



Therapeutic Effects of Photobiomodulation Therapy on Ovarian Structure and GDF9, BMP15 and BMP4 Expression in the Spinal Cord Injury Female Rat Model

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Abstract

Introduction: Spinal cord injury (SCI) often results in severe neurological deficits and secondary complications, including disruptions in female reproductive health. Current treatment options are limited in addressing both neurological recovery and reproductive outcomes. This study investigated the impact of photobiomodulation therapy (PBMT) on spinal cord healing and ovarian health in a female rat model of SCI.

Methods: in this study 18 rats were divided into three groups (Control, contusion and PBMT) and SCI was induced by a weight drop model. Real-time PCR use for gene expression (GDF9, BMP15 and BMP4), histological analyses for ovarian tissues by hematoxylin and eosin (H&E), cell counting and ovary volume by stereology and estrogen level measured by ELISA kit.

Results: Real-time PCR results showed that PBMT treatment significantly reduced inflammation, evidenced by lower levels of tumor necrosis factor alpha (TNF- α), and facilitated tissue repair in the ovary. Additionally, stereology results showed that ovarian assessments revealed improved follicular structure and overall enhancement of ovarian function in the PBMT-treated group compared to the contusion group, and this result is statistically significant.

Conclusion: This research highlights the dual benefits of PBMT in supporting neurological recovery and safeguarding reproductive health in female rats following SCI. These findings point to the potential of PBMT as a novel therapeutic approach to improve outcomes for women affected by SCI.

Keywords: Spinal cord injury; Photobiomodulation therapy; Ovary; Inflammation.



Introduction

Spinal cord injury (SCI) is a debilitating condition that causes severe neurological impairments, profoundly affecting the quality of life for those impacted.¹⁻³ Millions of people worldwide experience SCI, yet effective treatment options remain scarce.^{4,5} The pathophysiology of SCI involves two primary phases: the initial mechanical trauma causing the primary injury and the secondary injury characterized by complex biological processes such as inflammation, oxidative stress, and cell death. These secondary mechanisms exacerbate tissue damage and create significant barriers to neural repair, complicating the recovery process.⁶⁻⁹ Current therapeutic approaches for neurodegenerative disorders are largely aimed at managing symptoms and preventing further damage rather than facilitating true recovery

Traditional treatments, including pharmacological interventions, surgical methods, cell therapy, stem cell differentiation and inflammation and autophagy control, have shown limited success due to the multifactorial nature of neurodegenerative disorders.¹⁰⁻¹² This limitation underscores the need for innovative therapeutic strategies that actively promote tissue regeneration and functional restoration. photobiomodulation therapy (PBMT), also known as low-level laser therapy (LLLT), has emerged as a promising non-invasive technique that employs low-intensity laser light to enhance cellular activity and promote healing.¹³⁻¹⁷ Unlike high-energy lasers used for surgical applications, PBMT operates at lower energy levels, enabling tissue penetration without causing damage.¹⁸⁻²⁰ The proposed mechanism involves the absorption of light by cellular chromophores, particularly

within mitochondria, which boosts ATP production, reduces reactive oxygen species (ROS), and activates signaling pathways essential for cell proliferation and repair.^{21,22} Preclinical studies have demonstrated the neuroprotective and regenerative properties of PBMT in various models of neurological injury,²³⁻²⁵ including traumatic brain injury,²⁶ stroke,^{27,28} and SCI.²⁹ By reducing inflammation, mitigating oxidative stress, and supporting neural recovery, PBMT shows great promise as a potential therapeutic option for SCI. Nevertheless, to strengthen this approach, it is imperative to delve deeper into the underlying mechanisms and optimize treatment parameters tailored specifically to the effects on both neurological and reproductive health outcomes. Despite promising results, the specific mechanisms of PBMT and its effects on reproductive health outcomes remain inadequately explored, especially in female populations affected by SCI. This study aims to fill this research gap by assessing the potential therapeutic benefits of PBMT in a female rat model of SCI. Furthermore, understanding the implications of SCI on female reproductive health is critical, as recent studies indicate that SCI significantly disrupts the neuroendocrine system. This disruption can lead to reproductive challenges, such as alterations in ovarian structure, impaired follicular development, and hormonal imbalances.³⁰⁻³² SCI can impact the neuroendocrine system, leading to challenges in reproductive function, including alterations in ovarian structure, impaired follicular development, and hormonal imbalances.³³ Studying these effects in the context of PBMT is essential, considering the interconnected nature of neurological and reproductive health. Therefore, this study focuses on evaluating the therapeutic potential of PBMT in a female rat model of SCI, specifically analyzing ovarian histology, follicular health, and inflammatory markers to explore the relationship between SCI-induced inflammation and reproductive function. By addressing reproductive outcomes alongside neurological recovery, this research seeks to provide a more holistic understanding of PBMT's therapeutic potential in SCI, highlighting the need for inclusive treatment strategies that account for both neurological and reproductive health.

