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Photobiomodulatory Effects of Low-Power Pulsed Laser on Oxidative Stress, Apoptosis, and Inflammatory Responses in the Testicular Tissue of Cisplatin-Treated Adult Male Mice

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Abstract

Introduction: Cisplatin is widely used as a key treatment for solid tumors. Despite being a highly effective chemotherapy, the use of cisplatin is restricted by several side effects during treatment, such as germ cell depletion and testicular atrophy. Photobiomodulation (PBM) has emerged as a promising strategy for tissue repair. The present study seeks to assess the effects of PBM on improving antioxidant and anti-inflammatory responses in the testicular tissue of adult male mice subjected to cisplatin treatment.

Methods: Twenty-four adult male NMRI mice were randomly assigned to three groups (n = 8 each): an untreated Control group (Cont), an Injury group that received a solitary intraperitoneal (IP) injection of Cisplatin at 7 mg/kg (Cis), and a Treatment group that received Laser treatment at 4 J/cm^2 following the Cisplatin injection (Treat). Subsequently, the testes and epididymides were extracted. The right testis was designated for histological and stereological analysis, while the left testis was preserved at –80 °C for molecular and biochemical assessments.

Results: PBM conferred notable protective effects after treatment, significantly improving the total sperm count and sperm motility in the Treat group ($P \leq 0.0001$). Following laser irradiation, a marked reduction in the TNF-α and IL-1β levels was observed in the Treat group ($P = 0.0008$, $P \leq 0.0001$). Moreover, there was a significant rise in the expression of the *Bcl-2* gene in the Treat group ($P = 0.0004$).

Conclusion: Notwithstanding the constraints of the current research, this study endorses the translational potential of PBM and establishes a robust basis for forthcoming investigations in the domain of toxicology and laser-assisted therapies.

Keywords: Apoptosis, Cisplatin, Photobiomodulation (PBM), Oxidative stress

Introduction

Currently, infertility impacts 15% of couples. It is estimated that one in six individuals will experience infertility at some point in their lives, with male infertility accounting for nearly half of all cases. The causes of male infertility are diverse, including disruptions in endocrine function, toxic exposures, lifestyle influences, chemotherapeutic agents, and both genetic and epigenetic alterations.[1,2] However, male factor infertility, often attributed to impaired spermatogenesis resulting from a disruption in germ cell proliferation and differentiation, is identified as the underlying cause in 25–50% of infertility cases, according to various studies.[3] Chemotherapy, while demonstrating substantial effectiveness in cancer treatment, is regarded as a primary contributor to testicular toxicity and degeneration, particularly affecting the gonads. This

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presents a significant risk for both permanent and temporary infertility.[4]

Cisplatin (CP) is a platinum-based chemotherapeutic agent that is widely used as a key treatment for solid tumors and hematologic malignancies. CP functions as a DNA alkylating agent and exerts its anticancer effects through various mechanisms, including DNA damage, apoptosis induction, and reactive oxygen species (ROS) generation. Despite its high efficacy as a chemotherapeutic agent, the use of cisplatin is restricted by several side effects during treatment, such as germ cell depletion, testicular atrophy, and dysfunction of both the kidneys and testes.[5,6] Testicular toxicity induced by CP has been extensively documented in various experimental studies.[7,8] In testicular tissue, CP causes oxidative damage to proteins, lipids, and DNA mediated through germ cell depletion, testicular atrophy, and disruption of the oxidant-antioxidant balance.[9] Mitochondrial DNA (mtDNA) is particularly susceptible, as CP tends to accumulate in mitochondria and form adducts with both mtDNA and mitochondrial proteins.

Mitochondria, essential for energy production through oxidative phosphorylation, are the primary endogenous source of ROS. Consequently, CP exposure leads to in excessive ROS production, resulting in oxidative stress, activation of inflammatory signaling pathways such as NF-κB, and the initiation of intrinsic apoptotic cascades.[10,11]

Over the past several decades, numerous approaches have been explored to mitigate CP-induced testicular toxicity. These include the application of various natural compounds, such as flavonoids, and phenolic acids, which have antioxidant and anti-inflammatory properties and may contribute to preventing CP-related testicular damage.[12] Moreover, photobiomodulation (PBM) has emerged as a promising noninvasive strategy for tissue repair. Unlike high-intensity lasers, PBM lacks harmful thermal effects and instead promotes photochemical reactions within cells, thereby enhancing mitochondrial function, stimulating ATP production, and supporting protein synthesis.[13,14] In the context of male infertility, PBM has demonstrated encouraging potential. Studies have reported that exposure to PBM can enhance sperm motility and viability, elevate testosterone levels, and, notably, reduce sperm cell death both in vivo and in vitro.[15] Furthermore, animal research indicates that PBM may support male reproductive health by promoting spermatogenesis, increasing testicular volume, and lowering ROS production.[16] Considering the high prevalence of chemotherapy-induced infertility and the current lack of effective preventive interventions, exploring innovative therapeutic strategies is of great importance. The present study seeks to assess the effects of PBM on enhancing antioxidant and anti-inflammatory responses in the testicular tissue of adult male mice that have been treated with cisplatin.

Methods

Animals and Ethics

Twenty-four adult male NMRI mice, weighing between 25 and 30 gr and aged 8 weeks, were sourced from a licensed animal facility. In compliance with NIH regulations, the mice were kept in a handled environment that provided them with unlimited access to food and water, maintained at 50% humidity, with a temperature set at 24 ± 2°C, and subjected to a 12-hour light cycle. Each cage was designed to hold a maximum of two mice. All experimental procedures were approved by the Research Ethics Committee at Shahid Beheshti University of Medical Sciences, which approved the protocol for this study under the code (IR.SBMU.LASER.REC.1403.022).

