



Effect of low-level laser therapy on healing of burn wounds grafted with decellularised bovine omentum in a rat model: A dose-response analysis[#]

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Abstract

The effect of Gallium–Arsenide laser therapy on burn wounds grafted with decellularised bovine omentum was evaluated in Wistar rats. Sixty rats with experimentally induced third-degree burns and grafted with decellularised bovine omentum were divided into four groups. Group I was treated as control group without laser therapy while Groups II to IV received laser doses of 2, 4 and 6 J/cm², respectively. Laser treated groups showed faster epithelialisation compared to control group, with significant reduction in the wound area, particularly in Groups III and IV. Histopathological findings demonstrated enhanced tissue regeneration in Groups III and IV, indicating superior wound healing quality. Overall, 6 J/cm² laser therapy produced the most advanced and qualitatively superior wound healing among the treatment groups.

Keywords: Wistar rats, burn wound, decellularised bovine omentum, low level laser therapy (LLLT)

Burns arise from exposure to chemical, physical, or biological agents and can cause tissue damage, infection, severe pain, and even death (de Moraes et al., 2013; Jeschke et al., 2020). They are classified on the basis of depth into first, second and third-degree burns (Afifah et al., 2019). Although less common, third-degree burns are the most severe, as they completely destroy the epidermis and dermis, leading to significant physiological stress (Vasheghani et al., 2008). Early excision and grafting are considered as gold standard for the management of burn wounds (Jeschke et al., 2020). Recent advances in burn wound management include skin grafts (Shevchenko et al., 2010), biological dressings (Saska et al., 2011) and collagen-based dressings for wound coverage, alongside adjunctive physical modalities such as low-level laser therapy (LLLT), which has gained increasing interest for its role in promoting healing (Renno et al., 2011; Fiório et al., 2014).

Hussain (2017) and Megha et al. (2022) investigated the decellularisation techniques, ultrastructural characteristics, and host tissue remodelling responses associated with bovine omentum. Decellularised bovine omentum

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has shown promising therapeutic potential, demonstrating enhanced healing of full-thickness burn wounds in rat models (Anjana, 2020; Reji, 2024).

Low-level laser therapy (LLLT) acts through the absorption of light by cellular photoreceptors, particularly cytochrome oxidase, leading to enhanced ATP production, increased cellular proliferation, and stimulation of tissue repair and angiogenesis (Bossini et al., 2009; Renno et al., 2011; Novaes et al., 2014). It has been effectively applied in veterinary medicine for managing chronic ulcerative wounds in small and wild animals (Reji, 2002; Reji et al., 2023).

Optimal outcomes with LLLT are highly dependent on energy density (fluence), which governs the biological response of tissues (Avci et al., 2013). Lower energy densities (around 1–4 J/cm²) are generally associated with stimulation of cellular activity, modulation of inflammation, and early wound healing (Renno et al., 2011; Núñez et al., 2013; Fiório et al., 2014; Novaes et al., 2014), whereas higher energy densities (20 J/cm²) tend to enhance fibroblast proliferation, collagen synthesis, and tissue remodelling (Meireles et al., 2008). However, excessive energy delivery may lead to diminished or inhibitory effects (Kana et al., 1981), reflecting the biphasic dose response of LLLT. Consequently, precise selection of energy density is critical for achieving effective burn wound healing. Hence the study was aimed to evaluate LLLT at three fluence levels in a rat model for the healing of third-degree burns grafted with decellularised bovine omentum.

Materials and methods

Sixty Wistar rats (either sex) were procured from Small Animal Breeding Station, Mannuthy and the



Fig. 1: Burn induction

Table 1. Grouping of Experimental Animals

Group	Treatments
Group I (n=15) (control group)	Rats grafted with decellularised bovine omentum.
Group II (n=15)	Rats grafted with decellularised bovine omentum subjected to laser energy density of 2 J/cm ² .
Group III (n=15)	Rats grafted with decellularised bovine omentum subjected to laser energy density of 4 J/cm ² .
Group IV (n=15)	Rats grafted with decellularised bovine omentum subjected to laser energy density of 6 J/cm ² .