Materials and Methods

Animal Selection and Ethical Considerations

This study utilized 18 adult female Wistar rats, each weighing between 250 and 300 g, to achieve its research goals. The animals were randomly assigned to three groups, with 6 rats per group, to ensure a balanced sample size across experimental conditions. The sample size was determined based on a priori power analysis using effect sizes reported in previous studies, which indicated that 6 rats per group would provide an 80% power to detect statistically significant differences at an alpha level of 0.05.³⁴

The animals were procured from the Laboratory Animal Center of Shahid Beheshti University, Tehran, Iran. All experimental protocols adhered to ethical standards and received approval from the Ethics Committee on Animal Experimentation at Shahid Beheshti University of Medical Sciences (Ethics Code: IR.SBMU.LASER.REC.1403.019). The rats were housed individually in standard laboratory cages under carefully controlled conditions, including regulated temperature, humidity, and a consistent light-dark cycle, to ensure uniformity throughout the study.

Spinal Cord Injury Model Development and Group Assignment

The 18 rats were randomly allocated into three experimental groups (n=6 per group): Group A (laminectomy only), group B (SCI induced by contusion), and group C (contusion+PBMT). Anesthesia was administered using ketamine (80 mg/kg) and xylazine (10 mg/kg). A laminectomy was performed at the T8 vertebral level to expose the spinal cord. The contusion injury was created following the New York University (NYU) impactor protocol, wherein a 10 g weight was dropped from a height of 2.5 cm onto the exposed spinal cord. After the procedure, the injury site was covered with muscle and fascia, and the incision was closed in layers. Postoperative care included manual bladder expression twice daily and intraperitoneal administration of gentamicin (0.01 mg/kg) for five days to prevent infection.^{35,36}

Photobiomodulation Therapy

The rats in the SCI+PBMT group underwent Photobiomodulation therapy for 14 consecutive days using a diode laser. Therapy was administered once per day, and during each session, the rats were positioned in a prone posture on a custom restraining apparatus to ensure consistent and optimal exposure of the dorsal spinal cord region. The laser parameters included a wavelength of 810 nm, continuous wave operation (Noura Instrument NILTR102), a power output of 150 mW, and an exposure time of 3000 seconds per day. The 810 nm wavelength was selected due to its superior tissue penetration and its established ability to stimulate mitochondrial activity in neural cells, which is critical for promoting neuroprotection and repair.³⁷ Similarly, the 150 mW power setting was chosen to deliver sufficient energy for therapeutic effect while minimizing the risk of thermal damage, and the prolonged exposure time of 3000 seconds ensured a cumulative low-level dose that effectively modulates inflammatory responses and enhances cellular metabolism.³⁸ The laser was applied to five specific points along the lesion site with a spot size of 0.3 cm², using a laser pen positioned perpendicular to the tissue. The tissue analysis confirmed that approximately 6% of the 810 nm wavelength penetrated the dorsal skin to reach the spinal cord. To minimize movement during

irradiation, we administered a reduced dose of anesthesia to the animals. The same anesthetic protocol was applied daily to all other groups to ensure consistency across the study.^{29,39} These parameter selections were based on prior studies demonstrating their efficacy in reducing spinal cord inflammation and promoting neural recovery, thereby providing a rationale for their use in the current experimental model.^{37,38,40-42}

Perfusion and Tissue Collection

At the conclusion of the study, the animals were deeply anesthetized with intraperitoneal injections of diazepam (10 mg/kg) and ketamine (80 mg/kg). Once anesthetized, the rats were perfused with 150 mL of saline, followed by 200 mL of 4% paraformaldehyde. The ovarian tissues were then processed according to standard histological procedures.

