

RESEARCH PAPER

The safety and efficacy of platelet-derived exosomes for grade II diabetic foot ulcers in a phase I clinical trial

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ABSTRACT

Objective(s): Exosomes are extracellular vesicles derived from various cell types. Platelet-derived exosomes contain growth factors and cytokines that contribute to tissue repair. This study assessed the safety and efficacy of platelet-derived exosomes in treating diabetic foot ulcers.

Materials and Methods: Platelet-derived exosomes were isolated by ultracentrifugation and subsequently characterized. Fourteen patients with diabetes and grade II diabetic foot ulcers were included in the study based on the inclusion and exclusion criteria. Exosome injections at a concentration of 50 µg/ml and a volume of 1.5 ml were administered at one-week intervals. Safety and efficacy were assessed through clinical evaluation, measurements of wound dimensions, and laboratory analyses.

Results: No significant adverse clinical events or changes in laboratory test results were observed after exosome injection in the patients. None of the patients' wounds in the control group healed completely within one month. However, a decrease in wound size was observed in 3 patients in the control group. In the intervention group, the wounds of two patients completely closed within one month after PLT-Exo injection, and the wound dimensions of 42.85% of the patients exhibited a decreasing trend.

Conclusion: Diabetic foot ulcers remain a significant wound-healing challenge among affected patients. Because limb amputation has serious consequences, these ulcers require considerable attention, and current treatment modalities often fail. The present study highlighted the safety, efficacy, and clinical feasibility of platelet-derived exosomes. For wider clinical application, further studies under varied conditions and with larger patient groups are recommended.

Keywords: Diabetic foot ulcers; Exosomes; Extracellular vesicles; Wound healing

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Abbreviations

PLT-Exos	Platelet derived Exosomes	SDS	Sodium dodecyl sulfate
DFU	Diabetic foot ulcer	DMEM	Dulbecco's Modified Eagle Medium
DLS	Dynamic light scattering	TBS	Tris-buffered saline
TEM	Transmission electron microscopy	PDGF	Platelet-derived growth factor
BCA	Bicinchoninic acid assay	VEGF	Vascular endothelial growth factor
PBS	Phosphate-buffered saline	PVDF	Polyvinylidene fluoride
ESR	Erythrocyte Sedimentation Rate	IL-10	Interleukin 10
Hb	Hemoglobin	CRP	C-reactive protein
CBC	Complete blood count	ALP	Alkaline phosphatase
FBS	Fasting Blood Sugar	eGFR	estimated glomerular filtration rate
ALT	Alanine transaminase	ROS	Reactive oxygen species
AST	Aspartate transaminase	YAP	Yes-Associated Protein
AKT	Protein kinase B	ERK	Extracellular signal-regulated kinase
NPWT	Negative-pressure wound therapy	HIF-1	Hypoxia-inducible factor-1
Nrf2	Nuclear factor erythroid 2-related factor 2	MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
TNF-α	Tumor necrosis factor alpha		

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INTRODUCTION

A diabetic foot ulcer is a serious complication of diabetes that usually develops on the soles of the feet in individuals with poorly controlled disease. The prevalence of this complication among individuals with diabetes is approximately 19-34% and is associated with high rates of disability, hospitalization, and amputation. Chronic hyperglycemia in diabetes leads to neuropathy, angiopathy, and metabolic disorders. Together, these factors contribute to diabetic foot ulcers, with or without infection and neuroarthropathy. The condition progresses through callus formation, subcutaneous involvement, and ulceration, respectively [1-3].

Impaired wound healing results from factors such as recurrent infections, insufficient epithelialization, impaired angiogenesis, tissue necrosis, excessive reactive oxygen species (ROS) production, a pro-inflammatory environment, and fluid leakage. Various treatment approaches are used, including debridement, oxygen therapy, dressings, negative-pressure wound therapy, immunomodulatory hydrogels, immunotherapy, and platelet therapy. Therefore, identifying innovative and effective methods to enhance wound healing in these patients is important [4, 5].

Exosomes are nanovesicles secreted for intercellular communication. These mediators are released by a diverse array of cells under both physiological and pathological conditions. Various metabolites, mRNAs, miRNAs, proteins, DNA fragments, and lipids are released from cells in the form of lipid-bilayer vesicles [6, 7].

Platelets contribute to multiple aspects of wound healing by releasing growth factors, cytokines, and various extracellular matrix regulators. These effects include modulation of endothelial cells and angiogenesis, effects on fibroblasts, repair of injured connective tissues, and support for mesenchymal cell differentiation [8].

Preclinical studies indicate that platelet-derived exosomes can enhance wound healing by modulating the inflammatory response through a shift in macrophage phenotype toward an anti-inflammatory form (M2 phenotype), promoting re-epithelialization through YAP activation, increasing the proliferation and migration of stem cells, and stimulating angiogenesis via the AKT and ERK signaling pathways [9,10]. Thus, the objective of this study was to examine the safety and efficacy of platelet-derived exosomes for diabetic foot ulcers in a clinical trial.

