

Assessment of Safety and Efficacy of Cellular Therapy on Foot Ulcer in Diabetic Animal: A Preclinical Investigation

Mehdi Fathima, Deepak Kumar Jha*, Saroj Kumar Sah, Soham Bhattacharjee

Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka, INDIA.

ABSTRACT

Aim and Objectives: Around 20% of diabetic patients are at increased risk of chronic foot ulcers. Current treatments mainly focus on glycemic control and supportive care, with limited effectiveness. Mononuclear Cell (MNC) therapy, containing hematopoietic, mesenchymal, and progenitor cells, is emerging as a novel and effective regenerative approach for Diabetic Foot Ulcers (DFU). The objective of the study is to evaluate the safety profile and healing efficacy of MNC-based therapy in a diabetic rat model of non-healing wounds. **Materials and Methods:** 36- male Wistar rats (150–200 g) were randomized into different groups ($n=6$): Normal Healthy Control, Diabetic Control (STZ + Ulcer control), Mupirocin, Insulin + Mupirocin, MNCs ($\sim 10 \times 10^6$ cells), and MNCs ($\sim 10 \times 10^6$ cells) + Insulin. A diabetic model was induced in experimental rats using STZ (50 mg/kg, i.p.). From each diabetic rat, 1.5 mL of blood was collected to isolate MNCs, which were resuspended in 0.5 mL of autologous plasma and injected once at the ulcer site. Insulin groups received 4 IU/day (s.c.). The end points of our study evaluated various parameters, including wound closure, serum insulin, blood glucose, lymphocyte/NLR profiles, inflammatory markers (TNF- α , IL-6), and histology. **Results:** The combination of insulin and MNC-based therapy was well tolerated and resulted in a significant reduction in hyperglycemia, reduced inflammation, and enhanced the wound closure rate. This combinatorial approach facilitated tissue homeostasis and aided in the remodeling of cells for functional restoration. MNC therapy combined with insulin resulted in a marked glycemic reduction ($p<0.001$) and pro-inflammatory cytokines TNF- α and IL-6 levels were significantly attenuated ($p<0.001$) compared to diabetic controls. The combination therapy enhanced wound closure ($\sim 95\%$ by day 14) relative to MNCs alone ($\sim 75\%$) and insulin + mupirocin ($\sim 80\%$) ($p<0.01$). Lymphocyte sets and the NL ratio were also restored, indicating immune stabilization. Histological analysis revealed increased collagen deposition, angiogenesis, and re-epithelialization in the MNC + insulin group. **Conclusion:** MNC-based therapy can serve as a promising candidate for treating diabetic patients with non-healing ulcers.

Keywords: Diabetes mellitus, Diabetic Foot Ulcer (DFU), Mononuclear Cells (MNCs), Wound healing, Immune Response and Tissue homeostasis.

Correspondence:

Dr. Deepak Kumar Jha

Associate Professor, Department of Pharmacology, Karnataka College of Pharmacy, Bangalore-560064, Karnataka, INDIA.

Email: deepakjha736@gmail.com

ORCID: 0000-0002-1979-7940

Received: 15-07-2026;

Revised: 24-08-2026;

Accepted: 04-09-2026.

INTRODUCTION

Diabetes mellitus is a chronic metabolic condition that leads to significant clinical challenges due to its complications associated with uncontrolled diabetes. These acute and/or chronic complications contribute to morbidity and mortality in diabetic patients. Among the countless complications, Diabetic Foot Ulcers (DFUs) are one of the most severe non-healing wounds, affecting around 20% of individuals with diabetes (Fathima and Jha, 2024). DFUs are mainly caused by neuropathy and ischemia, often leading to ulceration, infection, and, in some serious cases,

amputation or death. The impact of DFUs is not only physical but also cause economic and psychological distress, further worsening the overall impact of diabetes on those affected (Armstrong *et al.*, 2023). Current treatment strategies for diabetic complications, including DFUs, involve comprehensive management and include intensive blood glucose control, antihypertensive therapy and use of statin therapy. Despite these treatments, the efficacy of preventing or healing DFUs remains limited, and thus alternative therapeutic avenues are needed. One such groundbreaking avenue is the use of autologous Mononuclear Cell (MNC) therapy. Mononuclear cells comprise monocytes, lymphocytes, and a small proportion of stromal cells. These cells are of great importance in modulating immune response and contributing to accelerated wound healing. MNCs has shown to promote tissue reparations, as evidenced by few studies; hence, it could probably act as a remedy for DFUs. The significance of using diabetic rats to treat DFUs with autologous MNCs lies in cell's ability to boost immune responses, clearing necrotic debris, initiate tissue



DOI: 10.5530/ijpi.20270254

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repair and remodeling processes. The presence of innate and adaptive immune cells in MNC therapy offer a new and efficient way to treat DFUs, an important need that is not yet addressed in managing complications of diabetes (Fathima and Jha, 2024). This study therefore aimed to investigate the use of autologous MNCs to treat non healing DFUs.

