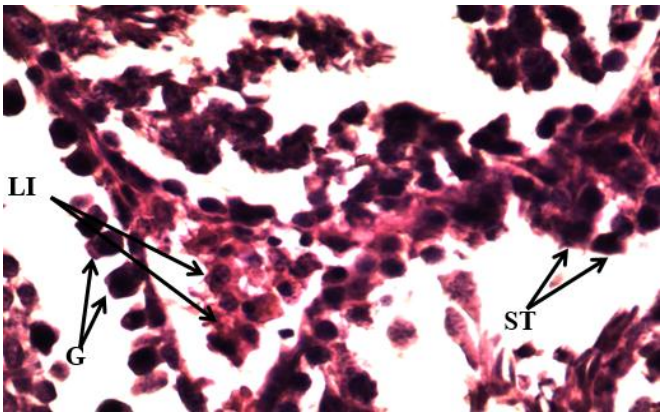

Figure 7. A cross section of the testis of male rats in the extract group (G3) showing a clear proliferation of Leydig cells (LI) in the interstitial tissue. Multiplication and large numbers of Spermatogonia (G) sperm, with large numbers of Sertoli (ST) cells. 40X H&E

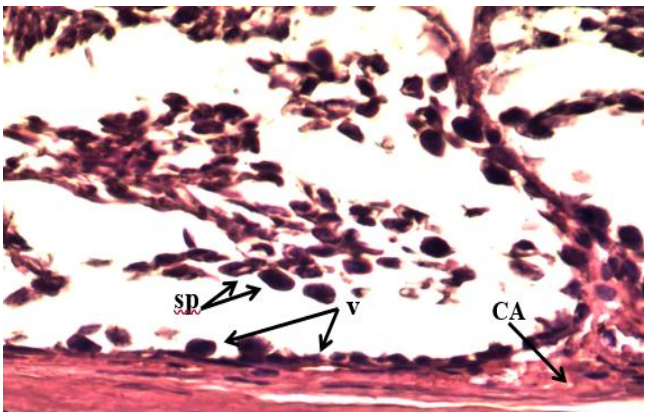

Figure 5. A cross section of the testis of male rats in the drug group cisplatin (G2) showing very few numbers of spermatids with bursting (v) with very few sperm cells (sp). The fibrous capsule surrounding the testicular tissue (CA) thickens, with pronounced hyperemia (C). 40X H&E

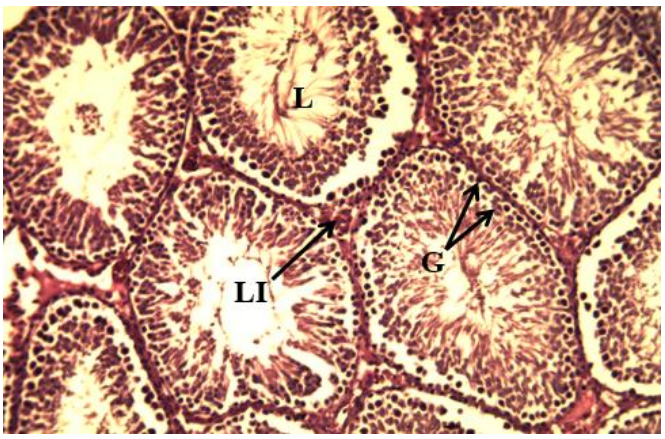

Figure 8.A cross section of the testes of male rats in the group of nano-extract (G4) showing the seminiferous tubules very dilated and full with a very narrow lining (L). The process of spermatogenesis within the tubule is complete with a clear proliferation of spermatids (G), reproduction It is evident in Leydig cells (LI). 10X H&E

