



Photobiomodulation Improves Testicular Structure and Antioxidant Defense in a Rat Model of Torsion/Detorsion

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Abstract

Introduction: Testicular torsion/detorsion (T/D) causes ischemia–reperfusion injury that disrupts spermatogenesis mainly through oxidative stress. Photobiomodulation (PBM), known for its antioxidant effects, may counteract such damage. However, its efficacy in T/D injury remains unclear. This study evaluated the potential of PBM to improve sperm quality, testicular structure, and redox balance.

Methods: Twenty-four adult male rats were randomly assigned to three groups: Sham (scrotal incision without torsion), T/D (left testis rotated 720° for 2 hours followed by detorsion), and T/D + PBM (T/D followed by transscrotal laser therapy). PBM was administered using an 810-nm diode laser at 100 mW, delivering 2 J/cm² per session for 20 seconds daily over six consecutive days. Epididymal sperm and testicular tissues were collected to assess sperm parameters, histological features, and oxidative stress markers, including superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde.

Results: T/D reduced sperm motility, viability ($P < 0.0001$), and count ($P < 0.05$). Histological analysis revealed decreased seminiferous tubule diameter and epithelial thickness. Moreover, antioxidant enzyme activities were diminished, whereas lipid peroxidation levels increased ($P < 0.001$). PBM intervention improved sperm motility and viability, restored seminiferous tubule architecture, and re-established the testicular redox balance by enhancing antioxidant defense and reducing lipid peroxidation ($P < 0.001$).

Conclusion: Collectively, these findings indicate that PBM effectively attenuates testicular damage induced by T/D by enhancing antioxidant defenses and restoring redox homeostasis. PBM promoted spermatogenic recovery, improved sperm quality, and preserved seminiferous tubule architecture following ischemia–reperfusion injury. These results highlight PBM as a promising, noninvasive adjunctive strategy for protecting male reproductive function after testicular ischemia insults.

Keywords: Testicular torsion/detorsion, Photobiomodulation, Oxidative stress, Spermatogenesis, Reperfusion injury



Introduction

The testis is fundamental to male reproductive function, serving as the site of spermatogenesis and steroid hormone production. Spermatogenesis is a continuous and highly energy-demanding process that involves the proliferation and differentiation of germ cells within the seminiferous tubules.¹ Sertoli cells provide structural and metabolic support to germ cells, while Leydig cells produce testosterone, essential for both spermatogenesis and secondary sexual development. Because this process occurs without interruption, the testis requires constant

blood flow, oxygen delivery, and nutrient supply. Disruption of this unique environment compromises testicular function and fertility potential.^{2,3}

Testicular torsion is a urological emergency characterized by twisting of the spermatic cord, which obstructs blood flow. Ischemia leads to depletion of ATP, ionic imbalance, and swelling of seminiferous epithelium, and prolonged hypoxia induces loss of germ cells.⁴ The gold-standard treatment is surgical detorsion, which restores blood supply. However, reperfusion is associated with a burst of reactive oxygen species (ROS), a process known as ischemia–

reperfusion injury.⁵ Excessive ROS production causes lipid peroxidation, protein oxidation, and mitochondrial dysfunction, ultimately leading to germ cell apoptosis and long-term impairment of spermatogenesis. Thus, while surgery relieves the mechanical cause of torsion, it does not fully prevent biochemical and structural damage.⁶

Given these challenges, Oxidative stress plays a central role in testicular torsion–detorsion (T/D) injury. Elevated ROS such as superoxide and hydroxyl radicals degrade DNA, lipids, and proteins.⁷ Malondialdehyde (MDA), a marker of lipid peroxidation, increases significantly after torsion, whereas antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) decline. This imbalance damages the seminiferous epithelium, disrupts the blood–testis barrier, and results in impaired sperm quality and quantity.^{8,9} These alternations underscore the need for therapeutic approaches to alleviate oxidative stress and preserve testicular integrity.

