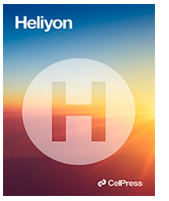
Heliyon 10 (2024) e39716

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Shengji ointment combined with bromelain promotes granulation of exposed tendons in diabetic foot ulcers: A multicenter, randomized, positive-controlled clinical trial

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ARTICLE INFO

Keywords:
Shengji ointment
Bromelain
Diabetic foot ulcer
Tendon injuries
Traditional Chinese medicine
Topical

ABSTRACT

Background: Exposed, infected and necrotic tendons often occur in the middle and late stages of diabetic foot ulcers (DFUs). The exposed tendon is both a potential source and route of infection, which prolongs the treatment period and affects recovery, leading to amputation and even death. Therefore, management of the exposed tendon in patients with DFU is the key to treatment. This study aimed to evaluate the clinical efficacy of Shengji ointment combined with bromelain in the treatment of DFU with tendon exposure and to provide clinical treatment options and evidence-based medicine.

Methods: This study was a multicenter, nonblinded, randomized, positive controlled clinical trial involving 180 patients with DFU with tendon exposure at four tertiary-grade A-class hospitals. The included patients were randomly assigned 1:1 to an observation group (n = 90) that received Shengji ointment combined with bromelain and a control group (n = 90) that received hydrocolloid dressing, with dressing changes once daily for 4 weeks. Patients in both groups continued with conventional treatments, such as blood glucose and blood pressure medication, lipid regulation, and antiplatelets. The primary outcome measure was wound coverage with granulation tissue. The secondary outcome measures included the wound healing rate, time to granulation, Maryland foot score, time to debridement of necrotic tendon tissue, and granulation tissue score. We performed measurements before enrollment and after the end of treatment for comparison.

Results: There was no significant difference in the baseline data between the two groups before treatment ($P > 0.05$). After treatment, the primary outcome indicators of the two groups were compared, and the wound granulation tissue coverage rate of the treatment group was greater than that of the control group ($P = 0.003$). For the secondary outcome outcomes, the wound healing rate, time to granulation, Maryland foot function score, time to debridement of necrotic

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https://doi.org/10.1016/j.heliyon.2024.e39716
Received 11 January 2024; Received in revised form 10 October 2024; Accepted 22 October 2024
Available online 31 October 2024

tendon tissue, and granulation tissue score in the treatment group were significantly better than those in the control group ($P < 0.05$). There was no significant difference in the incidence of adverse reactions between the two groups ($P = 0.444$).

Conclusions: Shengji ointment combined with bromelain effectively promotes the removal of exposed necrotic tendons in patients with DFU, promotes the regeneration of healthy granulation tissue, accelerates wound healing, and protects the limb and its function. It also appears to be safe as an intervention for the treatment of patients with DFU.

Trial registration: The study protocol was registered in the Chinese Clinical Trial Registry (ChiCTR) under the code ChiCTR2000039327 on October 23, 2020. The public title is "Study on evidence-based evaluation and therapeutic mechanism of integrated Chinese and Western medicine for treatment of diabetic foot - An evidence-based evaluation of The combination of Rubber Shengji Paste and compound bromelain to promote the healing of the exposed wound of diabetic foot tendon".

1. Introduction

Diabetic foot ulcer (DFU) is one of the most serious complications of diabetes mellitus (DM). It is essentially a chronic refractory wound caused by diabetic neuropathy, vascular disease, and bacterial infection [1]. With changes in lifestyle, such as dietary habits, diabetes has shown an epidemic trend, and the incidence of diabetic foot (DF) has also increased [2]. Approximately 25 % of the diabetic population is estimated to develop foot ulcers, which are the main cause of hospitalization for patients with diabetes [3]. Approximately 50 %–60 % of these ulcers become infected, and approximately 20 % of moderate to severe infections lead to lower limb amputation, with a mortality rate of over 70 % in patients with major amputations [4].

The occurrence of DFU is affected by a variety of factors, including foot deformities, peripheral neuropathy, and arterial diseases [5]. Therefore, multidisciplinary cooperation and comprehensive treatment have become the clinical consensus for the management of DFU, and the practice of active prevention and management principles can help reduce the amputation rate [6,7]. Even with this comprehensive regimen, the healing rate of DFU and the control of recurrence rates are still not satisfactory [3]. DFU not only imposes a considerable economic burden and heavy psychological pressure on patients but also seriously consumes medical resources [8,9]. Therefore, exploring a positive and effective clinical diagnosis and treatment plan for DF is a common challenge faced by doctors and researchers.

Patients with DFU often develop varying degrees of tendon exposure, infection, and necrosis in the middle to late stages. The degenerated and necrotic tendon in the wound has lost its normal physiological function, which affects wound drainage or causes infection to spread along the tendon tissue to the proximal end of the limb, resulting in adverse outcomes such as toe amputation and foot amputation. Degenerated and necrotic tendons breed bacteria to a certain extent, which further aggravates wound infection. The exposed tendon is both a potential source and route of infection, prolonging the treatment cycle and compromising healing [10]. Therefore, the management of tendons in patients with DFU is the key to treatment and determines the effectiveness of DFU therapy. At present, direct excision, which can remove the exposed tendon tissue completely, is mostly used for degenerated and necrotic tendons in the wounds of patients with DFU. However, this approach not only leads to partial loss of foot function but also may destroy the newly established collateral microcirculation in the periphery. Therefore, when using this approach, it is very important to find a "gentle" and effective treatment to remove the necrotic tendon.