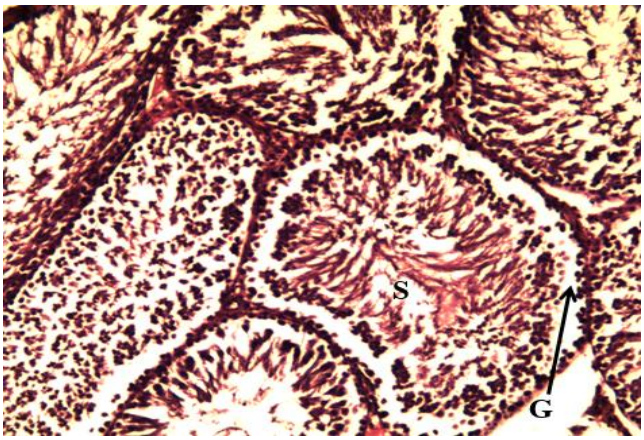

Figure 6. A cross-section of the testes of male rats in the extract group (G3) showing the seminiferous tubules large, dilated and filled with sperm (S), and the process of spermatogenesis inside the tubule (Spermatogenesis) appears complete, where the proliferation of spermatids (G) is observed, with large numbers of cells spermatozoa; 10X H&E

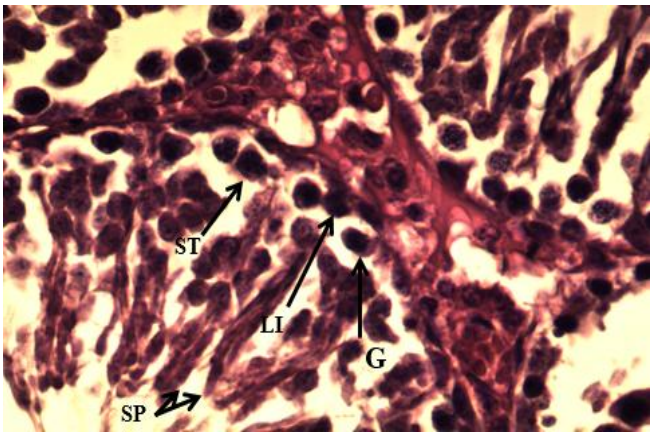

Figure 9. A cross section of the testis of male rats in the nano-extract group (G4) showing a clear proliferation of Lidick cells (LI) in the interstitial tissue, with a clear proliferation of spermatids (G), very clear and large Sertoli cells (ST) with many sperms. and long extending into the endothelium (SP). 40X H&E

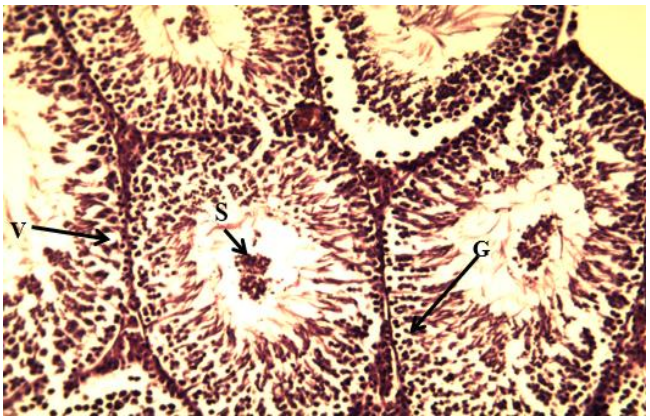

Figure 10. A cross-section of the testes of male rats in the group of extract and drug (G5). We notice a clear proliferation of spermatids (G). With large numbers of sperm cells, the sperm collect in the lining of the tubules (S), very small rupture in the spermatids (V).). 10X H&E

