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The Extract of *Lycium depressum* Stocks Enhances Wound Healing in Streptozotocin-Induced Diabetic Rats

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Abstract

In diabetes, impaired wound healing and other tissue abnormalities are considered major concerns. The aim of the present study was to assess the wound-healing activity of methanolic extracts of the extract of *Lycium depressum* leaves. A total of 60 healthy male Wistar diabetic rats weighing approximately 160 to 180 g and 7 weeks of age were randomized into 10 groups for incision and excision wound models: sham surgery group (SHAM), including creation of wounds and no treatment; base formulation group (FG) with creation of wounds and application of base formulation ointment; treatment group 1 (TG1) with 1 g of powder extract of the plant material in ointment; treatment group 2 (TG2) with 2 g; and treatment group 4 (TG3) with 4 g of powder extract of the plant material in ointment. A wound was induced by an excision- and incision-based wound model in male rats. The mature green leaves of *L depressum* were collected and authenticated. Extractions of dried leaves were carried out. For wound-healing activity, the extracts were applied topically in the form of ointment and compared with control groups. The healing of the wound was assessed based on excision, incision, hydroxyproline estimation, and biomechanical and biochemical studies. The extract of *L depressum* leaves enhanced wound contraction, decreased epithelialization time, increased hydroxyproline content, and improved mechanical indices and histological characteristics in treatment groups compared with SHAM and FG ($P < .05$). These findings permit the conclusion the extract of L depressum benefits parameters of wound healing in a diabetes induced model

Keywords

methanolic extract, *Lycium depressum*, wound healing, full-thickness wounds, diabetes, antioxidant

In diabetes, impaired wound healing and other tissue abnormalities are considered major concerns.[1] The biochemical mechanisms involved in the healing process are mainly associated with disorders in collagen production, which consequently leads to delayed re-epithelialization in wounds, compromised migration, and proliferation of keratinocytes and fibroblasts.[2] Various treatments have been adopted to solve this complex clinical problem; however, only a few have been proven to be effective.[3]

The prevalence of diabetes has become a major clinical problem and a serious issue for public health. Impaired wound healing in diabetic patients is one of the complications.[4] Lack of cellular and molecular signals required for normal wound repair processes such as angiogenesis, granulation tissue formation, epithelialization, and remodeling are encountered in diabetic patients and contribute to poor healing of the diabetic wound. The normal healing process in healthy individuals occurs at an optimal rate; however, it is usually delayed or even completely compromised in diabetic patients.[4]

There is an increasing interest in the potential of traditional and complementary medicines to be used in the area of wounds that has led to investigations of a range of plant extracts and other products that are traditional wound-healing agents.[5] These agents usually influence one or more phases of the healing process and are also involved in disinfection and provide a moist environment to encourage the establishment of a suitable environment for the natural healing process.[5]

The focus has been on India and China for recognizing vulnerary agents from natural products.[6] wound-healing properties of some medicinal plants used in Indian medicine has been discussed by others.[7] However, there are plants that could possess clinically important compounds all over the world.[6] For example, plant extracts from Nigeria and Japan

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have been examined for wound-healing properties.[8,9] Plant products such as *Hibiscus rosa sinensis*, *Pterocarpus santalinus* Linn (family *Fabaceae*), and *Schrebera swietenioides* Roxb (*Oleaceae*) have been used for the treatment of a variety of diseases, and their wound-healing properties have been escribed.[10,11]

Plant extracts with wound-healing properties have the potential for antioxidant, chelation, and antimicrobial activities and may act by one or more of these mechanisms.[12] Natural antioxidants have been reported to play a major role in blocking the oxidative stress induced by free radicals.[13] Therefore, it is very important to discover new sources of safe antioxidants of natural origin. Recently, many researchers have shown interest in edible and medicinal plants for their phenolic contents and related total antioxidant activities.[14,15]

Wild edible plants with antioxidant activities are important constituents of traditional diets in Iran. Some of these plants have not been screened for their wound-healing potential. This information is necessary to validate the safety of traditional plants and may be used to establish databases and discover new natural remedies with wound-healing potential.

Lycii fructus has been used as a remedy since ancient times in many countries because of its diuretic, antipyretic, tonic, aphrodisiac, hypnotic, and hepatoprotective effects. The genus *Lycium* (*Solanaceae*) is represented by various species, including *L depressum* Stocks, which is an edible plant in Iran, locally called *Khashk*, with antioxidant properties.[16]

A literature survey reveals that no systematic study has been done on the wound-healing activity of the extract of *L depressum* leaves. In the present work, the wound-healing activity of the methanolic extract of *L depressum* leaves was investigated in an ointment form in 3 concentrations. Assessment of the healing process was based on excision, incision, hydroxyproline estimation, and biomechanical and biochemical studies.

Materials and Methods

Our study protocol was reviewed and approved by the Urmia University of Medical Sciences ethical committee (Ref. UUMS-95/10/12). All animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85–23, revised 1985).

Induction of Diabetes

For insulin-deficient diabetes, rats were fasted overnight before receiving a single intraperitoneal injection (50 mg/kg in 0.9% sterile saline) of streptozotocin (STZ). Hyperglycemia (15 mmol/L or greater) was confirmed 2 days later by measurement of tail-vein blood glucose concentration (Ames Glucostix; Myles, Elkhart, IN).[17] The rats underwent the procedures 3 days after induction of diabetes.

