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EVALUATION OF THE WOUND HEALING PROPERTIES OF *MIMOSA PUDICA* LINN. IN STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN RATS

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ABSTRACT: *Mimosa pudica* Linn. (Fabaceae) It is traditionally used to treat wounds and diabetes mellitus. The study was designed to evaluate the wound healing properties of ethyl acetate and ethanolic extracts of seeds of *Mimosa pudica* in streptozotocin-induced diabetes mellitus in rats at dose levels of 100, 200 and 400 mg/kg body weight. Both extracts decrease the fasting blood glucose in a dose-dependent manner. A significant decrease in blood glucose level was also observed in the ethanol and ethyl acetate extract treated rats at different dose levels. The maximum percentage contraction in wound area (25.52 to 97.37, 23.65 to 93.12) was observed in diabetic rats treated with ethanolic extract at 400 mg/kg and 200 mg/kg dose respectively and with a significant percentage contraction in wound area (21.34 to 96.87, 20.71 to 95.22) was observed in diabetic rats treated with ethyl acetate extract at 400 mg/kg and 200 mg/kg dose respectively on 15th day. The results exhibit that *Mimosa pudica* possesses considerable antidiabetic and wound healing activity in diabetic rats.

INTRODUCTION: *Mimosa pudica* Linn. (Fabaceae) Is mainly known as a sensitive plant, humble plant sleeps sleeping, honteuse, dormidera, morivivi, touch-me-not, marie-honte, mayhont, grass, timawi and having many other sleep names ¹⁻³. It is a small and transient, prostrate or growing bush. It can reach a maximum height of 1 m together with another support. It has curved spines. The implant has a dummy system with large fibrous radical nodules. The color of the flower is pink with globose heads. The pods have groups and have from two to four brown seeds ²⁻⁴.

Mimosine, mimosinamine, tyrosine 3, 4-dihydroxypyridine, D-glucuronic acid 4-O-(3, 5-dihydroxybenzoic acid)-b-D glucuronide, mimosine acid, tubulin, C-glycosylflavones, phenolic ketone are the chemical constituents present in the plant ⁵⁻⁸. Also, the plant has also demonstrated medicinal activities such as antimicrobial, antidiabetic, anti-virulent, antifertility, neutralizing poison, antidepressant, hepatoprotective, anti-oxidant, hypoglycemic, lipid-lowering and wound healing ⁹⁻²³.

Ethanolic plant extracts were reported to reduce the blood glucose level in streptozotocin-induced diabetic rats. This indicates that *Mimosa pudica* has a significant antidiabetic activity ²⁰. Chloroform and methanolic root, shoot extracts showed wound healing activity when applied topically with a significant increase in the hydroxyproline content, tear strength and dry weight of the granulation

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tissue in the wound incision pattern²¹⁻²³. Because of the therapeutic activity and the presence of phenolic constituents mentioned above, the plant was chosen to study the wound healing activity in diabetic rats.

MATERIALS AND METHODS:

Plant Collection: Seeds of *Mimosa pudica* Linn. (Fabaceae) were collected from the local market of Sonapat, Haryana, India in December 2014. The herbarium was prepared using collected plant materials and authenticated by Dr. (Mrs.) Sunita Garg (Chief Scientist, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India) under voucher specimen no. NISCAIR/RHMD/ Consult/ 2015/2564-143-1 dated 06-01-2015. A voucher is also retained in the university department for further reference.

Plant Extracts Preparation: Ethyl acetate and ethanol extracts were extracted from 500 g of shade-dried seeds of *Mimosa pudica* by continuous hot percolation method by using Soxhlet extractor, in which, ethanol and ethyl acetate were used as solvents to collect the extracts. Both ethyl acetate and ethanol extracts were dried under vacuum below 40 °C to obtain a semi-solid consistency mass and were separately kept in desiccators for further use.

Animals: 150 - 200 g weighing wistar albino rats were used for experimentation. IAEC (Institutional Animal Ethical Committee) approval number was 360-73 dated 05-05-2016. Pathogenic free conditions were provided to the rats. The rats were housed, fed and treated as per the international guidelines principles of laboratory animal use and care. The animals were maintained in polypropylene cages under standard conditions (25 ± 2 °C, 12 h light and dark cycle) with pelleted food (Purina), while tap water was available *ad libitum*²⁴.

