Chapter: 6

Comparative evaluation of GL/PCL-LTZ-NAR and PVA/CH-AgNP-LZNP nanofiber with market cream Luligel™ for treatment of *Candida* infected diabetic wounds.

6 Comparative evaluation of GL/PCL-LTZ-NAR and PVA/CH-AgNP-LZNP nanofiber with market cream Luligel™ for treatment of *Candida* infected diabetic wounds.

6.1 Plan of study

1. *In-vivo* characterization
    a. Wound closure study
    b. Histopathological examination
    c. Laser doppler examination

6.2 Materials

PVA/CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR nanofibers were freshly prepared. LuliGel™ (Commercial cream) was procured from market.

6.3 Methods

6.3.1 *In-vivo* wound healing study

The wound healing activity of PVA/CH-AgNP-LZNP NF and PCL-GL-LTZ/NAR nanofibers were compared with the healing ability of LuliGel™ against *C. albican* infected full-thickness wounds using 200–250 g of Sprague-Dawley (S.D) rats as per the IAEC application number **IIT(BHU)/IAEC/2023/II/005** and literature [286]. Diabetes was induced in S.D rats as per protocols previously described earlier chapters. The diabetic animals were divided into four distinct groups: Untreated, LuliGel™ cream, PVA/CH-AgNP-LZNP, and GL-PCL-LTZ/NAR respectively, with each group containing three animals. Then a precise full-thickness wound of ~1.5 diameter was made over the dorsal part of each rat and a fresh *Candida* fungus suspension was inoculated over the wound to mimic the infection. All animals with wounds were kept in a highly humid environment to further enhance the fungus infection for 4 days. After fungal infections, each group received respective treatments. Post treatment wounds condition of each groups animal were monitored regularly, images of wound were captured and granulated tissues were collected from wounds at day 12 and day 18 of study.

6.3.2 Histopathological study

For histopathological study, the granulated tissue collected during the study were washed with phosphate-buffered saline (PBS) to remove any debris or contaminants and further stored in a formalin solution (10% w/v). For tissue sectioning and staining, each tissue samples were fixed on wax moulds and precise transverse section of 20µm thickness were cut using a microtome. Then cut pieces were fixed onto glass slides and subjected to H&E staining. Finally, the stained slides were carefully examined under a Dewinter optical microscope using a 10 X objective lens, and images were captured using Dewinter BioWizard™ software for further analysis.

6.3.3 Laser doppler study

The blood flow across the wound surface were assessed using laser doppler study [237]. For this, rats were anesthetized with ketamine and xylazine, and blood flow assessment were done by placing the wound site under the path of a laser doppler camera and analysed using OSMOZONE™ software.

6.4 Results

6.4.1 *In-vivo* wound healing study

The gauze-treated group had a poorest wound healing with wound area of 74.07±3.34% on day 12 and 46.25±2.44% on day 18 (Figure 6.1). The Luligel™, PVA-CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR NF group demonstrated excellent wound healing with wound area of 14.38±3.7%, 16.96±2.14%, and 13.08±2.8% on Day 12; and 7.9±2.4%, 2.14±1.1% and 1.25±0.8% on Day 21, respectively. The PVA-CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR NF group showed a significant high wound healing response with p value < 0.0001 (Figure 6B) in comparison to untreated or gauze treated group proving nanofiber effectiveness in preventing microbial infection and providing optimal conditions for wound healing. Furthermore, the GL-PCL-LTZ/NAR and PVA/CH-AgNP-LZNP NF group

showed a statistically significant high (p-value < 0.05) wound closure response compared to the Luligel™ group on day 18 indicating effectiveness of prolonged release luliconazole and antioxidant agents in improving wound healing response.