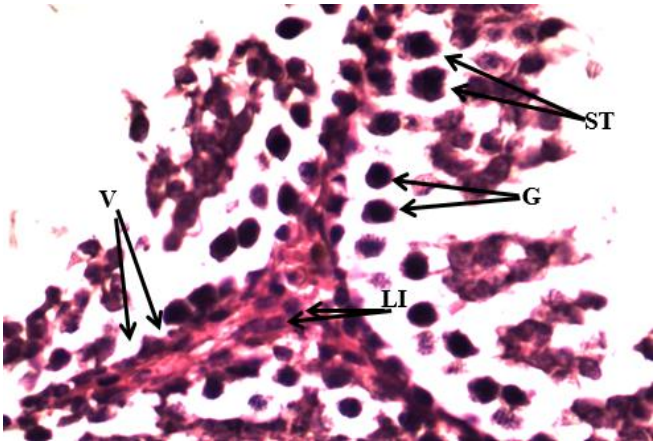

Figure 11. A cross section of the testis of male rats in the extract and drug group (G5). We notice a clear proliferation of spermatozoa (G). With the proliferation of Sertoli cells (ST), with the proliferation of Leydig cells (LI) with a slight rupture in spermatids (V).. 40X H&E

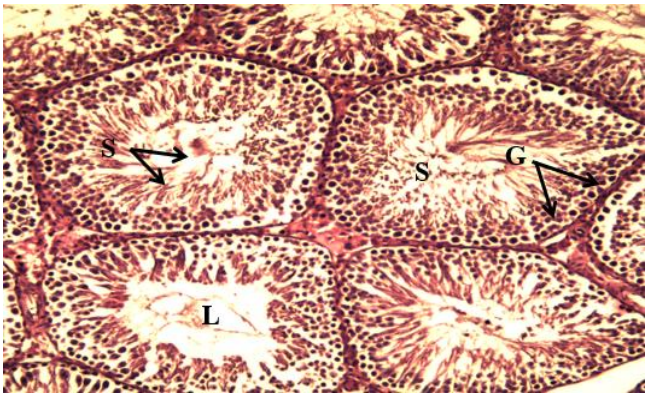

Figure 12. A cross-section of the testis of male rats in the group of nano-extract and drug (G6). We notice a significant expansion of the seminiferous tubules, which appear filled with sperm (S) and with a very narrow lining with a multiplication of spermatozoa (G), a clear proliferation of Leydig cells in the tissue interstitial (LI). 10X H&E

Discussion

The aim of our study is to know the protective effects of the normal and nano-extract of resveratrol and to reduce the toxic effects of cisplatin on the reproductive system of male albino rats. the other. Where the study showed a significant and clear increase in the study criteria represented by hormonal changes and the number of sperm cells. The histological study of the sections taken from the testes and epididymis confirmed a clear improvement in the diameters of the seminiferous tubules of the nano-extract group (G4) over the other groups. This was helped by the nanoloading of the extract due to the improvement of the bioavailability of the extract, its effective distribution, enhancing its concentration in target cells, increasing its solubility, stability and protection from toxicity and oxidative stress caused by the drug [15]. This retention and biological distribution of the extract due to the nanosize enhanced its pharmacokinetic action and the reason for its accumulation preferably in the target site, which increased its distribution and therapeutic effect [16]. An essential feature of an effective drug delivery device is the drug release rate to increase efficacy and therapeutic activity. Therefore, nanoparticles are multifunctional with the development of complex structures for them [17] Nowadays, nanocarriers are one of the most important frequent uses of drug delivery, as it has the advantage of maintaining a stable and effective concentration of the drug at the target site [18]. Chitosan is one of the lines used for loading. It is a polysaccharide with positive properties obtained from chitin and can be used to encapsulate drugs and biologically active molecules [19]. Thus encapsulation or coupling with nanostructures allows protecting and maintaining the structural integrity of polyphenol compounds as well as increasing their water solubility [20].

