Original Research

Sida cordifolia Linn. accelerates wound healing process in type 2 diabetic rats

Rajesh Singh Pawar*, Shweta Kumar, Fedelic Ashish Toppo, Lakshmi PK, Pratibha Suryavanshi

Pharmacognosy and Phytochemistry Laboratory, VNS Group of Institutions, Faculty of Pharmacy, VNS Campus, Vidyavihar, Neelbud, Bhopal, Madhya Pradesh 462044, India

Received 15 September 2015; revised 16 July 2016; accepted 29 August 2016

Available online 2 November 2016

Abstract

Objective: Sida cordifolia Linn. (Hindi—kungyi; Sanskrit—bala) is a shrub (family Malvaceae) grown all over India. In Ayurveda, the crushed leaves of the plant is used for the treatment and dressing of wounds. Traditionally, root juice is also used to promote the healing of wounds. This study was designed to evaluate the wound healing potential of hydrogel of methanolic extract of Sida cordifolia Linn. (MeOHSC) in diabetic rats.

Methods: The methanolic extract in the form of hydrogel was applied topically for the management of wounds in fructose-fed streptozotocin-induced diabetic rats. The % wound contraction, period of epithelialization, hydroxyproline content, and histopathological examination were evaluated using the excision wound model, and tensile strength was measured using the incision wound model.

Results: The results showed that in both models, hydrogel of MeOHSC was found to have significant wound healing activity compared with diabetic wound control in terms of % wound contraction \((p<0.01)\), period of epithelialization \((p<0.01)\), and hydroxyproline content \((p<0.01)\) in the excision model, and tensile strength \((p<0.01)\) in the incision model. The presence of collagen fibers and stronger growth of epithelial cells were observed in hydrogel of MeOHSC than in the diabetic wound control group.

Conclusion: Our findings suggest that hydrogel of MeOHSC has a potential benefit in enhancing the wound healing process in diabetic condition. This is possibly because of the presence of phenolic compounds and confirmation of gallic acid in the extracts.

Keywords: excision model; HPLC fingerprinting; incision model; Sida cordifolia Linn.; wound healing

1. Introduction

In the past few years, there has been an exponential growth in the field of herbal medicine because of the natural origin and fewer side effects of these resources. India is the largest producer of medicinal herbs and is called as the botanical garden of the world.1 Natural products are a source of synthetic and traditional herbal medicine, and are still the primary health care system.2 Herbal medicines are generally available as a mixture of more than one plant constituent.3 Wound healing is the process of regeneration of ruptured tissue after injury to the skin or other soft tissue. Following injury to the skin, the inflammatory responses begin, and cells below the dermis begin to generate collagen (connective tissue) production. The inflammatory responses involve phagocytosis of foreign protein. Then, epithelial cells are regenerated and differentiated into skin layers.4 In healthy individuals, the acute wound healing process is maintained and completed through the integration of multiple signals (in the form of cytokines and chemokines) released by keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. During wound-induced hypoxia, VEGF (vascular endothelial growth factor) released by macrophages, fibroblasts, and epithelial

* Corresponding author. Pharmacognosy and Phytochemistry Laboratory, VNS Group of Institutions, Faculty of Pharmacy, VNS Campus, Neelbud, Bhopal, 462044 Madhya Pradesh, India.

E-mail address: dr_pawar14@rediffmail.com (R.S. Pawar).
cells induce the phosphorylation and activation of endothelial nitric oxide synthase (eNOS) in the bone marrow, resulting in an increase in NO levels, which triggers the mobilization of bone marrow Endothelial Progenitor Cells (EPCs) to the circulation. The chemokine SDF-1α promotes the homing of these EPCs to the site of injury, where they participate in neovasculogenesis. In diabetic patients, eNOS phosphorylation is impaired, which hinders the movement of EPCs from bone marrow into circulation. It has also been shown that SDF-1α expression is decreased in epithelial cells and myofibroblasts in diabetic wounds, which prevents EPCs from homing to wounds and therefore limits wound healing.5

Sida cordifolia Linn. (Hindi—kungyi; Sanskrit—bala), a shrub belonging to family Malvaceae, is grown in tropical and subtropical regions worldwide and all over India.6 In Ayurvedic practices, Sida cordifolia (S. cordifolia) has three common applications: Mashabaladi Kvatha, where the plant seeds are mixed with other ingredients to relieve muscular pain; Balataila, a process for the treatment of nervous system complaints and stomach problems and as a cardiac tonic; and root juice is also used to promote wound healing.7 This study was designed to explore the wound healing activity of the topically applied hydrogel of the methanolic extract of S. cordifolia (MeOHSC) in diabetic rats.

