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Evaluation of wound healing activity in streptozotocin induced diabetic rats by ethanolic extract of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth

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Abstract
Wound healing is a complex process delayed by metabolic disorders like diabetic mellitus,it remains a crippling global health problem today leading to amputation of limbs and digits. *Blepharis maderaspatensis* (*L.)* B.Heyne ex Roth is said to be used for wound healing in many ethanobotanical studies. So, it was decided to check the efficacy of the same on streptozotocin induced diabetic rats. The extract was formulated as given in the literature by formulating a paste with 10gs, 15gs, 20gs of extract and 60gs black gram powder which was mixed with egg white and 2 or 3 drops of lime juice q.s. The wound healing efficiency by different concentrations of the extract was monitored by the excision wound healing method. The healing with the standard Framycetin Sulphate 1%w/w was taken as the positive control. The wound was completely healed by the 18th day in the 20% extract while it took 20 days for complete healing in positive control and 23 days for complete healing in negative control. Thereby confirming the reports in the ethanobotanical studies with regard to the wound healing activity of the plant.

Keywords: Diabetic rats, wound healing, Excision, *Blepharis maderaspatensis* (L.) B.Heyne ex Roth

1. Introduction
Blepharis maderaspatensis (L.) B.Heyne ex Roth is prostrate herb which is a common flora of the western ghats and hence the significance of its use. Many ethanobotanical [1, 2, 3, 4] reports support the fact that the leaves of this plant are used for wound healing activity. Rajsekaran *et al*. [5] have proved the wound healing activity of the ethanolic extract, which was a determining factor in the take up of the study. Amputation of limbs due to chronic wounds in diabetes has proved to be a serious issue in the current therapeutic scenario, so, in the current study an effort was made to test the wound healing properties of the ethanolic extracts of *Blepharis maderaspatensis* (L.) B.Heyne ex Roth in streptozotocin induced diabetic rats.

2. Materials and methods
2.1. Collection and preparation of Plant formulation.
The leaves of *Blepharis maderaspatensis* were collected from the medicinal plant gardens of Anugraha Herbals, Kuruppanthara, Kottayam. It was identified, authenticated and the voucher specimen was deposited in the Pharmacognosy Department at Department of Pharmaceutical Sciences, Mahatma Gandhi University, Cheruvandoor Campus, Ettumanoor, Kottayam, Kerala. The leaves collected were shade dried and powdered in a mechanical grinder to give a course powder. The powdered drug was macerated in 95% ethanol (R.S.) for 72 hrs. Then filtered and the solvent evaporated using the rotary vacuum evaporator. The final extract obtained from about 1 kg of the powdered drug was about 130grams. Paste formulation[6]was prepared by taking10gs, 15gs, 20gs of the extract and mixed with 10gs of egg white powder, 1ml of lime juice and 60gs of Black gram powder and water q.s. to produce 100gs of smooth paste formula.

2.2. Excision wound healing method[7]
Healthy adult wistar albino male rats were purchased from Govt. Vetinary College, Munoothy and housed at the animal house of Department of Pharmaceutical Sciences, Puhtuppally, Kottayam for two days before the experiment for acclamatisation. The animals were kept in hygienic as well as controlled conditions of temperature (25 ± 3°C) and humidity (50 ± 5 %) and a 10-12 hrs of light and dark cycles were observed. The animals were housed individually in polypropylene cages with saw dust bedding as approved by IEAC of Department of Pharmaceutical Sciences, Puthuppally till the end of the study. The animals were maintained on normal diet and water ad libitum.

2.2.1 Induction of Diabetes Mellitus in rats[8]

After overnight fasting 30 rats were induced Diabetes Mellitus by giving a single injection of streptozotocin (45mg/Kg i.p.) prepared by dissolving in 0.9% ice cold citrate buffer (4.5 pH). Fasting blood glucose level was estimated using glucometer (Accu-Chek, ROCHE, India) after 24hrs of the injection by taking blood from tail vein. Rats showing blood glucose level of 250-300mg/dl were included in the wound healing activity.

