Wound healing property of *Saraca indica* (roxb.) de Wild on the streptozotocin induced diabetic rat model

Sarita Solanki[1], Deepti Jain[2], Surendra Jain[3*], Ummed Singh Solanki[4]

[1]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal (M.P.), India
[2]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal (M.P.), India
[3*]Truba Institute of Pharmacy, Bhopal (M.P.), India
[4]Department of Biochemistry, Jhalawar Medical College, Jhalawar (Raj.), India
***Corresponding Author : Surendra Jain**

Abstract

Aim: The current study aimed to evaluate the wound healing property of *Saraca indica* (roxb.) de Wild on the streptozocin-induced diabetic rat model.

Method: Streptozotocin (40 mg kg^{-1} BW), dissolved in citrate buffer (pH 4.5), was injected intraperitoneally (i.p.) into fasted rats to induce diabetes. Evaluation of wound contraction, along with biochemical status during wound healing in diabetic rats was performed by comparing the control and treatment groups.

Result: In this study, *S. indica* significantly increased wound contraction in oral and topical treatment groups compared to the diabetic control group. The findings demonstrated that oral administration of *S. indica* extracts in diabetic rats reduced blood glucose levels on the first, eighth, and fifteenth days compared to the diseased control group. The significant changes was also observed in other parmeters like insulin, cholesterol etc due to biochemical and enzymatic status changes.

Conclusion: According to the results of this investigation, *S. indica* can potentially treat diabetic wounds and hence can be used as a therapeutic agent in developing drugs to treat diabetic wounds.

Keywords: Blood glucose; Diabetes; Ethanol extract; *S. indica;* Streptozocin; Wound healing

DOI: 10.53555/ecb/2023.12.6.309

Introduction

Diabetic complications are a substantial public health issue across the world. There were 366 million diabetics in 2011, projected to rise to 552 million by 2030. The prevalence of diabetes is expected to increase most rapidly during the next 19 yearsin poor and middle-income nations like India [1]. Wound healing mechanisms are disrupted in diabetes patients due to defective angiogenesis, neovascularization, matrix metalloproteinases (MMPs), keratinocytes, and fibroblasts. Because of this, diabetic individuals are at increased risk for developing infections and ulcers, which may lead to gangrene [2].

A wound is a cut or break in the continuity of the skin due to an accident or surgery, and its recovery presents a substantialmedical and social problem that can affect the quality of life for most people [3,4]. In the average case, wounds would heal in two to four weeks. Due to local pressureand vascular insufficiency, diabetic wounds often take longer to heal. Different forms of clinical presentation, symptoms, location, origin, depth, and other criteria contribute to categorizing wounds into distinct groups, such as stomach ulcers,diabetic wounds, bites, cuts, and duodenal ulcers [5]. Several connective tissue defects are associated with prolonged inflammation, slowed neovascularization, decreased collagen synthesis, increased proteases, and aberrant macrophage activity, all of which restrict the healing of diabetic wounds. 50–70 percent of all exclusions are performed to treat "diabetic wounds," and it is believed that one person in the United States loses a leg to amputation every 30 seconds. Because of the complicated linkage of cellular and molecular idiosyncrasies, individuals with persistent diabetes wounds need hospitalization, and treatment choices remain contradictory. Early detection and treatment can lessen the severity of symptoms and the likelihood of complications for these individuals [6,7].

Diabetic wounds fail to heal because of increased oxidative stress in the tissues, which in turn causes a redox imbalance [8]. Clinical studies revealed that individuals with long-standing type 2 diabetes had a highly oxidizing environment connected to hyperglycemia and tissue hypoxia, slowing wound healing. Several drugs for diabetic wound care are available. Still, they each come with their benefits and drawbacks, including high costs, risks of side effects, allergies, and drug resistance [9]. Thus, effective therapy for diabetic wounds must be a top concern. Traditional wound care often makes use of a variety of medicinal herbs and plant-

based preparations. Because of their high antioxidant content, herbal medicines are a valuable source for developing novel therapies for diabetic wounds [10-14].

A member of the Caesalpiniaceae family, *Saraca indica* (SI) is a tiny evergreen tree native to India. It is also known as the Ashoka tree. The blossoms have a vibrant orange and yellow hue. Since antiquity, it has been stated that it can cure many conditions within the conventional medical system. Internal piles, diabetes, dyspepsia, indigestion, burning feeling, blood problems, fractures, tumors, inflammation, and uterine diseases are just a few of the many conditions it has been used to treat [15]. Scientific investigations have shown it to have several biological actions, including antioxidant,[16] antibacterial,[17] antiulcer,[18] cardioprotective [19], and hypoglycemic, in addition to these applications in traditional medicine. A recent study found antidiabetic effects in SI flowers [20] but didn't check for the wound-healing efficacy of the plant.Hence, the current study aimed to evaluate the Wound healing property of *Saraca indica* (roxb.) de Wild on the streptozocin-induced diabetic rat model.

1. Material and methods

2.1 Plant Collection

In February and March 2021, Ashoka flowers were collected from the local region from Bhopal (M.P.), India, and taxonomically categorized. The flowers were then separated, washed, and air-dried at room temperature.

2.2 Plant extracts preparation

The collected flowers were crushed into a powder form and extracted in a Soxhlet apparatus with ethanol extract (60-80°Celsius) at a temperature no higher than 60°Celsius.The sections were concentrated at decreased pressure in a rotary evaporator to produce a rough semi-solid substanceutilized after drying.

2.3 Phytochemical screening

Preliminary phytochemical screening was done to check the existence of sterols,flavonoids, carbohydrates,tannins, triterpenoids, and glycosides in the flower extract.

2.4 Preparation of ointment

The hydrophobic ointment of *S. indica* extract was manufactured as per the procedure previously adevised by prior researchers, with only some minor modifications. The ingredients that go into making an ointment are detailed in the product monograph (Table 1).

Table 1: Steps involved in making an ointment containing an ethanolic extract of *S. indica*

w/w ointment	Material required	Quantity
5% w/w ethanolic extract ointment (5% EO)	Ethanolic Extract of *S. indica*	5.0 g
	Methyl Paraben	1-2 drops
	Liquid Paraffin	10.0 g
	Petroleum Jelly	70.0 g
	Polyethylene Glycol-6000	5.0 g
	Cetostearyl Alcohol	10.0 g
	Total Weight	100 g
10% w/w ethanolic extract ointment (10% EO)	Ethanolic Extract of *S. indica*	10.0 g
	Methyl Paraben	1-2 drops
	Liquid Paraffin	10.0 g
	Petroleum Jelly	65.0 g
	Polyethylene Glycol-6000	5.0 g
	Cetostearyl Alcohol	10.0 g
	Total Weight	100 g

2.5 Dose calculation for oral extract

The planning and creation of an oral intervention stock solution.

