EVALUATION OF WOUND HEALING PROPERTIES OF PSORELIYA COROLIFOLIA LINN IN DIABETIC RATS

Prashant M. Tayade., Sunita N.Borde., N. Chandrasekar., Shrikant A.Jagtap., Abhay S. Joshi

Address: *Corresponding Auther
1.Prashant M. Tayade*
Yash Institute of Pharmacy,
South city, Bajaj Nagar,
Waluj Road, Ph No: 9028033912
Aurangabad-431134 E-Mail: Pranay2424@yahoo.com
Maharashtra,
India.
2. Sunita N.Borde
Department of Zoology,
Dr. Babasaheb Ambedkar Marathwada University,
Aurangabad-431134
Maharashtra,
India.
3. N. Chandrasekar
J.S.S. college of Pharmacy
Oouty
Tamilnadu, India.
4. Shrikant A.Jagtap
Yash Institute of Pharmacy,
South city, Bajaj Nagar,
Waluj Road,
Aurangabad-431134
Maharashtra,
India.
5. Abhay S. Joshi
Yash Institute of Pharmacy,
South city, Bajaj Nagar,
Waluj Road,
Aurangabad-431134
Maharashtra,
India.

Summary

According to ayurveda pc was used widely in medicine for the treatment of wound and as an immunomodulatory. However its mechanism of action is not clear. In the present study we investigate the effect of pc on wound healing activity in streptozotocin induced diabetic rats. Incision by using tensiometer and excision models were employed rats. Significant wound healing activity was observed with the ointment formulation prepared by using ethanol extract at 1% concentration on the mentioned models. The results of histopathological examination supported the outcome of both incision and excision wound models. The wound healing effect was comparatively evaluated with a reference ointment. The experimental data demonstrated that *Psoreliya corolifolia* L. displayed remarkable wound healing activity.

Key words: *Psoreliya corolifolia,* Excision, Incision, Tensiometer, Wound healing.

Introduction

Adiponectin is an adipose-derived protein that is abundant in plasma in various oligomeric forms. It has a number of properties in various tissues, such as improvement of insulin sensitization and anti-inflammatory and antiatherogenic effects. Plasma protein levels of adiponectin have been shown to be de- creased in obesity and type 2 diabetes, thus epidemiologically implicating that it modulates some complications accompanying diabetes such as insulin resistance, atherosclerosis, and cardiac injury. Adiponectin also binds to early apoptotic cells through calreticulin and is important for their clearance. Although a number of studies have examined the effects of adiponectin on several types of cells and tissues, its effects on an impaired wound healing process, which is common in diabetic patients, is unclear[1].

The normal wound healing process is a highly regulated bio- chemical event that involves re-epithelialization and granulation tissue formation phases, with keratinocytes playing a major roll in re-epithelialization.

Upon injury, keratinocytes not only proliferate and migrate to cover the wound but also express numerous molecules, including growth factors, chemokines and inflammatory cytokines, which affect fibroblasts and granulation tissue formation. As for the impaired wound healing seen in patients with diabetes, it has been suggested that these regulations are dysfunctional leading to such problems as hyperkeratinization and callosity around diabetic ulcers.

Psoralea corylifolia L. has been used traditionally as medicine in ayurveda and recommended for the treatment of wound, stomachic, deobstruent, anthelmintic, diuretic and also certain skin diseases, e.g., leucoderma, psoriasis and leprosy [2,3]. Few reports, however, have addressed the anti- oxidant activity of Psoreliya corolifolia L.

Herein we repot the in vivo wound healing activity of psoralea corylifolia L. in streptozotocine induced diabetes in rats.

Materials and Methods

Plant materials:

Psoralea corylifolia L. plant was collected from Andhari village, Aurangabad, India in May 2008 and the plant was authenticated in the Department of Biology, Dr. Babasaheb Ambedkar Marathwada Univesity, Aurangabad. The plant material was identified according to criteria given in Flora of India. A voucher specimen (TYP 1019) was deposited in the Herbarium of Faculty of Biology, Dr. BAMU University, Aurangabad, India.

