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Cerium Oxide Nanoparticles Protect against Oxaliplatin Induced Testicular Damage: Biochemical, Histological, Immunohistochemical, and Genotoxic Study

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Abstract

Oxaliplatin is a chemotherapeutic drug used for colorectal cancer treatment. The testicular toxic effect is one of its recorded toxicities which resulted in a few studies. Oxidative stress could be a direct cause of this testicular toxicity. Cerium oxide nanoparticles (CONPs) are optimistic antioxidants for applications in medicine. The aim of the work is to study the protective effect of CONPs on testicular toxicity induced by oxaliplatin in rats. Forty adult male albino rats were divided into 4 groups: Control group, CONPs group (60 mg/kg, 5 times/week), Oxaliplatin group (4 mg/kg, twice/week), and Oxaliplatin & CONPs group, for 4 weeks. Seventy-two hours after the last administration, blood samples were taken for hormonal levels and testes were used for both histopathology and immunohistochemical microscopic examination. Sperm smears were also performed and their results were statistically analyzed to detect any sperm abnormalities. Oxaliplatin increased MDA levels. SOD and GPx activity was decreased. GSH levels were decreased. Also, it decreased the sperm cell count and serum testosterone, and anti-Müllerian hormon. In the testicular sections, significant histopathology changes were seen and immunohistochemical examination confirmed these results. Upon supplementation of CONPs with oxaliplatin decreased MDA levels. SOD and GPx activity was increased, and GSH did not change. In testicular sections, normal morphology was seen. Also, there was an increase in the sperm cell count and serum testosterone anti-Müllerian with significant improvement of testicular architecture, and immunohistochemical examination confirmed these results. The utilization of CONPs produced significant protection against all of the above-mentioned changes.

Keywords

Cerium Oxide Nanoparticles, Oxaliplatin, Oxidative Stress, Testis, Toxicity

1. Introduction

Platinum agents are excessively used in the testicular neoplasm chemotherapy protocol. Although, adverse drug reactions and resistance to these agents have restricted their use in anti-neoplastic treatment. Oxaliplatin is an anticancer agent, mainly used as an initial therapy line for the colorectal cancer [1]. Oxaliplatin testicular toxicity and its gonadal function adverse effects are not discovered yet [2]. Mattan *et al.* recorded that both the clinical and pre-clinical settings indicated that oxaliplatin exerted moderate transient gonadal toxicity.

Oxaliplatin induced testicular impact remains as a complicated adverse drug reaction in digestive oncology therapeutic protocols. Up till now, no preventive or curative protocol has been elucidated to be beneficial against these effects because the main mechanism is still unclear [3].

Oxidative stress may be a direct cause of this testicular toxicity as chemotherputic and antineoplastic agents that induce tumor cells apoptosis through prompting of reactive oxygen species (ROS). These ROS affect all diseased and normal cells and may lead to testicular toxicity and other organ toxicities [4].

Cerium oxide nanoparticles (CONPs), act as free radical scavengers, and exert direct antioxidants activity. They represent properties as that of peroxidase, dismutase, and catalase enzymes. They are potent against oxidative stress species and inflammation process. CONPs are applicable in medicine because they are well incurred *in vitro and in vivo* biological patterns [5].

Aim of this work is to study the protective role of CONPs against the toxic effects of oxaliplatin on the testes of adult albino rats.

Ethics Approval:

This study was approved by the local ethical committee of the Faculty of Medicine, Zagazig, Egypt. The study has been reviewed and approved by ZU-IACUC committee. Approval number: ZU-IACUC/3/F/239/2022.

2. Material

2.1. Chemicals

1) Oxaliplatin: It was purchased as pharmaceutical preparation (100 mg/vial), manufactured by Actavis Italy S.P.A. Co. (Milano, Italy).

2) Cerium oxide nanopowder: was purchased from Sigma/Aldrich chemical company, USA in the form of white nanopowder, <25 nm particle size and purity 99.95%.

3) Sodium citrate solution (2.9% - 3%): It was used for Epididymal spermatozoa examination; it was obtained from El-Nasr Co., Egypt.

4) Physiological saline solution (0.9%): It was used for Epididymal spermato-

zoa examination; it was obtained from El-Nasr Co., Egypt.

2.2. Kits

1) Roche Diagnostic GmbH, D-68298 Kits, Mannheim, for USA: US distributor for estimation of testosterone hormones.

2) MDA, SOD enzyme, GPx enzyme and GSH kits: they were purchased from Bio-diagnostic chemical company in Egypt.

2.3. Characterization of CONPs by Transmission Electron Microscope

Detection of primary particle size and morphology of CONPs done by transmission electron microscope (TEM) analysis (JEOL JEM-1400) JEOL Ltd., Tokyo, Japan, operating at an acceleration voltage 80 kV. This characterization was obtained by the team of Electron Microscopy Unite, faculty of agriculture research park, Cairo university (FARP). The CONPs sample was suspended in distilled water. Then the sample was sonicated. A drop of suspension was treated by 400 mesh Copper grid covered with a thin layer of carbon and dried by air dry prior to measurement.

2.4. Animals

This study was carried out on 40 adult male albino rats, each is weighing 150 - 200 grams. The study had been designed in the Faculty of Medicine, Zagazig University. All experimental procedures were ethically approved by The Ethical committee for scientific research of faculty of Medicine, Zagazig university. All animals received care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with "The Guide for the Care and Use of Laboratory Animals" [6]. In order to exclude fallacies, the following environmental conditions were standardized accordingly to Cuschier and Backer [7].

1) The climate conditions in the animal house and in the cage were free from any source of chemical contamination under room temperature (22˚C ± 2˚C), relative humidity 50% ± 5% and a 12 h light cycle with free access to tap water with proper ventilation.

2) Intensity, quality, periodicity and duration of light were that of the natural light.

3) Bedding and wood shavings were usually kept in galvanized iron-mesh cages with solid bottoms that were changed frequently to keep the animal clean.

4) Overcrowding was avoided as well as isolation was avoided so, 10 rats per cage were put.

5) Low noise level was maintained as noise may affect the behaviour of the animals.

The rats were kept in this environment for two weeks before starting experimentation to be adapted to any possible stress secondary to transportation procedure from the animal supplier or due to sudden environmental modification

and to exclude any diseased animals. The rats received balanced food, rich in all stuffs necessary to maintain their health before and during drug administration. It consisted of *ad libitum*. Distilled water was offered in separate clean containers [8].

Study design:

The study was done for 4 weeks and the rats were randomly divided into 4 groups each of ten rats:

After housing acclimatization, the rats were divided into 4 groups as following:

Control group (I) (10 rats): Each rat received only regular diet and distilled water to determine the basic values of performance. These rats were left without intervention to measure the basic parameters.

CONPs group (II) (10 rats): Each rat was given CONPs (60 mg/kg body weight 5 times/week) that diluted in distilled water by intraperitonial injection [9].

Oxaliplatin group (III) (10 rats): Each rat was given Oxaliplatin (4 mg/kg body weight) twice/week by intraperitoneal injection. It was diluted in distilled water until a concentration of 2 mg/ml. LD50 of intraperitoneal injection of oxaliplatin = 14.3 mg/kg body weight [10].

Oxaliplatin & CONPs group (IV) (10 rats): Each rat was given oxaliplatin injection (4 mg/kg, IP, twice/week) & CONPs were injected IP (60 mg/kg) five times/weeks. CONPs were injected initially, then after 4 hours oxaliplatin was injected [11].

One month injection in rats is equivalent to 24 months in human being [12].

Rats of all groups were used to measure:

1) Hormonal levels assay
2) Antioxidant markers
3) Sperm count
4) Histopathology study of the testis.
5) Immunohistochemical study of the testis
6) Genotoxic study (comet assay)

3. Methods

After 72 h of the last administration, the rats were anesthetized by ether inhalation, blood samples were taken from retro orbital plexuses into tubes containing EDTA for hormonal analysis, and then they were sacrificed. For histological study, both testes from each animal, were used for light microscopy and the epididymis was used to prepare sperm smears. The right testes were fixed in Bouin's fixative, then processed to prepare 5-mm-thick paraffin sections and stained with hematoxylin and eosin according to Bancroft and Gamble [13]. The epididymis was minced (using a sharp blade) in 1 ml of 0.9% saline solution. The resulting sperm solution was filtered to remove tissue fragments and used to prepare sperm smears, which were stained with 1% Eosin-Y and taken for sperm morphology examination. Quantitative analysis was done as the sperm smears

were seen with the light microscope to count sperm abnormalities. Sperms were examined by 20 high power field, sperm head and tail morphological abnormalities were listed. Each abnormality percentage was compared to that of the control group [14].