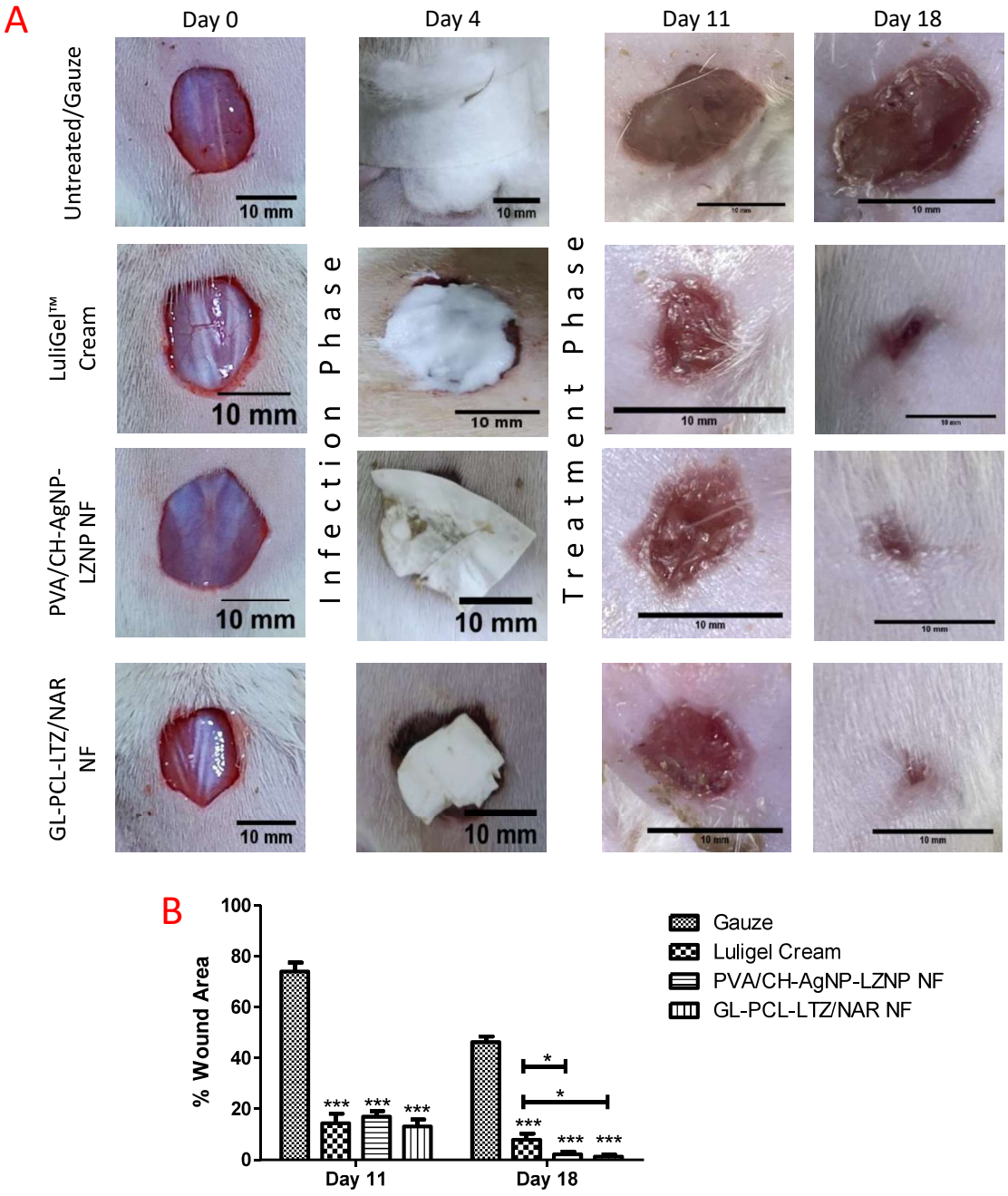

Figure 6.1 (A) Wound closure study image on different time points (B) Bar graph showing % wound area on Day 11 and Day 18 (Vertical bars in graph represent standard deviations (n=3), * represent p value < 0.05, ** represent p value < 0.01 and *** represent p value < 0.001).

6.4.2 Histopathological study

The histological images of the Gauze-treated, Luligel™ cream, PVA/CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR NF groups for days 11 and 18 are shown in Figure 6.2. The Luligel™ Cream, PVA/CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR nanofiber groups does not showed any signs of any infections, but the gauze-treated group had signs of infection. The gauze-treated group showed minimal reepithelialisation, but Luligel^{TM} cream, PVA/CH-AgNP/LZNP NF and GL-PCL-LTZ/NAR NF groups showed good signs of reepithelization and granulation on days 11 and 18, respectively. Additionally, on day 11 and 18, the GL-PCL-LTZ/NAR and PVA/CH-AgNP-LZNP group exhibited a higher neoangiogenesis signs compared to the gauze-treated and Luligel^{TM} cream groups.