3. Change of Hormones

Oxidative damage and elevated ROS may cause changes in the H-P-A axis and lead to elevated cortisol levels, which leads to inhibition of LH and FSH secretion by inhibiting GnRH from the hypothalamus and decreasing sensitivity of the pituitary gland to GnRH [21]. Testosterone is an important sex androgen that plays an important role in the growth, reproduction and balance of organ functions, as it is synthesized in Lydec cells and is a source of a variety of hormone biosynthesis enzymes [22]. The results of the current study indicated a significant decrease in the levels of testosterone hormones T, LH and FSH. In the group of animals treated with the chemical drug cisplatin

G2), these results were similar to previous studies such as the study of Shokri et al. of animals exposed to oxidative stress by the chemical drug cisplatin, and for studies [23] which found that cisplatin injections can reduce the level of sex hormone (FSH, LH and testosterone) in laboratory animals. The anterior pituitary gland secretes LH in response to GnRH, which in turn stimulates the Leydig cells to produce testosterone. Therefore, the low level of testosterone may be due to the direct chemical effect of the drug on Leydig cells and the effect of the epithelial layers of germ cells on the LH-Leydig cell axis or indirectly by reducing the sensitivity of the Leydig cell The pituitary gland axis negative feedback. For example, cisplatin stimulates the inhibitory neuronal circuit that controls the feedback mechanism including arginine vasopressin (AVP) in the central nervous system [24]. This in turn stimulates corticotrophin (CRH) and AcRHfrom the medulla region of the adrenal gland. This can inhibit the H-P-G axis and lead to a decrease in GnRH secretion from the hypothalamus. The change in the results of hormones and their increase compared to the drug group and the improvement of its quality is attributed to the role of the extract through stimulating estrogen receptors and stimulating the pituitary gland and the gonadotropin axis. Or because of its antioxidant effect, it exerts a protective effect on the hypothalamus and pituitary gland (Shati, 2019) or the extract can inhibit the decline of antioxidants and the rise of endogenous antioxidants (GSH), which stimulate enzymes that convert cholesterol into pregnonlone, which is the first step in the manufacture of steroids, including testosterone. These results were consistent with the results of and as in the chart below.