2.2.2Making of the excision wound

The animals were anesthetized with ketamine (1%) and the hair shaved from the dorsal thoracic central region. One excision wound was inflicted by marking the area of 300mm² and cutting the full thickness of skin from the predetermined area. The excess blood was absorbed with a sterile cotton swab dipped in isotonic saline. Animals were divided into five groups of 6 each. The negative control group was left without treatment. Positive control group was treated with framycetin sulphate (1%w/w) cream twice every day, morning and evening respectively. The other groups were treated with the different concentrations of extract paste twice daily till the wound heals completely. Each animal was housed separately till the end of the test. The wound healing activity was measured by Percentage wound contraction rate. The wound area was traced using a butter paper and measured with a scale graph paper.

$$\% \text{ wound Contraction} = \frac{\text{Initial Wound size - Specific day wound size} * 100}{\text{Initial Wound Size}} \quad (1)$$

3. Results & Discussion

Rajshekhran *et al* have reported the wound healing activity in normal rats by 10% *Blepharis maderaspatensis* extract. In the current study the work on diabetic rats, the wound healing capacity was evaluated by analyzing the percentage of wound contraction calculated as per Eq(1).It is indicated in the table as parenthesis. The streptozotocin induced diabetic rats showed complete healing by the 18th day with 20 % hydroalcoholic extract of *Blepharis maderaspatensis* (L.) B.Heyne ex Roth prepared in a similar manner as that prescribed in the literature reports. The 10 % extract as well as the 15% extract showed complete healing by the 20th day. The negative control took 23 days for complete healing where as the positive control took 20days for complete healing.

3.1 Table

The wound area(mm^2)as well as the calculated percentage of wound contraction calculated as per Eq.(1) for the strepotozotocin induced diabetic rats for the different concentrations of the hydroalcoholic extract of *Blepharis maderaspatensis* prepared by the indigenous formula as per the literature are shown in the table no.1.

Table 1: Effect of paste prepared from different concentrations of hydroalcoholic extract of Blepharis maderaspatensi(L.) B.Heyne ex Roth on wound healing activity in streptozotocin induced diabetic rats.

Post wounding days	Wound area(mm^2)(mean ± SEM) and percentage of wound contraction in parantheses				
	Negative Control	Positive control (Framycetin)	10% Extract	15% Extract	20% Extract
0	277.83±5.32	280.67±7.16	280.67±9.48	280.67±9.48	286.67±8.11
III	278±8.23 (-0.06)	232.50±3.55 (17.16)	267.00±8.09 (15.38)	237.50±4.61 (4.86)	280.67±9.48 (2.09)
VI	283.50±8.29 (-2.04)	189.33±3.64 (32.07)	230.17±5.17 (30.99)	193.67± 2.33 (17.99)	144.17± 5.22 (49.70)
IX	273.50±6.12 (1.55)	96.33±3.52 (65.67)	207.83± 4.43 (65.08)	98.00± 3.27 (25.95)	68.67± 5.63 (76.02)
XII	198.50±5.77 (28.55)	47.33±2.53 (83.13)	147.67±17.45 (82.36)	49.5± 2.11 (47.38)	15.17± 1.42 (94.70)
XV	94.67±3.52 (65.92)	9.00±1.44 (96.79)	67.67±10.12 (97.14)	8.00± 0.577 (75.88)	0.833± 0.31 (99.70)
XVIII	45.83±1.83 (83.50)	0.83±0.31 (99.70)	11.00± 1.46 (96.08)	0.33± 0.21 (99.64)	(100)
XX	9.00± 1.44 (96.76)	(100)	(100)	(100)	-
XXIII	(100)		-	-	-

4. Conclusions

It is clearly indicated by the study that the plant *Blepharis maderaspatensis* (L.) B.Heyne ex Roth possess wound healing activity even in diabetic rats. The indigenous formula has proved its efficiency it is seen that the paste was non irritant to the as the did not show any extra signs of scratch ing when compared to the control group of rats. This has opened up the avenue of developing up the plant drug for formulation and sales in ayurveda.

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