Although current treatments for diabetic foot ulcers focus on glycemic control and wound care, they often fail to promote complete healing, especially in chronic cases. Autologous Mononuclear Cells (MNCs), which include immune and stromal cell populations, have the potential to enhance tissue repair, reduce inflammation, and support new blood vessel formation. However, the effects of combining MNC therapy with systemic interventions such as insulin remain unclear. In this study, we hypothesize that administering autologous MNCs, alone or together with insulin, will accelerate wound closure, modulate inflammatory responses, and improve tissue regeneration in a streptozotocin-induced diabetic rat model. The main objectives were to assess the safety and effectiveness of these treatments by monitoring wound healing, metabolic indicators, inflammatory markers, and histological outcomes.

Mononuclear Cells (MNCs) offer a composite approach to treat Diabetic Foot Ulcers (DFUs) owing to the presence of diverse cellular makeup including monocytes, lymphocytes, and a relatively small proportion of stromal cells. Stromal cells particularly secrete angiogenic factors such as VEGF and PDGF and facilitate in the development of new blood vessels and promote blood supply to the wound area, thereby accelerating the tissue repair process. MNCs control inflammation by regulating the levels of pro and anti-inflammatory cytokines in the microenvironment. Additionally, these cells provide local defense and clears up infection. Furthermore, fibroblasts and keratinocytes in the local microenvironment get activated by these MNCs, which are essential for granulation tissue creation as well as the healing process of a wound. MNCs also help in Extracellular Matrix (ECM) remodelling by Modulating Matrix Metalloproteinases (MMPs), maintaining a balanced ECM degradation/production process that supports structural integrity during healing. MNCs therapy is a multifaceted approach to treat DFUs because it addresses DFUs delayed healing and offers a regenerative solution, especially in cases where traditional and allopathic therapies prove ineffective. Enhancing blood vessel formation, modulating immune responses, and facilitating tissue regeneration, make MNCs a novel candidate to treat non-healing DFUs. It has been proven in clinical studies that MNC therapy can greatly promote wound closure and reduce the risk of infection and promote tissue regeneration with a favorable safety profile. However, standardizing the treatment protocols and navigating regulatory approval is still a major challenge. Future research should be directed to the optimization of treatment protocols, additional combination therapies and extension of MNCs therapy

to other chronic conditions. Such a progress could eventually lead to greater effectiveness in clinical practice and thereby improving the outcomes of patients with DFUs and other chronic wounds (Yang *et al.*, 2024).

This study provides a significant pharmaceutical advantage by demonstrating a cost-effective, autologous, cell-based therapy using Mononuclear Cells (MNCs) that can enhance the healing of DFUs, a major unmet clinical need. Unlike advanced biologicals or engineered cell products, MNCs can be isolated from a small volume of the patient's own blood without requiring complex manufacturing, reducing the risk of immune rejection and minimizing regulatory hurdles. The combination of MNC therapy with insulin further highlights a translationally feasible approach that integrates regenerative treatment with routine diabetes management. This strategy has the potential to accelerate wound closure, reduce inflammation, and improve tissue regeneration, ultimately lowering the burden of chronic ulcers, preventing amputations, and decreasing healthcare costs associated with long-term DFU management.

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats (150-200 g) were used in the study. All animals were acclimatized for one week under standard laboratory conditions in accordance with CPCSEA guidelines and were provided a standard diet. The experimental protocols were approved by the IAEC (KCP/IAEC/14/23-24/06/28/03/24).

Experimental Design

STZ-Induced Type 1 Diabetes Foot Ulcer (DFU) Model

- Group I served as the normal healthy control group, and received drinking water *ad libitum*.
- Group II, designated as the disease control group, received a single intraperitoneal injection of STZ at 50 mg/kg, + foot ulcer (all the groups received STZ injection to induce diabetes and foot ulcer except the normal healthy control).
- Group III received standard treatment, which included topical antibiotic (Mupirocin 2%, q.s.) ointment.
- Group IV received insulin (4 U/kg) and topical antibiotic (Mupirocin) ointment.
- Group V received insulin (4 U/kg, s.c.) and MNCs ($\approx 10 \times 10^6$ cells). Group VI received MNCs ($\approx 10 \times 10^6$ cells, single injections localized at ulcer sites) (Jha *et al.*, 2019).

Method of Inducing Foot Ulcer

On the day of wound induction (Day 0), diabetic rats (glucose level ≥ 300 mg/dL) were anaesthetized with an i.p. of 40 mg/kg phenobarbital sodium to ensure complete immobility. The wound site was meticulously cleaned with a 70% ethanol wipe to minimize the risk of infection. A full-thickness wound was then induced by carefully excising the outlined skin using a scalpel. Following the procedure, the rats were returned back to their cages and closely monitored for any signs of distress or infection. They were provided with standard food and water. To prevent any secondary infections during the experiment, the wounds were regularly observed and cared for, with detailed observations recorded throughout the process (Lau *et al.*, 2008).

