

Berberine improves diabetic foot ulcer healing after tibial transverse osteotomy by inhibiting the PDGF-B/PDGFR β signaling pathway

Rudong Zhang and Huanzhe Jiang*

Department of Orthopedics, Xianju County Traditional Chinese Medicine Hospital, Taizhou, Zhejiang, 317300, China

Abstract: Background: Tibial transverse osteotomy (TTO) is a promising but limited surgical option for diabetic foot (DF). Berberine, a natural compound from *Coptis chinensis*, exhibits anti-inflammatory and pro-angiogenic effects relevant to metabolic diseases. **Objectives:** This study aimed to evaluate whether adjunctive berberine enhances the therapeutic outcomes of TTO in DF patients and to explore its potential association with the platelet-derived growth factor-B (PDGF-B)/PDGF receptor- β (PDGFR β) signaling pathway. **Methods:** This was a prospective, single-center, randomized, placebo-controlled, parallel-group, blinded trial. Of 200 screened DF patients, 96 eligible participants (Wagner grade 2–4) scheduled for TTO were randomized 1:1 to receive either TTO plus local berberine injections (n=48) or TTO plus local saline placebo (n=48). An additional non-randomized diabetic reference group (n=10) and healthy control group (n=10) were included for exploratory cellular studies only. The primary outcome was the total effective rate of ulcer healing at 3 months. Secondary outcomes included changes in Ankle-Brachial Index (ABI), ulcer area, Visual Analogue Scale (VAS) pain score and serum inflammatory markers (hs-CRP, IL-6). Exploratory outcomes comprised serum VEGF, bFGF and PDGF-B levels, as well as fibroblast apoptosis in wound tissue. **Results:** All 96 randomized participants completed the 3-month follow-up, with no dropouts. The berberine group demonstrated a significantly higher total effective rate than the surgery-alone group (87.5% vs. 68.8%; risk difference, 18.7%; 95% CI, 1.2% to 36.2%; $P < 0.05$). At 3 months, the berberine group also showed greater improvements in ABI (mean difference, 0.13; 95% CI, 0.08 to 0.18; $P < 0.01$) and ulcer healing rate (mean difference, 16.7%; 95% CI, 9.5% to 23.9%; $P < 0.01$), along with more significant reductions in hs-CRP and IL-6. Berberine treatment was associated with reduced fibroblast apoptosis in wound tissue (mean difference, -1.10%; 95% CI, -1.56% to -0.64%; $P = 0.001$) and lower protein expression of PDGF-B and PDGFR β . No severe adverse events related to berberine injections were reported. **Conclusion:** In DF patients undergoing TTO, adjunctive local berberine therapy significantly improves the total effective rate and promotes ulcer healing, with benefits associated with reduced inflammation and fibroblast apoptosis and downregulation of the PDGF-B/PDGFR β pathway. These findings are correlative and require further mechanistic validation. Larger multi-center trials are needed to confirm efficacy and safety.

Keywords: Berberine; Diabetic foot; PDGF-B/PDGFR β ; Tibial transverse osteotomy

Submitted on 14-11-2025 – Revised on 24-01-2026 – Accepted on 07-03-2026

INTRODUCTION

Diabetic foot (DF) is one of the most severe chronic complications of diabetes, characterized by foot ulcers, infection and tissue necrosis that may ultimately lead to amputation (McDermott *et al.*, 2023). The global prevalence of diabetes continues to rise, increasing the incidence and burden of DF worldwide (Zhang *et al.*, 2024). The core mechanisms underlying delayed healing of DF wounds involve persistent inflammatory responses, microcirculatory disorders, impaired angiogenesis and fibroblast dysfunction (Littig *et al.*, 2023). Current treatment approaches—including wound debridement, offloading, vascular reconstruction and biological dressings—remain less effective for patients with severe ischemia or extensive tissue necrosis (OuYang *et al.*, 2024). In recent years, tibial transverse osteotomy (TTO) has emerged as a novel orthopedic regenerative therapy for ischemic diabetic foot (Liu *et al.*, 2024). This technique involves lateral displacement of a small tibial bone segment, creating sustained mechanical tension that activates endogenous tissue repair mechanisms and

promotes angiogenesis—an effect known as "tension-induced angiogenesis" (Liu *et al.*, 2022). However, TTO alone has limitations, including relatively slow onset of effect, insufficient response to complex or infected wounds and limited regulation of diabetes-related molecular dysregulation (Liu *et al.*, 2025). Therefore, exploring adjunctive therapies that can enhance TTO efficacy at the molecular level is of significant importance.

Berberine, an isoquinoline alkaloid extracted from *Coptis chinensis* (Huanglian), has well-documented anti-hyperglycemic, anti-inflammatory and antioxidant activities (Kadhim *et al.*, 2025). It exerts protective effects in various diabetic complications by activating pathways such as AMPK and Nrf2 (Yanru *et al.*, 2025; Hu *et al.*, 2024). Notably, the platelet-derived growth factor-B (PDGF-B) and its receptor β (PDGFR β) signaling pathway plays a crucial role in regulating vascular maturation and tissue repair. Its abnormal activation is closely associated with impaired healing of diabetic chronic wounds (Li *et al.*, 2023). However, it remains unclear whether berberine can modulate this pathway in the context of TTO.

*Corresponding author: e-mail: jiangbigai650@163.com

Building on the mechanobiological effects of TTO in promoting tissue regeneration, this study hypothesizes that the combined application of berberine can further modulate the PDGF-B/PDGFR β signaling pathway, thereby alleviating inflammation and fibroblast apoptosis and synergistically promoting angiogenesis and ulcer healing. This study aims to verify whether the combination of TTO and berberine yields superior therapeutic outcomes compared to surgery alone and to explore its potential mechanisms. Abbreviations are listed in table 1.

MATERIALS AND METHODS

Study subjects and recruitment and grouping

This study is a prospective, randomized controlled clinical trial. Clinical data and pathological specimens of 200 patients with diabetic foot (DF) ulcers who received surgical and pharmacological interventions were selected from the case system of Xianju County Traditional Chinese Medicine Hospital. All patients visited Xianju County Traditional Chinese Medicine Hospital's outpatient clinics or inpatient wards between December 2023 and December 2024, with preliminary assessments performed by qualified podiatrists or endocrinologists. Eligible patients were randomly assigned to different treatment groups and all patients provided informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Age 40–75 years; meeting the World Health Organization (WHO) diagnostic criteria for type 2 diabetes (Cosentino *et al.*, 2020), with a disease duration ≥ 5 years; Diagnosed with Wagner grade 2–4 diabetic foot ulcers (i.e., deep ulcers with or without abscess, osteomyelitis, or localized gangrene) (Camilleri *et al.*, 2020). Ankle-brachial index (ABI) between 0.4 and 0.9, indicating moderate to severe lower limb ischemia. Ulcer duration ≥ 4 weeks, with no significant healing tendency after at least 4 weeks of standard medical treatment (including glycemic control, wound debridement and anti-infection therapy). Patients provided informed consent and were willing to cooperate with all follow-up procedures.

Exclusion criteria: Severe cardiac, hepatic, or renal dysfunction (e.g., NYHA functional class III – IV heart failure, Child-Pugh class C liver dysfunction, or estimated glomerular filtration rate < 30 mL/min/1.73 m²). Active malignant tumors or immune system diseases. Acute deep vein thrombosis or complete occlusion of major arteries in the affected limb with no possibility of revascularization. Known allergy to berberine or its formulation components. Pregnant or lactating women. Severe psychiatric disorders or cognitive impairment that would hinder compliance with treatment and follow-up.

Study grouping and design

A total of 96 eligible patients were randomized in a 1:1 ratio using a computer-based random number generator.

They were allocated into the following two groups: Surgery group (n= 48): Patients received standard Tibial Transverse Osteotomy (TTO) surgery only, followed by local injections of an equal volume of sterile saline solution around the incision postoperatively. Berberine group (n= 48): Patients underwent TTO surgery and additionally received local injections of berberine postoperatively. The CONSORT flow diagram for participant enrollment, allocation, follow-up and analysis is presented in Fig. 1.