Plant Material and Extract Preparation

The plant samples were collected from the Western provinces of Iran in April and May 2015. The values for moisture and ash were 78.4 ± 2.3 and 20.8 ± 0.7, respectively. Specimens from the plant material were deposited and authenticated at the Department of Botany Sciences, the Hamadan Research Agricultural and Natural Resources Center, Hamadan, Iran. The leaves of the plant were used in the study. The plant material crisp was powdered in an electric blender. For the methanolic extraction, 150 g of the fine powder was extracted with 600 mL of 80% methanol at 37°C for 3 hours. The sample was then centrifuged at 4500 rpm for 15 minutes, and the supernatants were used. The filtrate was placed in an oven to dry at 40°C. The clear residue obtained was used for the study. Moisture and ash contents were determined using standard methods.[18]

Phenolic Contents, Flavonoid Contents, and Antioxidant Activity of Methanolic Extract of L depressum

The radical scavenging capacity of the extract (DPPH) was estimated using the Brand-Williams method.[19] The ferric reducing ability of plasma (FRAP) assay was performed based on a method described by Benzie and Strain[20] with a slight modification. The total phenolic content (TPL) of the extract was determined using the Folin-Ciocalteu reagent based on a procedure described by others.[21] The TPL was expressed as gallic acid equivalents in milligram per gram of chloroform extract. The Trolox equivalent antioxidant capacity (TEAC) assay was based on the ability of the extract to scavenge the stable 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radicals.[22] The sample was mixed with 3.0 mL of ABTS solution, and the absorbance was then measured at 734 nm. For the lipid peroxidation inhibition assay, the extract (40 mL) was mixed with 4.1 mL linoleic acid in absolute ethanol (2.51%), 8 mL of phosphate-buffered saline (pH = 7.0; 0.05 M), and 3.9 mL of distilled water. The mixture was then placed in an oven at 40°C. Then, 70% ethanol (9.7 mL) and 30% ammonium thiocyanate (0.1 mL) were added to the mixture (0.1 mL). The absorbance of red color was measured at 500 nm 3 minutes after addition of 0.1 mL of ferrous chloride (0.02 M in 3.5% hydrochloric acid) to the reaction mixture.[23] The total flavonoid content of the extract was measured using a method described by others.[24] The ferric reducing power of the extract was measured based on the method of others.[25]

Formulation of the Ointment

The base formulation consisting of Eucerin (30%) and Vaseline (70%) in about 1: 2 proportions was prepared. The topical application form was prepared comprising 1, 2, and 4 g of powder extract of the plant material in ointment.

Excision Wound Model and Planimetric Studies

For the excisional wound-healing model, 30 healthy male Wistar rats weighing approximately 160 to 180 g and 7 weeks of age were randomized into 5 groups of 6 rats each: sham surgery group (SHAM), including creation of wounds and no treatment; base formulation group (FG), with creation of wounds and application of base formulation ointment; treatment group 1 (TG1) with 1 g of powder extract of the plant material in ointment; treatment group 2 (TG2) with 2 g; and treatment group 4 (TG3) with 4 g of powder extract of the plant material in ointment. After induction of anesthesia with Xylazine HCl 2% (5 mg/kg/IP, Alfasan International, Woerden, Holland) and ketamine HCl 10% (60 mg/kg/IP, Alfasan International, Woerden, Holland) rats were fixed in a ventral position on a surgery table. Following shaving and aseptic preparation, a circular excision wound was made by cutting away approximately 115 mm^2 full thickness of predetermined area on the anterior-dorsal side of each rat. All the test formulations were applied for 10 days starting from the day of wounding. Wound-healing property was evaluated by wound contraction percentage and wound closure time. Photographs were taken immediately after wounding and on days 0, 7, 14, and 21 postoperation using a digital camera while a ruler was placed near the wounds. The wound areas were analyzed by Measuring Tool of Adobe Acrobat 9 Pro Extended software (Adobe Systems Inc, San Jose, CA), and wound contraction percentage was calculated using the following formula:

$$\text{Percentage of wound contraction} = [(A_0 - A_t) / A_0] \times 100,$$

where A_0 is the original wound area and A_t the wound area at the time of imaging.[26] The animals were left in separate cages for 4 days at room conditions for acclimatization. Animal houses were in standard environmental conditions of temperature (22°C ± 3°C), humidity (60% ± 5%), and a 12-hour light/dark cycle. The animals were maintained on standard pellet diet and tap water. All rats were closely observed for any infection, and if they showed signs of infection, they were separated, excluded from the study, and replaced.

Determination of Hydroxyproline Levels

On day 21 after surgery, a piece of skin from the healed wound area was collected and analyzed for hydroxyproline content. As a major part of collagen, hydroxyproline has an essential role in collagen stability. Collagen is the major component of extracellular tissue, which gives support and strength. The hydroxyproline contents were estimated using a method described by others.[27] Briefly, tissues were dried in a hot air oven at 60°C to 70°C to constant weight and were hydrolyzed in 6N HCl at 130°C for 4 hours in sealed tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to chloramine-T oxidation for 20 minutes. The reaction was terminated by addition of 0.4 M perchloric acid, and color was developed with the help of Ehrlich reagent at 60°C and measured at 557 nm using a UV-visible spectrophotometer (CamSpec M330, Cambridge CB2 4BG, UK).

