Characterization of Wound Healing Potential and Antioxidant Activity of *Cardiospermum helicacabum* in Wistar Albino Rats

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**Abstract:** *Cardiospermum helicacabum*, known as the balloon plant which belongs to the Sapindaceae (soapberry) family. The present study was carried out for the phytochemical analysis and to evaluate the wound healing activity of ethanolic leaf extract of *Chelicacabum*. Preliminary screening of the ethanolic leaf extract of *C. helicacabum* for the presence of phytochemical compounds. Twenty four male Wistar albino rats (200-220gm) two months of age were used as experimental animals and were divided into four groups. The phytochemical assay reveals the presence of alkaloids, flavanoids, saponins, tannins, steroids, glycosides, carbohydrates and proteins. In excision wound model, simple ointment of ethanolic leaf extract of *C. helicacabum* at a proportion of 10% (w/w) and 20% (w/w) were applied for the contraction of wounds in rats and compared the effects with an antiseptic agent, Povidone Iodine ointment and normal control. The treated animals showed a significant result in period of epithelization and wound contraction.

**Key words:** *Cardiospermum helicacabum* • Phytochemicals • Wound healing • Excision mode

**INTRODUCTION**

India is one of the twelve mega-biodiversity countries of the world having rich vegetation with a wide variety of medicinal plants and a tradition of plant-based knowledge distributed amongst a vast number of ethnic groups [1]. Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and local communities in many developing countries [2]. Medicinal components from plants play an important role in conventional western medicine [3].

*Cardiospermum helicacabum* L. commonly known as Balloon vine or Love in a puff. *Cardiospermum* is the combination of the Latin words cardio, meaning heart and sperma, meaning seed and refers to the white heart-shaped pattern on the seed. *Heliacabum* is derived from the Latin word halicacabus, a plant with inflated fruits. It has been examined for anti-diarrhoeal as well as homeopathic medicinal properties [4]. *Cardiospermum helicacabum* L. has been used in the treatment of rheumatism, nervous diseases, stiffness of the limbs and snakebite. Young leaves can be cooked as vegetables [5]. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind [6].

A wound is one in which the skin or another external surface is torn, pierced, cut, or otherwise broken. It can be classified into two type; open wound and a closed wound. There are many different types of wounds ranging from mild to severe to potentially fatal. Contusions, small incisions and abrasions tend to be non-threatening, though some may pose the risk of infection. Deep punctures, avulsions and amputations, however, may be life threatening. In most cases, the risks posed by all types of wounds differ in severity based on the instrument causing them, the ease of blood flow and the cleanliness or jaggedness of the edges of the damaged skin. Abrasions, Avulsions, Contusions, Crush wounds, Cuts, Incised wound, Lacerations, Penetrating wound, Punctures are few examples of types of wound [7].

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 [8].

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical and microbial agents. It is triggered by the release of
chemical mediators from injured tissues and migrating cells [9, 10]. Phytochemical components like alkaloids, tannins and cardiac glycosides as antimicrobial effect of lemon grass. Natural products such as hebs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community [11, 12]. Pedalium murex showed significant increase in percentage of closure by enhanced the epithelization. The observation revealed that tissue regeneration was closely related to the treated group compared to the treated rats showed new blood vessels formation, extracellular matrix synthesis. Pedalium murex promoted the wound healing activity significantly in excision wound model [13].

Experimental Animals: The experiments were conducted after obtaining approval from Animal Ethical Committee.

Groupings: Twenty-four male Wister rats (200–220 gm) of approximately two months of age were used as experimental animals and were divided into four groups of six rats. The animals were caged individually after wounding for treatment till completion of wound healing. The animals were housed in standard environmental conditions of temperature (22 ± 3°C), humidity (60 ± 5%) and a 12 h light/dark cycle. During the course of the experiment the rats were administered a standard pellet diet and water.

Excision Wound Model: Excisions of wound were made as described [14]. Animals were anesthetized with anesthetic ether and placed in operation table in its natural position. The dorsal area from the scapula to the ilium were then scrubbed and prepared for surgery. An excision wound was inflicted by cutting away approximately 1.5 cm circular, full thickness of the predetermined area on the anterior-dorsal side of each rat. Using this excision wounding method, the epidermal, dermal and hypodermal and panniculus, carnosus layers were removed completely. The grouping table shown in Table 1.

Wound healing property was evaluated by wound contraction percentage and closure time. The wound area was measured every second day by placing a transparent paper over the wound and tracing it out; area of this impression was calculated using graph sheet and wound contraction. Wound closure time was recorded when total wound healed.