2. Materials and methods

2.1. Plant collection

The selection of plant was based on traditional use such as normal wound healing7 and hypoglycemic activities.8 S. cordifolia was collected from Vindhya Herbal Quality Testing and Research Laboratory Bhopal (MP, India). S. cordifolia was identified and authenticated by the botany section of Vindhya Herbal Quality Testing and Research Laboratory Bhopal (MP, India). The aerial parts of S. cordifolia were dried initially under shade and later preserved in a tightly closed container.

2.2. Preparation of plant extract

The powdered aerial part of S. cordifolia was defatted using petroleum ether and later on extracted by methanol and hydroalcohol. Methanolic extract was concentrated separately under vacuum, and the resulting dried extract was kept in refrigerator.

2.3. Phytochemical screening

The MeOHSC extract of the aerial parts was analysed for the presence of phytoconstituents such as glycosides, flavonoids, carbohydrates, protein and amino acids, lipids, resins, alkaloids etc.9

2.4. Determination of total phenolic content

The concentration of phenolics in the plant extract was determined using a spectrophotometric method. The methanolic solution of the extract at a concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL methanolic solution of the extract, 2.5 mL of 10% Folin-Ciocalteu reagent dissolved in water, and 2.5 mL of 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu reagent dissolved in water, and 2.5 mL of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45°C for 45 minutes. The absorbance was determined using a spectrophotometer at λmax = 765 nm.10

2.5. Determination of flavonoid concentration

The content of flavonoids in the examined plant extract was determined using the spectrophotometric method. The sample contained 1 mL methanol solution of the extract at a concentration of 1 mg/mL and 1 mL of 2% AlCl3 solution dissolved in methanol. The samples were incubated for 1 hour at room temperature. The absorbance was determined using a spectrophotometer at λmax = 415 nm.10

2.6. High-performance liquid chromatography analysis

Chromatographic analyses of samples were performed using the Shimadzu high-performance liquid chromatography (HPLC) system (Shimadzu Corp., Kyoto, Japan) consisting of an LC-20AD pump and an SPD-M 20A photodiode array detector. The C-18 column (dimension, 4.6 mm × 150 mm; particle size, 5 μm) was used as the stationary phase. HPLC-grade acetonitrile and water (Thermo Fisher Scientific India Pvt. Ltd., Mumbai) were used as the mobile phase at a ratio (water/acetonitrile/acetic acid) of 90:10:0.01. The flow rate was 1 mL/min, the injection volume was 20 μL, and the temperature was set at 40°C. Detector wavelength (280 nm) was fixed on the basis of UV—Visible spectrophotometer (Shimadzu Corp.) scan reports of the MeOHSC.

2.7. Sample preparation for HPLC analysis

Gallic acid (3,4,5-trihydroxybenzoic acid) was used as standard to verify its presence and absence in the MeOHSC extract. To prepare different concentrations of the standard solution, 10 mg standard was weighed, dissolved, and made up the volume with methanol in a 10-mL volumetric flask. Then, 0.2 μg/mL, 0.4 μg/mL, 0.6 μg/mL, and 0.8 μg/mL concentrations of standard solution were prepared. The same procedure was followed for preparing the 10 μg/mL sample of MeOHSC extract, and samples were filtered through a nylon filter with a pore size of 0.45 mm (Rankem, RFCL Ltd., New Delhi).
2.8. Preparation of hydrogel formulations