Experimental Design

Animals were allocated to the experimental groups at random by utilizing a six-sided die. Rolls resulting in 1 or 2 were designated for the untreated Control group (Cont), rolls of 3 or 4 were allocated to the Injury group receiving a solitary intraperitoneal (IP) injection of Cisplatin at 7 mg/kg (Cis), and rolls of 5 or 6 were assigned to the Treatment group receiving the same cisplatin dose followed by Laser 4 J/cm^2 (Treat). This allocation process persisted until the target sample size of eight animals per group was achieved. Each group comprised eight mice. The sample size was established by referencing earlier published research that utilized similar models of cisplatin-induced testicular injury and had comparable quantitative outcome metrics. Ethical considerations, in accordance with the 3Rs principles (Replacement, Reduction, and Refinement), were also taken into account to minimize animal use while maintaining adequate statistical power to detect biologically meaningful differences.[17] Animals were excluded from the study if any of the following conditions were observed: the existence of pre-existing diseases or abnormal baseline clinical or laboratory parameters; a weight loss exceeding 20% of the initial body weight during the experimental period; severe systemic toxicity following the administration of Cisplatin, which manifested as persistent lethargy, dehydration, an inability to eat or drink, or signs of distress that did not respond to supportive care; technical or procedural errors, including incorrect dosing, improper injection routes, or malfunctions of the laser device; or the development of unexpected pathological conditions that were not related to the study protocol. Animals that fulfilled these criteria were swiftly excluded from the study and humanely euthanized in compliance with the protocols established by the institutional animal care and use committee (IACUC) as well as international regulations for laboratory animal welfare.

PBM Protocol

Mice were anesthetized with an intraperitoneal injection of ketamine and xylazine before each PBM session, administered every other day. In the Cis + PBM (Treat) group, laser irradiation was applied transcutaneously to both testes, targeting the anterior surface directly above the testicular parenchyma. Before each treatment session, the scrotal hair was removed using a depilatory cream to

ensure uniform light penetration. The laser probe was positioned at a right angle to the scrotal surface, lightly touching the skin. One irradiation point was applied to each testis. The reported irradiation area of 0.5 cm^2 corresponds to the laser spot area in direct contact with the skin. The initial laser session was conducted 24 hours after single-dose cisplatin administration, while subsequent sessions were carried out every other day for 35 consecutive days (one spermatogenic cycle),[18,19] resulting in a total of 17 treatment sessions. Skin and testicular temperatures were recorded immediately prior to and immediately following each irradiation session using a non-contact infrared thermometer, ensuring that the temperature increase did not exceed 1 °C. This measure was taken to guarantee safety and to prevent any thermal effects. For photobiomodulation (PBM), an 810-nm pulsed infrared diode laser was employed. This laser produced pulses at a frequency of 50 Hz and a pulse width of 180 µs, resulting in a duty cycle of 1.44%. The area of the irradiation spot was 0.5 cm^2, which yielded a surface fluence of 4 J/cm^2. Each irradiation point received a total energy of 2 J over a 20-second exposure; thus, each testis received 2 J per session, leading to a cumulative energy of 34 J per testis throughout the entire treatment duration (2 J × 17 sessions). The average output power was measured at 100 mW, corresponding to a power density of 200 mW/cm^2. The calculated peak power was approximately 6.9 W.[20] The laser output at the skin surface was verified before the initiation of the experimental protocol using a calibrated optical power meter. The laser device was adjusted in accordance with the work guidelines before the initiation of the experimental sessions. It was recalibrated at regular intervals during the study to maintain consistent output stability. Optical losses through the scrotal skin and superficial tissues were considered during treatment planning, and identical irradiation conditions were maintained for all treated animals to ensure experimental consistency. The same trained operator performed all PBM sessions to confirm uniformity and reduce procedural variability. During the experimental period, no interventions were conducted in the Control group. Mice assigned to the Cis group received a single intraperitoneal injection of cisplatin, without receiving PBM or any sham laser treatment. The PBM protocol was exclusively implemented for the animals in the Cis + PBM (Treat) group, following the irradiation conditions outlined previously.

Tissue Gathering

At the end of the therapeutic duration, the animals were anesthetized using ketamine (100 mg/kg) and xylazine (5 mg/kg) before being sacrificed. The testis and epididymides were then extracted. The right testis was weighed and preserved in Bouin's solution for subsequent histological and stereological examination. Meanwhile, the left testis was snap-frozen and stored at –80 °C for molecular and biochemical assessments. Additionally, an analysis of the sperm was conducted.

Sperm Analysis

For sperm analysis, sperm count and motility were evaluated. Sperm were collected from the cauda epididymis of the left epididymis. The cauda epididymis was immediately transferred into a Petri dish containing 1 mL of Ham's F10 medium (Sigma-Aldrich, Product No. N6635), gently minced using a sterile needle, and incubated at 37°C for 20 minutes to allow sperm to swim out into the medium. No additional dilution of the sperm suspension was performed before sperm analysis. The resulting sperm suspension was gently mixed, and 10 µL was loaded into a Neubauer hemocytometer for sperm counting. Each sample was analyzed in three technical replicates using a Neubauer chamber, and sperm were counted in five microscopic fields for each replicate. Sperm motility was assessed manually under a light microscope and classified according to standard criteria. A total of 200 spermatozoa per animal were evaluated for motility analysis. The investigator performing sperm analysis was blinded to the experimental groups throughout the evaluation. All sperm analyses were performed by the same experienced investigator under identical experimental conditions to minimize inter-observer variability.

