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EVALUATION OF WOUND HEALING POTENTIAL OF AERIAL PARTS OF *CARDIOSPERMUM HALICACABUM* LINN

P. Udaya Chandrika*[,1], A. Srinivas Rao[2], M. Sri Rama Chandra[2], NVBLA Baby kambampati[2]

[1]Department of Pharmacognosy and [2]Department of pharmacology, Bhaskar pharmacy college, Yenkapally village, Moinabad, Rangareddy-501504, India

***Corresponding author e-mail:** chandrika.uday.18@gmail.com

ABSTRACT

Cardiospermum halicacabum (Linn) popularly called as balloon wine belongs to Sapindaceae family. Phytochemical constituents such as flavones, apigenin, triterpenoidal glycosides fatty acids and volatile ester have been reported in *C. halicacabum.* In the present study the aerial parts of *C. halicacabum* were studied for wound healing activity by incorporating extract in simple ointment base B.P. in concentration of 2% (w/w) and 4% (w/w) and studied in three types of model in rats viz. excision, incision and burn wound model. The statistical data indicated that the wound with ointment containing 4% w/w alcoholic extract exhibited significant ($P < 0.001$) wound contracting ability and period of epithelization. Significant tensile strength was observed with both the ointment formulations 2% w/w and 4% w/w. The experimental data demonstrated that *C. halicacabum* displayed remarkable wound healing activity.

Keywords: *C. halicacabum*; Alcoholic extract; Excision wound model; Incision wound model; Burn wound model; Nitrofurazone

INTRODUCTION

A wound may be defined as a "Disruption of normal tissue structure and function" and can be categorized by its etiology, location, or duration. Wound healing involves a chain of well orchestrated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling.

India has a rich tradition of plant-based knowledge on healthcare. Many of the synthetic drugs are associated with problems like allergy, drug resistance and so on making the scientists to seek alternative drug. A large number of plants/plant extracts/decoctions or pastes are equally used by tribal and folklore traditions in India for treatment of cuts, wounds, and burns. [1]

Cardiospermum halicacabum (Linn) belongs to Sapindaceae family. It is popularly known as Balloon Vine. The whole plant contains saponins, traces of alkaloids, flavonoids, apigenin and phytosterols. Ethanol root extract of *C. halicacabum* was reported to contain active principle cardiospermine for its anti-anxiety activity. [2] Phytochemical constituents such as flavones, triterpenoidal glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of *C. Halicacabum.* [3]

The plant *Cardiospermum halicacabum* was traditionally used as anxiolytic and as anticonvulsant. The whole plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific. [4] It is used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite. [5] The leaves are rubefacient and used in the treatment of rheumatism. [6]

A tea made from them is used in the treatment of itchy skin. [7] Salted leaves are used as a poultice on swellings. The leaf juice has been used for the treatment of earache. The root is diaphoretic, diuretic, and laxative. The plant was reported to possess

antiulcer, [8] Antiparasitic, [9] Antimalarial, [10] Antifilarial, [11] Antipyretic, [12] and Anxiolytic activity. [13] Experimental pharmacological studies have shown Analgesic, Anti-inflammatory, Antidiarrhoeal and Vasodepressant activities of this plant. [14]

A survey of literature revealed that no scientific study on the wound healing activity of aerial parts of the plant. So the objective of the present study was to evaluate the effect of alcoholic extract of aerial parts *C.halicacabum* on different parameters related to wound healing activity on albino rats.

METHODOLOGY

Collection of plant material: The aerial parts of *Cardiospermum halicacabum* were collected from Tirupathi, India. The plant material was dried, powdered and stored in air tight containers for further studies.

Preparation of plant extract: The air-dried crude drug was pulverized to obtain coarse powder. The powdered drug was extracted with methanol in a soxhlet extractor. The extract thus obtained was concentrated by recovering the solvent by Rotary Flash Evaporator. The concentrated extract was then evaporated to dryness in vacuum oven at temperature not more than 50ºC. The dried extract was stored at 2–8ºC in refrigerator and kept in tightly stoppered bottle under refrigeration.

Animals: Healthy Wistar Rats between 2-3 months of age and weighing 180-200g were used for the study.