Ointment Preparation: For the animal model studies simple ointment of Ethanolic leaf extract of C. helicacabum was formulated in white soft paraffin base at a proportion of 10 % (w/w) and 20 % (w/w) using a ceramic mortar and pestle.

Statistical Analysis: All values are reported as mean ± S.E.M. The statistical differences among groups were assessed using one way ANOVA followed by Dunnett’s test. A value of P < 0.05 was considered significant.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Group</th>
<th>Intervention</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Reference standard</td>
<td>Povidone Iodine Ointment</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>Test-I</td>
<td>10% (W/W) of C. helicacabum leaf extract</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>Test-II</td>
<td>20% (W/W) of C. helicacabum leaf extract</td>
</tr>
</tbody>
</table>

Test formulations were applied to respective groups twice a day for 7 days starting from the day of wounding.
RESULTS AND DISCUSSION

Phytochemical Screening: Preliminary phytochemical screening of the ethanolic extract of *C. helicacabum* reveals the presence of alkaloids, flavanoids, saponins, tannins, steroids, glycosides, carbohydrates and proteins are shown in the Table 2.

The preliminary work revealed the presence and absence of phytochemicals present in ethanolic leaf extract of *C. helicacabum* shown in Table 2.

In the previous research it was stated that the acetone leaf extract of *C. helicacabum* showed the presence of the phytochemical compounds such as tannins, flavanoids. In the Diethyl ether extract of leaf contains tannins, flavonoids, terpenes and also the presence of flavanoids, terpenoids, cardiac glycosides was shown in the chloroform leaf extract of *C. helicacabum*. In the ethanolic leaf and stem extract of *C. helicacabum* showed the presence of steroids, phenols, saponins, aminoacids, tannins, flavonoids, glycosides [15].

The wound is one in which the skin or another external surface is torn, pierced, cut, or otherwise broken. With the initiation of the experiment the animal is made to create wound for the 1\textsuperscript{st} day. The created wound (1.5 cm in diameter) in the body of the rat shown in the Figure 1.

The 1\textsuperscript{st} day wound created in albino wistar rats was applied with ethanolic leaf extract of *C. helicacabum* in the form of an ointment.

In the 3\textsuperscript{rd} day of the experiment the animal wound reduced by applying ethanolic leaf extract of *C. helicacabum* shown in the Fig. 2.

The Figure 3 shows the wound reduction on 5\textsuperscript{th} day. The 7\textsuperscript{th} day of the wound showed highest percentage of wound contraction shown in the Figure 4. The wound contraction was noticed on 1\textsuperscript{st}, 3\textsuperscript{rd}, 5\textsuperscript{th} and 7\textsuperscript{th} day intervals. The size of the wound was reduced day by day by the application of the ointment. On 7\textsuperscript{th} day the treated wound exhibit dryness of wound margins with epithelialization.

Table 2: Phytochemical analysis of ethanolic extract of *C. helicacabum*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Presence of phytochemical, -: Absence of phytochemical

Wound Healing Activity of Ethanolic Leaf Extract of *C. helicacabum*

Fig. 1: Wound creation in 1\textsuperscript{st} day

Fig. 2: 3\textsuperscript{rd} Day status of the experimental animal

Fig. 3: 5\textsuperscript{th} Day status of the experimental animal

Fig. 4: 7\textsuperscript{th} Day status of the experimental animal

The 1\textsuperscript{st} group is the control animals in which simple ointment is applied. The 2\textsuperscript{nd} Group treated with povidine iodine which is the drug used for wound healing activity. To the 3\textsuperscript{rd} group animals 10% and to the 4\textsuperscript{th} group animals 20% ethanolic leaf extract of *C. helicacabum* were applied.
Table 2: Wound healing activity of *C. helicacabum* extracts in rats by Excision model

<table>
<thead>
<tr>
<th>Group</th>
<th>1 day</th>
<th>3 day</th>
<th>5 day</th>
<th>7 day</th>
<th>Epithelialization period (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple ointment (Control)</td>
<td>10.92±0.15</td>
<td>21.8±0.6</td>
<td>43.23±0.57</td>
<td>55.3±0.14</td>
<td>19.76±0.26</td>
</tr>
<tr>
<td>Povidine iodine</td>
<td>17.73±0.53**</td>
<td>38.63±0.3**</td>
<td>67.6±0.62**</td>
<td>84.02±0.84**</td>
<td>13.48±0.24**</td>
</tr>
<tr>
<td><em>C. helicacabum</em> (10%)</td>
<td>11.23±0.2</td>
<td>18.40±0.6**</td>
<td>52.62±0.58**</td>
<td>70.50±0.52**</td>
<td>17.9±0.17*</td>
</tr>
<tr>
<td><em>C. helicacabum</em> (20%)</td>
<td>12.23±0.28*</td>
<td>37.21±0.4**</td>
<td>62.82±0.52**</td>
<td>73.12±0.46**</td>
<td>16.40±0.32**</td>
</tr>
</tbody>
</table>