MATERIALS AND METHODS

Plateletpheresis

Plateletpheresis products were collected from volunteer blood donors (two women and five men; mean age, 56 years) at the Iranian Blood Transfusion Organization (IBTO) according to standard protocols. Acid-citrate dextrose (ACD) was used as an anticoagulant.

Exosome isolation

Initially, the sample was centrifuged twice, and the supernatant was isolated ($1000 \times g$ for 5 minutes, followed by $671 \times g$ for 15 minutes). The supernatant obtained from the previous centrifugation step was diluted with PBS at a 1:2 ratio. Ultracentrifugation was performed in two stages at the same speed ($100000 g$ for 30 minutes). The pellet produced at each stage was used in the subsequent procedure. In the final step, the exosome suspension was stored at -80°C for subsequent experimental procedures.

Characterization of exosomes

DLS

The sample was diluted 20-fold with PBS. Approximately $300 \mu\text{L}$ of platelet-derived exosome suspension was used to evaluate nanoparticle size using a HORIBA device (HORIBA, JAPAN). The measurement was performed at 25°C .

TEM

The procedures included mixing the exosome suspension with 2% PFA (paraformaldehyde), fixing it on a Formvar carbon-coated 300-mesh copper grid (EMS-USA), wetting it with PBS, adding 1% glutaraldehyde for chemical fixation, applying uranyl oxalate solution at $\text{pH}=7$ for standard contrast, and adding UA-methylcellulose for positive-negative contrast according to a specific protocol. Finally, the sample was examined using a TEM (Zeiss, EM10C) at an accelerating voltage of 100 kV.

Flow cytometry

A $100\mu\text{L}$ aliquot of exosomes (0.7 mg/ml) was incubated with $5\mu\text{L}$ of CD41 antibody (EXBIO Praha, a.s, Vestec, Czech Republic) at 4°C in the dark for 30 minutes. After the instrument (BD FACSCalibur II, BD Biosciences, USA) was calibrated using beads, the results were analyzed.

Western blotting

Initially, the sample was lysed using a lysis buffer containing 10% SDS (100 mM Tris, 2% SDS,

50 mM DTT, and 300 mM NaCl) and supplemented with a protease inhibitor cocktail. Next, the proteins were separated by electrophoresis on a polyacrylamide gel containing SDS (SDS-PAGE) and then transferred to a PVDF membrane. In the next stage, 1% non-fat dry milk (NFD) in Tris-buffered saline containing 0.1% Tween 20 detergent (TBST) was used for blocking. The membrane was incubated with a CD63 mouse monoclonal antibody (MX-49.129.5; sc-5275) at a 1/1200 dilution. In the next step, a peroxidase-conjugated secondary antibody (anti-rabbit IgG-HRP; sc-2357) was used. Hydrogen peroxide in TBS and 0.5 mg/ml diaminobenzidine (DAB) substrate were used for detection. A limitation of this study was the absence of an appropriate internal/loading control for certain targets, which may affect the robustness and quantitative interpretation of the protein expression data.

BCA protein assay

Total protein in the sample was quantified using the Pierce BCA Protein Assay Kit according to the manufacturer's protocol (Thermo Fisher Scientific, USA).

Real-time PCR

Following culture and passage of the HDF-1 cell line (human dermal fibroblasts) in DMEM containing 10% FBS, the cells were treated with 50 µg/ml of exosomes for 24 hours. HDF-1 cells without exosome treatment were used as a negative control. In the subsequent steps, RNA extraction was performed using a kit (Qiagen, Germany), and cDNA synthesis was performed using a cDNA synthesis kit (TaKaRa, Japan) according to the manufacturers' protocols. VEGF and PDGF primers were designed and validated before use in this study. An Amplicon PCR Kit containing SYBR Green master mix and the ABI StepOnePlus detection system (Applied Biosystems, Foster City, CA, USA) was used to assess the expression levels of the target genes.

Study design

A clinical trial of exosome therapy for DFUs using nanovesicles derived from platelets was conducted in 14 patients with diabetes. The sample size was determined based on practical constraints, including limited resources and the availability of eligible participants. This study was conducted at a specialized clinic for diabetes and metabolic diseases (Tehran, IRAN) after ethics approval was obtained from Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1403.099) and the

study was registered in the Iranian Registry of Clinical Trials (IRCT20200413047063N7). Written informed consent was obtained from all patients. Convenience sampling was used, whereby participants were selected based on their availability and willingness to participate. Both the treating physician and the patient were blinded to the administered substance, resulting in a double-blind design.

Inclusion criteria

Inclusion criteria were male or female sex; age ≥ 18 years; type 1 or type 2 diabetes; clinically non-ischemic and non-infected wounds; $TpO_2 = 30$ mmHg or ABI (Ankle Brachial Index) $= 0.7$; chronic wounds not healed within 4 weeks before exosome injection; exclusion of a mechanical cause; and $HbA_{1c} = 7-9\%$.