Considering the role of oxidative stress in testicular torsion, therapeutic modalities with antioxidant capacity are of particular interest. Photobiomodulation (PBM), also known as low-level laser therapy, has emerged as a promising noninvasive intervention. PBM uses light in the red to near-infrared range to modulate cellular activity without thermal injury.¹⁰ Its main molecular target is cytochrome c oxidase in the mitochondrial respiratory chain. PBM enhances mitochondrial respiration, increases ATP production, and stabilizes membrane potential, thereby reducing ROS overproduction.¹¹ In addition, PBM modulates apoptotic pathways, lowers pro-inflammatory cytokines, and improves redox balance. These effects collectively protect tissues vulnerable to oxidative and ischemic damage.¹² Thus, PBM has demonstrated beneficial effects on reproductive tissues, where oxidative stress critically affects sperm and seminiferous tubules.

In reproductive models, PBM enhances sperm parameters, restores seminiferous tubule integrity, and normalizes oxidative stress biomarkers.¹³ Its multimodal actions suggest potential benefits for testicular T/D injury. Nevertheless, the specific capacity of PBM to mitigate ischemia–reperfusion damage in the testis remains insufficiently clarified.

Considering the significant role of T/D in producing high levels of ROS within tissues and the established effectiveness of laser therapy in mitigating it, and given the absence of studies directly assessing the impact of this noninvasive treatment on the condition, this study was initiated to explore the therapeutic potential of laser therapy in a testicular T/D injury model. The study aimed to assess the effects of PBM on the structural and functional recovery of the testis following testicular torsion by evaluating sperm parameters, seminiferous tubule histomorphometry, and oxidative stress markers (SOD, CAT, GPx, and MDA). The findings of this

research offered insights into the potential role of PBM as a supplementary therapy for maintaining male fertility.

Materials and Methods

Animal Preparation and Care

This study utilized 24 male Wistar rats, each weighing approximately 200 ± 10 g and aged 8 weeks, sourced from the Pasteur Research Institute in Tehran, Iran. In accordance with NIH guidelines, the animals were maintained in a controlled environment with unrestricted access to food and water, 50% humidity, a temperature of $24 \pm 2^\circ\text{C}$, and a 12-hour light cycle. Each cage, constructed from transparent polycarbonate, was lined with large-sized rice straw bedding and included environmental enrichment in the form of nesting material, which was consistent across all groups. Each cage housed only two rats.

Study Design

The rats were allocated into various groups through a simple randomization technique, utilizing a dice-throwing method. If the dice showed a number below or equal to 2, the rat was placed in the sham group; a result between 2 and 4, inclusive, assigned the rat to the T/D group; and a number above 4 placed the rat in the T/D+PBM group. Each group consisted of 8 rats. The sample size was determined using standard calculations, taking into account the number of groups and quantitative endpoints. In the sham group, the left testis was exposed through a scrotal incision without torsion. In the T/D group, the left testis was subjected to unilateral torsion for 2 hours, followed by detorsion. In the T/D+PBM group, rats underwent T/D, as in the previous group, and subsequently received laser therapy according to the protocol described below.

Testicular Torsion Procedure

Rats were anesthetized using an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) and positioned on their backs. Throughout the surgical procedure, the rats' condition was monitored by tracking their respiratory rate (70–110 breaths/sec), heart rate (260–500 beats/min), and body temperature (35.9 – 37.5°C). To maintain warmth, sterile pads were placed on the upper body of the rats.

A midline incision was made in the scrotum to access the left testis. The testis was then removed and twisted two full counterclockwise rotations (720°) around the spermatic cord from its anatomical position. As the rotation concluded, increased resistance was noted, indicating complete torsion of the spermatic cord. The testis was held in this twisted position with a clamp for 2 hours. To reverse the testicular torsion, the testicle was returned to its original position, and the scrotal incision was closed using 4-0 absorbable sutures.¹⁴

PBM Therapy

To reduce stress and ensure precise laser delivery, rats were anesthetized with an intraperitoneal injection of ketamine and xylazine. In the T/D+PBM group, laser irradiation was applied transcutaneously over the anterior surface of the affected testis, directly above the testicular parenchyma. The laser probe was positioned perpendicular to the scrotal surface in gentle contact with the skin. The first laser session was delivered immediately after detorsion, while subsequent sessions were administered once daily for six consecutive days. Skin and testicular temperatures were monitored in experiments and did not increase by more than 1 °C, ensuring safety and avoiding thermal effects.