Isolation of MNCs

Blood samples (approximately 1.5 mL) were collected from diabetic rats with open foot wounds via cardiac puncture (under anesthesia sodium phenobarbital) using sterile needles and syringes. Initially, the blood sample was diluted with an equal volume of normal saline, and the diluted blood was carefully layered on top of the density gradient media (HiSep), using a sterile pipette, in a ratio of 1:3. The tubes were centrifuged at 1500 rpm for 20 min at Room Temp. After centrifugation, the plasma and platelet-rich upper layer was gently transferred to a fresh tube using a sterile pipette, while the MNC layer at the interface was preserved. The MNC layer was transferred gently into a new tube and washed the MNCs with 10 mL of normal saline. These cells were pelleted at 1500 rpm for 10 min. The washing step was repeated once more to ensure complete removal of HiSep. After the final centrifugation, the supernatant was discarded, and the cells were resuspended in 0.5 mL of their respective plasma samples (Puleo *et al.*, 2017).

Treatment Protocol

Once diabetes was established, all groups except the normal control were subjected to a full-thickness wound surgically on the foot pad of each rat. Following one-week period for the wound to develop, peripheral blood was drawn out from each rat using the cardiac puncture method. The collected blood (1.5 mL) was processed to isolate MNCs; the isolated cells were injected back into the same animal directly at the wound site, targeting the area of tissue damage to promote localized healing. Post MNCs injections, the wound areas were meticulously bandaged to prevent secondary infections and to ensure that the injected cells remained concentrated at the site of injury.

The diabetic rats were continuously monitored for any adverse effects or complications. Over time, assessments included monitoring body weight, blood glucose levels, serum insulin levels (measured using a sandwich ELISA assay) (Ali *et al.*, 2024), and pro-inflammatory cytokine levels in plasma (TNF- α and IL-6 levels) (Quantified using a sandwich ELISA) (Hassan *et al.*, 2024).

Additionally, the healing of DFUs was closely monitored, with detailed observations of wound closure, reduction in ulcer size, and tissue histology.

Statistical Analysis

All data are expressed as mean $\pm$ SEM ($n=6$). One-way ANOVA followed by Tukey's *post hoc* test was used for statistical analysis ($p<0.05$).

RESULTS

Safety assessment

The normal healthy control group showed normal behavior and physiological parameters with no mortality. In contrast, the disease control (STZ-treated + Ulcer control) observed abnormal behavior, increased water intake and urination, reduced food consumption, and a 33% mortality rate. All treatment groups, including Mupirocin, Insulin + Mupirocin, Insulin + MNCs, and MNCs alone, maintained normal behavior, normal intake and urination patterns, and showed 0% mortality, indicating good safety and therapeutic tolerance.

Efficacy Assessment

The normal healthy control animals maintained consistently higher body weights throughout the study. In contrast, the diabetic (STZ-treated + Ulcer control) group showed a significant post-treatment reduction in body weight, reflecting the characteristic weight loss (Figure 1). But it remained unchanged with the treatment's mupirocin, insulin, and MNCs vs. diabetic control. This indicates that while these interventions may have helped prevent further weight loss, they did not effectively reverse the diabetes-induced reduction in body weight. Blood glucose levels were markedly elevated in the DC group compared with normal controls, indicating successful induction of diabetes. Mupirocin treatment alone had no significant effect on glucose levels compared with the DC group (ns). However, the groups receiving insulin alone, along with mupirocin, and in combination with MNCs showed a significant reduction in blood glucose (Figure 2). Figure 3 shows serum insulin levels. The DC group had reduced insulin (10 mcU/mL) compared to normal controls (40 mcU/mL). Treatment with MNCs plus insulin increased insulin to 32 mcU/mL, while MNCs alone raised it to 25 mcU/mL, suggesting both treatments help improve insulin production. Figure 4 shows TNF- α levels. The disease control group had elevated TNF- α (90 pg/mL), indicating increased inflammation. Treatment with MNCs and insulin significantly reduced TNF- α to normal levels (12–15 pg/mL), demonstrating a strong anti-inflammatory effect. Figure 5 shows IL-6 levels, which were elevated in the disease control group (17 pg/mL), indicating an inflammatory response. Treatment with MNCs plus insulin significantly reduced IL-6 to 4 pg/mL, demonstrating strong anti-inflammatory effects. Overall, the combinatorial approach

(autologous MNCs + insulin) stabilized blood glucose and reduced inflammation in diabetic rats. Combination therapy also lowered blood glucose, decreased pro-inflammatory cytokines (TNF- α , IL-6), and promoted collagen deposition, re-epithelialization, and angiogenesis. Figure 6 illustrates lymphocyte populations across the groups. Disease controls showed a significant decrease in lymphocytes compared to normal controls. Treatments with mupirocin + insulin and MNCs + insulin restored lymphocyte levels, while mupirocin alone and MNCs alone did not show significant improvement, indicating that combination therapy supports immune stabilization. Figure 7 depicts the Neutrophil-to-Lymphocyte (NL) ratio. The disease control group had a higher NL ratio than normal controls, whereas combination therapy significantly reduced the NL ratio, reflecting its anti-inflammatory effect. No significant changes were observed with the individual treatments compared to the disease control. Figure 8 compares wound healing in STZ-induced diabetic rats. The disease control group showed minimal healing with persistent tissue damage.