"Dosage in mg = Animal body weight (g)/1000 g × dose (mg)"

2.6 Animals

Wistar albino rats weighing 150 – 200 g were involved in the study. All the animals were kept in plastic cages (at a temperature of 25°C, with 45 – 55% tack and (12/12) hour day/night cycles in a typical habitat) with unrestricted access to food and water.

2.7 Oral Acute Toxicity and Dose Optimization

For determining an appropriate dosage for the oral acute toxicity test, Wistar albino rats were used that were around 6-7 weeks old & in good health, as recommended by "OECD guidelines 423".The first day began with overnight fasting for all the animals before they were treated with medication.More specifically, 250, 500, 750, and 1000 mg/kg of the ethanolic extract, diluted in 50% dimethyl sulfoxide, was given orally once daily for 16 days. The control group of rats received dimethyl sulfoxide (2 ml/kg) once a day during the trial.Daily observations of the animals' behavior, intake of food and water, signs of toxicity, convulsions, and deaths were made for up to 16 days.The optimum dose was selected accordingly. For a detailed toxicity analysis on the 15th day, all animals were starved overnight and sacrificed by cervical dislocation. The blood samples were collected and screened for various hematological parameters (Hb,WBC & RBC). Internal organs like Kidneys, Brains & livers were also processed for further analysis.

2.8 Acute skin irritation

An acute skin irritation study was conducted to identify any sensitive response on the skin induced by the ointment. The experiment followed all guidelines laid up by OECD- 402.On the first day, the animals' dorsal area hairs were removed, and test ointment was periodically applied there in a variety of concentrations (0% w/w, 5% w/w, 10% w/ w, 15% w/w, 20% w/w, 25% w/w, and 30% w/w). The animals were monitored for adverse effects, like skin responses, including itchiness, skin redness, inflammation, erythema, irritation, and edema, until the 16th day.

2.9 Study Design

- **Induction of diabetes**

  For two weeks, animal groups were given a 10% fructose solution instead of water. Streptozotocin (40 mg kg^{-1} BW), dissolved in citrate buffer (pH 4.5), was injected intraperitoneally (i.p.) into fasted rats to induce diabetes.A portable glucometer was used to measure the rats' Non-Fasting Blood Glucose (NFBG) levels. Animals with more than 200 mg/dl of blood glucose were considered diabetic.

- **Induction of serve wound (Excision wound model):**

All surgical operations were carried out in a sterile and hygienic environment. Animals were sedated with intraperitoneal injections of ketamine (40 mg/kg) before their dorsal hair was shaved off. After the hair had been shaved off, the region was cleansed with 90% ethanol. The excision wound was made on the side of the dorsal midlineusing biopsy punch pliers; it was 2.4 cm thick and 0.2 cm deep.

2.10 Treatment protocol

Nine groups were formed to experiment (each group consistedof 6 animals, Total = 54 rats).

- Group 1(NC): Normal rats + administered oral (0.9%w/v; 1ml/kg/daily) normal saline
- Group 2(DWC): Diabetic rats + wound + administered oral (0.9%w/v; 1ml/kg/daily) normal saline
- Group 3(TOT150): Diabetic rats + wound +test oral treatment (150 mg/kg/daily) ethanolic extract.
- Group 4(TOT300): Diabetic rats + wound +test oral treatment (300 mg/kg /daily) ethanolicextract.
- Group 5(SOT): Diabetic rats + wound + standard oral treatment (100 mg/kg daily), Aloe vera juice
- Group 6(TTT5): Diabetic rats + wound + test topical treatment of (5%w/w/daily) EO ointment.
- Group 7(TTT10): Diabetic rats + wound + test topical treatment of (10%w/w/daily) EO ointment.
- Group 8(STT): Diabetic rats + wound +standard topical treatment(5%w/w/daily) Povidone Iodine ointment
- Group 9(TOTT): Diabetic rats + wound + test oral (300 mg/kg/daily) ethanolic extract +topical treatment of (10%w/w/daily) EO ointment.

2.11 Following Parameters were analyzed

- **Wound area measurement:** The diameter of the wound was measured at 1,4, 8, 12, and 15-days post-surgery, and the wound closure % was calculated using the following formula:

  "Wound closure rate on day X (%) = [(wound diameter on day 0 – wound diameter on day X)/(wound diameter on day 0)] × 100"
- **Physiological measurement:** Body weight on days 1, 8, and 15 was measured.
- **Serum insulin and Blood glucose analysis:** Glucose, Cholesterol, HDL,and Insulin were determined on days 1, 8, and 15 using a fully automatic analyzer
- **Analysis of Inflammatory markers:** C-reactive protein, plasma fibrinogen, and Cytokines were also analyzed by ELISA.

2.12 Statistical analysis

The quantitative data was recorded into an Excel spreadsheet, analyzed using GraphPad Prism, and then categorized into roughly equivalent tables. The mean and standard deviation were used to describe all continuous values. One-way ANOVA followed by Bonferroni's post hoc test was used to establish a statistically significant difference.

2. Result

3.1 Preliminary phytochemical screening

The presence of tannins, alkaloids, carbohydrates,phenols, saponins, cardiac glycosides, steroids, flavonoids, and proteins in the ethanolic extract of *Saraca indica* was substantiated by phytochemical analysis.*Saraca indica* extracts phytochemical screening results are shown in Table 2; where + indicates presence, ++ indicates significant presence, and - indicates absence.

Table 2.The findings of the phytochemical screening of *Saraca indica* extract

Chemical Class	Test Performed	Ethanolic extract
Steroids	Liebermann's Reagent	--
	Salkowaski Test	-
Carbohydrates	Fehling's Test	+
	Molisch's Test	++
Alkaloids	Wagner's reagent	−
	Mayer's reagent	-
	Dragendorff's reagent	+
Glycosides	Borntrager's test	++
	Legal test	+
	Keller killiani Test	+
Phenolic compounds Tannins	Lead acetate test	++

3.2 Acute oral toxicity

No significant differences in hematological parameters (RBCs, WBCs, and hemoglobin), body weights, or organs were seen between groups of rates until the 1000 mg/kg dosage of *S.indica* (table 3).