Exclusion criteria

Exclusion criteria were bone involvement on X-ray images; a clear indication for surgery (vascular reconstruction or skin grafting); 3 or more ulcers on one leg; osteomyelitis; clinical signs of wound infection; wound necrosis not removable by debridement at week 0; malnutrition (Albumin < 2.5 gr/dl); wounds caused by electrical, chemical, or radiation burns; pregnancy or breastfeeding; participation in another clinical trial or receipt of another investigational product within 30 days before treatment; severe kidney failure (eGFR < 30 ml/min); hemodialysis; peripheral vascular disease (PVD); autoimmune disorders; severe heart failure; a current cancer diagnosis; severe liver disease; immunodeficiency disease; hepatitis; active tuberculosis; bleeding disorders; hemophilia; thrombocytopenia; blood dyscrasias; a known mental, physical, emotional, or social disorder; and $Hb < 8$ gr/dl in males or $Hb < 7$ gr/dl in females.

Wound size-related variability was controlled to minimize potential bias in treatment response. A fixed, predetermined dose of exosomes was administered uniformly to all participants regardless of ulcer size (wounds smaller than 5 cm²). Cases requiring modification of the injection volume or administration protocol because of unusually large wounds were excluded. This approach was adopted to maintain homogeneity among the study groups and ensure comparability of outcomes.

Patients

Fourteen patients with type 1 or type 2 diabetes and diabetic ulcers on the soles or toes were studied: seven in the intervention group and seven in the control group. All patients received standard

treatment, including debridement of necrotic tissue and wound dressing. The intervention group received four exosome injections (1.5 ml, 50 µg/ml) at weekly intervals, whereas the control group received a placebo. According to a previous study, the optimal dose for greater efficacy was 50 µg/ml [11]. The exosomes were injected directly around and into the wound center. Intrawound injections were administered in superficial and deeper areas.

Follow-up

The participants in this study were monitored for 4 months, starting from the initial injection and evaluation. Laboratory tests, including CBC, FBS, HbA1C, eGFR, Albumin, ALT, AST, ALP, CRP, Urea, creatinine, and ESR, were performed before study enrollment and at three-month intervals after injection. Participants were instructed to follow wound-care guidelines and avoid putting pressure on the wound. Four injections were administered by a physician at one-week intervals at the wound site (around and in the center of the wound, to a depth of 0.5 cm). Wound examinations were performed in the first, second, third, and fourth weeks and in the fourth month.

At each examination, the following data were documented: a comprehensive patient history, including fever, pain, treatment-related adverse effects, and signs of allergic reactions.

Wound assessment and treatment documentation involved measuring wound dimensions (length, width, and depth) and checking for signs of infection, such as fever, swelling, redness, and purulent exudation, at each scheduled examination. To evaluate wound size and healing

progress, the PUSH system (Pressure Ulcer Scale for Healing) was used; this system considers key factors such as wound area, exudation, and tissue type.

In the intervention group, standard wound care, including debridement of necrotic tissue and appropriate wound dressing, was performed in addition to exosome injection.

Statistical analysis

All analyses were performed using SPSS version 26 (SPSS, Inc., Chicago, IL, USA). Data normality was assessed using the Kolmogorov-Smirnov test. A paired-samples t test or Wilcoxon test was used to examine changes in laboratory test results before and after exosome injection. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Confirmation of exosome identity

Exosome identity was confirmed using different characterization techniques. The mean size of these extracellular vesicles was 88.62 ± 5.10 nm, and the zeta potential and PD index were -30mV and 0.2, respectively (Figure 1). Their spherical morphology was observed using TEM (Figure 3).

The main exosome marker, CD63, was confirmed by Western blotting, and the primary platelet marker, CD41, was confirmed by flow cytometry (Figures 2 and 4). After fibroblasts were treated with PLT-Exos, real-time PCR was used to evaluate VEGF and PDGF gene expression. The results showed that VEGF and PDGF gene expression increased significantly by approximately 3-fold and 4-fold, respectively (Figure 5).

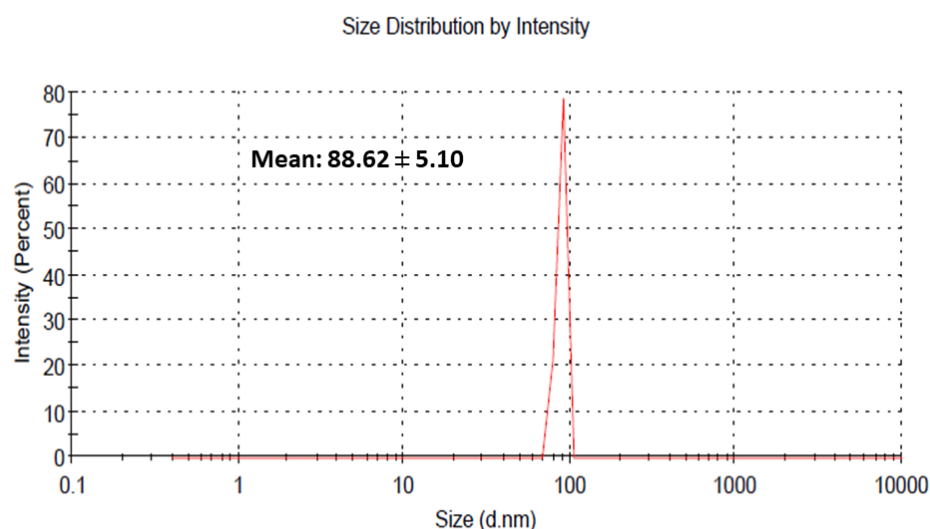


Fig. 1. Characterization of PLT-Exosomes: The result of size measurement by DLS. The mean size of exosomes was 88.62 ± 5.10 .