3.1. Biochemical Assays

1) Hormonal levels assay:

a) Measurement of serum testosterone hormon level

For each animal, about 2 ml of blood was collected into a glass tube for testosterone hormone levels. Serum separator tube (SST) permits samples (whole blood) to be clotted for 30 minutes before centrifugation for 15 minutes at 1000 ×g. Remove serum and put samples at −20˚C. Avoid repeated freeze that will disturb the samples. The Testosterone ELISA is a competitive immunoassay for measurement of testosterone in rat and mouse serum [15].

b) Measurement of serum Anti-Müllerian hormone (AMH) level

Anti-Müllerian hormone was measured by ELISA using (CUSABIO®) [16].

2) Methods used for epididymal spermatozoa examination:

Spermatozoa collection was done as described by Kuriyama *et al*. [17]. Epididymal fluid from each rat was collected by slipping the tail of epididymis and squeezing it to collect the fresh semen in a clean Petri dish and incubated at 37˚C for half an hour for liquefaction to allow sperm count.

Sperm count:

Sperm cell count was detected according to the method reported by Pant and Srivastava [18]. The hemocytometer pipette was used to remove semen to the mark 0.1 and pipette was completed to the mark 101 by normal saline stained with eosin. A cover slide was put over the counting chamber and a diluted semen drop was distributed between the haemocytometer chambers and its cover. Then sperms in 5 large squares that contain 80 small squares were estimated using high power of microscope (400×).

3) Antioxidant markers:

a) Reduced glutathione (GSH) level in testes:

This was determined by the method of Ellman [19] measuring yellow colour when 5, 5-dithio-bis-2 nitro benzoic acid (DTNB) was added with testes homogenates in phosphate buffer, then centrifuged. The absorbance was measured spectrophotometrically by ErbaChem 7.

b) Malonyldialdehyde (MDA) level in testes:

This was detected in the testes homogenates by the method of Draper and Hadley [20] following the reaction of thiobarbituricacid (TBA) with malonyldialdehyde (MDA). The pink color produced and detected spectrophotometrically by ErbaChem 7.

c) Antioxidant enzymes activities in testes:

*Catalase activity (CAT): This was detected following Aebi [21] by adding the homogenate hydrogen superoxide (H_2O_2) with phosphate buffer; the changes in absorbance were recorded spectrophotometrically by ErbaChem 7.

*Superoxide dismutase activity (SOD): This was estimated according to Beauchamp and Fridovich [22] by adding potassium phosphate buffer, EDTA, L-methionine, riboflavin and nitro blue tetrazolium (NBT). Blue color in the reaction was measured spectrophotometrically by ErbaChem 7.

*Glutathione peroxidase activity (GPx): This was detected following Flohe and Günzler [23] procedure. The mixture of phosphate buffer, glutathione (GSH), sodium aside, H_2O_2 and testes homogenates was prepared. Tubes were centrifuged and the supernatant was collected. The absorbance was recorded spectrophotometrically by ErbaChem 7.

4) Histopathological study of the testes:

At the time of sacrifice, (after 4 weeks), all animals were anesthetized by ether inhalation, the testes were immediately dissected out and grossly inspected to assess any gross abnormalities. Both testes were fixed in Bouin's solution. One hundred and fifty mL of the previous mixture was added to 50 ml 40% formalin solution and 10 mL of glacial acetic acid [24]. After fixation, testes were put in paraffin blocks and prepared of 5 μm thickness sections.

Testes specimen were subjected for light microscope examination: the specimens were immersed in 10% neutral-buffered formalin, washed, dehydrated, cleared, and embedded in paraffin. Sections of 5 μm thickness were submitted to Haematoxylin and Eosin (H&E) stain as a routine method for studying the general histological structure of the testes (Prophet *et al.* 1992) and for Masson's trichrome stain for collagen fibers detection [25].

5) Immunohistochemical Study of the testes:

Immunohistochemical Staining for PCNA: Immunohistochemical staining was carried out using primary antiserum to PCNA (Clone PC 10, DAKO A/S Denmark). The primary antibody was diluted in Trisbuffered saline with a dilution of 1:50, as determined by the data sheet. The sections were incubated with the primary antibody overnight at + 4˚C. The binding of the primary antibody was observed using a commercial avidinbiotin-peroxidase detection system recommended by the manufacturer (DAKO, Carpenteria, USA). A mouse monoclonal antibody was applied in place of the primary antibody to act as a negative control. Sections from the small intestine were used as a positive control. Then the slides were stained with diaminobenzene (DAB) as the chromogen and counterstained with hematoxylin [26].

Immunohistochemical staining for caspase-3: Immunohistochemical studies were done to detect the (caspase-3) to examine the apoptotic cellular changes Caspase-3 antibody, which is a rabbit poly-clonal antibody (CPP32) Ab-4 (Thermo Fisher Scientific, Fermont, California, USA). A standard avidin-biotin peroxidase complex system was used for detection of caspase-3 using according to the kit used (Neomarkers). Brown cytoplasmic staining is considered positive reaction. Slides stained with secondary antibody IgG only were used as negative control. Specimens from palatine tonsil were used as positive control [27].

Morphometric study:

The image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) at the Image Analyzing Unit of Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used to measure the area percent of collagen fibers and area percent of immunoreaction for caspase 3 at a magnification ×400. The area percent was measured using the interactive measure menu. The measuring frame of a standard area equal to 118476.6 mm^2 was chosen so that the brown positive immune reaction could be seen and masked by blue binary colour to be measured. Examination of ten readings from five non-overlapping sections from each rat of all groups was done.

PCNA-Labeling Index (PCNA-LI) [28]: Slides were examined under the light microscope with a magnification X 200. Then sections were evaluated for PCNA immunostaining. Microscopic fields were chosen at random. Five fields per slide and five slides per animal were evaluated. Only the basal germ cells of these tubules were counted, because they are the cells where active DNA synthesis took place**.** The PCNA-LI for each seminiferous tubule was estimated as a percentage of immune-labeled cells to all basal cells. For each specimen, the mean + SD was calculated. Then, the total PCNA-LI for all groups was estimated accordingly.

6) Genotoxic study (comet assay)

The comet assay was performed in concordance with the premises established by Singh *et al.* [29].

Preparation of base slides:

Low melting point agarose (LMPA 0.5%) and normal melting agarose (NMA 1.0%) were prepared. Slides were desiccated and downwarded to one third of frosted area during that NMA is hotted, the slides then were dried.

Testicular cell isolation:

A small piece of the testes was immersed in 1 ml cold Hank's Balanced Salt Solution "HBSS" containing 20 mM EDTA/10% Dimethylsulfoxide "DMSO", divided into small pieces, removed 5 - 10 µl of this mixture is mixed with 75 µl LMPA. Slides were put side by side on the gel box close together as possible. The buffer were diluted until fully covered the slides for 20 min to permit for DNA untangling and alkali-induced damage expression. Slides were stained with 80 µl IX Ethidium Bromide "EtBr" for 5 min and then dropped in cold distilled water to remove any more stain. The slides were covered by cover slip and scored.

Evaluation of DNA damage:

For inspection of DNA damage, fluorescent microscope was used for EtBr-stained DNA using × 40 objective. A Komet 5 image assay and analysis software featured by Kinetic Imaging, Ltd. (Liverpool, UK), linked to a CCD camera that obtained to assess the quantitative and qualitative DNA damage in the cells by recording the length of DNA migration (tail length) and the percentage of migrated DNA in the tail (tail DNA%). Then, the program calculated tail moment

(correlation between tail length and tail DNA%). Generally, images of 100 (50 × 2) randomly chosen from cells were tested per sample. The mean value (for 100 cells) was calculated.

d) Statistical analysis:

The collected data were expressed as Mean ± SD. The statistical analysis was performed using a Statistical Package for Social Sciences (SPSS version 20.0). Quantitative data were done by student t test (*t*-test). Qualitative data were done by One-way analysis of variance (ANOVA) was used, followed by Post hoc least significant difference (LSD). The Fisher's exact test was used as appropriate to assess differences in sperm abnormalities between the groups. Propability (P value) was set as P value of > 0.05 indicates non-significant results, <0.05 for significant results, <0.001 for high significant result and <0.001 for very high significant results.