Incision Wound Model and Biomechanical Testing

30 healthy male Wistar rats weighing approximately 160 to 180 g and 7 weeks of age were randomized into 5 groups of 6 rats each (see above). All animals in the 4 groups were anesthetized as mentioned above, and a paravertebral long incision of 4 cm length was made through the skin and cutaneous muscle at a distance of about 1.5 cm from the middle on the right side of the depilated back. After the incision was made, the 2 ends of the wound were sutured at 0.5 cm intervals with 3/0 nylon. The formulations were applied the same way in the excisional wound model. Ointments were applied once daily for 9 days. On day 9, sutures were removed, and a strip of skin, 7 cm long, with the same widths of wound diameter, in the manner that the wound was located at the middle of the strip, was removed using a double-blade scalpel. The skin was then wrapped in Ringer's soaked gauze, aluminum foils, and plastic bags and kept at −20°C in the freezer until mechanical testing. The TA.XTPlus Texture Analyzer mechanical test device was used for the assessment (Stable Micro Systems, Surrey GU7 1YL, UK). The samples were fitted with appropriate clamps, with the distance between the clamps at the start of testing being 4 cm. The strips were loaded with a 0 to 30 kg load cell, with strain rate of 1 cm/min, and the load elongation curves were obtained. Yield strength (yield point; kg), ultimate strength (kg), maximum stored energy (kg/cm), and stiffness (kg/cm) were measured from the load elongation curves.

Histological Preparation and Quantitative Morphometric Studies

The tissue samples were taken on days 7, 14, and 21 after surgery from the periphery of the wound along with normal skin and fixed in 10% buffered formalin, dehydrated and embedded in paraffin wax, sectioned at 5 μm, and stained

Table 1. Area (mm^2) and WCP (mm^2/d) in Animals of Experimental Groups.[a]

Groups	Day 0	Day 7	Day 14	Day 21
SHAM; WCP	114.72 ± 2.56; 00.00 ± 00	83.35 ± 2.18; 24.78 ± 2.46	60.79 ± 2.69; 46.38 ± 2.23	4.39 ± 1.76; 87.53 ± 2.28
FG; WCP	115.76 ± 1.33; 00.00 ± 00	81.25 ± 2.15; 25.12 ± 2.11	57.38 ± 2.48; 48.31 ± 2.57	3.72 ± 1.84; 89.82 ± 2.54
TG1; WCP	115.32 ± 2.44; 00.00 ± 00	62.38 ± 2.12[b]; 46.08 ± 2.46[b]	41.48 ± 2.07[b]; 64.27 ± 2.81[b]	0.00 ± 0.00[b]; 100.00 ± 0.00[b]
TG2; WCP	114.58 ± 2.52; 00.00 ± 00	61.37 ± 2.56[b]; 47.88 ± 2.41[b]	40.59 ± 2.77[b]; 63.46 ± 2.20[b]	0.00 ± 0.00[b]; 100.00 ± 0.00[b]
TG3; WCP	115.03 ± 2.17; 00.00 ± 00	62.55 ± 2.25[b]; 46.09 ± 2.28[b]	42.85 ± 2.68[b]; 63.78 ± 2.17[b]	0.00 ± 0.00[b]; 100.00 ± 0.00[b]

Abbreviations: WCP, wound contraction percentage; SHAM, sham surgery group; FG, base formulation group; TG, treatment group.
[a]Values are given as mean ± standard error of the mean. The treated groups are compared using the Student *t* test with other groups.
[b]The mean difference is significant at the .05 level versus SHAM and FG groups (Kruskal-Wallis test).

with hematoxylin and eosin and Masson's trichrome stains. Photomicrographs were obtained under light microscope to assess the predominant stage of wound healing. Three parallel sections were obtained from each specimen. Cellular infiltration, including the number of mononuclear cells, polymorphonuclear cells, and fibroblastic aggregation, were quantitatively evaluated. Acute hemorrhage, congestion, vascularization, epithelialization, collagen production, and density were also evaluated qualitatively. Morphological findings were scored using image analyzing software (Image-Pro Express, version 6.0.0.319, Media Cybernetics, Silver Springs, MD). The histological parameters were classified into 5 levels according to the intensity of occurrence (−, absence; +, discrete; ++, moderate; +++, intense; ++++, very intense).[27]

Biochemical Analyses

The frozen samples at −80°C were homogenized in phosphate-buffered saline and centrifuged at 5°C. The supernatant was used for analysis of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione S-transferase (GST), and carbonyl proteins. GST and SOD analyses were performed based on methods described by others.[28,29] Carbonyl proteins were analyzed based on a protocol described by others.[30] The biochemical data were quantified based on the Bradford method and normalized in relation to total protein levels in the supernatant.[31]

Statistical Analysis

Differences among groups in the excisional model for hydroxyproline levels were evaluated by Kruskal-Wallis variance analysis. When the *P* value from the Kruskal-Wallis test statistics was statistically significant, multiple comparison tests were used to determine the differences. Student's *t*-test was used for evaluation of other test results. Comparison among days was assessed using the Mann-Whitney *U*-test. The Bonferroni correction was applied for all possible multiple comparisons. SPSS 11.5 (SPSS Inc, Chicago, IL) was used for statistical analysis. *P* value was set at .05.