As an important part of China's medical and health system, traditional Chinese medicine (TCM) plays an irreplaceable role in diagnosing and treating diseases [11]. TCM has a long history, and after thousands of years of use, it possesses a solid practical foundation and an extensive theoretical system [12]. Hua Fu Sheng Ji is an important TCM treatment for DFU. The "Hua Fu Sheng Ji" method uses Shengji ointment combined with bromelain to remove degenerated and necrotic tendon tissue from the wound, thereby providing a clean wound bed and ultimately promoting wound healing. The choice of enzymes is based on years of practice and summarized findings of biologically targeted decay removal. Compared with chymotrypsin and trypsin, bromelain has more economic advantages and positive clinical efficacy [13]. The application of the "Hua Fu Sheng Ji" method restores microenvironmental homeostasis for wound repair, which may lead to the reversal of degeneration of tendon tissues and stimulate the growth of granulation tissues to cover the tendon tissues, preserving part of the tendon and preserving the foot function to a certain extent.

Relevant animal experiments were conducted by this group in the preliminary stage. The topical application of Shengji ointment combined with bromelain can inhibit the overexpression of the inflammation-related factors iNOS and interleukin-1β and shorten the inflammatory response time in diabetic ulcer model rats [14]. In the wound healing stage of diabetic ulcer model rats, the combination of Shengji ointment and bromelain promoted the expression of Arg-1, CD206, and DECTIN-1 [15]. These findings suggest that the healing mechanism of bromelain in combination with Shengji ointment may be related to the promotion of M2-type macrophage polarization. Compared with the application of Shengji ointment or bromelain alone, the combination of Shengji ointment and bromelain significantly promoted the expression of vascular endothelial growth factor at the wound healing stage, shortened the healing time and increased the healing rate in rats [16]. The results of the present study demonstrated that the combination of Shengji ointment and bromelain inhibited the excessive inflammatory response and promoted wound healing in diabetic ulcer model rats. However, relevant clinical evidence is still lacking. Therefore, a comprehensive evaluation of the clinical efficacy of this method is highly important for enriching the theoretical system of external TCM treatment.

A prospective study of the predictive factors and clinical efficacy of Shengji ointment in the treatment of DFU in elderly individuals

was published in *Frontiers in Pharmacology* [17]. This multicenter randomized controlled trial evaluated the clinical efficacy of Shengji ointment combined with bromelain in the treatment of DFU patients with tendon exposure and confirmed the clinical efficacy of the "Hua Fu Sheng Ji" method in promoting granulation tissue growth and maximizing tendon tissue preservation and foot function.

2. Materials and methods

2.1. Participants

From December 2020 to December 2021, 180 patients with DFU and tendon exposure in four different hospitals were enrolled in this study (Table 1). All enrolled patients voluntarily signed informed consent before enrollment. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (under code: 2020-006-01).

3. Eligibility criteria

3.1. Inclusion criteria

Patients who met the following criteria were included.

(1) Patients who met the diagnostic criteria of DF [18] and Wagner grade 3–4 [19] (see Table 2) with tendons exposed;
(2) Age between 18 and 85 years;
(3) Fasting blood glucose $\leq$10 mmol/L;
(4) Targeted ulcer debridement area between 1 and 20 cm^2 (for patients with multiple lesions, the largest ulcer will be the target lesion);
(5) An ankle–brachial index $\geq$0.5 on the side of the limb with the ulcer;
(6) The ulcer has blood, pus, or sticky secretions;
(7) Voluntary participation and signing an informed consent form.

3.1.1. Exclusion criteria

Patients who met any of the following criteria were excluded.

(1) DFU caused by electrical, chemical, radioactive, neoplastic, or varicose veins, among other reasons, or malignant lesions within the ulcer;
(2) There were clinical signs of a systemic infection, such as cellulitis, fever, increased white blood cells, or a positive bacterial culture;
(3) Severe uncontrolled hypertension with a systolic blood pressure of $\geq$160 mmHg or a diastolic blood pressure of $\geq$110 mmHg;
(4) Serum albumin $<$28 g/L;
(5) Hemoglobin $<$90 g/L;
(6) Platelet count $<50 \times 10^9$/L;
(7) Severe heart, liver, or kidney injury, in which case medical treatment may seriously affect patient safety;
(8) Women who are pregnant, lactating, recently pregnant, or planning to get pregnant in the near future;
(9) Patients with cognitive impairment who could not fully understand the research content or give informed consent;
(10) Patients who were allergic to some components of the study drug;
(11) Participation in other types of clinical trials within the last month;
(12) Poor compliance, inability to complete the study or failure to comply with the study regulations.

Treatment of Adverse Events: Adverse events during the study will be reported on the Case Report Form (CRF) and evaluated for relevance to the study.

Table 1
Hospitals participating in this study.

Name	Location (city)
The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine	Tianjin
Affiliated Hospital of Liaoning University of Traditional Chinese Medicine	Shenyang
Affiliated Hospital of Shanxi University of Traditional Chinese Medicine	Taiyuan
Tianjin Binhai New Area Hospital of Traditional Chinese Medicine	Tianjin

Table 2
Wagner classification of diabetic foot.

Grade	Clinical manifestation
0	High-risk foot, with risk factors for foot ulcers but no ulcers
1	Superficial skin ulcers were observed without infection
2	Deep ulcers, often associated with soft tissue inflammation, without abscess or bone infection
3	Deep ulcers with abscesses or osteomyelitis
4	Localized gangrene (toe, heel, dorsum)
5	Most or all foot ulcers

3.2. Preparation of the medication

3.2.1. Shengji ointment

The Shengji ointment was purchased from Tianjin Darentang Jingwanhong Pharmaceutical Co., LTD., Tianjin, China. Size: 30 g/box. National Medicine Approved Number: Z12020345. Batch number: 206600.