Histological Analysis with H&E Staining and stereology

Ovarian tissues were stained with hematoxylin and eosin (H&E) for histological assessment. Microscopic analysis and imaging were conducted to evaluate tissue structure. Ovarian tissue volume, cellular integrity, was examined, and relevant measurements were obtained using stereology technic.³⁹

Measurement of 2,7-Dihydrodichlorofluorescein (DCF), Reduced Glutathione (GSH) and Glutathione Disulfide (GSSG)

Quantification of ROS in Ovarian Tissue Using DCF Fluorescence, in this procedure ovarian tissue homogenates were reacted with 10 μ M DCFH-DA (37 °C, 30 minutes, dark), converting intracellular ROS to fluorescent DCF. Absorbance was measured at 502 nm (SpectraMax M5) with parallel fluorescence detection (Ex/Em 485/530 nm). Values were normalized to total protein (Bradford assay) and compared against a H₂O₂ standard curve (0-100 μ M). The levels of GSH and GSSG were measured using a mixture of 5,5'-Dithiobis (DTNB, Sigma-Aldrich), Tris (Sigma-Aldrich), and distilled water. The working solution was analyzed spectrophotometrically. A 10 μ L tissue lysate sample was added to 990 μ L of the 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 extracted from cellular samples, and its purity was assessed using UV spectrophotometry (NanoDrop). We measured the absorbance at 260 nm to determine RNA concentration, and the A260/A280 ratio was calculated to evaluate purity; an A260/A280 ratio of approximately 2.0 indicated high-quality RNA suitable for downstream applications. Reverse transcription was then performed according to the cDNA synthesis kit protocol,

and gene expression levels of tumor necrosis factor alpha (TNF- α), GDF9, BMP15 and BMP4 were quantified using real-time PCR.

Statistical Analysis

Statistical analysis was performed using SPSS version 18. Quantitative data are expressed as mean $\pm$ standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for normally distributed data. A *P* value of < 0.05 was considered statistically significant.

Results

Ovarian Parameters

Our analysis of ovarian function revealed significant declines in ovarian volume, follicular count, and oocyte viability in the SCI group compared to the control group. SCI was associated with a marked reduction in the number of growing follicles and a decrease in oocyte viability, reflecting substantial damage to female reproductive health post-SCI. Histological examination of ovarian tissue (Figure 1) confirmed these findings, showing that SCI exhibited disrupted ovarian morphology with diminished follicle numbers and enlarged interstitial spaces. However, in the contusion + PBMT group, these parameters were significantly improved. Notably, the contusion + PBMT group demonstrated a higher follicular count, larger ovarian volume, and greater oocyte viability than the contusion group, highlighting the therapeutic potential of PBMT in alleviating SCI-induced ovarian damage. Histological analysis (Figure 1) also showed partial restoration of ovarian structure and follicular development in the contusion + PBMT group.

Ovarian Volume

Stereological analysis revealed a significant reduction in ovarian volume in the contusion group compared to the control group (***P* < 0.001), suggesting potential ovarian atrophy as a result of SCI-induced tissue damage. In contrast, ovarian volume was significantly greater in the contusion + PBMT group than in the contusion group, indicating that PBMT may help preserve or partially restore ovarian tissue integrity following SCI (***P* < 0.01). Quantitative analysis of ovarian volume (Figure 2) demonstrated a marked reduction in group B (SCI by contusion) and a partial recovery in group C (contusion + PBMT), further emphasizing the potential of PBMT to mitigate SCI-induced ovarian damage.

Number of Ovarian Cells

The number of primordial follicle count, pre-antral follicle count, and antral follicle count was significantly reduced in the SCI group compared to the control group, but the number of atretic follicle count increased (*****P* < 0.0001). This indicates that SCI negatively impacts follicular development and oocyte health.