Mupirocin alone resulted in partial healing, while mupirocin plus insulin improved wound closure and reduced inflammation. MNCs, along with the insulin group, produced the most effective healing, with near-complete wound closure and minimal inflammation. MNCs alone also enhanced healing but were less effective than the combination therapy. These results indicate that MNCs combined with insulin provide superior wound healing in diabetic conditions. Figure 9 shows histological sections of wound tissue. The disease control group (A) exhibited disrupted tissue architecture, chronic inflammation, necrosis, and minimal healing. Mupirocin alone (B) showed partial re-epithelialization and collagen formation, with persistent inflammation. Mupirocin plus insulin (C) improved tissue organization and granulation and reduced inflammation. MNCs + Insulin (D) demonstrated substantial tissue regeneration, restored epithelium, increased collagen deposition, and minimal inflammatory infiltrates, indicating superior recovery. MNCs alone (E) showed moderate tissue repair with reduced inflammation but were less effective

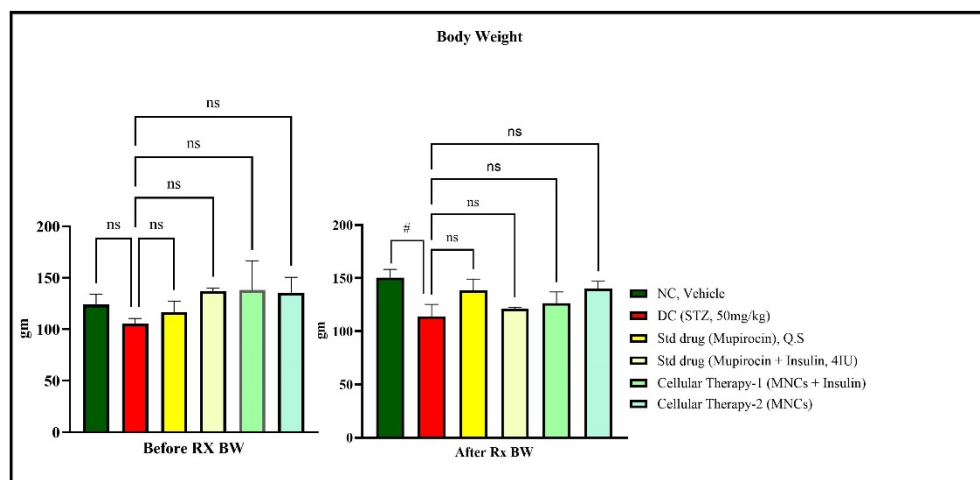


Figure 1: Changes in Body weight in Streptozotocin induced diabetic foot rat treated with Autologous peripheral blood mononuclear cells. Data are expressed as mean $\pm$ SEM ($n=6$ per group). ns: not significant, $p>0.05$ compared with disease control (STZ + wound control); # $p<0.05$ compared with normal healthy control.

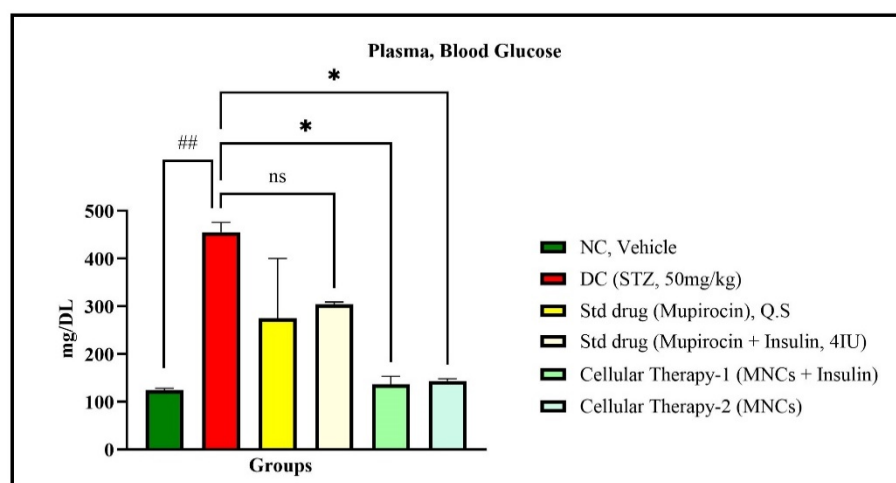


Figure 2: Effect of Peripheral Blood Mononuclear Cell Therapy on Blood Glucose Levels in Diabetic Rats with Foot Ulcers. Values are expressed as Mean $\pm$ SEM, ($n=6$ rats in each group), ## $p<0.01$ compared to normal healthy control, * $p<0.05$ compared to disease control (STZ + wound control).