An 810-nm pulsed infrared diode laser was used for photobiomodulation therapy. The laser emitted pulses at a frequency of 50 Hz with a pulse width of 180 µs, resulting in a duty cycle of 1.44%. The irradiation area was 0.5 cm², delivering a surface fluence of 4 J/ cm². Each irradiation point received a total energy of 2 J during a 20-second exposure (12 J across all treatment periods), with an average output power of 100mW and corresponding power density of 200 mW/cm². The calculated peak power was approximately 6.9 W.^{15,16}

All laser irradiation sessions were performed by the same trained operator to ensure consistency and minimize procedural variability. The laser device was calibrated according to the manufacturer's instructions prior to the initiation of experimental sessions.

Sampling

At the conclusion of the experiment, the rats were humanely euthanized under deep anesthesia using ketamine and xylazine. Once it was confirmed that there were no motor or sensory responses to the tail, an incision was made in the lower abdomen to access the testicular and epididymal tissues. The four left testicular tissues were placed in a fixative solution for histological evaluation, while the other was stored at -70°C for molecular analysis. The epididymal tail was carefully removed and placed in an incubator for both quantitative and qualitative sperm analysis. Samples exhibiting any signs of swelling, inflammation, or bruising in the scrotum were excluded from the study. All laboratory analyses were conducted by personnel who were blinded to the identity of the samples throughout the entire process.

Sperm Parameters Analysis

Following the removal of the epididymal tail, the tissue was swiftly immersed in 1 ml of Ham's F-10, which had been pre-equilibrated in a 37°C incubator, and then dissected. The sample was then placed back in the incubator at 37°C for a duration of 20 minutes before the examinations were initiated. Sperm parameters, including sperm motility, viability, and count, were assessed according to the World Health Organization guideline.¹⁷

Sperm Motility

Sperm motility was evaluated under a light microscope at 400× magnification. Ten µl of epididymal sperm suspension was placed on a glass slide, and at least 200 sperm per sample were analyzed. Motility was classified as progressive, non-progressive, or immotile to determine functional quality.

Sperm Count

The sperm count was determined using a Neubauer hemocytometer. Epididymal sperm were diluted 1:20 with phosphate-buffered saline (PBS), and sperm in at least five large squares were counted. The total sperm concentration was calculated and expressed as millions per milliliter.

Sperm Viability

Sperm viability was assessed by mixing 10 µL of sperm suspension with 10 µL of Eosin-Nigrosin stain on a clean slide. After smearing and air-drying, at least 200 sperm were examined under a light microscope at 1000× magnification. Unstained sperm were considered alive, whereas dead sperm absorbed the dye. Viability was calculated as the percentage of living sperm.

Histological Processing of Testicular Tissue

After euthanasia, the testis was excised and initially immersed in Bouin's solution for 24 hours. Subsequently, tissues were transferred to 10 % formalin for fixation. Standard tissue processing was performed, including dehydration, clearing, and embedding in paraffin blocks, followed by sectioning with a microtome (RM2235, Leica Microsystems, Germany). Paraffin-embedded testicular tissue sections (5µm) were deparaffinized in xylene, rehydrated through graded ethanol, stained with hematoxylin, differentiated and blued, counterstained with eosin, dehydrated, cleared, and mounted for light microscopic examination.

Measurement of Seminiferous Tubule Diameter

Seminiferous tubule diameters were assessed on H&E-stained sections at 10× magnification using ImageJ software. For each testis, 10 tubules were randomly selected from different areas of the section. Within each tubule, 16 points were identified and connected by eight straight lines, ensuring that the center of the tubule intersected all lines. These intersections were used to calculate the diameter in eight different directions, and the mean diameter was reported for each tubule. Only transversely cut tubules with a nearly circular cross-sectional shape (ratio of two axes close to 1:1.5) were included in the analysis.¹⁸

Measurement of Seminiferous Tubule Epithelial Thickness

The epithelial thickness of seminiferous tubules was

evaluated on H&E-stained sections using ImageJ software. For each testis, 10 transversely cut tubules were randomly selected from different areas of the section. At four evenly spaced points around the circumference of each tubule, measurements were taken perpendicular to the basement membrane. The mean of these was recorded as the epithelial thickness for each tubule.¹⁸

Johnsen Score

The Johnsen scoring system was used to assess spermatogenesis in seminiferous tubules, assigning scores from 1 to 10 based on the presence and maturity of germ cells and the tubular structure. For each sample, at least 50 tubules were evaluated (with $\times 400$ magnification), and the mean score was calculated to represent spermatogenic activity.

