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Therapeutic Effects of Low-Level Laser Therapy on Rat Spinal Cord Injury: Analysis of Inflammatory Markers and Testicular Function

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Abstract

Introduction: Spinal cord injury (SCI) is a severe condition often leading to lasting neurological impairment and associated reproductive health issues in males. The aim of this study was to investigate the therapeutic potential of photobiomodulation therapy (PBMT) during the inflammatory phase of SCI to prevent oxidative damage, reduce inflammation, and mitigate potential damage to testicular function.

Methods: Eighteen male rats were randomly divided into three groups: Group A (laminectomy only), group B (contusion), and group C (contusion + PBMT). Thirty minutes post-injury, group C received PBMT for two weeks. Histological and stereological analysis was evaluated using the hematoxylin and eosin test (H&E). ELISA and real-time PCR were performed for eight weeks post-SCI to assess testosterone and inflammatory and apoptotic changes. Statistical analysis was performed using SPSS with one-way ANOVA and Tukey's post hoc test, and statistical significance was set at $P<0.05$.

Results: The SCI group exhibited significant reductions in sperm count (mean ± SD: 14.2 ± 86.1), motility (34.8 ± 72.8), and viability (26.12 ± 43.9), with increased levels of inflammatory markers (IL-1 β: 4.4 ± 71.6, TNF-α: 3.14 ± 66) and damage to testicular structure. In contrast, animals treated with PBMT showed significant improvements in sperm parameters (sperm count: 48 ± 34, motility: 57.2 ± 18.5, viability: 52 .3 ± 88.2) and a marked reduction in inflammation (IL-1 β: 3.09 ± 14.2, TNF-α: 2.67 ± 74.1) compared to untreated SCI animals ($P<0.001$). Additionally, PBMT-treated animals demonstrated significant improvements in testosterone levels (1.57 ± 44.8) and a reversal of testicular cell loss ($P<0.001$).

Conclusion: These findings suggest that PBMT mitigates the negative effects of SCI on testicular tissue by reducing inflammation and preserving cellular integrity, thus supporting its use as a dual therapeutic approach to aid neurological recovery and maintain reproductive health.

Keywords: Spinal cord injury; Photobiomodulation therapy; Reproductive health; Inflammation.

Introduction

Spinal cord injury (SCI) is a severe condition causing profound neurological impairments, significantly affecting quality of life.[1-4] Millions worldwide suffer from SCI, with limited effective treatments available such as cell therapy.[5,6] SCI involves two phases: the primary injury from mechanical trauma and the secondary injury, characterized by inflammation, oxidative stress, and cell death, which worsen tissue damage and hinder neural repair.[7-10] Current therapeutic strategies for SCI predominantly focus on symptom management and damage prevention rather than promoting actual recovery.[11-13] Traditional interventions such as pharmacological treatments, surgical procedures, and rehabilitation have demonstrated some efficacy but often fall short due to the multifaceted nature of SCI.[11-13] This inadequacy has prompted the search for novel therapeutic modalities that can actively promote tissue regeneration and functional restoration. Photobiomodulation therapy (PBMT) a non-invasive treatment using low-intensity laser light, has emerged as a promising approach to stimulate cellular processes and promote healing.[14-18] Unlike high-energy lasers used

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in surgical contexts, PBMT employs lower energy levels, facilitating tissue penetration without causing harm.[19-21] It works by enhancing mitochondrial activity, increasing ATP production, reducing reactive oxygen species (ROS), and activating pathways that support cell repair. However, reducing cellular stress and controlling autophagy has recently been successful with other therapeutic approaches.[22-24] Preclinical studies have indicated that PBMT possesses neuroprotective and regenerative properties across various models of neurological injury,[25-27] including traumatic brain injury,[28] stroke,[29,30] and SCI.[31] Its ability to reduce inflammation, modulate oxidative stress, and promote neural recovery makes it a potential therapeutic for SCI. However, further research is needed to clarify its mechanisms, optimize treatment parameters, and evaluate its effects on various physiological systems. The timing of PBMT initiation is a crucial factor in determining its efficacy. Our study employed PBMT starting 30 minutes' post-injury, hypothesizing that early intervention during the acute inflammatory phase can modulate inflammatory responses and mitigate secondary injury processes. This timing was chosen to target the initial surge of inflammatory mediators that contribute to downstream tissue damage. While the acute effects of SCI may not fully manifest within 30 minutes, intervening early is anticipated to attenuate their severity and improve long-term recovery outcomes. In addition to the neurological implications of SCI, there is growing recognition of how SCI and its treatments affect reproductive health, particularly in males.[32-36] SCI can disrupt the neuroendocrine system, leading to reproductive challenges, including compromised testicular function, reduced sperm quality, and hormonal alterations.[37] Consequently, therapeutic approaches for SCI should also consider their potential effects on male reproductive health. Previous studies, have suggested that partial recovery of spermatogenesis occurs spontaneously in up to 50% of rats over six months post-SCI.[38] However, this study explores whether PBMT can enhance natural recovery by reducing inflammation and testicular damage during the acute and subacute phases of SCI. By focusing on early intervention, it aims to assess PBMT potential to prevent or mitigate long-term reproductive health deterioration. Using a male rat SCI model, the research evaluates testicular histology, sperm quality, and inflammatory markers to understand the link between SCI-induced inflammation and reproductive decline, providing insight into PBMT therapeutic potential.