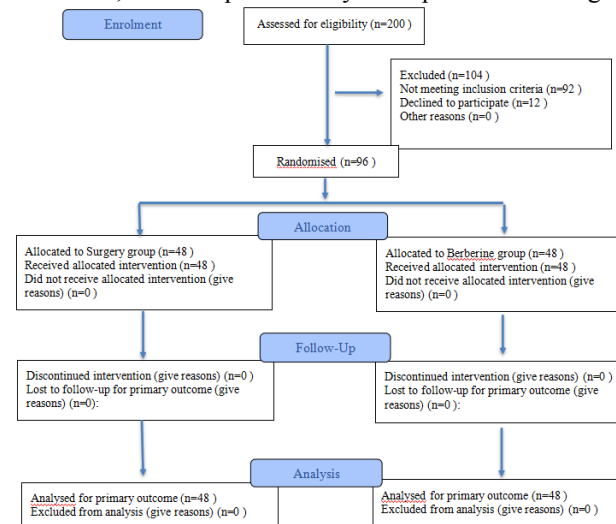


Fig. 1: CONSORT 2025 flow diagram

The randomization sequence was generated and kept sealed (allocation concealment) by an independent statistician who was not involved in patient recruitment or evaluation. Due to the nature of the local injection procedure, it was not feasible to blind the surgeons performing the injections. However, the patients were blinded to their group assignment (berberine vs. saline). All outcome assessors, including podiatrists who evaluated wound healing, technicians who performed ABI and Doppler ultrasound measurements and laboratory personnel who conducted ELISA, Western blot and TUNEL assays, were blinded to the group assignments. The blinding was maintained by having an independent research nurse (not involved in patient assessment) prepare the injectate (berberine or saline) in coded, opaque syringes labeled only with the patient's randomization number. The code was broken only after the final data analysis was completed.

Sample size calculation: Based on preliminary pilot data, with an effect size (d) of 0.8, $\alpha = 0.05$ and $\beta = 0.2$, a minimum of 45 patients per group was required. Accounting for an estimated dropout rate, 48 patients were ultimately enrolled per group.

To establish baseline references and provide tissue/cell samples for in vitro mechanistic studies (such as the TUNEL assay and primary fibroblast culture), two

additional non-randomized control groups were included for exploratory and reference purposes only and were not intended for direct statistical comparison of clinical efficacy with the randomized groups:

Diabetes group ($n = 10$): Patients meeting the DF diagnostic criteria (Wagner grade 2–4) (Camilleri *et al.*, 2020) but who did not undergo TTO surgery due to patient preference, relative surgical contraindications, or resource limitations. These patients continued to receive the same conventional medical treatment as before surgery.

Normal group ($n = 10$): Healthy volunteers without a history of diabetes, peripheral vascular disease, or skin diseases, recruited from the community and matched approximately by age and sex to the DF patient groups.

The demographic and baseline clinical characteristics of all patients in each group are summarized in table 2, with no statistically significant differences observed among the groups.

Methods

Intervening measure

Surgical procedure of tibial transverse osteotomy

All patients received prophylactic intravenous antibiotic therapy (cefazolin sodium, 2 g, 30 minutes before surgery), which continued until 48 hours postoperatively. Blood glucose levels were managed using an insulin pump or intensive subcutaneous insulin therapy, with the target of maintaining preoperative and postoperative glucose levels within 6.1–10.0 mmol/L for the first 72 hours. During the surgery, a proprietary precision bone transport intelligent management system (Registration No.: 9586806) was utilized to harvest a rectangular bone block measuring approximately 5 cm × 1.5 cm from the anteromedial cortex of the tibia. An external fixator was installed to ensure the stability of the bone block. The incision was covered with sterile dressings and inspected regularly.

Bone transport commenced on the 5th postoperative day, involving lateral transverse traction at a rate of 1 mm per day for 7 days. This was followed by medial return transport at the same speed, frequency and duration. The external fixator was worn for approximately 4 weeks in total (Fig. 2). During this period, regular wound dressing changes were performed to prevent infection.

Administration of berberine

Berberine (purity $\geq 98\%$, Sigma-Aldrich, Cat. No. B3251) was administered starting from the first day after TTO surgery. Under strict aseptic conditions, multiple subcutaneous injections were performed in the tissue approximately 1–2 cm around the surgical incision. The total dosage was 0.1 mg/kg, administered twice per week for a duration of 4 weeks. This dosing regimen was based

on previous animal studies investigating the topical application of berberine for promoting skin wound healing (Alkholifi *et al.*, 2023). The selected dose demonstrated good safety and effective local transdermal penetration in preliminary experiments. The injection volume was calculated according to the patient's body weight, with berberine dissolved in 1.0 mL of sterile saline as the solvent.

Patients in the surgery group received injections of an equal volume of sterile saline at the same time points and sites as a control.

Sample collection and processing

(1) Skin sample collection

On postoperative day 14, under local anesthesia, a 3 mm skin biopsy punch was used to collect a full-thickness skin tissue sample (approximately 3 × 3 mm in size) from a site 0.5 cm outside the ulcer edge. One sample was collected per patient. The sample was immediately divided into two parts: one part was fixed in 4% paraformaldehyde for histological analysis and the other part was placed in sterile preservation solution and transported to the laboratory within 2 hours at 4 °C for fibroblast isolation. All patients provided informed consent prior to surgery, agreeing to the use of their samples for research purposes.

(2) Primary culture and identification of fibroblasts

Based on a published method (Kisiel and Klar, 2019) with appropriate modifications. The brief procedure is as follows: After rinsing the skin tissue with D-Hanks solution containing antibiotics, the tissue was minced and sequentially digested with 0.2% neutral protease (Sigma, Cat. No. N0632) and 0.25% trypsin-EDTA (Gibco, Cat. No. 25200056) to obtain dermal cells. The cells were resuspended in DMEM medium supplemented with 10% fetal bovine serum (Gibco, Cat. No. 26140079) and cultured in a 37 °C, 5% CO₂ incubator. Cells from passages 3–5 were used for experiments. Identification was performed through immunocytochemical staining for Vimentin and Type I collagen expression. Cells with a positive rate $> 95\%$ were used for subsequent experiments.

Molecular biological detection

TUNEL assay

Skin samples from diabetic foot patients were collected for paraffin sectioning. The samples were de-waxed, rehydrated and treated with proteinase K to enhance cell permeability. The sections were then incubated with the TUNEL reaction mixture at a 1:9 ratio, under specified temperature conditions. After incubation, peroxidase (POD) was used as a converter and DAB was added to induce color development. The sections were counterstained with hematoxylin. Following routine dehydration and clarification, the sections were mounted with neutral resin.

Table 1: List of abbreviations

Abbreviation	English full name
ABI	Ankle-brachial index
bFGF	Basic fibroblast growth factor
DF	Diabetic foot
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
HIF-1 α	Hypoxia-inducible factor-1 α
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
IL-10	Interleukin-10
MMP	Matrix metalloproteinase
PDGF-B	Platelet-derived growth factor-B
PDGFR β	Platelet-derived growth factor receptor- β
PI	Pulsatility index
RI	Resistance index
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
TTO	Tibial transverse osteotomy
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VAS	Visual analog scale
VEGF	Vascular endothelial growth factor

Table 2: Clinical features

Group	n	Gender [n (%)]		Age (years, mean $\pm$ SD)
		Male	Female	
Surgery group	48	26(54.17)	22(45.83)	57.85 $\pm$ 6.58
Berberine group	48	28(58.33)	20(41.67)	59.38 $\pm$ 6.95
Diabetes group	10	5(50.00)	5(50.00)	57.90 $\pm$ 6.45
Normal group	10	6(60.00)	4(40.00)	59.10 $\pm$ 6.84
χ^2/F	-	0.383		0.155
P	-	0.960		0.283

**Fig. 2:** Pre- and post-modification incision conditions.

Negative control samples were included for each experiment. Five to ten high-power fields were randomly selected under the microscope and 1,000 cells were counted to assess the number of brown-stained nuclei. The apoptosis index (AI) was calculated as the

percentage of apoptotic cells out of the 1,000 cells observed. The experiment simultaneously established a positive control (sections treated with DNase I) and a negative control (the reaction system did not contain terminal deoxynucleotide transferase TdT).