3.2.2. Pineapple protease (bromelain)

The bromelain was from Shantou Olive Pharmaceutical Co., Ltd., Shantou City, Guangdong, China. Specification: 10,000 units. National Medicine Approved Number: H44024825. Batch number: 200913.

3.2.3. Comfeel® plus wound dressing

The Comfeel® Plus wound dressing was from Coloplast Group (Humblebaek, Denmark). Size: 25 g/piece. China Food & Drug Equipment (Jin) Word 2008 No. 3640515. Batch number: 103579.

3.3. Study design

This study was a multicenter, randomized controlled clinical trial conducted at 4 medical institutions in China. In this study, the wounds were measured, and statistical analysis of the data was performed in a blinded manner. The MagMinDA Clinical Trial Randomized Grouping System (https://open.magminda.com/login) was used to perform minimized dynamic randomization with a bias assignment probability of 1.0 to optimize the grouping efficiency according to the ratio of 1:1 between the observation group and the control group and to control the distribution balance between the groups to efficiently implement random concealment, thus avoiding the generation of selection bias.

The study was divided into an observation group and a control group. The basic conventional medical treatment of the two groups was implemented according to the Chinese Guidelines for the Prevention and Treatment of Diabetic Foot (2019 Edition) [10] (blood glucose control; antihypertensive, lipemic, antiplatelet and anticoagulant therapy; etc.). During the 4-week clinical trial, dressings were changed once daily or on the basis of wound exudation as needed. The data were collected and reported per the Consolidated Standards of Reporting Trials (CONSORT) guidelines [20] and recommendations for herbal formulation extensions [21].

The daily care of patients' wounds and surgical dressing changes were performed by outpatient or ward doctors. To ensure the consistency and standardization of operations at different centers, the standard operating procedure for the clinical external treatment of DFU with tendon exposure under nonblinded evaluation was developed as follows.

(1) After the outer dressing is removed from the wound, the inner dressing is then removed with forceps;
(2) First, sterile cotton wool is used to adsorb the exudate from the sore, and then the periwound or sore surface can be optionally sterilized with iodine povidone;
(3) Perform sharp tension-free removal of liquefied and floating tendon and fascia tissues in the wound, preserving tendons and fascia that have not yet become necrotic;
(4) In the observation group, "Hua Fu Sheng Ji" is applied in the following manner: bromelain powder is evenly spread on the exposed part of the tendon on the sore surface at approximately 0.2 g/cm^2. Shengji ointment is applied to a degreased cotton pad and then it is externally applied on the sore surface, with an application amount of 25 g/100 cm^2 [22], and the border is placed along the edge of the sore. The amount of medication used is calculated on the basis of weight after the area is measured and the medication is weighed for application. In the control group, Comfeel® Plus wound dressing is applied externally to the sores, and the amount of filling should not be higher than the level of normal skin around the sores;
(5) Stress reduction: Decompression during the whole experiment is provided by strengthening patient education and urging patients to use appropriate relief methods, such as crutches and wheelchairs.

3.4. Study outcomes

3.4.1. Primary outcome

Wound coverage with granulation tissue was the primary outcome. The development of granulation tissue was assessed as follows:

Wound coverage rate = The wound area covered by granulation tissue (mm^2)/whole wound area (mm^2) × 100%.

3.4.2. Secondary outcome

The secondary outcomes included the wound healing rate, granulation time and degeneration, necrosis and tendon tissue clearance time, and Maryland foot function score.

(1) Wound healing rate = (Original wound area - unhealed wound area)/Original wound area × 100 %.
(2) Granulation time (days): The period within which new granulation tissue appears within the wound.
(3) The Maryland Foot Score was used to evaluate foot function, including the presence or absence of pain. The maximum score is 100 points, with >89 points indicating excellent function, 75–89 points indicating good function, 50–74 points indicating average function, and <50 points indicating poor function.

3.4.3. Subgroup analysis

Subgroup analysis of all outcomes was performed according to the location of the disease (plantar, dorsal, or toe).

3.5. Wound measurement methods

The eKare inSight three-dimensional scanning program (eKare Inc., USA, specification: V1.12.2, lot number: 000354) was used to measure the wound. The modus operandi and procedure are described in the Supplementary Material (S1).

3.6. Sample size determination

The sample size was calculated using PASS 11.0 software (NCSS Statistical software https://www.ncss.com/software/pass). Preliminary clinical trials of intervention measures in the experimental group reported an effective rate of 71 %, and literature reports of intervention measures in the control group reported an effective rate of 50 %. Two-sided $\alpha = 0.05$ and $1\text{-}\beta = 0.9$ were set, and the sample size ratio of the two groups was 1:1. Seventy-two cases in each group were calculated via PASS 11.0, and a 20 % dropout rate was considered. Finally, the sample size of this trial was determined to be 90 patients in the experimental group and 90 patients in the control group, for a total of 180 patients.

3.7. Ethics

The ethical review of this multicenter clinical study was conducted in strict compliance with the World Medical Association Declaration of Helsinki [23] and the Ministry of Health's "Measures for Ethical Review of Biomedical Research Involving Human Beings (for Trial Implementation)" (Document No. 17, 2007). To ensure that this study was conducted in an orderly manner without violating medical ethics, the study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine with approval number 2020-006-01.

