Evaluation of Wound healing activity of leaves of *Passiflora foetida* L in Streptozotocin-induced diabetes mellitus and Diabetic Excision Model

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Abstract— *The present study aimed to evaluate the wound healing activity of the leaves of Passiflora foetida L. in Streptozotocin (STZ)-induced diabetes mellitus in rats. Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/Kg) in Wistar albino rats after overnight fasting. The diabetic rats were subjected to excision wounds on the dorsal side, which were treated with varying doses (100, 200, and 400 mg/Kg) of ethanol and ethyl acetate extracts of Passiflora foetida leaves for 21 days. The wound healing parameters, including wound contraction rate, epithelialization time, and scar area, were monitored and compared with the control group and standard treatment (Metformin 5 mg/Kg). The results revealed that both ethanol and ethyl acetate extracts exhibited significant wound healing activity, with the ethyl acetate extract (400 mg/Kg) showing the most potent effect. The extracts significantly enhanced wound closure, reduced epithelialization time, and promoted granulation tissue formation. The findings suggest that Passiflora foetida leaves possess substantial wound healing properties and could be a potential therapeutic candidate for the management of diabetic wounds*.

***Keywords— Passiflora foetida L, ethyl acetate extract, ethanol extract, Streptozotocin, antidiabetic acitivity*.**

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which often leads to complications such as delayed wound healing. Diabetic wounds are a major health concern, as they are prone to infection, poor healing, and prolonged recovery times. Traditional therapeutic approaches have limitations, thus prompting the need for alternative treatments that can promote wound healing in diabetic patients. *Passiflora foetida* L., commonly known as wild passionflower, has been traditionally used for its medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial effects. Recent studies have highlighted its potential in promoting tissue regeneration and wound healing. However, the wound healing activity of *Passiflora foetida* in the context of diabetic wounds remains underexplored.

In this study, we aimed to evaluate the wound healing potential of *Passiflora foetida* leaf extracts in a rat model of Streptozotocin (STZ)-induced diabetes mellitus. By assessing key wound healing parameters, such as wound contraction, epithelialization time, and granulation tissue formation, this study seeks to determine the efficacy of *Passiflora foetida* leaf extracts in promoting wound healing in diabetic conditions. The findings could provide valuable insights into the therapeutic potential of *Passiflora foetida* for managing diabetic wounds.

II. MATERIALS AND METHODS

Streptozotocin (Sigma–Aldrich Canada, Oakville, Ontario, Canada). All other chemicals and reagents used were of analytical grade.

2.1 Reagents:

Buffer (pH 5): 50 g citric acid monohydrate, 12 ml glacial acetic acid, 120 g sodium acetate trihydrate, and 34 g sodium hydroxide added to distilled water up to 1000 ml.

2.2 Animals:

Wistar albino rats weighing 150–200 g were used for experimentation. The rats were procured from the disease-free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Reg. No. 1669/GO/abc/12/CPCSEA Dated 08/04/2013). Pathogen-free conditions were provided to the rats. The rats were housed, fed, and treated as per the international guidelines and principles of laboratory animal use and care. The animals were maintained in polypropylene cages under standard conditions (25±2°C, 12 hrs light and dark cycle) with pelleted food (Purina), while tap water was available ad libitum (Hedrich HH, 2006). They were maintained on the standard pellet diet and water ad libitum for 2 weeks to acclimatize prior to the investigation. All experimental procedures and protocols were approved by the Institutional Animals Ethics Committee, Department of Pharmaceutical Sciences, M.D. University, Rohtak (1767/GO/Re/S/14/CPCSEA, 18/07/2014).

2.3 Diabetes Induction:

2.3.1 Streptozotocin-induced diabetes mellitus:

After overnight fasting, streptozotocin (STZ; 50 mg/Kg, i.p.) (Sigma–Aldrich Canada, Oakville, Ontario, Canada), prepared in citrate buffer (0.1M, pH 4.5), was administered to rats to induce diabetes (Junod A et al., 1969). 24 hours after the injection, fasting blood glucose levels were determined using a Glucometer (Accu-Chek® Extra Care, Roche Diabetes Care India Pvt. Ltd., 601B, Silver Utopia, Chakala Road, Andheri (East), Mumbai, Maharashtra) with glucose oxidase reagent strips after withdrawing blood from the retro-orbital plexus. Animals with a glucose level greater than 250 mg/dl were used for the study, 7 days after streptozotocin injection.