3.2. Results

No deaths were recorded during the study period.

A) Biochemical results:

1) Hormonal level assay:

i) Serum testosterone hormone level:

In CONPs treated group "II" in comparison with group I, there was non significant difference in the mean values of serum testosterone along the duration of the study ($p < 0.05$) (Table 1).

When serum testosterone mean values of the Oxaliplatin treated group "III" were compared to group "I", showed a highly significant decrease after 4 weeks of Oxaliplatin treatment ($p < 0.001$) (Table 2).

When serum testosterone mean values of Oxaliplatin + CONPs treated group "IV" were compared to group I, there was non significant difference along the duration of the study ($p < 0.05$) (Table 3).

When serum testosterone mean values of the Oxaliplatin + CONPs treated group "IV" were compared to Oxaliplatin group "III", showed a highly significant increase after 4 weeks of CONPs with Oxaliplatin treatment ($p < 0.001$) (Table 4).

Table 1. Astatistical comparison between (control group "I" and CONPs treated group "II") as regard mean values of hormonal data by *t*-test.

Group	control Group (I)	CONPs Group (II)	*P*
Serum testosterone Mean ± SD	6.4 ± 0.65	6.63 ± 0.5	0.92[#]
Serum AMH Mean ± SD	5.86 ± 0.26	5.49 ± 0.29	0.34[#]

[#]Non significantly different. Student's t-test. M ± SD = mean ± Standard Deviation. P = value of significance. AMH = Anti-Müllerian hormone. CONPs = cerium oxide nanoparticles.

Table 2. A statistical comparison between (Oxaliplatin group "III" and control group "I") as regard mean values of hormonal data by *t*-test.

Group	Oxaliplatin Group (III)	control Group (I)	*P*
Serum testosterone Mean ± SD	2.58 ± 1.5	6.4 ± 0.65	<0.001**
Serum AMH Mean ± SD	9.09 ± 0.21	5.86 ± 0.26	<0.001**

**Highly significant different $p < 0.05$. Student's t-test. M ± SD = mean ± Standard Deviation. P = value of significance. AMH = Anti-Müllerian hormone.

Table 3. Astatistical comparison between (-ve control group "I" and Oxaliplatin & CONPs treated group "IV") as regard mean values of hormonal data by *t*-test.

Group	control Group (I)	Oxaliplatin & CONPs Group (IV)	*P*
Serum testosterone Mean ± SD	6.4 ± 0.65	6.6 ± 0.5	0.64#
Serum AMH Mean ± SD	5.86 ± 0.26	5.99 ± 0.20	0.51#

#Non significantly different. Student's t-test. M ± SD = mean ± Standard Deviation. P = value of significance. AMH = Anti-Müllerian hormone. CONPs = cerium oxide nanoparticles.

Table 4. Astatistical comparison between (Oxaliplatin group "III" and Oxaliplatin & CONPs treated group "VI") as regard mean values of hormonal data by *t*-test.

Group	Oxaliplatin Group (III)	Oxaliplatin & CONPs Group (IV)	*P*
Serum testosterone Mean ± SD	2.58 ± 1.5	6.6 ± 0.5	<0.001**
Serum AMH Mean ± SD	9.09 ± 0.21	5.99 ± 0.20	<0.001**

**Highly significant different $p < 0.05$. Student's t-test. M ± SD = mean ± Standard Deviation. P = value of significance. AMH = Anti-Müllerian hormone. CONPs = cerium oxide nanoparticles.

ii) Serum Anti-Müllerian hormone (AMH) level:

In CONPs treated group "II" in comparison with group I, there was non significant difference in the mean values of serum AMH along the duration of the study ($p < 0.05$) (Table 1).

When serum AMH mean values of the Oxaliplatin treated group "III" were compared to group "I", showed a highly significant increase after 4 weeks of Oxaliplatin treatment ($p < 0.001$) (Table 2).

When serum AMH mean values of Oxaliplatin + CONPs treated group "IV"

were compared to group I, there was non significant along the duration of the study ($p < 0.05$) (Table 3).

When serum AMH mean values of the Oxaliplatin + CONPs treated group "IV" were compared to Oxaliplatin group "III", showed a highly significant decrease after 4 weeks of CONPs with Oxaliplatin treatment ($p < 0.001$) (Table 4).

2) Epididymal semen analysis data:

Epididymal semen analysis data was sperm cell count, percent of sperm motility and percent of normal and abnormal sperm forms.

In CONPs treated group "II" in comparison with group I, there was non significant difference in the mean values of epididymal semen analysis data along the duration of the study ($p < 0.05$) (Table 5).

When epididymal semen analysis data mean values of the Oxaliplatin treated group "III" were compared to control group "I", showed a highly significant increase in abnormal forms after 4 weeks of Oxaliplatin treatment ($p < 0.001$) (Table 6).

When epididymal semen analysis data mean values of the Oxaliplatin+ CONPs treated group "IV" were compared to group "I", showed non significant difference after 4 weeks of treatment ($p < 0.05$) (Table 7).

When epididymal semen analysis mean values Oxaliplatin + CONPs treated group "IV" were compared to Oxaliplatin treated group III, there was highly significant decrease abnormal forms of sperms along the duration of the study ($p < 0.001$) (Table 8).

Table 5. A statistical comparison between the control group "I" and CONPs group "II" as regard mean values of semen analysis {sperm count, percent (%) of sperm normal form, percent of sperm motility, percentage of abnormal head and percentage of abnormal tail} by Fisher's test.

	control Group (I)	CONPs Group (II)	*P*
Sperm count (106/mm^3) Mean ± SD	139.7 ± 4.3	139.1 ± 4.1	0.83$^{\#}$
Percentage of (%) Normal sperm	88.8%	86.4%	0.051$^{\#}$
Percent (%) of sperm motility Mean ± SD	88.3 ± 9.1	89 ± 8.9	0.9$^{\#}$
Percentage of abnormal head (amorphous head, head without hook, and double head)	4.9%	5.7%	0.489$^{\#}$
Percentage of abnormal tail (coiled, angulated, broken, and double tail)	6.7%	7.9%	0.34$^{\#}$

M ± SD = mean ± Standard Deviation. P = value of significance. $^{\#}$non significantly different. CONPs = cerium oxide nanoparticles.

Table 6. A statistical comparison between the Oxaliplatin group "III" and -ve control group "I" as regard mean values of semen analysis {sperm count and the percent (%) of sperm motility, percent of normal forms, percentage of abnormal head and percentage of abnormal tail} by Fisher's test.

	Oxaliplatin Group (III)	control Group (I)	*P*
Sperm count (106/mm^3) Mean ± SD	53 ± 2.8	139.7 ± 4.3	<0.001**
Percentage of Normal sperm	52.5%	88.8%	<0.001**
Percent (%) of sperm motility Mean ± SD	40 ± 2.4	88.3 ± 9.1	<0.001**
Percentage of abnormal head (amorphous head, head without hook, and double head)	13%	4.9%	<0.001**
Percentage of abnormal tail (coiled, angulated, broken, and double tail)	34.5%	6.7%	<0.001**

**Highly significant different $p < 0.05$. M ± SD = mean ± Standard Deviation. P = value of significance.

Table 7. A statistical comparison between the control group "I" and Oxaliplatin & CONPs group "IV" as regard mean values of semen analysis {sperm count and the percent (%) of sperm motility, percent of normal forms, percentage of abnormal head and percentage of abnormal tail} by Fisher's test.

	control Group (I)	Oxaliplatin & CONPs Group (IV)	*P*
Sperm count (106/mm^3) Mean ± SD	139.7 ± 4.3	139.3 ± 5	0.5$^{\#}$
Percentage of Normal sperm	88.8%	86.7%	0.77$^{\#}$
Percent (%) of sperm motility Mean ± SD	88.3 ± 9.1	90.7 ± 7.5	0.89$^{\#}$
Percentage of abnormal head (amorphous head, head without hook, and double head)	4.9%	5.1%	0.34$^{\#}$
Percentage of abnormal tail (coiled, angulated, broken, and double tail)	6.7%	6.9%	0.60$^{\#}$

M ± SD = mean ± Standard Deviation. P = value of significance. $^{\#}$non significantly different. CONPs = cerium oxide nanoparticles.