To prepare the hydrogel of MeOHSC, sodium metabisulfite, methyl paraben sodium, and propyl paraben sodium were dissolved in water. A gelling agent (Carbopol 934) was added, and the mixture was stirred continuously until it became swollen completely. Triethanolamine was slowly added to the dispersion with continuous stirring that resulted in a stiff gel. Next, MeOHSC (10%) was added, and stirring continued for 15 minutes. Volume was made up with water, and the mixture was stirred continuously until it became dissolved in water. A gelling agent (Carbopol 934) was added, and the mixture was stirred continuously until it became a uniform gel was formed.\(^\text{11,12}\)

2.9. Evaluation

- **pH**: 1 gr of gel was weighed accurately and dissolved in 100 mL purified water. The pH of the dispersion was measured using a digital pH meter.\(^\text{12}\)
- **Viscosity**: the viscosity of the formulation was determined as such without dilution by R/S CPS plus Rheometer (Brookfield AMETEK, Massachusetts, USA) using spindle 05 at 0.1 g.\(^\text{12}\)
- **Homogeneity**: the formulation was tested for homogeneity by visual inspection. It was also tested for physical appearance and presence of any aggregates.\(^\text{12}\)
- **Test of skin irritancy**: this test is performed by patch test. The test substances were applied to an area of approximately 6 cm\(^2\) of skin and covered with a gauze patch. The patch was loosely held in contact with skin by means of a semicocclusive dressing for 4 hours and was then removed. Observation was recorded 1 hour after the removal of the patch. No visible reaction or erythema or intense erythema with edema and vesicular erosion should occur; a good hydrogel base shows no visible reaction.\(^\text{12}\)

2.10. Animals

Healthy adult albino rats (Wistar strain) of either sex with a body weight between 150 g and 250 g were selected for the study. The animals were kept under 12:12-hour day and night schedules with the temperature set between 18°C and 20°C. They were housed in large spacious hygienic cages during the experimental period. Animals were allowed free access to water and standard pellet diet up to the end of the study. The animal study was conducted in VNS Group of Institutions, Faculty of Pharmacy, Pharmacology Division, Bhopal (MP, India), with due permission from the Institutional Animal Ethical Committee (CPCSEA protocol no. PH/IAEC/VNS/2K14/006).

2.11. Experimental protocol

The animals were divided into three groups, with each group containing six animals for excision and incision wound models. In the excision wound model and incision wound model, Group I was considered as normal wound control and treated with only hydrogel base, Group II was denoted as diabetic wound control and treated with only hydrogel base, and Group III received topically hydrogel of MeOHSC. They were applied daily, and the healing property was assessed in terms of physical, biochemical, and histopathological studies.

2.12. Induction of type 2 diabetes

Wistar albino rats were made diabetic by administering them with 10% fructose solution and a single injection of streptozotocin (STZ) prepared in citrate buffer (0.1M, pH 4.5), 40 mg/kg (intraperitoneally), after overnight fasting. Blood glucose was drawn from the tail vein, and glucose levels were determined using a glucometer. Wistar albino rats having an elevated blood glucose (>250 mg/dL) were selected for creation of excision and incision wounds. Blood glucose levels were estimated at the time of creation of the wounds and after the treatment.\(^\text{13}\)

2.13. Excision diabetic wound model

An excision wound model was done by cutting away 100 mm\(^2\) full thickness area on the depilated back of the rats. After confirmation of diabetes, fasting blood glucose of >250 mg/dL by glucometer on the 7\(^{th}\) day wound induction was started. The rats were anesthetized via administration of ketamine (5 mg/kg body weight) and xylazine (50 mg/kg body weight). The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 100-mm\(^2\) full thickness of skin from a predetermined shaved area. Wounds were left undressed to the open environment.

In this model, % wound contraction, epithelialization period, and hydroxyproline content were evaluated, and histopathological examination was done. The % wound contraction was measured every 2\(^{nd}\) day, and the period of epithelialization was calculated on the complete removal of scar tissue. Hydroxyproline content was measured and histopathological examination was performed on Day 12 after wounding.\(^\text{14}\)


All animals were anesthetized prior to wound creation, and two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from the midline on each side of the depilated back of rats. Both edges were kept together and stitched with black silk surgical thread (no. 000), and a curved needle (no. 11) was used for stitching. The sutures were removed on Day 3 after wounding, and breaking strength was measured by continuous water flow techniques.\(^\text{5}\)

2.15. Wound healing evaluation parameters

2.15.1. Percentage wound contraction and period of epithelialization

Wound contraction in individual animals from the control and treatment groups was measured for each 2-day interval
using a transparent graph sheet, and the rate of wound healing was expressed as percentage contraction.