Table 3: Primer sequences

Gene		Primer sequence (5'-3')	Product length
PDGF-B	Forward	5'-CTGAGCTGGACTTGAACATGA-3'	380 bp
	Reverse	5'-CACTACTGTCTCACACATTGCAGG-3'	
PDGFR-β	Forward	5'-TCGTCCTCAA-GATTTCGAGC-3'	397 bp
	Reverse	5'-TCATAGGGTACATGTAGGGGGAT-3'	
c-fos	Forward	5'-GTAGAGCCAGCTATCTCCTGA-3'	496 bp
	Reverse	5'-TCCACATCTGGCACAGAGC-3'	
β-actin	Forward	5'-CATGTACGTTGCTATCCAGGC-3'	250 bp
	Reverse	5'-CTCCTTAATGTCACGCACGAT-3'	

Results were considered valid only when typical apoptotic staining occurred in the positive control and no staining was observed in the negative control.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Cat. No. 15596018). Reverse transcription was performed using the PrimeScript RT reagent kit (Takara, Cat. No. RR047A). RT-qPCR was carried out with SYBR Premix Ex Taq II (Takara, Cat. No. RR820A) on a QuantStudio 3 system. Primer sequences are listed in table 3. All primers were designed using Primer-BLAST (NCBI) and validated for specificity in GenBank. Amplification efficiency was confirmed to be between 90% and 110% via standard curves. β-actin was used as the internal reference gene and the relative expression of target genes (*PDGF-B*, *PDGFRβ*, *c-fos*) was calculated using the $2^{-\Delta\Delta C_t}$ method.

Western blot

Total protein was extracted from cell lysates. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% skimmed milk, membranes were incubated with primary antibodies at 4°C overnight. The primary antibodies used were as follows: rabbit anti-human PDGF-B antibody (Abcam, Cat. No. ab23914, 1:1000), rabbit anti-human PDGFRβ antibody (CST, Cat. No. 3169, 1:1000) and mouse anti-human β-actin antibody (Sigma, Cat. No. A5441, 1:5000). After washing, membranes were incubated with corresponding HRP-conjugated secondary antibodies at room temperature for 1 hour, followed by ECL detection. Band intensity was analyzed using ImageJ software and semi-quantification was performed by calculating the ratio of the target protein grayscale value to that of β-actin.

Masson staining

Tissue samples were fixed in 4% paraformaldehyde, followed by dehydration and paraffin embedding. Masson's trichrome staining was used to assess collagen accumulation. For immunohistochemical staining, antigen retrieval was performed by heating,

permeabilization was achieved with 1% Triton X-100 and nonspecific binding sites were blocked with 5% fetal bovine serum. The sections were incubated with anti-HIF-1α and anti-PDGFR-β antibodies (1:100) at specific temperatures. After rinsing with phosphate-buffered saline (PBS), the tissue sections were treated with Alexa Fluor 488-labeled secondary antibodies and incubated for one hour at room temperature. Following this, the nuclei were counterstained using DAPI and the samples were visualized and imaged under a fluorescence microscope. At the same time, a negative control (replacing the primary antibody with phosphate buffered saline PBS) was established to rule out non-specific staining. All staining steps are carried out simultaneously to ensure consistent conditions.

Study outcomes

The primary outcome of this trial was the total effective rate of ulcer healing at 3 months post-intervention, as defined by the comprehensive evaluation criteria (see below).

Secondary outcomes assessed at baseline, 2 weeks and 4 weeks post-intervention included: 1) clinical symptom scores (color, size, pain, exudate); 2) Ankle-Brachial Index (ABI); 3) ulcer area; 4) serum levels of inflammatory markers (hs-CRP, IL-6, IL-10); and 5) dorsal foot artery hemodynamic parameters (pulsatility index, resistance index, vessel diameter and blood flow velocity).

Exploratory outcomes included: 1) changes in serum levels of VEGF and bFGF; 2) protein expression levels of PDGF-B, PDGFRβ and c-fos in wound tissue (assessed by Western blot); 3) fibroblast apoptosis rate in wound tissue (assessed by TUNEL assay); and 4) histological analysis of collagen deposition (Masson staining). These exploratory outcomes were not powered for formal hypothesis testing and are reported to generate hypotheses for future research.

Clinical efficacy evaluation

Clinical efficacy evaluation criteria

Referring to the internationally recognized consensus on the healing assessment of diabetic foot ulcers (Lipsky *et al.*, 2020), a comprehensive evaluation was conducted 3 months after treatment: (1) Cured: Complete healing of

the ulcer wound, with full alleviation and disappearance of coldness and numbness in the limbs. (2) Significant: Healing progress of the ulcer area reaches 85% or more, with significant improvement in coldness and numbness in the limbs. (3) Effective: Healing degree of the ulcer is between 45% and 85%, with improvement in coldness and numbness in the limbs. (4) Ineffective: Healing of the ulcer area is less than 45%, with no improvement in coldness and numbness in the limbs. The total effective rate was calculated as (cured + significantly improved + effective)/total number of cases $\times$ 100%.

Clinical symptom scoring

The responsible laboratory personnel evaluated the color, size, pain degree and exudate of foot ulcers before treatment and at the 2nd and 4th weeks of treatment using a self-made scale. The standards were as follows: (1) Color assessment: Healthy red color, 0 points; pale red, 2 points; dark red, 4 points; purplish dark, 6 points. (2) Size assessment: Reduction of 90%–100%, 0 points; 60%–90%, 2 points; 30%–60%, 4 points; less than 30%, 6 points. (3) Pain assessment: No pain, 0 points; mild or intermittent pain, 2 points; noticeable pain but not affecting sleep, 4 points; severe pain affecting sleep, 6 points. (4) Exudate assessment: No exudate, 0 points; less than 5 ml/day, 2 points; 5–10 ml/day, 4 points; more than 10 ml/day, 6 points. Although this scale has not been extensively validated, the assessment items (color, size, pain, exudation) are commonly used dimensions in clinical wound evaluation. To minimize bias, all assessments were independently conducted by two researchers blinded to patient group assignments. The average of their scores was taken as the final rating. Inter-observer consistency was evaluated by calculating the intraclass correlation coefficient (ICC), with an ICC > 0.85 indicating good reliability.

Ankle-brachial index (ABI) measurement

Before treatment and at the 2nd and 4th weeks of treatment, the Ankle-Brachial Index (ABI) was measured using a fully automatic arteriosclerosis detector (Vasera VS-1500, Fukuda Denshi, Japan). The measurements were performed in a quiet, temperature-controlled environment (22–25°C). After the patient rested in a supine position for 10 minutes, the procedure was conducted by a trained technician who was blinded to the patient's group assignment. The average of three consecutive stable measurements was recorded.

Ulcer area measurement

Ulcer area measurement method: Transparent film tracing combined with computer image analysis software (ImageJ, NIH) was used for measurement. Two independent observers, blinded to the group assignments, performed the measurements separately. The inter-observer correlation coefficient (ICC) was calculated to assess reliability. The final ulcer area was taken as the average of the two measurements.

Serum inflammatory factor determination

Fasting venous blood was collected at baseline and at the 2nd and 4th weeks post-treatment. Serum levels of hs-CRP and IL-6 were measured using commercial ELISA kits (hs-CRP: R&D Systems, Cat. No. DCRP00; IL-6: R&D Systems, Cat. No. D6050). All procedures were performed strictly in accordance with the manufacturer's instructions.

Dorsal foot artery hemodynamic measurement

A color Doppler ultrasound diagnostic device (GE Logiq E9, equipped with a 9L linear array probe) was used to measure the pulsatility index (PI), resistance index (RI), intravascular diameter and peak systolic velocity of the dorsalis pedis artery on the affected side. The measurements, including intravascular diameter and peak systolic velocity, as well as the calculation of the pulsatility index and resistance index, were performed by an experienced ultrasound physician who was blinded to the patients' group assignments. The average of three measurements was recorded.

Detection of serum VEGF and bFGF levels

Fasting venous blood (5 mL) was collected from all subjects in the morning at baseline and at the 2nd and 4th weeks post-treatment. The concentrations of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in serum were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits. The human VEGF ELISA kit was purchased from R&D Systems (USA), catalog number DVE00. The human bFGF ELISA kit was purchased from R&D Systems (USA), catalog number DFB50. All procedures were performed strictly according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6. Continuous data are presented as mean $\pm$ standard deviation. Categorical data are expressed as proportions and analyzed using the chi-square (χ^2) test. For the primary outcome (total effective rate at 3 months), the chi-square test was used, with the risk difference and its 95% confidence interval (CI) calculated. For key secondary outcomes (ABI and ulcer healing rate), analysis of covariance (ANCOVA) was applied to compare changes from baseline to 3 months between the two randomized groups, adjusting for baseline values, with effect estimates accompanied by 95% CIs. For repeated measures data (clinical scores, ABI, serum markers, hemodynamic parameters at baseline, 2 weeks and 4 weeks), a linear mixed-effects model was employed, including group, time and group-by-time interaction as fixed effects and participants as random effects. This approach accommodates missing data under the missing-at-random assumption without

imputation; no missing data occurred for the primary outcome at 3 months. For comparisons among multiple groups at a single time point, one-way ANOVA was used (Tukey's post hoc test for equal variances, Games-Howell for unequal variances); for two-group comparisons, independent samples t-tests were applied. For repeated measures with significant interaction, Bonferroni's post hoc test was used. All in vitro experiments were independently repeated at least three times (biological replicates). Western blot band intensities were analyzed using ImageJ, with semi-quantification as the ratio of target protein to β -actin. All statistical tests were two-sided, with $P < 0.05$ considered significant. Given the exploratory nature of secondary and mechanistic analyses, no adjustment was made for multiple comparisons; results with P-values near 0.05 should be interpreted cautiously.