Evaluation of Testicular Oxidative Stress Markers

To evaluate oxidative damage and the antioxidant defense system in testicular tissue, the activities of SOD, CAT, GPx, as well as the levels of MDA, were measured using standard methods. The left testis was extracted, weighed, and then homogenized. To examine the markers, 70-100 mg of homogenized tissue was used. Subsequently, it underwent centrifugation at 3000 rpm for 15 minutes. The resulting supernatant was utilized to assess oxidative stress.

CAT Activity Assay

The activity of this enzyme in the testis was measured according to Aebi.¹⁹ Kit Nactaz™- Catalase Activity Assay Kit was used for this evaluation. A reaction mixture (3.00 mL) was prepared using 50 mM potassium phosphate, 0.036% (w/w) hydrogen peroxide, and 10 units of catalase. The spectrophotometer, equipped with an appropriate thermostat, was set to A240 and 25°C. It was then calibrated against a quartz cuvette filled with a phosphate buffer. A quartz cuvette was filled with 2.90 ml of a hydrogen peroxide solution and placed in the spectrophotometer. The bottom surface was allowed to reach an equilibrium at 25 °C. Subsequently, 0.10 ml of the catalase solution was introduced into the cuvette. The mixture was promptly inverted, and the decrease in absorbance was recorded every second for 180 seconds.

Assessment of SOD Activity

Total SOD activity in the samples was evaluated using the method described by Sun et al.²⁰ To assess SOD levels, the Nasdox™-Superoxide Dismutase Assay Kit-Non-Enzymatic was employed. The control group wells were prepared by combining 200 μ L of the initial reagent, 50 μ L of deionized water, and 50 μ L of the secondary reagent. For the sample group, 50 μ L of lysed tissue was added along with the reagents, mirroring the control group setup. The samples were then incubated for 5 minutes at room temperature, shielded from light. Subsequently, a

microplate reader measured the optical absorbance of the samples at a 405-nm wavelength. The resulting readings were utilized as the sample OD in the designated formula:

$$SOD\ activity = \frac{OD\ Test}{OD\ Control} \times 200$$

Measurement of GPx Activity

GPx activity in the rat testis was determined employing the method of Paglia and Valentine.²¹ The samples were combined in a solution containing 1 mM Na2EDTA, 2 mM glutathione reductase, 2 mM NADH, 4 mM NaN3, and 1000 U glutathione reductase with 50 mM Tris buffer (pH = 7.6). They were mixed for 5 minutes and maintained at 37°C. The reaction was initiated using 8.8 mM hydrogen peroxide, and NADPH absorption was measured at 340 nm for 3 minutes. The Nagpix™Glutathione Peroxidase Assay Kit was utilized for this procedure.

Determination of MDA

The Nalondi™-Lipid peroxidation Assay Kit-MDA was employed to assess lipid peroxidation as an indicator of oxidative stress. Following the protocol of the kit, a standard curve was initially established using various dilutions prepared through the fluorimetric method. Subsequently, 200 μ L of the sample was combined with 800 μ L of a solution prepared according to the instructions of the kit. This mixture was then placed in a Bain-Marie at 95 degrees for 45 minutes. After a 10-minute cooling period, the samples underwent centrifugation, and the resulting supernatant was utilized for measurement at a wavelength of 553/532.²²

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism (version 10.2.3, GraphPad Software, San Diego, CA, USA). Differences between the groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test to compare all pairs of groups. A p-value < 0.05 was considered statistically significant.

Results

Sperm Parameters

Sperm parameters, including motility, viability, and count, were evaluated to assess the effect of PBM on testicular T/D injury.

Sperm motility was considerably lower in the torsion group than in the sham group ($P < 0.0001$). PBM significantly enhanced motility relative to the T/D group ($P = 0.0003$) (Figure 1a).

The total sperm count showed a significant difference between the T/D and sham groups ($P < 0.05$). Laser therapy resulted in a slight increase in the sperm count

compared to the T/D group, although this change was not statistically significant ($P=0.4$) (Figure 1b).

Sperm viability was reduced in the T/D group in comparison with the sham group ($P<0.0001$). PBM notably improved the percentage of live sperm relative to the T/D group ($P<0.007$) (Figure 2a-b).