Materials and methods

Animal Selection and Ethics Approval

A total of 18 adult male Wistar rats, each weighing between 250 and 300 g, were selected for this study to meet the research objectives. The animals were sourced from the Laboratory Animal Center at Shahid Beheshti University in Tehran, Iran. The experimental procedures complied with ethical guidelines and were approved by the Ethics Committee on Animal Experimentation at Shahid Beheshti University of Medical Sciences (Ethics Code: IR.SBMU.LASER.REC.1402.046). The rats were housed individually in standard laboratory cages and maintained under controlled environmental conditions, ensuring consistent temperature, humidity, and light cycles.

Establishment of Spinal Cord Injury Model and Group Allocation

The study animals were randomly divided into three groups: Group A (laminectomy only), group B (SCI by contusion), and group C (SCI followed by administration of PBMT). Anesthesia was achieved with ketamine (80 mg/kg) and xylazine (10 mg/kg). A laminectomy was performed at the T8 vertebral level, exposing the spinal cord. A contusion injury was then induced using the New York University (NYU) impactor protocol, where a 10 g weight was dropped from a height of 2.5 cm onto the exposed spinal cord. Following injury, the lesion site was covered with muscle and fascia, and the incision was closed in layers. Postoperative care included manual bladder expression twice daily, and gentamicin (0.01 mg/kg) was administered intraperitoneally for five days to prevent infection.[39,40]

Photobiomodulation Therapy

Rats in the SCI + PBMT group received treatment for 14 consecutive days with a diode laser (Noura instrument NILTR102, IRAN). The laser settings included an 810 nm wavelength, continuous wave, with a power of 150 mW for 3000 seconds per day. The laser was applied at five points along the lesion site with a spot size of 0.3 cm^2 using a laser pen held perpendicular to the tissue. The analysis confirmed that the 810 nm wavelength penetrated the tissue, with approximately 6% reaching the spinal cord through the dorsal skin. To minimize movement during irradiation, animals received a reduced anesthetic dose. The other groups also received this anesthetic dose daily to maintain consistency across the study.[31,41] The PBMT parameters were selected to ensure safe, efficient energy transmission to the spinal cord.[42,43]

Testosterone Measurement

Serum testosterone levels were measured using blood samples collected via a cardiac puncture under deep anesthesia. The samples were allowed to clot, centrifuged at 5500 rpm for 5 minutes, and stored at -80 °C until the analysis using a specific ELISA kit.

Perfusion and Tissue Collection

At study completion, animals were deeply anesthetized using intraperitoneal diazepam (10 mg/kg) and ketamine (80 mg/kg). Under anesthesia, rats were perfused with 150

ml of saline followed by 200 mL of 4% paraformaldehyde. Testicular tissues were then processed following standard histological protocols.

Histological Analysis With Hematoxylin and Eosin Staining

Testis tissues were stained with hematoxylin and eosin (H&E) for histological evaluation. Microscopic examinations and digital imaging were performed to assess tissue structure. Testicular tissue morphology, including cellular integrity, was analyzed and relevant measurements were obtained using stereology.

Measurement of Reduced Glutathione and Glutathione Disulfide

Levels of glutathione (GSH) and glutathione disulfide (GSSG) were determined using 5,5'-Dithiobis (DTNB, Sigma-Aldrich), Tris (Sigma-Aldrich), and distilled water. The working solution was analyzed spectrophotometrically. A tissue lysate sample (10 µL) was added to a 990 µL DTNB mixture, incubated for 5 minutes at room temperature, and analyzed at 412 nm to quantify GSH through the formation of 2-nitro-5-thiobenzoic acid.