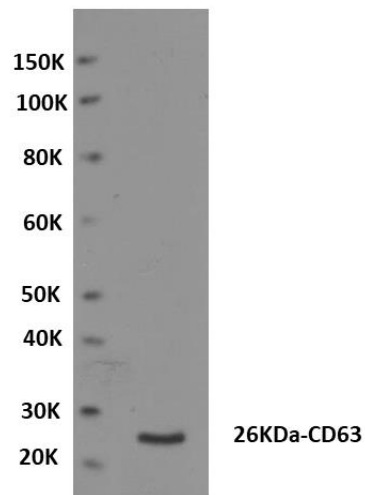


Fig. 2. Characterization of PLT-Exosomes: the expression of CD63 by western blotting

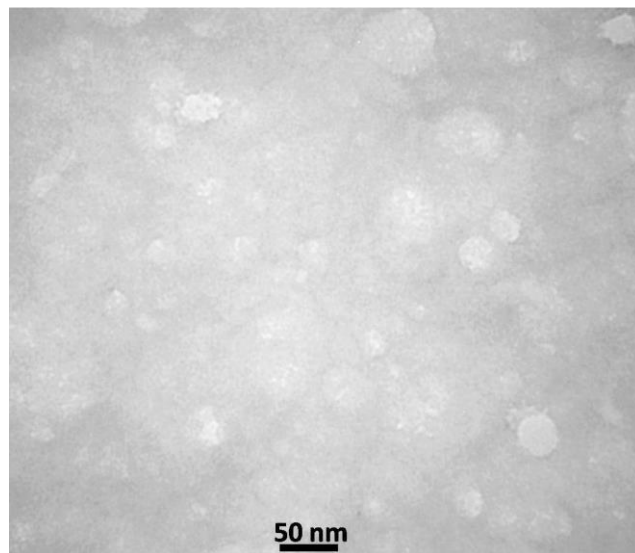


Fig. 3. Characterization of PLT-Exosomes: round morphology of exosomes was confirmed by the TEM method.

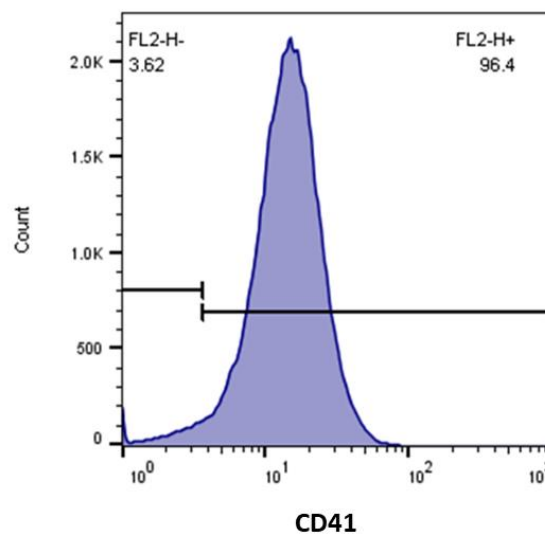


Fig. 4. Characterization of PLT-Exosomes: the expression of CD41 was done by flow cytometry.

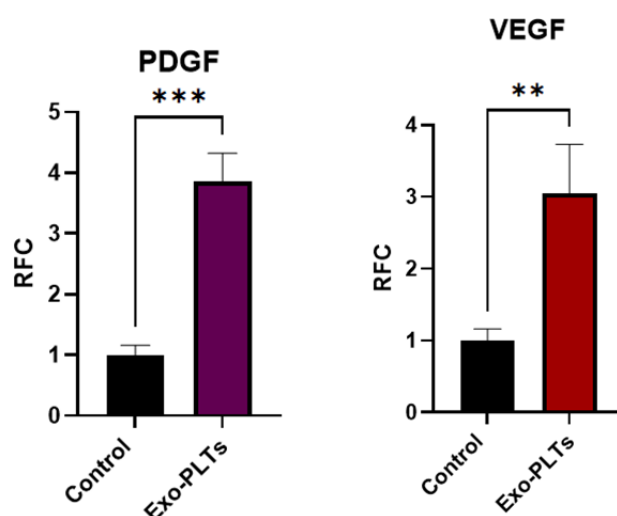


Fig. 5. The results of the gene expression of PDGF and VEGF by Real-Time PCR in Fibroblast cells after treatment with 50 µg/ml of exosomes for 24 hours. The experiments were repeated 3 times, and then the graphs were drawn with the collected data (mean±SE). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Screening of patients

From 2023 to 2025, in accordance with the inclusion and exclusion criteria, 14 patients with grade II DFUs (according to the Wagner criteria) participated in the study. Participants were randomly allocated to the intervention and control groups after providing informed consent. Their mean age was 62 years (age range, 40 to 80 years). In addition, 10 patients were male and 4 were female. All participants received standard care for DFU management. Before each injection, tissue debridement was performed, and a sterile Mepilex foam dressing was applied after PLT-Exo injection.