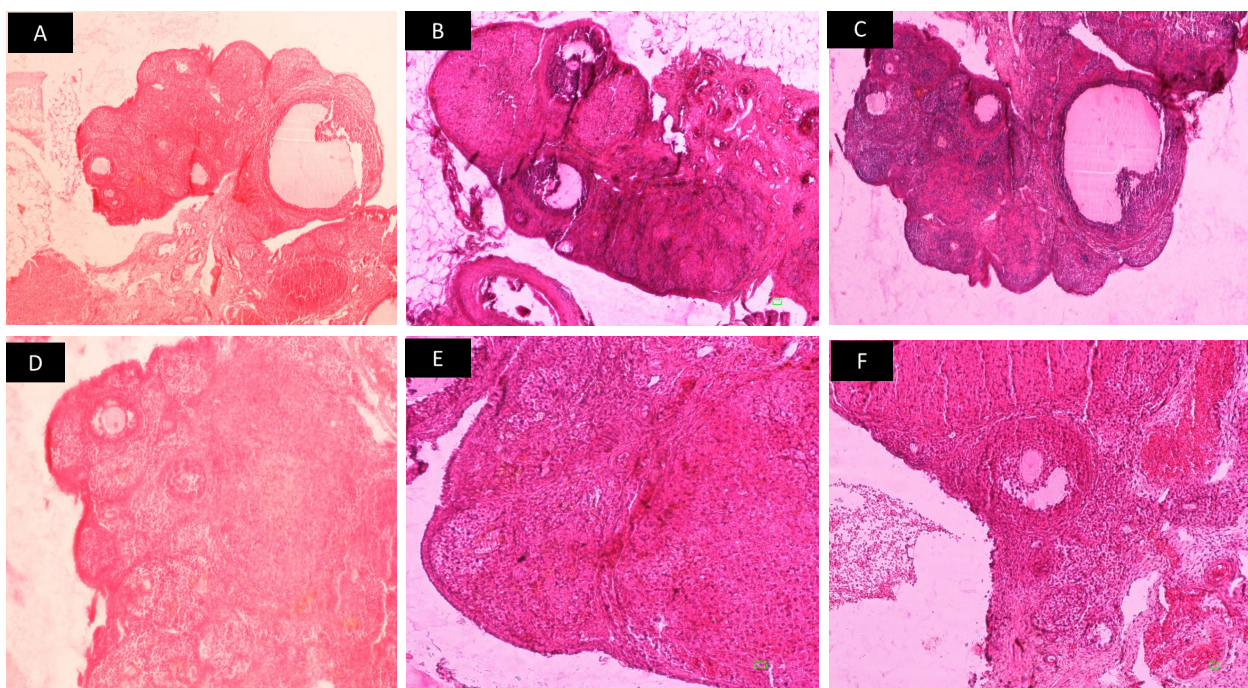


Figure 1. Representative Images of Hematoxylin and Eosin (H&E) Staining of Ovarian Tissue From the Experimental Groups: (A, D) Group A (Control) with normal ovarian architecture and intact follicles, (B, E) Group B (SCI by contusion) showing disrupted ovarian morphology, and (C, F) Group C (contusion + PBMT) exhibiting improved ovarian structure with restored follicles.

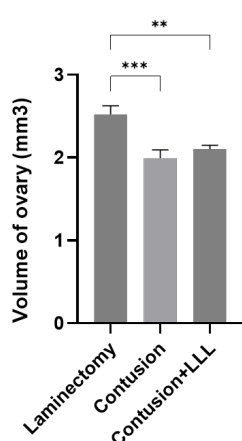


Figure 2. Quantitative Analysis of Ovarian Parameters Across Experimental Groups: Ovarian Volume (mm³). Significant reductions were observed in Group B (contusion), while partial restoration was noted in Group C (contusion + PBMT), suggesting the beneficial effects of PBMT on ovarian volume integrity.

Specifically, there was a marked reduction in pre-antral follicle count and primordial follicle count in the SCI group (Figure 3). However, the contusion + PBMT group exhibited significant improvements in these cell populations, suggesting that PBMT may support cellular recovery and preserve ovarian function following SCI. This was particularly evident in the partial restoration of primordial follicles and pre-antral follicles, further highlighting the restorative potential of PBMT in mitigating SCI effects on follicular populations. Additionally, estrogen levels were assessed

(Figure 4), showing significantly reduced levels in the SCI group compared to the Control group. In contrast, the contusion + PBMT group demonstrated significant improvements in estradiol levels, suggesting that PBMT helps counteract the negative effects of SCI on ovarian endocrine function (** $P < 0.001$, * $P < 0.05$ receptively).