Real-Time PCR for Gene Expression

Total RNA was isolated from testis samples and assessed for purity. Reverse transcription followed cDNA synthesis kit protocols, and gene expression levels of miRNAs were quantified using SYBR GREEN Real-Time PCR.

Statistical Analysis

Statistical analysis was performed using SPSS version 18. Data are presented as mean ± standard deviation (SD). One-way ANOVA, followed by Tukey's post hoc test, was conducted for normally distributed data. Statistical significance was set at $P<0.05$.

Results

Sperm Parameters

Our evaluation of sperm metrics revealed substantial declines in sperm count, motility, and viability in the SCI group compared with the Control group ($P<0.01$). Specifically, the SCI group exhibited significant reductions in sperm count (14.2 ± 86.1), motility (34.8 ± 72.8), and viability (26.12 ± 43.9). In contrast, animals treated with PBMT showed significant improvements in sperm parameters (sperm count: 48 ± 34, motility: 57.2 ± 18.5, viability: 52 .3 ± 88.2) compared to untreated SCI animals ($P<0.001$). Group B exhibited disrupted seminiferous tubule morphology, with a mean spermatogenesis index, significantly lower than group A ($P<0.001$). In contrast, the SCI + PBMT group demonstrated substantial improvements; sperm count, motility, and viability were significantly higher than those in group B ($P<0.001$) and approached levels observed in group A. Histological evidence from group C highlights partial restoration of seminiferous tubule structure and spermatogenesis. The spermatogenesis index in group C was significantly higher than that in group B ($P<0.001$), but it remained slightly lower than that in group A (Figures 1 and 2).

Testis Volume

Stereological analysis revealed that the testicular volume in the SCI group (14.8 ± 81.9) was noticeably reduced compared to that in the Control group (24.6 ± 32.9, $P<0.0001$). This reduction could signal testicular atrophy, possibly due to tissue damage or cellular depletion caused by SCI. In contrast, testis volume in the SCI + PBMT group (19.8 ± 59.8) was significantly greater than that observed in the SCI group ($P<0.0001$). Sertoli cell and Leydig cell counts followed a similar trend, with a significant reduction in group B (Sertoli cell: 26.4 ± 44.1, Leydig cell: 13.77 ± 84.9) and partial restoration in group C (Sertoli cell: 28.63 ± 21.7, Leydig cell: 18.36 ± 22.7), highlighting the therapeutic potential of PBMT in mitigating SCI-induced testicular damage ($P<0.0001$) (Figure 3).

Number of Testicular Cells

Quantitative assessment of testicular cell populations showed that the SCI group exhibited significantly fewer spermatogonia ($10.93 \pm 45.9 \times 10^6$), spermatocytes ($13.69 \pm 32.8 \times 10^6$), and spermatids cells ($12.73 \pm 15.2 \times 10^6$), compared to group A ($P<0.0001$). This decrease highlights the adverse effects of SCI on germ cell production, which is critical for maintaining normal reproductive function. The SCI + PBMT group, however, demonstrated a significant increase in these cell populations ($17.12 \pm 43.8 \times 10^6$, $27.39 \pm 91.4 \times 10^6$ and $34.91 \pm 22.6 \times 10^6$ receptively) compared to the SCI group ($P<0.0001$), indicating that PBMT may support cellular recovery and sustain spermatogenesis following SCI (Figure 4).

Testosterone Level

ELISA-based measurement of serum testosterone showed that levels were considerably lower in the SCI group (0.82 ± 77.9) relative to the Control group (2.21 ± 87.3) ($P<0.0001$), reflecting impaired endocrine activity in response to SCI. This reduction in testosterone might exacerbate reproductive dysfunction and disrupt the hormonal environment necessary for normal spermatogenic function. However, in the SCI + PBMT group (1.47 ± 72.4), testosterone levels were significantly elevated compared to the SCI group without laser intervention ($P<0.01$), suggesting that PBMT may enhance testosterone synthesis and contribute to hormonal balance in SCI cases (Figure 5).