Safety evaluation

Within 48 hours after each exosome dose, no significant clinical complications, including pain, fever, redness, inflammation, or edema, were observed.

No statistically significant changes were observed in laboratory parameters ($P>0.05$), suggesting no detectable systemic toxicity within the study's monitoring period (Tables 1 and 2).

Efficacy assessment

Among the 7 patients in the intervention group, after 4 exosome doses were administered at regular intervals over 1 month, the wounds of two patients healed completely after two and four injections, respectively (Figures 6 and 7).

A notable decrease in wound size was observed in three patients after four doses administered over one month. Wound dimensions did not change in one patient and increased in another patient (Figures 6 and 7). The final PUSH scores were provided at various stages of the treatment process to monitor wound-healing progress (Figure 7).

Table 1. The results of laboratory tests in involved patients before PLTs-Exo injection and 1 month after injection. Statistical analyses were performed using paired T-Test or Wilcoxon test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

CRP	Albumin (gr/dL) P=0.5	ALP (U/L) P=0.07	AST (U/L) P=0.8	ALT (U/L) P=0.2	eGFR (ml/min) P=0.1	Creatinine (mg/dL) P=0.5	BUN (mg/dL) P=0.5	Urea (mg/dL) P=0.6	HbA1c (%) P=0.1	FBS (mg/dL) P=0.6	PLT ($\times 10^3/\mu\text{L}$) P=0.7	RBC ($\times 10^6/\mu\text{L}$) P=0.9	WBC ($\times 10^3/\mu\text{L}$) P=0.4	PLT-Exo injection group
Neg	4.3	130	31	33	101	0.67	11	21	7.5	108	235	5.2	5.3	Before Patient1
Neg	5.1	110	31	30	125	0.78	13	26	7.3	110	233	5.4	4.9	After Patient1
Neg	5.5	109	10	15	91	1.24	17	19	6.4	75	189	3.99	4.76	Before Patient2
Neg	5.4	121	12	18	92	1.19	18	23	6	90	200	4.2	5.2	After Patient2
Neg	5.3	140	10	8	92	1.3	18	17	7.2	124	309	5	8.6	Before Patient3
Neg	4.9	122	11	10	95	1.25	17	18	7	130	333	4.9	7.7	After Patient3
Neg	2.5	149	24	23	98	0.84	15	26	8.3	151	345	6.01	9.78	Before Patient4
Neg	2.7	131	25	25	94	0.9	14	22	8.6	130	320	5.8	9.1	After Patient4
Neg	4.7	134	27	20	121	1.21	19	18	7.4	144	257	6.32	7.15	Before Patient5
Neg	4.6	119	25	22	133	1.11	17	24	7.1	130	240	5.9	7.21	After Patient5
Neg	3.1	145	20	20	110	0.85	16	31	7.6	97	245	5.02	7.9	Before Patient6
Neg	3	135	19	21	116	0.77	19	29	7.2	80	250	5.1	7.7	After Patient6
Neg	3.1	122	19	25	144	0.78	15	21	7.5	89	201	5.01	5.5	Before Patient7
Neg	3.4	111	24	28	140	0.76	16	17	7.5	100	189	5.3	6.1	After Patient7

Table 2. The results of laboratory tests in involved patients before PLTs-Exo injection and 4 months after injection. Statistical analyses were performed using paired T-Test or Wilcoxon test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

CRP	Albumin (gr/dL) P=0.08	ALP (U/L) P=0.7	AST (U/L) P=0.9	ALT (U/L) P=0.3	eGFR (ml/min) P=0.3	Creatinine (mg/dL) P=0.2	BUN (mg/dL) P=0.1	Urea (mg/dL) P=0.4	HbA1c (%) P=0.5	FBS (mg/dL) P=0.2	PLT ($\times 10^3/\mu\text{L}$) P=0.3	RBC ($\times 10^6/\mu\text{L}$) P=0.8	WBC ($\times 10^3/\mu\text{L}$) P=0.9	PLT-Exo injection group
Neg	4.3	130	31	33	101	0.67	11	21	7.5	108	235	5.2	5.3	Before Patient1
Neg	3.9	145	39	28	121	0.61	12	25	7	120	280	5.1	6.1	4 months Patient1
Neg	5.5	109	10	15	91	1.24	17	19	6.4	75	189	3.99	4.76	Before Patient2
Pos	4.1	130	25	22	97	0.9	15	23	7	101	163	4.5	5.9	4 months Patient2
Neg	5.3	140	10	8	92	1.3	18	17	7.2	124	309	5	8.6	Before Patient3
Neg	4.1	135	11	13	102	0.99	15	20	7.2	121	290	4.8	7.5	4 months Patient3
Neg	2.5	149	24	23	98	0.84	15	26	8.3	151	345	6.01	9.78	Before Patient4
Neg	3.8	139	27	30	100	1.01	16	27	7.9	141	288	5.9	7.8	4 months Patient4
Neg	4.7	134	27	20	121	1.21	19	18	7.4	144	257	6.32	7.15	Before Patient5
Neg	3.6	130	21	19	110	0.89	19	20	7.5	127	179	5.96	5.8	4 months Patient5
Neg	3.1	145	20	20	110	0.85	16	31	7.6	97	245	5.02	7.9	Before Patient6
Neg	3.8	151	27	27	95	0.89	20	28	6.9	102	201	5.3	9.1	4 months Patient6
Neg	3.1	122	19	25	144	0.78	15	21	7.5	89	201	5.01	5.5	Before Patient7
Neg	4.1	120	24	20	104	0.9	18	22	7.3	95	220	5.1	6.8	4 months Patient7