Table 8. A statistical comparison between the Oxaliplatin group "III" and Oxaliplatin & CONPs group "IV" as regard mean values of semen analysis {sperm count and the percent (%) of sperm motility percent of normal forms, percent of normal forms, percentage of abnormal head and percentage of abnormal tail} by Fisher's test.

	Oxaliplatin Group (III)	Oxaliplatin & CONPs Group (IV)	*P*
Sperm count (106/mm^3) Mean ± SD	53 ± 2.8	139.3 ± 5	<0.001**
Percentage of Normal sperm	52.5%	76.7%	<0.001**
Percent (%) of sperm motility Mean ± SD	40 ± 2.4	90.7 ± 7.5	<0.001**
Percentage of abnormal head (amorphous head, head without hook, and double head)	13%	7%	<0.001**
Percentage of abnormal tail (coiled, angulated, broken, and double tail)	34.5%	16.3%	<0.001**

**Highly significant different p < 0.05. M ± SD = mean ± Standard Deviation. P = value of significance. CONPs = cerium oxide nanoparticles.

3) Antioxidant markers:

a) Reduced glutathione (GSH) level in testes:

There was highly significant decrease of testicular tissue (GSH) content in Oxaliplatin treated rats group when were compared with the control, and CONPs & Oxaliplatin groups (Table 10 and Table 12). There was non-significant difference of testicular tissue (GSH) content in CONPs treated rats group when were compared with the control group (Table 9). There was non-significant difference of testicular tissue (GSH) content in CONPs & Oxaliplatin treated rats group when compared with the control group (Table 11).

b) Malonyldialdehyde (MDA) level in testes:

There was highly significant increase of testicular tissue (MDA) content in Oxaliplatin treated rats group when were compared with the control, and CONPs & Oxaliplatin groups (Table 10 and Table 12). There was non-significant difference of testicular tissue (MDA) content in CONPs treated rats group when were compared with the control group (Table 9). There was non-significant difference of testicular tissue (MDA) content in CONPs & Oxaliplatin treated rats group when compared with the control group (Table 11).

c) Antioxidant enzyme activities:

Testicular tissue antioxidant enzyme(SOD) and (GPx) activities significantly decreased in the Oxaliplatin treated group as they were compared to the control, CONPs and CONPs & Oxaliplatin groups, in testicular homogenates of (Tables 9-12).

Table 9. Statistical comparison between control group (I) and CONPs group (II) as regard levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx) & reduced glutathione (GSH) by *t*-test.

Parameters	control Group (I)	CONPs Group (II)	*P*
MDA Mean ± SD	118.2 ± 13.7	119.9 ± 12.8	0.45[#]
SOD Mean ± SD	5218.5 ± 1167.7	5785.7 ± 905.4	0.89[#]
GPx Mean ± SD	129.7 ± 26.7	116.7 ± 38.9	0.33[#]
GSH Mean ± SD	74.7 ± 36.3	68.5 ± 28.6	0.12[#]

M ± SD = mean ± Standard Deviation. P = value of significance. [#]non significantly different. CONPs = cerium oxide nanoparticles.

Table 10. Statistical comparison between negative control group (I) and Oxaliplatin group (III) as regard levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx) & reduced glutathione (GSH) by *t*-test.

Parameters	Oxaliplatin Group (III)	control Group (I)	*P*
MDA Mean ± SD	188.4 ± 50.2	118.2 ± 13.7	<0.001**
SOD Mean ± SD	7544.3 ± 1524.9	5218.5 ± 1167.7	<0.001**
GPx Mean ± SD	151.4 ± 51.8	129.7 ± 26.7	<0.001**
GSH Mean ± SD	36.9 ± 13.4	74.7 ± 36.3	<0.001**

**Highly significant different $p < 0.05$. M ± SD = mean ± Standard Deviation. Student's t-test. P = value of significance.

B) Histopathological changes of the testes:

1) Macroscopic examination: the testes of both control and treated groups revealed normal appearance and size, with no cystic changes or abnormal masses were noitced. Cut sections were normal.

2) Light microscopic examination:

Hematoxylin and eosin-stained (H&E) sections of testis of the control and Cerium oxide nanoparticles groups showed that the testicular parenchyma was formed of densely packed seminiferous tubules which were lined by stratified germinal epithelium and separated by a narrow interstitium containing clusters

Table 11. Statistical comparison between negative control group (I) and Oxaliplatin & CONPs group (IV) as regard levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx) & reduced glutathione (GSH) by *t*-test.

Parameters	control Group (I)	Oxaliplatin & CONPs Group (IV)	*P*
MDA Mean ± SD	118.2 ± 13.7	119.8 ± 27.4	0.37[#]
SOD Mean ± SD	5218.5 ± 1167.7	5491.6 ± 1793.9	0.71[#]
GPx Mean ± SD	129.7 ± 26.7	131.8 ± 45.7	0.40[#]
GSH Mean ± SD	74.7 ± 36.3	69.3 ± 12.8	0.16[#]

M ± SD = mean ± Standard Deviation. P = value of significance. [#]Non significantly different. CONPs = cerium oxide nanoparticles. Student's t-test.

Table 12. Statistical comparison between Oxaliplatin group (III) and Oxaliplatin & CONPs group (IV) as regard levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx) & reduced glutathione (GSH) by *t*-test.

Parameters	Oxaliplatin Group (III)	Oxaliplatin & CONPs Group (IV)	*P*
MDA Mean ± SD	188.4 ± 50.2	119.8 ± 27.4	<0.001**
SOD Mean ± SD	7544.3 ± 1524.9	5491.6 ± 1793.9	<0.001**
GPx Mean ± SD	151.4 ± 51.8	131.8 ± 45.7	<0.001**
GSH Mean ± SD	36.9 ± 13.4	69.3 ± 12.8	<0.001**

**Highly significant different $p < 0.05$. M ± SD = mean ± Standard Deviation. P = value of significance. CONPs = cerium oxide nanoparticles. Student's t-test.

of interstitial cells and blood vessels (**Figure 1(a)**). Seminiferous tubules were lined by many layers of spermatogenic cells arranged in the form of spermatogonia, primary spermatocytes, spermatids and sperms, separated by Sertoli cells with large euchromatic nuclei resting on the basement membrane. The interstitial spaces contained clusters of Leydig cells (**Figure 1(b)**). H&E sections of the testis of oxaliplatin treated group showed many distorted seminiferous tubules. Congested blood vessel was seen in between a wide interstitium. Some tubules showed marked reduction in the thickness of the germinal epithelium. Homogenous acidophilic material was observed in between the tubules (**Figure 2(a)**). Separation of the lining spermatogenic cells from the underlying basement

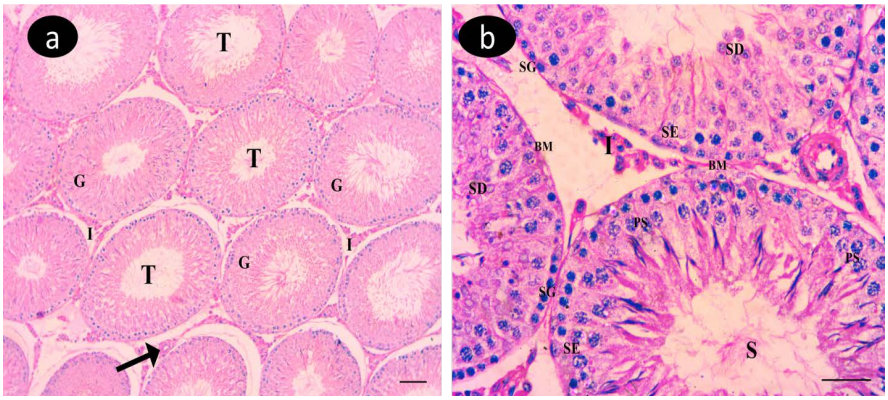


Figure 1. A photomicrograph of a section in the testis of control group showing (a): The testicular parenchyma to be formed of densely packed seminiferous tubules (T) which are lined by stratified germinal epithelium (G) and separated by a narrow interstitium containing clusters of interstitial cells (I) and blood vessels (arrow). (b): Seminiferous tubules lined by many layers of spermatogenic cells arranged in the form of spermatogonia (SG), primary spermatocytes (PS), spermatids (SD) and sperms (S), separated by Sertoli cells (SE) with large euchromatic nuclei resting on the basement membrane (BM). The interstitial spaces contain clusters of Leydig cells (I). (H&E (a) ×100 scale bar 50 μm. (b) ×400 scale bar 20 μm).