Table 3. In acute oral toxicity research, body parameters including white blood cells (WBCs), red blood cells (RBCs), hemoglobin, and organ percent.					
HEMATOLOGICAL PARAMETERS	**Normal Control Group**	**EtOH Extract (250mg/kg)**	**EtOH Extract (500mg/kg)**	**EtOH Extract (750mg/kg)**	**EtOH Extract (1000mg/kg)**
WBCs	12.57±0.25	10.90±0.21	11.94±0.04	12.7±0.8	13.57±0.56
RBCs	08.27±0.8	8.07±0.4	9.53±0.7	9.91±0.5	08.17±0.3
Hemoglobin(g/dl)	18.37±0.80	19.47±1.2	18.47±1.1	18.87±0.9	19.59±1.4
Liver (g%)	2.81±0.00	2.83±0.01	2.76±0.01	9.77±0.11	2.67±0.02
Brain (g%)	0.63±0.01	0.64±0.01	0.57±0.02	18.3±0.11	0.55±0.01
Kidney (g%)	0.55±0.01	0.56±0.01	0.54±0.01	17.63±0.11	0.53±0.01

3.3 Acute skin irritation study

The study found that up to 30% w/w of *Saraca indica* ointment, no signs of skin irritation, inflammation,swelling, erythema, itching, and mortality were reported.

3.4 Wound area measurement

In the diabetes control group G2, wound decrease was 39.50 percent after 15 days. On the other hand, 63.90 percent was recorded in the G4 (150 mg/kg) treatment group. Similar reductions (79.41 percent) in wound size were seen in the other treated group G6 (5% w/w ointment), although this treatment was deemed less effective than the oral treatment in group G8. When compared to the experimental groups G8 and G9, the results of using the conventional drugs methanolic extract (100 mg/kg) and povidone-iodine (5% w/w ointment) were shown to be more significant (83.70% and 90.86% respectively). The combination treatment of oral extract administration and topical ointment application (i.e., povidone-iodine (150 mg/kg) + ethanolic extract (5 w/w)) showed significantly greater efficacy against the healing of diabetic wounds (table 4, fig 1 & 2).

Table 4: Effect *Saraca indica* extracts (oral and topical) on percentage of wound contraction on excision wound healing in low fructose-streptozotocin induced diabetic rats.		
Groups	**Wound area (sq. cm)**	**% of wound**

	Day 1	Day 4	Day 8	Day 12	Day 15	contractions on the 15th day
Group 1(NC)	4.44±0.01	4.47±0.01	4.34±0.01	2.55±0.01	2.45±0.02	55.18%
Group 2(DWC)	4.43±0.01	4.35±0.03	4.17±0.03	1.66±0.02	1.75±0.01	39.50%
Group 3(TOT150)	4.44±0.02*	4.49±0.01*	4.55±0.01*	2.58±0.02*	2.47±0.01*	55.63%
Group 4(TOT300)	4.46±0.01	4.53±0.02	4.62±0.01	2.63±0.02	2.85±0.03	63.90%
Group 5(SOT)	4.46±0.03**	4.62±0.06**	4.74±0.01**	3.36±0.03**	3.27±0.03**	73.31%
Group 6(TTT5)	4.47±0.04	4.76±0.01	4.75±0.02	3.56±0.02	3.55±0.02	79.41%
Group 7(TTT10)	4.45±0.02*	4.55±0.02*	4.64±0.02*	3.16±0.08*	3.18±0.02*	71.46%
Group 8(STT)	4.48±0.04*	4.77±0.01*	4.86±0.01*	3.66±0.02*	3.75±0.02*	83.70%
Group 9(TOTT)	4.49±0.02**	4.87±0.01**	4.94±0.02**	4.15±0.05**	4.08±0.03**	90.86%

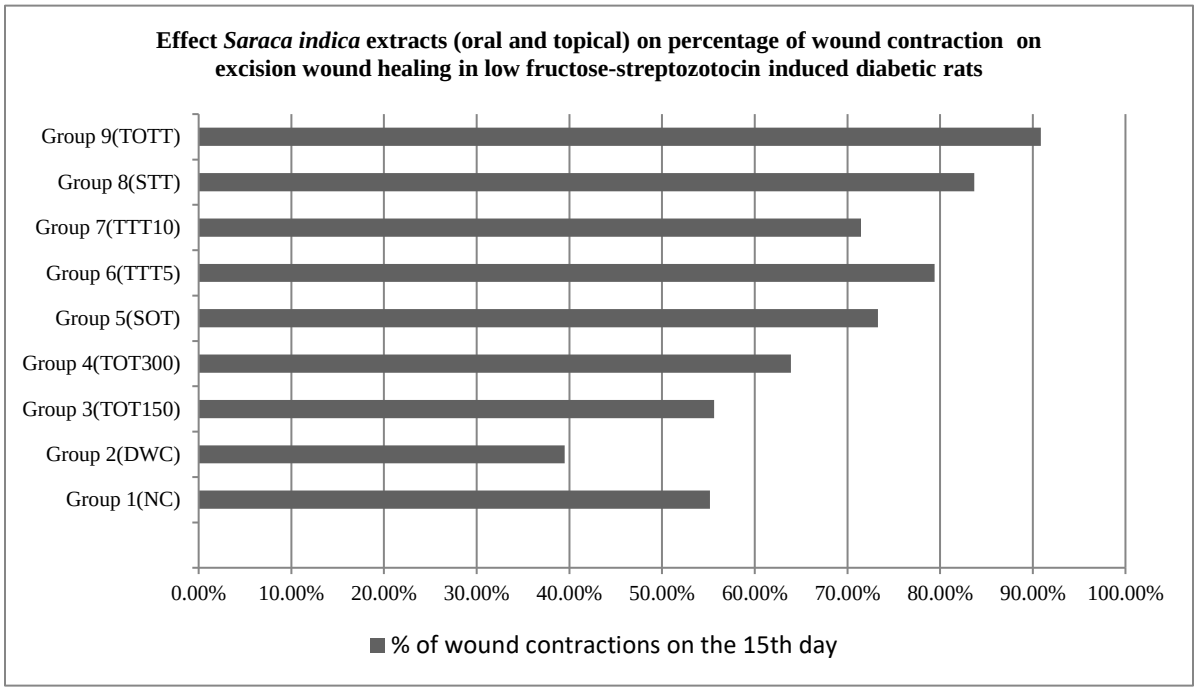


Figure 1 : Effect *Saraca indica* extracts (oral and topical) on percentage of wound contraction on excision wound healing in low fructose-streptozotocin induced diabetic rats