2.4 Diabetic Excision Model for Wound Healing Activity:

2.4.1 Surgical Procedures and Treatment:

On the 7th day after diabetes induction, excision wounds were created. These wounds were used for biochemical parameters study and for the rate of wound contraction. Using thiopentone sodium (40 mg/Kg i.p.), animals were anesthetized, and each rat was shaved from the right side. Ethanol 70% v/v was used for disinfection of the shaved area. From the shaved area on the dorsal middle line, excision wounds of size 4 cm^2 were made by cutting a 2 cm x 2 cm piece of skin. For 21 days, ethanol and ethyl acetate extracts in concentrations of 100 mg/Kg, 200 mg/Kg, and 400 mg/Kg were orally given. The control group received an equal amount of vehicle (citrate buffer).

2.5 Excision Wound:

The epithelialization time (Villegas LF et al., 1997) was noted when no raw wound was left behind and when the scar fell off. Excision wounds on a transparent paper with a millimeter scale were traced to determine the rate of wound contraction. The percentage of wound area healed was calculated using the change in wound size. The number of days taken for complete epithelialization was expressed as the period of epithelialization (when no raw wound was left behind).

2.5.1 Excision Wound Model:

As mentioned above, the excision wound model was performed. Parameters like percentage contraction in the wound, the period of epithelialization, and granulated tissue scar area were evaluated (Nayak BS et al., 2007). Every third day, photographs were taken, and the wound boundaries were traced on transparent paper to measure the area of wounds in all groups.

2.6 Parameters Monitored:

2.6.1 Rate of Wound Contraction:

At 0 days, before extract treatment and after wounding on days 3, 6, 9, 12, 15, and 18, excision wounds were traced on a transparent paper with a millimeter scale. On every third day, the change in wound size was calculated as the percentage of wound area that had healed. The percentage contraction of the wound was calculated using the formula:

$$\%\{\text{wound contraction}\} = (A0 - At) / A0 * 100 \quad (1)$$

Where (A0) is the original wound area and (At) is the area of the wound at a specific time period after wounding (Yates CC et al., 2007; Rashed AN et al., 2003).

2.6.2 Epithelialization Period:

Epithelialization period is the number of days required for the scar to fall off without any raw wound left behind. The epithelialization period of the wound was expressed as the number of days taken for complete epithelialization (when no raw wound was left behind) (Dinesh M et al., 2010).

2.6.3 Animals Grouping:

Nine groups of animals, each consisting of six rats, were made. Rats were given extracts for 21 days. Among all the extracts, ethyl acetate and ethanol extracts were selected for the study of pharmacological activities. Ethyl acetate and ethanol extracts of different plant species showed the maximum number of potent chemical constituents determined by qualitative phytochemical analysis and chromatographic profiles. For these reasons, ethyl acetate and ethanol extracts in different doses were selected for further study. In the literature survey of plants, it is clearly mentioned that the above-mentioned two extracts are safe at a dose level of 2000 mg/Kg; the dose level was selected as 100 mg/Kg (1/20th), 200 mg/Kg (1/10th), and 400 mg/Kg (1/5th) of the safe dose, i.e., 2000 mg/Kg (Vikram PK et al., 2012; Bhide NK, 1962).

- Group I: Standard (Metformin 5 mg/Kg)
- Group II: Diabetic rats with wound without treatment (normal control group)
- Group III: Diabetic rats without wound (for diabetes only)
- Group IV: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose of 100 mg/Kg
- Group V: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose of 200 mg/Kg
- Group VI: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose of 400 mg/Kg
- Group VII: Diabetic rats with wound treated with ethanol extract by oral route at a dose of 100 mg/Kg
- Group VIII: Diabetic rats with wound treated with ethanol extract by oral route at a dose of 200 mg/Kg
- Group IX: Diabetic rats with wound treated with ethanol extract by oral route at a dose of 400 mg/Kg

2.7 Statistical Analysis:

Wound area was measured as the percentage contraction in wound size. Analysis of data was performed using Dunnett's t-test with GraphPad Prism 7.0. When $P < 0.05$ compared with control, the data is considered significant.