Group 1: Simple ointment treated control group

Group 2: Animals treated with Standard (Nitrofurazone 0.2% w/w)

Group 3: Animals treated with ACHLD 2% w/w (2g extract in 100g simple ointment) (Alcoholic extract ointment of low dose 2% w/w)

Group 4: Animals treated with ACHHD 4% w/w (4g extract in 100g simple ointment) Alcoholic extract ointment of *Cardiospermum* high dose 4% w/w

Acute dermal toxicity studies: This study was carried out on rabbits and rats. The skin of the animal was shaved at three different positions on the dorsal side, each about 500 mm^2. The 1st area was kept as control, to which vehicle was applied. 2nd area was applied with ACHLD 2% w/w and the 3rd area treated with ACHHD 4% w/w. After 4 hr, the skin was observed for signs of inflammation. [15]

Selection of dose and treatment period: Two types of ointment formulations with different concentration of the extract were prepared viz. 2% (w/w) ointment, where 2 g of extract was incorporated in 100 g of simple ointment base (Anonymous, 1953); 4% (w/w) ointment where, 4g of extracts of the aerial parts were incorporated in 100g of simple ointment base B.P. Nitrofurazone ointment (0.2% w/w) obtained from Smith Kline– Beecham Pharmaceuticals Bangalore, India, was used as standard drug for comparing the wound healing potential of the extract in different animal model.

Excision wound model: The rats were depilated on the back and a predetermined area of 500 mm^2 full thickness skin was excised in the dorsal inter scapular region. The drugs were topically applied daily till the complete epithelization starting from the day of operation. The parameters studied were wound closure and time of epithelization. The wounds were traced on mm^2 graph paper on the days of 4th, 8^{th}, 12^{th} and 16^{th}. The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelization time that indicates the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelization. [16, 17]

Wound closure % = Wound area on day 0 - Wound area on day n/ Wound area on day 0 x 100 where *n* =number of days 4^{th}, 8^{th}, 12^{th} and 16^{th}day.

Incision wound model: The rats were anesthetized by administering ketamine (0.5 ml/kg b. w. i.p.). Incision wounds of about 6 cm in length and 2mm in depth were made with sterile scalpel on the shaved back of the rats. Four groups with six animals in each group were anaesthetised and two paravertebral-long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. All the groups were treated in the same manner as mentioned in the case of the excision wound model. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5-cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. Sample drugs along with simple ointment (control) and standard drug were administered once daily for 9 days; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured with a tensiometer. [18, 19]

Tensiometer: The tensiometer consists of a 6 x 12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board. The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp with 1cm width was tied on the tip of the other arm by a fishing line in such a way that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with a polyethylene bottle on the other end. The tensile strength of a wound represents the degree of
wound healing. Usually wound healing agents promote again in tensile strength. The instrument used for measurement is called a tensiometer, as explained above. One day before performing the experiment (measurement of tensile strength) the sutures were removed from the stitched wound.

Determination of tensile strength: Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue.
Sutures were removed on the day 9 after wound creation and the tensile strength was measured. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer. [20] The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound. The mean determination of tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of the extract-treated wounds were compared with controls.

Burn wound model: Partial thickness burn wounds were inflicted on overnight starved animals under pentobarbitone (30mg/kg b. w. i.p.) anesthesia by pouring hot molten wax at 80°C. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^2 circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drugs or vehicle was applied topically as mentioned above. [21]

Statistical analysis: The values are represented as mean ± S.E.M for six rats. Unpaired *t*-test was used for reporting the P-value and significance with respect to the control group.