Values were mean±SEM, n=6, *P*<0.05, **P*<0.01 Vs control (one way ANOVA followed by Dunnett’s test)

![Wound Healing Activity Graph](image1)

Fig. 5: Wound healing activity of *C. helicacabum* extracts in rats by Excision model

![Epithelialization Period Graph](image2)

Fig. 10: Epithelialization period for wound healing activity of *C. helicacabum*

respectively. In the control animals wound contraction 55.3% on 7th day. 84.2% wound reduced on 7th day by applying povidine iodine. By comparing ethanolic leaf extract of *C. helicacabum* 10% and 20% concentrations, 20% is more effective than the 10% Concentration of the extract. On the first day wound contraction was 12.23 in 20% extract. On 7th day 73.12% wound was reduced. In the case of 10% concentration of plant extract, the wound contraction is 70.5%. So 20% is more effective than the 10% concentration of plant extract. The wound contraction of ethanolic leaf extract of *C. helicacabum* is nearest to the action of drug. So this plant is effective to use in the wound healing activity.

Epithelialization period is differ in the four groups of animals. Epithelialization period of control animals is 19.76 days. The drug has the epithelialization period 13.48 days. By comparing 10% and 20% of *Cardiospermum* leaf extracts 20% has more activity than 10%. Epithelialization period is 16.40 days for 20% and 17.9 days for 10% concentrations. The activity of plant extract is close to those of standard drug. So the Ethanolic leaf extract of *C. helicacabum* is more effective for wound healing activity.

The above graph shows the wound healing activity of *C. helicacabum* extracts in rats by Excision model in different day intervals. The epithelialization of the experimental animal model in various period.
This graph shows the period of epithelialization in different groups of animals. This graph shows that the epithelialization period for standard drug and *C. helicacabum* 20% extract are closely related. So it is an effective plant for wound healing activity.

*C. helicacabum* leaves can be used as a food source [16]. In a recent study showed significant activity as an analgesic and as an antiulcer treatment [17].

As an anti-filarial, tested on the helimenth, *Brugiapahangi*, extracts in high concentration of *C. halicacabum* have a small but direct macro falaricidal effect [18].

*C. halicacabum* L. has been used in the treatment of rheumatism, nervous diseases, stiffness of the limbs and snakebite. Young leaves can be cooked as vegetables [19].

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind [20]. Natural products are known to play an important role in both drug discovery and chemical biology [21]. Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. Any part of the plant may contain active components [22].

India has a rich tradition of plant-based knowledge on healthcare. 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 [23].

Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins [15].

*Cardiospermum halicacabum* possesses various phytochemicals and active biopolymers, which play a major role in the treatment of cancer. Many plants have been examined to identify new and effective anticancer compounds, as well as to elucidate the mechanism of cancer prevention and apoptosis [24].

Phytochemicals such as the steroids and saponins are responsible for the activities of the Central Nervous System. Steroids and triterpenoids shown to have analgesic properties. The terpenoids have shown to decrease blood sugar level in animals studies [25]. The saponins possess hypocholesterolemic and antidiabetic properties [26]. It has been found that more highly oxidized phenols are more inhibitory to microorganisms [27]. Flavonoid compounds inhibit multiple viruses. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity and a wide range of anti-infective actions have assigned to tannins [28]. Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [25, 29].

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Krishnamurthy Naik revealed that stem extract of *C. helicacabum* in different solvents promoted wound healing activity. The epithelization of wound in case of mice treated with extract was found to be quite earlier than control [30].

**CONCLUSION**

In this work, we have demonstrated that the wound healing effect of *C. helicacabum* leaf used as traditional plant. In the present research, the findings demonstrate the potential value bioactive compounds that characterized the wound healing property to *C. helicacabum* leaf. Moreover, the ethanolic fraction of *C. helicacabum* leaf are the most promising to isolate the active wound healing principles in *invivo* models.

However, the concern and difficulties related to the investigation of herbal medicines have precluded the financial incentives that could be provided to pharmaceutical industries.

**REFERENCES**


