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Research Article

INVESTIGATION OF WOUND HEALING ACTIVITY OF ETHYL ACETATE EXTRACT OF FLOWERS OF *CALOTROPIS GIGANTEAN*

SM. Patil* and R. Saini

Department of Pharmacy, Shree Dev Bhoomi Institute of Education, Science and Technology, Dehradun, Uttarakhand, India.

ABSTRACT

Calotropis gigantea is used in herbal medicine for the treatment of skin itches, as antiseptic for wound, expectorant, asthma, anti viral, antimicrobial and useful in leprosy scabies ring worms of the scalp activity. The objective of our study is to investigate the effect of wound healing activity of ethyl acetate extract of flowers of *Calotropis gigantea* in rats. The animals were treated with the ethyl acetate extracts of *Calotropis gigantea* (400mg/kg/day and 800mg/kg/day) topically. Healing was assessed by the rate of wound contraction, period of epithelialization. The plant showed a definite, positive effect on wound healing in rats.

Key words: *Calotropis gigantea*, wound healing, leprosy, antimicrobial, epithelialization.

INTRODUCTION

Wounds generally termed as physical injuries that result in an opening or breaking of the skin. There are different types of wounds which range from mild to potentially fatal. Wound healing is the body's natural process of regenerating dermal and epidermal tissues. The healing cascade is activated when platelets come into contact with exposed collagen leading to platelet aggregation and the release of clotting factors resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing[1]. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collegenation and tissue remodeling[2].

The manufacture and clinical evaluation of herbal remedies and/or their isolates have made it increasingly feasible to transform traditional medicine into a modern industrial enterprise capable of making significant contribution to both healthcare deliveries and economic growth of developing countries[3]. Today, traditional medical practice has been recognized by the World health Organization (WHO) as a building block of primary healthcare[4]. But it emphasizes the fact that safety should be the overriding criterion in the selection of herbal remedies for use in healthcare[5]. There is no longer any doubt regarding the value and potential of traditional remedies[3].

Calotropis gigantea Linn. (Asclepiadaceae) is a glabrous or hoary, laticiferous shrubs or small trees, commonly known as THE SWALLOW-WORT or MILKWEED[6]. The flowers of the plant contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin and uscharidin; gigantin. The flower also contains the protease calotropin DI and DII and calotropin FI and FII[7]. The flowers contains some poisonous constitute due to which it has somewhat caustic effect on the mucous membrane and tender skin, and may secondary dermatitis[8]. The aim of this present study is to evaluate the wound healing activity of ethyl acetate extracts the

flowers of the *Calotropis gigantea* Linn by means of in vivo circular excision and linear incision wound healing models.

MATERIALS AND METHODS

Plant materials

The flowers of *Calotropis gigantea* Linn (Figure 1) used in this study were obtained from the local area of Uttarakhand and were identified based on its physical characteristics. The flowers were dried and crushed to small pieces using pestle and mortar and powered in an electric grinder.

Phytochemical Screening

The powder of the flowers of *Calotropis gigantea* Linn was subjected to successive extraction with different solvents in increasing order of polarity of solvents[9]. The dry extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents[10].

Preparation of Ethyl acetate Extracts

The shade dried flowers were crushed into small pieces and powdered. The powder was loaded into soxhlet extractor and was subjected to extraction for about 25–30 h with ethyl acetate. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency. Then it was dried in the dessicator. The yield was about 6%.

Acute Oral Toxicity Study[11]

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), registered under (CPCSEA), ministry of Social Justice and Empowerment, Government of India. Healthy adult albino rats of either sex, fasted overnight, were divided into 6 groups (n=6 per cage) and were fed with increasing dose (1, 2, 4 and 8g/kg body wt.) of ethyl acetate extract. The total ethyl acetate extract, administered orally in doses of up to 8g/kg body wt., did not produce any sign of toxicity or mortality in rats up to 14 days after administration.