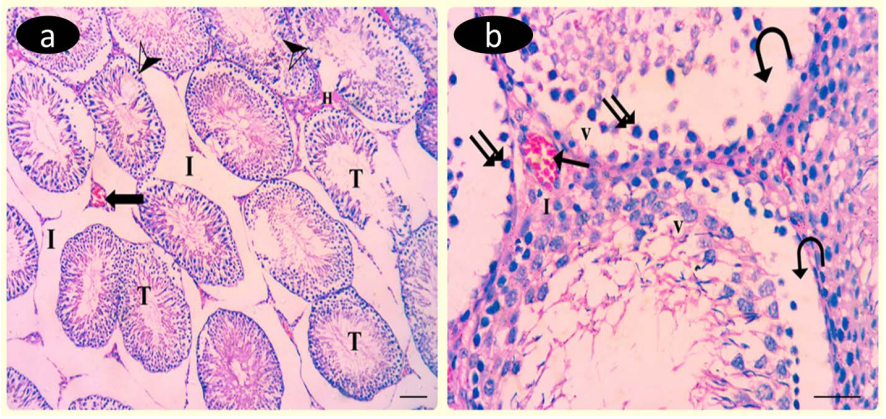


Figure 2. A photomicrograph of a section in the testis of oxaliplatin-treated group showing (a): Many distorted seminiferous tubules (T). Congested blood vessel (arrow) is seen in between a wide interstitium (I). Some tubules show marked reduction in the thickness of the germinal epithelium (head arrows). Homogenous acidophilic material (H) is observed in between the tubules. (b): Separation of the lining spermatogenic cells from the underlying basement membrane is observed (curved arrow). Seminiferous tubules show darkly stained nuclei of spermatogenic cells (double arrows) and multiple vacuoles (V). Congested blood vessel (arrow) and cellular infiltration (I) are seen in the interstitium. (H&E (a) ×100 scale bar 50 μm. (b) ×400 scale bar 20 μm).

membrane was observed. Seminiferous tubules showed darkly stained nuclei of spermatogenic cells and multiple vacuoles. Congested blood vessel and cellular infiltration were seen in the interstitium (**Figure 2(b)**).

Hematoxylin and eosin-stained sections of the testis of Oxaliplatin & CONPs group showed apparently normal seminiferous tubules lined by germinal epithelium with narrow interstitium containing little acidophilic material (**Figure 3(a)**).

Seminiferous tubules were lined by Spermatogonia, primary spermatocytes, spermatid, sperms and Sertoli cells. Few homogenous acidophilic material was noticed in the interstitium (Figure 3(b)).

Light microscopic examination of Masson's trichrome stained sections of testis of control and Cerium oxide nanoparticles groups showed few blue stained collagen fibers in tunica albuginea surrounding the testis (Figure 4(a)). The

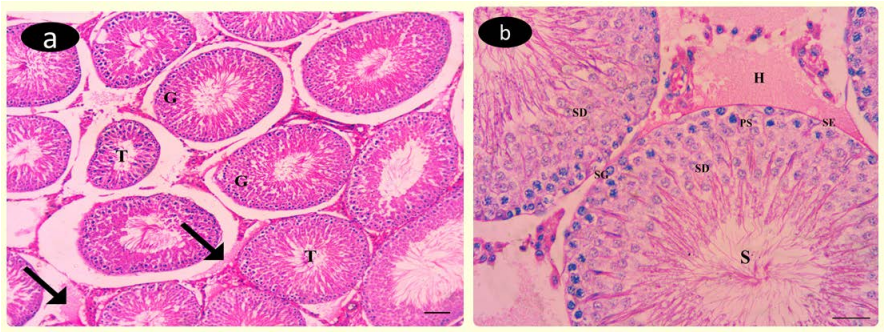


Figure 3. A photomicrograph of a section in the testis of Oxaliplatin & CONPs-treated group showing (a): Apparently normal seminiferous tubules (T) lined by germinal epithelium (G) with narrow interstitium containing little acidophilic material (arrows). (b): Seminiferous tubules lined by Spermatogonia (SG), primary spermatocytes (PS), spermatid (SD), sperms (S) and Sertoli cells (SE). Few homogenous acidophilic material (H) is noticed in the interstitium. (H&E (a) ×100 scale bar 50 μm. (b) ×400 scale bar 20 μm).

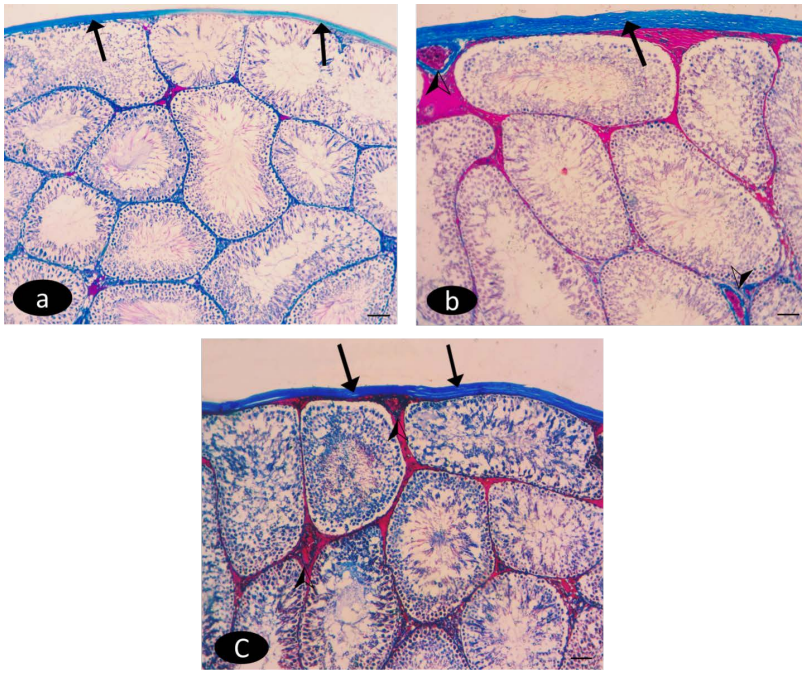


Figure 4. Photomicrographs of Masson's trichrome stained sections in testis of adult albino rats showing (a): Few blue stained collagen fibers in tunica albuginea (arrows) surrounding the testis in control group. (b): Marked collagen fibers are seen in tunica albuginea (arrows) and around blood vessels (arrow heads) in oxaliplatin-treated group. (c): Moderate collagen fibers are seen in tunica albuginea (arrows) and around blood vessels (arrow heads) in oxaliplatin & CONPs-treated group. (Masson's trichrome, ×100 scale bar 50 μm).

oxaliplatin treated group showed marked collagen fibers in tunica albuginea and around blood vessels (Figure 4(b)). The Oxaliplatin & CONPs group showed moderate collagen fibers in tunica albuginea and around blood vessels (Figure 4(c)).

Light microscopic examination of immunohistochemical stained sections for Proliferating cell nuclear antigen (PCNA) of control, Cerium oxide nanoparticles and Oxaliplatin & CONPs groups (in 80% of rats of that group) showed positive nuclear immunoreaction in spermatogonia and primary spermatocytes (Figure 5(a) & Figure 5(c)). The oxaliplatin treated group showed few positive nuclear immunoreaction in spermatogonia (Figure 5(b)).

Light microscopic examination of immunohistochemical stained sections for caspase 3 of control and Cerium oxide nanoparticles groups showed mild cytoplasmic immunoreactivity in germinal cells and interstitial cells (Figure 6(a)). The oxaliplatin treated group showed extensive cytoplasmic reaction (Figure 6(b)). The Oxaliplatin & CONPs group showed moderate cytoplasmic reaction (Figure 6(c)).