% Wound contraction = \( \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100 \)

Epithelialization period was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind.\(^{15}\)

2.15.2. Hydroxyproline measurement

Wound tissues were analyzed on the 12th day for hydroxyproline content, a basic constituent of collagen. Tissues were vacuum-dried to a constant weight and hydrolyzed in 6N HCL at 130°C for 3 hours in sealed tubes. The hydrolysate was neutralized to pH 7, and then subjected to Chloramine-T oxidation for 20 minutes. The reaction was terminated by the addition of 0.4M perchloric acid, and developed color with Ehrlich reagent at 60°C was read at \( \lambda_{\text{max}} = 557 \text{ nm} \) in UV spectrophotometer (Shimadzu).\(^{16}\)

2.15.3. Tensile strength

For measurement of tensile strength, the sutures of the wound were removed on 3rd postwound day, and the skin breaking strength was measured using the continuous water flow technique described by Lee.\(^{17}\)

2.16. Histopathological studies

In histopathological studies, tissue samples were collected on the 12th postwound day and fixed in 10% formalin solution for 24 hours and dehydrated with a sequence of ethanol—xylene series of solution. Then, they were filtered and embedded with paraffin (40–60°C), and microtome sections were taken at 5-mm thickness. The sections were processed in alcohol—xylene series and stained with hematoxylin–eosin dye. The histological samples were observed under a Digital microscope (Labomed Inc., USA).\(^{18}\)

2.17. Statistical analysis

The results were expressed as mean ± standard error mean. The statistical significance was assessed using one-way analysis of variance followed by Dunnett’s test.

3. Results

3.1. Phytochemical studies

This study revealed the presence of phytochemicals, which are considered active medicinal chemical constituents, as shown in Table 1. Important medicinal phytochemicals such as

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Phytoconstituents</th>
<th>Identification test</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Proteins and amino acids</td>
<td>Barford test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Fehling's test</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>Benedict test</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Alkaloids</td>
<td>Millions test</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Lipids</td>
<td>Biuret test</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Resins</td>
<td>Ninhydrin test</td>
<td>+++</td>
</tr>
</tbody>
</table>

Table 1 Qualitative analysis of phytoconstituents of *S. cordifolia*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolics (mg of GAE/g of extract)</th>
<th>Total flavonoids (mg of RE/g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>0.024 mg of GAE/g of extract</td>
<td>0.032 mg of RE/g of extract</td>
</tr>
</tbody>
</table>

GAE = Gallic acid equivalent; RE = Rutin equivalent.

Table 2 Content of total phenolics and flavonoids in plant extract of *S. cordifolia*.\(^{19}\)
carbohydrate, protein amino acid, flavonoids, glycosides, lipid, resins, and some traces of alkaloids were present in the MeOHSC extract.

3.2. Determination of phenolic compound

The total phenolic content in the MeOHSC extract using the Folin–Ciocalteau reagent is expressed in terms of gallic acid equivalent or GAE (standard curve equation: $y = 0.0011x - 0.0867$, $r^2 = 0.996$). The values obtained in the concentration of total phenols are expressed as mg of GAE/g of extract (Table 2). The total phenolic content in the MeOHSC extract was found to be 0.24 mg GAE/g.

3.3. Determination of flavonoid content

The concentration of flavonoids in the MeOHSC extract was determined using the spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (RE), mg of RE/g of extract (Table 2). The concentration of flavonoids in MeOHSC extract was found to be 0.32 mg/g.