study was conducted in the Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy and the study was approved by Institutional Animal Ethics Committee. Gamma irradiated decellularised bovine omentum was procured from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. The surgical site (thoraco-lumbar area) was prepared aseptically for burn infliction under general anaesthesia which was induced by intraperitoneal cocktail injection of inj. xylazine hydrochloride (5mg/kg) and inj. ketamine hydrochloride (50mg/kg). A 1 cm² burn wound was induced by using a temperature-controlled burn inducing device (Anjana et al., 2022) (Fig. 1). Seventy-two hours following burn induction, the eschar was debrided aseptically under general anaesthesia and grafted with decellularised bovine omentum. The rats were divided randomly into four groups with fifteen animals each. Group I was considered as control group. The animals in Group II, III and IV were subjected to laser irradiation as given in Table 1.

A Gallium Arsenide (GaAs) laser (BMS DIGI LASER-Pro®, India) with a 650 nm red probe (2–100 mW) was used to irradiate burn wounds. The device featured a graphic LCD for controlling treatment parameters, with automatic calculation of power, energy, and energy density (0.01–100 J/cm²). Safety measures were taken during laser irradiation (Fig. 2)

Healing of burn wound was monitored based on parameters such as gross appearance, digital wound planimetry, rectal temperature, haematological analyses and histopathological evaluation.

Results and discussion

The present study evaluated burn wound healing



Fig. 2: Laser application

following grafting with decellularised bovine omentum at the following laser energy densities: 2, 4, and 6 J/cm². Lanzafame & Stadler (2017) suggested that majority of the treatment protocols for LLLT used energy densities between 1 and 10 J/cm² which was supposed to be the therapeutic window for laser therapy.

Gross morphological evaluations were done on days- 3rd, 6th, 9th, 12th, 15th and on complete epithelialisation (Fig. 3). There were distinct variations in healing patterns among the four groups. The control group exhibited a relatively slow and typical secondary intention healing pattern, with prolonged inflammation up to sixth day, delayed contraction and epithelialisation, and complete healing only by 23rd day with noticeable scar formation, consistent with the general wound healing sequence described by Natarajan et al. (2000), Eming et al. (2007) and Gurtner et al. (2008). In Group II (2 J/cm²), earlier reduction in inflammation, improved wound contraction from 9th day, and faster epithelialisation leading to closure by day 19 were observed, aligning with reports by Medrado et al. (2003) and Mester (2013) who used a similar dose for wound healing. There was moderate scar formation in this group. Group III (4 J/cm²) demonstrated a more accelerated healing response, with early modulation of inflammation by third day, rapid progression to the proliferative phase by sixth day, substantial contraction and epithelialisation by days 9th to 12th, and near-complete healing with minimal scarring by around days 15th to 18th, corroborating with the findings of Gupta et al. (2015) and Nekha et al. (2021). The most enhanced healing was observed in Group IV (6 J/cm²), characterised by minimal inflammation, rapid epithelialisation, early scab detachment, and superior cosmetic outcome with minimal scar formation. Corazza et al. (2007) and Bossini et al. (2009) observed that the scar formation was reduced when higher energy densities were used. Also, studies by Karu (1999), Posten et al. (2005), Hawkins & Abrahamse (2006), Peplow et al. (2010) and Novaes et al. (2014), indicated a more pronounced therapeutic effect at higher energy densities.

Muñoz-Torres et al. (2025) scoring system was adopted for gross morphological evaluation which took into consideration of following parameters: granulation