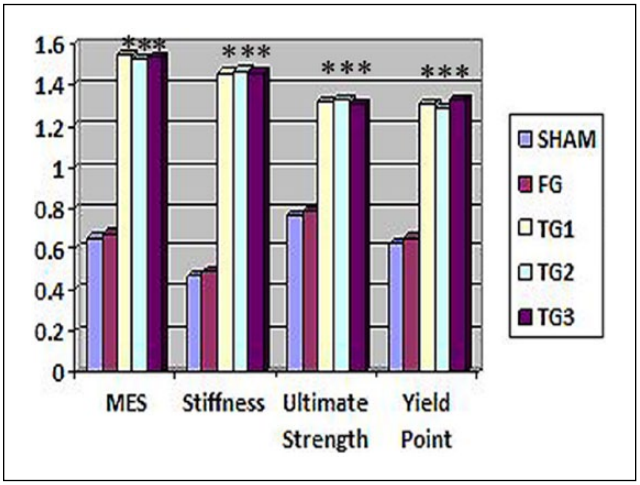

Figure 1. Bar graph indicating biomechanical parameters assessed for each of the experimental groups. Data are presented as mean ± standard error of the mean. *$P < .05$ versus SHAM and FG groups.
Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group; MES, maximum stored energy.

Results

Phenolic Contents, Flavonoid Contents, and Antioxidant Activity

The methanolic extract of *L depressum* demonstrated a TPL of 53.29 ± 0.64 mg of gallic acid (equivalents/g of dry weight), total flavonoid content of 29.16 ± 0.31 mg catechin (equivalents/g of dry weight), antioxidant activity using the FRAP assay 426.0 ± 3.39 µmol (Fe(II)/g of dry weight), antioxidant activity using the ABTS assay (711.0 ± 9.58 µmol Trolox equivalent/g of dry weight), DPPH radical scavenging activity (87.49% ± 2.08%), reducing power (714.0 ± 6.84 µmol Trolox equivalents/g of dry weight), and inhibition against lipid peroxidation (79.45% ± 2.03%).

Reduction in Wound Area

Wound contraction percentages in different groups during the course of the study are shown in Table 1. The healing

Table 2. Intensity of Histological Parameters Assessed in the Experimental Animals.[a]

Groups	Days	Histological Parameters				
		Acute Hemorrhage	Congestion	Vascularization	Epithelialization	Collagen
SHAM	7	+++	+++	+	−	+
	14	++	++	++	+	+
	21	−	−	++	++	++
FG	7	++	+++	+	+	+
	14	+	+	++	++	++
	21	−	−	++	++	++
TG1/TG2/TG3	7	+[b]	+[b]	++[b]	+[b]	+[b]
	14	−	−	+++[b]	++[b]	++[b]
	21	−	−	+++[b]	++++[b]	+++[b]

Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group.
[a]Classification of histological parameters according to the intensity of occurrence: −, absence; +, discrete; ++, moderate; +++, intense; ++++, very intense. Histopathological damage was assessed as explained under Materials and Methods on days 7, 14, and 21 of lesion.
[b]$P < .05$ versus SHAM and FG groups.

rate of the extract-treated groups was significantly different when compared with the control group ($P < .05$). However, time had a significant effect on wound contraction of all wounds ($P = .034$).

Hydroxyproline Content of the Wounds

Proline is hydroxylated to form hydroxyproline after protein synthesis. Hydroxyproline contents in the groups SHAM, FG, TG1, TG2, and TG3 were found to be 41.18 ± 2.92, 44.13 ± 3.17, 63.41 ± 4.54, 64.76 ± 4.75, and 63.28 ± 3.98 mg/g, respectively. Hydroxyproline contents were increased significantly in the extract-treated groups, which implies more collagen deposition compared with SHAM and FG groups ($P = .001$).

Biomechanical Findings

The biomechanical indices, maximum stored energy, stiffness, ultimate strength, and yield strength obtained for the extract-treated groups were significantly higher than those obtained for the SHAM and FG groups ($P = .003$; Figure 1).

Histological and Morphometric Findings

There were significant differences in comparisons of the extract-treated and non–extract-treated groups, particularly in terms of cellular infiltration, acute hemorrhage, congestion, edema, collagen production and density, reepithelialization, and neovascularization. During the study period, scores for reepithelialization and neovascularization were significantly higher in animals of the extract-treated groups than SHAM and FG groups ($P = .001$). Polymorphonuclear and mononuclear cell count and fibroblast cell proliferation as well as mean rank of the qualitative study of acute hemorrhage, edema, and collagen production score in the extract-treated groups were significantly higher than those of SHAM and FG groups ($P = .001$; Table 2; Figures 2-5).

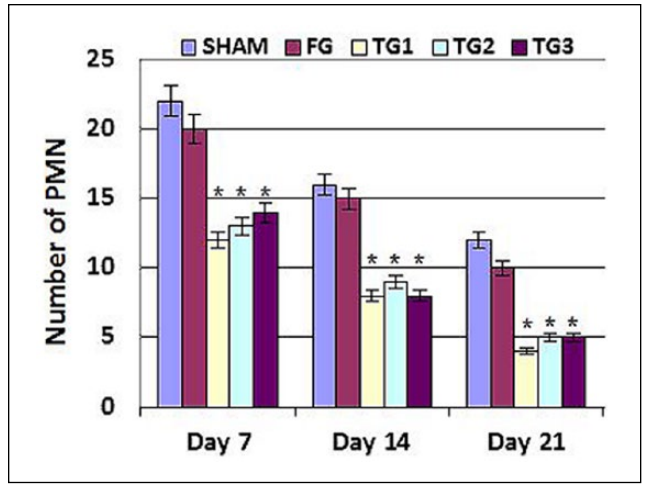

Figure 2. Bar graph indicating number of polymorphonuclear cells (PMN) in excisional model of the rat skin in experimental groups. Results are expressed as mean ± standard error of the mean. *$P < .05$ versus other experimental groups.
Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group.