3.4. HPLC analysis

The HPLC profile of MeOHSC extract is presented in Table 3 (Figure 1). The standard gallic acid ($y = 60.409x + 82.464$, $r^2 = 0.946$) revealed one peak with a retention time of 4.832 minutes. The methanolic extract showed two peaks, in which Peak 1 is a minor peak with a retention time of 4.833 minutes. It closely resembles that of standard gallic acid peak, indicating that they are of similar chemical structure, which may be responsible for the biological activity. Meanwhile, Peak 2 is a major peak, which may be another phytoconstituent or active medicinal constituent present in the MeOHSC extract. This is possible owing to the qualitative analysis of the MeOHSC extract.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Sample concentration (MeOHSC) (μg/mL)</th>
<th>Area</th>
<th>Retention time (min)</th>
<th>Concentration of gallic acid in MeOHSC extract (μg/10 mg extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>127,351</td>
<td>4.833</td>
<td>74</td>
</tr>
</tbody>
</table>

HPLC = high-performance liquid chromatography; MeOHSC = methanolic extract of S. cordifolia.

Figure 1. HPLC chromatograms of (A) standard gallic acid and (B) gallic acid in hydrogel of MeOHSC. HPLC = high-performance liquid chromatography; MeOHSC = methanolic extract of S. cordifolia.
3.5. Evaluation of hydrogel

The parameters to be evaluated for the hydrogel are shown in Table 4. The hydrogel of MeOHSC was prepared and subjected to evaluation of the parameters. The hydrogel of MeOHSC was greenish black in color, uniform, and nonirritant, and formed the smooth gel. The pH of the formulation was 4.44 ± 0.05, which was constant throughout the study. The viscosity hydrogel of MeOHSC was found to be 2.980 Pa.s at 0.1 g and did not show variation during the study.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Hydrogel</th>
<th>pH</th>
<th>Viscosity Pa.s</th>
<th>Homogeneity</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanolic extract</td>
<td>4.44 ± 0.05</td>
<td>2.980</td>
<td>Good</td>
<td>Greenish black</td>
</tr>
</tbody>
</table>

Table 5

Effect of MeOHSC hydrogel on rate of % wound contraction in excision wound model.

<table>
<thead>
<tr>
<th>Postwounding day</th>
<th>Group I (normal control)</th>
<th>Group II (diabetic control)</th>
<th>Group III (diabetic wound control + MeOHSC extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2nd</td>
<td>21.2 ± 4.1</td>
<td>13.25 ± 4</td>
<td>10 ± 0</td>
</tr>
<tr>
<td>4th</td>
<td>29.2 ± 4.3</td>
<td>22.5 ± 12</td>
<td>15 ± 5.7</td>
</tr>
<tr>
<td>6th</td>
<td>45.8 ± 10</td>
<td>38.7 ± 10</td>
<td>25 ± 5.7</td>
</tr>
<tr>
<td>8th</td>
<td>52.2 ± 11</td>
<td>41.25 ± 11</td>
<td>27.5 ± 9.5</td>
</tr>
<tr>
<td>10th</td>
<td>71.8 ± 12</td>
<td>56.5 ± 7</td>
<td>70 ± 8.1</td>
</tr>
<tr>
<td>12th</td>
<td>95 ± 1.0*</td>
<td>66.5 ± 7</td>
<td>97 ± 0.8</td>
</tr>
<tr>
<td>14th</td>
<td>100 ± 0*</td>
<td>74.6 ± 5</td>
<td>100 ± 0**</td>
</tr>
<tr>
<td>16th</td>
<td>—</td>
<td>80 ± 8.1</td>
<td>—</td>
</tr>
<tr>
<td>18th</td>
<td>—</td>
<td>85.9 ± 6.1</td>
<td>—</td>
</tr>
<tr>
<td>20th</td>
<td>—</td>
<td>96.7 ± 0.9</td>
<td>—</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error mean.

\( n = 6 \) albino rats/group.

* \( p < 0.05 \), ** \( p < 0.01 \), comparison of Groups II and III.

MeOHSC = methanolic extract of \( S. \) cordifolia.

3.6. Determination of percentage wound contraction

The percentage decrease in the wound surface area was found to increase in a time-dependent manner in all groups and is shown in Table 5 (Figure 2). In this study, the topical application of hydrogel of MeOHSC in diabetic rats significantly (\( p < 0.01 \)) enhanced the rate of % wound contraction on the 12th day, and the normal wound control group showed significantly (\( p < 0.05 \)) similar result on the 14th day as compared with diabetic wound control group, which showed poor wound healing on 20th postwound day.