3.3. Morphometric Statistical Results

1) Sperm smears morphology:

Light microscopic examination of sperm smears, from control group, revealed that most (88.8%) of the sperms appeared with normal structure; however, a

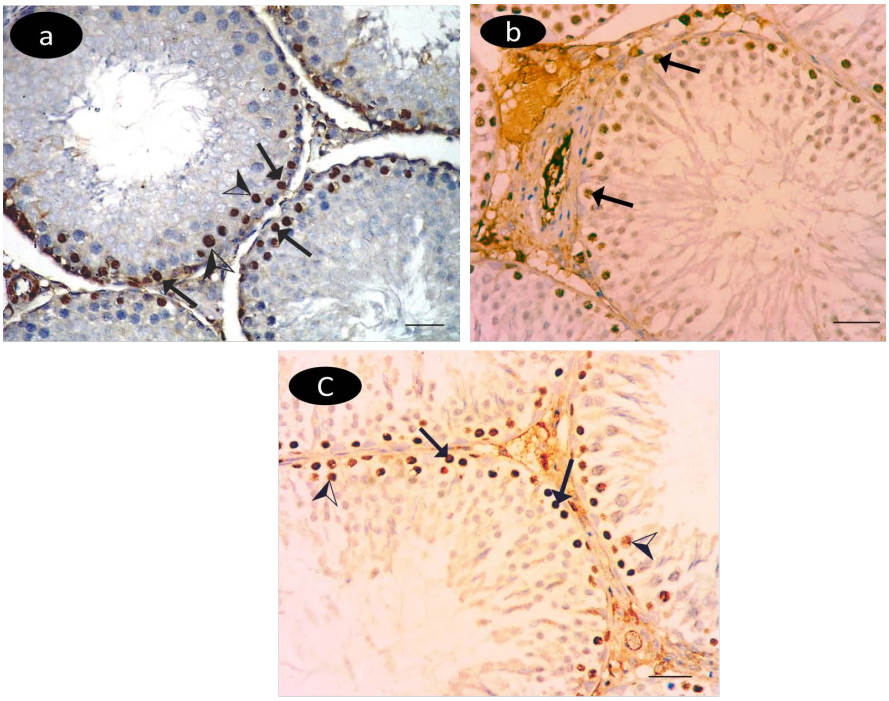


Figure 5. Photomicrographs of PCNA immunostained testicular sections showing (a): Control group & (c): Oxaliplatin & CONPs-treated group illustrating positive nuclear immunoreaction in spermatogonia (arrows) and primary spermatocytes (arrow heads). (b): Oxaliplatin-treated group showing few positive nuclear immunoreaction in spermatogonia (arrows). (PCNA immunoreaction, ×400 scale bar 20 μm).

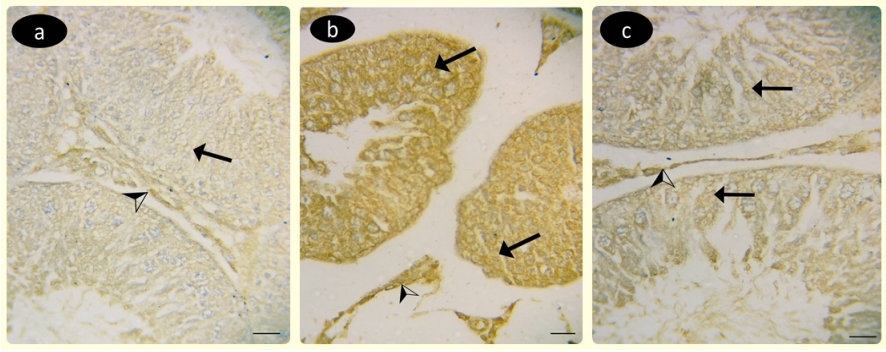


Figure 6. Photomicrographs of caspase 3 immunoreactivity for testicular tissue showing (a): mild caspase 3 cytoplasmic immunoreactivity in germinal cells (arrow) and interstitial cells (arrow head) of control group. (b): Extensive cytoplasmic reaction in both germinal cells (arrow) and interstitial cells (arrow head) in oxaliplatin-treated group. (c): Moderate cytoplasmic reaction in germinal cells (arrow) and interstitial cells (arrow head) in Oxaliplatin & CONPs-treatedgroup. (Caspase 3 immunoreaction, ×400 scale bar 20 μm).

small percentage represented abnormal structured heads (4.9%) and abnormal structured tails (6.7%; **Table 5**). Cerium oxide nanoparticles group: the percentage of normal structured sperms was 86.4%, whereas that of the abnormal structured heads was 5.7% and abnormal structured tails was 7.9% (**Table 5**). Results of the sperm morphology test of oxaliplatin treated group revealed that 47.5% of the counted sperms showed structural abnormalities of which 13% showed abnormal structured heads and 34.5% showed abnormal structured tails (**Table 6**). Moreover, the sperm morphology test of Cerium oxide nanoparticles and Oxaliplatin group revealed that the percentage of normal structured sperms was 86.7%, whereas that of the abnormal structured heads was 5.1% and abnormal structured tails was 6.9% (**Table 7**).

2) Morphometric results:

a) Area percentage (%) of collagen fibers:

Statistical analysis of the area % of collagen fiber content showed a highly significant increase in the oxaliplatin treated group (III) as compared to control group (I). However, there was a non-significant increase in the cerium oxide nanoparticles and oxaliplatin group (IV) as compared to control group (I) (**Table 13**; **Histogram 1**).

b) PCNA-LI Results:

Comparing total PCNA-LI values of the different experimental groups to that of the control group. The values were decreased in the testes of oxaliplatin treated group than those of the control group. There was non-significant improvement of the PCNA-LI values of cerium oxide nanoparticles and oxaliplatin group (**Table 13**; **Histogram 1**).

c) Area percentage (%) of caspase 3 immunoexpression:

Statistical analysis of the area % of caspase 3 positive immunoexpression showed a highly significant increase in the oxaliplatin treated group (III) as compared to control group (I). However, there was a non-significant increase in the cerium oxide nanoparticles and oxaliplatin group (IV) as compared to control group (I)

(Table 13; Histogram 1).

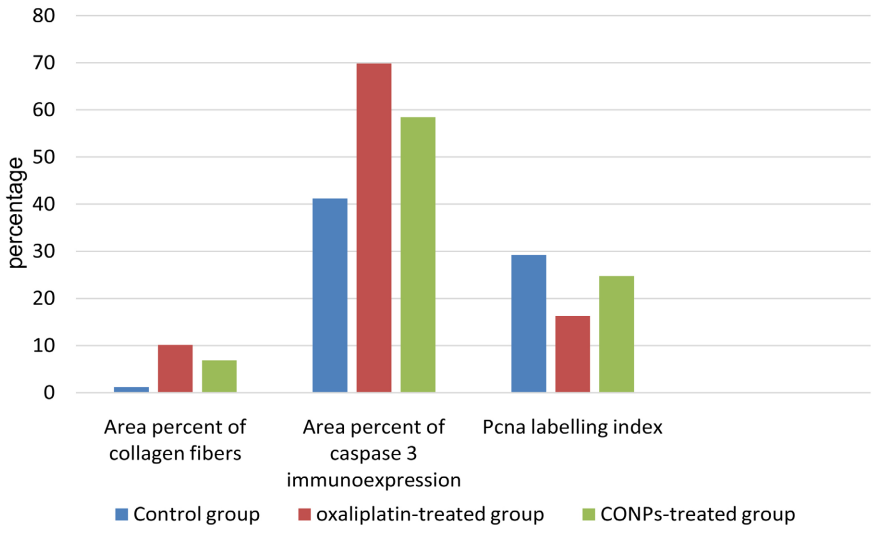
d) Single cell gel electrophoresis (comet assay):

No significant differences were observed regarding mean values of comet tail length, percentage of tail DNA (tail DNA%), and tail moment among control, CONPs and CONPs & oxaliplatin groups by Student's *t* test ($p > 0.05$) (Table 14 and Table 15). Table 16 and Table 17 show significant difference among control group, Oxaliplatin and Oxaliplatin & CONPs treated groups regarding comet tail length (μm), tail DNA% and tail moment by Student's t test ($p < 0.001$). Figure 7 shows normal nuclei and undamaged cells in control and CONPs groups, while abnormal tailed nuclei and damaged cells in Oxaliplatin and Oxaliplatin & CONPs treated group were detected.

Table 13. Morphometric and statistical analysis of testis specimens among the studied groups.