RESULTS

Human skin fibroblast apoptosis rate

The apoptosis rate of skin fibroblasts in the diabetic foot group was the highest among all groups ($6.59 \pm 0.75\%$), $P < 0.05$. The rate was lower in the surgery group compared to the diabetic foot group, while the apoptosis rate was significantly lower in the berberine group ($2.76\% \pm 0.13\%$) than in the surgery group ($3.86\% \pm 0.42\%$; $t = 7.162$, $P = 0.001$) (Fig. 3).

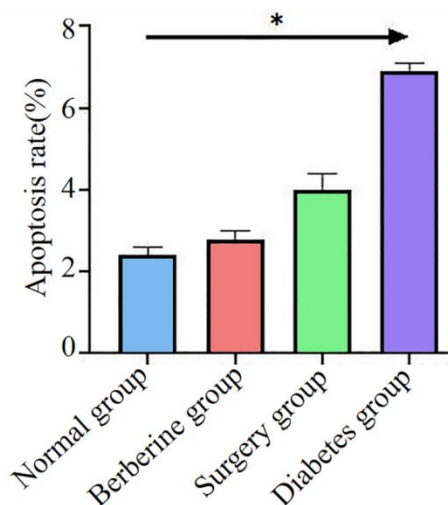


Fig. 3: Human skin fibroblast apoptosis rate ($n = 5$). Note: Data are expressed as mean $\pm$ standard deviation, * $P < 0.05$ Compared with the normal group and the differences between groups are statistically significant.

Tibial transverse osteotomy improves diabetic foot with higher treatment efficacy of berberine

The TTO group achieved a total effective rate of 68.8% (33/48) (Figs. 4A-E), indicating a positive therapeutic effect on diabetic foot. In comparison, the berberine group demonstrated a higher total effective rate of 42

out of 48 cases (Figs. 4A-E). During treatment, the local color and pain scores of the affected limbs in the surgery group gradually decreased and the exudate and ulcer area scores also decreased over time (Figs. 5A-D). The reduction was most significant in the berberine group, $P < 0.05$. Furthermore, ABI and ulcer area were significantly improved, with the berberine group showing a greater improvement in ulcer area compared to the surgery group (Fig. 6A) and the ABI in the berberine group increased significantly compared to the surgery group, $P < 0.05$ (Fig. 6B).

Berberine reduces inflammatory factor expression to improve hemodynamics of the dorsal foot artery

As the treatment duration increased, the levels of inflammatory factors decreased in both groups (Figs. 7A-C). In terms of hemodynamic indicators of the dorsal foot artery, the pulsatility index and vessel diameter gradually increased in both groups, $P < 0.05$, with the most significant improvement in the berberine group, $P < 0.05$ (Figs. 8A-D).

Adverse events and safety monitoring

All 96 randomized participants completed the 4-week injection course and 3-month follow-up. No serious adverse events (anaphylaxis, systemic toxicity, severe local infection, or osteomyelitis) occurred in either group. In the berberine group, mild local adverse events were reported in 6 patients (12.5%): transient injection-site pain ($n=4$) and mild local erythema ($n=2$), all resolving spontaneously within 24–48 hours. In the placebo group, 4 patients (8.3%) reported transient injection pain. The difference was not statistically significant ($P > 0.05$, chi-square test). No patient withdrew due to adverse events. Routine liver and renal function tests at baseline and 4 weeks showed no clinically meaningful changes in either group (data not shown).

Berberine inhibits the PDGF-B/PDGFR β signaling pathway to promote healing of diabetic foot

After berberine administration, Masson's staining revealed a disordered tissue structure, with uneven collagen fiber distribution and potential infiltration of inflammatory cells. On the seventh day post-treatment, tissue structure in the surgery group had improved, with collagen fibers beginning to realign, although inflammation persisted. In the berberine group, collagen fibers are arranged more orderly and there is less infiltration of inflammatory cells (Fig. 9).

At the molecular level, VEGF expression was lower in the berberine group, while bFGF levels showed a marked increase following treatment (Figs. 10A-B). Furthermore, the protein expression of PDGF-B and its receptor PDGFR β decreased in the berberine group compared to the surgery group ($P < 0.05$) (Figs. 10C-D).

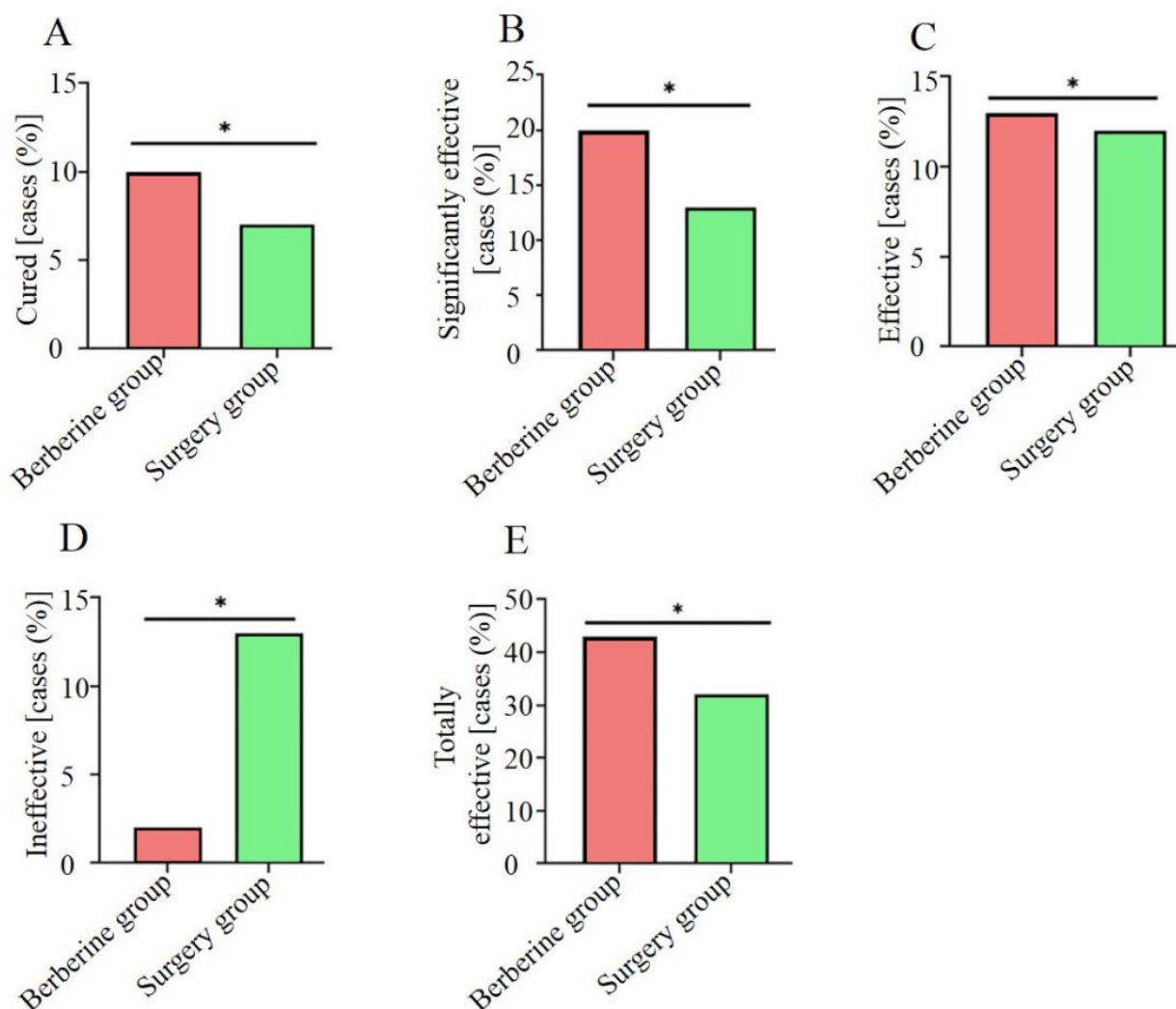


Fig. 4: Therapeutic efficacy evaluation of diabetic foot after treatment (n = 48).