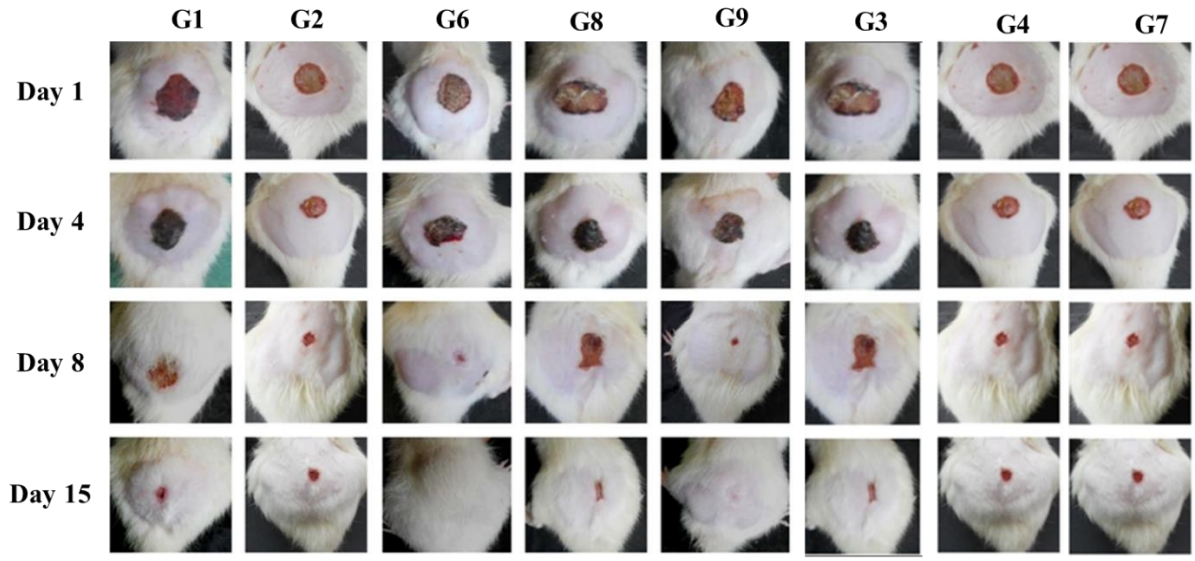


Figure 2 : Photographic depiction of the effect of *S. indica* on the wound healing process in an excision wound modelin fructose- streptozotocin-induced diabetic rats

3.5 Body Weight

When comparing diabetic rats to healthy control, a statistically significant (P <0.0001) reduction in body weight was seen. Compared to the diabetic wound control group, there was a statistically significant (P <0.0001) rise in body weight in groups 9, 8, and 6. The results are shown in (Table 5).

Table 5. Effect of *S. indica* extracts on body weight. (Mean±SEM)

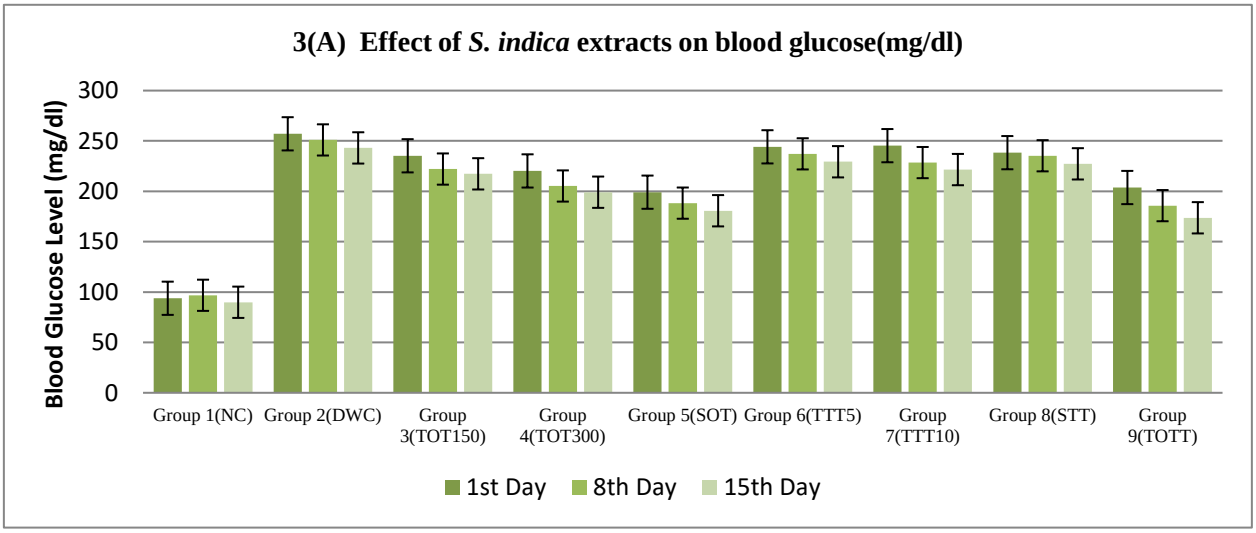
Body Weight	1st Day	8th Day	15th Day
Group 1(NC)	253.85±0.024	256.81±0.025	259.88±0.026
Group 2(DWC)	248.08±0.032	251.02±0.031	245.09±0.035
Group 3(TOT150)	259.27±0.012	262.21±0.013	257.25±0.014
Group 4(TOT300)	262.44±0.034	265.49±0.035	269.32±0.036
Group 5(SOT)	269.74±0.024	263.78±0.025	266.73±0.026
Group 6(TTT5)	274.87±0.027	277.81±0.028	279.86±0.029
Group 7(TTT10)	265.55±0.019	268.52±0.020	271.59±0.021
Group 8(STT)	278.37±0.015	275.31±0.016	277.29±0.017
Group 9(TOTT)	283.77±0.031	285.78±0.032	283.72±0.033

