Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Wound healing activity of ethanolic extract of leaves of *Eclipta alba*

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ABSTRACT

Ethanolic extract of leaves *Eclipta alba* was evaluated for its wound healing activity in ether anaesthetized Wistar rats at two different doses (150 and 300 mg/kg) using incision, excision, and dead space wound model. Significant increase in skin breaking strength, granuloma breaking strength, wound contraction, hydroxyl proline content and dry granuloma weight and decreased in epithelization period was observed. A supportive study made on granuloma tissue to estimate the levels of catalase and superoxide-dismutase recorded a significant increase in the level of these antioxidant enzymes. Granuloma tissue was subjected to histopathological examination to determine the pattern of lay-down for collagen using Van Gieson and Masson Trichome strains. Enhanced wound healing activity may be due to free radical scavenging action of the plant and the enhanced level of antioxidant enzymes in granuloma tissue. Better collagenation may be because of improved antioxidant studies.

KEY WORDS:*Eclipta alba,* Ethanolic extract, wound healing activity, free radical scavenging activity.

INTRODUCTION

Eclipta alba (Linn.) Hassk, family asteraceae, grows as a common weed throughout India, ascending up to 6,000 ft. on the hills. An erect or prostrate much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate; the flower-heads are white. The herb contains wedelolactone and dimethyl wedelolactone possessing potent antihepatotoxic properties. The herb is a rich source of ascorbic acid. It also contains an alkaloid, ecliptine. The plant is a good source of thiophene derivatives, which are active against nematodes. *Eclipta alba* antifungal and antimicrobial activity has already been established (1-5).

MATERIALS AND METHODS

Plant material

Leaves of *Eclipta alba* were collected from medicinal garden of Dravyaguna department of D.G.M. Ayurvedic Medical College & Hospital, Gadag, Karnataka during the flowering stage and its herbarium was deposited. Plant was authenticated by Mr. Suresh H.J. Botanist of Alva's Ayurvedic Medical college, Moodbidri.

Phytochemical screening

Preliminary phytochemical screening (6) was done to study the presence of steroids, triterpenoids, essential oil, flavonoids, tannins, carbohydrates, and amino acids in the leaves of the plant.

Preparation of ethanol extract

The shade dried powdered leaves (600g) were exhaustively extracted with 7.5 litre of ethanol (95%) using a soxhlet apparatus and concentrated in vacuo (yield 200g).

Animals

Healthy Wistar albino rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard chow diet (Pranav Agro Ind. Limited, Sangli, Maharashtra) and water *ad libitem*. They were housed in polypropylene cages maintained under standard conditions (12/12 hr light/dark cycle; 25^0C $\pm$ 3^0 C, 35-60% RH). The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (Reg. no 918/ac/05/CPCSEA).

Acute toxicity studies

Healthy adult albino rats of either sex, starved overnight, were divided into 11 groups (n=6) and were orally fed with increased dose of 100 mg/kg - 2000mg/kg of ethanol extract. Total ethanol extract administered orally in doses of up to 2g/Kg, did not produce any sign of toxicity and mortality in rats when observed for 14 days after administration.

Selection of Dose

The studies were carried out using ether-anaesthetized rats in three different wound models at two different doses (150mg/kg and 300 mg/Kg body wt) of ethanolic

extract of leaves of *Eclipta alba*. by using topical route. *Eclipta alba* ethanol extract 5 % ointment as low dose and 10% as high dose was applied topically in excision, incision and dead space and wound model. The treatment period was considered 10 days for incision and dead space wound model and the treatment period was considered till scar falling of wound in case of excision model.

Wound models

Wound healing activity was studied using three models viz. incision wound model, excision wound model and dead space wound model.

Incision wound

Wister male albino rat weighing between 150-250 gm body weigh were divided into three groups, each group consisting of 6 rats and each animal kept separately under laboratory condition. They had free access to commercial pellet diet and *ad libitum*. Group I: Control group: animal of this group received no treatment.

