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RESEARCH ARTICLE

Comparison of three different dosages of low-level laser therapy on expression of cell proliferation and inflammatory markers following ovariohysterectomy in rats

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ABSTRACT

The objective of the current study was to evaluate Low-level laser therapy (LLLT) on the healing of incisional wounds following ovariohysterectomy in rats, by means of subjective histopathological and immunohistochemical analysis. A total of 72 female Wistar rats were categorised into four treatment groups (Group I; sacrification 4 hours following only one LLLT application, Group II; sacrification 7 days following only one LLLT application, Group III; sacrification 4 hours after two LLLT applications, and Group IV; sacrification 7 days after two LLLT applications). Each group was further divided into four different doses subgroups (Group Control [C, off mode LLLT application], L_1 [1 J/cm^2], L_3 [3 J/cm^2], and L_6 [6 J/cm^2]), with equal representation in each subgroup. Ovariohysterectomy was employed using two 2-cm-length midline abdominal incisions in the left and right sides of line alba. The Group C was assigned to the left side incision to each rat in the study. After irradiation, the tissue was subjected to histopathological analysis to determine the extent of mononuclear cell infiltration, edoema, and epithelialization. Additionally, immunohistochemical analysis was performed to evaluate the expression of proliferating cell nuclear antigen (pCNA) and inducible nitric oxide synthase (iNOS). Group L_1 and L_3 significantly decreased mononuclear cell infiltration compared with Group C in all treatment groups ($p < 0.05$). Group L_3 significantly decreased edoema compared with Group C in all groups except for treatment Group I ($p < 0.05$). Group L_2 and L_3 significantly increased epithelization in treatment Group IV ($p < 0.05$). Moreover, Group L_2 and L_3 significantly increased pCNA in all groups, while L_2 and L_3 significantly decreased iNOS expression in treatment Group II, III, and IV ($p < 0.05$). However, no statistical difference was found between subgroups of treatment Group I in iNOS expiration ($p > 0.05$). The results of the current examination demonstrated that LLLT can modulate mononuclear cell infiltration and edoema, and improve epithelization, as well as increase pCNA expression, whereas decrease iNOS expression during the wound healing process, therefore enhancing wound healing following ovariohysterectomy in rats.

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Low-level laser therapy; ovariohysterectomy; photobiomodulation; pCNA; iNOS

1. Introduction

The skin also called the outer layer of living tissue, is responsible for protecting the inner body from the external environment and maintaining homeostasis as a preventive barrier. Thus, the wound healing process must occur successfully and rapidly for the body to remain healthy. The wound healing process occasionally may not produce the desired outcomes due to excessive inflammation, severe injuries, and infection [1]. For these reasons, several therapy methods are used to enhance the wound healing process and quality of healing tissue [2,3].

The incisional wound healing model in rats is a commonly employed experimental model for investigating the different phases of wound healing and assessing the effectiveness of potential interventions for wound healing. The proposed methodology entails inducing a regulated wounds on the dermis of a rat specimen, followed by the systematic observation of the ensuing recuperative indeed. The utilisation of the incisional wound healing model in rats offers a regulated and consistent platform for investigating different aspects of wound healing, including cellular reactions, extracellular matrix reorganisation, and the impacts of interventions [4–6]. Additionaly midline incision with abdominal approach commonly used for ovariohyterectomy [7–10].

Acceleration of the wound healing is a critical part of patients in returning to a normal life style earlier following surgery. Wound healing is a complex process that includes the induction of an acute inflammatory process, migration and proliferation of parenchymal and connective tissue cells, synthesis of extracellular matrix proteins, remodelling of

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connective tissue and parenchymal components, and finally colagenization and wound strength acquisition [11,12]. Following the third day, granulation tissue begins to invade the incision area and collagen fibres appear [13]. After day 5, neovascularization has reached its peak, and fibroblasts are proliferating. Collagen collection and fibroblast growth persist during the second week [14].

Low-level laser therapy (LLLT) or defined as Low-intensity laser therapy, is an increasingly popular therapy modality, used for the treatment of various diseases, including fracture healing [15,16], tendinitis [17], rheumatoid arthritis [18], and wound healing [9]. Although the action mechanism of LLLT is unclear, it reduces edoema and inflammation, and increases wound epithelization, granulation tissue, fibroblastic proliferation, extracellular matrix synthesis, neovascularization, tissue oxygenation, and nutrition [17–19].

Previous studies conducted on rodents have indicated the advantageous effect of LLLT on biosynthesis and collagen metabolism in experimental models of dermal skin injury [20,21]. Nevertheless, a number of other conflicting information regarding LLLT's impact on wound healing has been documented in the literature, including a decrease in collagen synthesis [22,23]. This discrepancy of result could be due to different energy density and intensity, irradiation time, and irritation modes [20,24]. Furthermore, a number of studies on various cell types have also demonstrated that the beneficial effect of LLLT depends on light parameters including wavelength (nm), energy dose (J/cm^2), and intensity (W/cm^2) [16,24,25].

Previously studies have demostrated that application of LLLT at different doses has the potential to influence the recuperative process of burn injuries and bone fractures in rats [26, 27]. For this reason, the aim of the current study was to investigate the effect of the different treatment protocols and dosage of LLLT on the incisional wound healing model following ovariohysterectomy in rats. We hypothesised that LLLT will produce positive results in wound healing following ovariohysterectomy in a dose-dependent manner.

2. Materials and methods

2.1. Animals

Atatürk University Local Board of Ethics Committee for Animal Experiments, which depend on the guidelines proposed by the recommendations of National Institutes of Health Guide for Care and Use of Laboratory Animals, approved the current study (Decision no: 2019/110).

A total of seventy-two female Wistar rats (120 days of age and average weight of 300 g) were randomly obtained from the Medical Experimental Application and Research Centre of Atatürk University. The animals were housed six per cage prior to the beginning of the study and were acclimated for ten days in the experimental room. The humidity ranged from 40 to 60%. A uniform temperature of 22 ± 2 °C was maintained during a 12-h light/dark cycle. The rats were allowed access to water and a standard pelleted diet *ad libitum* during the study.