Diabetes Induction: Streptozotocin (STZ; 50 mg/kg, i.p.) (Sigma-Aldrich Canada, Oakville, Ontario, Canada), prepared in citrate buffer (0.1 M, pH 4.5) was used to make rats diabetic by a single dose after overnight fasting²⁵. Blood was taken out from the rat's orbital plexus 24 h after the injection, and the fasting blood glucose level was estimated after the 7th day of Streptozotocin injection (with

glucose oxidase reagent strips) using Glucometer (Accu-check®) and animals with glucose levels preminent than 250 mg/dl were used for the study. At the time of creation of the wounds, blood glucose levels of rats were studied.

Surgical Procedures and Treatment: Wounds were created on the 7th day after the induction of diabetes. Excision wound healing method was used to calculate the wound healing potential of different extracts in experimental rats. Different biochemical parameters and the rate of wound contraction were studied. Rats were anesthetized with thiopentone sodium at a dose level of 40 mg/Kg i.p. and each rat was shaved on the right side. 4 cm² sized excision wound was created by cutting out a 2 cm × 2 cm piece of skin from the shaven area. Ethyl acetate and ethanol extracts were given orally in concentrations of 100 mg/kg, 200 mg/kg, and 400 mg/kg for 21 days. Citrate buffer was given in an equal amount to the control group.

Wound Healing Activity:

Excision Wound: When no raw wound is left behind and when scar falls off, that period in days was noted as epithelialization time²⁶. Excision wounds on a transparent paper having a millimeter scale were traced to determine the rate of wound contraction and percentage of wound area healed was calculated using change in wound size. The number of days taken for complete epithelialization was expressed as the period of epithelialization (so that no raw wound was left behind).

Grouping of Animals: Animals were divided into nine groups, each group consisting of six rats. The extracts were administered for 15 days. Group I: standard (Metformin 5 mg/kg), Group II: Diabetic rats with wound without treatment as 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 100 mg/kg, Group V: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose 200 mg/kg, Group VI: Diabetic rats with wound treated with ethyl acetate extract by oral route at a dose 400 mg/kg, Group VII: Diabetic rats with wound treated with ethanol extract by oral route at a dose 100 mg/kg, Group VIII: Diabetic rats with wound treated with ethanol extract by oral route at a dose 200 mg/kg, Group

IX: Diabetic rats with wound treated with ethanol extract by oral route at a dose 400 mg/kg.

The Rate of Wound Contraction and Period of Epithelialization: At 0 day, before extract treatment and after wounding on 3, 6, 9, 12, 15, 18 and 21 days excision wounds were traced on a transparent paper having mm scale and on every third-day change in wound size were calculated as the percentage of wound area that has healed. The percentage contraction of the wound was calculated using:

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

Where A_0 is the original wound area and A_t is the area of the wound at a specific period after wounding²⁷.

Statistical Analysis: Wound area was measured as percentage contraction in wound size. Analysis of data was statistically done by Dunnett's t-test using Graph pad prism 7.0. When $P < 0.05$ compared with control, the data is considered significant.

RESULTS AND DISCUSSION: The present study is the preliminary assessment of the antidiabetic and wound healing activity of the ethyl acetate and the ethanol seeds extracts of *Mimosa pudica*. There is a dose-dependent fall in blood glucose level in streptozotocin-induced diabetic rats and a decrease in wound size in both the

extracts. Due to alteration in connective tissue metabolism in Diabetes mellitus, diabetic patients have difficulty in wound healing. Decrease in the level of production or increase in the catabolism of newly synthesized collagen occurs due to loss of collagen in diabetic patients²⁶.

The decrease in blood glucose level was observed when *Mimosa pudica* L. extracts were administered to glucose loaded normal rats fasted for 18 h. A significant increase in blood glucose level in the diabetic control group at the end of the 14th-day experimental period and an increase in wound healing by excision wound healing method was observed for 21 days. After administration of the extracts, there is a significant decrease in fasting blood glucose level and percentage area of wound contraction.