Reactive Oxygen Species Formation

Analysis of oxidative stress, as indicated by ROS levels,

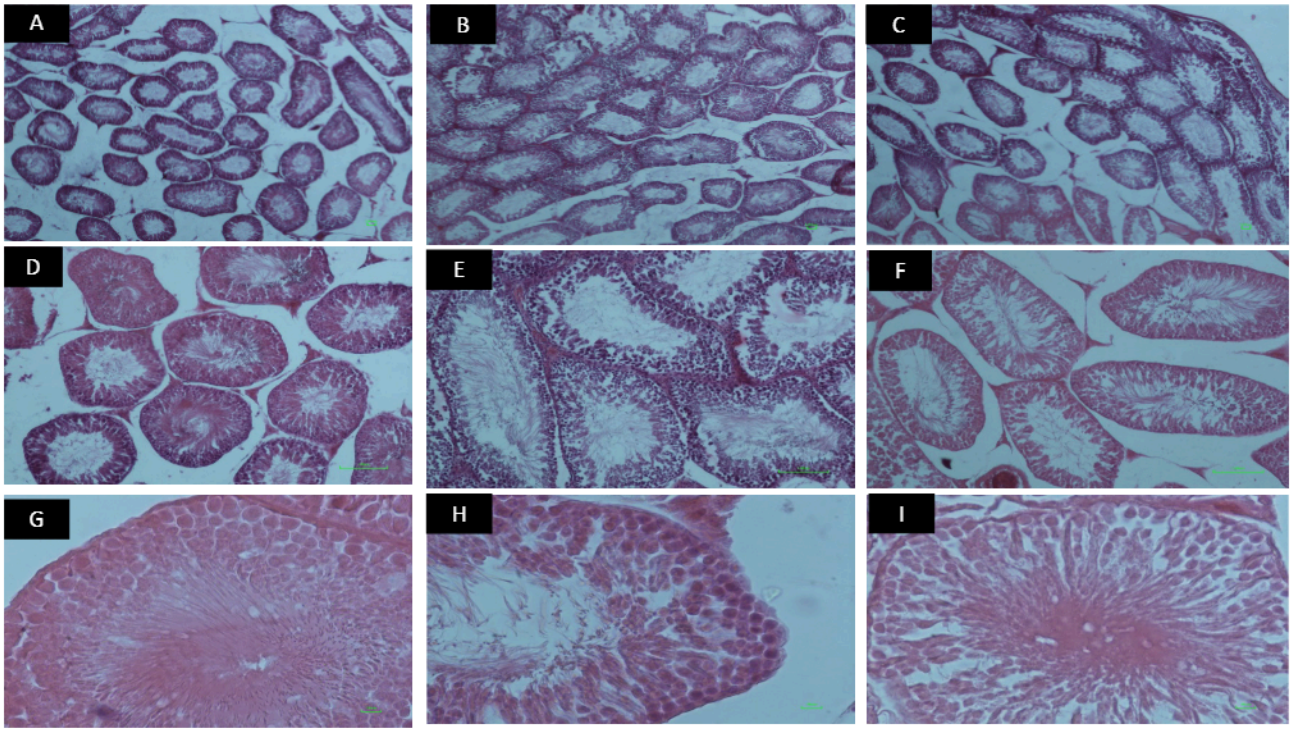

Figure 1. Representative Images of Hematoxylin and Eosin (H&E) Staining of Testicular Tissue From the Experimental Groups. (A, D, G) Group A (laminectomy only) shows normal testicular architecture with intact seminiferous tubules and active spermatogenesis. (B, E, H) Group B (SCI induced by contusion) exhibits disrupted seminiferous tubule morphology, reduced spermatogenesis, and increased interstitial spaces. (C, F, I) Group C (SCI + PBMT) demonstrates improved seminiferous tubule structure and partially restored spermatogenesis compared with group B. The spermatogenesis index in group C was significantly higher than that in group B ($P < 0.001$). These histological findings align with observed sperm parameter trends, highlighting PBMT role in mitigating the negative effects of SCI on reproductive health (magnification: 4, 10, 20)