Biochemical Findings

The MDA values were significantly reduced in the extract-treated groups in comparison with FG and SHAM groups on day 14 ($P = .0032$). On day 21, the MDA levels in TG1, TG2, and TG3 groups were significantly decreased compared with FG and SHAM groups ($P = .001$). The SOD levels were significantly higher in the extract-treated animals compared with FG and SHAM groups on day 7 ($P = .001$). The SOD levels in TG1, TG2, and TG3 groups were

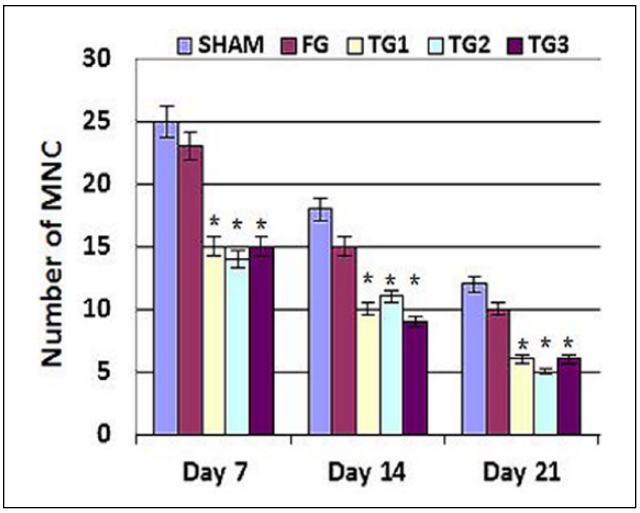

Figure 3. Bar graph indicating number of mononuclear cells (MNCs) in excisional model of the rat skin in experimental groups. Results are expressed as mean ± standard error of the mean. *$P < .05$ versus other experimental groups.
Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group.

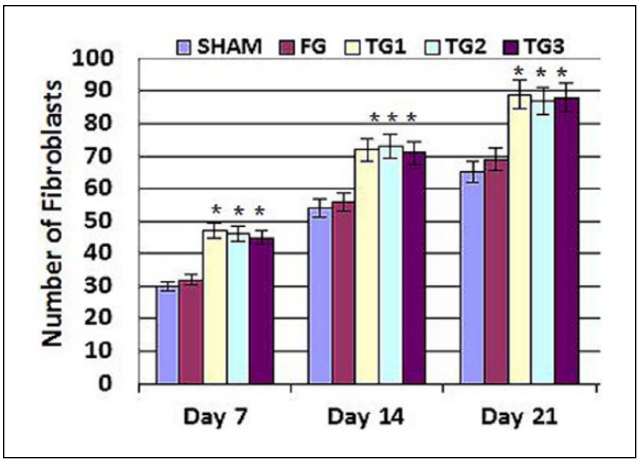

Figure 4. Bar graph indicating number of fibroblasts in excisional model of the rat skin in experimental groups. Results are expressed as mean ± standard error of the mean. *$P < .05$ versus other experimental groups.
Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group.

significantly decreased compared with FG and SHAM groups on day 14 ($P = .0034$). There were no significant differences in GST levels among groups during the study period ($P > .05$). Carbonyl proteins were significantly lower in the extract-treated animals compared with FG and SHAM groups during the study period ($P = .001$; Table 3).

Discussion

One of the most serious complications of diabetes is the formation of diabetic foot syndrome. Herbal extracts with high antioxidant properties can be used to treat the resulting consequences of the syndrome.[32] The wound-healing process in diabetic patients is impaired and delayed because of high blood glucose levels. High blood glucose hampers proliferation of cells and decreases collagen production, which results in decrease in chemotaxis and phagocytosis.[25] Elevated blood glucose level, a reduction in the levels of growth factors, and the inhibition of fibroblast proliferation have all been suggested to contribute to the observed impairment in wound healing.[33] STZ-induced diabetes in rats is one of the most extensively studied models of diabetes. In this study, therefore, STZ-induced diabetic rats were used as the model of diabetes to study diabetic wound healing.

Collagen, the major component that strengthens and supports extracellular tissue, is composed of the amino acid hydroxyproline, which has been used as a biochemical marker for tissue collagen.[34] In the present study, in the excisional wound model, in the extract-treated animals, there was a significant decrease in wound area. This indicated improved collagen maturation by increased cross-linking. The balance between synthesis and breakdown and, therefore, deposition of collagen is important in wound healing and development of wound strength.[35]

Hydroxyproline is a major component of collagen that permits the sharp twisting of the collagen helix. It helps provide stability to the triple-helical structure of collagen by forming hydrogen bonds. Hydroxyproline is found in only a few proteins other than collagen. For this reason, hydroxyproline content has been used as an indicator to determine collagen content.[36] The measurement of hydroxyproline has been reported in other studies assessing the effect of extracts on wound healing, and it has been concluded that because hydroxyproline is a direct estimate of collagen synthesis, the results permit the conclusion that the treatment works at molecular levels.[7,10] In the present study, increase in hydroxyproline content in the extract-treated groups indicated increased collagen content because hydroxyproline yields a direct estimate of collagen synthesis.