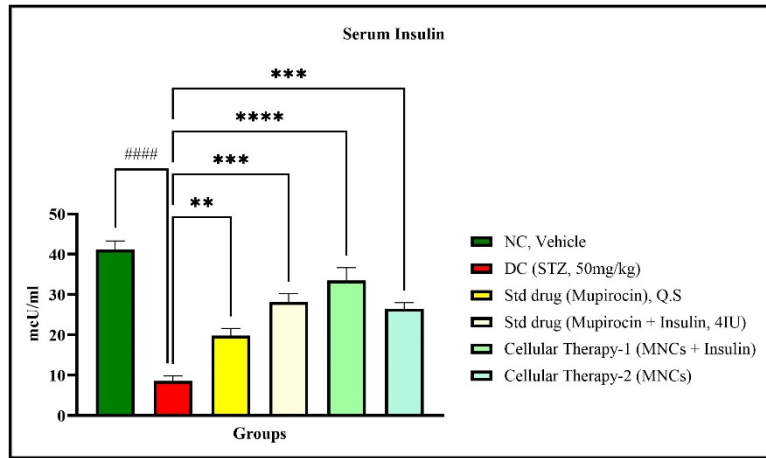


Figure 3: Changes in Serum Insulin Levels Following Peripheral Blood Mononuclear Cells Therapy in Diabetic Rats with Foot Ulcers. Values are expressed as Mean±SEM, (n=6 rats in each group), #### $p<0.0001$ compared to normal healthy control and Test drug (MNCs + Insulin) vs disease control (STZ + wound control), *** $p<0.001$, ** $p<0.01$ compared to disease control (STZ group).

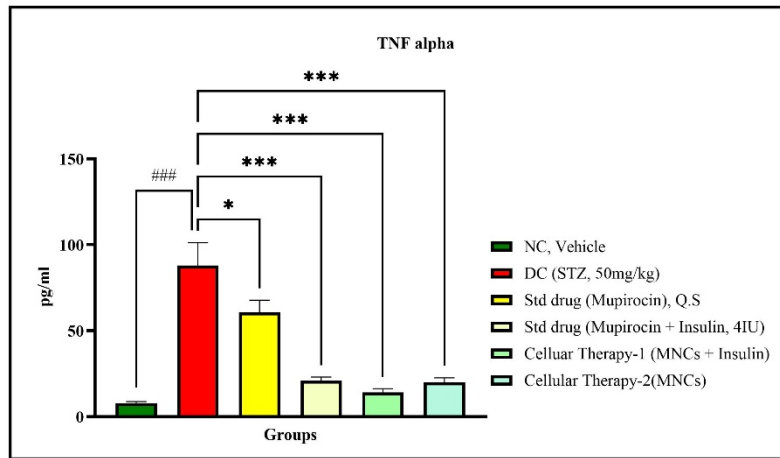


Figure 4: Modulatory Effect of PBMCs on TNF- α in Diabetic Rats with Foot Ulcers. Values are expressed as Mean±SEM, (n=6 rats in each group), ### $p<0.001$ compared to normal healthy control and disease control group. * $p<0.05$ compared to disease control (STZ + wound control).

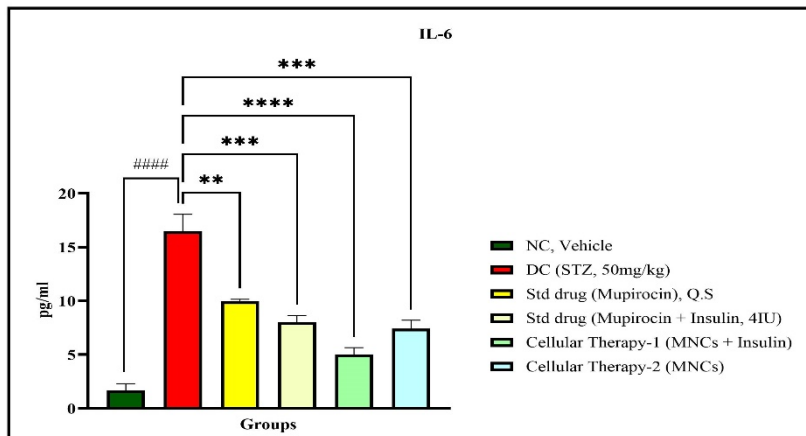


Figure 5: Effect of Peripheral Blood Mononuclear Cells Therapy on IL-6 Levels in Diabetic Rats with Foot Ulcers. Values are expressed as Mean±SEM, (n=6 rats in each group), #### $p<0.0001$ compared to normal healthy control and Test drug (MNCs + Insulin) vs disease control (STZ + wound group), *** $p<0.001$ and ** $p<0.01$ compared to disease control (STZ + wound control).