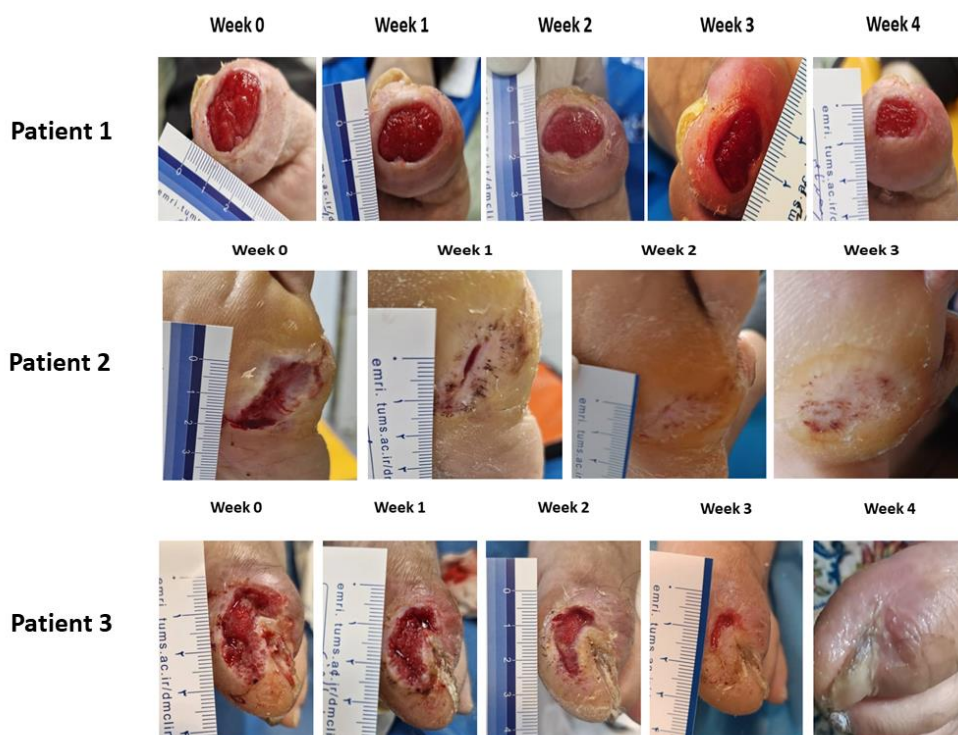
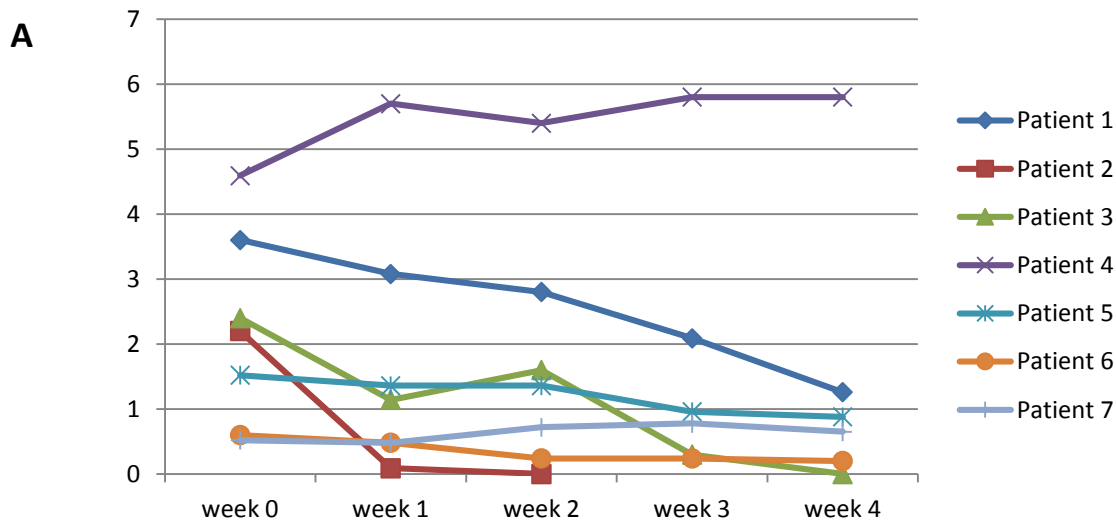


Fig. 6. The results of wound size in 3 patients of the intervention group at 5 weeks of follow-up.