Mechanical testing is sensitive to changes that occur during the progression of wound healing and can be used as a tool to measure the quality of healing. Mechanical property data provide a clinically relevant and functional assessment of wound-healing quality. Histological analyses highlight cellular and connective tissue adaptation at the ultrastructural level in the repair process.[37] In the present study, when compared with other experimental groups, the extract-treated animals showed a statistically significant difference in biomechanical parameters.

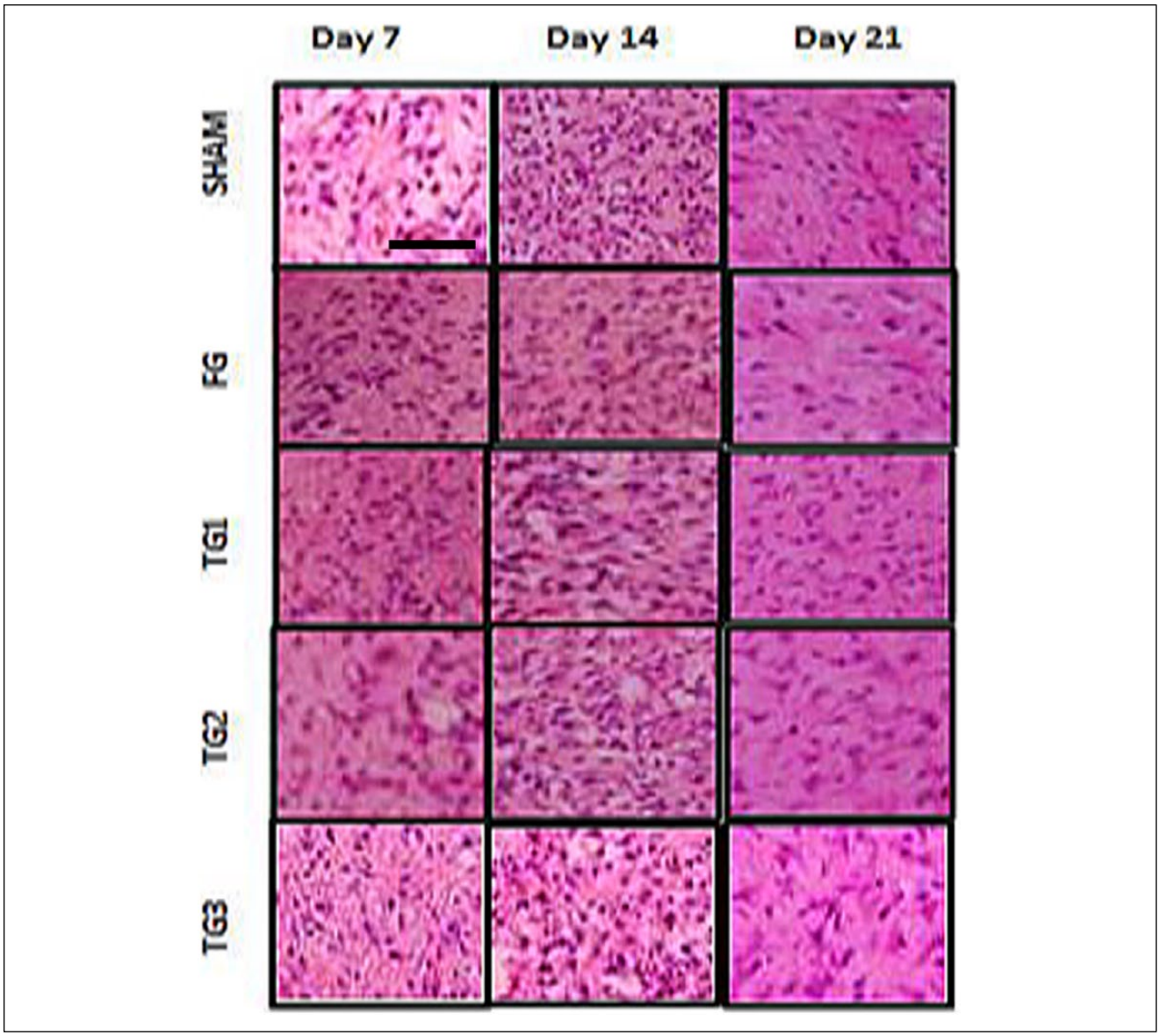

Figure 5. Serial photographs of wounds at 3 time points in the experimental groups. Scale: 50 μm.
Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group.

It has been reported that the ethanol extract of *H rosa sinensis* L (*Malvaceae*) significantly increased the rate of wound contraction, wound breaking strength, rate of epithelialization, and weight and hydroxyproline content of the granulation tissue.[10] It has also been discussed that granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test-treated animals suggests higher collagen content.[10]

In the present study, histopathological examination and scoring revealed that there was a significant difference by means of wound-healing scores in the extract treated groups compared with other experimental groups. The extract decreased the maturation time of granulation tissue and wound contraction, which means that it enhanced re-epithelialization with significant effects on inflammatory infiltration and number of fibroblasts in time-dependent activity.