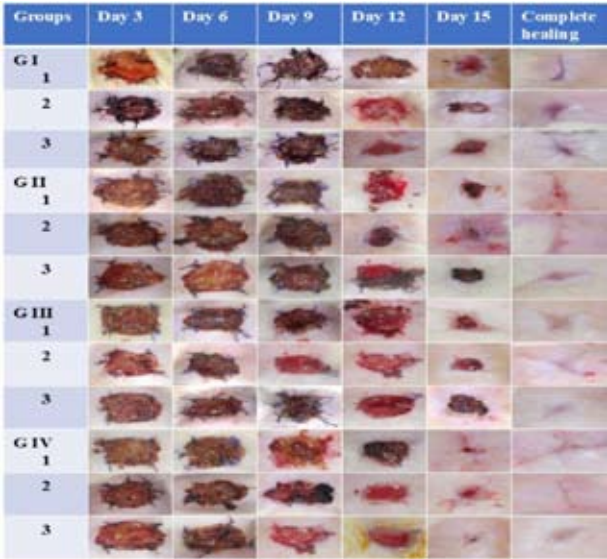


Fig. 3: Gross appearance of wound

tissue formation, redness or oedema, presence of necrotic tissue, wound appearance, quantity and type of exudate and the condition of the perilesional skin and wound edges. The total score ranged from ‘8’ to ‘32’ where the lower scores indicated better clinical improvement as shown in Table 2. Group IV animals showed the lowest scores, followed by Group III, while Groups I and II had comparatively higher values. By day nine, wounds in Groups III and IV had already improved to a score of 10, whereas similar improvement was seen only by day 15th in the control and 2 J/cm² groups, indicating slower healing. Although differences became non-significant by day 15th, earlier recovery in higher-dose groups remained clinically relevant. By day 21st, all laser-treated groups approached near-complete healing, suggesting that higher energy densities accelerated wound healing through enhanced photobiomodulatory effects.

Complete epithelialisation occurred on an average by days 23 (Group I), 19.17 (Group II), 17.83 (Group III), and 17 (Group IV) as shown in Fig. 4, indicating a dose-dependent effect of laser therapy, consistent with de Moraes et al. (2013). The laser treated groups healed faster than the control group. Similar findings were

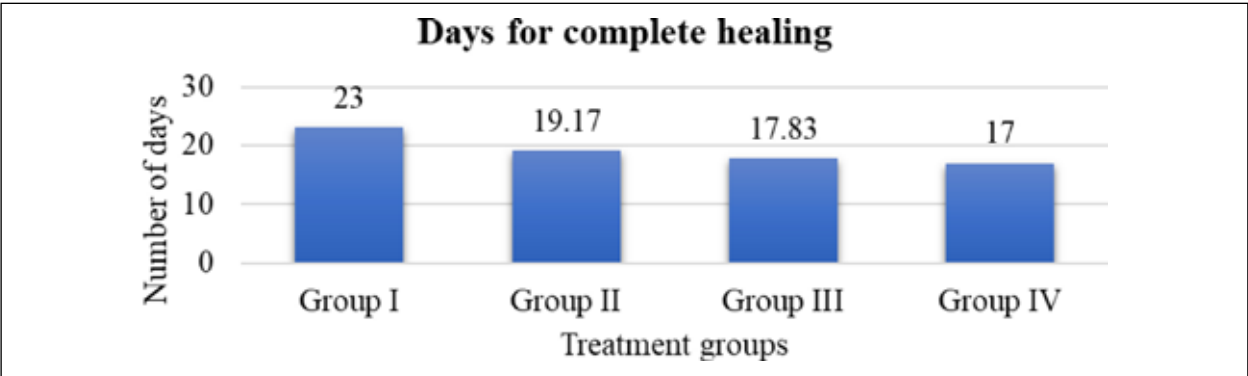


Fig. 4: Average number of days taken for complete healing

Table 2. Gross morphological scoring

Day	Group I	Group II	Group III	Group IV	χ^2 -value (p-value)
Day 3	23.67 ^{aA} $\pm$ 0.49	23.17 ^{aA} $\pm$ 0.48	18.83 ^{bA} $\pm$ 0.48	15.17 ^{bA} $\pm$ 0.70	19.42** (<0.001)
Day 6	22.50 ^{aA} $\pm$ 0.43	21.67 ^{aA} $\pm$ 0.49	15.00 ^{bA} $\pm$ 0.58	11.50 ^{bAB} $\pm$ 0.43	19.84** (<0.001)
Day 9	19.50 ^{aAB} $\pm$ 0.43	19.50 ^{aAB} $\pm$ 0.56	11.67 ^{bAB} $\pm$ 0.76	8.83 ^{bBC} $\pm$ 0.31	19.11** (<0.001)
Day 12	16.00 ^{aABC} $\pm$ 0.58	17.00 ^{aABC} $\pm$ 0.58	9.33 ^{bBC} $\pm$ 0.49	8.17 ^{bC} $\pm$ 0.17	18.90** (<0.001)
Day 15	13.67 ^{aBCD} $\pm$ 0.42	14.17 ^{aBCD} $\pm$ 0.6	8.33 ^{bBC} $\pm$ 0.21	8.17 ^{bC} $\pm$ 0.17	18.61** (<0.001)
Day 21	9.67 ^{aCD} $\pm$ 0.56	8.50 ^{abCD} $\pm$ 0.5	8.00 ^{bC} $\pm$ 0	8.00 ^{bC} $\pm$ 0	13.32** (<0.001)
Day Final	8.00 ^D $\pm$ 0	8.00 ^D $\pm$ 0	8.00 ^C $\pm$ 0	8.00 ^C $\pm$ 0	0.000 ^{ns} (1.000)
F-value (p-value)	35.82** (<0.001)	35.82** (<0.001)	34.59** (<0.001)	32.90** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