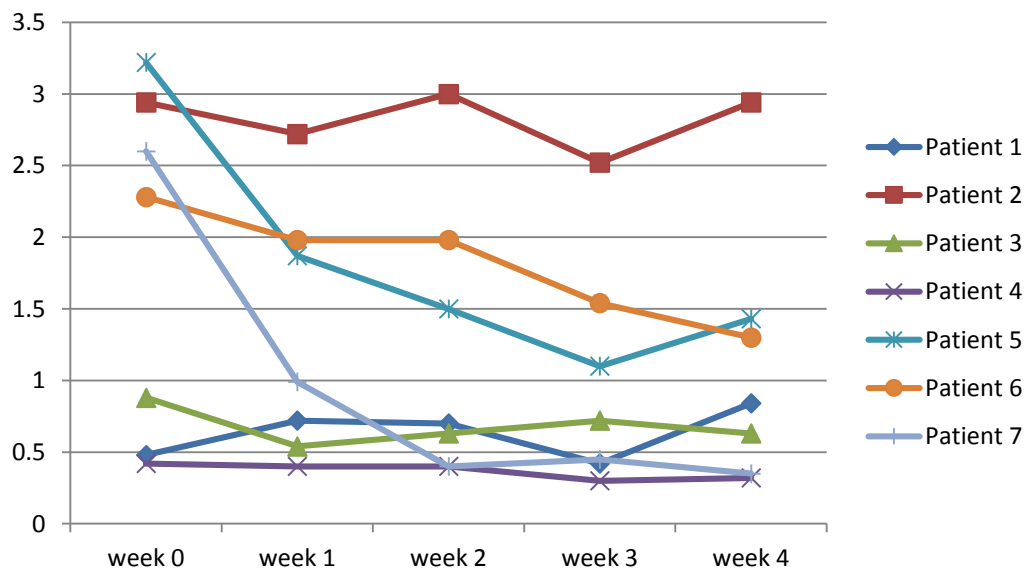
B

Patient	Week 0	Week 1	Week 2	Week 3	Week 4
1	8	8	7	7	6
2	8	2	0	0	0
3	8	7	5	3	0
4	9	9	9	9	9
5	7	7	7	4	4
6	4	4	2	2	2
7	4	4	5	4	3

Fig. 7. (A) The graphs of wound area (cm^2) as length and width in 5 weeks of follow-up after injection of PLT-Exo in the intervention group. (B) The wound score was evaluated using the PUSH system during treatment with exosomes.

Patient 1**Patient 2**

Fig. 8. The results of wound size in 2 patients of the control group at 5 weeks of follow-up.

A

B

Patient	Week 0	Week 1	Week 2	Week 3	Week 4
1	4	5	5	3	4
2	8	7	7	6	6
3	4	3	3	4	3
4	3	3	3	3	3
5	9	7	7	5	5
6	7	6	6	6	6
7	8	6	5	3	3

Fig. 9. (A) The graphs of wound area (cm²) as length and width in 5 weeks of follow-up in the control group. (B) The wound score was evaluated using the PUSH system in the control group.

The wound tissue of patients 1 and 4 remained granulated throughout treatment, whereas the wound tissue of patient 6 was granulated during the first two weeks and that of patient 7 was granulated during the first three weeks. The wound tissue of patient 3 contained slough during the first two weeks, and the wound tissue of patient 5 contained slough during the first three weeks. The wounds of all patients showed no exudate throughout treatment.

In contrast, the wound-healing process in the control group was inadequate within the one-month follow-up period, and none of the wounds closed completely. However, a reduction in wound size was observed in 3 patients (Figures 8 and 9).

DISCUSSION

Diabetic foot ulcers are among the most common and costly complications of diabetes and are often associated with hospitalization, amputation, and reduced quality of life. Various dressings, debridement, blood glucose management, antibiotics for infection control, oxygen therapy, NPWT, offloading, and cell therapy are used to treat this disorder [5,12-14]. However, several challenges remain, including an inadequate response in chronic wounds, high costs, prolonged treatment duration, resistance to treatment, and a lack of targeted solutions [15, 16]. Consequently, there is a clear need to develop innovative and effective treatments, such as exosome therapy.

The beneficial effects of platelets have been confirmed in the management of various diseases. More recently, platelet therapy has also been considered for treating different types of wounds. Platelets and platelet-derived exosomes contain important components, including growth factors [17-20].

Initially, a size difference was observed between the methods used in our study to confirm exosome identity. Measurement of exosome size using DLS and TEM showed a difference of approximately 40 nm. This difference could be due to various reasons, including differences in sample preparation protocols or exosome aggregation caused by high-

speed centrifugation, and it does not preclude validation of the results, as similar discrepancies have been reported in other studies [21]. In addition, CD41 is one of the main platelet markers, and we used flow cytometry to confirm its presence in the isolated exosomes, in accordance with other studies [22].

This study investigated the expression of two important tissue-repair factors, PDGF and VEGF, in vitro, and the results showed significant increases in their expression compared with the control group. VEGF is involved in angiogenesis in injured tissues [23]. PDGF is an important factor in osteogenesis, remodeling, and tissue regeneration during wound healing [24].

White *et al.* demonstrated that VEGF-PIGF-2, PDGF-BB-PIGF-2, and HB-EGF-PIGF-2, when used in combination, played an effective role in treating chronic diabetic ulcers [25]. Therefore, one mechanism contributing to wound healing in our patients may involve the effects of growth factors such as PDGF and VEGF on fibroblasts at the wound site.