Patients were provided with written informed consent before participation in the study; they were informed of the study purpose, study procedures, possible benefits, and risks; and they were asked if they wished to participate in the study and informed them of their right to withdraw from the study at any time. If either the patient or his/her legal representative could not read, an impartial witness was required to assist and witness the informed consent. The medical ethics committee supervised the clinical trial process.

3.8. Data collection and management

Data collection and management programs are described in Supplementary Material 2(S2).

3.9. Statistical analysis

(1) SAS 9.4 software was used for analysis.
(2) All significance tests and resulting *p* values were two-sided, with an alpha level of 0.05.

3.9.1. Statistical description

The measurement data are presented as the median, mean (standard deviation) or interquartile range according to their distribution. The frequency of occurrence and percentage were calculated for count and grade data.

3.9.2. Statistical inference method

(1) Analysis of demographic and baseline information: Count data were analyzed by the chi-square test. The measurement data conforming to a normal distribution and passing the homogeneity of variance test were analyzed by a *t*-test, the data failing the homogeneity of variance test were analyzed by a corrected *t*-test, and data with a skewed distribution were analyzed by a rank sum test.

(2) Analysis of clinical treatment: Wound coverage rate, wound healing rate, granulation time and degeneration, necrosis and tendon tissue clearance time and Maryland foot function score were analyzed using ANOVA to compare the differences between groups.

3.9.3. Security analysis

(1) Adverse events (Treatment Emergent Adverse Event, TEAE)/reactions during the trial, Serious Adverse Events/Reactions, Incidence of Adverse Events/Reactions leading to Dislodgement during the study were analyzed.
(2) A detailed list of adverse events/reactions (serious) that occurred during the trial and a list describing the causes of adverse events/reactions (serious) that led to dropout was collected.
(3) For safety indicators, differences between groups were analyzed using a centrally stratified CMHX2 test, and values are presented as descriptive results.

4. Results

A total of 236 patients with Wagner grade 3–4 tendon-exposed wounds from four hospitals were screened from December 2020 to December 2021. After multiple layers of screening, a total of 180 people participated in this study. These patients were randomly assigned to the experimental group or the control group, with 90 patients in each group. During the clinical trial, there were 7 patients in the observation group and 8 patients in the control group that dropped out, as shown in Fig. 1.

4.1. Analysis of demographic information

As shown in Table 3, in terms of the sex distribution of the included patients, there were significantly more male patients than female patients (see Fig. 2). The distribution of work status differed between groups, and between-group comparisons were made by removing other categories, with significantly more manual workers than mental workers. The distribution of educational attainment also differed between groups, and between-group comparisons were made by removing unspecified categories, with those with less than a high school education being significantly more common than those with more than a high school education.

4.2. Analysis of baseline information

Table 4 provides a detailed summary of the characteristics of the 165 patients included in the analysis. There were no significant differences between the two groups at baseline ($P > 0.05$). The demographic data of the two groups were balanced and comparable.

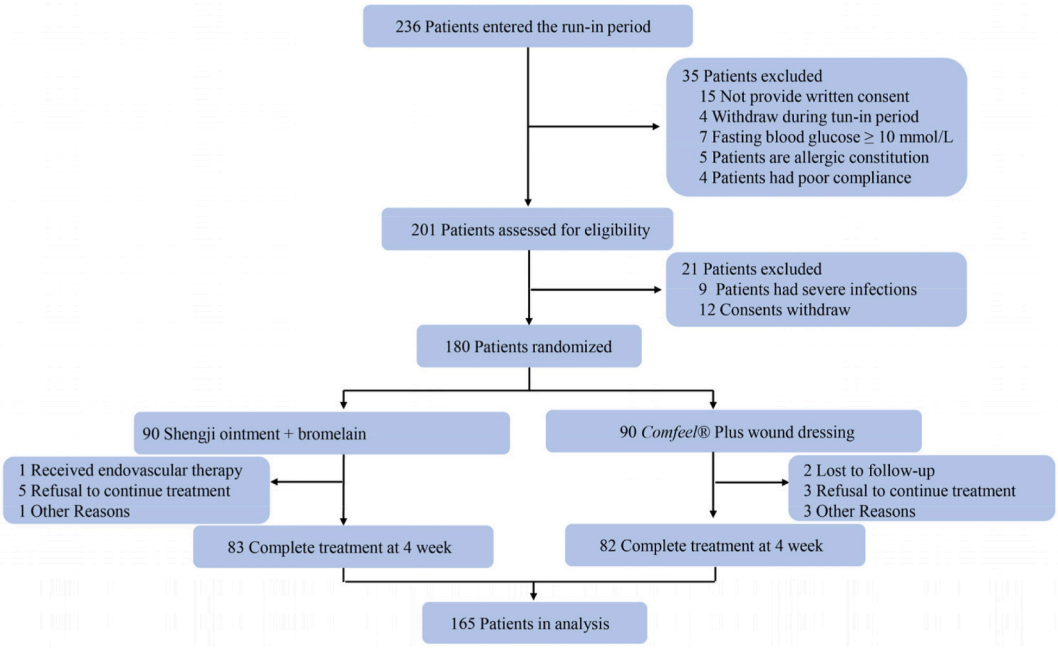

Fig. 1. Study flowchart of patient enrollment.