Preparation of plant extact:

Whole plant of *psoralea corylifolia* L was collected and shed dried. Furocoumarins were isolated from the whole plant of *Psoralea corylifolia* with ethanol. The air-dried plant of *Psoralea corylifolia* (2 kg) were powdered, then defatted with hexane at room temperature and soaked in 75% ethanol in water for a period of 3 days. The filtrate was concentrated under reduced pressure to an aqueous ethanol extract (132 g). The extract was re-dissolved in 95 % ethanol at 60 °C water- bath, and kept quietly until needle crystals were separated out (09 g). The purity of furocoumarin crystals was checked by TLC and HPLC to be at least 95% pure [4,5].

Biological activity tests

Animals

Male, Sprague-Dawley rats (160-180 g) and Swiss albino mice (20-25 g) were obtained from the animal breeding laboratories of Yash Institute of Pharmacy, Aurangabad. India. The animals were left for 3 days at room conditions for acclimatization. They were maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group, otherwise described in procedure. The study was permitted by the Institutional Animal Ethics Committee and was performed according to the international rules considering the animal experiments and biodiversity right.

Preparation of test samples

Excision and incision wound models were used to evaluate the wound healing activity. For the in-vivo wound models, test samples were prepared in ointment base consisting of glycol stearat, 1,2 propylene glycol, liquid paraffin (3:6:1) in 1% concentration 0.5 mg of each test ointment was applied topically on the wounded site immediately after wound was created by a surgical blade. The vehicle group of animals was treated with the ointment base only. Whereas the reference drug group of animals were treated with 0.5 g of Cipladine (Cipla Ltd., MNB/87/08).
Cipladine contains 5% of Povidone- iodine.

Linear incision wound model

All the animals were anaesthetized with 10% Kethamin HCl and the back hair of the rats were shaved by using a shaving machine. Five cm long, two linear paravertebral incisions were made with a sterile surgical blade through the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the vertebral column [6]. The wounds were closed with three surgical interrupted sutures of 1 cm apart. The animals were divided into 4 groups. The extracts, the reference drug (Cipladine) and the vehicle were topically applied once in a day throughout 9 days. The fourth group, negative control group of animals, was not treated with any material. All the sutures were removed on the 9^{th} post wound day. On day tenth all the animals were killed under anesthesia. One linear- paravertebral incised skin was measured using tensiometer for its tensile strength, the other incised skin was sent for histopathological examination [7,8]. Tensiometer measures the breaking strenght in N (Newton), which is called tensile strength.

$$\% \text{ Tensile strength (TS) of } \textbf{extract} = \frac{\text{TS of } \textbf{extract} \text{ group - TS of vehicle group}}{\text{TS of vehicle}} \times 100$$

$$\% \text{ Tensile strength of } \textbf{reference} = \frac{\text{Tensile strength of } \textbf{reference} \text{ - TS of vehicle group}}{\text{TS of vehicle}} \times 100$$

Table 1: Comparison of contraction values of the extracts of psoralea corylifolia L, vehicle and reference material on linear incision wound model

Groups	Wound healing processes	
	Statistical mean ± SEM	**% Tensile strength**
Vehicle	19.82 ± 1.81	26.10
Test extract	26.53 ± 1.89	49.6***
Standard (Cipladine)	29.28 ± 1.12	58.3***

*P<0.05; **P<0.01; ***P<0.001; SEM; Standard Error Mean.

The extract and the reference drug were compared with vehicle group.

Excision wound model

This model was used to monitor wound contraction and wound closure time. Each group of animals (six animals each) was anaesthetized by 0.01 cc Ketalar. The back hairs of the mice were depilated by shaving. The circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 5 mm biopsy punch; wounds were left open [9]. The extracts, the reference drug (Cipladine) and the vehicle were applied topically once a day till the wound was completely healed. The progressive

changes in wound area were monitored by a camera (Sony cyber shot, India) every other day. Later on, wound area was evaluated by using AutoCAD program. Wound contraction was calculated as percentage of the reduction in wounded area. A specimen sample of tissue was isolated from the healed skin of each group of mice for the histopathological examination [10].

Table 2: Effect of the extract of *Psoralea corylifolia* L. on excision wound model.