Reactive Oxygen Species Formation

Our analysis of oxidative stress revealed improvements in GSH activity in the contusion + PBMT group compared to the contusion group, supporting the antioxidant effects of PBMT (**** $P < 0.0001$). Additionally, ROS levels were significantly higher in the SCI group compared to the control group (** $P < 0.001$), indicating increased oxidative stress in ovarian tissue, which can impair cellular function and promote tissue damage. In contrast, the contusion + PBMT group displayed significantly lower ROS levels, suggesting that PBMT may reduce oxidative stress and protect ovarian cells (** $P < 0.01$). Furthermore, GSSG levels were improved in the contusion + PBMT group, further demonstrating the ability of PBMT to counteract oxidative stress and restore redox balance in ovarian tissue (** $P < 0.001$, * $P < 0.01$) (Figure 5).

Gene Expression

Real-time PCR analysis revealed that TNF- α expression (Figure 6) was significantly elevated in the contusion group compared to the Control group (**** $P < 0.0001$), indicating a heightened inflammatory response. TNF- α is a pro-inflammatory cytokine involved in immune

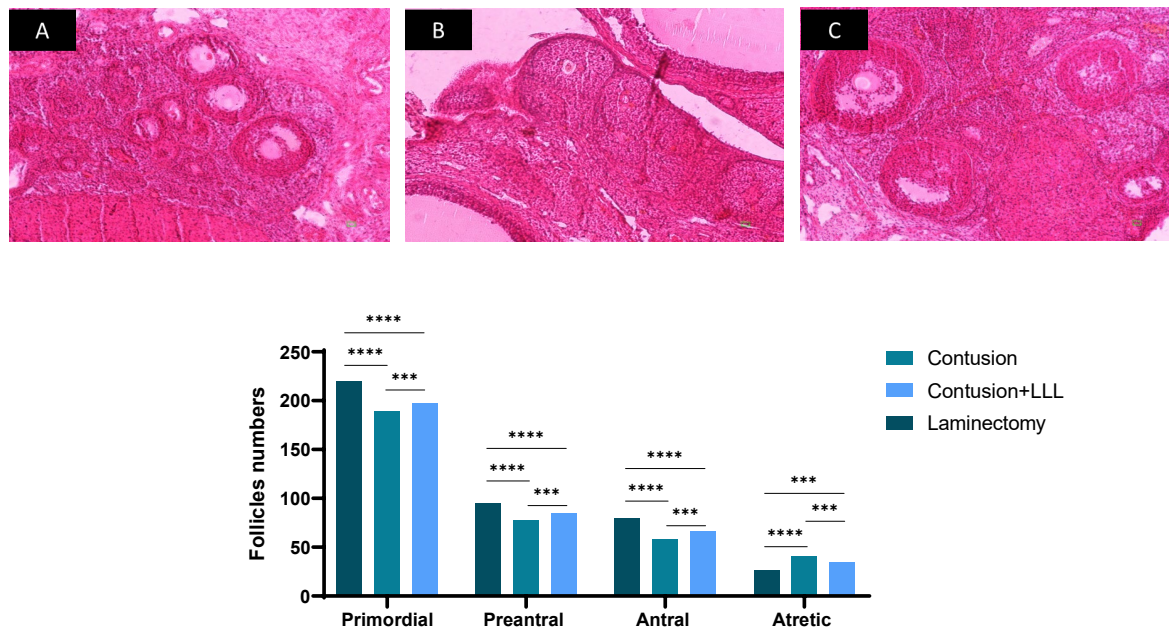


Figure 3. Histological (Upper Row: A: Control, B: Contusion, C: Contusion+PBMT) and Quantitative Assessment (Lower Row) of Ovarian Cell Populations: Primordial Follicle Count ($\times 10^6$), Preantral Follicle Count ($\times 10^6$), Antral Follicle Count ($\times 10^6$), and Atretic Follicle Count ($\times 10^6$). Significant decreases were observed in Group B(contusion), with partial restoration in Group C (contusion + PBMT), supporting the potential of PBMT to recover ovarian cellular integrity (**** $P < 0.0001$, *** $P < 0.001$ receptively).