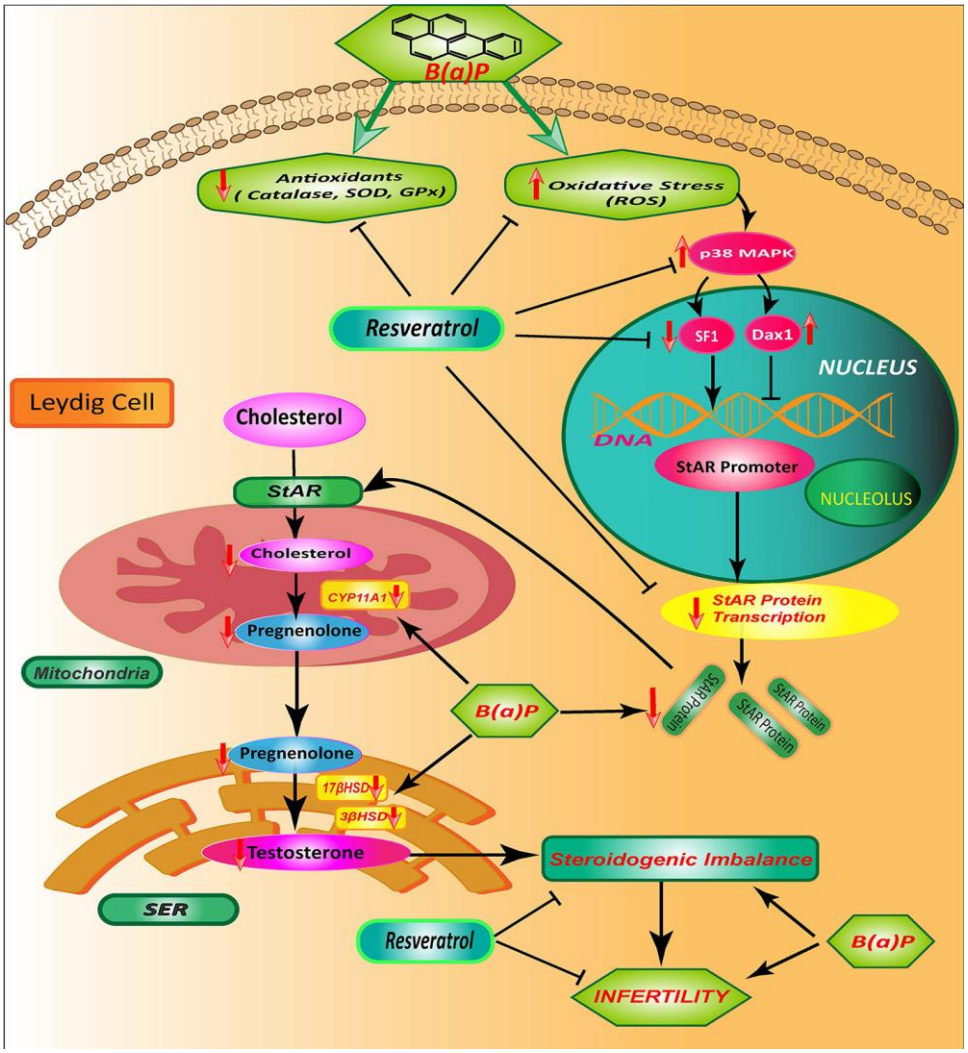

Figure 13. Schematic diagram showing the mode of protective action of Res on Benzo(a)pyrene [B(a)P] induced by Leydig cell dysfunction. Resveratrol inhibits oxidative stress and p38 MAPK-related activation. It also enhances StAR expression and SF1 binding to the StAR promoter. Thus, resveratrol protects against B(a)P.Changes in the numbers of sperm-forming cells and the diameters of the seminiferous tubules.

Cisplatin caused damage at the level of testicular tissues in the group treated with the drug due to its toxic effects and a significant decrease in the numbers of spermatogenetic cells (sperm progenitors, primary spermatoblasts and spermatoblasts) in male albino rats, and this was in agreement with the findings of the researchers [25], that treatment with chemical drugs disrupts the stages of sperm development through damage to some cell components of sperm such as proteins and lipids and damage to their membranes, as well as an imbalance in energy metabolism or by changing In the structure of its acid, and this in turn negatively reflects the stages of cell division and leads to its death and the occurrence of sterility or impairment of fertility [26].

There are many reasons for the occurrence of these cases, including what was indicated by Garcia et al. that the drug leads to an increase in reactive oxygen species in Leydek cells in the testis, and this in turn inhibits the expression of the mRNA gene of CYP11A1 that leads to the construction of testosterone. As well as a defect in the construction of testicular steroids and cause infertility. [26], and the current study showed damage to the germ layer, which in turn leads to azoospermia as a result of chemotherapy, and this direct effect of Lydec cells caused clear damage to them and Sertoli cells as it appeared in the tissue sections [22] The change in the distances can be attributed to the damage to the blood-testicular barrier by ROS, which leads to the entry of excess water and toxic agents between the sperm-producing cells [27].