Histological Analysis of Testicular Tissue

Evaluation of histologic parameters provides direct insight

into the structural integrity and functional capacity of the testis, reflecting the effectiveness of PBM in preserving spermatogenesis after T/D injury.

Seminiferous tubule diameter was moderately reduced in the injured group compared with the sham group ($P=0.0001$). Laser treatment restored the tubule diameter relative to the injured group ($P<0.05$) (Figure 3a,c).

Spermatogenic epithelium thickness decreased in the T/D group versus the sham group ($P<0.0001$). Following PBM,

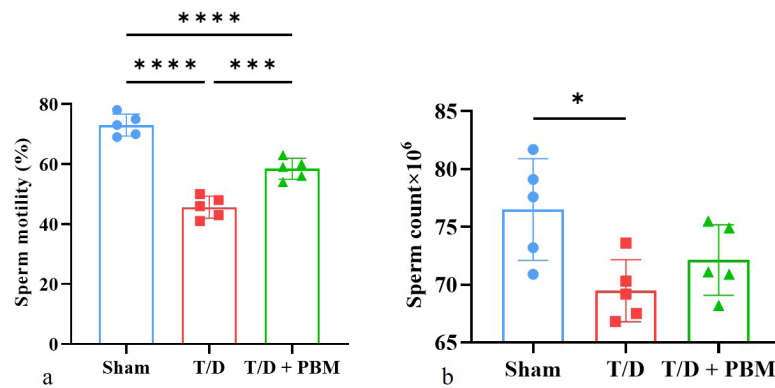


Figure 1. Effect of laser therapy on sperm parameters in the rat induced by T/D. a) sperm motility and b) sperm count. Data are expressed as mean $\pm$ SD of five independent samples ($n=5$ /group). Statistical significance is indicated as * $P<0.05$, *** $P<0.001$, and **** $P<0.0001$

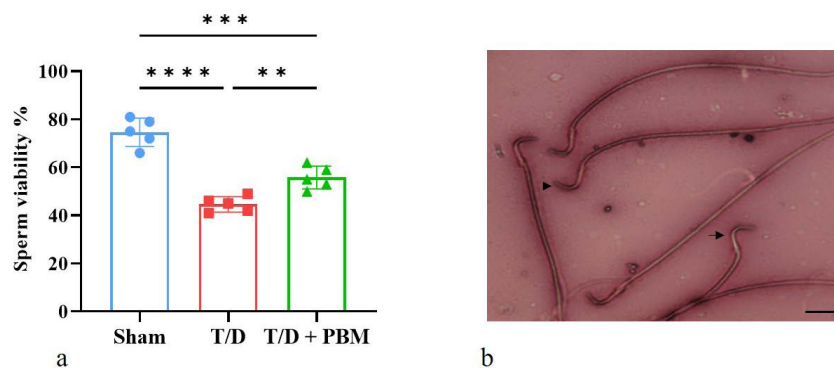


Figure 2. Effect of PBM on sperm viability in the rat testicular T/D model. a) Sperm viability and b) photomicrographs of the eosin-nigrosin stained sperm at $\times 100$ magnification (bar: 50 μ m). The arrowhead indicates a live sperm, and the arrow indicates a dead sperm. Data represent the mean $\pm$ SD of five independent samples ($n=5$ /group). Statistical significance is indicated as ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$

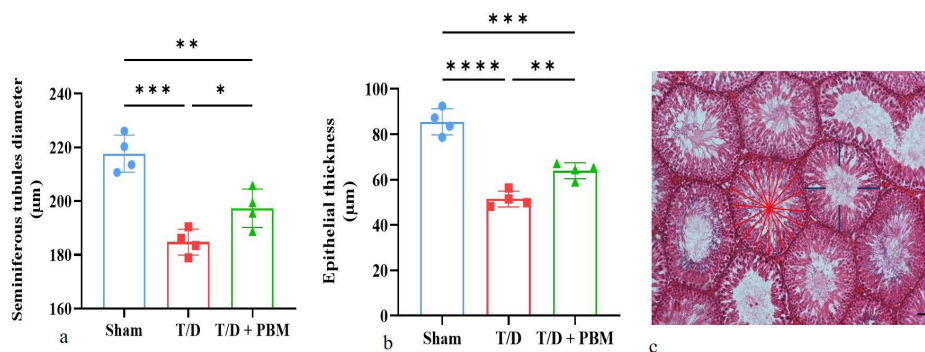


Figure 3. Effect of laser intervention on histological parameters in the rat subjected to T/D. a) diameter of seminiferous tubules, b) epithelial height, and c) a photomicrograph of testicular tissue at $\times 10$ magnification demonstrates the method for measuring the thickness of the epithelium (blue lines) and the diameter of the tubules (red lines) (bar: 50 μ m). Data are presented as mean $\pm$ SD of four independent samples ($n=4$ /group). Statistical significance is indicated as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$