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Means having different capital letter as superscript differ significantly within a column

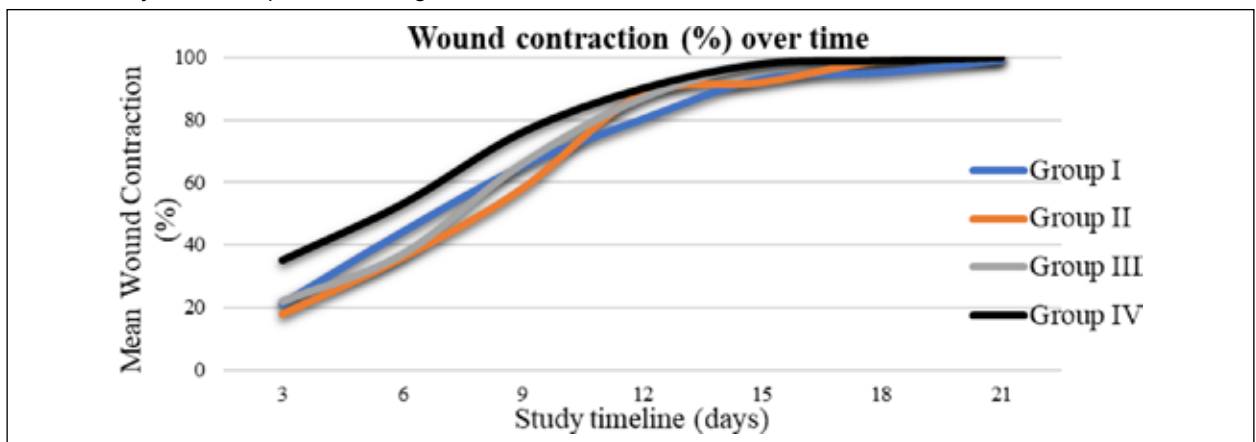
observed by Reji (2002) in the healing of chronic wounds in dogs which were treated with He-Ne laser.

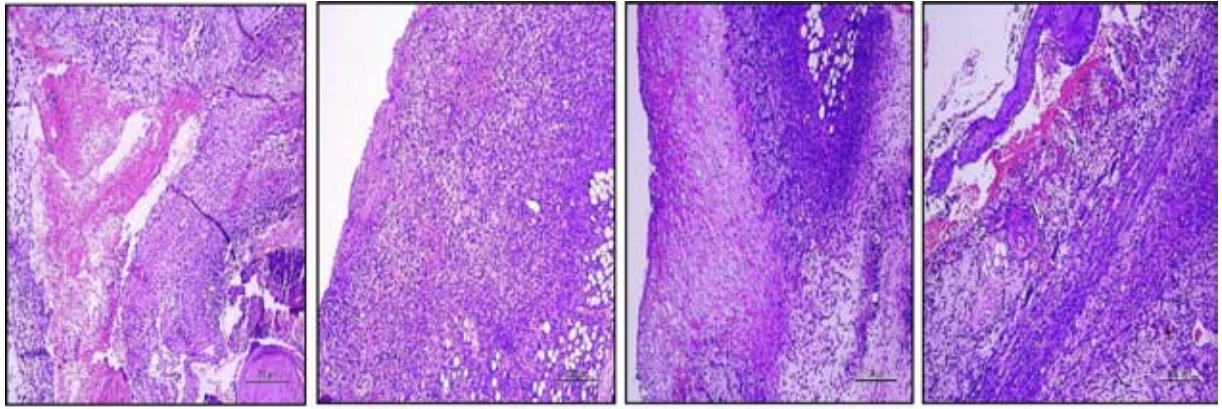
Wound planimetry was done by using smartphone application (imitoMeasure) as described by Giggin et al. (2024). Wound contraction was calculated using the formula $(A_0 - A_t)/A_0 \times 100$ (Ahamed & Sastry, 2011), with peak contraction observed by day 15, likely due to fibroblast differentiation into myofibroblasts (Reji, 2024). Consistent with Gonçalves et al. (2010) and Nekha et al. (2021), LLLT promoted progressive reduction in wound size. In the present study, maximum contraction was observed in Group IV by day 12, followed by Groups III and II, while Group I showed slower healing (Fig. 5). These findings aligned with Melo et al. (2011) and Yang et al. (2022), who reported accelerated wound closure and epithelialisation with LLLT when compared with the control group.