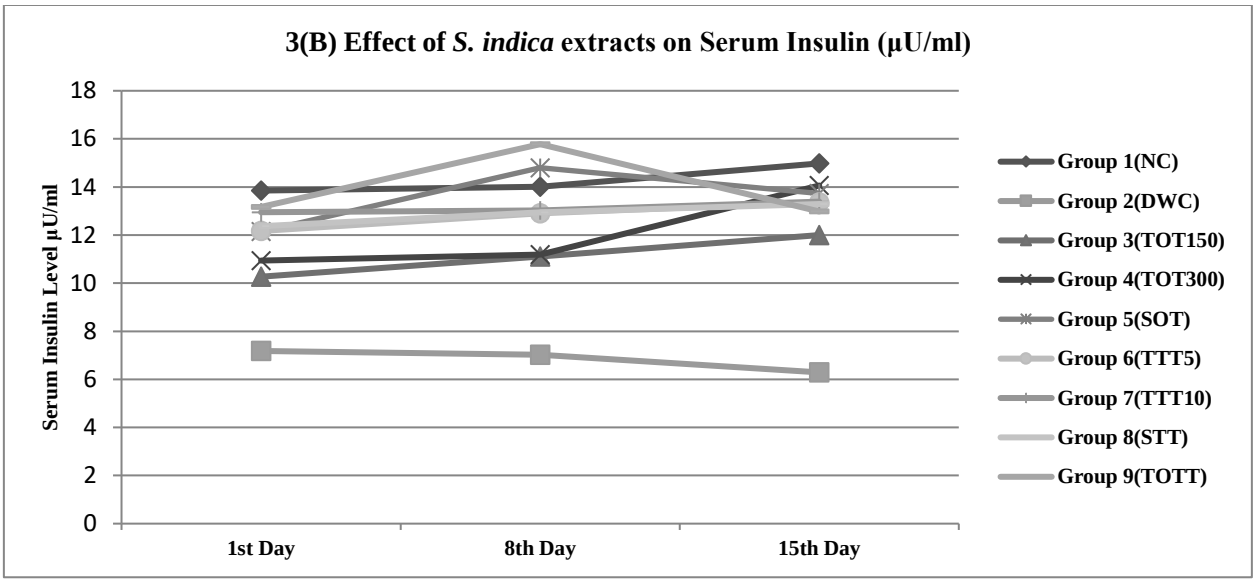
3.6 Biochemical analysis (Blood glucose, Serum insulin, Cholesterol, HDL)

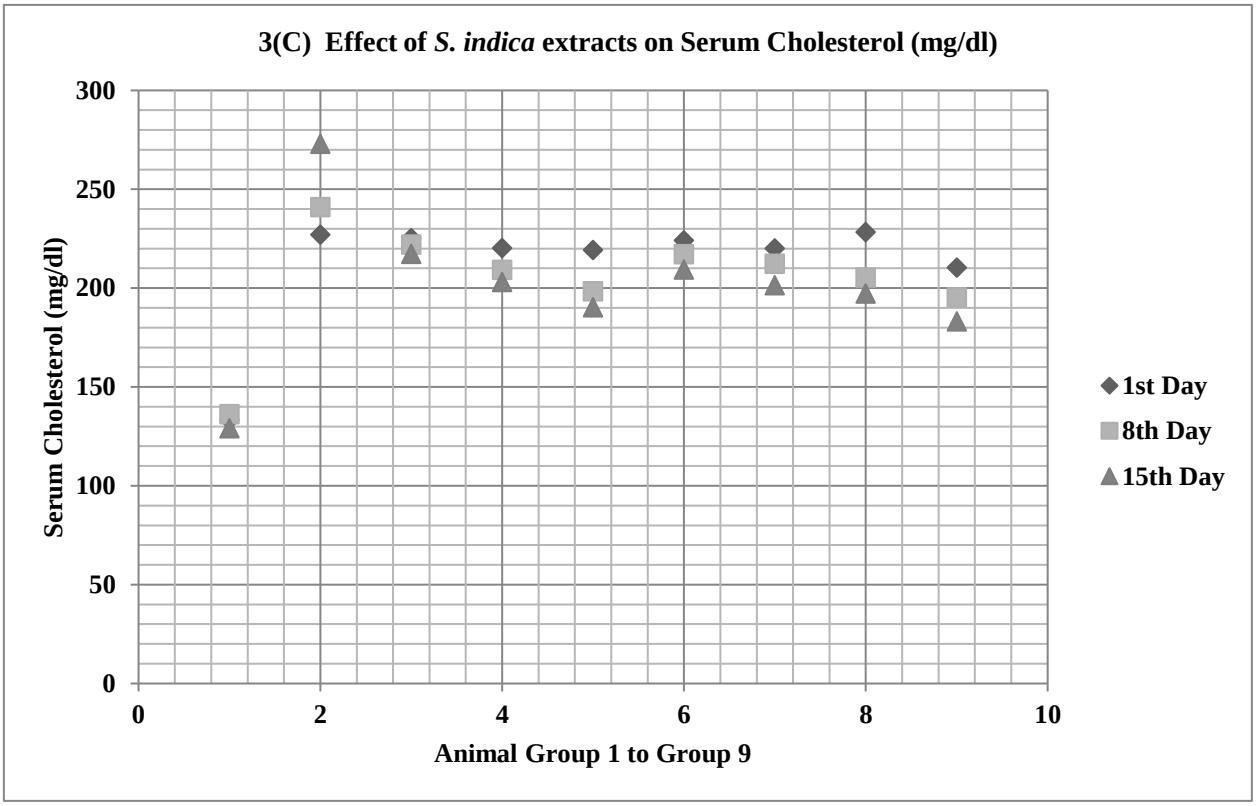
When comparing diabetic wounded rats to healthy control, there was a statistically significant rise in blood glucose level (P <0.0001). The blood glucose level was considerably reduced after 15 days of administration of the combination extract (P <0.0001). . Serum blood glucose levels were found to be significantly decreased in the Group 5 and Group 9 (**p <0.01 and ***p <0.001, respectively) . There was no statistically significant downregulation in groups 3, 6 and 7 compared to the diabetic control group.(Table 6, Fig.3A). Fig. 3; B,C,D demonstrates the changes in insulin, cholesterol and HDL level.