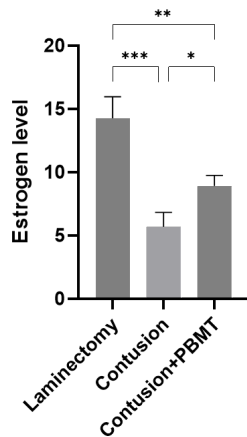


Figure 4. Serum Estrogen Levels in Rats Across Experimental Groups. ELISA-based measurement of serum estrogen showed significantly lower levels in the SCI group compared to the control group. In the contusion+PBMT group, estrogen levels were significantly elevated compared to the SCI group (*** $P < 0.001$, ** $P < 0.001$, * $P < 0.05$ receptively).

regulation and inflammation, and its overexpression suggests tissue damage and a pro-inflammatory microenvironment caused by SCI. Conversely, the expressions of GDF9 (growth differentiation factor 9), BMP15 (bone morphogenetic protein 15), and BMP4 (bone morphogenetic protein 4) were markedly reduced in the contusion group (**** $P < 0.0001$). These factors play critical roles in ovarian follicle development and oocyte health. GDF9 and BMP15 are oocyte-derived growth factors essential for follicular maturation, granulosa cell function, and ovarian tissue integrity, while BMP4 is involved in ovarian cell differentiation and proliferation. Their reduced expression indicates a disruption in ovarian

function and impaired follicular development due to SCI. In the contusion + PBMT group, TNF- α expression was significantly reduced, reflecting a dampened inflammatory response. Additionally, GDF9, BMP15, and BMP4 expression levels significantly increased compared to the contusion group (**** $P < 0.0001$), suggesting that PBMT supports the recovery of ovarian function by promoting follicular development and enhancing granulosa cell and oocyte health. These findings highlight the potential of PBMT to mitigate the adverse effects of SCI by modulating inflammation, restoring growth factor expression, and promoting cell survival and ovarian tissue recovery. PBMT emerges as a promising therapeutic strategy to preserve and restore ovarian function following SCI.

Discussion

SCI results in severe neurological deficits and hampers recovery, presenting significant therapeutic challenges despite progress in medical research.⁴³ One potential treatment option is PBMT, which has shown promise in promoting tissue repair, reducing inflammation, and improving functional recovery following SCI.^{43,44} As a non-invasive intervention, PBMT utilizes low-energy light to stimulate cellular processes, providing an alternative to conventional treatments that often fail to address the complex pathology of SCI.⁴⁵ At the cellular and molecular levels, SCI triggers a cascade of damaging events, such as inflammation, oxidative stress, and glial activation, which exacerbate tissue injury and impede recovery. Pro-inflammatory cytokines like TNF- α play crucial roles in the inflammatory response, contributing to secondary damage and the progression of SCI.^{46,47}

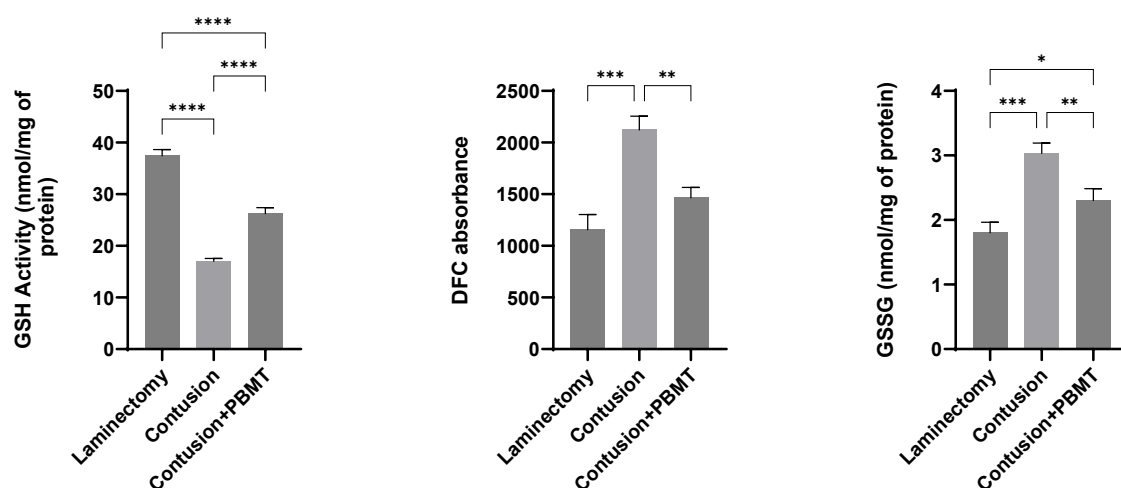


Figure 5. Reactive Oxygen Species Formation and Antioxidant Activity in the Experimental Groups. The result showed that increased GSH activity in the contusion + PBMT group in comparison with contusion group and decrease DCF levels in the contusion + PBMT group in comparison with SCI group, and decreased GSSG levels in the contusion + PBMT group in comparison with contusion group (**** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$ receptively).