Antioxidants have been reported to play a significant role in improving the wound-healing process and protecting the tissues from oxidative damage.[38] Wound-healing mechanisms may contribute to stimulating the production of antioxidants at the wound site and provide a favorable

Table 3. Comparison of the Activities of MDA, SOD, GST, and Carbonyl Proteins in the Tissue Samples Taken From Experimental Groups on Days 7, 14, and 21.[a]

Variables	SHAM	FG	TG1	TG2	TG3
Day 7					
MDA (nmol/mg protein)	0.075 ± 0.019	0.068 ± 0.015	0.062 ± 0.015[b]	0.064 ± 0.013[b]	0.065 ± 0.016[b]
SOD (U/mg protein)	0.28 ± 0.05	0.82 ± 0.07	5.83 ± 0.19[b]	5.92 ± 0.16[b]	5.85 ± 0.18[b]
GST (µmol/min/g)	0.47 ± 0.15	0.45 ± 0.12	0.46 ± 0.11	0.44 ± 0.12	0.45 ± 0.15
Carbonyl proteins (nmol/mL)	75.44 ± 6.17	72.38 ± 5.57	23.37 ± 4.18[b]	24.42 ± 4.57[b]	23.89 ± 4.65[b]
Day 14					
MDA (nmol/mg protein)	0.082 ± 0.14	0.073 ± 0.011	0.054 ± 0.018[b]	0.056 ± 0.014[b]	0.055 ± 0.011[b]
SOD (U/mg protein)	0.62 ± 0.03	0.65 ± 0.04	1.23 ± 0.12[b]	1.24 ± 0.14[b]	1.25 ± 0.18[b]
GST (µmol/min/g)	0.44 ± 0.12	0.47 ±0.10	0.45 ± 0.13	0.45 ± 0.12	0.46 ± 0.17
Carbonyl proteins (nmol/mL)	72.63 ± 5.84	73.69 ± 4.35	25.31 ± 4.48[b]	25.83 ± 4.11[b]	24.67 ± 4.74[b]
Day 21					
MDA (nmol/mg protein)	0.093 ± 0.021	0.084 ± 0.012	0.036 ± 0.015[b]	0.034 ± 0.011[b]	0.035 ± 0.012[b]
SOD (U/mg protein)	0.21 ± 0.02	0.23 ± 0.06	0.22 ± 0.19	0.24 ± 0.34	0.23 ± 0.28
GST (µmol/min/g)	0.45 ± 0.14	0.45 ± 0.15	0.46 ± 0.18	0.44 ± 0.12	0.44 ± 0.15
Carbonyl proteins (nmol/mL)	25.36 ± 4.79	24.78 ± 4.17	14.54 ± 3.28[b]	15.76 ± 3.31[b]	15.50 ± 3.98[b]

Abbreviations: SHAM, sham surgery group; FG, base formulation group; TG, treatment group; MDA, malondialdehyde; SOD, superoxide dismutase; GST, glutathione S-transferase.
[a]Data are expressed as mean ± SD.
[b]$P < .05$ versus SHAM and FG groups.

environment for tissue healing.[39] Increased oxidative damage might directly interfere with skin tissue repair.[39] In the present study, the extract-treated animals showed a significant increase in SOD levels compared with other groups, demonstrating the antioxidant effect of the extract at all concentrations. It could be concluded that the antioxidant effect of the extract provided an important anti-inflammatory response, which could be associated with stimulation of antioxidant enzymes, particularly SOD, which remained at high levels in the extract-treated animals. Furthermore, lower levels of MDA and carbonylated proteins, which are important markers of tissue stress, were observed in the extract-treated animals.

Although the present study showed the promising effect of the extract of *L depressum* on wound healing in rats, data regarding the molecular mechanisms leading to its action remain to be investigated in depth. Molecular evidence for the action of the extract was not provided, which may be considered a limitation of this study.

The present study demonstrated that methanolic extract of *L depressum* leaves had properties that render it capable of promoting accelerated wound healing by comparing it with controls.

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Declaration of Conflicting Interests

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References

1. de Almeida SA, Salomé GM, Dutra RA, Ferreira LM. Feelings of powerlessness in individuals with either venous or diabetic foot ulcers. *J Tissue Viability*. 2014;23:109-114.
2. Lee CH, Chang SH, Chen WJ, et al. Augmentation of diabetic wound healing and enhancement of collagen content using nanofibrous glucophage-loaded collagen/PLGA scaffold membranes. *J Colloid Interface Sci*. 2015;439:88-97.
3. Romero-Cerecero O, Zamilpa A, Díaz-García ER, Tortoriello J. Pharmacological effect of *Ageratina pichinchensis* on wound healing in diabetic rats and genotoxicity evaluation. *J Ethnopharmacol*. 2014;156:222-227.
4. Greenhalgh DG. Wound healing and diabetes mellitus. *Clin Plast Surg*. 2003;30:37-45.
5. Deshmukh PT, Gupta VB. Embelin accelerates cutaneous wound healing in diabetic rats. *J Asian Nat Prod Res*. 2013;15:158-165.
6. Lindblad WJ. Considerations for determining if a natural product is an effective wound-healing agent. *Int J Low Extrem Wounds*. 2008;7:75-81.
7. Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: a review. *Int J Low Extrem Wounds*. 2003;2:25-39.
8. Chah KF, Eze CA, Emuelosi CE, et al. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *J Ethnopharmacol*. 2006;104:164-167.
9. Kimura Y, Sumiyoshi M, Sakanaka M. Effects of *Astilbe thunbergii* rhizomes on wound healing: part I. Isolation of promotional effectors from *Astilbe thunbergii* rhizomes on burn wound healing. *J Ethnopharmacol*. 2007;109:72-77.