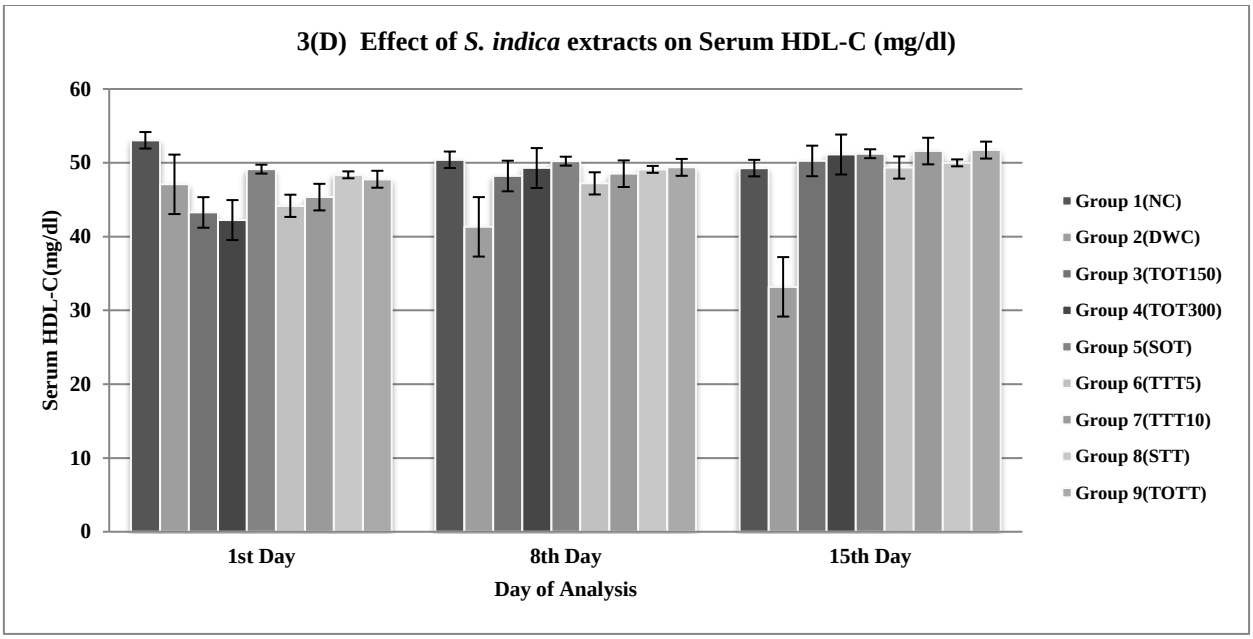
Table 6. Effect of *S. indica* extracts on blood glucose(mg/dl). (Mean±SEM)

Groups	1st Day	8th Day	15th Day
Group 1(NC)	93.85±0.024	96.81±0.025	89.88±0.026
Group 2(DWC)	257.08±0.012	251.02±0.011	243.09±0.035
Group 3(TOT150)	235.27±0.022	222.11±0.013	217.35±0.017
Group 4(TOT300)	220.24±0.034	205.29±0.035	199.12±0.036
Group 5(SOT)	199.14±0.024	188.28±0.025	180.73±0.026
Group 6(TTT5)	244.17±0.027	237.21±0.028	229.36±0.029
Group 7(TTT10)	245.35±0.019	228.52±0.020	221.59±0.021
Group 8(STT)	238.37±0.015	235.31±0.016	227.29±0.017
Group 9(TOTT)	203.77±0.031	185.78±0.032	173.72±0.033




3(B) Effect of S. indica extracts on Serum Insulin (µU/ml)
Serum Insulin Level µU/ml
18
16
14
12
10
8
6
4
2
0
1st Day
8th Day
15th Day
Group 1(NC)
Group 2(DWC)
Group 3(TOT150)
Group 4(TOT300)
Group 5(SOT)
Group 6(TTT5)
Group 7(TTT10)
Group 8(STT)
Group 9(TOTT)


3(C) Effect of S. indica extracts on Serum Cholesterol (mg/dl)
Serum Cholesterol (mg/dl)
300
250
200
150
100
50
0
0
2
4
6
8
10
Animal Group 1 to Group 9
1st Day
8th Day
15th Day



`Figure 3 : (A) Glucose level in all groups (B) Cholesterol level in all groups (C) HDL level in all groups (D) Insulin level in all groups

3.7 Inflammatory markers

C-reactive protein (CRP) is a member of the acute phase protein family produced by hepatocytes and whose levels significantly increase in response to pro-inflammatory cytokines (interleukin-1 and 6, TNF-α), tissue infection, damage, and other inflammatory diseases. A rise in these proteins' levels reliably predicts the inflammatory disease's existence. Therefore, C-reactive protein, Fibrinogen, and other inflammatory markers were evaluated in this research section. In comparison to the diabetic control group, Fibrinogen, and C-reactive protein levels were significantly lower in the groups that were given either the *S. indica* extract orally, the standard drug povidone-iodine, and *S. indica* ethanolic extract ointment topically (150 mg/kg) table 7 & 8. Fig. 4 demonstrates changes in IL-1 and TNF-α.

Table 7. Effect of *S. indica* extracts on Serum CRP(mg/dl). (Mean±SEM)

Groups	1st Day	8th Day	15th Day
Group 1(NC)	0.71±0.024	0.79±0.025	0.90±0.026
Group 2(DWC)	2.88±0.012	2.96±0.011	3.09±0.035
Group 3(TOT150)	2.27±0.022	2.01±0.013	1.95±0.017
Group 4(TOT300)	2.24±0.034	1.99±0.035	1.62±0.036

Group 5(SOT)	1.84±0.024	1.22±0.025	1.03±0.026
Group 6(TTT5)	1.82±0.027	1.71±0.028	1.36±0.029
Group 7(TTT10)	1.35±0.019	1.12±0.020	1.09±0.021
Group 8(STT)	1.37±0.015	1.11±0.016	1.10±0.017
Group 9(TOTT)	1.30±0.031	1.28±0.032	1.09±0.033

Table 8. Effect of *S. indica* extracts on Serum Fibrinogen (gm/L). (Mean±SEM)

Groups	**1st Day**	**8th Day**	**15th Day**
Group 1(NC)	2.71±0.024	2.79±0.015	2.90±0.026
Group 2(DWC)	2.98±0.012	3.06±0.041	3.49±0.035
Group 3(TOT150)	2.77±0.032	2.41±0.013	2.05±0.037
Group 4(TOT300)	2.74±0.034	2.39±0.035	2.02±0.036
Group 5(SOT)	2.44±0.044	2.12±0.035	1.93±0.026
Group 6(TTT5)	2.82±0.027	2.71±0.028	2.36±0.069
Group 7(TTT10)	2.35±0.019	2.12±0.020	1.99±0.021
Group 8(STT)	2.47±0.025	2.11±0.046	1.90±0.017
Group 9(TOTT)	2.40±0.031	2.28±0.042	1.87±0.033