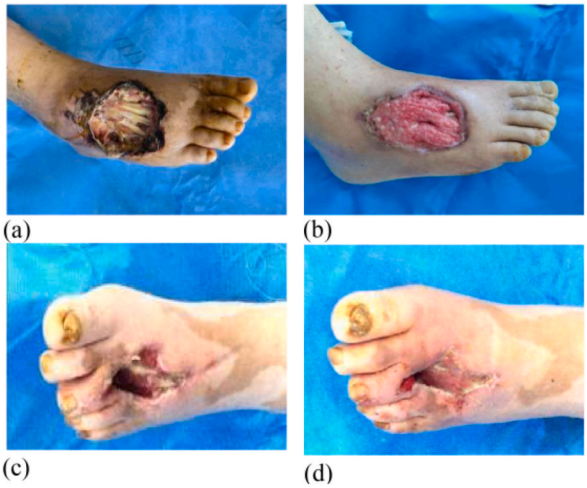

Fig. 2. (a, b) Treatment group before treatment. (c, d) Control group after treatment. Pictures a and b are from the same patient. Pictures c and d are from the same patient.

Table 3
Demographic data distribution characteristics analysis.

Variables		N (%)	x^2	*P* value
Sex	Male	95 (57.58)	7.58	0.006
	Female	70 (42.42)		
Working conditions	Brainwork	29 (17.58)	133.26	< 0.001
	Physical work	112 (67.87)		
	Other	24 (14.55)		
Educational level	Junior high school and below	110 (66.67)	136.86	< 0.001
	High school and above	12 (7.27)		
	Unknown	43 (26.06)		

Table 4
Baseline characteristics of the patients.

Variables	Observation group (n = 83)	Control group (n = 82)	x^2/t (z)	*P* value
Sex				
Male [n (%)]	49 (59.04)	46 (56.10)	0.15	0.703
Female [n (%)]	34 (40.96)	36 (43.90)		
Age, mean (SD), years	62.60 (10.24)	65.56 (10.69)	−1.82	0.071
Weight, mean (SD), kg	69.41 (11.09)	68.89 (12.91)	0.28	0.779
Height, mean (SD), cm	169.11 (7.47)	167.96 (7.96)	0.96	0.340
Duration, median (Q1, Q3), months	3(1.3,7.4)	3.1(1.5,6.9)	0.34	0.731
Location of disease				
Dorsal [n (%)]	21 (12.73)	21 (12.73)	1.68	0.432
Plantar [n (%)]	39 (23.64)	45 (27.27)		
Toe [n (%)]	23 (13.94)	16 (9.69)		
Comorbidities				
Coronary heart disease [n (%)]	58 (69.88)	50 (60.98)	0.30	0.585
Hypertension [n (%)]	52 (62.65)	55 (67.07)	0.15	0.702
Cerebrovascular disease [n (%)]	22 (26.51)	27 (32.93)	0.82	0.367
Diabetic nephropathy [n (%)]	18 (21.69)	30 (36.59)	0.17	0.684
Diabetic retinopathy [n (%)]	27 (32.53)	14 (17.07)	0.10	0.755
Area, median (Q1, Q3), cm^2	1.4(0.3,4.2)	1.4(0.3,4.2)	−0.54	0.590
Volume, median (Q1, Q3), cm^3	1.2(0.4,4.3)	1.4(0.3,4.2)	−0.11	0.910
Depth overall, median (Q1, Q3), cm	0.4(0.1,0.7)	0.4(0.2,0.8)	0.25	0.806
Fasting blood glucose, mean (SD), mmol/L	6.84 (0.17)	6.86 (0.18)	0.01	0.991
2h postprandial blood glucose, mean ± SD, mmol/L	10.64 ± 0.19	10.29 ± 0.17	−1.02	0.307
Wagner classification				
Grade 3	54 (65.06)	55 (67.07)	0.08	0.785
Grade 4	29 (34.94)	27 (32.93)		

ABI ankle–brachial index, standard deviation (SD).

4.3. Primary outcome

After 4 weeks of treatment, the wound granulation coverage rate of the treatment group increased by 48.21 ± 29.81 % compared with that of the baseline period, and that of the control group increased by 29.16 ± 33.66 % compared with that of the baseline period. The difference between the two groups was statistically significant ($P < 0.0001$). The curative effect in the treatment group was better than that in the control group. There was no significant difference in the wound granulation coverage rate between the two groups at baseline ($P > 0.05$), but there was a significant difference between the two groups at the end of the fourth week ($P = 0.003$) (Table 5). These findings suggest that the combination of Shengji ointment and bromelain is effective in promoting the growth of granulation tissue and covering exposed tendon tissue. Representative images of the observation group and the control group before and after treatment are displayed in Figures [2(a - d)].

4.4. Secondary outcome

The wound healing rate in the experimental group was 58.55 ± 31.52 %, whereas that in the control group was 45.85 ± 32.72 %. The wound healing rate of the treatment group was greater than that of the control group ($P = 0.010$) (Table 5).

The median time to granulation was 6 days in the treatment group, whereas it was 10 days in the control group. The difference in granulation time between the two groups was statistically significant ($P < 0.0001$), with the treatment group having a shorter time to granulation than the control group (Table 5).

As shown in Table 6, there was no significant difference between the Maryland Foot Function Scores of the two groups before treatment ($P = 0.126$). After four weeks of intervention, the Maryland Foot Function Score of the treatment group improved by 8.76 ± 11.72 points compared with that of the pretreatment group, that of the control group improved by 6.24 ± 9.93 points, and the difference in efficacy between the two groups was statistically significant ($P = 0.041$). At the end of the treatment cycle, the Maryland foot function score of the treatment group was significantly greater than that of the control group ($P = 0.021$).

The median time to clear degenerated and necrotic tendon tissue was 14 days in the treatment group compared with 21 days in the control group. The difference in the time to clear degenerated and necrotic tendon tissue between the two groups was statistically significant ($P < 0.0001$). The clearance time of degenerated and necrotic tendon tissue was shorter in the observation group than in the control group (Table 5).