Measurement of Sperm Chromatin Dispersion (SCD)

To conduct this test, a semen sample was diluted using PBS. A volume of 20 µl from the diluted sample was combined with 80 µl of 1% low melting point agarose. Subsequently, 50 µl of this mixture was applied to a slide that had been previously coated with 65% regular agarose. The slide was then placed on top of it. After allowing for 5 minutes of incubation in the refrigerator, the slide was carefully removed. Following the protocol of the Halosperm kit, the first step involved incubating with acid denaturing solution A for 7 minutes in the dark to denature the nuclear DNA proteins. This was followed by 15-minute incubation with lysing solution B at room temperature to halt the denaturation process, and finally, dehydration was carried out using increasing concentrations of alcohol. The staining solutions provided in the kit were then utilized. To estimate the extent of sperm DNA fragmentation, one hundred sperm from each sample were analyzed under a light microscope at 100* magnification. Sperm cells exhibiting fragmented DNA were identified by the absence of halos or the presence of small halos, while those with medium and large halos were classified as healthy and free from DNA fragmentation.[21]

Stereological Analysis

At the end of the therapeutic duration, animals were sacrificed and the testes were carefully excised. Tissues were immediately fixed in Bouin's fixative for the required duration to ensure optimal preservation. Following fixation, the samples underwent dehydration and embedding in a tissue processor. Paraffin blocks were then sectioned at thicknesses of 5 µm and 10 µm, and the sections were subsequently dyed with hematoxylin and

eosin for histological examination.

Assessment of the total quantity of germ cells and somatic cells

The total quantity of germ cells and somatic cells was determined using the Optical Disector method, applying the formula[22]:

$$N_V = \frac{\sum Q}{h \times \sum P \times a/f} \times \frac{t}{B.A}$$

where ΣQ is the total number of counted cells, ΣP is the number of points intersecting the selected fields, a/f is the frame area, h is the disector height, BA is the microtome section thickness, and t is the actual measured section thickness.

Assessment of the Diameter of the Seminiferous Tubules

To determine the diameter of the seminiferous tubules, a random selection of 10 tubes was made initially. Subsequently, 16 points, which were interconnected by 8 lines that intersect at the center of the tube, were chosen within each tube. The mean length of these 8 lines was regarded as the diameter of the tube. Cross sections were additionally utilised for measurement.[23]

Measurement of Pro-Inflammatory Cytokines

To assess the levels of TNF-α and IL-1β in testicular tissue, an ELISA was performed (Milenia Biotech, Nauheim, Germany). In summary, the test kit functioned as a solid-phase ELISA on a microplate that has been coated with a monoclonal antibody specific to TNF-α and IL-1β. After the introduction of a second polyclonal horseradish peroxidase-labeled antibody that targeted a different epitope of the TNF-α and IL-1β molecules, a sandwich complex was created, which consisted of both antibodies along with TNF-α and IL-1β. The chromogenic substrate used was 3.3,5.5-tetramethyl benzidine (TMB), and the optical density of the resulting colored product was measured using a microplate reader at 450 nm.[24]

Gene Expression Analysis

Tissue samples were quickly immersed in the RNAlater solution to maintain RNA integrity and were stored at −80 °C until they were processed. Total RNA was extracted using the RNX-Plus RNA Isolation Kit (CinnaGen Co., Tehran, Iran) in accordance with the manufacturer's guidelines. Any residual genomic DNA was removed through treatment with DNase I (Roche, Mannheim, Germany). The concentration and purity of RNA were assessed spectrophotometrically by measuring absorbance at 260 and 280 nm, with only samples exhibiting an A260/A280 ratio between 1.8 and 2.0 being utilized. The integrity of RNA was further validated through 1.5% agarose gel electrophoresis, which displayed intact 28S and 18S rRNA bands. First-strand cDNA was synthesized from 1 μg of total RNA using a commercial cDNA synthesis kit (YektaTajhiz, Tehran, Iran), adhering to the manufacturer's protocol.

Relative mRNA expression levels of Bax, Bcl-2, and Caspase-3 were quantified using SYBR Green real-time PCR, employing exon–exon junction-specific primers that were designed with Primer3Plus software and validated through NCBI Primer-BLAST. The complete primer sequences and amplicon lengths are provided in Table 1. The specificity of the primers was confirmed by the observation of a single peak in the melt-curve analysis. The amplification efficiency for each gene was assessed using serial dilutions of cDNA, which ranged from 95% to 102%. No-template controls (NTC) and no-reverse-transcription controls (NRT) were included in all runs to check for potential contamination.

GAPDH was utilized as the endogenous reference gene for normalization. Testicular tissue samples from six mice per experimental group (n = 6 biological replicates) were analyzed, with each sample being run in technical triplicates. Replicates with a Ct standard deviation greater than 0.3 were excluded. The mean threshold cycle (Ct) values were employed to calculate relative expression using the 2-ΔΔCt method.

Measurement of Oxidative Stress Marker

After excision, testicular tissues were processed to minimize ex vivo changes in ROS production. The tissues were finely minced and enzymatically dissociated using trypsin–EDTA to obtain a single-cell suspension. The cells were then washed twice with ice-cold phosphate-buffered saline (PBS) and centrifuged at 1,200 rpm for 5 minutes at 4°C. To reduce the possibility of artifactual ROS generation during sample preparation, all the procedures were performed using pre-cooled reagents, the samples were kept on ice whenever possible, and the processing time was standardized for all experimental groups. Intracellular ROS levels were subsequently assessed by incubating the cell suspensions with 20 μM DCFDA at 37°C for 45 minutes in the dark. The fluorescence intensity was assessed using a spectrofluorometer with an excitation wavelength of 488 nm, serving as a marker for intracellular ROS levels.