2.2. Surgery

Animals were anaesthetised with intramuscular 11.4 mg/kg xylazine (Xylazin Bio 2%, 50 ml Bioveta) and 114 mg/kg ketamine (Ketasol 10%, 10 ml Interhas-Richter Pharma, Wels, Austria) with mixed in the same syringe. Each animal was placed with dorsoventral recumbency, and the skin was shaved and washed with 70% ethanol and povidone iodine, respectively. Ovariohysterectomy was performed using two midline abdominal incisions (2 cm in length) in the left and right sides of line alba. The ovarian ligament and cervix were ligated with 4-0 silk (Dogsan, Turkey), using a single-clamp method. The abdominal wall was closed with four interrupted sutures using 2.0 vicryl suture (Ethicon, Johnson & Johnsonand) then, four interrupted sutures were placed in the skin using 3.0 nylon for each side. The left-side incision was used for the control groups (Group C), while the right-side incision was used for the treatment groups.

2.3. Groups

Animals were randomly assigned to one of four groups ($n = 18$, Group I; sacrification 4 h following only one LLLT application, Group II; sacrification 7 days following only one LLLT application, Group III; sacrification 4 h after two LLLT applications, and Group IV; sacrification 7 days after two LLLT application). These groups were categorised into 4 subgroups consisting of 6 rats (Group C, L_1, L_3, and L_6, Figure 1). The end of the treatment protocols, rats were sacrificed with a lethal dose of an anaesthetic substance (Thiopental Sodyum, Pental Sodyum 1 g, I.E Turkey). Additionaly, Each group was treated with off-mode LLLT on the left side of the abdominal incision to avoid a placebo effect.

2.4. Laser irradiation

A mono-diode laser probe (MLA 1/25) and a GaAs (gallium arsenide) laser device (Lasermed 2200, Eme Phsio Italy) with a mean output power of 17 mW; spot size 1 cm^2; continuous mode; and wavelength (λ) of 904 nm were used with an exposure time of 40, 120, and 240 s in L_1, L_3, and L_6 groups, respectively. LLLT was applied immediately after the surgery and was performed transcutaneously upon a single point directly to the incision side. LLLT application was conducted by the same person and at the same time of day (10:00–11:00 am).

2.5. Histopathologic evaluation

Following the rats sacrificed, the skin wound was removed and fixed with a 10% neutral formalin solution. The tissues were taken into paraffin blocks after routine alcohol-xylol follow-up procedures. 3-µm thickness sections taken on slides with polylysine were stained with haematoxylin-eosin staining. The tissues were evaluated by light microscopy (Olympus BX 51, JAPAN) in terms of mononuclear cell infiltration, edoema, and epithelialization as absent (0), mild [1], moderate [2], severe [3], according to their histopathological property [28].