In addition, after the fourth week of intervention, the granulation tissue score of the patients' wounds in the treatment group was 3.6 ± 0.85 points, whereas in the control group, it was 3.21 ± 1.2 points. The difference between the two groups was statistically significant ($P = 0.0014$). The granulation tissue score of patients in the treatment group was significantly greater than that of patients in the control group (Table 5). These findings indicate that the intervention in the treatment group improved the growth of granulation tissue.

Shengji ointment combined with bromelain was safely administered. There were 2 cases of adverse events in the treatment group, with an incidence rate of 2.22 %, and 5 cases of adverse events in the control group, with an incidence rate of 5.56 %. There was no significant difference between the two groups ($P = 0.444$). In the control group, 1 patient experienced local fluctuations during treatment, which was a sign of wound infection (Table 9). During the course of the study, five patients in the observation group and two patients in the control group developed infections in their wounds, accompanied by elevated leukocyte counts. These patients were treated with antibiotics without affecting the normal implementation of the treatment program.

4.5. Subgroup analysis

The ulcer site (plantar, dorsal, or toe) was used as a stratifying factor for subgroup studies (Table 6, Table 7, Table 8). In the

Table 5
Comparison of the primary and secondary outcomes between the two groups.

Outcomes	Visit	Observation group (n = 83)	Control group (n = 82)	x^2/t	*P* value
Primary outcome					
Wound granulation coverage rate, mean (SD), %	Baseline	29.31 (25.55)	36.83 (27.20)	3.35	0.069
	4 weeks	77.52 (19.66)	65.99 (28.01)	9.39	0.003
	Difference efficacy	48.21 (29.81)	29.16 (33.66)	12.38	<0.001
Secondary outcomes					
Wound healing rate, mean (SD), %	4 weeks	58.55 (31.52)	45.85 (32.72)	6.85	0.010
Granulation time, median, day		6	10	16.09	<0.001
Maryland foot function score, mean (SD), point	Baseline	57.16 (24.07)	51.39 (24.06)	2.37	0.126
	4 weeks	65.92 (22.61)	57.63 (23.01)	5.44	0.021
	Difference efficacy	8.76 (11.72)	6.24 (9.93)	4.24	0.041
Degenerated, necrosis tendon tissue clearance time, median, day		14	21	29.32	<0.001
Granulation tissue score, mean (SD), point	4 weeks	3.60 (0.85)	3.21 (1.20)	6.18	0.014

Table 6
Subgroup analysis of ulcer site (plantar) as a stratification factor.

Outcomes	Visit	Observation group (n = 21)	Control group (n = 21)	x^2/t	*P* value
Primary outcome					
Wound granulation coverage rate, mean (SD), %	Baseline	31.1 (27.04)	36.90 (26.39)	0.50	0.485
	4 weeks	74.02 (22.14)	56.38 (31.53)	4.40	0.042
Secondary outcomes					
Wound healing rate, mean (SD), %	4 weeks	54.25 (45.72)	35.59 (31.83)	1.92	<0.001
Granulation time, median, day		7	10	5.18	0.023
Maryland foot function score, mean (SD), point	Baseline	61.19 (26.93)	40.24 (22.61)	7.46	0.009
	4 weeks	65.90 (25.32)	47.57 (23.63)	5.88	0.020
Degenerated, necrosis tendon tissue clearance time, median, day		14	21	6.18	0.013
Granulation tissue score, mean (SD), point	4 weeks	3.52 (0.87)	2.81 (1.36)	3.94	0.055

Table 7
Subgroup analysis of ulcer site (dorsal) as a stratification factor.

Outcomes	Visit	Observation group (n = 20)	Control group (n = 18)	x^2/t	*P* value
Primary outcome					
Wound granulation coverage rate, mean (SD), %	Baseline	30.05 (28.50)	36.50 (29.50)	0.47	0.498
	4 weeks	78.39 (14.52)	57.53 (27.00)	9.05	0.005
Secondary outcomes					
Wound healing rate, mean (SD), %	4 weeks	59.68 (21.45)	28.62 (31.42)	14.23	<0.001
Granulation time, median, day		6.50	11.50	9.41	0.002
Maryland foot function score, mean (SD), point	Baseline	55.30 (25.15)	54.33 (22.73)	0.02	0.902
	4 weeks	61.40 (23.20)	60.56 (18.41)	5.88	0.903
Degenerated, necrosis tendon tissue clearance time, median, day		14	25	15.07	<0.001
Granulation tissue score, mean (SD), point	4 weeks	3.65 (0.67)	2.78 (1.11)	10.08	0.003

Table 8
Subgroup analysis of the ulcer site (toe) as a stratification factor.

Outcomes	Visit	Observation group (n = 42)	Control group (n = 43)	x^2/t	*P* value
Primary outcome					
Wound granulation coverage rate, mean (SD), %	Baseline	28.07 (23.86)	36.93 (27.25)	2.54	0.114
	4 weeks	78.86 (20.68)	74.22 (24.46)	0.89	0.348
Secondary outcomes					
Wound healing rate, mean (SD), %	4 weeks	60.15 (27.22)	58.08 (29.15)	0.07	0.792
Granulation time, median, day		5	10	3.78	0.052
Maryland foot function score, mean (SD), point	Baseline	56.02 (22.37)	55.60 (24.08)	0.01	0.934
	4 weeks	68.07 (21.12)	61.33 (23.45)	1.94	0.167
Degenerated, necrosis tendon tissue clearance time, median, day		14	21	10.50	0.001
Granulation tissue score, mean (SD), point	4 weeks	3.62 (0.94)	3.58 (1.05)	0.03	0.865

Table 9
Incidence of adverse events.