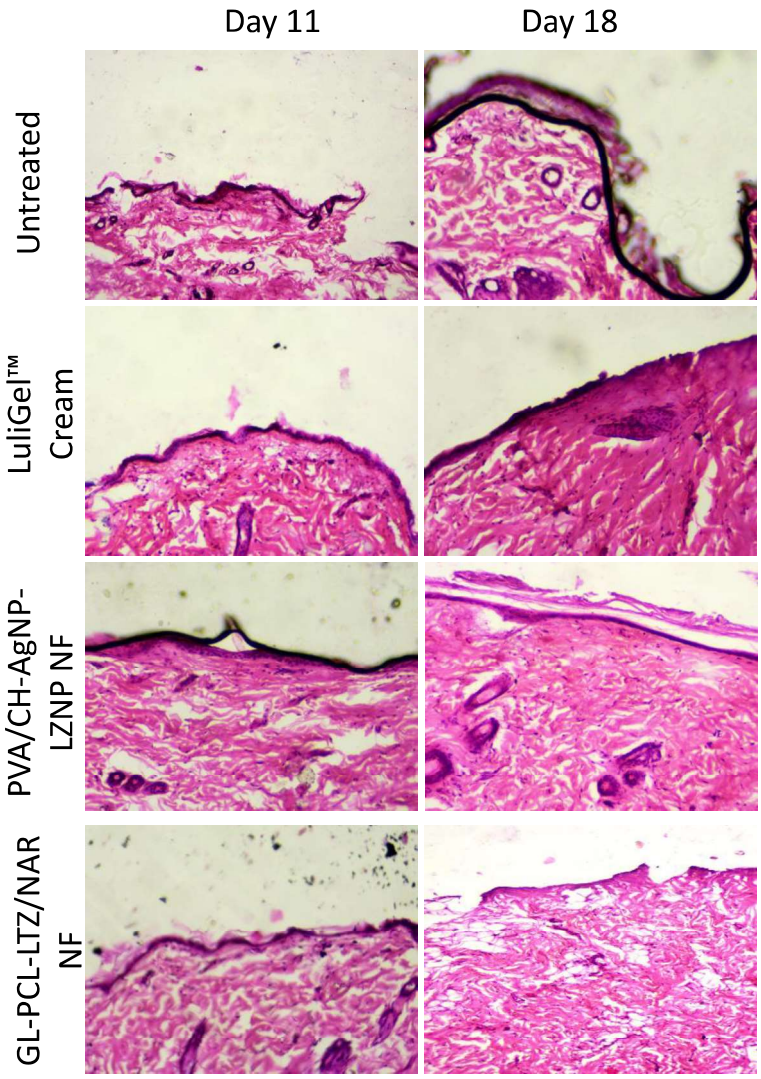

Figure 6.2 H&E histological data of different treatment groups on day 11 and 18

6.4.3 Laser doppler study

The blood flow across wound sites in different groups, including Gauze/untreated-treated, Luligel™ cream, GL-PCL-LTZ/NAR, and PVA/CH-AgNP-LZNP nanofiber groups, are shown in Figure 6.3 A. The Luligel™, GL-PCL-LTZ/NAR NF, and PVA/CH-AgNP-LZNP NF treatment group showed a statistically significant increase in blood flow around the wound area compared to the gauze/untreated treatment group, with a p-value of less than 0.001 on day 11 and 18. A similar pattern were observed between the Luligel™ and nanofibers groups. The GL-PCL-LTZ/NAR NF and PVA/CH-AgNP-LZNP NF group

exhibited a higher blood flow across the wound on day 11 and day 18, with a p-value of less than 0.05 (Figure 6.3B). These finding could be due to antioxidant activity of AgNP and naringenin present within the matrix of nanofiber.