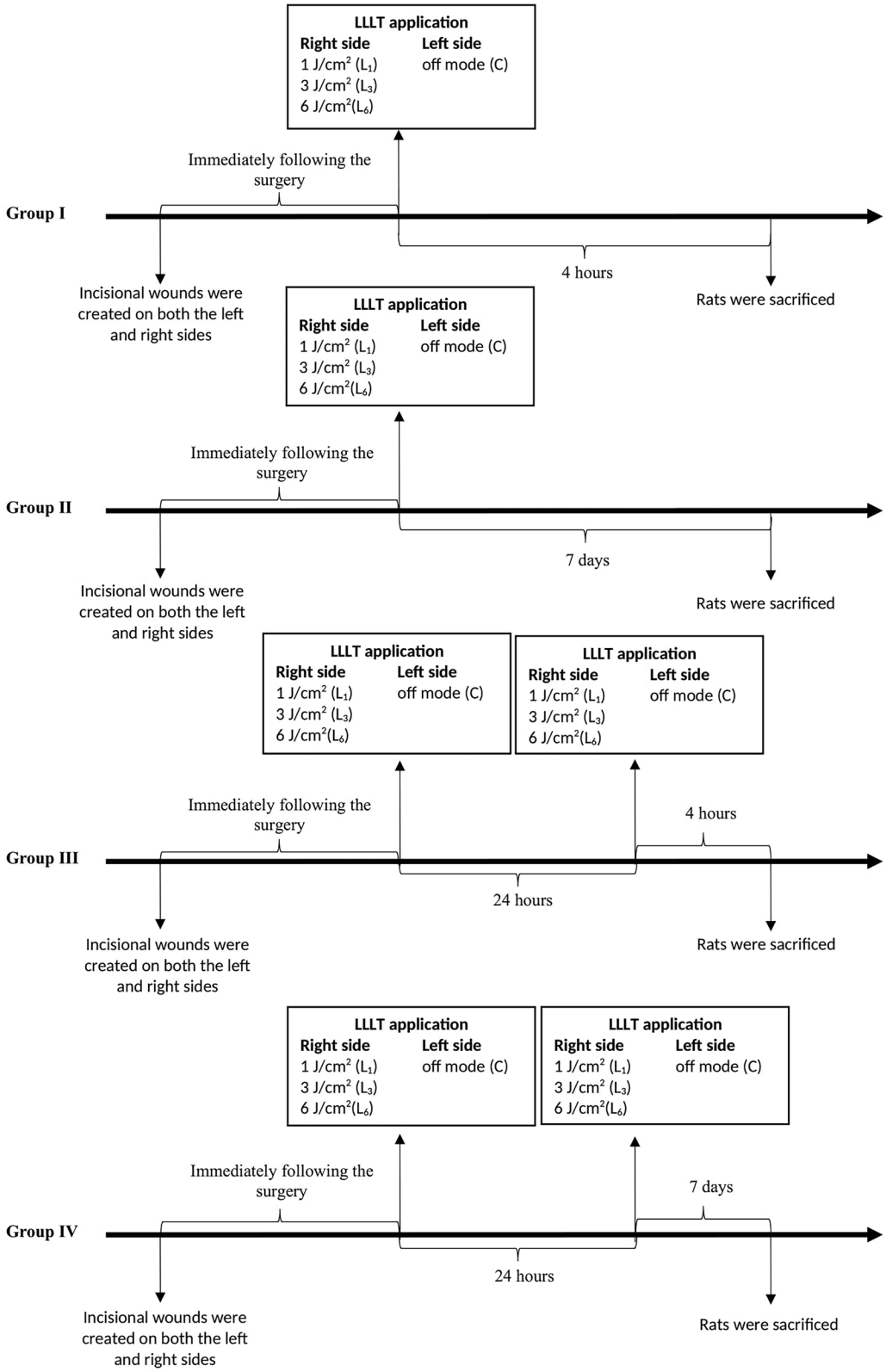

Figure 1. Timeline overview of the experimental study. C; (Group Control; LLLT application with off mode in the left side of incisional wound), L_1; (LLLT application with 1 J/cm^2 dosage in right side of incisional wound), L_3; (LLLT application with 3 J/cm^2 dosage in right side of incisional wound), and L_6; (LLLT application with 6 J/cm^2 dosage in right side of incisional wound).

2.6. Immunohistochemical evaluation

Following the rats' sacrificed, the skin wound was removed and fixed with a 10% neutral formalin solution. The tissues were taken into paraffin blocks after routine alcohol-xylol follow-up procedures. 3-µm thickness sections taken on poly-lysine slides were passed through xylol and alcohol series, after washing with phosphate-buffered saline (PBS) for 10 min in 3% H_2O_2, and endogenous peroxidase inactivation was achieved. To reveal the antigen in the tissue, they were

treated with antigen retrieval solution for 2×5 min at 500 watts. The tissues washed PBS after protein blocking were incubated with inducible nitric oxide synthase (iNOS, Abcam, Cat. No: ab15323 1/200 dilution ratio) and proliferating cell nuclear antigen (pCNA, Abcam, Cat No: ab92552, 1/200 dilution ratio) primary antibodies at room temperature for 20 min. Seconder antibodies; anti-Polyvalnet, HRP (Thermofischer, Cat No: TP-125-HL) were used as recommended by the manufacturer and 3,3′-Diaminobenzidine (DAB) was used as chromogens in tissues. Following counterstaining with Mayes's Haematoxylin, it was covered with Entellan and examined under a light microscope. The examination was performed as no immunopositivity in skin tissue (–), mild (+), moderate (++), and severe (+++). The stained sections were examined using a light microscope (Zeiss, Axio, Germany) [28].

2.7. Statistical analysis

Statistical analysis was used by the SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Prior to analysis, the data distribution was checked using the Shapiro–Wilk test. The Kruskal-Wallis test was used to compare each group in non-normally distributed data, followed by the Mann-Whitney U test as a *post hoc* test. Descriptive data were addressed as mean ± standard deviation (SD). The significant level was set to 0.05 for all tests.

3. Results

There was a significant difference between the treatment and LLLT groups in mononuclear cell infiltration, edoema, and epithelization ($p < 0.05$, Table 1). In the Group I skin tissues, severe mononuclear cell infiltration was found in Group C (2.83 ± 0.40). Moderate mononuclear cell infiltration was identified in Group L_1 (2.16 ± 0.32) and L_3 (2.12 ± 0.14), whereas mild mononuclear cell infiltration was observed in Group L_6 (1.16 ± 0.40), (Figure 2(a)).

In the Group II skin tissue, severe mononuclear cell infiltration and moderate edoema were found in Group C (2.83 ± 0.40). Moderate mononuclear cell infiltration and edoema were observed in Group L_1 (2.10 ± 0.22). Moderate mononuclear cell infiltration and severe edoema were detected in Group L_3 (2.13 ± 0.12 and 2.83 ± 0.22, respectively). Mild mononuclear cell infiltration and edoema were observed in Group L_6 (2.16 ± 0.40 and 1.16 ± 0.38, respectively). Moderate epithelization was shown in all groups (Figure 2(b)).

In Group III C and L_1 skin tissue, severe mononuclear cell infiltration (2.83 ± 0.40 and 2.70 ± 0.14, respectively) and edoema (2.80 ± 0.13 and 2.73 ± 0.22, respectively), and mild epithelization (1.31 ± 0.51 and 1.39 ± 0.72, respectively) were observed. Moderate Mononuclear cell infiltration (2.12 ± 0.16) and mild edoema (1.26 ± 0.34), and epithelization (1.16 ± 0.40) were found in Group L_3, whereas mild mononuclear cell infiltration (2.16 ± 0.40), edoema (1.16 ± 0.21) and epithelization (1.10 ± 0.34) were observed in Group L_6 (Figure 2(c)).

In Group IV C and L_1 skin tissue, moderate mononuclear cell infiltration (2.84 ± 0.18 and 2.83 ± 0.30, respectively), edoema (2.22 ± 0.23 and 2.19 ± 0.52, respectively) and epithelization (2.56 ± 0.51 and 2.39 ± 0.62, respectively) were detected. Severe epithelization (2.88 ± 0.22) and moderate mononuclear cell infiltration (2.11 ± 0.31) and edoema (2.16 ± 0.22) were observed in

Table 1. Comparison of mononuclear cell infiltration, edoema, and epithelization levels in all treatment groups.