Animal selection

The acute toxicity study was carried out using healthy Wistar albino rats of either sex weighing between 150 and 200 g. While for wound healing activity, healthy male Wistar albino rats, weighing between 150 and 200 g were selected so as to avoid possible interference of female sex hormones with the process of wound healing.

Wound models

The studies were carried out using ether-anesthetized rats and their back was shaved, in two different wound models, at two different dose levels of 400 and 800 mg/kg body wt.

Incision wounds

Two, 6-cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat[12]. Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding[13].

Excision wounds

A circular skin piece of full thickness (approximately 500 mm^2) was removed from a predetermined dorsal area[14]. The wounds were traced on 1- mm^2 graph paper on the day of wounding and subsequently on alternate days until healing were complete. The entire wound was left open. The treatment was done topically in all the cases. The extract was applied at a dose of 400mg/kg/day and 800mg/kg/day. Changes in the wound area were calculated, giving an indication of the rate of wound contraction.

Statistical Analysis

Results, expressed as mean ± SE, were evaluated using one-way ANOVA with-*post hoc* Scheffe's *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In the present study, wound healing effects of the ethyl acetate extract of the flowers of the *Calotropis gigantea* Linn were

evaluated. Wound healing involves various phases which include granulation, collagenation, collagen, maturation and scar maturation. Preliminary phytochemical screening revealed the presence of cardiac glycosides, calotopin, uscharin, calotoxin, calactin and gigantin. The acute toxicity studies show that the drug was safe up to a maximum dose of 800g/kg body wt. of the animal.

In the incision wound model, a significant increase was observed in the skin tensile strength of the ethyl acetate extract-treated group on the tenth post-wounding day, at both dose levels shown in **Table 1**. In studies using the excision wound model, animals treated with the ethyl acetate extract of *Calotropis gigantea* Linn showed a significant decrease in the epithelization period for the eschar compared to control. The extract also facilitated the rate of wound contraction significantly at both dose levels (**Table 2**).

CONCLUSION

Thus, the plant extract might be useful as a wound healing agent. The potent wound healing capacity of the extract of *Calotropis gigantea* Linn as shown from the wound contraction and increased tensile strength.

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Fig. 1: Photograph of flower of *Calotropis gigantea* Linn

Table 1: Effect of ethyl acetate extract of *Calotropis gigantea* Linn on wound healing in the incision wound model

Wound Parameter studied	Incision model Breaking Strength (g)
Control	216.40±8.05
Ethyl acetate extract of *Calotropis gigantea* 400mg/kg body wt.	374.21±12.25 [a]
Ethyl acetate extract of *Calotropis gigantea* 800mg/kg body wt.	344.21±18.34 [a]

[a] *P < 0.05 compared to Control. Values are mean ± S.E.M. (n = 6)*

Table 2: Effect of ethyl acetate extract of *Calotropis gigantea* Linn on wound healing in the excision wound model

Wound model	Excision % of Wound contraction							
Parameters studied	Epithelization period (days)	2days	4days	6days	8days	10days	12days	14days
Control	22.40±0.25	18.76 ±1.4	38.30 ±0.8	49.23 ±0.35	54.46 ±0.7	72.40 ±2.5	84.18 ±0.25	90.38 ±0.65
Ethyl acetate extract 400mg/kg body wt.	15.46±1.64[a]	24.34 ±1.36[a]	43.34 ±1.65[b]	45.34 ±0.65[a]	58.66 ±1.45[a]	79.40 ±0.78[a]	88.76 ±0.34[a]	96.05 ±0.85[a]
Ethyl acetate extract 400mg/kg body wt.	18.34±0.84[a]	25.37 ±1.41[a]	34.38 ±0.75[b]	55.34 ±1.24[a]	71.60 ±1.12[a]	90.47 ±0.82[a]	96.36 ±0.74[a]	98.86 ±0.42[a]

[a] P < 0.05 compared to control.[b]P < 0.05 compared to 800mg. Values are mean ± S.E.M. (n = 6).

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