Measurement of GSH Levels

Glutathione (GSH) levels were assessed using a spectrofluorometric method. Testicular cell suspensions

Table 1. Primer design

Genes	Primer sequences	Product size (bp)
Bax	**F:** GCTACAGGGTTTCATCCA	155 bp
	R: GTATCCACATCAGCAATCAT	
Bcl-2	**F:** GAACTGGGGGAGGATTGTGG	186 bp
	R: GGGGTGACATCTCCCTGTTG	
Caspase-3	**F:** AAGCACTGGAATGTCA	175 bp
	R: AACACTATCCATCAATTCC	
Gapdh	**F:** AGTGCCAGCCTCGTCTCATA	153 bp
	R: TGAACTTGCCGTGGGTAGAG	

(0.5 mL) were treated with the fluorescent probes O-phthalaldehyde (OPA) and N-ethylmaleimide (NEM). After staining, the cells underwent centrifugation at 1000 rpm for 1 minute, and the resulting pellet was resuspended in 2 mL of a fresh culture medium. To remove any remaining fluorescent dye, the cells were washed twice. The fluorescence intensity was subsequently measured with a Shimadzu RF-5000U spectrophotometer, with excitation and emission wavelengths set at 495 nm and 530 nm, respectively..[25]

Statistical Analysis

Data analysis was conducted using SPSS v27. The Shapiro–Wilk test was employed to evaluate normality. For parametric data, comparisons were made using one-way ANOVA, while nonparametric data were analyzed with the Kruskal–Wallis test. Multiple comparison tests were performed using Tukey's test and Dunn's test. Graphical representations were created utilizing Prism software (version 10). Results are presented as mean ± SD, with $P < 0.05$ considered statistically significant.

Results

PBM Improved Sperm Count and Sperm Motility

Cisplatin administration induced severe impairment in evaluated sperm parameters, including total sperm count and sperm motility in semen fluid, which reflected profound testicular toxicity. PBM exhibited considerable protective effects post-treatment, resulting in a significant increase in total sperm quantity and motility relative to the Cis group. (Figure 1; $P \leq 0.0001$).

PBM Reduced Sperm Chromatin Dispersion (SCD)

The evaluation of the SCD test indicated that cisplatin had a substantial impact on the integrity of chromatin in sperm found in the semen. Nevertheless, following PBM, the DNA repair mechanism in the Treat group notably reverted to its baseline condition when compared to the Cis group (Figure 1; $P = 0.0074$).