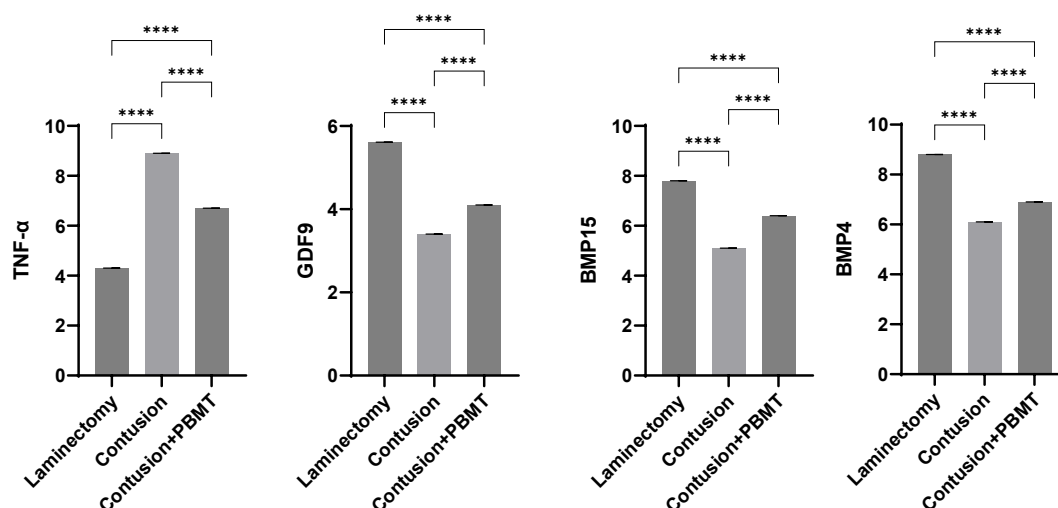


Figure 6. Gene Expression Analysis. Real-time PCR demonstrated a significant elevation in TNF- α expression in the contusion group, highlighting increased inflammation. Conversely, the expressions of GDF9, BMP15, and BMP4 were markedly reduced in the SCI group, indicating impaired ovarian function. Notably, the contusion + PBMT group showed a substantial increase in BMP15, BMP4, and GDF9 expression levels, suggesting that PBMT may mitigate inflammation and promote the recovery of ovarian tissue function.

Furthermore, excessive ROS production leads to oxidative stress, overwhelming the body's antioxidant defenses and causing further spinal cord damage.^{48,49} Recent studies suggest that PBMT may alleviate these harmful processes by modulating inflammatory responses, reducing oxidative stress, and regulating cellular functions, thereby promoting a more favorable environment for tissue repair and regeneration.^{44,50-53} Our findings revealed that PBMT treatment significantly reduced TNF- α expression in the contusion group, consistent with its role in mitigating the inflammatory response. Furthermore, real-time PCR analysis demonstrated significant upregulation of ovarian-specific factors, including BMP4, BMP15, and GDF9, in the contusion + PBMT group. These proteins are critical for follicular development and oocyte maturation, and their restoration suggests that PBMT not only

reduces inflammation but also supports ovarian recovery by improving cellular signaling pathways involved in reproductive health.⁵⁴ The selection of these specific ovarian genes was based on their well-documented roles in regulating folliculogenesis and maintaining ovarian function. Emerging evidence indicates that dysregulation of miRNAs can adversely affect the expression of key genes such as BMP4, BMP15, and GDF9, potentially contributing to reproductive dysfunction following SCI.⁵⁵⁻⁵⁹ Real-time PCR analysis showed that PBMT treatment effectively modulated the inflammatory response, as evidenced by the significant reduction in pro-inflammatory cytokines in treated rats.^{54,60} This observation is consistent with other studies demonstrating the efficacy of PBMT in regulating inflammatory responses after SCI. Additionally, rats treated with PBMT exhibited improved oxidative stress