Groups	Mononuclear cell infiltration	Edoema	Epithelization
Group I			
C	2.83 ± 0.40[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
L_1	2.16 ± 0.32[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
L_3	2.12 ± 0.14[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
L_6	1.16 ± 0.40[c]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Group II			
C	2.83 ± 0.40[a]	2.10 ± 0.33[a]	1.83 ± 0.34[a]
L_1	2.10 ± 0.22[b]	2.16 ± 0.21[a]	1.90 ± 0.18[a]
L_3	2.13 ± 0.12[b]	2.83 ± 0.22[b]	2.01 ± 0.30[a]
L_6	2.16 ± 0.40[b]	1.16 ± 0.38[c]	2.16 ± 0.40[a]
Group III			
C	2.83 ± 0.40[a]	2.80 ± 0.13[a]	1.31 ± 0.51[a]
L_1	2.70 ± 0.14[a]	2.73 ± 0.22[a]	1.39 ± 0.72[a]
L_3	2.12 ± 0.16[b]	1.26 ± 0.34[b]	1.16 ± 0.40[a]
L_6	2.16 ± 0.40[b]	1.16 ± 0.21[b]	1.10 ± 0.34[a]
Group IV			
C	2.84 ± 0.18[a]	2.22 ± 0.23[a]	2.56 ± 0.51[a]
L_1	2.83 ± 0.30[a]	2.19 ± 0.52[a]	2.39 ± 0.62[a]
L_3	2.11 ± 0.31[b]	2.16 ± 0.22[a]	2.88 ± 0.22[b]
L_6	2.16 ± 0.40[b]	1.16 ± 0.38[b]	2.83 ± 0.44[b]

The different letters in the same column indicate significant difference ($p < 0.05$). Data were expressed as mean ± SD. Group I (LLLT application once and sacrification following 4h); II (LLLT application once and sacrification following 7 days); III (LLLT application twice with 24 intervals and sacrification 4h following the last application), and IV (LLLT application twice with 24 intervals and sacrification 7 days following the last application). C; (Group Control; LLLT application with off mode in left side of incisional wound), L_1; (LLLT application with 1 J/cm^2 dosage in right side of incisional wound), L_3; (LLLT application with 3 J/cm^2 dosage in right side of incisional wound), and L_6; (LLLT application with 6 J/cm^2 dosage in right side of incisional wound).

Table 2. Comparison of proliferating cell nuclear antigen (pCNA) and inducible nitric oxide synthase (iNOS) expression levels in all treatment groups.

Groups	pCNA	iNOS
Group I		
C	1.33 ± 0.51[a]	2.93 ± 0.32[a]
L_1	1.16 ± 0.41[a]	2.85 ± 0.14[a]
L_3	2.16 ± 0.33[b]	2.60 ± 0.51[a]
L_6	2.83 ± 0.23[c]	2.49 ± 0.34[a]
Group II		
C	0.16 ± 0.02[a]	2.83 ± 0.34[a]
L_1	1.17 ± 0.18[b]	2.66 ± 0.49[a]
L_3	2.13 ± 0.34[c]	1.33 ± 0.55[b]
L_6	2.83 ± 0.28[d]	1.16 ± 0.14[b]
Group III		
C	1.33 ± 0.11[a]	2.16 ± 0.22[a]
L_1	1.16 ± 0.44[a]	2.80 ± 0.44[b]
L_3	2.16 ± 0.29[b]	2.78 ± 0.82[b]
L_6	2.83 ± 0.38[c]	1.15 ± 0.42[c]
Group IV		
C	1.19 ± 0.14[a]	2.81 ± 0.42[a]
L_1	2.22 ± 0.18[b]	2.66 ± 0.55[a]
L_3	2.14 ± 0.44[b]	1.33 ± 0.31[b]
L_6	2.80 ± 0.30[c]	1.11 ± 0.12[b]

The different letters in the same column indicate significant differences ($p < 0.05$). Data were expressed as mean ± SD. Group I (LLLT application once and sacrification following 4h); II (LLLT application once and sacrification following 7 days); III (LLLT application twice with 24 intervals and sacrification 4h following the last application), and IV (LLLT application twice with 24 intervals and sacrification 7 days following the last application). C; (Group Control; LLLT application with off mode in left side of incisional wound), L_1 (LLLT application with 1 J/cm^2 dosage in the right side of incisional wound), L_3 (LLLT application with 3 J/cm^2 dosage in the right side of the incisional wound), and L_6 (LLLT application with 6 J/cm^2 dosage in right side of incisional wound).

