International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

R E S E A R C H A R T I C L E

Evaluation of Wound Healing Activity of Ethanolic Extract of Areial Parts of Cardiospermum Helicacabum Linn in Albino Rats

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A B S T R A C T

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 5% (w/w) in rats through excision wound model. The statistical data indicated that the wound with ointment containing 5% w/w alcoholic extract exhibited significant ($P < 0.001$) wound contracting ability and period of epithelization. The experimental data demonstrated that C. halicacabum displayed remarkable wound healing activity.

Keywords: C. halicacabum; Alcoholic extract; Excision wound model, wound clousure, tissue regeneration, soframycin.

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PAPER QR-CODE

Article History: Received 21 March 2018, **Accepted** 27 April 2018, **Available Online**15 July 2018

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Citation: Dinesh.A, *et al.* Evaluation of Wound Healing Activity of Ethanolic Extract of Areial Parts of Cardiospermum Helicacabum Linn In Albino Rats. *Int. J. Curnt. Tren. Pharm, Res., Res*., 2018, 6(4): 114-118.

C O N T E N T S

1. Introduction

Traditional medicine, play a significant element in the cultural heritage, still remains the main choice for a large majority of people for treating various diseases and ailments. More than 1000 medicinal plants (89.93%); 58 minerals, metals, or ores (5.25%) and 54 animal and marine products (4.86%) are used to control various forms of diseases like diabetes, cardiovascular disorders, hepatoprotective, antibacterial, antifungal and the wound healing. [1]Wounds are defined as “skin defects caused by mechanical, thermal, electrical and chemical injuries, or by the presence of an underlying medical or physical disorder” by Thomas in1990. [2-10] The wounds are classified into the following three categories:

a) Chronic wounds are those that involve longer healing times ranging from months to years, for example pressure sores and leg ulcers. The chronic wounds fail to heal even though all efforts are undertaken to aid healing and become persistent or reoccur after a period of time. [11, 12] Commonly, it is known that if any wound does not heal within three months, it is considered as a chronic wound.

b) Acute wounds are defined as disruptions in the integrity of the skin and underlying tissues that progress through the healing process in a timely and uneventful manner. [13]

c) Post-operative wounds are intentional acute wounds. [5]

Wounds are the unpreventable events of life. It may be produced by physical, chemical, thermal, microbial or immunological damage to the tissue. [14] It results in the destruction of epithelium and also underlying connective tissue. [15,16] Current estimates indicate that worldwide nearly 6 million people suffer from chronic wounds. [17] Unhealed wounds always produce pain and inflammation at the wound site. Chronic wounds may even lead to multiple organ failure or death of the patient. [18] Wound healing process includes constant interactions between cell–cell and cell–matrix that proceed in to three overlapping phases viz. inflammatory phase(0–3days), cellular proliferation or proliferative phase (3–12 days) and remodeling phase (3–6 months). [19-21]Healing requires the synergistic efforts of various tissues and cell lineages. [22] It involves accumulation of platelets, clotting of blood, fibrin formation and an inflammatory response to injury, modification in the ground substances, angiogenesis and re-epithelialization. Disrupted wound healing places are firmly amalgamated by collagen. [23] The basic principle behind optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition to tissue and moist wound healing environment to restore the anatomical continuity and function of the wound. [24] Wound healing process comprises coagulation, inflammation, proliferation, formation and accumulation of fibrous tissues, collagen deposition, epithelialization, contraction of wound with formation of granulation tissues, remodeling and maturation. [25] The plant Cardiospermum halicacabum linn (sapindaceae) was traditionally used as anxiolytic and as anticonvulsant. The whole plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific. [26] It is used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite. [27] The leaves are rubefacient and used in the treatment of rheumatism. [28]

2. Materials and Methods

Collection of Plant Material:

The whole plant of Cardiospermum *helicacabum linn*.collected from local areas of Tirupati in Chittoor district of AP, India, during the month of January 2017.The plant material was dried, powdered and stored in air tight containers for further studies.