profiles, with lower ROS and oxidized glutathione (GSSG) levels and higher reduced glutathione (GSH) levels, suggesting enhanced antioxidant defenses and reduced oxidative damage to ovarian tissue. This improvement in oxidative stress suggests that PBMT helps create a more favorable environment for neuronal survival and tissue repair following SCI.^{54,60} Beyond its impact on spinal cord recovery, this study also assessed the effects of PBMT on female reproductive health. Ovarian tissue analysis revealed improved ovarian structure and enhanced function in the PBMT-treated rats, indicating that PBMT may help preserve reproductive function following SCI. Overall, PBMT multi-faceted effects, including reducing inflammation, alleviating oxidative stress, and promoting tissue regeneration, position it as a promising therapeutic approach for SCI. Moreover, several key studies support the mechanistic pathways underlying our findings. For instance, Chung et al and Hashmi et al demonstrated that LLLT using an 810 nm wavelength not only reduces inflammation but also enhances cellular metabolism and neuroregeneration in various models.^{37,61} Similarly, Gambacciani and Palacios (2017) provided evidence that laser therapy can improve reproductive function,⁶² while Vafaei-Nezhad et al reviewed the safety and challenges of PBMT in SCI.²⁹ Additionally, a systematic review by da Cruz Tobelem et al reinforced the neuroprotective and anti-inflammatory effects of PBMT in experimental SCI models,⁶³ and Oubiña et al showed that LLLT modulates ovarian function in mature female mice.⁶⁴ Our results are in strong agreement with those of Oubiña et al. Specifically, both studies demonstrate that PBMT significantly improves ovarian structure and function. Our stereology result showed contusion+ PBMT group increased follicular counts, improved ovarian volume, and enhanced expression of key ovarian genes (BMP4, BMP15, and GDF9), which are critical for follicular development and oocyte quality. Similarly, Oubiña et al observed modulation of folliculogenesis, a reduction in apoptosis, and improved oocyte quality following LLLT. These parallel findings validate our approach and underscore PBMT potential as a dual-action therapy for mitigating both neurological deficits and reproductive dysfunction induced by SCI. These studies collectively validate our approach and support the mechanistic pathways observed in our study. Despite these promising findings, it is important to note that functional studies directly validating the proposed molecular mechanisms of PBMT are currently limited. Future investigations should include targeted functional assays to confirm how PBMT modulates both inflammatory and ovarian signaling pathways in SCI models. Furthermore, when compared with other treatment modalities such as pharmacological anti-inflammatory agents and antioxidant therapies, PBMT offers a distinct advantage as a non-invasive, multi-targeted approach that not only mitigates inflammatory

and oxidative damage but also supports the recovery of ovarian function. This comparative perspective underscores the novelty and clinical significance of our findings, highlighting PBMT's potential as a dual-action therapy for both neurological and reproductive recovery following SCI.^{37,65} Future research should focus on optimizing PBMT parameters such as wavelength and treatment duration, as well as investigating its long-term effects on both neurological and reproductive health. Clinical trials will be essential to determine the therapeutic efficacy of PBMT and translate these promising results into practical applications for SCI patients. SCI profoundly impacts neurological functions and ovarian health, with limited therapeutic options available. This study demonstrates the therapeutic potential of PBMT in mitigating SCI-induced damage to ovarian structure and function. PBMT treatment significantly reduced pro-inflammatory cytokine TNF- α levels, highlighting its anti-inflammatory effects. Moreover, PBMT led to marked improvements in the expression of ovarian-specific factors such as BMP4, BMP15, and GDF9, which are essential for follicular development and oocyte quality.

Conclusion

PBMT effectively counteracts the inflammatory and oxidative damage caused by SCI, supports ovarian tissue recovery, and preserves reproductive function. As a non-invasive therapeutic option, PBMT shows promise for addressing the dual challenges of SCI-related neurological and reproductive health impairments. Future studies should aim to optimize treatment protocols and evaluate long-term outcomes to establish PBMT as a clinically viable therapy for preserving reproductive health in SCI patients.

Authors' Contribution

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Methodology: Hojjat-Allah Abbaszadeh.

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Supervision: Hojjat-Allah Abbaszadeh.

Validation: Hojjat-Allah Abbaszadeh.

Visualization: Hojjat-Allah Abbaszadeh, Reza Mastery Farahani.

Competing Interests

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 of Medical Sciences (IR.SBMU.LASER.REC.1403.019).

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