PBM Reduced TNF-α and IL-1β Levels in the Testicular Tissue

The administration of Cisplatin resulted in a notable

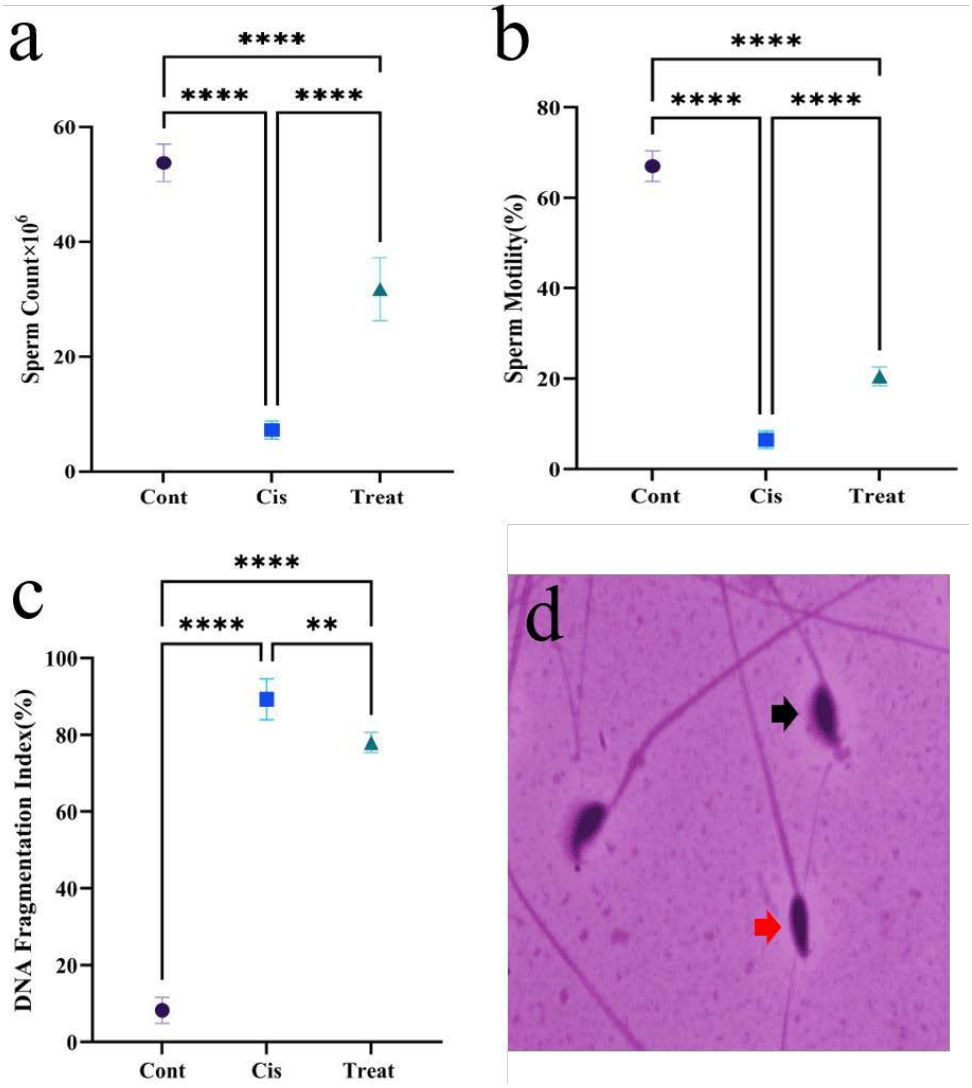

Figure 1. a-c. The Mean ± SD of total sperm count, sperm motility and Sperm Chromatin Dispersion (SCD) in different groups. Groups: 1. Control (Cont); 2. Cisplatin (Cis); 3. Treatment (Treat). *P* values < 0.05 (*), *P* values < 0.01 (**), and *P* values < 0.001 (***), *P* values < 0.0001 (****). d. Micrographs presenting the results of the SCD test with × 100 magnification. Sperm cells with fragmented nuclear DNA: no halo (red arrow). Sperm cells without fragmented nuclear DNA: halo (black arrow)

increase in TNF-α and IL-1β levels, which contributed to inflammation within the testicular tissue. Nevertheless, following laser irradiation, a marked reduction in the levels of these factors was observed compared to the Cis group ($P=0.0008$, $P\leq0.0001$, respectively). Additionally, notable differences in both cytokines were identified between the Treat and Cont groups (Figure 2; $P\leq0.0001$).

PBM Reduced Caspase-3 and Bax Genes and Increased Bcl-2 Genes in the Testicular Tissue

Cisplatin administration led to a significant reduction in the expression of the anti-apoptotic *Bcl-2* gene, with a corresponding notable increase in the pro-apoptotic genes *Caspase-3* and *Bax*. After laser treatment, there was a significant rise in the expression of the *Bcl-2* gene ($P=0.0004$). In comparison, the *Caspase-3* gene showed a significant decrease relative to the Cis group, while the expression levels of the *Bax* gene in the Treat group did not exhibit any significant changes when compared to the Cis group (Figure 3; $P\leq0.0001$ & $P=0.0501$, respectively).

PBM Reduced Stress Markers and Increased Antioxidant Markers in the Testicular Tissue

The ROS levels in testicular germ cells exhibited notable upregulation after exposure to cisplatin-induced toxicity. Conversely, the antioxidant marker GSH experienced a significant reduction due to cisplatin-induced toxicity. PBM effectively lowered the ROS levels in the germ cells of the Treat group when compared to the Cis group by modulating the mitochondrial respiratory cycle. In comparison, the levels of GSH in the Treat group showed a notable rise when contrasted with the Cis group (Figure 2; $P\leq0.0001$ & $P\leq0.0001$, respectively).

PBM Enhanced the Diameter of Seminiferous Tubules in the Testicular Tissue

The seminiferous tubules consist of the germinal epithelium and the peritubular tissue, both of which can be significantly affected by the toxic effects of cisplatin. In this study, we noted a marked reduction in the diameter of the seminiferous tubules in the Cis group relative to the Cont group. However, we also observed a notable enhancement in the Treat group in relation to the Cis group after the application of PBM (Figure 4; $P=0.0279$).