III. RESULTS AND DISCUSSION

There is a reduction in blood glucose level when extracts of *Passiflora foetida* L. were administered to glucose-loaded normal rats fasted for 18 h. In our study, the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in the diabetic control group at the end of the 14th day experimental period along with healing of wounds by excision wound healing method observed for 21 days. There is a significant decrease in the fasting blood glucose level and increase in the percentage contraction of wound area when extracts were administered to diabetic rats.

The rich fiber content of *Passiflora foetida* L. may be responsible for the activity. Dietary fibers lower the level of blood glucose by decreasing the rate of absorption of carbohydrate from the intestine and so are beneficial for type II diabetic patients (Khan A and Safdar M, 2003).

On 0 day, 7th day, and 14th day, there is a significant decrease in the level of plasma glucose in the ethanolic extract at the dose of 400 mg/Kg and 200 mg/Kg as illustrated in Table 1. There is an increase in the percentage area of wound contraction from 27.60% to 98.51%, 22.18% to 96.90% respectively on 15th day in ethanolic and ethyl acetate extract respectively at the dose of 400 mg/Kg. There is not much increase in the percentage contraction in the wound area in the lower doses (100 mg/Kg and 200 mg/Kg) in ethyl acetate and ethanol extract [1] as illustrated in Table 2. Complete wound healing is shown by ethyl acetate and ethanol extract at the dose level of 400 mg/Kg on 17th day. 100 mg/Kg and 200 mg/Kg showed complete healing of wounds on 18th day.

Decrease in the blood glucose level may be due to these active constituents present in the extract. The flavonoids present in ethyl acetate extract 400 mg/Kg and 200 mg/Kg showed the hypoglycemic activity on 0 days, 7th day, and 14th day.

Phenolic compounds present in plant extract may be beneficial in diabetes and many other diseases as reported from earlier studies. Therefore, the activity of the plant may be due to these phenolic compounds (Vasco C et al., 2008; Ahmad N and Mukhtar H, 1999).

3.1 Antidiabetic activity of leaves of *Passiflora foetida* L. in streptozotocin-induced diabetes mellitus:

TABLE 1
ANTIDIABETIC ACTIVITY OF LEAVES OF *PASSIFLORA FOETIDA* L. IN STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

S. No	Group	Plasma glucose level (mg/dl)		
		0 day	7thday	14thday
1	Standard (Metformin)	275.83±4.945	151.66±3.626*	160.33±2.21*
2	Diabetic Control with wound	285.16±2.072	296.33±3.412	304.16±6.263
3	Diabetic Control without wound	281.66±5.420	284.16±4.490	285.00±5.721
4	Ethyl acetate extract 100mg/Kg	286.00±7.000	200.50±4.500	221.50±8.500
5	Ethyl acetate extract 200mg/Kg	282.50±5.500	181.5±4.500*	201.00±5.100
6	**Ethyl acetate extract 400mg/Kg**	**285.50±4.500**	**165.50±7.500***	**192.50±4.500***
7	Ethanol extract 100 mg/Kg	279.50±12.500	226.50±4.500	235.00±7.000
8	**Ethanol extract 200mg/Kg**	**284.50±11.500**	**185.02±3.000***	**224.50±6.500***
9	**Ethanol extract 400mg/Kg**	**283.50±9.500**	**163.00±7.000***	**211.00±9.000***

Values are expressed as mean±SEM, n=6, p<0.05 versus diabetic control group (Dunnett's t-test after analysis of variances)