Note: (A) Number of cured cases; (B) Number of significantly improved cases; (C) Number of effective cases; (D) Number of ineffective cases; (E) Total number of effective cases. Compared with the surgery group, the data were expressed as mean $\pm$ standard deviation, *P < 0.05, **P < 0.01, and the differences between the groups were statistically significant.

DISCUSSION

The impaired healing of diabetic foot (DF) ulcers is a complex pathological process involving persistent inflammation, microcirculatory disorders, impaired angiogenesis and dysfunction of reparative cells (Chen *et al.*, 2022). This study evaluated the clinical efficacy of Tibial Transverse Osteotomy (TTO) combined with Berberine in the treatment of DF and explored its potential association with the modulation of the PDGF-B/PDGFR β signaling pathway.

Our results demonstrate that, compared with TTO surgery alone, combined treatment with Berberine significantly improved the overall response rate, ankle-brachial index (ABI), ulcer healing rate and alleviated pain in DF patients. As a mechanical intervention, TTO is believed to promote

local neovascularization through the "tension-induced angiogenesis" effect (Yu *et al.*, 2025; Nie *et al.*, 2020). The addition of Berberine appears to further optimize this reparative microenvironment, as evidenced by enhanced clinical outcomes. These findings align with previous research reporting the anti-inflammatory and pro-reparative activities of Berberine (Wang *et al.*, 2024).

At the cellular level, we observed that Berberine treatment was associated with a significantly lower rate of fibroblast apoptosis in wound tissue compared to the surgery-alone group. This protective effect on a key reparative cell type may be partly attributed to the more pronounced decrease in systemic inflammatory markers (hs-CRP and IL-6) observed in the Berberine group, consistent with its known anti-inflammatory properties (Hu *et al.*, 2023).

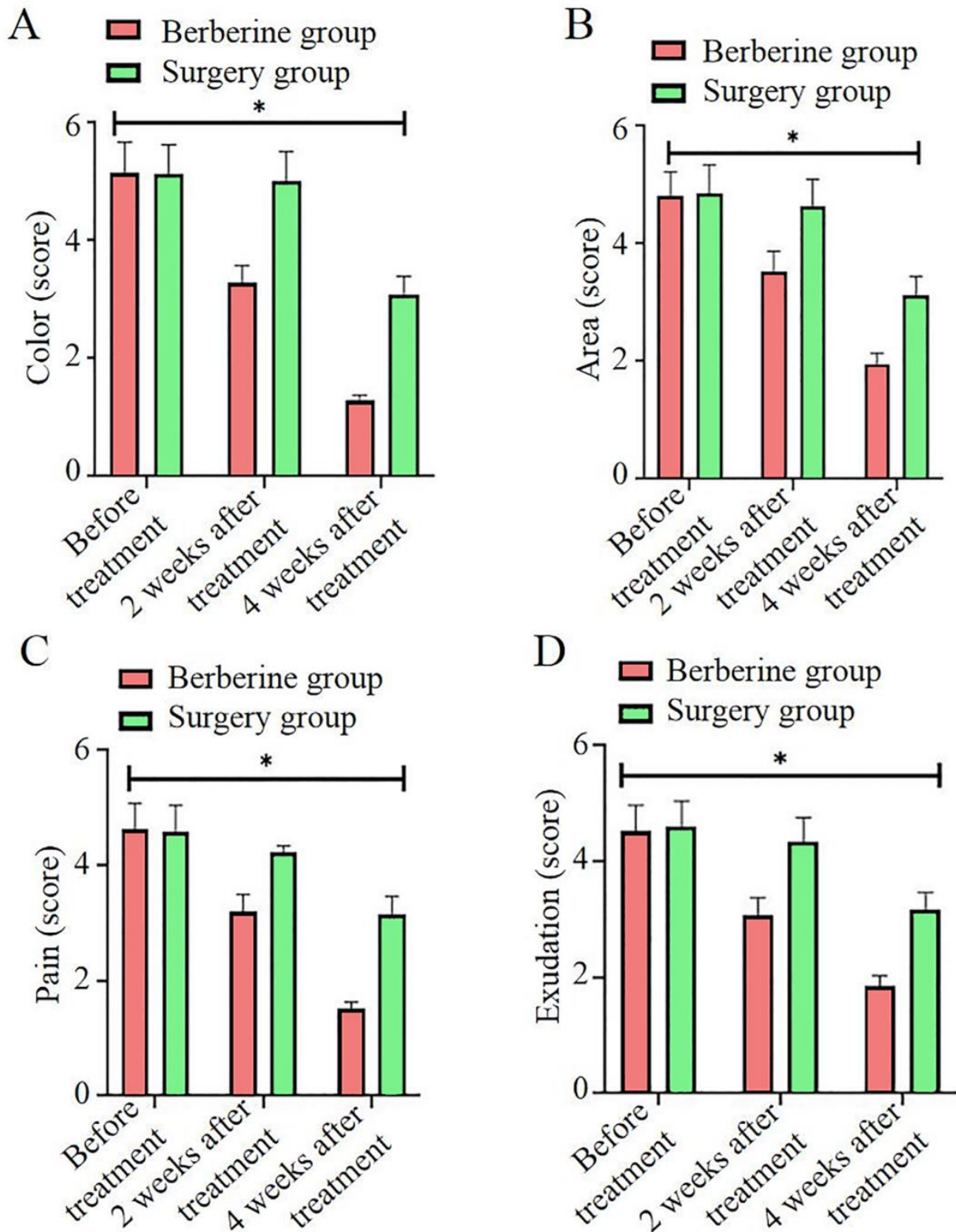


Fig. 5: Evaluation of pain, exudate and other conditions of diabetic foot after treatment (n = 48).

Note: (A) Color score; (B) Area score; (C) Pain score; (D) Exudate score. Compared with the surgery group at 2 and 4 weeks of treatment, the data were expressed as mean $\pm$ standard deviation, * $P < 0.05$, ** $P < 0.01$, and the differences between the groups were statistically significant.

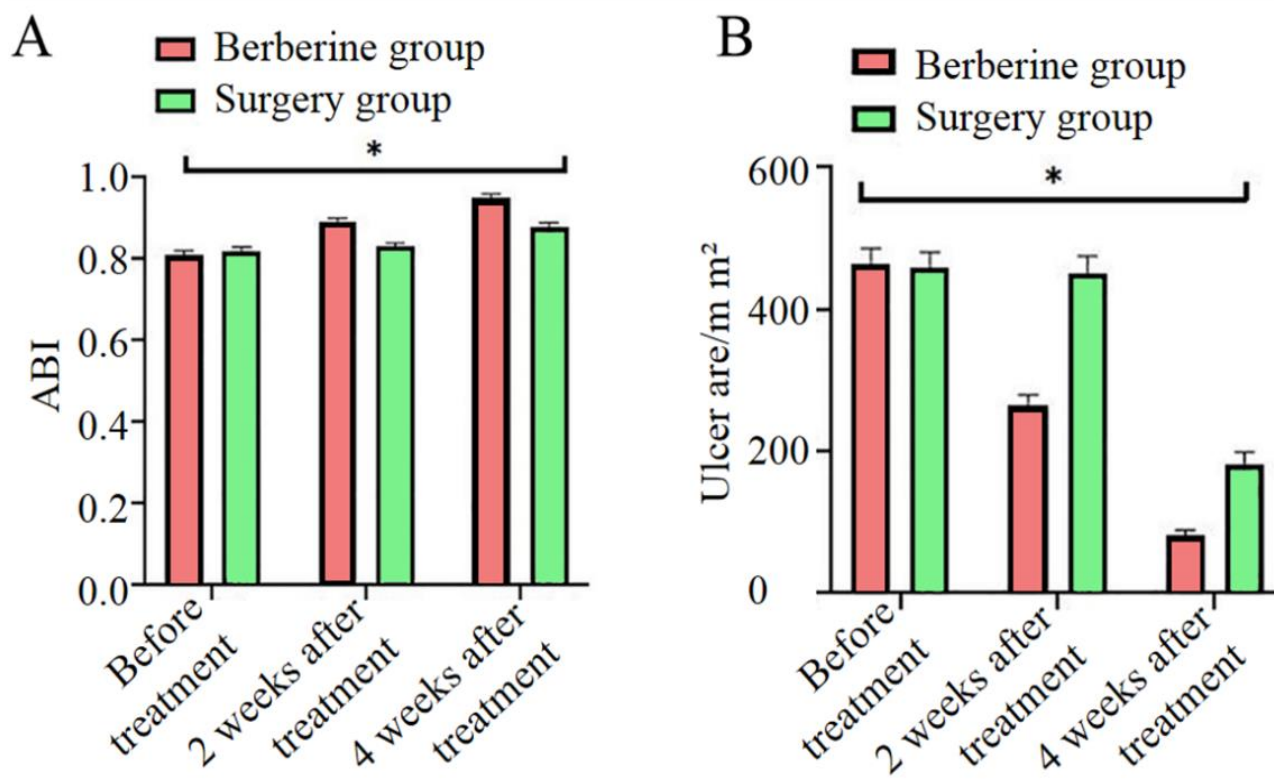


Fig. 6: Improvement of ABI and ulcer area (n = 48).