Rectal temperature remained within normal physiological limits throughout the study, indicating no systemic thermoregulatory disturbance following burn induction (Sharma et al., 2022). This was consistent with Reji (2024), who reported only a transient rise on day zero and Caldwell et al. (1989), who observed no febrile response even in larger TBSA burns. The minimal burn area (<1% TBSA) in the present study likely explains the absence of systemic temperature changes.

On haematology total erythrocyte and leukocyte counts exhibited only minor, non-significant fluctuations, reflecting a mild inflammatory response rather than severe systemic involvement, in contrast to reports of anaemia or leukocytosis in extensive burns (Jeschke et al., 2020; Reji, 2024). Lymphocytosis was observed throughout the study, suggested an active immune response, whereas a transient monocytosis on third day indicated early inflammatory activation and macrophage recruitment, consistent with Anjana (2020) and Jeschke et al. (2020). Granulocyte counts remained comparatively lower, with a notable reduction in laser-treated groups by ninth day, possibly due to the anti-inflammatory effects of photobiomodulation (Amini et al., 2023). Haemoglobin levels showed a mild decrease following burn induction, likely due to blood loss and haemolysis, as reported by Khalil et al. (2018) and Sen et al. (2019), while platelet counts remained elevated but stable, indicating maintained haemostatic function.

Histopathological evaluation showed a progressive improvement in wound healing from Group I to IV as shown in Fig. 6 to 10, with laser-treated groups demonstrating enhanced tissue repair. The control group exhibited prolonged inflammation, delayed epithelialisation, and incomplete adnexal regeneration, consistent with Guo & DiPietro (2010). Group II (2 J/