Authentication of Plants:

The whole plant of Cardiospermum *helicacabum linn* (Sapi ndaceae) was authentified by DR.K. Madhavachetty, Asst professor. Department of Botany, SriVenkateswara University, Tirupati.The voucher specimen is available in the herbarium file of Pharmacology Dept, Krishnateja Pharmacy College.

Preparation of Plant Extracts:

About 300 g of powder was subjected to solvent extraction using 70% hydro alcoholic solvent (ethanol and distilled water) in the ratio of 1:4 (drug: solvent) in soxhlet apparatus at a temperature of 50-60^{o}C for 72 hours. The extract thus obtained was dried under reduced pressure and temperature not exceeding 40^{o}C to get the crude extract.

Preliminary Phytochemical Screening (Kokate, 1994):

The ethanolic extract obtained is then subjected to qualitative phytochemical analysis in order to identify the nature of constituents present in the Cardiospermum *helicacabum linn.*

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 (Soframycin1% 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 5% w/w (4g extract in 100g simple ointment) Alcoholic extract ointment of Cardiospermum high dose 5% 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 5% w/w. After 4 hr, the skin was observed for signs of inflammation. [29]

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); 5% (w/w) ointment where, 4g of extracts of the aerial parts were incorporated in 100g of simple ointment base B.P. Soframycin ointment (1% 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 mm2 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 mm2 graph paper on the days of 4th, 8th , 12th and 16th. 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. [30,31] Wound closure % = Wound area on day 0 - Wound area on day n/

Wound area on day 0 x 100 where n =number of days 4th, 8th, 12th, 16th,18thday.

Measurement of wound contraction :

Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper on every alternate days.

Wound contraction was calculated using the following formula

$$\text{Wound contraction}(\%) = [(WD_0 - WD_t) / WD_0] \ 100$$

Where as,

WD_0 = wound diameter on day 0

WD_t = wound diameter on day t

Table 1: Ingredients for the preparation of simple ointment

Ingridents	Quantity (g)
Polysorbate 60	5.0
Whitesoft paraffin	25.0
Cetosteryl alcohol	4.0
Glycerin	12.0
Butylated hydroxyl anisole	0.02
Purified water	q.sto 100

Table 2: Ingredients for the preparation of extract of Cardiospermum helicacabum linn

Ingridents	Quantity (g)
Ethanolic extract of Cardiospe	5.0
Polysorbate 60	5.0
Whitesoft paraffin	25.0
Cetosteryl alcohol	4.0
Glycerin	12.0
Butylated hydroxyl anisole	0.02
Purified water	q.sto 100

Statistical analysis

Results are expressed as mean ± SE. The differences between experimental groups were compared by student t' test (control vs. treatment) and was considered statistically highly significant when $p<0.001$.

3. Results and Discussion

The phytochemical screening of the ethanolic extracts Cardiospermum helicacabum linn revealed the presence of tannins, saponins, steroids, terpenoids, flavonoids and alkaloids. It was reported that the flavonoids and saponins possess significant wound healing activity. [32] Rats of either sex applied with the extracts up to a dose of 2000 mg/kg for fourteen days did not produced any signs of toxicity and mortality. The animals were observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep behavioral and clinical abnormalities, gross lesions, body weight changes and coma from day 1 to 14 days. The animals were found to be physically active and they were consuming food and water in a regular way. The values presented in the table 3 represent wound area (mm2) and percentage of wound contraction at 2, 4, 6,8,10,12,14,16, and 18 days for control (simple ointment), standard ointment (soframycin 1%) and ethanol extract Cardiospermum helicacabum linn. A significant ($p< 0.001$) where the animals treated with standard and ethanol plant extract showed 93% & 92% on 14th day and 98% & 96% on 16th day respectively which is very closer to that of standard soframycin. The complete contraction of wound (100%) was observed on 18th day in group III& II where as group I showed 68% only (Table 3, Fig 2 & Fig 3).