Note: (A) ABI; (B) Lower limb ulcer area. Compared with the surgery group at 2 and 4 weeks of treatment, the data were expressed as mean $\pm$ standard deviation, *P < 0.05, **P < 0.01, and the differences between the groups were statistically significant.

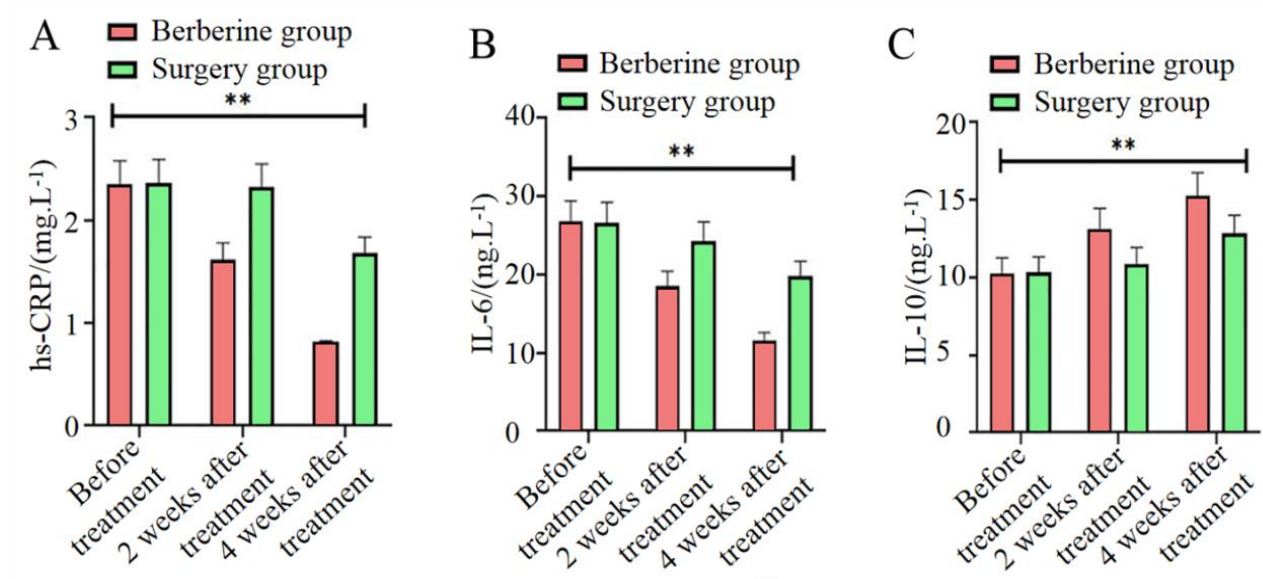


Fig. 7: Improvement of inflammatory indicators during treatment (n = 48).

Note: (A) hs-CRP level; (B) IL-6 level; (C) IL-10 level. Compared with the surgery group at 2 and 4 weeks of treatment, the data were expressed as mean $\pm$ standard deviation, *P < 0.05, **P < 0.01, and the differences between the groups were statistically significant.

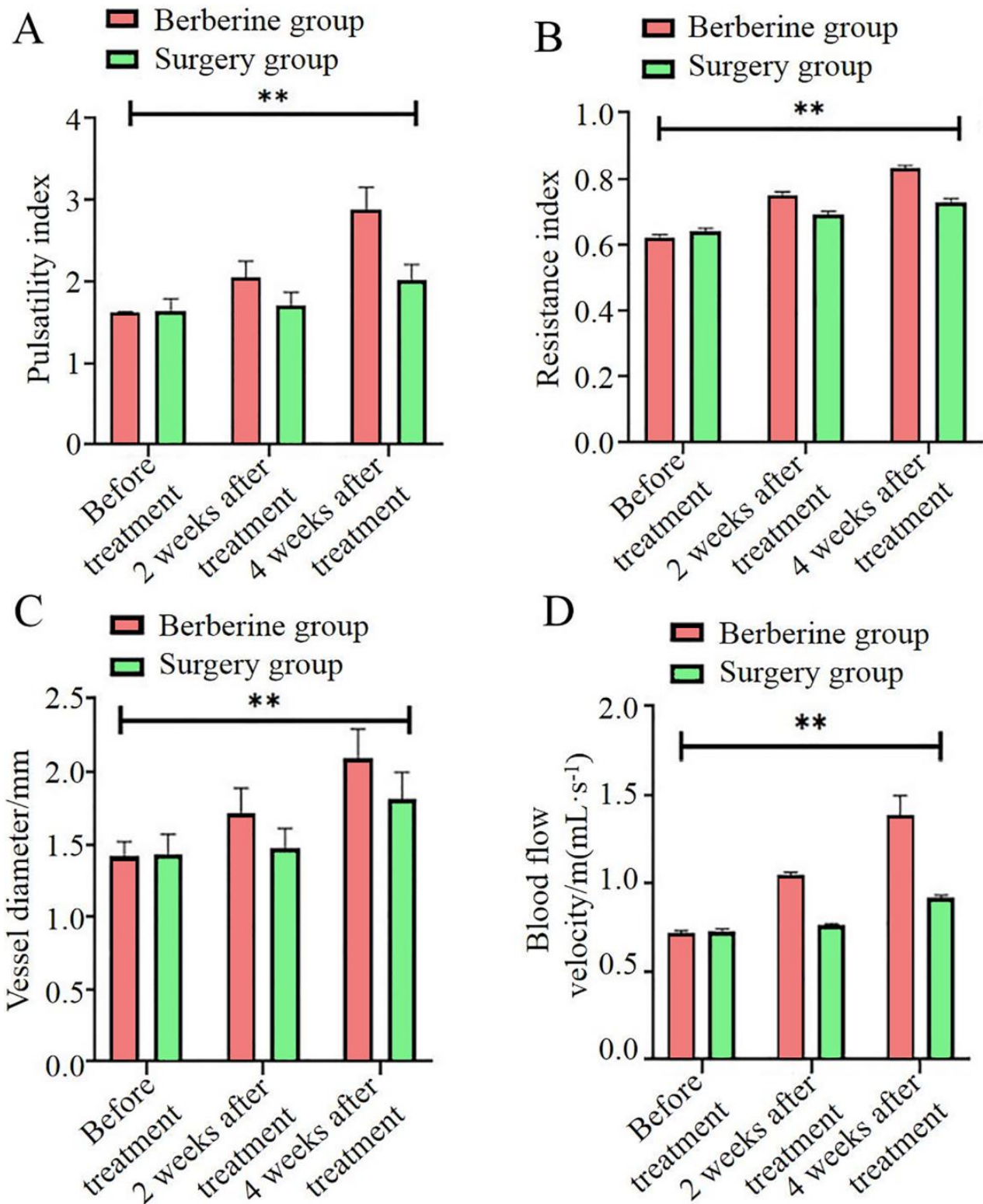


Fig. 8: Dorsal foot artery hemodynamics (n = 48).

Note: (A) Pulsatility index; (B) Resistance index; (C) Vessel diameter; (D) Blood flow velocity. Compared with the surgery group at 2 and 4 weeks of treatment, the data were expressed as mean $\pm$ standard deviation, * $P < 0.05$, ** $P < 0.01$, and the differences between the groups were statistically significant.

