Assessment of Wound Healing Potential of *Passiflora foetida* L. Stem in Streptozotocin-Induced Diabetes Mellitus

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Abstract— *The study investigates the wound healing potential of Passiflora foetida L. stem in a Streptozotocin (STZ)-induced diabetic rat model. Diabetes mellitus is a major contributor to delayed wound healing, and traditional plant-based treatments offer promising alternatives. In this experiment, diabetic wounds were induced in Wistar albino rats using STZ, and the healing process was evaluated following the topical application of Passiflora foetida L. stem extracts. The rats were divided into various treatment groups, with one group receiving the extract, while others were treated with a standard wound healing agent or a control. Parameters such as wound closure rate, histopathological changes, and biochemical markers associated with healing (collagen content, inflammatory mediators, and antioxidant levels) were assessed over a period of time. The results demonstrated a significant improvement in wound healing in the group treated with Passiflora foetida L. stem extract, showing accelerated wound closure and enhanced tissue regeneration compared to the control group. These findings support the traditional use of Passiflora foetida for wound healing and suggest that it may possess therapeutic potential for diabetic wound care. Further studies to isolate the active compounds and evaluate their mechanisms of action are warranted.*

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

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and is associated with a range of complications, including impaired wound healing. In diabetic patients, wounds, particularly diabetic ulcers, often heal at a slower rate due to factors such as poor circulation, immune dysfunction, and prolonged inflammation. The delayed healing of wounds in diabetic individuals poses a significant challenge to both patients and healthcare systems, necessitating the exploration of new therapeutic strategies.

Passiflora foetida L., a species of the passionflower, is traditionally used in various cultures for its medicinal properties, including wound healing. The plant is known for its diverse phytochemical profile, which includes alkaloids, flavonoids, and terpenoids, all of which have demonstrated potential biological activities such as antioxidant, anti-inflammatory, and antimicrobial effects. Despite its historical use, scientific evidence supporting its efficacy in wound healing, particularly in diabetic conditions, remains limited.

Streptozotocin (STZ)-induced diabetes is a widely used experimental model that mimics the pathophysiological features of type 1 diabetes in humans, including delayed wound healing. This model provides a valuable platform to investigate the effectiveness of various therapeutic agents, including plant extracts, in enhancing the wound healing process.

This study aims to assess the wound healing potential of Passiflora foetida L. stem in STZ-induced diabetic rats. By evaluating parameters such as wound closure rate, histopathological changes, and biochemical markers of healing, we aim to provide scientific evidence supporting the traditional use of Passiflora foetida for treating diabetic wounds and to identify its potential as an alternative therapeutic option for managing diabetic ulcers.

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 between 150–200 g, were used for the experiment. These rats were obtained from the disease-free small animal facility at Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Reg. No. 1669/GO/abc/12/CPCSEA Dated 08/04/2013). They were kept in pathogen-free conditions. The rats were housed, fed, and treated in accordance with international guidelines and principles for laboratory animal use and care. They were maintained in polypropylene cages under standard conditions (25±2°C, 12-hour light and dark cycle) and were provided with pelleted food (Purina), with tap water available ad libitum (Hedrich HH, 2006). The rats were acclimatized to the standard pellet diet and water for 2 weeks before the study began. All experimental procedures and protocols were approved by the Institutional Animal 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 over night 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:

$$\%\{\text{wound contraction}\} = (A_0 - A_t)\ /A_0 * 100 \quad (1)$$

Where (A_0) is the original wound area and (A_t) 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

The oral dose of the ethyl acetate and ethanol leaves extracts of Passiflora foetida had shown a dose-dependent effect on the blood glucose level and wound healing effect on the diabetic rats.

On the 0th day, 7th day, and 14th day, there was a significant decrease in plasma glucose levels in the ethanolic extract at the dose levels of 200 mg/Kg and 400 mg/Kg. This activity may be due to the various chemical constituents present in the extract. The hypoglycemic activity is attributed to the presence of flavonoids in the ethyl acetate extract.

There was an increase in the percentage area of wound contraction from 30.12% to 93.14% and 24.73% to 85.81% respectively on the 12th day in the ethanolic and ethyl acetate extracts at the dose level of 400 mg/Kg. There was not much increase in the percentage contraction in the wound area at the lower doses (100 mg/Kg and 200 mg/Kg) in both the ethyl acetate and ethanol extracts.

Complete wound healing was shown by the ethyl acetate and ethanol extracts at the dose level of 400 mg/Kg on the 14th day. The 100 mg/Kg and 200 mg/Kg doses showed complete healing of the wound on the 18th day.

The present study reveals that the ethyl acetate and ethanol extracts accelerate the healing of wounds in diabetic rats. The results suggest that the 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 the hypoglycemic activity (Rosenthal SP, 1968).