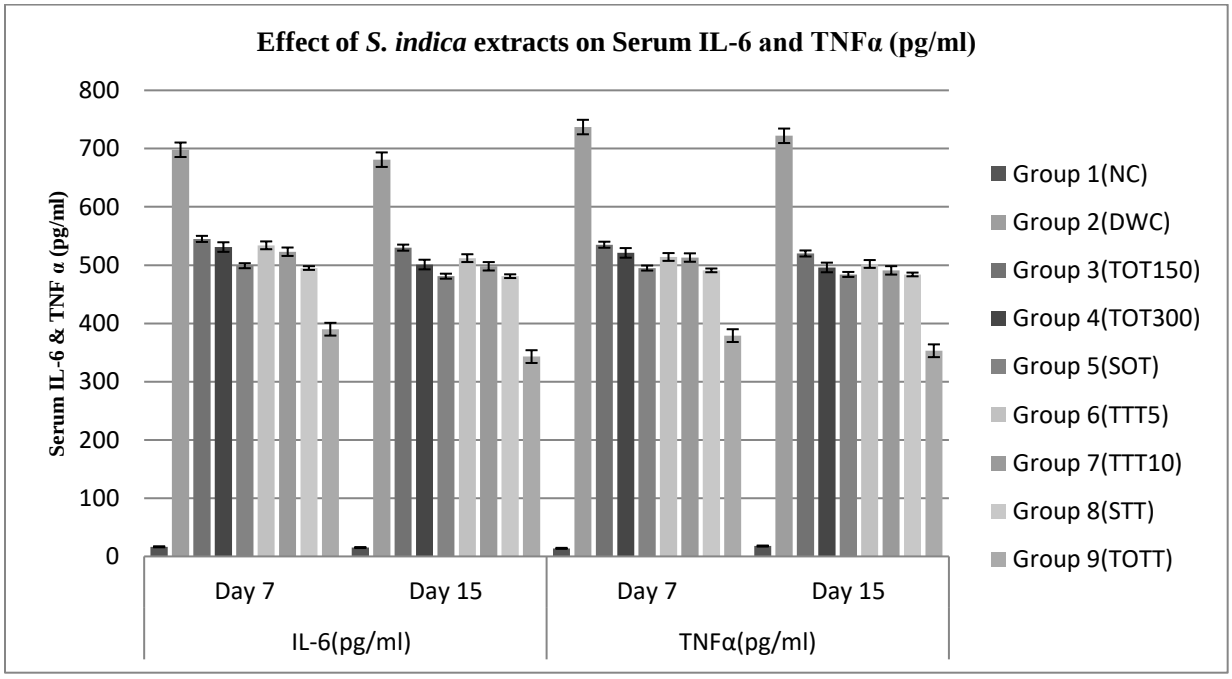


Figure 4 : Effect of *S. indica* (oral and topical) extract on Il-6 and TNF-α level

3. Discussion

Diabetes mellitus is the leading cause of poor angiogenesis and wound healing [21,22]. Deficient wound healing and ulcers contribute to gangrene and amputation in diabetic individuals [23].The three overlapping stages of wound healing—inflammation, cellular proliferation, and remodeling—make it a complicated yet ordered phenomenon [24,25]. When tissues are damaged, an immediate inflammatory response ensues, characterized by the recruitment of monocytes, mast cells, and neutrophils to the injured area and the release of cytokines [26]. The released cytokines encourage "keratinocytes, endothelial cells, and fibroblasts to proliferate and migrate to the wound." Wound closure and scar formation occur in the last stage due to extracellular matrix remodeling, angiogenesis, and epithelialization. Abnormalities cause impaired wound healing in diabetes patients in the inflammatory response [27], blood flow [28], angiogenesis [22], and fibroblast migration [29].Numerous flavonoids have gained popularity as potential remedies for conditions like diabetes [30].

Research reported before [31, 28] showed that administering *S. indica* to diabetic rats significantly reduced their blood glucose levels. After an injury, granulation tissue rapidly increases collagen production, the most abundant extracellular protein in the wound's granulation tissue [32,33].

The present study used an excision wound rat model of diabetes to examine the impact of *S. indica* (orally and topically) on wound healing in diabetic rats. This is the first research showing that *S. indica* improves wound healing in diabetic rats. Serum concentrations of oxidative indicators like MDA were elevated in diabetic rats used in the study. Diabetic control rats also had poor wound healing due to a lack of endogenous antioxidants like GSH, CAT, and SOD. Inhibition of LPO, increased collagen deposition, nitric oxide generation, and enhancement of endogenous SOD, CAT, and GSH all contributed to faster wound healing after treatment with povidone.LDLC and HDLC are affected by the oral and topical extract of *Saraca indica*and were found to be non-significant in contrast to the diabetes control group. The highest significance was seen in group 9 compared to the diabetic control group.

Acute toxicity and therapeutic dosage selection were performed in the current investigation using several doses. Up to 150 mg/kg/5% w/ w of S. indica ointment showed no symptoms of behavioral abnormalities, redness, inflammation, skin itching, edema, erythema, irritation, or death in the research. The primary skin irritation from the ointment was graded as 0, indicating that the formulation was well tolerated. The results agree with those found by Yadav et al. (2018) and Saha et al. (2021) [35, 36].

Polyphenols have been shown to decrease the production of inflammatory markers and pro-inflammatory cytokines, accelerating the granulation and collagen deposition stages of tissue maturation [35]. Because of this, the phenolic chemicals in *Saraca indica* may work together to speed up the healing process. Regarding diabetic wound healing, the groups who were given the combined group treatment showed considerable improvements. As stated in the research, oral administration of *Saraca indica* extract dramatically decreased blood glucose levels and plasma free radicals, suggesting that these factors may be responsible for the extract's wound-healing activity. Additionally, on day 15, the wound ulcer was much better healed, and Group 9 performed better than other groups altogether. Furthermore, this result agrees with the findings of Tuhin et al. (2017) [37].

4. Conclusion

The present study evaluated the ethanolic extract and ointment of *Saraca indica* for their ability to treat diabetic lesions. *Saraca indica* was more effective when used topically and orally than the conventional medicines aloe vera and povidone. Results suggest that *Saraca indica* polyphenols may help wound healing by increasing collagen production, speeding up wound contraction, and restoring dermal tissue. Numerous factors, including epithelialization, collagen, antioxidant defense system, and thecollagen turnover, are hypothesized to play a role in wound healing. However, the exact mechanism is not yet known. As a result, we are extending our investigation into this area to nail down the precise mechanism of action. This study has the potential to aid in developing *Saraca indica*-based herbal therapies for treating wounds in the future.

