Wound Healing Potential of *Celastrus paniculatus* Seed Oil in Rat Model

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**ABSTRACT**

*Celastrus paniculatus* is a vital herb in Ayurveda, reported to have immense health benefits. It is used to alleviate cognitive issues, promote the brain and enhances memory. The study was designed as a wound healing potential of *Celastrus paniculatus* seed oil in the rat. The wound healing activity of *C. paniculatus* seed oil (CPS) prepared in gel (5 and 10% w/w) was studied against excision and burn wound in rats, Betadine (10% w/w) for wound and Silver nitrate (0.2% w/w) for burn used as standard drugs. Percentage wound contraction, duration of epithelization was considered as a wound healing activity for 16 days. The skin of the wound area was dissected out for histological analysis. Treatment of CPS gel at two doses (5 and 10%) produces significant wound healing activity in comparison to diseases and standard control. In excision model, the wound contraction rate with CPS 5% gel was shown 99.98% and CPS 10% gel (96.35%), where disease control shows 82.45% only. In the case of burn model, the wound contraction rate with CPS 5% gel was 99.99% and CPS 10% gel (95.47%), where disease control shows 76.17% only. The histological analysis showed fibroblast cells, collagen fibers and new blood vessels present with CPS treated groups as compared to disease control. Finally, it was concluded that CPS 5% gel shown excellent wound healing ability as compared to standard groups. Test drug has shown improvement in fibroblast cells, collagen fibers and blood vessels highly but less with standard drugs.

**Key words:** *Celastrus paniculatus*, excision wound, burn wound, wound contraction, betadine and silver nitrate

**INTRODUCTION**

Wounds are one of the structural, cellular and functional disorders in the living body, which is happening due to the physical, thermal, chemical, bacteriological and immunological tissue offenses1,2. The tissue destruction as wound or burn may lead to stabilizing or decreasing organ capability leading to a decrease in biological functions3. It is classified on the evidence of blood releases and apparent bleeding such as an incised wound, abrasion, avulsions4,5 and blood escapes from the bloodstream but remains in the skin6. Such types of damaged tissues are the consequences of a substantial quantity of time in high or destructive stress7. The wound healing is a normal biological process of the living body that achieved through four precisely and highly programmed sequential process, i.e., hemostasis, inflammation, proliferation and tissue remodeling or resolution8. All these events
occurred through the integration of dynamic processes involving soluble mediators, blood cells and parenchymal cells. The method of tissue repair and regeneration consist of a sequence of molecular and cellular events which occurs after the onset of a tissue lesion to restore the damaged tissue. Chronic injuries are described by high amounts of anti-inflammatory cytokines like factor-α (TNF-α) or interleucine-1β (IL-1β), proteases (MMP) and leukocyte enzyme released from the body. These wounds have reduced metalloproteinase simultaneously protein inhibition levels. Platelet aggregation is a complicated rectification and damage closing method and competitive border restoration.

The plant Celastrus paniculatus Wild commonly known as Jyotishmati belonging to family Celastraceae is a primordial Ayurvedic herb used for the treatment of various human ailments and known for its immense health benefits. It is highly distributed in Punjab and Kashmir places above 3000 feet height and cultivated in October to November every year. Celastrus paniculatus seeds oil have been considered science ancient time in India as beneficial to the intellect and memory. Traditionally, the seed oil is used as an Unani and Ayurvedic medicine in Indian due to the presence of major phytoconstituents such as oleic acid, fatty acid, linoleic acid, palmitic acid, stearic acid, benzoic, acetic acids, volatile acid, celanapine, celapangine, celapague and celastrine. The present study was designed to prepare 5 and 10% gel formulation of Celastrus paniculatus seed oil and evaluate the wound healing potential of Celastrus paniculatus seed oil in the form of gel in the rat model.

MATERIALS AND METHODS

Plant collection and authentication: The Celastrus paniculatus seeds oil was procured from KBTC-Kashyap Brothers Trading Co. Ltd., (commodities-herbs-spaces oil); N-13, O-23, Bijasan Road, Bijasan Colony Indore (M.P.), India in the month of February 2019 and the experimental work was conducted in the month of March-May 2019 at Hygia Institute of Pharmaceutical Education and Research, Lucknow (U.P.), India.

Chemicals and reagents: The standard drugs Betadine 10% w/w and Silver nitrate 0.2% w/w were procured from the chemist shop and the other required reagents and chemicals obtained from the institute chemical store department HIPER, Lucknow.