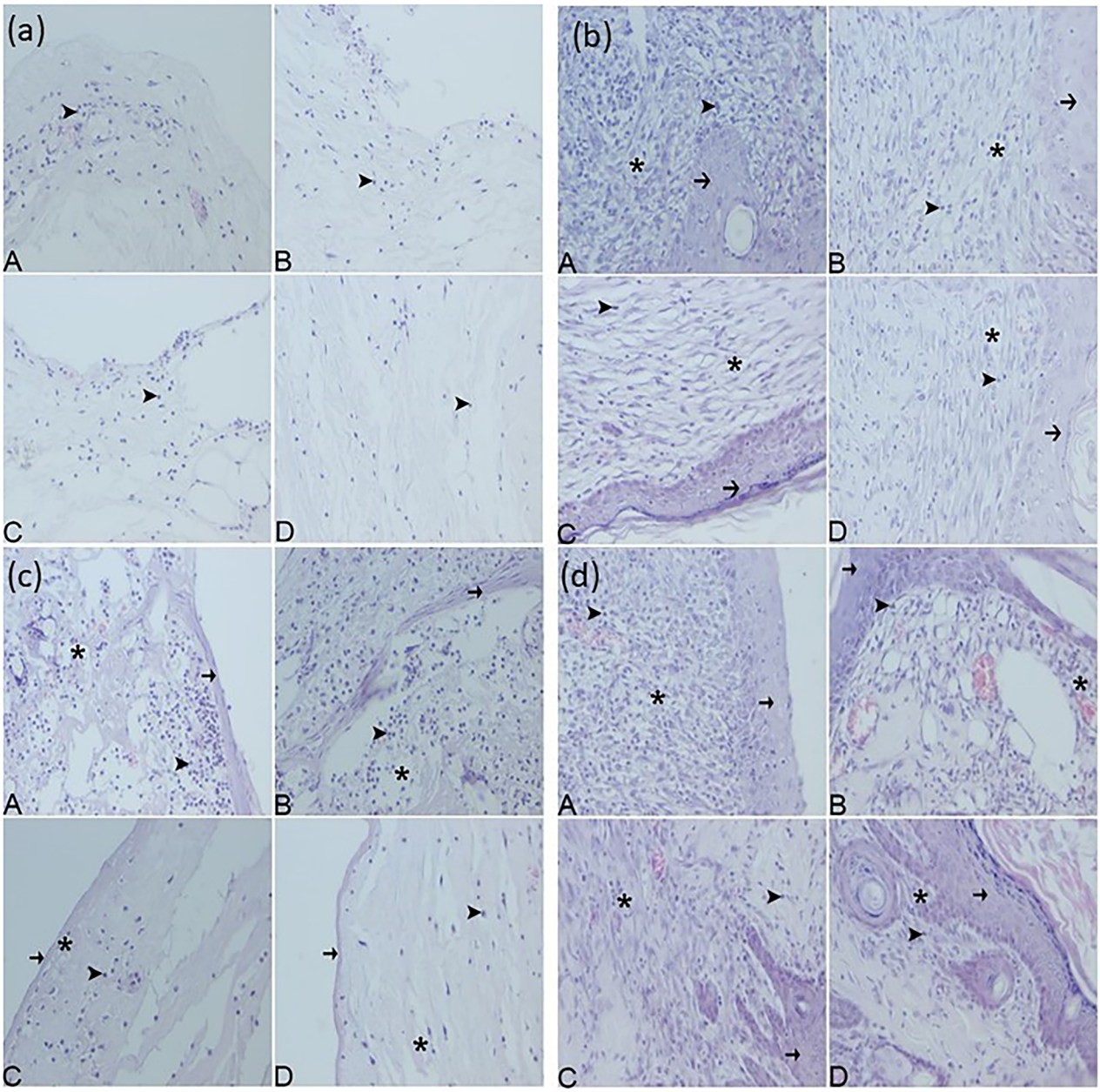

Figure 2. Mononuclear cell infiltration, edoema, and epithelization indicate as arrowhead, asterisks, and arrow, respectively. (a) Group I, (A) severe mononuclear cell infiltration in Group C, (B-C) moderate mononuclear cell infiltration in Group L_1 and L_3, (D) mild mononuclear cell infiltration in Group L_6; (b) Group II, (A) severe mononuclear cell infiltration, moderate edoema and mild epithelization in Group C, (B) moderate mononuclear cell infiltration, edoema and mild epithelization in Group L_1, (C) moderate mononuclear cell infiltration, severe edoema and mild epithelization in Group L_3, (D) moderate mononuclear cell infiltration (arrowhead), mild edoema and mild epithelization in Group L_6; (c) Group III, (A-B) mild epithelization, severe mononuclear cell infiltration, and edoema in Group C and L_1, (C) moderate mononuclear cell infiltration, mild epithelization and edoema in Group L_3, (D) mild mononuclear cell infiltration, edoema, edoema in Group L_6; (d) Group IV, (A) modarete edoema, severe mononuclear cell infiltration and epithelization in Group C, (B) modarete edoema, severe mononuclear cell infiltration and epithelizayion in Group L_1, (C) severe epithelization, moderate mononuclear cell infiltration and edoema in Group L_3, (D) modarete mononuclear cell infiltration, mild edoema, and severe epithelization in Group L_6. Skin-HE 40x;.

Group L_3, whereas severe epithelization (2.83 ± 0.44), moderate mononuclear cell infiltration (2.16 ± 0.40), and mild edoema (1.16 ± 0.38) were found in Group L_6 (Figure 2(d)).

There was a significant difference between the groups in iNOS and pCNA immunopositivity, edoema, and epithelization ($p < 0.05$, Table 2). In Group I skin tissue, mild pCNA expression was observed in Group C (1.33 ± 0.51) and L_1 (1.16 ± 0.41), while moderate and severe pCNA expression was in L_3 (2.16 ± 0.33) and L_6 (2.83 ± 0.23), respectively. Additionally, severe iNOS expression was found in all groups (Figure 3(a and b)).

In Group II skin tissue, there was no observed pCNA expression in Group C. Mild pCNA expression was found in Group L_1 (1.17 ± 0.18), while moderate and severe pCNA expression was in Group L_3 (2.13 ± 0.34) and L_6 (2.83 ± 0.38), respectively. Severe iNOS expression was observed in Group C (2.83 ± 0.34) and L_1 (2.66 ± 0.49), while moderate iNOS expression in Group L_3 (1.33 ± 0.55) and L_6 (1.16 ± 0.14), (Figure 4(a and b)).

In Group III skin tissue, mild pCNA expression was noted in Group C (1.33 ± 0.11) and L_1 (1.16 ± 0.44), whereas moderate