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

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

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

S. No	Group	Plasma glucose level(mg/dl)		
		0 day	7th day	14th day
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	277.00±12.000	181.50±4.500*	185.00±7.000*
5	**Ethyl acetate extract 200 mg/Kg**	**280.00±5.000**	**162.50±6.500***	**165.50±11.500***
6	**Ethyl acetate extract 400 mg/Kg**	**286.50±3.500**	**158.50±5.500***	**163.50±7.500***
7	Ethanol extract100 mg/Kg	295.00±4.000	165.50±6.500*	168.03±3.000*
8	**Ethanol extract 200 mg/Kg**	**282.50±6.500**	**157.50±5.500***	**163.50±6.500***
9	**Ethanol extract 400mg/Kg**	**277.00±12.000**	**152.50±3.500***	**161.50±6.500***

Values are expressed as mean±SEM, n=6, p<0.05 versus diabetic control group (Dunnett'st-test after analysis of variances)

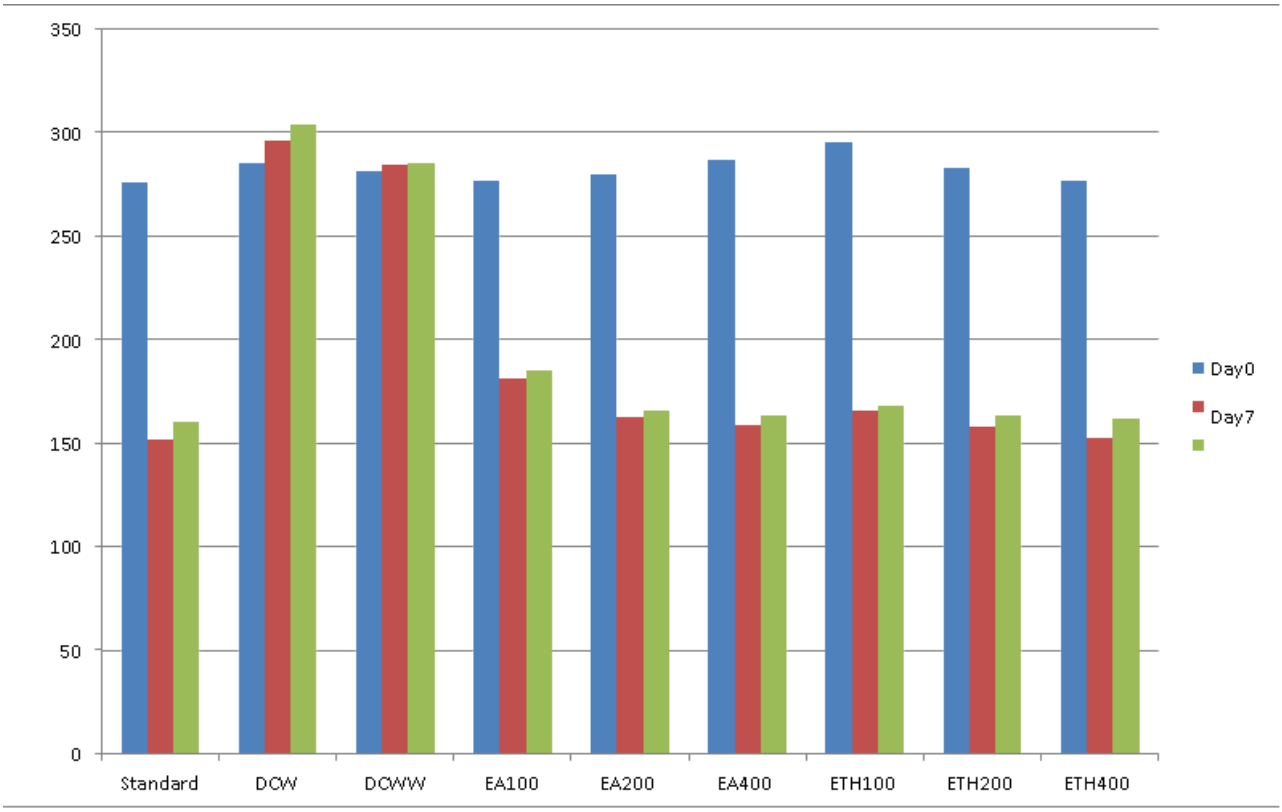

FIGURE 1: Effect of *Passiflora foetida* L. in streptozotocin-induced diabetes mellitus