Group II: Animal of this group given 150 mg/kg of ethanolic extract of *Eclipta alba*.

Group III: Animal of this group given 300 mg/kg of ethanolic extract of *Eclipta alba*.

Two paravertebral incisions (6 cm long) were made through the full thickness of the skin on either side of the vertebral column of the rat (7). Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the 7^{th} day. Wound breaking strength was measured on 10^{th} post wounding day (8).

Excision Wounds

Wister male albino rat weighing between 150-250 gm body weigh were divided into three groups, as followed in Incision wound model. A circular piece of full thickness (approximately 500mm^2) was cut off from a predetermined area on the back of the rat (9). Wounds were traced on 1mm^2 graph paper on the day of wounding and subsequently on alternate days until healing were complete. Changes in wound area were calculated giving an indication of the rate of wound contraction. Number of days required for falling of the escher without any residual raw wound gave the period of epithelization.

Dead Space and Wound

Wister male albino rat weighing between 150-250 gm body weigh were divided into three groups, as followed in Incision wound model. These wound were created by implanting two polypropylene tubes (2.5 cm), one on either side, in the lumbar region on the dorsal surface of each rat. On the 10^{th} post-wounding day, granuloma tissue formed on implanted tubes was dissected out carefully. Granuloma tissue from one tube was kept (at-64^{0}C) for estimation of antioxidant enzyme levels. The other tube was used for determination of tensile strength after which it was dried in an oven at 60 ^{0}C for 24 hrs and noted dry weight. Acid hydrolysate of dry tissue was used for estimation of hydroxyproline content in the tissue (10).

Biochemical attributes

Granuloma tissue from dead space model was homogenized in phosphate buffer saline (pH 7.0) and centrifuged under cold condition. The clear supernatant was spectrophotometrically estimated to determine the level of antioxidant enzymes, viz. superoxide dismutase and catalase (11).

Histopathology

A section of granuloma tissue was subjected to histopathological examination so as to determine the pattern of lay-down for collagen using two special strains i.e. Van Gieson and Masson Trichome (12).

Statistical analysis

Results were subjected to one-way ANOVA with post hoc Scheffe's test.

RESULTS AND DISCUSSION

Acute toxicity studies showed that drug was found to be safe up to maximum dose of 2g/Kg body weight of the animal. In incision wound model, significant increase was observed in the skin tensile strength of ethanol extract treated group on 10^{th} post wounding day at both the doses (Table 1). The drug treated animals of dead space wound model showed significant increase in dry granuloma weight, granuloma breaking strength and in the level of hydroxyproline content (Table 1) at both the dose levels. Histopathological study revealed increase in collagen deposition in the drug treated group (Figs 1,2) compared to control (Figs 3,4).

Studies on antioxidant enzymes revealed that the extract treated animals showed significant increase in the level of superoxide dismutase and catalase, the two powerful antioxidant enzymes of the body that are known to quench superoxide radicals (Table 2).

In studies using excision wound model, animals treated with ethanol extract of *Eclipta alba* showed a significant decrease in epithelization period as evidenced by shorter period for fall of escher as compared to control. The drug extract also facilitated the rate of wound contraction significantly at both the dose levels (Table 3).

Granulation, collagenation, collagen maturation and scar maturation are some of many phases of wound

Table 1: Effect of ethanolic extract of Eclipta alba on wound healing in incision and dead space wound models

Treatment	Incision breaking strength	Dead Space		
		Dry granuloma wt. (g/100g)	Breaking strength (g)	Hydroxyproline µg/100g
Control	221.50±9.09	16.30±1.10	156.03±35.4	975.49±175.38
Treated				
150 mg/Kg	412.05±18.25 [a]	27.01±2.79 [a]	387.54±8.14 [a]	1691.33±143.98
300 mg/Kg	378.25±14.90 [a]	48.25±2.04 [a]	357.34±42.93 [a]	1410.54±73.89 [a]

[a] significant at P<0.05 vs Control ; Values are mean ± SE of 6 replicates.