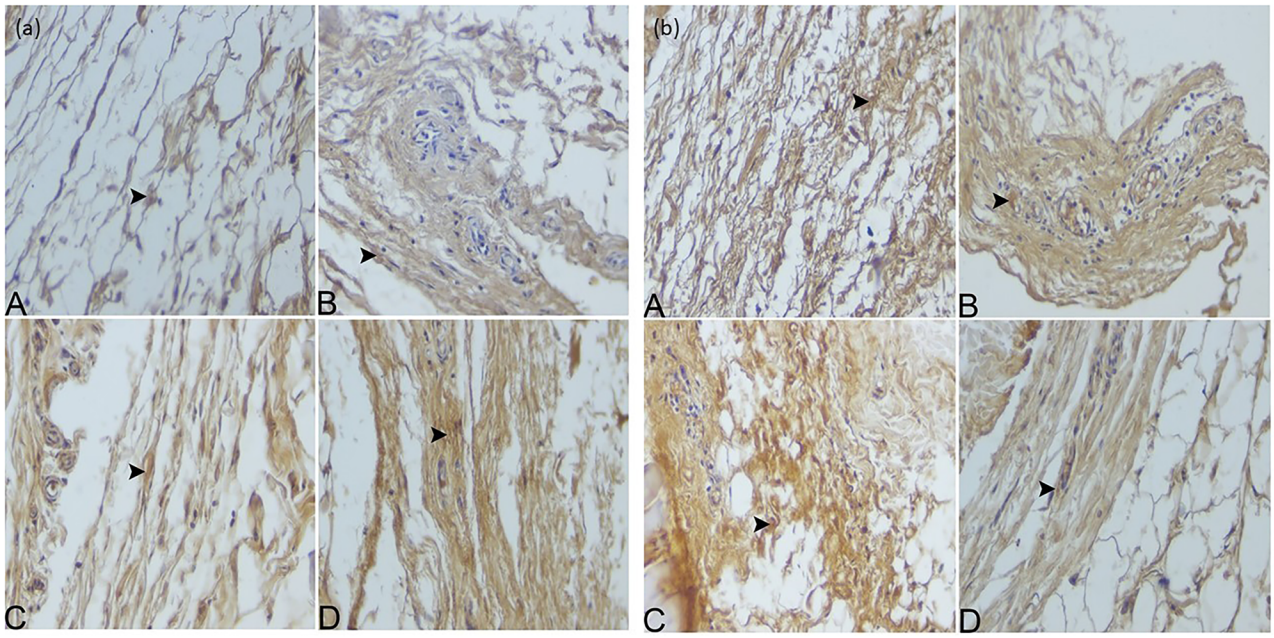

Figure 3. (a) Grup I, (A-B) mild pCNA expression (arrowhead) in Group C and L_1, (C) moderate pCNA expression in L_3, (D) severe pCNA expression in Group L_6; (b) Grup I (A-B-C-D) severe iNOS expression in all group (C, L_1, L_3, and L_6). Skin-IHC.

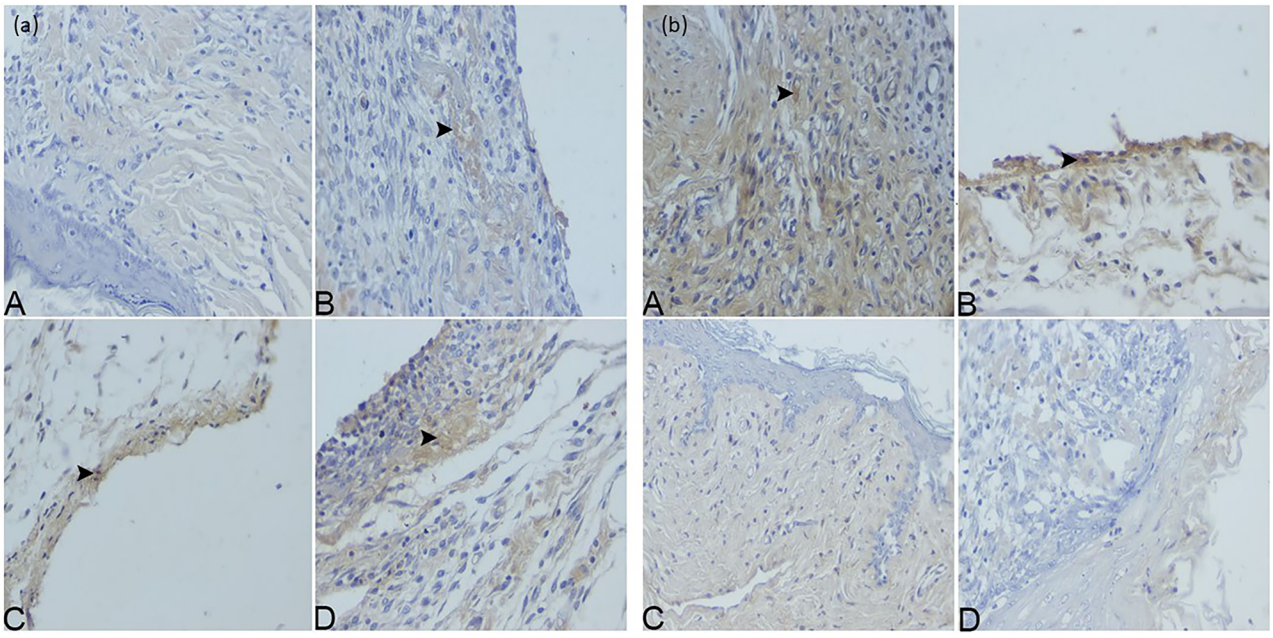

Figure 4. (a) Group II, (A) pCNA immunonegative in Group C, (B) mild pCNA expression (arrowhead) in Group L_1, (C) moderate pCNA expression in Group L_3, (D) severe pCNA expression in Group L_6; (b) Group II (A-B) severe iNOS expression (arrowhead) in Group C and L_1, (C-D) mild iNOS expression in Group L_3 and L_6. Skin-IHC.

and severe pCNA expression was detected in Group L_3 (2.16 ± 0.29) and L_6 (2.83 ± 0.38), respectively. Moderate iNOS expression was observed in Group C (2.16 ± 0.22). Severe iNOS expression was noted in Group L_1 (2.80 ± 0.44) and L_3 (2.78 ± 0.82), whereas mild iNOS expression in Group L_6 (1.15 ± 0.42), (Figure 5(a and b))

In Group IV skin tissue, mild pCNA expression was found in Group C (1.19 ± 0.14). Moderate pCNA expression was noted in Group L_1 (2.22 ± 0.18) and L_3 (2.14 ± 0.44), whereas severe pCNA was in Group L_6 (2.80 ± 0.30). Severe iNOS expression was noted in Group C (2.81 ± 0.42) and L_1 (2.66 ± 0.55), whereas mild iNOS expression was in Group L_3 (1.33 ± 0.31) and L_6 (1.11 ± 0.12), (Figure 6(a and b)).