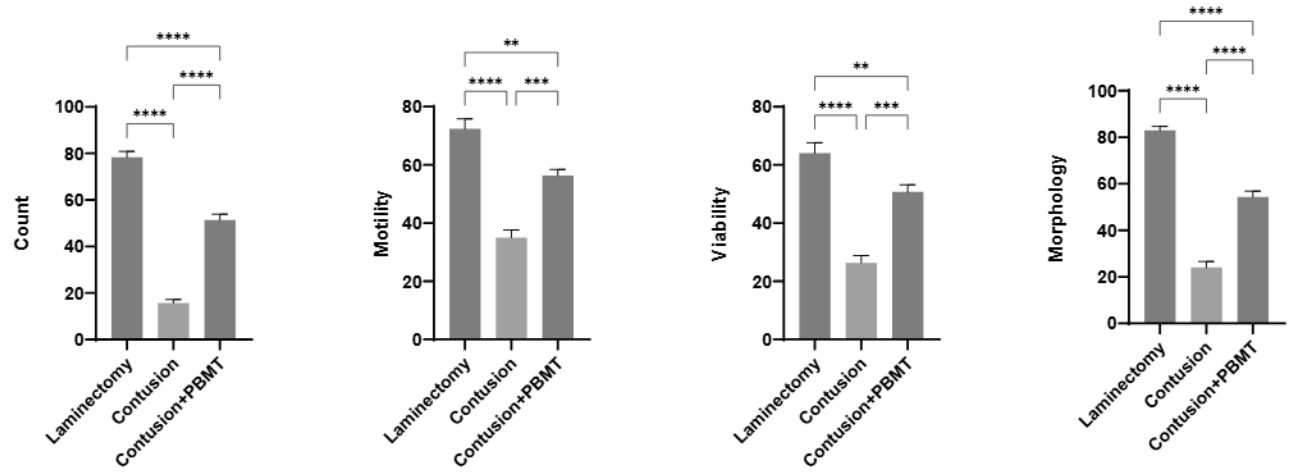

Figure 2. Sperm Parameters In Rats Across Experimental Groups. Sperm count, sperm motility, sperm viability, and sperm morphology were assessed. The SCI group showed significantly reduced sperm count, motility, viability, and morphology compared to the Control group. In contrast, the SCI + PBMT group exhibited significant improvements in these parameters, suggesting that PBMT may help counteract the negative effects of SCI on sperm health. (** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$)

revealed a pronounced increase in ROS formation in the SCI group compared to group A ($P < 0.0001$). This elevation in ROS suggests heightened oxidative stress within testicular tissue following SCI, which could impair cellular function and promote tissue damage. In the SCI + PBMT group, ROS levels were significantly lower than those in the untreated SCI group ($P < 0.0001$), indicating a potential antioxidant effect of PBMT that may help mitigate oxidative stress and protect testicular cells from damage. GSSG levels ($P < 0.001$) and GSH activity ($P < 0.001$) also supported these findings, showing improved oxidative balance in the SCI + PBMT group (Figure 6).

Gene Expression

To further investigate inflammatory responses and

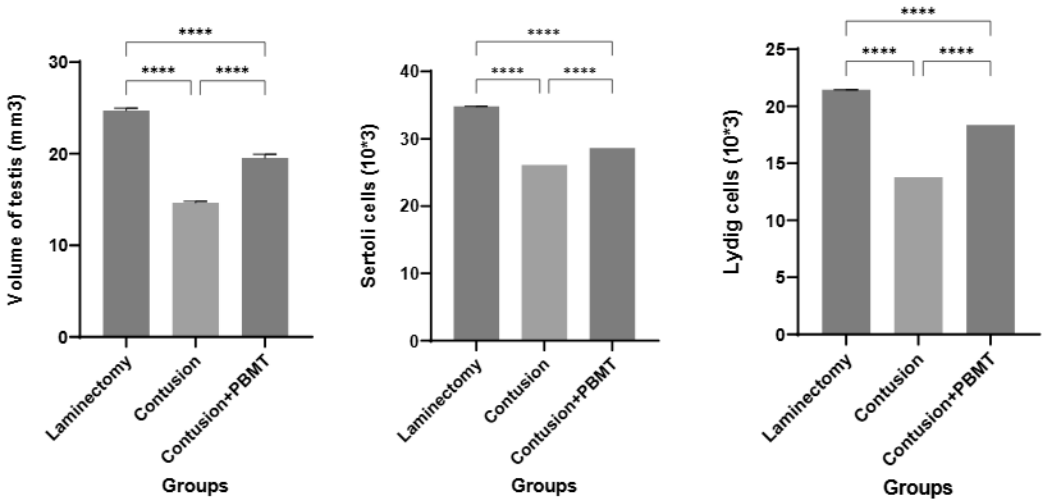

Figure 3. Testis volume and cell count analysis. Testis volume (mm^3) shows a significant reduction in group B compared with group A (**** $P<0.0001$), with partial restoration observed in group C. Sertoli cell count ($\times 10^3$) demonstrates a decrease in group B (****$P<0.0001$), with an improvement in group C. Leydig cell count ($\times 10^3$) shows similar trends, where group B exhibits a significant reduction (****$P<0.0001$) and group C displays partial recovery