Parameters	Group I Control group Mean ± SD	Group II CONPs group Mean ± SD	Group III Oxaliplatin-treated group Mean ± SD	Group IV Oxaliplatin & CONPs group Mean ± SD
Area percentage of collagen fibers	1.19 ± 0.03	1.8 ± 0.08	10.12 ± 3.1**	3.4 ± 0.5$^{\#}$
Area percentage of caspase 3 immunoexpression	41.2 ± 0.9	41.8 ± 0.7	69.8 ± 2.8***	42.3 ± 0.005$^{\#}$
PCNA-Labeling Index (PCNA-LI)	29.2 ± 8.8	28.8 ± 8.2	22.5 ± 5.6*	24.8 ± 7.9#

*: Significant ($p < 0.05$). **: Highly significant when comparing treated group with control group ($p < 0.01$). $^{\#}$: Non-significant difference when comparing recovery group with control group ($p > 0.05$). ***: Very highly significant ($p < 0.001$).



Histogram 1. Showing morphometric and statistical analysis of testis specimens among studied groups.

Table 14. Comet test statistical analysis (comet tail length (μm), Tail DNA percentage, Unit tail moment) in control, and CONPs treated groups by *t*-test.

Parameter	Group	control group M ± SD	CONPs group M ± SD	*P*
Comet test	Tail length (μm)	3.71 ± 0.26	3.69 ± 0.25	0.838#
	Tail DNA%	1.59 ± 0.26	1.48 ± 0.26	0.275#
	Unit tail moment	14.39 ± 1.5	15.01 ± 1.5	0.902#

N = number of rats M ± SD: Mean ± Standard Deviation μm: micrometre. P = value of significance. Student's t-test.

Table 15. Comet test statistical analysis (comet tail length (μm), Tail DNApercentage, Unit tail moment) in control and oxaliplatin & CONPs treated groups by *t*-test.

Parameter	Group	control group M ± SD	Oxaliplatin & CONPs group M ± SD	*P*
Comet test	Tail length (μm)	3.71± 0.26	4.11 ± 0.13	0.980#
	Tail DNA%	1.59 ± 0.26	1.98± 0.13	0.381#
	Unit tail moment	14.39 ± 1.5	15.46 ± 1.3	0.896#

N = number of rats M ± SD: Mean ± Standard Deviation μm: micrometre. # = non significant different. P = value of significance. CONPs = cerium oxide nanoparticles. Student's t-test.

Table 16. Comet test statistical analysis (comet tail length (μm), Tail DNA percentage, Unit tail moment) in control and oxaliplatin treated groups by *t*-test.

Parameter	Group	control group M ± SD	Oxaliplatin group M ± SD	*P*
Comet test	Tail length (μm)	3.71 ± 0.26	8.07 ± 0.10	<0.001**
	Tail DNA%	1.59 ± 0.26	6.88 ± 0.13	<0.001**
	Unit tail moment	14.39 ± 1.5	20.41 ± 1.3	<0.001**

N = number of rats M ± SD: Mean ± Standard Deviation μm: micrometre. P = value of significance. **Highly significant different $p < 0.05$. Student's t-test.

Table 17. Comet test statistical analysis (comet tail length (μm), Tail DNA percentage, Unit tail moment) in coxaliplatin and oxaliplatin & CONPs treated groups by *t*-test.

Parameter	Group	Oxaliplatin group M ± SD	Oxaliplatin & CONPs group M ± SD	*P*
Comet test	Tail length (μm)	8.07 ± 0.10	4.11 ± 0.13	<0.001**
	Tail DNA%	6.88 ± 0.13	1.98 ± 0.13	<0.001**
	Unit tail moment	20.41 ± 1.3	15.46 ± 1.3	<0.001**

N = number of rats M ± SD: Mean ± Standard Deviation μm: micrometre. **highly significant different $p < 0.05$. M ± SD = mean ± Standard Deviation. P = value of significance. CONPs = cerium oxide nanoparticles. Student's t-test.

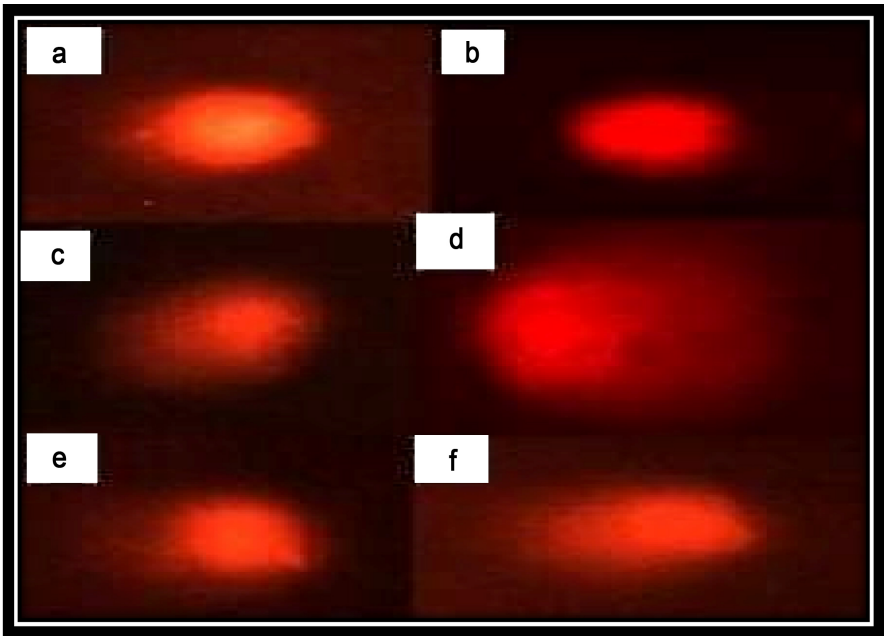


Figure 7. Fluorescent microscope photomicrographs of testicular cells obtained from rats showing ((a), (b)) Normal condensed type nuclei (without comet tail) in control group. ((c), (d)) the toxic effect of oxaliplatin treated group appears as damaged DNA represented by the tail. ((e), (f)) slightly damaged cell with comet tail in Oxaliplatin & CONPs-treated group.

4. Discussion

Platinum agents are broadly utilized in the chemotherapy protocol of testicular cancer. Nevertheless, adverse drug reactions and drug resistance to these agents have limited their demand in antineoplastic treatment. Chemotherapy-induced testicular toxicity evaluation depends mostly on traditional reproductive markers as semen analysis and hormonal level [30].

In our study, there were highly significant decrease in testosterone level and sperm cell counts with high significant increase AMH level in Oxaliplatin treated group indicating reproductive toxicity.

These results are in parallel with Behringer *et al.* [31] who conducted a study on mice concluded results same ours, decrease in humans and mice serums testosterone, abnormal sperm cell counts and increased AMH levels after platinum chemotherapy, submitted that germ cells damage was due to exogenous agent may promot dematuration or differentiation of Sertoli cells [32]. Male mice had low AMH level showed normal germ cell and spermatogenesis, whereas male mice expressed high AMH level, also showed weakened Leydig cell development and function [33].

In our study, there were disturbances in the levels and activities of oxidative stress markers in oxaliplatin treated rats. These results are in accordance with Qu *et al.* [34] who recorded that oxaliplatin caused a significant increase in the mitochondrial oxidative stress indexes lipid peroxidation and protein carbonyl. Alterations in antioxidants enzymes activities were also observed. Oxidative

stress causes main role in the cell mitochondrial toxicity of oxaliplatin. The intercell integrity between hepatocytes tissue is damaged by the reactive oxygen species-mediated lipid per-oxidation and protein carbonyl formation.

Further, The histopathological results of the current work supported the toxic effect of oxaliplatin on the testis as oxaliplatin-treated rats showed distorted seminiferous tubules, congested blood vessel within a wide interstitium and marked reduction in the thickness of the germinal epithelium. Homogenous acidophilic material was observed in between the tubules. Separation of the lining spermatogenic cells from the underlying basement membrane was observed. Seminiferous tubules showed darkly stained nuclei of spermatogenic cells and multiple vacuoles. Few proportion of PCNA-positive nuclei and high DNA damage by comet assay in oxaliplatin exposed rats tissues.

These results are in accordance with that recorded by Garcia *et al.* [35] histopathological changes caused by oxaliplatin injection were epididymal hypospermia, decrease spermatogenic cells counts in the testis. For explaining the previous findings, other researchers who studied the effect of oxaliplatin on offspring testes stated that the decrease in thickness of the germinal epithelium resulted from reduction in the divisions of spermatogonia B caused by an elongation of the G1 phase. Reduction in the thickness of the germinal epithelium has also been reported, in response to a Cisplatin treatment, as the result of decreased cellular divisions and oxidative stress. Another study indicated a decrease in tubular diameter and epithelial height as the result of Di-nbutyl phthalate (DnBP) administration, due to the loss of cells in the germinal layer [36].