PBM Did not Enhance the Quantity of Somatic Cells in the Testicular Tissue

Cisplatin toxicity did not influence the quantity of

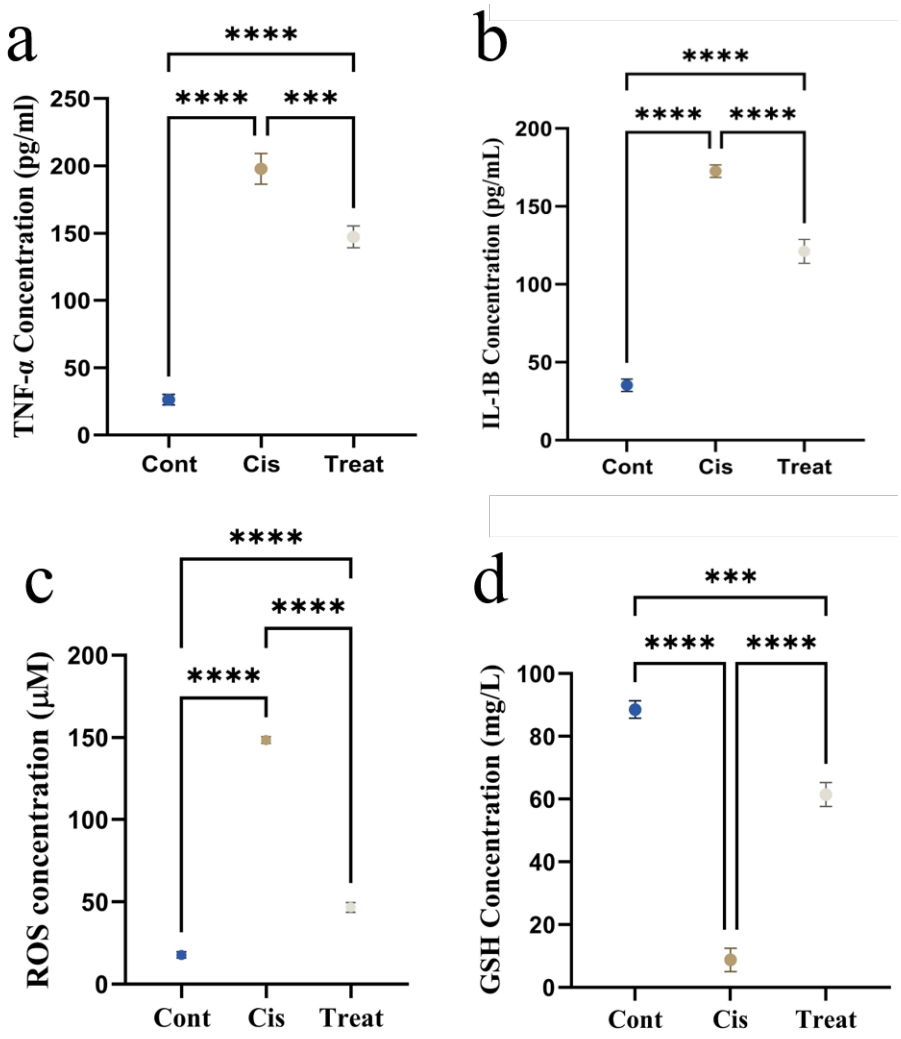

Figure 2. a-d The Mean ± SD of TNF-α, IL-1β, ROS and GSH in different groups. Groups: 1. Control (Cont); 2. Cisplatin (Cis); 3. Treatment (Treat). *P* values < 0.05 (*), *P* values < 0.01 (**), and *P* values < 0.001 (***), *P* values < 0.0001 (****)

somatic cells, such as Sertoli and Leydig cells, within the testicular tissue. Furthermore, following laser irradiation, there was no rise in the quantity of these cells in the Treat group in comparison to the Cis group (Figure 5; $P=0.06$ & $P=0.6607$, respectively).

PBM Improved the Total Quantity of Germ Cells in the Germinal Epithelium

The total quantity of germ cells was significantly affected by cisplatin toxicity due to high division, but immediately after laser irradiation, the number of these cells showed a notable rise compared to the Cis group (Figure 5; $P=0.0078$, $P=0.004$, & $P\leq 0.0001$, respectively).

Discussion

The present work demonstrates that PBM robustly prevents cisplatin-induced testicular damage in mice. The treatment not only restored key functional parameters, significantly improving sperm count and motility, reducing DNA fragmentation, and increasing germ cell populations, but also targeted the underlying pathophysiology. These recoveries were driven by a multi-faceted mechanism in which PBM rebalanced the testicular oxidative environment, restoring antioxidant capacity (GSH) and mitigating cisplatin-induced ROS.[26] This reduction in oxidative stress was tightly interlinked with key molecular shifts, including suppression of TNF-α and IL-1β, and a rebalancing of apoptotic regulators toward cell survival (decreased *Caspase-3*, increased *Bcl-2*). The beneficial effects of PBM on sperm function and chromatin integrity are consistent with established literature. Our findings align with the work of Babaei et al. and Shokoohi et al, who also demonstrated that antioxidant and anti-inflammatory agents like lycopene and N-acetylcysteine improve testicular health by reducing ROS and controlling apoptosis.[27,28] This suggests that PBM, though a physical modality, converges on the same critical cyto-protective pathways as pharmacological interventions.[28] Our findings suggest that PBM operates through these cyto-protective pathways. The reduction in sperm DNA fragmentation can be attributed to enhanced DNA repair and suppressed apoptotic activity, evidenced

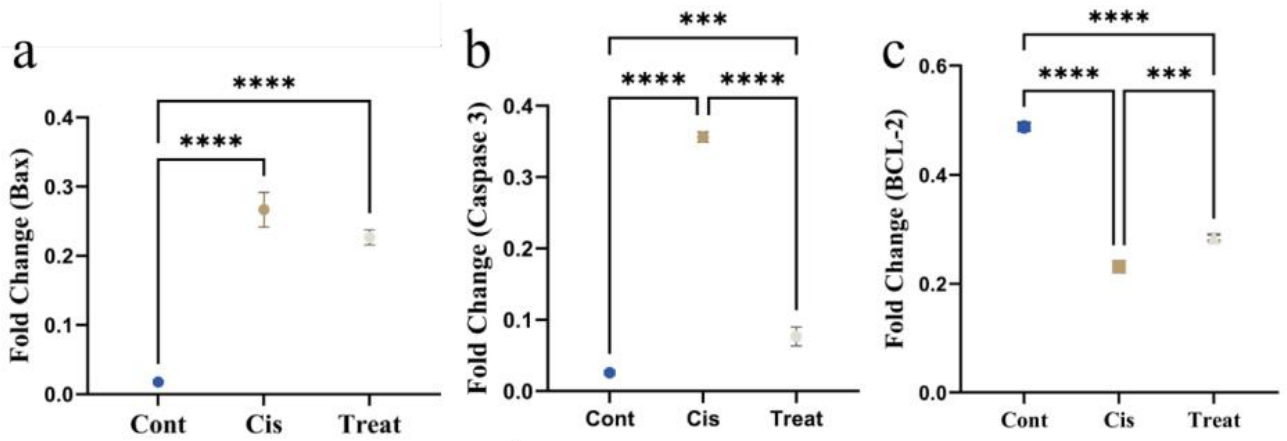

Figure 3. a-c The Mean ± SD of the relative gene expression of *Bax*, *Caspase-3* and *Bcl-2* in testis tissue in different groups. Groups: 1. Control (Cont); 2. Cisplatin (Cis); 3. Treatment (Treat). *p* values < 0.05 (*), *p* values < 0.01 (**), and *p* values < 0.001 (***), *p* values < 0.0001 (****)