	Treatment group [n (%)]	Control group [n (%)]	*P* value
adverse event	2 (2.22)	5 (5,56)	0.444
serious adverse event	0 (0)	1 (1.1)	

subgroup analysis in which the plantar technique was used as a stratification factor, the difference between the granulation tissue scores of the observation group and the control group was not statistically significant ($P = 0.055$). The remaining primary and secondary outcome indicators improved more in the observation group than in the control group ($P < 0.05$). In a subgroup analysis in which the dorsal area of the foot was used as a stratification factor, the difference between the Maryland foot function scores of the observation group and the control group was not statistically significant ($P = 0.903$). The remaining primary and secondary outcome indicators improved more in the observation group than in the control group ($P < 0.05$). A subgroup analysis in which the toe was used as a stratification factor revealed that the degenerated, necrotic tendon tissue clearance time was significantly shorter in the observation group than in the control group ($P = 0.001$). The differences between the remaining primary and secondary outcome indicators in the observation group and the control group were not statistically significant ($P > 0.05$).

5. Discussion

Most patients with DF have poor basic conditions, lack understanding of the disease, and delay treatment, resulting in complex and diverse conditions [24]. An analysis of the characteristics of the population included in this study revealed that males outnumbered females, and most of them were engaged in physical labor with generally lower educational levels. The mean body weight of the male patients was greater than that of the female patients, and they were subjected to greater force on their feet. Among the risk factors, smoking and other independent risk factors for peripheral vascular disease were more common, leading to a higher incidence of the disease. Workers engaged in physical labor are mostly in an upright position during work and have more repetitive activities, resulting in longer periods of force on the lower limbs and poorer blood flow return in the lower limb vessels. A low educational level can lead to inadequate understanding and awareness of the disease, failure to recognize the harm caused by the disease, and missing the appropriate timing for treatment, resulting in more severe conditions. The average age of onset is also related to their generally low level of education. During the process of inquiring about their educational level, many patients refused to disclose or gave vague answers, which may also be related to their lack of educational awareness.

In this study, the age of onset of the disease was greater, and the course of the disease was longer, with significant time differences, which reflects the uniqueness and complexity of the disease. All ulcers were in the stable necrosis stage without severe ischemia. The existence of a partial blood supply provides the basis for granulation tissue growth. Most ulcers were deep, with only a few having localized necrosis. Some necrotic tendons were non-functional, but some tendons retained vitality. The location of tendon exposure in patients with DF is mostly the plantar region, which is related to the anatomical structure of the foot.

The treatment process for diabetic foot tendon exposure ulcers is complex and requires the proliferation of surrounding granulation tissue to wrap around the tendons, close the tendon sheath, and cut off the path of infection that spreads along the tendon sheath. Therefore, granulation tissue growth is a key factor in wound healing. In previous clinical studies related to DFU, the focus was mostly on the endpoint event indicator of the wound healing rate. However, under the action of the “Hua Fu Sheng Ji” method, the key process is the gradual coverage and growth of exposed tendons by newly generated granulation tissue. Through our study, we found that the granulation growth in both groups after treatment with the “Hua Fu Sheng Ji” method was better than that at baseline, and the granulation coverage rate of the “Hua Fu Sheng Ji” therapy group was better than that of the control group.

The process of granulation coverage on the wound surface is angiogenesis, which refers to the formation of new microvessels from mature blood vessels and is a critical factor in wound healing [25]. The level of angiogenesis in diabetic wounds significantly affects normal blood flow to the wound [26]. Damage to the microcirculation in wounds leads to local hypoxia and insufficient nutrient supply. The decrease in angiogenesis caused by damage to the microcirculation obstructs the migration of inflammatory and repair cells to the wound, limiting the ability of local immune cells to produce different angiogenic factors and further inhibiting angiogenesis [27–29]. However, after the “Hua Fu Sheng Ji” method was applied, macroscopic observations revealed that granulation tissue growth accelerated, which promoted granulation tissue formation and the growth of healthy granulation tissue on the wound surface.

The key to intervention with the “Hua Fu Sheng Ji” method is to cover a tendon that has not completely lost its vitality with granulation tissue until it integrates with the surrounding blood supply. The coverage of granulation tissue on the wound surface results in vascularization of the tendon. TCM promotes improved circulation around the wound and mobilizes positive factors in the body to repair the wound. Through local blood vessel dilation and the growth of collateral channels, nutrients are infused to promote the exposure of the surface of the exposed tendon and gradually cover and fuse with it, ultimately leading to wound healing. This process promotes self-repair of the wound and is a key link in promoting wound healing. The indicators of the granulation coverage rate and granulation time are not endpoint indicators but rather substitute indicators for important intermediate states. The objective evaluation of granulation growth is highly important for standardizing research.

"Fu" refers to the pathological products that hinder wound healing during the “Hua Fu Sheng Ji” method. In this disease, it refers to degenerated and necrotic tendon tissue, which is a breeding ground for bacteria. When drainage is obstructed, other tissues become liquefied and cannot be discharged, leading to the production of many inflammatory factors and the occurrence of an inflammatory reaction. "Ji" refers to the newly formed granulation tissue. In the process of wound healing, "Fu" and "Ji" are opposite and conflicting processes that result in the struggle between positive and negative forces on the wound surface. The process of "Hua Fu" involves liquifying and removing substances that obstruct the formation of new muscle tissue. Therefore, the process is "Hua Fu Sheng Ji-Ji Sheng Fu Hua-Fu Hua Ji Sheng." “Hua Fu” is a necessary condition for tissue growth and can further accelerate the “Hua Fu” process. The two interact and achieve wound healing.