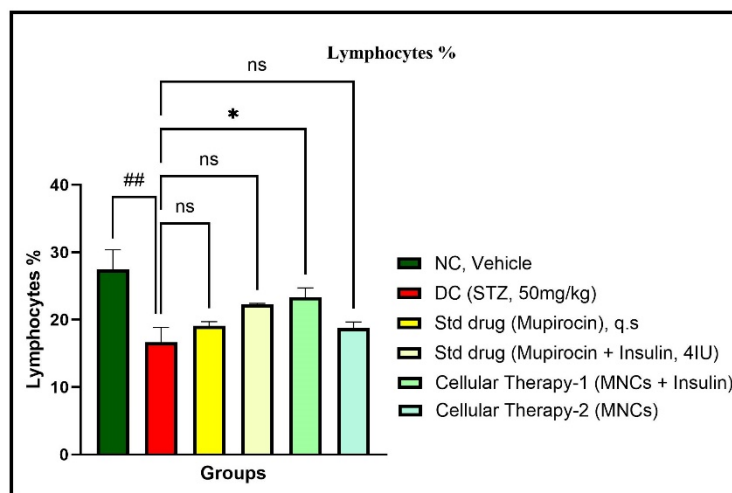


Figure 6: Evaluation of PBMC Therapy on Lymphocyte Profile in Diabetic Rats with Chronic Foot Ulcers. Values are expressed as Mean±SEM, (n=6 rats in each group), ## $p<0.01$ compared to normal healthy control, * $p<0.05$ and ns $p>0.05$ compared to disease control (STZ + wound control).

than the combination therapy. Overall, the combinatorial therapy of MNCs and insulin provided the most favorable outcomes in wound healing.

DISCUSSION

Plant-based treatments such as *Eriosema robustum* (Gael *et al.*, 2024) and *Trigonella foenum-graecum* (Salimabad *et al.*, 2023) have also shown benefits in diabetic wound healing by enhancing collagen production and exhibiting their antibacterial properties in diabetic rats. Additionally, curcumin (Tejada *et al.*, 2016), derived from turmeric, has also shown promising results in diabetic wound healing. Their findings demonstrated that applying curcumin nanoparticles topically accelerated wound closure and boosted the expression of growth factors in diabetic mice. Another promising plant-based remedy, *Momordica charantia* (Singh *et al.*, 2017), also known as bitter melon, has also been studied for its therapeutic effects on diabetic wound healing. The topical application of *M. charantia* fruit extract has been shown to significantly improve the wound healing in diabetic rats by promoting collagen synthesis and enhancing wound tensile strength. The present study demonstrates that the combinatorial therapy of Mononuclear Cells (MNCs) and insulin significantly enhances wound healing, reduces inflammation, and restores immune balance in diabetic rats. These findings align with previous studies reporting that diabetic wounds are characterized by chronic inflammation, impaired angiogenesis, and reduced collagen synthesis, which collectively delay healing processes (Barrientos *et al.*, 2008 and Brem *et al.*, 2007). In this study, 10 million MNCs were obtained from 1.5 mL of animal peripheral blood, which was validated using crystal violet (Jha *et al.*, 2025). These cells were then directly injected into the wound site, leading to significant progress in wound healing. This highlights the potential of cell-based therapy in accelerating the healing of DFUs and reinforces the therapeutic benefits of MNC

therapy. The effect of different treatments produced significant variations in the levels of serum insulin, blood glucose, TNF- α , and IL-6 and also in the body weight across all experimental groups. Among the treatment groups, the intervention of MNCs has shown improvement in the metabolic parameters as well as inflammatory cytokines. Hyperglycemia is known to upregulate inflammatory cytokines prolonging the inflammatory phase of wound healing (Xu *et al.*, 2013). Consistent with this, the disease control group in our study showed markedly elevated TNF- α (90 pg/mL), IL-6 (17 pg/mL), and an increased neutrophil-to-lymphocyte ratio, similar to patterns observed in STZ-induced diabetic models (Sanci *et al.*, 2024 and Nirejen *et al.*, 2023). Cell-based therapies, including mononuclear cells, mesenchymal stem cells, and progenitor cells, have previously been shown to improve wound healing by enhancing angiogenesis, modulating inflammation, and releasing reparative cytokines (Dash *et al.*, 2009; Falanga *et al.*, 2007; Maxson *et al.*, 2012). Our results support these mechanisms, with MNC treatment improving healing outcomes, though the greatest effect was seen in the combined MNCs + insulin group. Insulin is known to accelerate wound healing by promoting keratinocyte migration, fibroblast proliferation, collagen synthesis, and angiogenesis through PI3K/Akt-dependent signaling pathways (Greenhalgh *et al.*, 2003; Apikoglu *et al.*, 2010). Earlier studies have shown that topical insulin improves re-epithelialization, granulation tissue formation, and overall wound closure in diabetic animal models (Yang *et al.*, 2020). These findings are consistent with the improved healing responses observed in the insulin-containing treatment groups in the present study. The synergistic effect of MNCs and insulin observed here is especially noteworthy. While MNCs contribute regenerative and immunomodulatory benefits, insulin improves the metabolic environment, potentially enhancing MNC survival, paracrine activity, and tissue regeneration (Yu *et al.*, 2022; Hsieh *et al.*, 2022). Such combined metabolic and cellular approaches