10. Shivananda Nayak B, Sivachandra Raju S, Orette FA, Chalapathi Rao AV. Effects of *Hibiscus rosa sinensis* L (*Malvaceae*) on wound healing activity: a preclinical study in a Sprague Dawley rat. *Int J Low Extrem Wounds*. 2007;6:76-81.
11. Rasal AS, Nayak PG, Baburao K, Shenoy RR, Mallikarjuna Rao C. Evaluation of the healing potential of *Schrebera swietenioides* in the dexamethasone-suppressed wound healing in rodents. *Int J Low Extrem Wounds*. 2009;8:147-152.
12. Mallefet P, Dweck CA. Mechanisms involved in wound healing. *Biomed Sci*. 2008;7:609-615.
13. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables: the millennium's health. *Int J Food Sci Technol*. 2001;36:703-725.
14. Djeridanea A, Yousfia M, Nadjemib B, Boutassounaa D, Stockerc P, Vidalc N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. 2006;97:654-660.
15. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem*. 2006;94:550-557.
16. Tabaraki R, Nateghi A, Ahmady-Asbchin S. In vitro assessment of antioxidant and antibacterial activities of six edible plants from Iran. *J Acupunct Meridian Stud*. 2013;6:159-162.
17. Mizisin AP, Vu Y, Shuff M, Calcutt NA. Ciliary neurotrophic factor improves nerve conduction and ameliorates regeneration deficits in diabetic rats. *Diabetes*. 2004;53:1807-1812.
18. Süntar I, Küpeli Akkol E, Keles H, et al. Efficacy of *Daphne oleoides* subsp. *kurdica* used for wound healing: identification of active compounds through bioassay guided isolation technique. *J Ethnopharmacol*. 2012;141:1058-1070.
19. Jaitak V, Sharma K, Kaltia K, et al. Antioxidant activity of *Potentilla fulgens*: an alpine plant of western Himalaya. *J Food Comp Anal*. 2010;23:142-147.
20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Biochem*. 1996;239:70-76.
21. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern Med*. 2012;12:221.
22. Arts MJTJ, Dallinga JS, Vass HP, Haenen GRMM, Bast A. A critical appraisal of the use of the antioxidant capacity (TEAC) assay in defining optimal antioxidant structures. *Food Chem*. 2003;80:409-414.
23. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. *J Food Sci*. 1993;58:1407-1410.
24. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxides radicals. *Food Chem*. 1999;64:555-559.
25. Rehman ZU. Evaluation of antioxidant activity of methanolic extract from peanut hulls in fried potato chips. *Plant Foods Hum Nutr*. 2003;58:75-83.
26. Reddy BSR, Reddy KK, Naidu VGM, et al. Evaluation of antimicrobial, antioxidant and wound-healing potentials of *Holoptelea integrifolia. J Ethnopharmacol*. 2008;115:249-256.
27. Qiu Z, Kwon AH, Kamiyama Y. Effects of plasma fibronectin on the healing of full-thickness skin wounds in streptozotocin-induced diabetic rats. *J Surg Res*. 2007;138:64-70.
28. Nozaki N, Shishido T, Takeishi Y, Kubota I. Modulation of doxorubicin-induced cardiac dysfunction in toll-like receptor-2-knockout mice. *Circulation*. 2004;110:2869-2874.
29. Siddiqui A, Raisuddin S, Shukla Y. Protective effects of black tea extract on testosterone induced oxidative damage in prostate. *Cancer Lett*. 2005;227:125-132.
30. Jana K, Dutta A, Chakraborty P, et al. Alpha-lipoic acid, *N*-acetylcysteine protects intensive swimming exercise-mediated germ-cell depletion, pro-oxidant generation, and alteration of steroidogenesis in rat testis. *Mol Reprod Dev*. 2014;81:833-850.
31. Bradford M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal Biochem*. 1976;72:248-254.
32. Viswanathan V, Kesavan R, Kavitha KV, Kumpatla S. A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers. *Indian J Med Res*. 2011;134:168-173.
33. Gonçalves RV, Novaes RD, Sarandy MM, et al. 5α-Dihydrotestosterone enhances wound healing in diabetic rats. *Life Sci*. 2016;152:67-75.
34. Mehrtash M, Mohammadi R, Hobbenaghi R. Effect of adipose derived nucleated cell fractions with chitosan biodegradable film on wound healing in rats. *Wound Med*. 2015;10-11:1-8.
35. Dogan S, Demirer S, Kepenekci I, et al. Epidermal growth factor-containing wound closure enhances wound healing in non-diabetic and diabetic rats. *Int Wound J*. 2009;6:107-115.
36. Martin J, Zenilman M, Lazarus GS. Molecular microbiology: new dimensions for cutaneous biology and wound healing. *J Invest Dermatol*. 2010;130:38-48.
37. Brem H, Tomic-Canic M, Entero H, et al. The synergism of age and db/db genotype impairs wound healing. *Exp Gerontol*. 2007;42:523-531.
38. Fitzmaurice SD, Sivamani RK, Isseroff RR. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. *Skin Pharmacol Physiol*. 2011;24:113-126.
39. Shukla A, Rasik AM, Dhawan BN. Asiaticoside-induced elevation of antioxidant levels in healing wounds. *Phytother Res*. 1999;13:50-54.