Days	Wound area mm ± SEM		
	Vehicle	Test Extract	Standard
0	21.22 ± 1.86	19.98 ± 1.84	28.84 ± 2.11
2	19.02 ± 1.92	16.34 ± 1.38	14.33 ± 1.32
4	15.21 ± 1.52	12.57 ± 1.33	9.21 ± 1.39
6	12.26 ± 1.33	9. 58 ± 1.03	07.02 ± 1.02
8	9.63 ± 0.84	05. 04 ± 0.89	03.42 ± 0 .87
10	5.36 ± 0.52	03.54 ± 0.34	0.98 ± 0.23
12	3.12 ± 0.34	01.06 ± 0.28	00.00 ± 0.00

*P<0.05; **P<0.01; ***P<0.001; SEM; Standard Error Mean.
The extract and the reference drug were compared with vehicle group.

Table 3: Percent contraction of wound in excision wound model.

Days	% Contraction		
	Vehicle	Test Extract	Standard (Cipladine)
0	-	-	-
2	11.2	12.14	35.4
4	10.22	32.54	44.1*
6	22.8	64.11**	72.4*
8	38.9	89.24***	91.7***
10	44.2	92.34***	96.3***
12	46.3	94.23***	100***

*P<0.05; **P<0.01; ***P<0.001; SEM; Standard Error Mean
The extract and the reference drug were compared with vehicle group.

Histopathology

Sample tissues were fixed in 10% formalin and were embedded in paraffin wax. Serial sections (5 micrometer thickness) of paraffin embedded tissues were cut. The tissues were stained by haematoxylin and eosin, which were examined by light microscope Ulceration, necrosis and epithelisation were evaluated in the skin tissues. Also congestion, edema, polymorphonuclear leukocytes (PNL), mononuclear cells, fibroblasts and vascularisation were qualitatively evaluated by grading as (-), (+), (++), (+++).

Table 4: Histopathological evaluation of wound healing processes of vehicle, test extract and cipladine.

Groups	ED	N	NV	EP	U	PMN	FP
Vehicle	+	-	+	-	+	+	+
Test Extract	+	-	++	+	-	-	++
Cipladine	+	-	++	+	-	-	++

Hematoxylin and Eosine stained sections were scored as mild (+), moderate (++) and severe (+++) for epidermal and/or dermal remodeling. ED: Edema, PMN: polymorphonuclear cells, N: Necrosis, NV: Neovascularization, EP: Epitellization, U: Ulceration, FP: Fibroblast Proliferation.

Statistical Analysis of the data

The data on percentage wound healing was statistically analyzed using one-way analysis of variance (ANOVA). The values of $p \leq 0.001$ were considered statistically significant. Mann-Whitney-U, Kruskal Wallis and chi-square tests were used for the statistical analysis of the histopathological data.

Results and Discussion

The measurements of the progression of wound healing induced by the extracts, reference Drug and vehicle groups in the excision wound model are shown in Table 2. The Test drug extract treated groups of animals showed 64.11 % contraction on the wounds on the day six. On the twelfth day the extract demonstrated 94.23 % contractions, which were closed to contraction value of the reference drug Cepladine (100).The results of the measurements of tensile strength are shown in Table 1. Tensile strength of the animals treated with test extract showed the 49.6 % at day 10. Topical application of test extracts on the incision wound model demonstrated a significant improvement in wound tensile strength as compared to vehicle groups. On the histopathological examination of the wound sections, the main activity observed was the proliferation of fibroblasts, rather than their migration. In excision wound model, when compared to the control groups re-epitelialization capacity of test extract was the

highest with a value of 100%. The test drug demonstrated a similar highly efficient wound healing activity throughout the experiment displaying a significantly more reduction in wound area as compared to not only the normal control but also the standard treated wounds. Hence, we can infer that the test extract not only is actively promoting faster wound contraction, but is also acting as a potent agent in aiding the process of tissue granulation and remodeling in the first and second weeks of the healing process. The significant healing outcome can also be visualized in the histopzthological sections.

The test extract were found to possess the best wound healing activity. However, which phytochemical(s) of the extract are responsible for this effect, was not investigated in detail yet. Phytochemical studies are in progress, where the extract will be subjected to further fractionation and purification for the identification/isolation of the compound(s) responsible for activity.

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