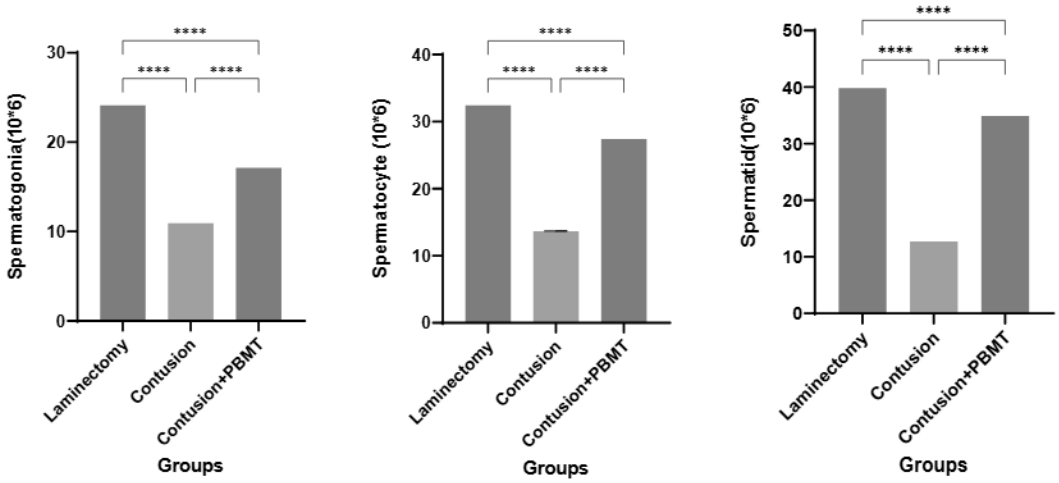

Figure 4. Quantitative assessment of testicular cell populations across experimental groups. Spermatogonia count ($\times 10^6$) highlighting a significant decline in contusion group, with partial recovery observed in SCI + PBMT group. Spermatocyte count ($\times 10^6$) showing a significant decrease in contusion group (****$P<0.0001$) compared with laminectomy group, with partial restoration in SCI + PBMT group. Spermatid count ($\times 10^6$) revealing a marked reduction in contusion group, and improvement in SCI + PBMT group.

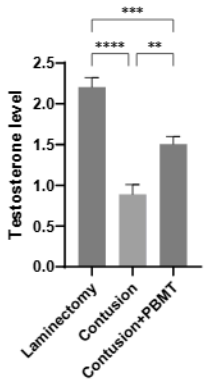

Figure 5. Serum Testosterone Levels in Rats Across Experimental Groups. ELISA-based measurement of serum testosterone showed significantly lower levels in the SCI group compared to the Control group. In the SCI+PBMT group, testosterone levels were significantly elevated compared to the SCI group, suggesting that PBMT may enhance testosterone synthesis and restore hormonal balance following SCI. ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$

apoptosis, we analyzed the expression of relevant genes using real-time PCR (Figure 7). Results indicated that TNF-α expression was significantly elevated in the SCI group compared to the Control group ($P<0.0001$), suggesting an imbalanced inflammatory response post-SCI. Caspase 3 expression was also upregulated in the SCI group, indicating increased apoptosis. In contrast, IL-1β expression was reduced in the SCI group, pointing to a dysregulated inflammatory response. In the SCI+PBMT group, TNF-α and Caspase expression were significantly reduced and CSIF increased, while IL-1β expression was higher than that in the untreated SCI group ($P<0.0001$), suggesting a modulating effect of PBMT on inflammation and cell survival. These gene expression patterns suggest that PBMT may promote cell survival and recovery of testicular function following SCI.