A significant decrease in plasma glucose level in the ethanolic extract at a dose level of 400 mg/kg and 200 mg/kg was observed for 0 days, 7th day and 14th day as illustrated in **Table 1**. Various chemical constituents present in the extract are responsible for the antidiabetic activity. Flavonoids present in ethyl acetate extract might have shown the hypoglycemic activity at the dose level of 400 mg/kg and 200 mg/kg on 0 days, 7th day and 14th day.

TABLE 1: ANTIDIABETIC ACTIVITY OF MIMOSA PUDICA LINN. IN STREPTOZOTOCIN INDUCED DIABETES MELLITUS

S. no.	Group	Plasma glucose level (mg/dl)		
		0 day	7 th day	14 th 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 100 mg/kg	284.00 ± 13.00	172.50 ± 2.500*	173.00 ± 6.000*
5	Ethyl acetate extract 200 mg/kg	281.50 ± 7.500	170.50 ± 5.500*	171.50 ± 4.500*
6	Ethyl acetate extract 400 mg/kg	278.00 ± 14.000	166.00 ± 10.000*	168.52 ± 4.500*
7	Ethanol extract 100 mg/kg	276.00 ± 13.000	168.50 ± 12.500*	167.05 ± 12.000*
8	Ethanol extract 200 mg/kg	280.50 ± 12.500	162.00 ± 7.000*	162.50 ± 4.500*
9	Ethanol extract 400 mg/kg	286.50 ± 7.500	155.50 ± 2.500*	161.50 ± 9.000*

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

There is an increase in the percentage area of wound contraction from 25.52% to 97.37% and 21.34% to 96.87% respectively on the 15th day in ethanolic and ethyl acetate extract 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 as shown in **Table 2**. Complete wound healing is shown by ethyl acetate and ethanol extract at the dose of 400 mg/kg on the 18th day.

TABLE 2: WOUND HEALING ACTIVITY OF *MIMOSA PUDICA* LINN. IN DIABETIC EXCISION MODEL

S. no.	Group	Percentage contraction in the wound area							Epithelization period (in days)
		3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day	21 st 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	Ethyl acetate extract 100 mg/kg	19.43 ±0.245	43.77 ±0.160*	66.84 ±0.180*	77.46 ±0.190*	92.48 ±0.065*	100	100	17.50 ±0.500
4	Ethyl acetate extract 200 mg/kg	20.71 ±0.340	48.27 ±0.150*	67.36 ±0.005*	77.98 ±0.090*	95.22 ±0.495*	100	100	17.00 ±0.00
5	Ethyl acetate extract 400 mg/kg	21.34 ±0.185	52.36 ±0.160*	69.00 ±0.225*	81.76 ±0.285*	96.87 ±0.050*	100	100	16.50 ±0.50
6	Ethanol extract 100 mg/kg	21.31 ±0.040	46.30 ±0.035*	67.10 ±0.445*	77.10 ±0.020*	91.05 ±0.430*	100	100	18.00 ±0.00
7	Ethanol extract 200 mg/kg	23.65 ±0.075	48.08 ±0.115*	72.26 ±0.040*	82.94 ±0.125*	93.12 ±0.305*	100	100	17.50 ±0.50
8	Ethanol extract 400 mg/kg	25.52 ±0.005	51.57 ±0.015*	78.42 ±0.300*	86.31 ±0.385*	97.37 ±0.500*	100	100	16.50 ±0.50

Apart from various other potent chemical constituents present in plants extracts, phenolic compounds may be beneficial to have the check on diabetes and many other diseases as reported from earlier studies^{28, 29}.

CONCLUSION: The current research study confirmed that the ethyl acetate and ethanol extract of *Mimosa pudica* seeds have properties to promote wound healing and antidiabetic activity. This study gives us excellent, precise facts that the extract may show potential harmonizing add-on in future after collecting supplementary scientific statistics for diabetic patients with wound healing defect.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding this study.

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