have been proposed in literature but are clearly demonstrated in the outcomes of this study. Histopathological examination further confirmed these effects, showing improved collagen deposition, organized epithelial layers, reduced necrosis, and decreased inflammatory cell infiltrates in the combination therapy group. These observations align with previous studies showing that cellular therapies paired with metabolic stabilization promote superior tissue regeneration in diabetic wounds (Wei *et al.*, 2023; Shao *et al.*, 2023). Overall, the biochemical, hematological, and histological findings indicate that the combination of MNCs and insulin addresses multiple pathophysiological deficits in diabetic

wound healing, including inflammation, immune dysfunction, impaired angiogenesis, and delayed tissue repair, resulting in a significantly enhanced therapeutic effect compared to individual treatments. The combined MNC-insulin approach addressed both systemic metabolic issues and localized wound healing. While plant extracts are effective for localized tissue repair, MNC therapy with insulin offers broader benefits by improving insulin sensitivity and reducing systemic inflammation. This suggests that MNC therapy provides a more comprehensive therapeutic approach, and future studies could explore this combinatorial approach for treating diabetic wounds.

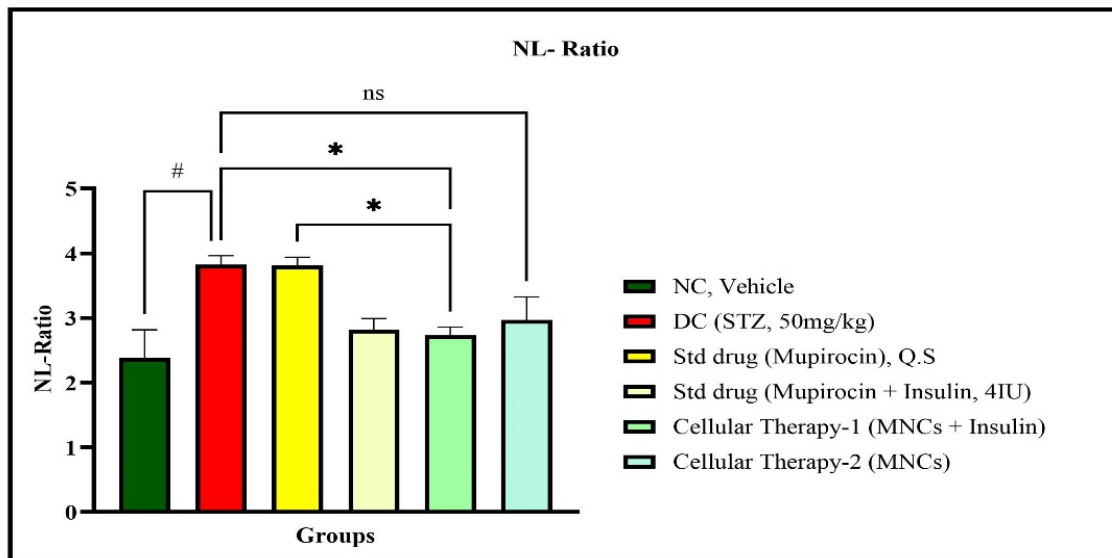


Figure 7: Evaluation of PBMC Therapy on NL Ratio in Diabetic Rats with Chronic Foot Ulcers. Values are expressed as Mean±SEM, (n=6 rats in each group), #p<0.05 compared to normal healthy control, *p<0.05 (compared to DC and Std drug, Mupirocin) and ns p>0.05 compared to disease control (STZ + wound control).

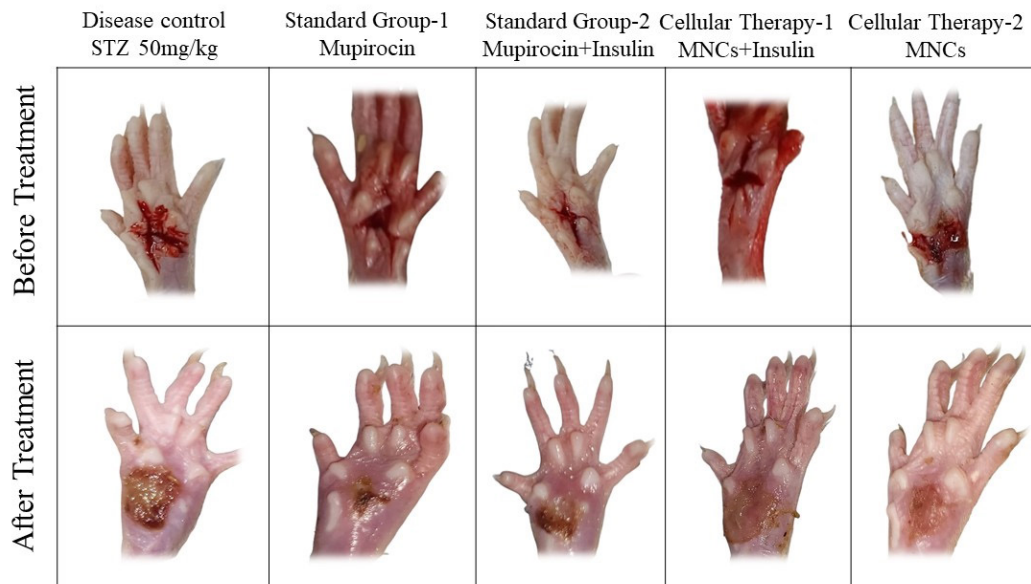


Figure 8: Comparison of foot ulcer in Diabetic Foot Rats Before and After treatment.