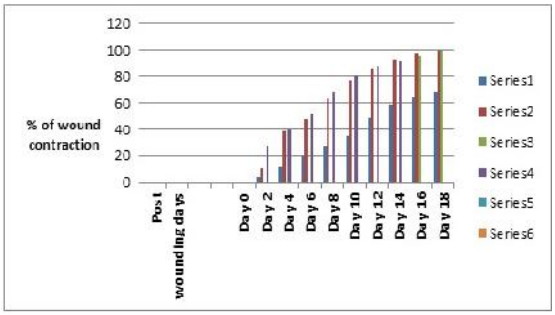

Figure 1(a): Effect of ethnolic extract of Cardiospermum on wound healing process in rats

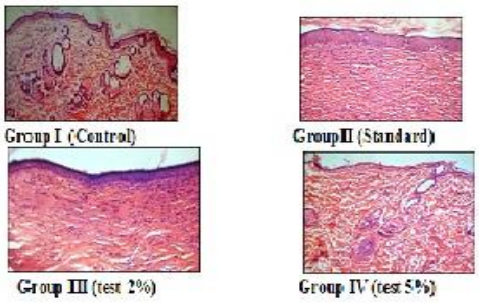

Figure 1(b): Effect of ethnolic extract of Cardiospermum on wound healing process in rats

Medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring to the patient. Some of these plants owe their effects to direct effect on the wound healing process. The present investigation showed that the ethanolic extract of Cardiospermum helicacabum linn has significant wound healing property. This plant was preferred for the evaluation of wound healing activity because of its nontoxicity and absence of unwanted side effects. The active ingredients present in this plant are anticipated to interfere with one or more phases of the wound healing process in a positive manner in proper sequence and at the right time frame to show improved efficacy. These secondary metabolites in plant extracts that could bind to cellular receptors at wound site to initiate modulation of wound healing process was recently reviewed. [33] Terpenoids are known to boost wound healing process due to their astringent and antimicrobial properties which seems to be responsible for wound contraction and epithelialization. [34] In the same way other bioactive compounds like flavonoids[35], anthocyanins[36] , phenolics[37], caffeic acid[38] , chlorogenic acid[39] , ferulic acid[40] , water soluble alkaloids like indole derivatives in Adhatoda vasica[41] and Adhatoda zeylanica[42] are reported to promote wound healing process appreciably. Topical

application of ethanol extract Cardiospermum helicacabum linn. (Sapindaceae) to an infected wound not only reduces the rise of further infection but also improves the healing activity. It also found to improve the different phases of wound repair, including collagen synthesis and maturation, wound contraction and epithelialization.

4. Conclusion

This study shows that ethanolic extract of Cardiospermum helicacabum linn has potential wound healing effect when formulated as ointment and suggests further study in this herb.

Table 1: Effect of ethanolic extract of C.helicacabum linn, soframycin and simple ointment on % of wound contraction of excision wound models

Post wounding days	Control (simple ointment)		Standard ointment (Soframycin 1%w/w)		Ethanol extract of *C.helicacabumlinn ((5%w/w)*	
	Wound area(mm2)	% of wound contraction	Wound area(mm2)	% of wound contraction	Wound area(mm2)	% of wound contraction
Day 0	530±33.6	0	516±36.8	0	514±21.0	0
Day 2	509±18.6	4	458±36.8	11	372±18.8	27
Day 4	465±13.8	12	318±12.6*	38	312±19.9*	39
Day 6	424± 30.1	20	270±14.7*	48	245±15.3*	52
Day 8	389±14.8	27	193±11.4**	63	162±12.5**	68
Day 10	345±23.6	35	110±8.6**	77	95±9.6**	81
Day 12	269±14.3	49	79±6.3**	85	66±7.4**	87
Day 14	215±11.3	59	36±1.6**	93	37±3.5**	92
Day 16	189±14.3	64	10±1.9**	98	19±0.8**	96
Day 18	171±15.1	68	0.0**	100	0.0**	100

Values are means ± S.E of 6 animals in each group. * Significant differences at $p<0.01$ when compared to control. **Significant differences at $p<0.001$ when compared to control by student t-test.

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