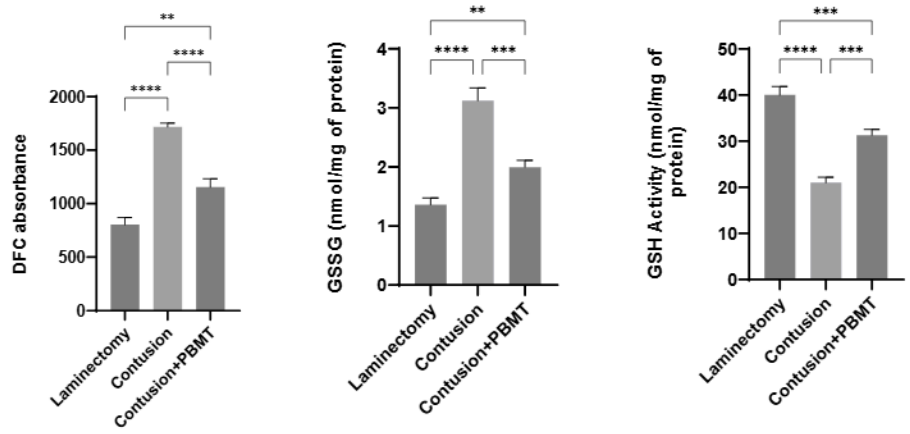

Figure 6. Reactive Oxygen Species Formation and Antioxidant Activity Across Experimental Groups. DFC absorbance shows elevated ROS levels in the SCI group compared to the Control group. GSSG levels (ng/mg of protein) indicate increased oxidative stress in the SCI group, while GSH activity (ng/mg of protein) was significantly lower in the SCI group, pointing to reduced antioxidant defense (** $P<0.01$; *** $P<0.001$; **** $P<0.0001$)

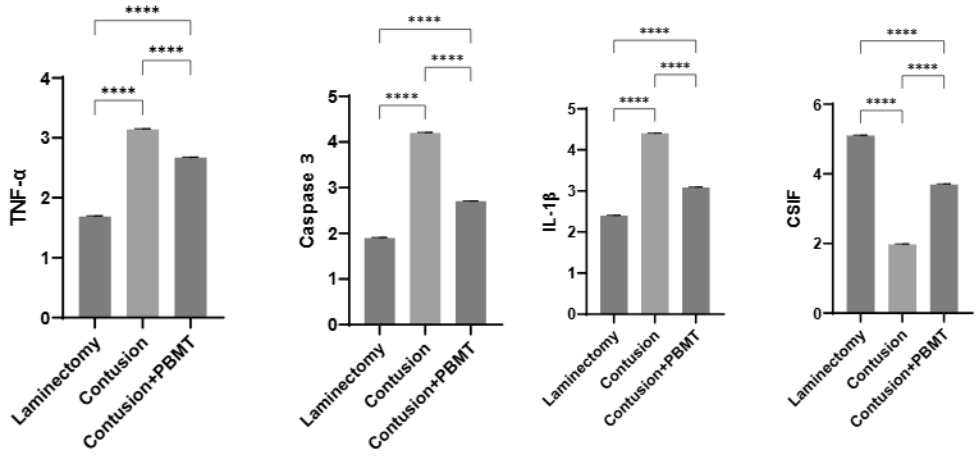

Figure 7. Gene Expression Analysis. Real-time PCR showed that TNF-α, Caspase 3 and IL-1β expressions were elevated, and CSIF expression was reduced in the SCI group compared to the Control group. In the SCI+PBMT group, TNF-α, IL-1β and Caspase expression decreased, while CSIF increased, suggesting that PBMT may modulate inflammation and promote cell survival following SCI.**** $P<0.0001$

Discussion

SCI presents significant challenges, leading to severe neurological deficits and impeded recovery.[41-44] Despite advancements in medical research, effective therapeutic interventions for SCI remain elusive. A promising therapeutic strategy is PBMT, which has shown potential in facilitating tissue repair, reducing inflammation, and enhancing functional recovery.[45,46] PBMT providing an alternative to traditional therapies that often fail to address the complex nature of SCI pathology.[47] Our study shows that SCI negatively impacts male reproductive health, significantly decreasing sperm count, motility, viability, and morphology. Specifically, the SCI group exhibited marked declines in sperm parameters compared to the control group, reflecting the detrimental effects of SCI on male fertility. These results align with previous findings that suggest SCI can lead to severe reproductive dysfunction, likely due to direct injury to the testes or alterations in the hormonal environment.[47] Histological analysis supported these findings, showing disrupted seminiferous tubule structure and a lower spermatogenesis index in the SCI group. In contrast, the SCI + PBMT group exhibited significant improvements in sperm count, motility, and viability, approaching levels observed in the Control group. These improvements were paralleled by histological evidence showing partial restoration of seminiferous tubule architecture and spermatogenesis. Our stereological analysis revealed that the testicular volume in the SCI group was significantly reduced compared to the Control group. This reduction may indicate testicular atrophy, a consequence of cellular damage and depletion following SCI. However, the SCI + PBMT group showed partial recovery of testicular volume, further supporting the protective effects of PBMT on testicular structure and function. Sertoli and Leydig cell counts followed a similar trend, with significant reductions in the SCI group and partial restoration in the PBMT-treated group, highlighting PBMT potential in preserving testicular cellular integrity post-SCI. SCI triggers a cascade of damaging events, including inflammation, oxidative