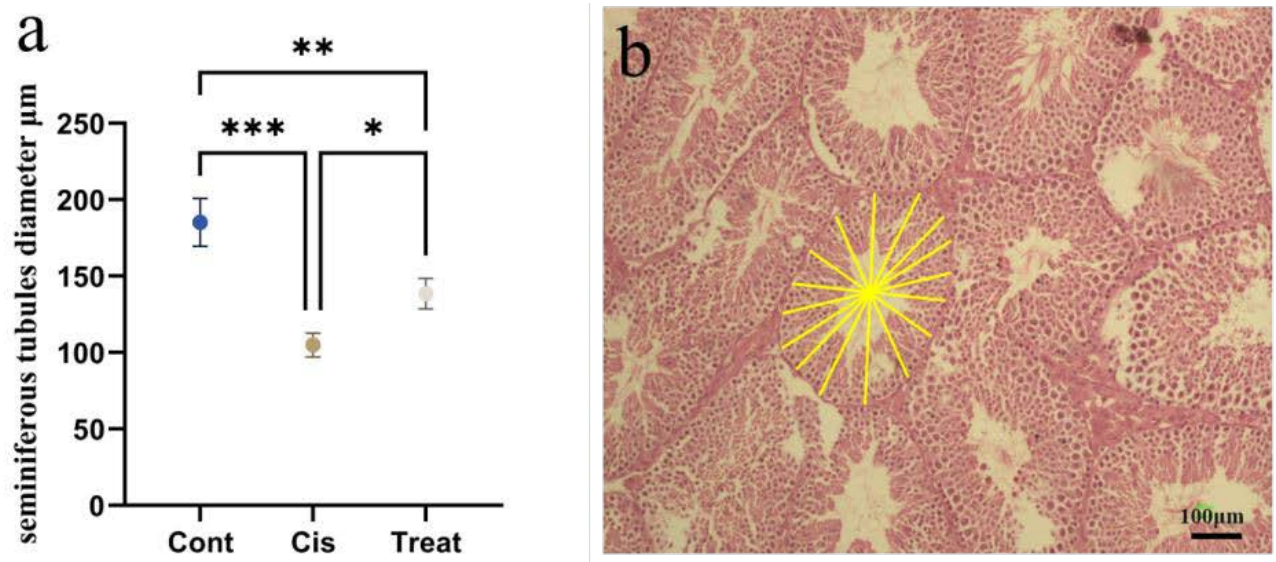

Figure 4. a The Mean ± SD of the diameter of the seminiferous tubule in different groups. Groups: 1. Control (Cont); 2. Cisplatin (Cis); 3. Treatment (Treat). *P* values < 0.05 (*), *P* values < 0.01 (**), and *P* values < 0.001 (***), *P* values < 0.0001 (****). **b** Photomicrograph of the diameter of the seminiferous tubule stained with H&E, × 10 magnification. Scale bar = 100 µm