Preparation of gel: The Celastrus paniculatus seed oil was prepared in gel form using white petroleum jelly by adding the seed oil with gentle shaking on the mental heater at a fixed temper, i.e., 45°C which appears transparent and clear, store in a tightly closed container for topical application. The developed gel was 5 and 10% w/w as 95 g of petroleum jelly and 5 g of Celastrus paniculatus seed oil were added in a beaker as 5% w/w. The 10% w/w gel was prepared by using the same processes with the addition of 10 grams of seed oil into 90 g of white petroleum jelly.

Toxicity study: The acute toxicity of seed oil was assessed by using a single oral dose of 2000 mg kg⁻¹ body weight in the rat as per mentioned in the OCED Guideline 423-2D method. The animals were only reserved water over the night during critical observation and then observed for two weeks. No sign of any illness and mortality was reported during and after the completion of this study, hence the selected dose was agreed for pharmacological research.

Phytochemical study: The preliminary phytochemical test of Celastrus paniculatus seed oil was subjected for the observation of alkaloids, glycosides, amino acids, phenolic compounds, tannins, fixed oil, carbohydrates, phenolic compounds, flavonoids, saponins, sterols and triterpenoids.

Experimental animals: Healthy Wister albino rats (150-250 g) were procured from the Institute animal house for the study. Animals were acclimatized for two weeks as providing proper ventilation in polypropylene cages and during the experiment, every two successive days, the bedding fabrics, cages washed and cleaned. The animals were fed with standard diet and water ad libitum in an appropriate room temperature (25±2°C), humidity (60±0.2%) and light zone for 12 h with an ecological situation for 12 h. The experiments were conducted after prior approval of protocol from IAEC (Support no. HIPER/AIEC/29/18/10). The research was carried out as accordance with CPCSEA regulations and instruction of institutional animals’ moral consent.

Experimental design: All the animals were randomly divided into eight groups (4+4) separately with six animals in each as designed for 21 days of innovative work as follows:

Excision wound
- Group I: Disease Control (Applied vehicle only on wound area)
- Group II: Standard Control (Applied Betadine 10% w/w on wound area)
- Group III: Lower dose test drug (Applied CPS Gel 5% w/w on wound area)
• **Group IV**: Higher dose test drug (Applied CPS Gel 10% w/w on wound area)

**Burn wound**

• **Group I**: Disease Control (Applied vehicle only on burn area)
• **Group II**: Standard Control (Applied Silver nitrate 0.2% w/w on burn area)
• **Group III**: Lower dose test drug (Applied CPS Gel 5% w/w on burn area)
• **Group IV**: Higher dose test drug (Applied CPS Gel 10% w/w on burn area)

**Procedure for excision wound**: The back skin of the animal was hairless and sanitized with 60% ethanol to approximately 3.5-4.0 cm. There was an impress on the dorsal thoracic area 1 cm since the vertebral pier and 5 cm from the ear side on the anesthetized rat. Wound sizes approximately 2.5-3.0 cm was produced by wounding a membrane cover from the rasp region. Then, the once-daily test and standard drugs were implemented for wound healing for 16 days.

**Procedure for burn wound**: The rat was hairless, sanitized with 60% ethanol approximately 3.5-4.0 cm. After 30 sec, a stainless steel rod (15 mm radius) was heated at 70°C over a most open fire. The warm, dripped wax was rubbed in a 1.5-2.0 cm spiral clock with the metal frame onto the animal’s spinal cord for the ventricle region chosen under moderate anesthesia. Followed by glue on the upper epidermis, the stainless steel coil has been separated and retained an apparent rectangular wound for burns after 7-10 min of crystallization. Then, once a day, testing and conventional medications were implemented for wound healing within 16 days.

**Investigational parameters**: All animals for measurement of wound contraction and wound area were observed in the course of the study at predetermined periods, i.e., 04, 08, 12 and 16 days. In the recommended moment interval, also wound healing (epithelization time) was noted.

**Histopathological analysis**: The creature instance of the membrane was isolated from the epidermis of each group at the end of the experiment for evaluation of histopathological changes. Further, it was processed for as per standard procedure to get a clear image of the slide through a light microscope at 40x magnification. In the context of repair of the single epithelioid, nuclear tissues link entrain hypertrophy and connective tissue formation in the dermis layer. The wound was treated in phases of epithelization, fibroblastic growth and capillary blood.