References

1. Whiting D, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311–21.
2. Tam J, Lau K, Liu C, To M, Kwok H, Lai K, Lau C, Ko C, Leung P, Fung K, Lau C. The in vivo and in vitro diabetic wound healing effects of 2-herb formula and its mechanisms of action. J Ethnopharmacol 2011;134:831–8.
3. Dong J, Chen L, Zhang Y, Jayaswal N, Mezghani I, Zhang W, Veves A. Mast cells in diabetes and diabetic wound healing. Advances in Therapy. 2020 Nov;37(11):4519-37.
4. Ezhilarasu H, Vishalli D, Dheen ST, Bay BH, Srinivasan DK. Nanoparticle-based therapeutic approach for diabetic wound healing. Nanomaterials. 2020 Jun 25;10(6):1234.
5. Getahun A, Kifle ZD, Ambikar D, Atnafie SA. In vivo evaluation of 80% methanolic leaves crude extract and solvent fractions of buddleja polystachya fresen (buddlejaceae) for wound healing activity in normal and diabetic mice. Metabolism Open. 2021 Sep 1;11:100110.
6. Patel S, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomedicine & Pharmacotherapy. 2019 Apr 1;112:108615.
7. Shao Y, Dang M, Lin Y, Xue F. Evaluation of wound healing activity of plumbagin in diabetic rats. Life Sciences. 2019 Aug 15;231:116422.
8. Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, Leaper D, Georgopoulos NT. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. International wound journal. 2017 Feb;14(1):89-96.
9. Cano Sanchez M, Lancel S, Boulanger E, Neviere R. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: a systematic review. Antioxidants. 2018 Jul 24;7(8):98.
10. Kim J, Lee CM. Wound healing potential of a polyvinyl alcohol-blended pectin hydrogel containing Hippophae rahmnoides L. extract in a rat model. International journal of biological macromolecules. 2017 Jun 1;99:586-93.
11. D'almeida RE, Carro RT, Simonetta S, Zampini IC, Simirgiotis M, Borquez J, Isla MI, Alberto MR. Flavonoid-enriched fractions from Parastrephia lucida: Phytochemical, anti-inflammatory, antioxidant characterizations, and analysis of their toxicity. South African Journal of Botany. 2020 Dec 1;135:465-75.
12. Das U, Behera SS, Pramanik K. Ethno-herbal-medico in wound repair: An incisive review. Phytotherapy Research. 2017 Apr;31(4):579-90.
13. Maver T, Maver U, Stana Kleinschek K, Smrke DM, Kreft S. A review of herbal medicines in wound healing. International journal of dermatology. 2015 Jul;54(7):740-51.
14. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Future Journal of Pharmaceutical Sciences. 2021 Dec;7(1):1-3.
15. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer Science & Business Media; 2008 Apr 22.
16. Sen S, Chakraborty R, Verma A, De B, Devanna N, Dey BK. Evaluation of antioxidant activity of Saraca indica leaves extracts using in vitro and ex vivo models. J Sci Ind Res 2014;73:157-62
17. Nayak S, Sahoo AM, Chakraborti CK, Haque MN. Antibacterial activity study of Saraca indica leaves extract. Int J Pharm Res Dev 2011;3:16.
18. Maruthappan V, Sakthi SK. Antiulcer activity of aqueous suspension of Saraca indica flower against gastric ulcers in albino rats. J Pharm Res 2010;3:17-20.
19. Viswanatha Swamy AH, Patel UM, Koti BC, Gadad PC, Patel NL, Thippeswamy AH. Cardioprotective effect of Saraca indica against cyclophosphamide induced cardiotoxicity in rats: a biochemical, electrocardiographic and histopathological study. Indian J Pharmacol 2013;45:44-8.

20. Preethi F, Fernandes J, Pricilla K. Hypoglycemic activity of Saraca indica Linn barks. J Pharmacol Res 2010;3:491.
21. Herrick S, Sloan P, McGurk M. Sequential changes in histologic pattern and extracellular matrix deposition during the healing of chronic venous ulcers. Am J Pathol 1992;141:1085–95.
22. Duraisamy Y, Slevin M, Smith N. Effect of glycation on basic fibroblast growth factor induced angiogenesis and activation of associated signal transduction pathways in vascular endothelial cells: possible relevance to wound healing in diabetes. Angiogenesis 2001;4:277–88.
23. Rossing P, Hommel E, Smidt UM, Parving HH. Reduction in albuminuria predicts a beneficial effect on diminishing the progression of human diabetic nephropathy during anti-hypertensive treatment. Diabetologia 1994;37:511–516.
24. Clark R, Henson P. Molecular and cellular biology of wound repair.New York: Plenum Press, 1996:46–52.
25. Martin A. The use of antioxidants in healing. Dermatol Surg 1996;4:156–160.
26. Trautmann A, Toksoy A, Engelhardt E, Brocker EB, Gillitzer R.Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo. J Pathol 2000;190:100–106.
27. McMurry J. wound healing with diabetes mellitus. Better glucose control for better wound healing in diabetes. Surg Clin North Am 1984;64:769–778.
28. Huysman E, Mathieu C. Diabetes and peripheral vascular disease.Acta Chir Belg 2009;109:587–594.
29. Hehenberger K, Kratz G, Hansson A, Brismar K. Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. J Dermatol Sci 1998;16:144–151.
30. Pattanayak SP, Sunita P. Wound healing, antimicrobial and antioxidant potential of Dendrophthoe falcate (L.f.) Ettingsh. J Ethnopharmacol 2008;120:241–247.
31. Jung E, Kim S, Hwang I, Ha T. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ db/db mice. J Agric Food Chem 2007;55:9800–4.
32. Chithra P, Sajithlal GB, Chandrakasan G. Influence of aloe vera on the healing of dermal wounds in diabetic rats. J Ethnopharmacol 1998;59:195–201.
33. Pradhan D, Panda P, Tripathy G. Wound healing activity of aqueous and methanolic bark extracts of Veronica arborei in wistar rats. Nat Prod Res 2009;8:6–11.
34. Graber AM. Wound management. General Surg 2002;22–34
35. Yadav E, Singh D, Yadav P, Verma A. Antioxidant and anti-inflammatory properties of Prosopis cineraria based phenolic rich ointment in wound healing. Biomedicine & Pharmacotherapy. 2018 Dec 1;108:1572-83.
36. Saha S, Sonar V, Soni B, Koladiya S, Chakraborty S, Pithawala M. Investigating wound healing potential of Typha angustata L. inflorescence in albino Wistar rats. Phytomedicine Plus. 2021 Nov 1;1(4):100054.
37. Tuhin RH, Begum M, Rahman M, Karim R, Begum T, Ahmed SU, Mostofa R, Hossain A, Abdel-Daim M, Begum R. Wound healing effect of Euphorbia hirta linn.(Euphorbiaceae) in alloxan induced diabetic rats. BMC complementary and alternative medicine. 2017 Dec;17(1):1-4.