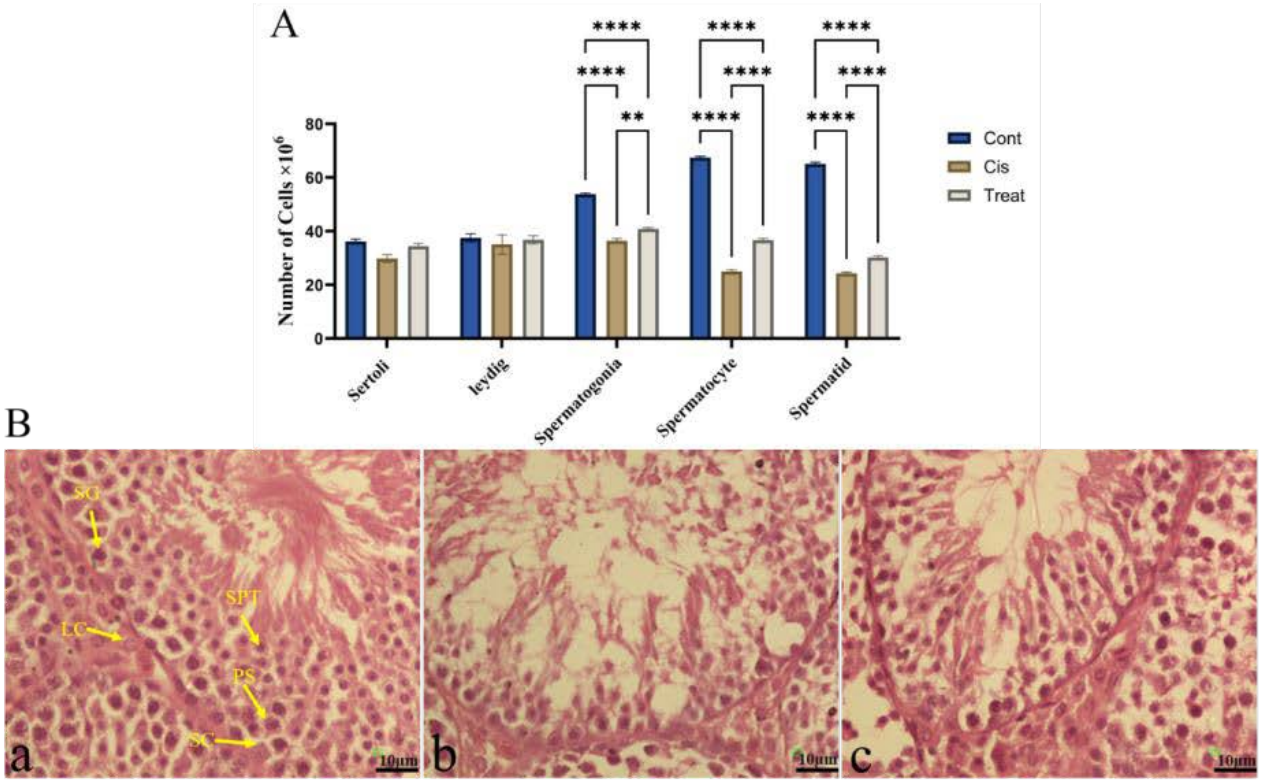

Figure 5. (a). The Mean ± SD of the total number of Spermatogonia, primary Spermatocytes, Spermatids, and Sertoli and Leydig cells in different groups. Groups: 1. Control (Cont); 2. Cisplatin (Cis); 3. Treatment (Treat). *P* values < 0.05 (*), *P* values < 0.01 (**), and *P* values < 0.001 (***), *P* values < 0.0001 (****). **(b). a-c** Photomicrograph of the testis stained with H&E, × 40 in different groups. a. Control (Cont); b. Cisplatin (Cis); c. Treatment (Treat). SG (spermatogonia), PS (primary spermatocyte), SPT (round spermatid), SC (Sertoli cell), LC (Leydig cell). Scale bar = 10 µm

by the dysregulation in *Caspase-3*. This aligns with the work of Ahn et al. and Yazdi et al, who demonstrated that PBM irradiation promotes sperm viability and minimizes cell death via mitochondrial modulation.[29,30]

Cisplatin exposure triggered a significant increase in TNF-α and IL-1β, initiating a cascade that stimulated ROS production. As supported by Kamel et al, this excessive ROS burden compromised membrane integrity, induced DNA damage, and directly activated *Caspase-3*, shifting the cellular balance toward apoptosis. The consequence of this molecular cascade was a marked depletion of germ cells in the seminiferous epithelium, demonstrating how dysregulated gene expression directly translates to tangible histopathological outcomes.[31]

The reversal of these cisplatin-induced damages by PBM through the specific downregulation of TNF-α, ROS, and *Caspase-3* and the upregulation of *Bcl-2* provides strong evidence that cellular restoration is driven by molecular reprogramming. This aligns with and extends the work of Farivar et al. [32]and Taha & Valojerdi[33] by delineating the specific anti-inflammatory, antioxidant, and anti-apoptotic pathways through which PBM rescues testicular architecture and cell viability.[34]

In addition to the notable recoveries in both functional and molecular aspects, we noted that laser treatment modifies the diameter of the seminiferous tubules while leaving the quantity of Sertoli and Leydig cells unaffected, suggesting a targeted restorative impact. This specificity aligns with earlier reports by Taha & Valojerdi [33]and Soltani et al.,[26] who similarly observed limited improvement in the somatic cell counts despite gains in germ cell parameters. These results indicated that PBM can improve the diameter of the seminiferous tubules by increasing the expansion and differentiation of germ cells.[34]

The pathogenic cascade begins when cisplatin triggers inflammatory signaling (TNF-α, IL-1β), stimulating ROS production. This oxidative stress can cause damage to both nuclear and mitochondrial DNA, thereby activating *Caspase-3* and initiating apoptosis. The consequent loss of germ cells directly manifests as impaired sperm count and motility. PBM intervenes by dampening this cascade at its source, suppressing both the initial inflammatory signals and the resulting oxidative stress. This shifts the molecular balance toward cell survival (e.g., *Bcl-2*) and facilitates the restoration of the germ cell population. This coherent chain of events demonstrates how molecular disruptions translate into cellular dysfunction and how targeted interventions can halt this cycle.[35,36]

The novelty of this study is in the comprehensive evaluation of sperm function, cellular subpopulations, oxidative stress markers, and gene-expression profiles, enabling the integration of molecular and cellular findings into a coherent and multidimensional framework. Animal models can only approximate the complexities of human reproductive responses, and the relatively short evaluation window may have missed longer-term tissue remodeling or hormonal feedback mechanisms. Additionally, while the lack of impact on tubule architecture and somatic cell numbers is noteworthy, it invites further research into

adjunct strategies such as matrix remodeling agents or combination therapy for full functional restitution.

In comparison to prior studies, the current results reinforce the general consensus that PBM offers a strong, non-drug-based method for reducing reproductive damage induced by chemotherapy. The findings show overall agreement with earlier reports, while also outlining specific limitations related to tissues and pinpointing opportunities for further methodological enhancement. One limitation of this research is the lack of a sham-irradiation group, which hinders the complete exclusion of possible procedural effects stemming from animal handling, anesthesia, and probe contact that are not related to PBM. Future investigations that incorporate an appropriate sham control would further solidify the interpretation of the observed therapeutic effects. As the field of PBM evolves, future research should emphasize the optimization of wavelength and dosage parameters, the expansion of cellular targets, and the combination of PBM with complementary biochemical or structural interventions to enable more comprehensive testicular repair.

Conclusion

This study contributes to the expanding body of knowledge by demonstrating that inflammation-driven ROS generation and apoptosis-related molecular alterations are associated with germ-cell depletion in cisplatin-induced testicular toxicity. PBM was associated with favorable modulation of these molecular markers, accompanied by the restoration of germ-cell populations and improvement of functional sperm parameters, as demonstrated by both molecular and cellular analyses. Although certain limitations remain, the overall findings provide further insight into the potential biological effects of PBM, support its translational potential, and provide a solid foundation for future investigations in reproductive toxicology and laser-based therapeutics.

Authors' Contribution

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Availability of Data and Material

Data are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

All authors consent to the publication of this manuscript.

Ethical Approval

All experimental procedures in this study were reviewed and approved by the Ethics Committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.LASER.REC.1403.022).

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