epithelium thickness improved notably when compared to the T/D group ($P=0.007$) (Figure 3b,c).

The Johnsen score analysis revealed a significant reduction in spermatogenic activity in the T/D group compared to the sham group ($P=0.0002$), indicating impaired spermatogenesis due to testicular torsion. Notably, treatment with the laser resulted in a marked improvement in this score relative to the injured group ($P<0.05$) (Figure 4a-d).

Oxidative Stress Markers

Oxidative stress plays a pivotal role in testicular injury after T/D. Therefore, the activities of antioxidant enzymes (SOD, CAT, GPx) and the level of MDA were examined to assess the effect of PBM.

CAT activity was notably reduced in the injured group compared with the sham group ($P<0.0001$). Subsequent to PBM, CAT activity was moderately restored relative to the injured group ($P=0.001$). (Figure 5a).

SOD activity was considerably lower in the T/D group than in the sham group ($P<0.0001$). After laser therapy, a significant improvement in SOD activity was observed relative to the T/D group ($P=0.006$) (Figure 5b).

GPx activity appreciably decreased in the torsion group versus the sham group ($P=0.0004$). Application of PBM led to a marked increase in GPx activity relative to the torsion group ($P<0.05$) (Figure 5c).

MDA levels were substantially elevated in the affected group compared with the sham group ($P=0.001$). A significant decline in MDA levels was observed following PBM treatment relative to the injured group ($P=0.003$). No significant difference was observed between the sham and treatment groups (Figure 5d).

Discussion

Testicular torsion interrupts blood flow, rapidly inducing hypoxia and tissue stress. Subsequent detorsion restores circulation. However, the abrupt reperfusion aggravates

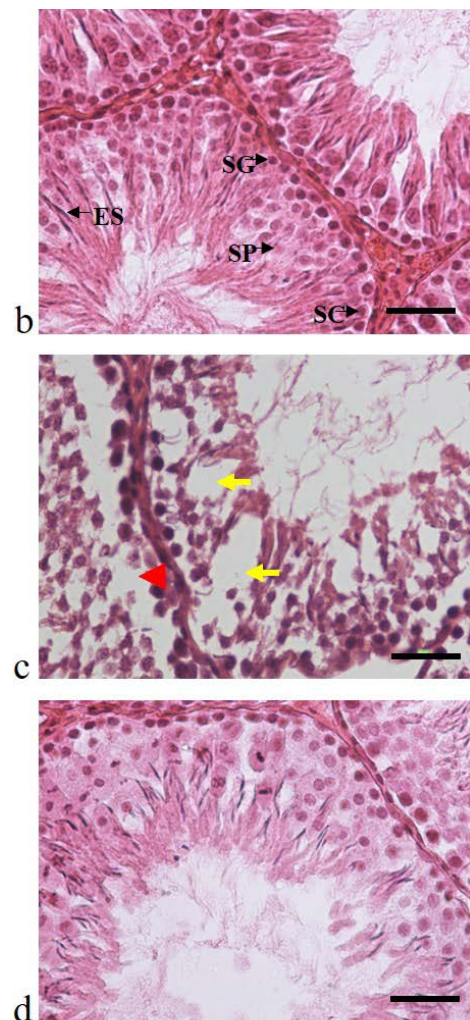
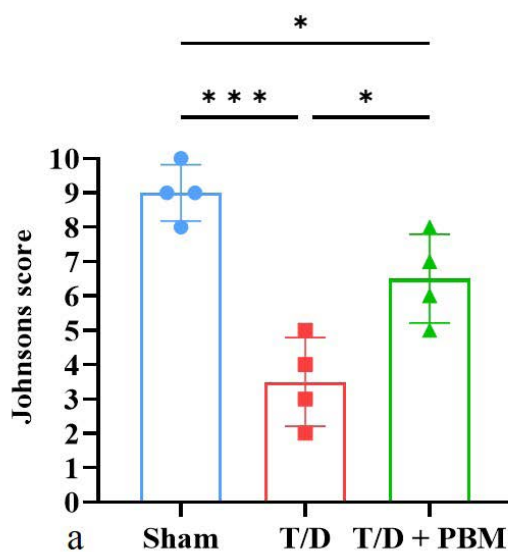