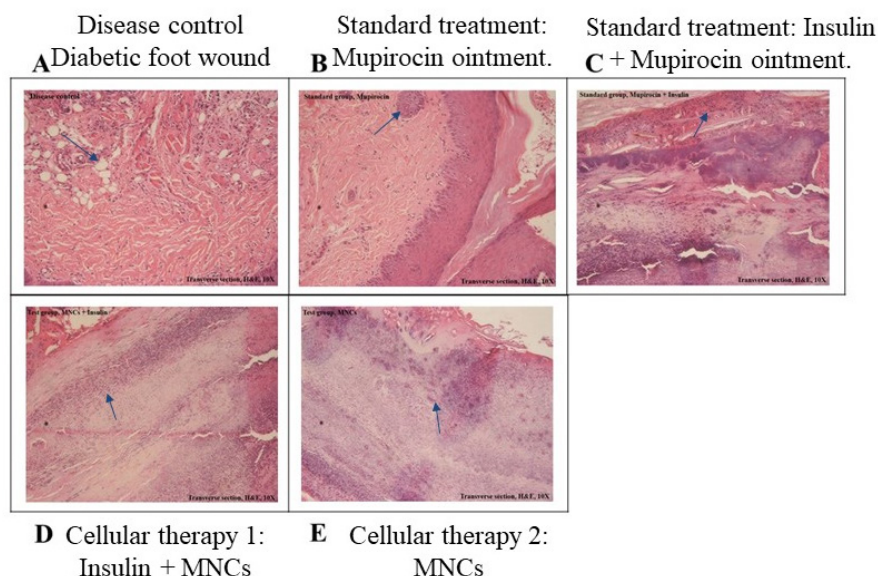


Figure 9: Histopathological Evaluations of Diabetic Foot Wounds in Rats Under Different Therapeutic Regimens.

CONCLUSION

In conclusion, combination therapy (MNCs + Insulin) demonstrated substantial promise for the treatment of DFU complications. The combined approach demonstrated multiple benefits. The treatment helps maintain glucose levels within optimal ranges and prevents tissue damage by minimizing systemic inflammation and cytokines. Additionally, histological analysis of DFU rat tissue showed signs of tissue homeostasis, active cellular proliferation, and remodelling, eventually leading to the normal tissue structure. The synergistic effect of cell-based therapy and insulin appears to tackle the intricate pathophysiology associated with DFUs more effectively than conventional treatments. This comprehensive approach addresses multiple underlying factors contributing to the development and persistence of these ulcers.

ACKNOWLEDGEMENT

The authors express gratitude to the Director and Department of Pharmacology, Advanced Laboratory of Karnataka College of Pharmacy, Bangalore, for providing the infrastructure for carrying out the research work.

ABBREVIATIONS

MNC: Mononuclear cells; **DFU:** Diabetic foot ulcers; **STZ:** Streptozotocin; **NLR:** Neutrophil-to-lymphocyte ratio; **TNF- α :** Tumor necrosis factor-alpha; **IL-6:** Interleukin-6; **VEGF:** Vascular endothelial growth factor; **PDGF:** Platelet-derived growth factor; **ECM:** Extracellular matrix; **MMPs:** Matrix metalloproteinases; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethics Committee; **i.p.:** Intraperitoneal; **s.c.:** Subcutaneous; **ELISA:** Enzyme-linked immunosorbent assay; **ANOVA:** Analysis of

variance; **SEM:** Standard error of the mean; **DC:** Disease control; **PBMC:** Peripheral blood mononuclear cell.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Dr. Deepak Kumar Jha: Conceptualization, study design, supervision, critical revision of the manuscript, and final approval. **Mehdi Fathima:** Literature review, Experimental work, data acquisition, formal analysis, and drafting of the original manuscript. **Saroj Kumar Sah:** Visualization, and assistance in experimental work and manuscript preparation. **Soham Bhattacharjee:** Assistance in experimental work, data acquisition, graphical representation, editing, and manuscript formatting.

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Cite this article: Fathima M, Jha DK, Sah SK, Bhattacharjee S. "Assessment of Safety and Efficacy of Cellular Therapy on Foot Ulcer in Diabetic Animal": A Preclinical Investigation. *Int. J. Pharm. Investigation*. 2027;17(1):215-23.