The current results in rats injected with cisplatin (G2) showed a significant decrease in the diameters of the seminiferous tubules. The results were in agreement with studies [28]. Many interpretations show that oxidative stress is one of the main causes and causes of tissue damage As a result of treatment with cisplatin, by the accumulation of the drug in the testis, especially in the mitochondria of cells, causing inhibition of the enzymes of the respiratory chain by the action of the active oxygen species ROS, such as superoxide radical, hydroxyl radical and hydrogen peroxide, and this was what appeared in the study of Tsunenari et al. The spermatic epithelium, the programmed death of germ cells causes damage to the seminiferous tubes and impairment of fertility and this was evident when treating animals with chemical drugs, including cisplatin, where the decrease in the process of cell proliferation in the seminiferous tubules and an increase in chromatin substance indicates a decrease in the thickness of the germ epithelium and is due to a decrease in mitosis In cells, mitosis and DNA changes decrease. [29] and these cells that suffer from failure to complete their division are removed by the process of programmed cell death [16]. While the groups treated with the normal and nano extract simultaneous with the drug showed an improvement and an increase in the number of cells compared to the positive control group treated with the drug, where resveratrol works to modify the expression levels of the main sperm-regulating proteins including clusterin, zona pellucida binding protein (ZPBP), heat shock proteins and centrin 1 indicating that the extract could indeed be able to interact with the molecular pathways critical to the proper production and function of sperm [11]. The extract also prevents the formation of lipid peroxidation caused by tributyl hydroxide in the sperm. Being lipophilic, it is able to inhibit the production of lipid peroxidation through the Fenton reaction [2], and it can reduce MDA and increase antioxidants in treatment groups by inhibiting lipid peroxidation. ROS Given the low antioxidants during spermatogenesis and its high sensitivity to ROS compared to somatic cells, therefore, they are susceptible to being affected by the reactive oxygen species of their cell membranes and organelles. Therefore, the extract protects the sperm and maintains the structure of their vital membranes. Being a polyphenol that possesses a hydroxyl group, it can pass the blood-testis barrier and protect the sperm DNA from oxidative stress. and Reval et al.indicated that the extract can prevent apoptosis and DNA damage caused by pyrene in sperm. The extract also acts directly on the gene expression of sirtuin 1 according to it [9], where resveratrol increased the expression of sirtuin 1, the neuronal nitric oxide synthase (n NOS) and protein expression (eNOS). The extract activates sirtuin 1 with subsequent activation of eNOS, which leads to enhanced synthesis of AMP guanosine via the nitric oxide/AMP guanosine pathway [12], This pathway leads to a decrease in the rate of programmed death and stimulates germ cell differentiation. [30] and the study agreed with the results of Avdatek et al. (2018), where the extract was able to activate the antioxidant system within rat sperm cells and protect them from DNA damage caused by glycophosphate. and speed up her recovery. It also agreed with [7].

Changes Temperament - Pathological

The results of the examination of histological sections were in agreement with previous studies [8] for the effect of the drug on testicular tissues, which caused edema and an empty lumen of spermatogenic cells. And necrosis in the seminal tube. The testes are frequently affected by many oxidizing agents such as cisplatin, which leads to the formation of ROS, which causes cell injury and tissue disorder. [16]. Therefore, the chemical drug causes the destruction of Lydk cells and reduces their numbers, which leads to a decrease in the level of testicular lipid secretion, a hormone responsible for the functions of the prostate gland and seminal vesicles, and its deficiency leads to a defect in the work of these organs and then testicular atrophy[14]. Free radicals are produced due to the oxidative stress of chemotherapy and cause cytotoxic effects such as their interaction with DNA, which leads to a defect in its replication and structure, and then a defect in the functions of cells and ends with their death. The generated radicals cause lipid peroxidation in the cell membrane and the energy houses of the cell, and this in turn leads to changes in the permeability of its membranes and a clear imbalance leading to a defect in the work of the cell and tissue.

On the other hand, the drug causes a decrease in the hormone testosterone, as it appeared in the current study, that one of the main factors affecting the growth and development of testicular and epididymal tissues depends on androgens that affect the work and building of the hormone. These results agreed with the study of Kohsaka et al. and results from Yucel et al.

The improvement in the tissue sections of the normal and nano extract groups simultaneous with the drug is due to the extract's role in scavenging free radicals and increasing hormone levels. The blood serum, tissue improvement and elimination of oxidative stress, also appeared through the restoration of the weight of the testicle and the shape of the seminiferous tubules, and this indicates the improvement of testicular tissue. The study agreed with the findings of Omur et al. that resveratrol inhibited the effect of aflatoxin on the levels of antioxidants in the testicles and protected the tissue from the toxic effect. It also agreed with the study of Singh et al.when the extract was used to protect the testicles as a result of treating animals with cisplatin. Reddy et al.showed that treatment with the extract at a dose of 20 mg/kg for 45 days was able to improve the activity of antioxidants and steroidogenesis and was reflected in the stages of spermatogenesis in rats treated with cisplatin, and it also agreed with [19].

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