Figure 4. a) Laser treatment improves the Johnsen score in the testis of the rat under the testicular T/D. Photomicrographs of tissue stained with hematoxylin and eosin (Magnification, $\times 40$) (bar: 50 μm). b) sham group, c) T/D group, and d) T/D + PBM. Data represent four independent samples ($n=4/\text{group}$). Yellow arrows indicate vacuolization, and red arrowheads demonstrate sloughing. SG: spermatogonia, SP: primary spermatocyte, Es: elongated spermatid, SC: Sertoli cell. Statistical significance is indicated as $*P<0.05$ and $***P<0.001$

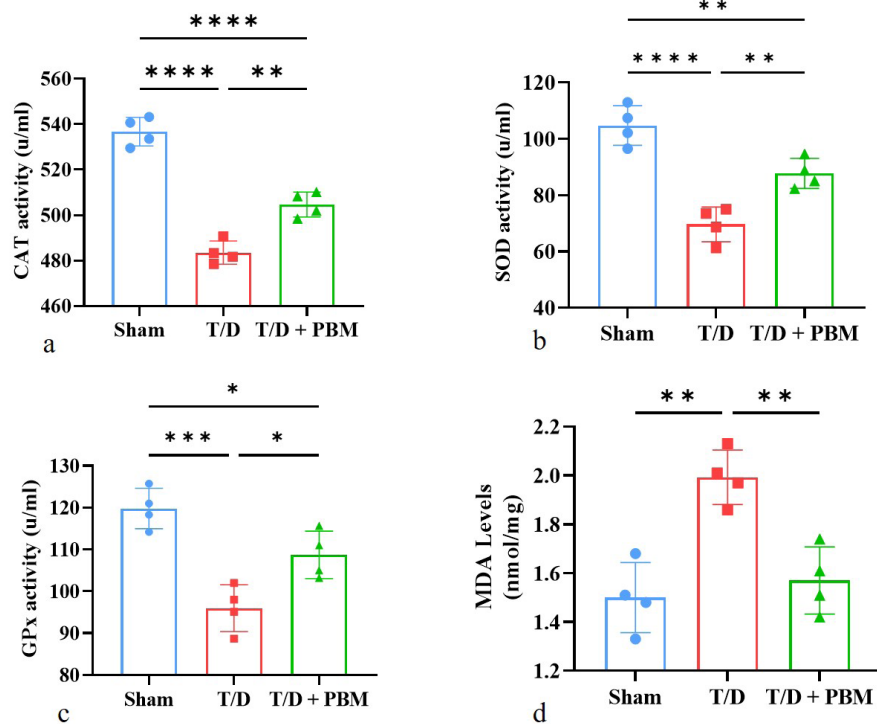


Figure 5. PBM therapy enhances testicular antioxidant defense in the rat after T/D. a) Catalase, b) superoxide dismutase, c) glutathione peroxidase, and d) malondialdehyde. Data are expressed as mean $\pm$ SD of four independent samples (n=4/group). Statistical significance is indicated as * P <0.05, ** P <0.01, *** P <0.001, and **** P <0.0001

injury by triggering excessive ROS generation. In line with this pathophysiology, our findings showed that testicular T/D resulted in a marked decline in sperm characteristics. Histological assessment confirmed structural deterioration in seminiferous tubules. These alterations were accompanied by the reduced activity of antioxidant enzymes SOD, CAT, and GPx, together with elevated MDA as a marker of lipid peroxidation. Importantly, laser therapy after detorsion restored tissue antioxidant balance and alleviated oxidative stress in the seminiferous tubules. This recovery of the testicular microenvironment supported spermatogenesis, ultimately leading to improved sperm parameters.