Table 2: Effect of ethanolic extract of Eclipta alba on the level of antioxidant enzymes in dead space wound model

Enzyme	Superoxide dismutase (IU/mg)	Catalase (k/sec/mg)
Control	0.1172±0.0119	$2.5 \times 10^{-2} \pm 2.8 \times 10^{3}$
Treated		
150 mg/Kg	0.2543±0.039	$5.01 \times 10^{-2} \pm 2.75 \times 10^{3}$ [a]
300 mg/Kg	0.171±0.018 [a]	$6.9 \times 10^{-2} \pm 5.99 \times 10^{3}$ [a]

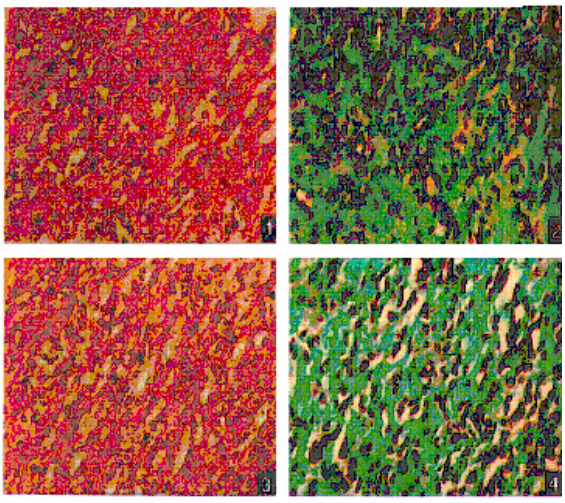
[a] significant at P<0.05 vs Control ; Values are mean ± SE of 6 replicates.

Table 3. Effect of ethanolic extract of Eclipta alba on excision wound model

Treatment	Epithelization period (Days)	Excision Wound Model (% of wound contraction by day)										
		2	4	6	8	10	12	14	16	18	20	22
Control	22.33 ±0.42	15.76 ±1.58	33.36 ±1.32	40.38 ±1.34	47.60 ±1.2	79.7 ±2.37	87.65 ±0.84	90.48 ±0.54	94.63 ±074	95.39 ±0.65	98.2 ±0.47	99.26 ±0.03
Treated												
150 mg/Kg	13.33 ±0.33	19.60 ±10.0	38.50 ±9.79	55.81 ±9.94	89.73 [a] ±10.3	95.12 [a] ±2.36	98.51 ±1.25	99.06 ±2.34				
300 mg/Kg	14.00 ±0.68	32.35[a] ±9.94	49.58 [a] ±12.4	64.14 [a] ±10.0	84.92 [a] ±6.60	95.72 [a] ±1.57	99.32 ±.0.52					

[a] significant at P<0.05 vs Control ; Values are mean ± SE of 6 replicates.

Fig: 1-4 Comparative Histopathological study of control group and treated group



Figs 1 and 2 shows increase in collagen deposition in the ethanolic extract treated group in comparison of Figs 3 and 4 of control group

healing which run concurrently, but independent of each other. Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing as drugs which, influence one phase may not necessarily influence another. Hence in our study we have used three models to assess the effect of leaf extract on various phases of wound healing.

The result of present study showed that ethanolic extract of *Eclipta alba* possesses a definite pro-healing action. This is demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelization. Significant increase was also observed in skin breaking strength and hydroxyproline content which was a reflection of increased collagen levels that was further supported by histopathological evidence and gain in granuloma breaking strength. This indicated improved collagen maturation by increased cross-linking while an increase in dry granuloma weight indicated higher protein content. An increase in the levels of antioxidant enzymes (superoxide dismutase and catalase) was observed in granuloma tissue of dead space wound model. These enzymes are known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals (13, 14).

Phytochemical screening revealed the presence of tannins and flavonoids. Flavonoids have been documented (15) to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing. Thus, the enhanced wound healing may be due to free radical scavenging action of the plant, and enhanced level of antioxidant enzymes in granuloma tissue. Better collagenation seen under the influence of this plant extract may because of improved antioxidant status.

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