4. Discussion

The experimental research demonstrated that dosage dependence of LLLT reduced mononuclear cell infiltration and edoema and increased epithelization in incision wound

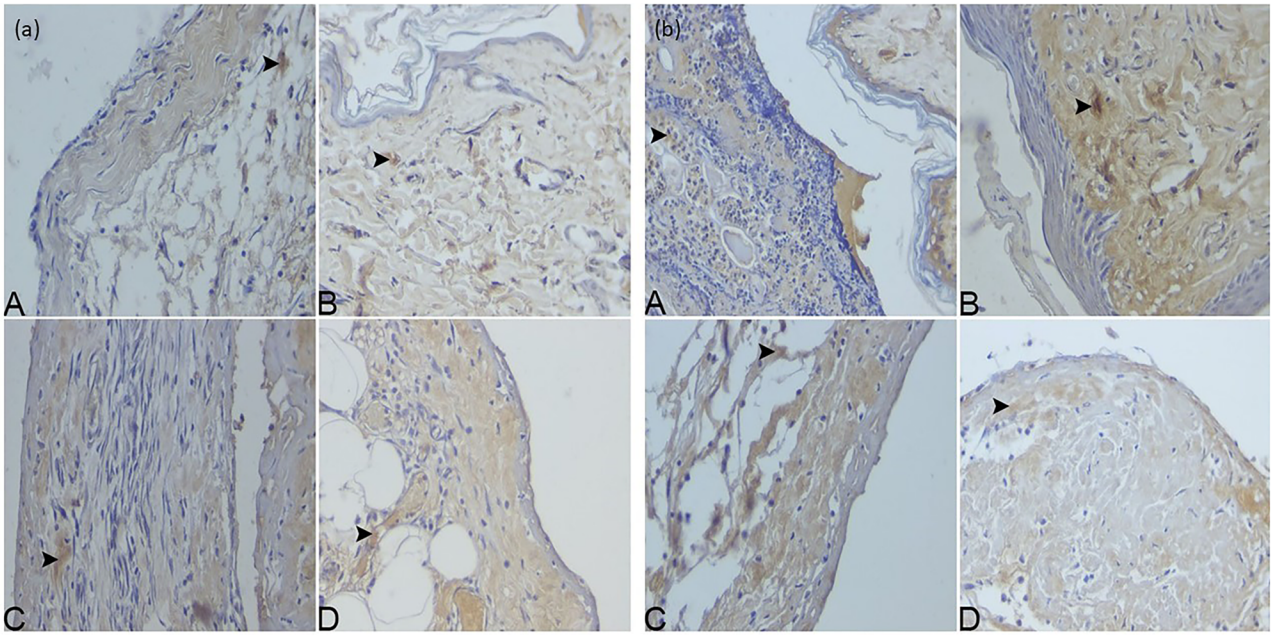

Figure 5. (a) Group III, (A-B) mild pCNA expression (arrowhead) in Group C and L_1, (C) moderate pCNA expression in Group L_3, (D) severe pCNA expression in Group L_6; (b) Group III, (A) moderate iNOS expression (arrowhead) in Group C, (B-C) severe iNOS expression in Group L_1 and L_3, (D) mild iNOS expression in Group L_6. Skin-IHC.

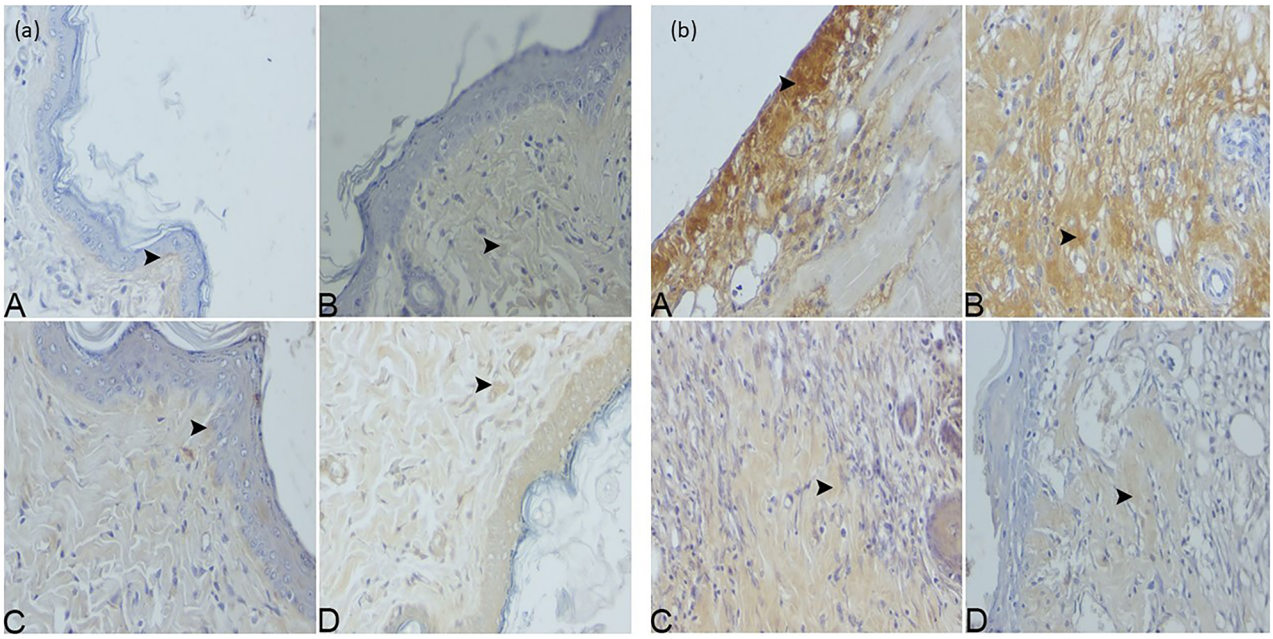

Figure 6. (a) Group IV, (A) mild pCNA expression in Group C, (B-C) moderate pCNA expression in Group L_1 and L_3, (D) severe pCNA expression in Group L_6; (b) Group IV, (A-B) severe iNOS expression (arrowhead) in Grup C and L_1, (C-D) mild iNOS expression in Group L_3 and L_6. Skin-IHC.

healing following ovariohysterectomy in rats. Additionally, dosage dependence of LLLT increased pCNA expression and reduced iNOS expression. Although all LLLT treatment groups were effective in wound healing, the most effective results were obtained in the L_6 (6 J/cm^2) groups in all groups. In physical medicine, a laser with a wavelength of 600–984 nm is typically employed, with 632.8 nm for a He-Ne laser and 904 nm for a Ga-As laser being the most common possibilities for wound healing. In the current study, we used a Ga-As laser with a 904 nm wavelength since red light is optimum for wound healing and Ga-As needs a lower dose owing to its strong pulse power and high penetration [29].