TABLE 2

WOUND HEALING ACTIVITY OF STEM 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	20.35±0.350	44.58±0.145*	59.02±0.445*	77.06±0.300*	91.99±0.425*	100	100	17.50±0.50
4	Ethylacetate extract 200 mg/Kg	21.83±0.215	46.06±0.420*	62.10±0.055*	79.45±0.545*	95.79±0.415*	100	100	16.50±0.50*
5	Ethylacetate extract 400 mg/Kg	**24.73±0.005**	**52.83±0.315***	**74.23±0.500***	**85.81±0.035***	**100**	**100**	**100**	**14.50±0.50***
6	Ethanolextract extract 100 mg/Kg	23.71±0.285	43.11±0.110*	67.09±0.090*	81.36±0.640*	92.09±0.090*	100	100	17.50±0.50
7	Ethanolextract extract 200 mg/Kg	27.73±0.175	54.72±0.410*	73.28±0.315*	87.95±0.145*	93.70±0.	100	100	17.50±0.50
8	Ethanolextract extract 400 mg/Kg	**30.12±0.120**	**55.71±0.770***	**78.47±0.470***	**93.14±0.510***	**100**	**100**	**100**	**14.00±0.00**

Values are expressed as mean ± SEM, n=6,p<0.05 versus diabetic control group (Dunnett'st-test after analysis of variances)

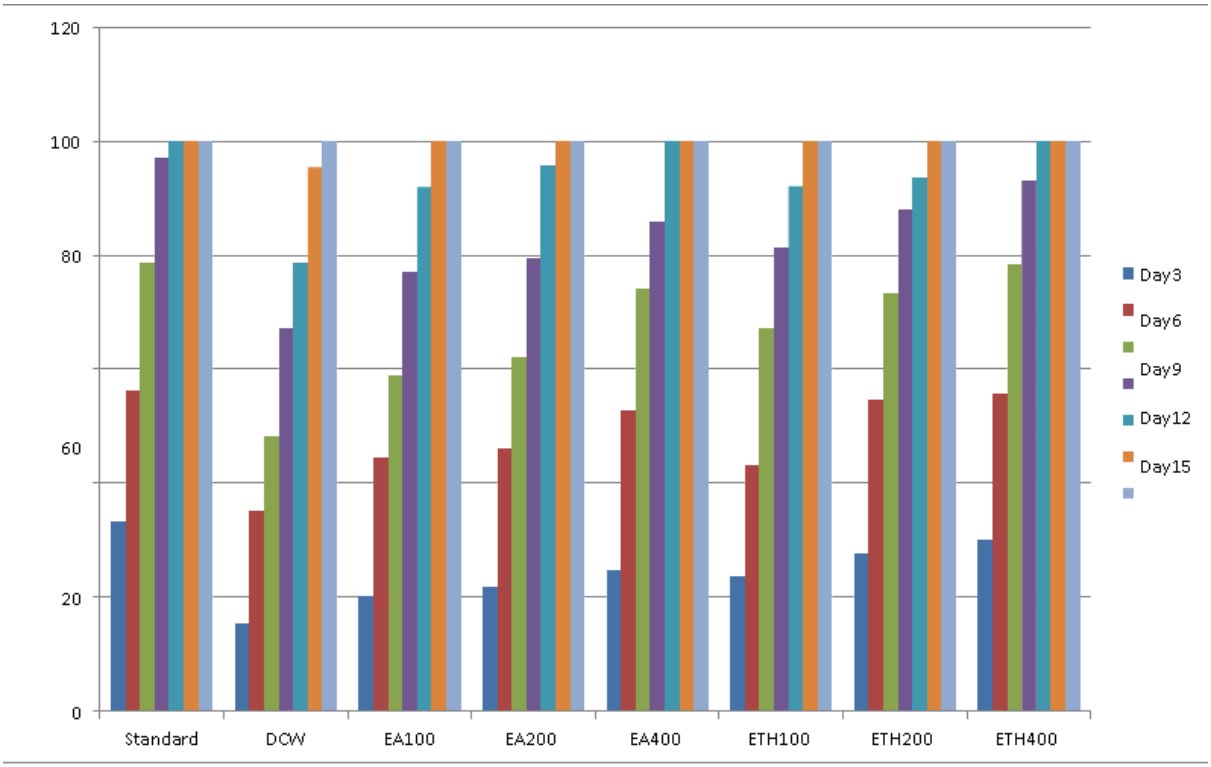

FIGURE 2: Wound healing activity of *Passiflora foetida* L. in diabetic excision model.

IV. CONCLUSION

This study demonstrates that the ethyl acetate and ethanol extracts promote faster wound healing in diabetic patients. The findings indicate that these extracts may positively influence various phases of wound healing. It is likely that the improved wound healing in diabetic rats is attributed to the hypoglycemic effects (Rosenthal SP, 1968).

The study supports the traditional use of Passiflora foetida leaves for treating diabetic wounds. This outcome encourages further research to isolate the active chemical components responsible and to more thoroughly assess the plant's effectiveness in promoting diabetic wound healing.

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