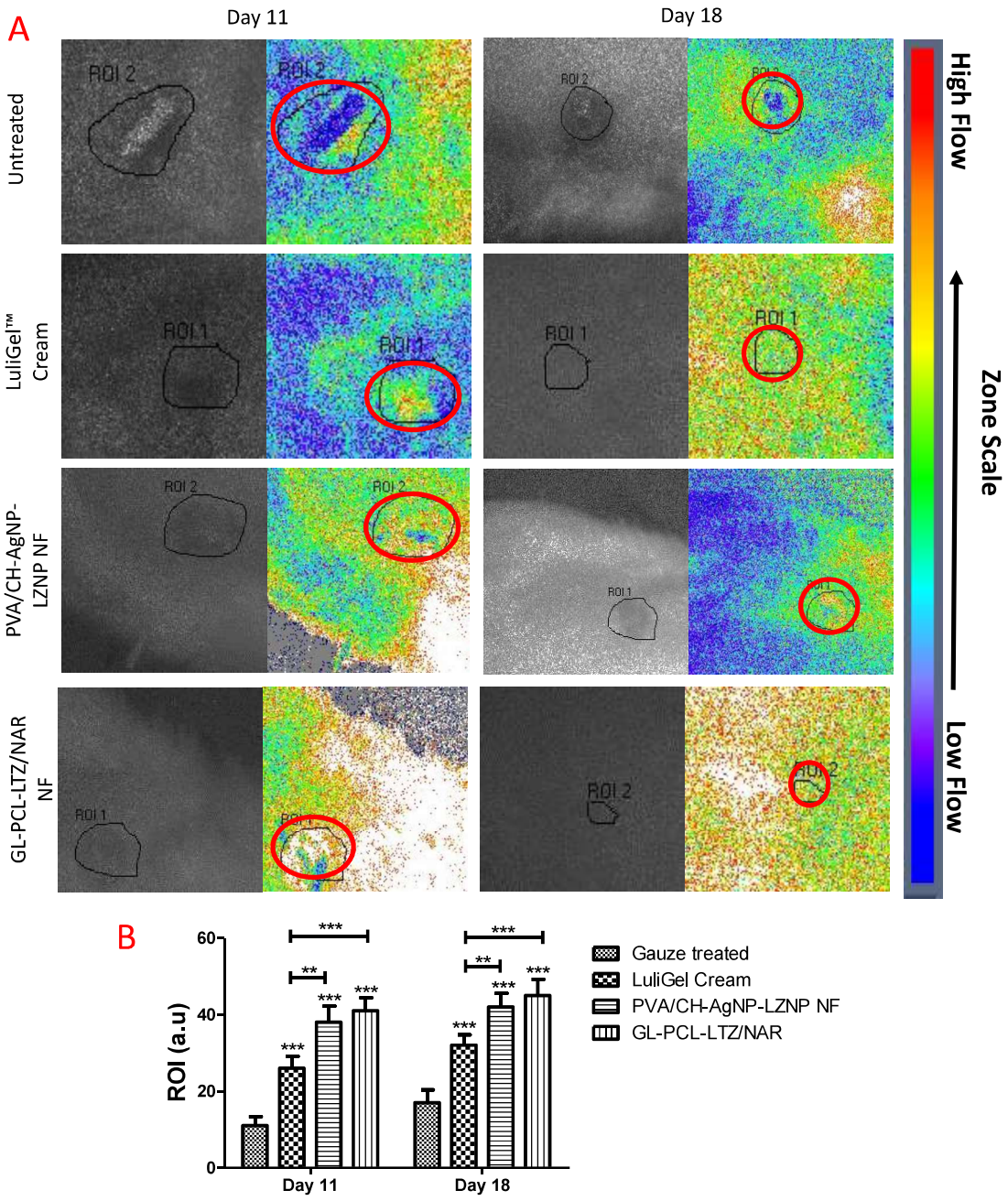

Figure 6.3 (A) Laser doppler image of different treatments; (B) Bar graph showing Blood flow around wound area in different treatment groups (Vertical bars in graph represent standard deviations (n=3), ** represent p value < 0.01, *** represent p value < 0.001).

6.5 Discussion and conclusions

The study demonstrated the significant impact of different wound treatments on wound healing outcomes in diabetic rats. The gauze-treated group exhibited the poorest wound healing response, while the Luligel™ group, PVA-CH-AgNP-LZNP NF group, and GL-PCL-LTZ/NAR nanofiber group demonstrated remarkable efficacy in promoting wound closure. The GL-PCL-LTZ/NAR and PVA-CH-AgNP-LZNP NF groups showed superior healing than LuliGel™ group highlighting the role of control release of drugs in improving wound healing responses. Histological data supported these findings and showed better reepithelialisation, granulation and neoangiogenesis responses in nanofibers treated groups. The laser Doppler study further supported the findings of histological study and reported significantly better blood flow (p value < 0.01) around wound area in GL-PCL-LTZ/NAR and PVA-CH-AgNP-LZNP NF treated groups in comparison to the untreated and marketed treatment (Luligel™ cream).

Overall the PVA/CH-AgNP-LZNP NF and GL-PCL-LTZ/NAR nanofibers treatment found to be superior in comparison to the untreated and marketed cream (Luligel™) treatment in infected diabetic rats wounds. This could be due to nanofibers morphological, prolonged drug release, and antioxidant activity of AgNP and naringenin present within the nanofiber matrix which ultimately leads to the improved blood flow around wound area and superior wound healing.