Wound healing is a complicated, active, and organised biological process. During wound healing, three stages have been identified: inflammation, proliferation, and maturation. Within the initial phase, inflammatory cells and keratinocytes release cytokines and growth factors that promote fibroblast development and chemotaxis. An increased intensity or

duration of this phase can cause healing to be delayed and angiogenesis and neocollagenesis to be impaired, ultimately leading to amputation procedures [12,30]. In this study, LLLT-treated groups decreased mononuclear cell infiltration. Similarly, a previous study reported that LLLT can help accelerate wound healing, possibly because acute inflammation is reduced quickly and the proliferation phase of healing occurs earlier [31]. Thus, the LLLT reduced the inflammatory response of wound healing. Furthermore, the LLLT reduces inflammatory cell infiltration, enhances wound healing processes by increasing collagen production, and provides rapid closure of the wound tissue [32,33]. A study conducted on rats demonstrated that LLLT irradiation (4 J/cm^2) reduced inflammatory cells in the wound area, which is in agreement with this study [34].

LLLT has been shown to regulate the inflammatory process as well as the immune reaction [35]. This treatment has the potential to normalise or decrease inflammatory reactions such as edoema and inflammatory cell migration [36,37]. The current study noted that Group L_6 (6 J/cm^2) was the most effective group in reducing edoema in all groups. Many studies have shown that LLLT can help reduce edoema and inflammatory signs [38–40]. Unexpectedly, Group II L_3 increased edoema in the current study. This discrepancy may be explained as a result of symptomatic pain reduction caused by LLLT, leading to enhanced activity that causes more edoema [41].

Epithelization is crucial because it restores the integrity of the skin, making it less susceptible to infection [42]. Our findings indicated that Group L_3 (3 J/cm^2) and L_6 (6 J/cm^2) in all groups increased wound healing, as demonstrated by the statistical results of epithelization following 7 days when compared with that in Group C in all groups. A similar result was previously reported in rats, which seem LLLT modulation epithelization in rat with third-degree burn wounds [43]. Accelerated keratinocyte proliferation and migration, as a result of LLLT's local or systemic effects, could be a possible explanation for rapid and better epithelization [44]. Additionally, LLLT enhances the transmembrane electrochemical proton gradient in the mitochondria, which causes an antiport process to release calcium from the mitochondria into the cytoplasm, triggering or stimulating biological mechanisms including RNA and DNA synthesis, cell mitosis, protein secretion, and cell proliferation [45,46]. Interestingly, Group L_3 in all groups did not change epithelization formation compared with Group C in all groups. A possible explanation for this result is that the combination of given energy density or fluency (J/cm^2) and a number of exposures is insufficient for LLLT stimulant effects on epithelization.

The indicator of pCNA is a well-established marker for detecting cellular proliferation during wound healing. pCNA is a nuclear protein that contributes to cell proliferation by modulating DNA polymerase and it is clinically reliable diagnostic for proliferation [46]. A previous study described that rats exposed to LLLT showed a higher number of pCNA-positive cells, indicating enhanced cell proliferation [47]. According to the study by Wu et al. (2010) [48], increased pCNA expression causes cellular proliferation during early tendon healing. This research also indicated that pCNA may be an effective marker for assessing cellular proliferation throughout the development of healing. In another study conducted by Gupta et al. [49], on a laboratory model of partial-thickness cutaneous abrasion, increased pCNA expression led to enhanced proliferation after treatment with 635-nm and 819-nm LLLT, which is in agreement with the current study.

The iNOS are not normally found in cells but rather are released in response to stimuli involving cytokines including interleukin-1, tumour necrosis factor-α, and γ-interferon [50, 51]. Overload of nitric oxide, according to elevated iNOS expression, may contribute to pre-existing oxidative and nitrosative stress by reacting with reactive oxygen species to produce peroxynitrite [52]. A previous study reported that LLLT significantly decreased iNOS expression, as well as improved histological abnormalities and reduced collagen concentration [29]. According to the study by Young et al., wavelengths between 660 nm and 870 nm induce macrophages to produce substances that promote fibroblast growth, but a wavelength of 880 nm suppresses this release [52]. Additionally, Albertini et al., indicated that LLLT that demonstrated suppression of iNOS, and hence a decrease in nitric oxide production, resulted in an extensive decrease in nerve regeneration [53]. However, unexpected results were obtained in our study. This study reported that Group L_6 (6 J/cm^2) decreased iNOS in all groups. Group L_3 (3 J/cm^2) decreased iNOS in Group II and IV, whereas increased in Group III. However, Group L_1 (1 J/cm^2) did not increase iNOS in any group. Although the reason for the increase in iNOS in L_1 and L_3 groups is unknown, it can be explained by an insufficient dosage of LLLT.

The current study had some limitations. It is possible that a more extensive sample size may be required to comprehensively establish the advantages of this therapeutic approach. Quantitative wound healing evaluation is preferred over wound site evaluation. Instead of subjective wound site evaluations, quantitative wound healing assessment approaches are recommended [54]. Unfortunately, the current investigation centred its attention on the histological assessment. In order to ascertain the therapeutic effectiveness of LLLT in forthcoming investigations, it is advisable to undertake a quantitative evaluation of wound healing, encompassing measurements such as the area and distance of epithelial migration from the wound edge. This assessment should be complemented by a histological examination.

Conclusion

The current study findings would propose that although all of the LLLT-treated groups decrease mononuclear cell infiltration and edoema, and promote epithelialization, more effective results are obtained in a dosage of 6 J/cm^2 (L_6). Additionally, LLLT increased pCNA expression and decreased iNOS expression, thereby improving wound healing following ovariohysterectomy in rats.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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