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Wound healing activity of *Achyranthes aspera* (Linn.) in experimental rats

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Abstract

***Achyranthes aspera* Linn. which is commonly known as chirchita is a herb used in indigenous system of medicine in India. Looking to the wide application of this plant, it was proposed to investigate the anti-inflammatory activity leading to the wound healing and repairing of the damaged tissue activity in the plant extract. The aerial part of *Achyranthes aspera* collected from Hathidhal or Udaipura tehsil in Raisen district of M.P. during flowering season was extracted in Soxhlet apparatus with 90% alcohol. Healthy wistar rats (20) weighing between 150-200 gm were used for experimental work.. The study revealed that when maximum dose 8 mg/mm² of *Achyranthes aspera* suspension was applied to wound model, a significant increase was observed in the skin tensile strength for 15 days.**

Keywords: *Achyranthes aspera Linn., Wound healing, Rats*

Introduction

Naturally occurring biologically active compounds are wide spread in plants. *Achyranthes aspera* Linn. which is commonly known as chirchita is a herb used in indigenous system of medicine in India for the cure of heart diseases, asthma (Saxena, 2003) as well as antimicrobial agent (Sharma *et al.* 2006). Looking to the wide application of this plant it was proposed to investigate the anti-inflammatory activity leading to the wound healing and repairing of the damaged tissue activity in the plant extract.

Materials and Method

Plant material

The aerial part of *Achyranthes aspera* was collected and extracted in Soxhlet apparatus with 90% alcohol from Hathidhal or Udaipura tehsil in Raisen district of M.P. in India during flowering season. The crude was concentrated in reduce pressure and low temperature that gave 30 gm gummy material which was further made to a suspension with gum accacia. This suspension was used in wound healing of Albino rat and the crude extract was taken for detailed phytochemical analysis using chromatographic and spectral technique.

Animal material

Healthy wistar rats (20) weighing between 150-200 gm were used for experimental work. Before wounding animals were housed in wire topped cages with husk bedding. During the experimental period the animals were housed individually and resuscitation was done with Ringer locke (0.1 ml/100mg) daily.

Wounding Procedure

Rats were anaesthetised with pentobarbitone (30 mg/kg) and the hair on the back was clipped with electric clippers. Burn wounds were created by pouring hot molten wax at 80 °C into metal cylinder placed on the back of the rat.

The metal cylinder was having 300 mm area of circular openings and capacity to hold 4.6 gm of wax. On solidification of wax (8min.) the metal cylinder with wax adhered to skin was removed which left distinctly demarked circular wounds of 300 mm^2, after this each animal was placed in a separate cage for full recovery from anaesthesia before being returned to holding rooms. No local or systemic chemotherapeutic agents were given. Animals showing signs of infection were excludcd from the study. Actual amount of heat by molten wax to create burn wound was collected by the following formula.

$$\frac{\Delta H}{\Delta A} = \frac{MS\,(T_2 - T_1)}{\text{Area of skin exposed to molten wax (300 mm}^2\text{)}}$$

$\Delta H/\Delta A$ = Amount of heat delivered by molten wax to mm^2 of exposed skin
M = Mass of molten
T_1 = Initial temperature
T_2 = Room temperature
S = Specific heat

The following parameters were studied:

(a) Epithelization period

It was monitored by noting the number of days required for eschar to fall away and leaving no raw wound behind.

(b) Wound contraction

To monitor this progressive changes, wound area were followed planimetrically, leaving the wounding day, wounds were traced, on a transparent paper on alternate days the animal was restrained in proper position during tracing, They were then transferred to 1 mm^2 graph sheet from this wound area and the percent of wound contraction was calculated taking the initial size of the wound (300mm.^{2}) as 100%.

Results and Discussion

The results of the study are shown in Table 1-3. Ethenolic extract and its suspension was used for the healing of wound. It was observed that the breaking strength was much significant than that of the control animal. Therefore it shows that the different concentration of *Achyranthes aspera* may help in wound healing.Chemisty of the biologically active compound in purified fraction of *Achyranthes aspera* showed the presence of Saponin like compound which caused epithelization of the damage tissue.

Table-1: Percentage yield of *Achyranthes aspera* by Soxhlet extraction at 40 °C

Solvent used	Wt. Of powder (gm)	Wt. Of extract (gm)	Yield of crude drug %
n-Hexane	260	5.62	2.620
Chloroform	700	6.42	3.210
Ethyl acetate	200	2.85	1.425
70% Methanol	200	5.30	2.650
Water	200	15.43	7.660

*Both the solutions gave polar compound

Table-2: TLC of crude drug of *Achyranthes aspera*

Solvent system	Fraction	Behavior			RF value of each spot
		Spot No.	Visible	UV light	
$CHCl_3$:MeOH:H_2O (2:2:1)	A1	Spot 1	Dark green	Dark green	0.31
$CHCl_3$:CCl_4:H_2O (2:2:1)	A2	Spot1 Spot 2 Spot 3	Light green Light yellow Dark green	Yellow Brown green	0.42
$CHCl_3$:MeOH:H_2O	A3	Spot 1 Spot 2	Light brown Yellow brown	Dark brown brown	0.56

Table-3: MIC value of the different fraction of Saponin of *Achyranthes aspera* against *Staphylococcus aureus.*

Saponin Fraction	Microorganism minimum inhibitory concentration
Fraction 1	0.63
Fraction 2	0.31
Fraction 3	0.15

The study revealed that when maximum dose 8 mg/mm^2 of *Achyranthes aspera* suspension was applied to wound model, a significant increase was observed in the skin tensile strength for 15 days. Diffcrent dose's of the plant extract was applied and showed good results. The drug treated animal of dead space wound model showed significant increase in dry granulosa weight. Granulosa breaking strength was found to be improved. The collegen fibres were found to be deposited in the drug treated animals more fastly as compared to the control, Varshney *et al.* (1997) have reported the wound healing activity in Bovine saliva. Similarly, Shriwaikar *et al.* (2003) have also reported the wound haeling property of ethenolic extract of leaves of Hyptis sceaveloerrs with supportive role of antioxidant enzymes.

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