The removal times of degeneration, necrosis, and tendon tissue clearance indicate that the “Hua Fu Sheng Ji” method has clinical advantages for the removal of denatured and necrotic tendon tissue. The rot produced by this disease can be divided into tangible rot and intangible rot. Tangible rot refers to necrotic tissue such as tendons, whereas intangible rot affects the parabiotic tissue involved in wound healing through immune, metabolic, and other factors, as well as a potential impact on surrounding tissues. When the necrotic tissue cannot be shed, there is no blood supply to the trunk or microvessels, antibiotics cannot act directly, and the necrosis itself becomes a place for bacteria to breed, making the new granulation tissue unable to grow.

If the exposed tendon is left untreated, necrosis will occur, and drainage will be affected, requiring mechanical debridement, causing loss of function. The exposed tendons of wounds with less secretion and relatively dry surfaces are mostly shriveled, and the surface is covered with a layer of gray–black dry scabs. Bromelain could not dissolve these tendons. With the help of the Shengji ointment, the wound secretion gradually increased and thickened, and the tendon also expanded. The loose structure of the tendon helps the granulation tissue grow inside it. When the upper layer of the tendon is removed layer by layer, granulation tissue can be found in the lower layer of the tendon. Under the action of the drug, the granulation tissue can gradually fill the base and then grow until the remaining tendon is completely wrapped and the wound has healed. Therefore, the debridement effect of the “Hua Fu Sheng

Ji" method is not to regenerate the muscle after all removal but rather to take into account both the removal and retention functions and act and promote each other at the same time to maximize the retention of tendon tissue to prepare a material basis for the long-term retention of the limb and protection of function.

For functional scoring, the study period was 4 weeks, and there was no systematic recording of long-term function. However, during the study period, there were certain differences between the groups while promoting healing, which initially confirmed that the "Hua Fu Sheng Ji" method had a certain role in preserving function and limb salvage, but the long-term effect still needs to be confirmed by follow-up.

In this study, no treatment-related adverse reactions were observed. The adverse reactions to external TCM or external medicine generally affect the local area, which is more intuitive and easier to distinguish. The common adverse reactions of topical TCM include contact dermatitis and skin allergic reactions. The long-term and large-scale application of some toxic herbs can cause liver and kidney toxicity, cancer, and even death. None of the above was present in this study.

6. Limitations

Although this study has obvious advantages, its potential limitations are worth considering. First, under the guidance of TCM theory, this study selected the combination of Shengji ointment and bromelain for treatment, which cannot be used to judge the specific efficacy of a single drug and can only be comprehensively analyzed. Second, there is a lack of follow-up data on foot function in this clinical study, and the long-term treatment and rehabilitation effects of the "Hua Fu Sheng Ji" method need to be further confirmed. Moreover, we only researched the clinical efficacy of Shengji ointment combined with bromelain, and the underlying mechanisms have not been investigated. However, our primary aim was to provide a clinical protocol for the treatment of patients with Wagner grade 3–4 DF with tendon-exposed wounds. This study demonstrated significant clinical efficacy in Wagner grade 3–4 DF patients with tendon-exposed wounds. Further studies will be conducted in the future to elucidate the mechanism of this effect.

7. Conclusion

In conclusion, this study demonstrated that in the treatment of DF with tendon-exposed wounds, the "Hua Fu Sheng Ji" method can increase the wound coverage rate and wound healing rate, shorten the granulation time and the necrosis and tendon tissue clearance time, and increase the Maryland foot function score and granulation tissue score. It can promote granulation tissue growth and wound healing and maximize tendon tissue preservation and foot function.

CRediT authorship contribution statement

Xu Sun: Writing – original draft. **Jinpeng Jing:** Writing – review & editing, Writing – original draft. **Rui Dai:** Writing – original draft. **Chaojun Zhu:** Methodology, Investigation, Formal analysis, Data curation. **Yuzhi Sun:** Methodology, Investigation, Formal analysis, Data curation. **Junchao Sun:** Methodology, Investigation, Formal analysis, Data curation. **Dayong Li:** Methodology, Investigation, Formal analysis, Data curation. **Xin Li:** Methodology, Investigation, Formal analysis, Data curation. **Xiaoli Zhang:** Methodology, Investigation, Formal analysis, Data curation. **Xiaoliang Li:** Methodology, Investigation, Formal analysis, Data curation. **Yue Shi:** Methodology, Investigation, Formal analysis, Data curation. **Tingting Liu:** Methodology, Investigation, Formal analysis, Data curation. **Rui Gao:** Supervision, Project administration, Conceptualization. **Zhaohui Zhang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Ethics statement

All patients agreed to participate in this study and signed written informed consent. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (under code: 2020-006-01).

Data availability statement

The raw data of this study did not show in a publicly available database. But the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Funding

This work was supported by the National key research and development plan funding support (NO.2019YFC1709303).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their special thanks to the Scientific Research Section of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine for their support during the implementation of the project, and to the postgraduate students (Yiming Wang, Mengge Ma, Hanchi Sun, Chenghao Ren, Yuqing Guo, Jingxin Yan) of Traditional Chinese Medicine Surgery of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine for their help.

Abbreviations

DF	Diabetic Foot
DFU	Diabetic Foot Ulcer
DM	Diabetes Mellitus
TCM	Traditional Chinese Medicine

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39716.

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