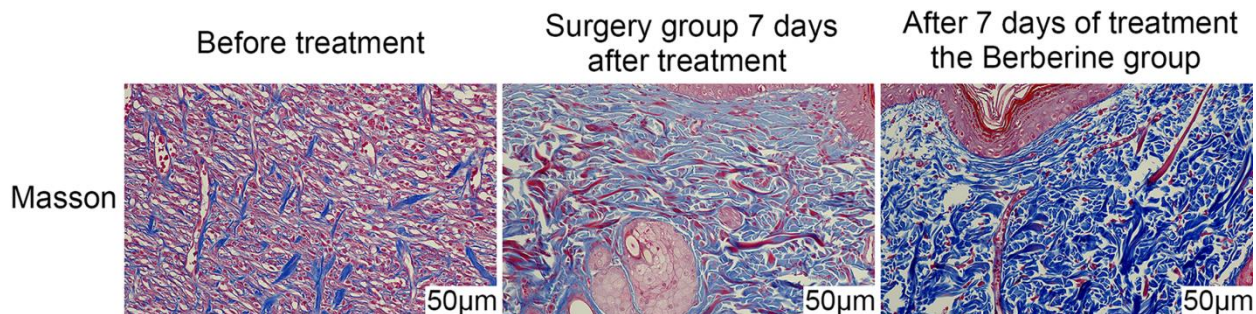


Fig. 9: Masson staining of wound tissue ($\times 200$).
Note: Collagen fibers are blue and muscle fibers are red.

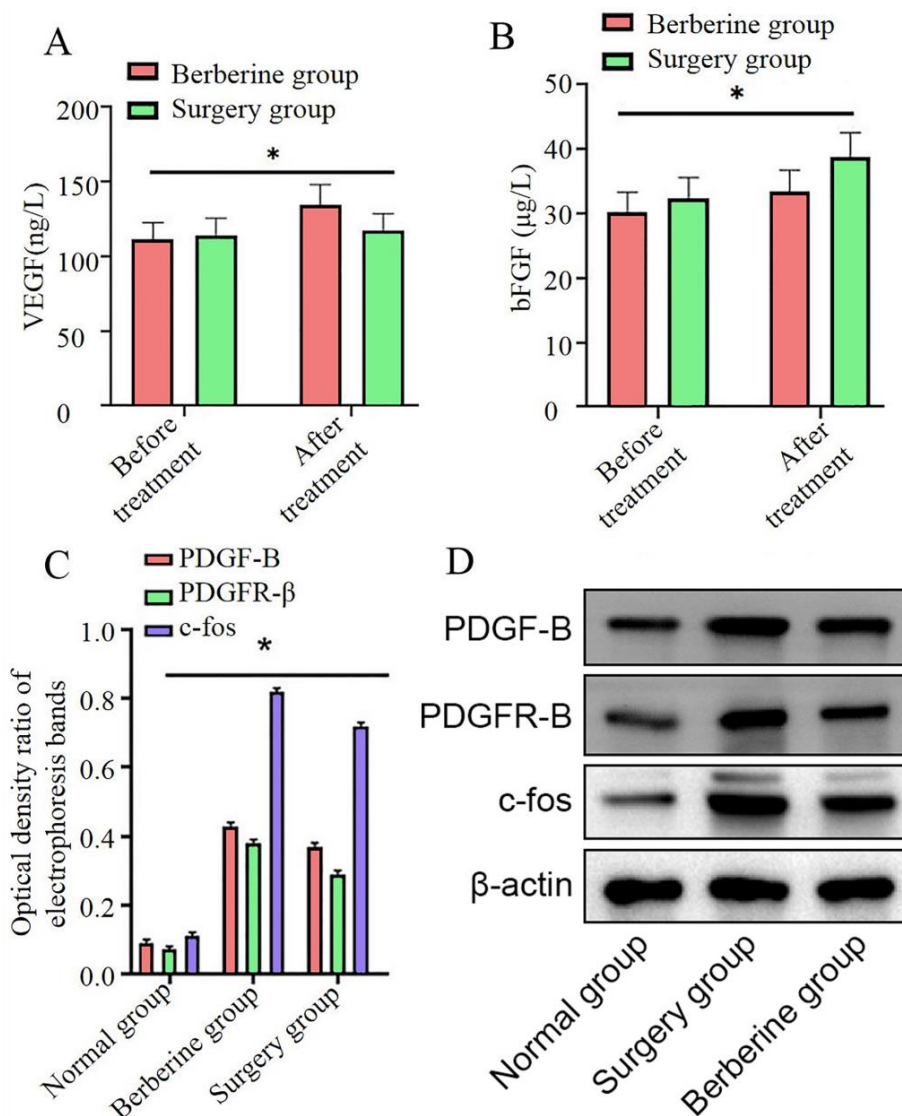


Fig. 10: Levels of VEGF, bFGF and protein expression of PDGF-B and PDGFR β .
Note: (A) VEGF level ($n = 48$); (B) bFGF level ($n = 48$); (C) Electrophoresis band density ratio ($n = 5$); (D) B-actin, PDGF-B, PDGFR- β , c-fos protein bands ($n=5$). Compared with the surgery group before and after treatment, the data were expressed as mean $\pm$ standard deviation, * $P < 0.05$, ** $P < 0.01$, and the differences between the groups were statistically significant.

This provides a cellular-level explanation for the accelerated ulcer healing observed with the combined therapy. Angiogenesis is another critical component of wound healing. Interestingly, while clinical hemodynamic indicators (such as dorsalis pedis artery flow) improved significantly in the Berberine group, serum VEGF levels were lower compared to the surgery group, whereas bFGF levels were significantly elevated. We speculate that this might reflect a potential "normalization" of the angiogenic process. In diabetic chronic wounds, the VEGF pathway can be dysregulated, leading to the formation of leaky and immature vessels (Liu *et al.*, 2024). A relative decrease in VEGF, coupled with a substantial increase in bFGF (a promoter of vascular maturation), could theoretically steer the process toward the formation of a more functional and stable vascular network. However, this interpretation remains hypothetical, as our study lacks direct functional assessments of vascular maturity, such as pericyte coverage or vascular permeability assays.

The initial hypothesis of this study was that Berberine might modulate the PDGF-B/PDGFR β pathway. Our Western blot results confirmed that, compared to the TTO surgery group, Berberine treatment was associated with significantly lower protein expression of both PDGF-B and PDGFR β in the wound tissue. PDGF signaling plays a complex role in tissue repair and its sustained activation under chronic diabetic conditions may be linked to pathological fibrosis and inflammation (Hayes *et al.*, 2022; Cao *et al.*, 2023). The observed downregulation of this pathway, along with a similar trend in the downstream early-response gene *c-fos*, suggests a potential mechanism by which Berberine may help create a more favorable environment for repair. It is crucial to emphasize, however, that these findings demonstrate a correlation, not causation. Without specific pathway inhibition or activation experiments (e.g., using siRNA or agonists), we cannot confirm that the inhibition of PDGF-B/PDGFR β is the primary driver of Berberine's therapeutic effects.

However, several limitations of this study should be considered when interpreting the results. First, the non-randomized control groups (Normal and Diabetes groups) were small and not randomly assigned. Their primary purpose was to provide baseline pathological reference and cell sources for *in vitro* studies. Consequently, any direct comparisons between these groups and the randomized intervention groups should be interpreted with caution due to the high risk of selection bias. Second, the method of berberine administration (repeated local subcutaneous injections) is not a standard clinical practice for this compound. While the dose was based on prior animal studies, we did not conduct formal pharmacokinetic studies in humans, nor did we systematically collect comprehensive safety data beyond routine clinical observation. The potential for local irritation, allergic reactions, or systemic effects from this regimen remains incompletely characterized and requires further

investigation. Third, our mechanistic insights are primarily based on correlative molecular data from tissue and serum. The interpretation of serum growth factor levels as a direct reflection of the wound microenvironment is an oversimplification. Furthermore, our hypothesis regarding "angiogenic normalization" lacks validation from functional vascular assays. Fourth, from a statistical perspective, multiple comparisons were performed without adjustment for the potential inflation of Type I error. While we have presented all data transparently, the significant findings, particularly those with P-values near 0.05, should be viewed as exploratory and requiring confirmation. Finally, as a single-center study with a relatively short (3-month) follow-up period, the generalizability of our findings and the long-term efficacy and safety of the combined treatment remain to be validated in larger, multi-center trials with extended follow-up.