**Fig. 5:** Rate of wound contraction

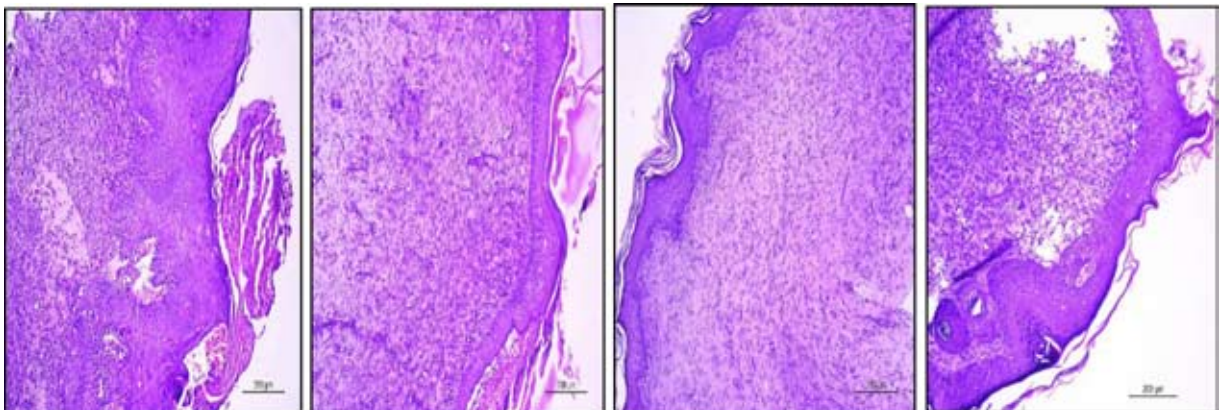


Group I- Haemorrhage and scab

Group II- Marked inflammatory cell infiltration and moderate adipocytes

Group III- Necrosis of superficial dermis, moderate fibroblasts and adipocytes

Group IV- Mild re-epithelialisation and haemorrhage

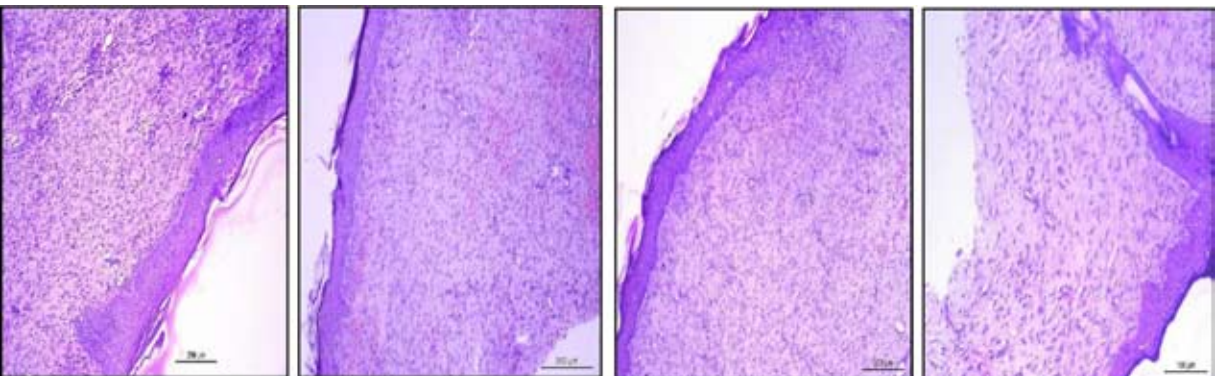
Fig. 6: Histopathological evaluation- Day 3 (H&E, X100)

Group I- Re-epithelialisation, presence of scab and mild collagen deposition

Group II- Moderate re-epithelialisation, presence of scab and immature keratin formation

Group III- Immature keratin formation, moderate collagen deposition & re-epithelialisation

Group IV- Moderate re-epithelialisation, mature keratin and presence of hair follicle

Fig. 7: Histopathological evaluation- Day 9 (H&E, X100)

Group I- Multilayered epithelium, immature keratin & moderate collagen

Group II- Multilayered epithelium, mature keratin & moderate collagen

Group III- Multilayered epithelium, mature keratin, marked fibroblasts & moderate collagen

Group IV- Multilayered epithelium, mature keratin, marked fibroblasts & collagen (H&E, X200)

Fig. 8: Histopathological evaluation- Day 15 (H&E, X100)

cm²) showed improved healing with earlier inflammatory resolution, moderate collagen deposition, and epithelial maturation, aligning with Karu (1999) and Avci et al. (2013). Group III (4 J/cm²) demonstrated more organised

granulation tissue, increased fibroblast proliferation, neovascularisation, and enhanced epithelialisation, which was in accordance with Gupta et al. (2015). Group IV (6 J/cm²) exhibited the most advanced healing, with dense