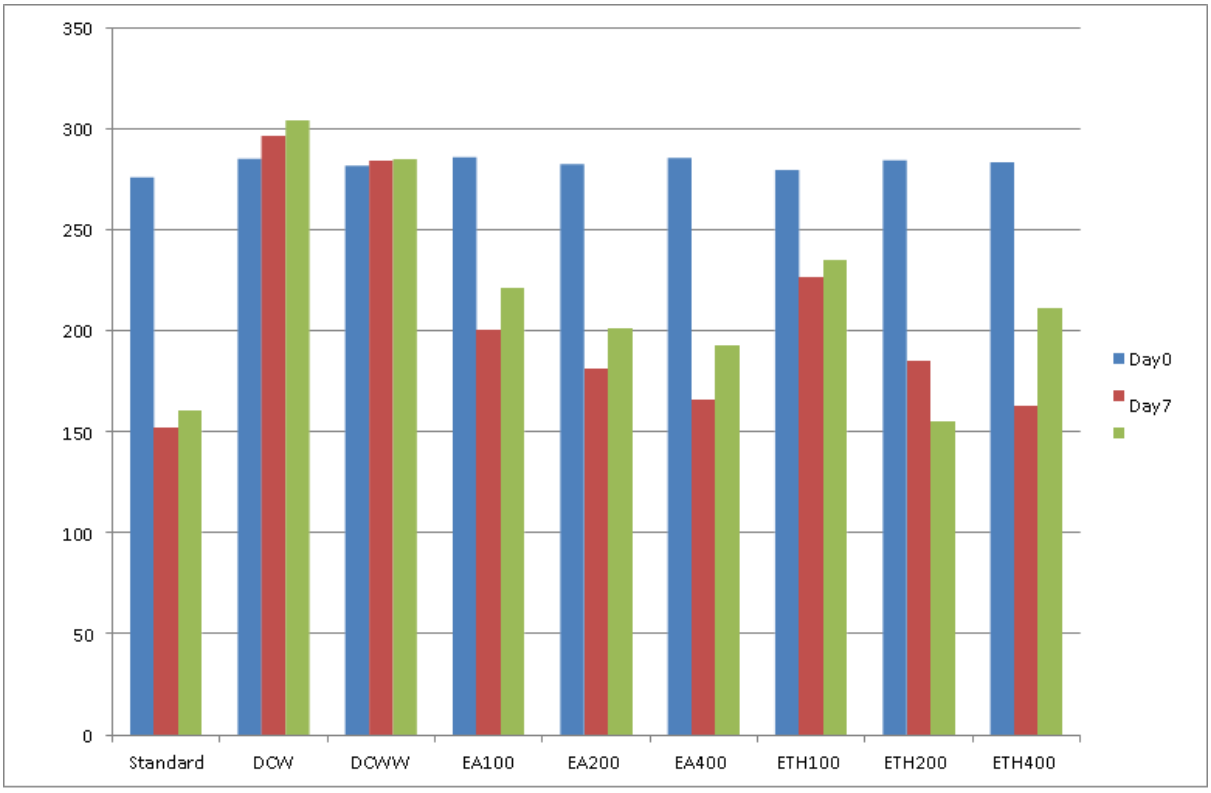

FIGURE 1: Effect of *Passiflora foetida* L. in streptozotocin-induced diabetes mellitus

TABLE 2

WOUND HEALING ACTIVITY OF LEAVES OF *PASSIFLORA FOETIDA* L. IN DIABETIC EXCISION MODEL

S. No.	Group	Percentage contraction in wound area							Epithelization period (in days)
		3rd day	6th day	9th day	12th day	15th day	18th day	21st day	
1	Standard (Metformin)	33.30±0.304	56.34±0.432*	78.71±0.354*	96.96±0.692*	100	100	100	14.86±0.307*
2	Control with wound	15.54±0.164	35.06±0.284	48.37±0.189	67.19±0.276	78.62±0.392	95.45±0.761	100	20.50±0.365
3	Ethylacetate extract 100 mg/Kg	19.09±0.180	38.98±0.75	56.50±0.250	81.43±0.185	92.82±0.395	100	100	17.50±0.50
4	Ethylacetate extract 200 mg/Kg	19.49±0.020	39.26±0.735	60.50±0.020	85.31±0.050	94.92±0.195	100	100	17.50±0.50
5	Ethylacetate extract 400 mg/Kg	**22.18±0.380**	**42.81±0.260***	**67.09±0.075***	**95.29±0.085***	**96.90±0.035***	**100**	**100**	**16.50±0.50***
6	Ethanol extract extract 100 mg/Kg	20.66±0.145	39.73±0.315	62.52±0.420	81.91±0.190	90.68±0.685	100	100	17.50±0.50
7	Ethanol extract extract 200 mg/Kg	24.13±0.335	43.56±0.185*	68.50±0.250*	86.87±0.100*	93.96±0.310*	100	100	17.00±0.00*
8	Ethanol extract extract 400 mg/Kg	**27.60±0.495**	**54.69±0.105***	**73.74±0.305***	**91.15±0.195***	**98.51±0.225***	**100**	**100**	**16.00±0.00***