The ischemia–reperfusion events following testicular T/D directly affect epididymal sperm quality. In our study, T/D significantly reduced sperm motility and viability, indicating impaired membrane integrity and compromised mitochondrial function in epididymal sperm. Masoumi et al.²³ demonstrated similar reductions in motility and viability following T/D injury, with antioxidant intervention partially restoring these parameters. Although no prior studies have evaluated PBM in this injury, related research in a scrotal hyperthermia model has shown that PBM improves spermatogenesis and sperm quality.²⁴ Laser therapy after detorsion in our study improved both motility and viability, likely by enhancing mitochondrial ATP production, stabilizing sperm membranes, and reducing

oxidative stress in the local microenvironment. The up-regulation of antioxidant enzymes and the reduction in lipid peroxidation further support the protective effect of PBM on sperm quality indices.²⁵

Alongside the effects on epididymal sperm, significant structural changes were observed in the testis, particularly in the seminiferous tubules. Here, T/D caused a noticeable reduction in tubule diameter and germinal epithelium thickness, reflecting disrupted spermatogenesis and cellular stress in the testis. These alterations are consistent with earlier investigations on T/D-induced histological damage. Aktoz et al.²⁶ demonstrated degeneration of the germinal epithelium, manifested as thinning of the epithelium and reduced seminiferous tubule diameter, following testicular T/D. The damage likely results from oxygen deprivation during torsion, followed by ROS-mediated injury upon detorsion, compromising Sertoli cell function and germ cell survival.⁷ PBM intervention after detorsion improved both tubule diameter and epithelial thickness, suggesting enhanced tissue recovery, likely by promoting proliferation and differentiation of testicular cells, thereby restoring normal architecture. A previous report in transient scrotal hyperthermia models has shown that PBM can restore spermatogenesis and improve testicular structure through enhanced microcirculation, reduced oxidative stress, preservation of blood–testis barrier integrity, and up-regulation of antioxidant defenses.²⁷

The histological regeneration observed in this study aligns with the improvements in sperm motility and viability, highlighting that PBM not only preserves cellular function but also maintains the structural framework necessary for ongoing spermatogenesis.

Following the histological observations, testicular redox status was further assessed. In the T/D group, a marked decline in the activity of antioxidant enzymes, including SOD, CAT, and GPx, was detected alongside a significant rise in MDA levels. These findings are consistent with previous studies.²⁸ Ischemia through the disruption of complexes I and III of the electron transport chain within the mitochondria of germinal epithelial cells, particularly Sertoli cells, leads to a reduction in antioxidant capacity within the tissue, leading to lipid degradation and oxidative stress.²⁹

After PBM intervention, the enzymatic profile shifted favorably: activities of SOD, CAT, and GPx were restored, and MDA accumulation was significantly reduced. These improvements may be attributed to multiple mechanisms. PBM appears to target the abundant mitochondria within Sertoli cells, where photon absorption enhances mitochondrial activity, leading to greater ATP production and reduced ROS leakage. Improved Sertoli cell function subsequently strengthens their nutritional and structural support for differentiating germ cells. Collectively, these effects mitigate lipid peroxidation, reinforce cellular defenses, and maintain the redox balance crucial for ongoing spermatogenesis.¹¹ These findings are in agreement with the report by Aghajpour et al.³⁰ and Bayat et al.³¹ that showed that laser therapy has the potential to restore testicular tissue by reducing ROS-induced damage.

Given these outcomes, some limitations of the study can be considered. First, the study did not assess apoptotic pathways, including key markers such as Caspase-3, Bax, and Bcl-2, which could provide further insight into cellular mechanisms of damage and recovery. Second, the evaluation was limited to a one-week period. Although this duration captures early responses, longer-term studies are needed to determine whether PBM effects on spermatogenesis and redox balance are sustained over time.

Conclusion

In summary, laser therapy after testicular T/D effectively improved epididymal sperm motility and viability, restored seminiferous tubule diameter and germinal epithelium thickness, and enhanced antioxidant defense by up-regulating SOD, CAT, and GPx while reducing lipid peroxidation (MDA). Present findings suggest that PBM can serve as a promising therapeutic approach to rapidly restore testicular function after ischemia-reperfusion injury, even when the total sperm count remains unaffected.

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Competing Interests

The authors declare no conflicts of interest related to this study.

Ethical Approval

All experimental procedures and protocols were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.LASER.REC.1403.023).

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