In our study, Light microscopic examination of Masson's trichrome stained sections of testis of control and CONPs groups showed few blue stained collagen fibers in tunica albuginea surrounding the testis. The oxaliplatin treated group showed marked collagen fibers in tunica albuginea and around blood vessels. The Oxaliplatin & CONPs group showed moderate collagen fibers in tunica albuginea and around blood vessels. These results are in parallel with Soudeh *et al.* [37]. Similar findings were observed by other researcher who studied the effect of cyclophosphamide (anticancer chemotherapeutic drug) on testis [38]. She attributed the excess collagen fibers to either over production of the fibers or decrease in the rate of collagen remodeling by fibroblasts.

PCNA immunostaining was detected exclusively in the nuclei of testicular cells, however no staining was detected in the cytoplasm. In control and CONPs group, high proportion of PCNA-positive nuclei and low morphologic alterations were observed. This results confirmed by Yokozawa *et al.* [39] who stated that proliferating cell nuclear antigen (PCNA) is an intranuclear polypeptide that is involved in DNA replication, excision and repair. Its synthesis and expression is linked to cell proliferation. Since spermatogenesis is a complex cell cycle of rapidly proliferating cells ending with liberation of sperms, PCNA was used in this study to quantitatively analyze spermatogenesis.

PCNA immunohistochemical staining has evidenced to be worthy. In general,

cell proliferation significantly impacted to the degree of dangerous effects where the less differentiated cells showed a stronger ability to proliferate, thus promoting abnormal structure and function. Bonetti *et al.* [40] stated that PCNA expression was significantly related to the degree of hepatocellular cells differentiation and that numerous PCNA-positive nuclei were especially observed in tumor and areas of extracapsular tumor proliferation. In conclusion, these results clarify that PCNA staining is a reliable tool of cell (tumor) proliferation but not in the damaged cells.

In our study, immunohistochemical stained sections for caspase 3 of control and CONPs groups showed mild cytoplasmic immunoreactivity in germinal cells and interstitial cells. The oxaliplatin treated group showed extensive cytoplasmic reaction. The Oxaliplatin & CONPs group showed moderate cytoplasmic reaction. These results were in accordance with Hayward *et al.* [41].

Similar findings were observed by previous investigators who studied the histopathological changes caused by another chemotherapeutic agent; Cisplatin in wister rats. Caspase-3 is a member of intrinsic protein factors for programmed cell death (apoptosis). It plays a vital role in seminiferous tubules in apoptosis regulation. Its activation leads to DNA degradation, chromatin condensation and membrane protein destruction [42].

The current study shows that histological damage in testis is associated with increase in testicular lipid peroxidase. Several studies have shown that platinum agents toxicity in kidney is mediated by depletion of anti-oxidants and elevations of lipid peroxidase. Platinum agents have generated free radicals by interaction with DNA. So, overproduction of free radicals has main role in testicular injury associated with platinum agents treatment [43].

The cytotoxicity of oxaliplatin is thought to hinder DNA synthesis. The bulky DACH ring in oxaliplatin structure can result in the formation of platinum-DNA adducts. The platinum-DNA-protein cross-links influence nuclear metabolism and organization and outface DNA replication and repair. Also, oxaliplatin induces apoptosis in a variety of normal and carcer cells (testis, colorectum, liver) through different mechanisms [44].

Chater *et al.* [45] showed that oxaliplatin created an increase of p53 expression and a decrease apoptosis proteins inhibitors (IAP) in mouse. Hayward *et al.* recorded that Bcl-xL down-expression intensified oxaliplatin-induced apoptosis through the p53- and Bax-mediated apoptotic route.

Gourdier *et al.* [46] demonstrated oxaliplatin disinhibits DNA replication and transcription, it chiefly affects organs with rapid cell mitosis and divisions such as the testes.

In our study, co-administration of CONPs causes improvement in levels of testosterone, AMH levels and sperm cell counts in rats, it was nearly similar to control group levels. In this group, pretreatment with CONPs increase the testosterone level, decrease AMH level and decrease semen analysis impairement, also improve oxidative stress markers levels and activities, beside the improve-

ment of histopathological changes and genetic damage of testes.

As, CONPs can decrease NO released by suppressing both gene expression and enzymatic activity of iNOS. The chief effect is on anti-inflammatory pathways. The professed valuable effect of CONPs might be mainly obtained through its antioxidant properties by decreasing the MDA content in the testicular tissues by scavenging free radicals [47].

These results are in parallel with that results recorded by Rahimi *et al.* [48] that showed improvement of sperm count, abnormality, viability rate, and motility, epithelial thickness, seminiferous tubules diameter, and serum testosterone level in N-acetyl cysteine (antioxidant) treated group. In addition of improvement of histological feature and immunoreactivity in the form of normal spermatogenic cells (spermatogonia, spermatocyte, and spermatid), somatic testicular cells (sertoli and leydig cells), and accurate spermatogenesis with abundant spermatids were observed in mice treated with N-acetyl cysteine with mild immunoreactivity level of caspase-3 in the spermatogonial cells.

Oxidative stress may give to infertility due to defective sperm function Agarwal *et al.* [47]. The oxidative stress plays a main role as trigger testis destruction by induction of lipids, proteins and DNA structural impairment and pathways that power physiological functions impairment. Spermatozoa are armed with antioxidant defense mechanism and are ready to scavenge ROS, so protecting gonadal cells and mature spermatozoa from oxidative toxic stress. However, in risk and pathological agent exposure, excess ROS release, resulting in oxidative toxic stress that gives to decreased sperm motility, viability and count [49].

It was shown that in the groups treated with CONPs, semen parameters improved compared with the toxic group. This result is in accordance with the role of CONPs as antioxidant. CONPs has been contributed to be a superoxide dismutase mimetic enzyme. The CONPs exist in both Ce^{3+} and Ce^{4+} state. Karakoti *et al.* have recorded that, CONPs reduce super-oxide-produced hydrogen peroxide (H_2O_2). Ce^{4+} oxidizes H_2O_2 to O_2 and regenerates Ce^{3+}, and Ce^{3+} is also oxidized to Ce^{4+}. It can form an autoregenerative redox cycle on the surface of CONPs between Ce^{3+} and Ce^{4+}, and create oxygen defects to attract the free radicals. Other researchers showed that CONPs increased total thiol and total antioxidant power. Similarly, CONPs inhibit lipid per-oxidation and increase TAC and TTG levels in testis tissue. Although there are many proofs which confirm CONPs antioxidant properties, it was shown CONPs may presenting toxic effects, it is generally shown that toxicity rises as the nanoparticles size becomes smaller. The smaller nanoparticles size the larger surface area they have per unit of mass in so they are possibally more active. Also, the cell nanoparticles uptake increases with the smaller sizes so raises the amount of nanoparticles in the tissues and blood vessels. CONPs have a higher surface area to volume ratio, and these leads to a larger surface Ce^{3+}/Ce^{4+} ratio, this could be related with a more toxicity for smaller CONPs. These other reports showed various beneficial as well as toxic effects for CONPs. Regardless all the conflicting evidence on CONPs toxicity, these nanoparticles have very beneficial applications [50].

5. Conclusion

This study provides evidence that oxaliplatin adversely affects male reproductive organ tissues and sperm characters through increasing the oxidative damage. The cerium oxide nanoparticles administration is highly effective in preventing the testes against oxaliplatin toxicity. Further studies are recommended on larger populations and longer duration and to assess the toxicity on the female reproductive system.

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Ethical Approval

This study was approved by the local ethical committee of the Faculty of Medicine, Zagazig, Egypt. The study has been reviewed and approved by ZU-IACUC committee. Approval number: ZU-IACUC/3/F/239/2022.

Author Contributions

Experiments were created and designed by Dalia M. Amin. They were performed by Marwa T. Abaza. Biochemical analyses, statistics, and discussion were performed by Dalia M. amin, Samar Reda, and Ghada El sammak. All authors contributed to writing and revising the manuscript.

Data Availability Statement

Dalia Mohamed Amin: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no conflict of interests in relation to this article.

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