stress, and apoptosis. Pro-inflammatory cytokines such as TNF-α and IL-1β play key roles in exacerbating secondary damage following SCI.[48,49] Real-time PCR analysis revealed significantly elevated TNF-α and Caspase 3 expression in the SCI group, indicating heightened inflammation and apoptosis. However, PBMT treatment significantly reduced these markers, while enhancing cell survival indicators such as CSIF. These findings suggest that PBMT can modulate inflammatory responses, promoting cell survival and facilitating tissue recovery following SCI. PBMT ability to regulate inflammatory cytokine levels aligns with previous studies that demonstrate its capacity to reduce pro-inflammatory cytokines and enhance anti-inflammatory responses, which is essential for preventing further tissue damage post-SCI.[24,50] Oxidative stress, arising from excessive production of ROS, further damages spinal cord tissue by overwhelming the body's antioxidant defenses.[51,52] Our analysis of ROS levels demonstrated that PBMT significantly reduced ROS and GSSG (oxidized glutathione) levels while increasing GSH (reduced glutathione) activity, indicating that PBMT enhances antioxidant defenses and mitigates oxidative damage. These findings align with studies showing that PBMT effectively reduces oxidative stress and promotes antioxidant activity, creating a more favorable environment for tissue repair and neuronal survival following SCI.[46,53-56] These findings underscore the potential of PBMT to reduce oxidative damage and protect testicular cells from further harm, supporting its use as a therapeutic tool for mitigating SCI-related oxidative stress. The hormonal environment plays a crucial role in maintaining reproductive function, and SCI-induced reductions in testosterone levels can further impair spermatogenesis and fertility. Our ELISA-based measurement of serum testosterone levels showed significantly lower testosterone in the SCI group compared to the control group. However, PBMT treatment led to a significant increase in testosterone levels, suggesting that PBMT may enhance testosterone synthesis and restore hormonal balance post-SCI. These findings are consistent with previous studies demonstrating PBMT role in promoting endocrine recovery and mitigating reproductive dysfunction following SCI. The restoration of testicular function observed in our study further emphasizes the multifaceted therapeutic potential of PBMT. Our findings suggest that PBMT offers a promising therapeutic approach for SCI, with significant benefits in reducing inflammation, alleviating oxidative stress, and improving testicular health. The positive impact of PBMT on reproductive health further underscores its potential to improve overall physiological outcomes in SCI patients.

Conclusion

This study demonstrates PBMT therapeutic potential in reducing SCI-induced testicular damage. PBMT lowered inflammatory cytokines, improved sperm quality, motility, viability, and testosterone levels, and preserved testicular structure. These findings highlight PBMT's ability to mitigate inflammation and structural damage, supporting its use as a strategy to counter SCI-induced reproductive decline.

Authors' Contribution

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Formal analysis: Foozhan Tahmasebinia, Marzieh Abedi, Sama Abbasi.
Investigation: Hadise Taheri, Hamid Reza Mosleh, Shima Jahanbaz.
Methodology: Hojjat-Allah Abbaszadeh.
Project administration: Hojjat-Allah Abbaszadeh.
Resources: Hadise Taheri, Hamid Reza Mosleh, Shima Jahanbaz.
Software: Marzieh Abedi, Sama Abbasi.
Supervision: Hojjat-Allah Abbaszadeh.
Validation: Abbas Aliaghaei, Hojjat-Allah Abbaszadeh.
Visualization: Hojjat-Allah Abbaszadeh.
Writing–original draft: Hadise Taheri, Hojjat-Allah Abbaszadeh.
Writing–review & editing: Hojjat-Allah Abbaszadeh.

Competing Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was approved by the Medical Research Ethics Committee at Shahid Beheshti University (IR.SBMU.LASER.REC..1402.046).

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