Figure 2. Effect of hydrogel of MeOHSC on rate of wound contraction in excision wound model. MeOHSC = methanolic extract of \( S. \) cordifolia.
3.7. Period of epithelialization

The period of epithelialization is shown in Table 6. The period of epithelialization was shown to exhibit extremely significant ($p < 0.01$) results in the hydrogel of MeOHSC and also showed significant ($p < 0.05$) results in the normal wound control group in comparison with a diabetic wound control group, which showed poor epithelialization.

3.8. Hydroxyproline measurement

The hydroxyproline content in wound tissue treated with hydrogel of MeOHSC and normal wound control as well as diabetic wound control are shown in Table 6. The hydroxyproline content in the hydrogel of MeOHSC-treated diabetic rats, and the normal wound control group showed significantly ($p < 0.01$) better result compared with the diabetic wound control group.

3.9. Tensile strength

The hydrogel of MeOHSC was observed to exhibit significantly ($p < 0.01$) good tensile strength, whereas the normal wound control group showed significantly ($p < 0.01$) similar as well as good tensile strength when compared with the diabetic wound control group, which seemed to exhibit poor tensile strength (Table 6).

3.10. Histopathological study

The result of the histopathological examination is shown in Figure 3. The hydrogel of MeOHSC-treated rats and the normal wound control group showed stronger epithelial cell growth with greater collagen content than the diabetic wound control group. Fewer macrophages and fibroblasts were observed in the diabetic control group when compared with the hydrogel of MeOHSC-treated rats as well as the normal wound control group.

4. Discussion

Diabetic foot is the most common complication of diabetes, even greater than retinopathy, nephropathy, heart attack, and stroke combined. Infection frequently follows, and unless there is aggressive intervention, amputation becomes the end
point. The prevalence of diabetes among those older than 65 years of age was last estimated by the Centers for Disease Control and Prevention using 2008 data from the National Health Interview Survey. It is about 19.9% among those aged 65–74 years, and 17.1% among those older than 75 years of age. These estimates are based on self-reports and are thought to underestimate the true prevalence of one-third. In addition, between 10% and 15% of those with diabetes can expect to develop a foot ulcer at some point in their lives. Both foot ulcer and amputation vary by geographic location.20 The aim of this study is to evaluate the phytochemical and pharmacological effects of the methanolic extract of S. cordifolia for its wound healing potential in diabetic rats.

The qualitative analysis showed the presence of various phytoconstituents, which may participate individually or give the synergetic effect in wound healing. The total phenolic content (0.24 mg GA/g) and flavonoids content (0.32 mg RU/g) found to be present in the extract may be responsible for the biological activity of the hydrogel of MeOHSC. The HPLC analysis of MeOHSC extract also confirms the presence of gallic acid (74 µg/10 mg of extract), which is an active medicinal constituent. Gallic acid, a polyphenol natural product, is known to have antioxidant, anti-inflammatory, antimicrobial, and radical scavenging activities.21 It is a food component that is especially abundant in tea, and is an antimutagenic, anticarcinogenic, and anti-inflammatory agent.22 It also helps in the management of hyperglycemia, hyperlipidemia, and cardiac dysfunction associated with STZ-induced type 1 diabetes in rats.23

The histopathological examination of hydrogel of MeOHSC-treated rats and normal wound control group showed greater epithelial cell growth with higher collagen content than diabetic wound control. In the present study, we observed excellent results in treating diabetic wounds using the hydrogel of MeOHSC. However, we continue to conduct further studies to assess the effect of hydrogel of MeOHSC on various phases of wound healing, including granulation, collagenation, and epithelialization at the cellular and molecular levels.

5. Conclusion

In our study, we observed excellent results in treating diabetic wounds using the hydrogel of MeOHSC. We are also very thankful to the Faculty of Pharmacy, VNS Group of Institutions, Bhopal, India, for providing a good platform for research work.

Acknowledgments

We acknowledge “All India Council of Technical Education (AICTE), New Delhi India” for providing grants under the Research Promotion Scheme (RPS). We are also very thankful to the Faculty of Pharmacy, VNS Group of Institutions, Bhopal, India, for providing a good platform for research work.

References