RESULTS AND DISCUSSION

The results of wound healing activity by excision wound model were presented in Table 1. It was observed that the wound contracting ability of the extract ointment in both concentrations were significantly greater than that of the control (i.e. simple ointment treated group). The wound contracting ability of animals treated with ointment containing 4% (w/w) alcoholic extract was found to be highly significant ($P < 0.001$) on day 16 (93.33 ± 0.4258) as compared to the control group. Treatment with ACHHD produced significant ($P<0.001$) reduction in the period of epithelization (20.80 ± 0.563).
The results are presented as mean weight in gram ± SEM, the measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 2. The tensile strength of the extract ointment (ACHLD 2% w/w, ACHHD 4% w/w) treated groups showed maximum significant $P<0.001$ breaking strength (515.8 ± 6.554, 542.9 ± 6.756) and the nitrofurazone ointment (0.2% w/w) treated group (584.4 ± 32.675) showed significant higher breaking strength, $P<0.001$ compared with control group. Thus both the concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.
The results of wound healing activity by burn wound model are presented in Table 3. There was a significant increase in percentage contractibility from day 4 onwards in ACHHD treated rats and also on later days the closure rate is much faster when compared with control rats. The wound contracting ability of animals treated with ointment containing (4% w/w) alcoholic extract was found to be significantly higher ($P < 0.001$) on 16th day (77.75 ± 1.808) when compared to the control group. A better healing pattern and reduction in period of epithelization was observed in ACHHD 4% w/w treated group showed highly significant ($P<0.001$) activity (24.10 ± 1.260).

CONCLUSION

According to the results reported here, the alcoholic extract of *Cardiospermum halicacabum* was found to have dose dependant wound healing activity in the experimental models compared to control. Flavonoids of this plant might have contributed to the wound healing process, along with other phytochemical contents of the aerial parts. However, it needs further evaluation in clinical settings before consideration for the treatment of wounds.

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Table 1: Effect of Alcoholic extracts of *Cardiospermum halicacabum* on wound contraction of excision wound

Groups	Treatment	Dose	Day 4	Day 8	Day 12	Day 16	Period of epithelization
Group 1	Control (simple ointment)	-	2.783 ± 0.0298	18.55 ± 0.03688	42.33 ± 0.4944	64.77 ± 0.3607	24.60 ± 0.1449
Group 2	Standard (Nitrofurazone)	0.2% w/w	13.00 ± 1.335***	33.66 ± 2.119***	62.17 ± 0.443***	95.00 ± 0.4221***	17.90 ± 0.446***
Group 3	ACHLD	2% w/w	7.800 ± 2.173**	25.12 ± 4.313	52.83 ± 1.754**	82.00 ± 2.506**	21.83 ± 0.6033**
Group 4	ACHHD	4% w/w	7.8 ± 0.2451**	23.08 ± 0.6614**	65.47 ± 0.6507**	93.33 ± 0.4258***	20.80 ± 0.563***

n = 6 animals in each group. The treated groups are compared by Student t-test with the control group.*** $P < 0.001$,** $P < 0.01$, * $P < 0.05$.

Table:2 Effect of *Cardiospermum halicacabum* extract and standard drug on incision wound model in rats

Groups	Treatment	Dose	Tensile strength(g) Mean weight in gram ±S.E.M
Group 1	Control (Simple ointment)	-	335.9 ± 2.694
Group 2	Standard (Nitrofurazone)	0.2% w/w	584.4 ± 2.675***
Group 3	ACHLD	2% w/w	515.8 ± 6.554***
Group 4	ACHHD	4% w/w	542.7 ± 6.756***

n = 6 animals in each group.
The treated groups are compared by Student t-test with the control group.*** $P < 0.001$,** $P < 0.01$, * $P < 0.05$.

Table 3: Effect of Alcoholic bark extracts of *Cardiospermum halicacabum* on wound contraction of burn wound
Mean percentage of wound contraction ±SEM

Groups	Treatment	Dose	4th day	8th day	12th day	16th day	Period of epithelization
Group 1	Control (simple ointment)		21.17 ± 0.6009	34.00 ± 1.975	53.87 ± 2.160	63.67 ± 1.533	28.80 ± 0.765
Group 2	Standard (Nitrofurazone)	0.2% w/w	27.33 ± 2.541	52.83 ± 0.863***	71.33 ± 0.434***	81.45± 0.456***	22.20 ± 0.2400***
Group 3	ACHLD	2% w/w	23.25 ± 3.037	43.17 ± 2.548**	50.78 ± 1.650**	70.67 ± 1.308*	25.60 ± 0.565**
Group 4	ACHHD	4% w/w	27.56 ± 0.956**	32.73 ± 2.321	62.33 ± 0.955**	77.75 ± 1.808***	24.10 ± 1.260***

n = 6 animals in each group. The treated groups are compared by Student t test with the control group. *** $P < 0.001$,** $P < 0.01$, * $P < 0.05$.

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