CONCLUSION

In conclusion, this study demonstrates that the adjunctive application of Berberine on the basis of Tibial Transverse Osteotomy (TTO) can more effectively improve limb ischemia, promote ulcer healing and alleviate pain in patients with Diabetic Foot (DF). The observed benefits are associated with a range of biological effects, including reduced inflammation, protection of fibroblasts from apoptosis and coordinated changes in angiogenic factors. Notably, the combined therapy was linked to the downregulation of the PDGF-B/PDGFR β signaling pathway in the wound tissue. However, as this is a correlative finding, further research is essential to: 1) confirm these results in larger, multi-center randomized controlled trials with long-term follow-up to establish definitive efficacy and safety; 2) utilize advanced cellular and animal models with gene manipulation techniques to functionally validate the role of the PDGF-B/PDGFR β pathway and identify the direct molecular targets of Berberine; and 3) rigorously evaluate the pharmacokinetics, optimal dosing and safety profile of different berberine administration routes for this indication.

Acknowledgments

We gratefully acknowledge the Xianju County Traditional Chinese Medicine Hospital for providing the necessary equipment for this study.

Author's contributions

Rudong Zhang: Conceptualization, Methodology, Investigation, Data Curation, Writing – Original Draft.
Huanzhe Jiang: Supervision, Project Administration, Funding Acquisition, Writing – Review & Editing.
All authors have read and approved the final version of the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

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

Ethical approval

The study protocol (Protocol Number: ZJ2025065) has been reviewed and approved by the Ethics Committee of Xianju County Traditional Chinese Medicine Hospital (Approval Number: ZJ2025065). All patients and healthy volunteers participating in this study were fully informed of the research objectives, procedures, potential risks and benefits prior to enrollment and provided written informed consent. Human skin tissue samples used in this study (for primary fibroblast culture) were sourced from patients who had provided the aforementioned informed consent. The collection, preservation and use of all samples strictly adhered to the regulations specified in the ethical approval. This study was performed in adherence with the CONSORT guidelines. See supplementary file for the CONSORT checklist.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

- Alkoholifi FK, Aodah AH, Foudah AI and Alam A (2023). Exploring the Therapeutic Potential of Berberine and Tocopherol in Managing Diabetic Neuropathy: A comprehensive approach towards alleviating chronic neuropathic pain. *Biomedicines.*, **11**(6): 1726.
- Camilleri A, Gatt A and Formosa C (2020). Inter-rater reliability of four validated diabetic foot ulcer classification systems. *J Tissue Viability.*, **29**(4): 284–290.
- Cao Z, Liu Y, Wang Y and Leng P (2023). Research progress on the role of PDGF/PDGFR in type 2 diabetes. *Biomed Pharmacother.*, **164**: 114983.
- Chen X, Peng Y, Xue H, Liu G, Wang N and Shao Z (2022). MiR-21 regulating PVT1/PTEN/IL-17 axis towards the treatment of infectious diabetic wound healing by modified GO-derived biomaterial in mouse models. *J Nanobiotechnology.*, **20**(1): 309.
- Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Juni P, Lettino M, Marx N, Mellbin LG, Ostgren CJ, Rocca B, Roffi M, Sattar N, ESC Scientific Document Group (2020). 2019 ESC Guidelines on diabetes, pre-diabetes and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J.*, **41**(2): 255–323.
- Hayes AJ, Farrugia BL, Biose IJ, Bix GJ and Melrose J (2022). Perlecan, a multi-functional, cell-instructive, matrix-stabilizing proteoglycan with roles in tissue development has relevance to connective tissue repair and regeneration. *Front Cell Dev Biol.*, **10**: 856261.
- Hu F, Hu T, Qiao Y, Huang H, Zhang Z, Huang W, Liu J and Lai S (2024). Berberine inhibits excessive autophagy and protects myocardium against ischemia/reperfusion injury via the RhoE/AMPK pathway. *Int J Mol Med.*, **53**(5): 49.
- Hu Z, Zhao K, Chen X, Zhou M, Chen Y, Ye X, Zhou F, Ding Z and Zhu B (2023). A berberine-loaded bletilla striata polysaccharide hydrogel as a new medical dressing for diabetic wound healing. *Int J Mol Sci.*, **24**(22): 16286.
- Kadhim RA, Erfanparast A, Tamaddonfard E, Amirkashani D and Imani M (2025). Berberine ameliorates acute hyperglycemia and hypoinsulinemia induced by ketamine/xylazine in rats: Role of α 2 adrenergic receptors, oxidative stress and inflammatory suppressing mechanisms. *BMC Vet Res.*, **21**(1): 560.
- Kisiel MA and Klar AS (2019). Isolation and culture of human dermal fibroblasts. *Methods Mol Biol.*, **1993**: 71–78.
- Li Y, Li D, You L, Deng T, Pang Q, Meng X and Zhu B (2023). dCas9-Based PDGFR- β activation ADSCs accelerate wound healing in diabetic mice through angiogenesis and ECM remodeling. *Int J Mol Sci.*, **24**(6): 5949.
- Lipsky BA, Senneville E, Abbas ZG, Aragon-Sanchez J, Diggle M, Embil JM, Kono S, Lavery LA, Malone M, van Asten SA, Urbancic-Rovan V, Peters EJG and International Working Group on the Diabetic Foot (IWGDF) (2020). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev.*, **36**: e3280.
- Littig JPB, Moellmer R, Agrawal DK and Rai V (2023). Future applications of exosomes delivering resolvins and cytokines in facilitating diabetic foot ulcer healing. *World J Diabetes.*, **14**(1): 35–47.
- Liu J, Yao X, Xu Z, Wu Y, Pei F, Zhang L, Li M, Shi, M, Du X and Zhao H (2024). Modified tibial cortex transverse transport for diabetic foot ulcers with Wagner grade $\geq$ II: A study of 98 patients. *Front Endocrinol (Lausanne).*, **15**: 1334414.
- Liu J, Zhang Y, Liu C, Jiang Y, Wang Z, Guo Z and Li X (2024). A single dose of VEGF-A circular RNA sustains in situ long-term expression of protein to accelerate diabetic wound healing. *J Control Release*, **373**: 319–335.
- Liu N, Dai S, Liu D, Sun X, Ren G, Pei L, Ye S and Huang J (2025). Application of transverse tibial bone transfer in diabetic foot patients: A comprehensive evaluation of postoperative prognostic factors and risk factors. *Orthop Surg.*, **17**(9): 2708–2716.
- Liu Z, Xu C, Yu YK, Tu DP, Peng Y and Zhang B (2022). Twenty years development of tibial cortex transverse transport surgery in PR china. *Orthop Surg.*, **14**(6): 1034–1048.
- McDermott K, Fang M, Boulton AJM, Selvin E and Hicks CW (2023). Etiology, epidemiology and disparities in

- the burden of diabetic foot ulcers. *Diabetes Care.*, **46**(1): 209–221.
- Nie X, Kuang X, Liu G, Zhong Z, Ding Y, Yu J, Liu J, Li S, He L, Su H, Qin W, Zhao J, Hua Q and Chen Y (2020). Tibial cortex transverse transport facilitating healing in patients with recalcitrant non-diabetic leg ulcers. *J Orthop Translat.*, **27**: 1–7.
- OuYang H, Yang J, Wan H, Huang J and Yin Y (2024). Effects of different treatment measures on the efficacy of diabetic foot ulcers: A network meta-analysis. *Front Endocrinol (Lausanne).*, **15**: 1452192.
- Wang K, Yin J, Chen J, Ma J, Si H and Xia D (2024). Inhibition of inflammation by berberine: Molecular mechanism and network pharmacology analysis. *Phytomedicine.*, **128**: 155258.
- Yanru Y, Jia C and Yan Y (2025). Berberine in diabetic nephropathy: Mechanistic overview. *Diabetol Int.*, **17**(1): 9.
- Yu X, Zhang D, Yu L, Zhao S, Hu R and Li X (2025). Study on effectiveness and changes in immunoglobulin levels of transverse tibial transport in treatment of Wagner grade 3-4 type 2 diabetic foot ulcer. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.*, **39**(8): 1030–1036.
- Zhang Y, Liu H, Yang Y, Feng C and Cui L (2024). Incidence and risk factors for amputation in Chinese patients with diabetic foot ulcers: A systematic review and meta-analysis. *Front Endocrinol (Lausanne)*, **15**: 1405301.