The primary aim of this study was to investigate the safety of platelet-derived exosomes. The safety of exosomes was investigated from various perspectives, including the examination of primary reactions, secondary reactions, and possible exosome toxicity in different organs. Various immunopathological reactions may cause skin complications, such as delayed hypersensitivity reactions and chronic granulomatous inflammation. These reactions could be due to the source of the exosomes, residual cellular debris during exosome preparation, the presence of endotoxins, or bacterial byproducts [26-30].

In our study, no clinical complications, such as fever, redness, inflammation, swelling, increased heart rate, or increased respiratory rate, were observed within 48 hours after injection. Post-treatment serum biochemistry (ALT, AST, and creatinine) showed no statistically significant deviations from baseline values ($p > 0.05$), indicating no detectable systemic organ toxicity. Jancy Johnson *et al.* reported the safety of platelet-

derived exosomes in a study of 11 patients with chronic wounds [31]. The safety of exosomes derived from different cell types in wound healing has been reported in numerous studies [32]. However, skin reactions after injection of biological agents have been reported. For example, multiple foreign-body granulomas were reported shortly after dermal injection of mesenchymal stem cell-conditioned medium [28]. Nevertheless, platelet-derived exosomes appeared safe under the conditions of this study and warrant further clinical investigation.

In addition to evaluating the safety of exosomes, the rate of wound healing was also assessed in terms of wound size, time to wound closure or shrinkage, and reduction in wound depth.

Clinical studies have reported shorter healing times and the effectiveness of PRP in reducing the size of diabetic foot ulcers [33].

Chen *et al.* demonstrated a key role for MALAT1 in PLT-Exo-mediated healing of DFUs. In primary DFU fibroblasts, they reported downregulation of MALAT1 and overexpression of miR-374a-5p. Following PLT-Exo treatment, however, MALAT1 expression increased and miR-374a-5p expression decreased. MALAT1 may inhibit pyroptosis and apoptosis in DFU fibroblasts [34].

Jayasuriya *et al.* reported increased TNF- α and IL-6 as pro-inflammatory factors and decreased IL-10 as an anti-inflammatory factor. MALAT1, through a positive feedback mechanism involving Nrf2 and HIF-1 α , enhances VEGF expression and may thereby facilitate angiogenesis [35].

An important factor in the chronicity of diabetic ulcers is chronic hyperglycemia. This condition causes inflammation, neuropathy, and impaired angiogenesis [36]. In this study, blood glucose levels were assessed throughout the study, including before and after injection, in all patients. The wound of one patient closed completely after two injections with HbA1c:6.4%, and the wound of another patient closed after four injections with HbA1c:7.2% (Figures 6 and 7). The American Diabetes Association recommends controlling blood glucose by maintaining HbA1c below 7%. Numerous studies indicate that elevated HbA1c delays wound healing in patients with diabetes [37]. The post-injection HbA1c level in one patient whose wound area did not decrease and whose wound dimensions ultimately increased over time was 8.3% (Figure 7 and Table 1). The study group included two patients with well-controlled blood glucose (HbA1c:6.4) and one patient with poorly controlled blood glucose (HbA1c:8.3), which may be considered a source of bias in this study group. The results for these patients also indicate that blood

glucose control has an important effect on the wound-healing process. Therefore, regulation of blood glucose may have a crucial impact on wound healing.

Malnutrition is frequently observed in patients with diabetic foot ulcers and is associated with longer hospital stays. Conditions specific to patients with diabetes, including hyperglycemia, increased resting energy expenditure, protein loss, and nitrogen imbalance, increase their susceptibility to malnutrition [37, 38]. The nutritional status of patients in this study was similar to that of individuals without diabetes, and they did not receive diets tailored to their medical conditions. Therefore, inadequate nutrition may have contributed to the limited treatment response in some patients.

Another problem faced by the patients in this study was their inability to relieve pressure on the wound during the injection phase, largely because of work-related factors affecting most patients; this may have significantly affected wound-healing outcomes.

This clinical trial had several limitations, including a small sample size, differences in diabetic diets, the influence of glycemic control on wound healing, and the inability of some participants to adhere to offloading because of occupational demands.

CONCLUSION

In conclusion, the present study primarily demonstrates the favorable safety profile of platelet-derived exosomes in wound management. Throughout the study period, no serious adverse events, local complications, or treatment-related toxicities were observed following exosome administration. These findings suggest that platelet-derived exosome therapy can be considered a safe therapeutic approach for wound treatment under the conditions evaluated in this study. Nevertheless, larger-scale clinical studies with extended follow-up and standardized protocols are necessary to confirm these safety outcomes and further investigate potential therapeutic efficacy in different patient populations and wound conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTION

A M: writing, review and editing of article, data collection and analysis, **M S and E NE:** data collection, supervision, formal analysis, methodology, validation ; **M SZ and Sh A:** data collection, validation and formal analysis ; **C R and M R:** data collection

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AI STATEMENT

The authors declare that no artificial intelligence (AI) tools were used in the preparation, writing, or revision of this manuscript.

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