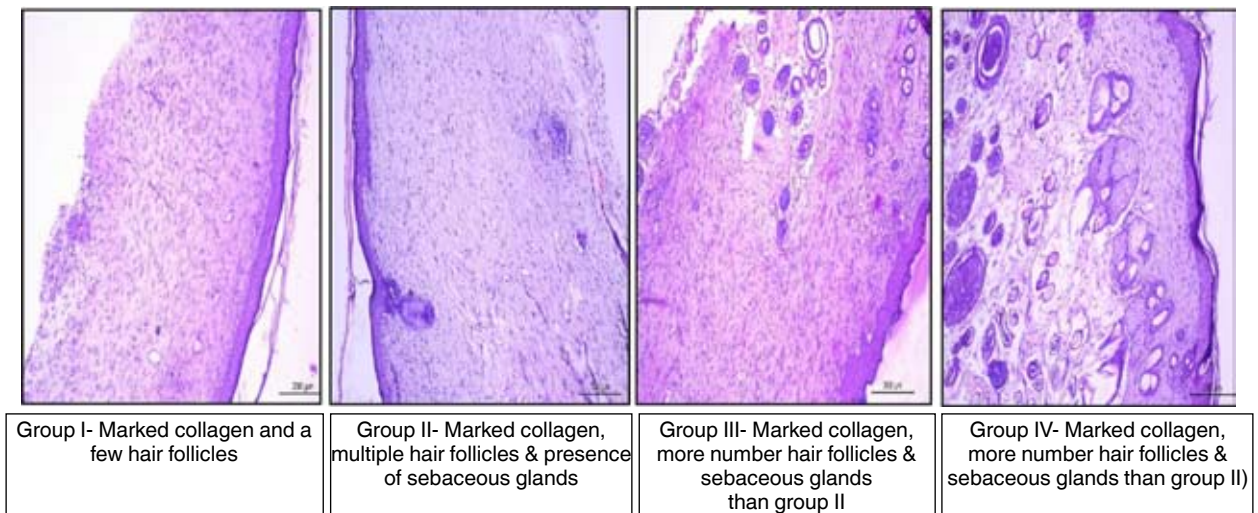


Fig. 9: Histopathological evaluation- Day 21 (H&E, X100)

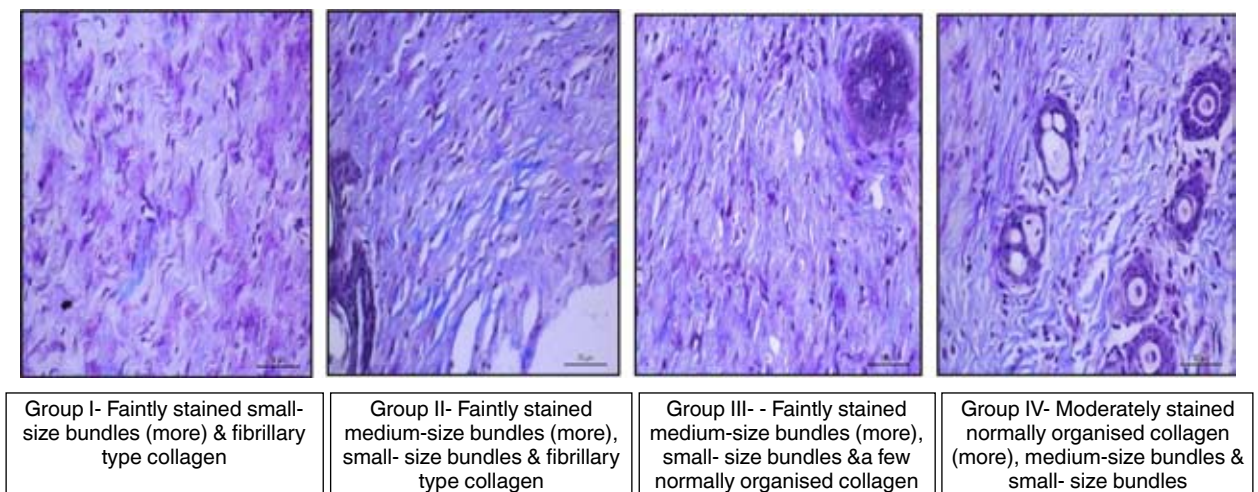


Fig. 10: Histopathological evaluation- Day 21 (Masson's trichrome, X400)

collagen organisation, minimal inflammation, early re-epithelialisation, and adnexal regeneration, indicating a fluence-dependent improvement in tissue repair. Similar findings were reported by Meireles et al. (2008).

Conclusion

Low-level laser therapy (Gallium–Arsenide) significantly enhanced burn wound healing in rats grafted with decellularised bovine omentum, with higher energy densities (particularly 4 and 6 J/cm²) showing superior outcomes. Combining a physical modality with biological scaffold reduced inflammatory response, promoted faster wound contraction, and improved tissue regeneration, indicating its potential as an effective adjunct therapy in burn management.

Conflicts of interest

The authors declare that they have no conflict of interest.

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