Values are expressed as mean ± SEM, n=6, p<0.05 versus diabetic control group (Dunnett's t-test after analysis of variances)

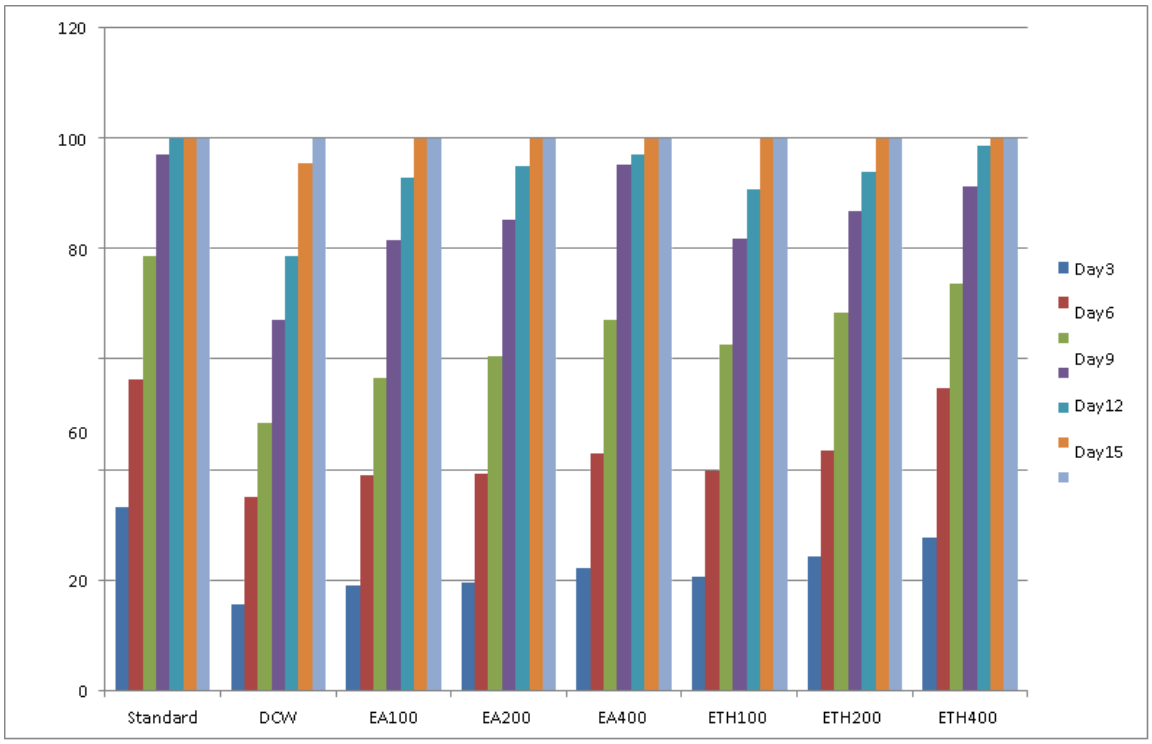

FIGURE 2: Wound healing activity of *Passiflora foetida* L. in diabetic excision model.

IV. CONCLUSION

The present study reveals that the ethyl acetate and ethanol extracts accelerate healing of wounds in diabetic patients. The results suggest that extracts may have a beneficial effect on wound healing phases. It is quite possible that the increase in the healing of wounds in diabetic rats is due to hypoglycemic activity (Rosenthal SP, 1968).

The study confirms the traditional use of *Passiflora foetida* leaves for the treatment of diabetic wounds. This result motivates us to carry out a vast study to isolate responsible potent active chemical constituents and to better evaluate the diabetic wound healing activity of the plant.

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