**Statistical analysis**: The data was analyzed by using mean±SEM (n=6) for all parameters. The statistical analysis was done by using one way ANOVA followed by Bonferroni multiple comparison test. The **p<0.05** shows statistically significant against disease control and **p<0.05** against standard control.

**RESULTS AND DISCUSSION**

**Phytochemical screening**: The preliminary phytochemical study was conducted for observation of potent phytoconstituents present in *Celastrus paniculatus* seed oil that contains saponins, terpenoids, alkaloids, tannins, flavonoids, steroids and absence of glycosides and phenols (Table 1).

The phytoconstituents present in *C. paniculatus* seed oil are highly responsible for the improvement of the wound due to its good antimicrobial activity, immunomodulators and maturation of newly generated cells. The alkaloid plays a beneficial role in the maturation of tissues and protects the damage of blood vessels of the wound area. The flavonoids reversed the increased expression of enzyme modulators and improved biological function. Tannins are useful in preventing further injuries of the new cells, while the steroids play a crucial role in the improvement of immunity. Saponins possess a fantastic anti-inflammatory with antioxidative properties, which plays a protective role during wound epithelization. Terpenoids are an excellent therapeutic agent to prevent further microbial infection. This data is precisely supported through a research study conducted by Shashank *et al.*

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Phytochemical</th>
<th>Observation</th>
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<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Phenols</td>
<td>-</td>
</tr>
</tbody>
</table>

* + sign indicates present and - absent
Pharmacological screening: The result observed from physical parameters shows that the animal treated with low dose of *C. paniculatus* seed oil prepared in gel form (CPS 5% gel) found as statistically significant (p<0.05) improvement in body weight (158±5.65 g), body temperature (97±2.00°F) and daily food intake (90±1.59%) as comparison to disease control and standard treatment group. Whereas, the higher dose of treatment (CPS 10%) group also shows significant (p<0.05) improvement as compared to disease control such as body weight (145±8.15 g), body temperature (101±1.82°F) and daily food intake (73±1.85%) and the result was almost comparable with standard control (Table 2).

Excision wound contraction and epithelialization: It was observed that the significant improvement in excision wound contraction in the animals treated with CPS gel as compared to disease and standard control whereas the lower dose of CPS (5% gel) shows highly significant (p<0.05) wound contraction (99.96%) and tissue epithelialization (09.48±0.27 days) at the end of study as compared to disease control and standard group (Table 3).

This experiment has been also supported same as the study conducted by Bhanumathy et al., on triterpene compound lupeol isolated from *Celastrus paniculatus* leaves extract that screened for excision and incision wound activity. In lupeol treated group wound healing activity was more significant than the standard control and also the epithelialization of the incision wound was faster with a high rate of wound contraction.

The wound healing area in the different treatment groups for 16 days study confirms that the test drug (CPS gel) produced considerable wound contraction and significant tissue epithelization at the end of the study. Furthermore, it was observed that the lower dose of CPS (5% gel) produced complete and statistically significant (p<0.05) closure of the wound and packed tissue epithelization as compare to other treatment groups (Fig. 1).

Burn wound contraction and epithelialization: There was a significant improvement in burn wound contraction observed in the animal treated with CPS gel as compared to disease and standard control. It was noted that the lower dose of CPS (5% gel) shows highly significant (p<0.05) improvement in burn wound contraction (99.98%) and tissue epithelialization (09.61±0.35 days) at the end of the study as compared to disease control and standard treatment groups (Table 4).

This activity has been supported by Harish et al., which reported that the *Celastrus paniculatus* extract shows a high wound contraction rate that associated with burn wound contraction and epithelialization activity.

### Table 2: Effect of *C. paniculatus* gel on the physical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Body temperature (-F)</th>
<th>Daily food intake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>145±8.15</td>
<td>101±1.82</td>
<td>73±1.85</td>
</tr>
<tr>
<td>SC</td>
<td>148±6.05</td>
<td>100±2.08</td>
<td>81±2.05</td>
</tr>
<tr>
<td>CPS-5</td>
<td>158±5.65**</td>
<td>97±2.00**</td>
<td>90±1.59**</td>
</tr>
<tr>
<td>CPS-10</td>
<td>156±6.82**</td>
<td>98±1.08**</td>
<td>86±2.96**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=6). The **p<0.05 shows statistically significant against disease control and *p<0.05 against standard control. CPS = *C. paniculatus* Seed, DC = Disease Control and SC = Standard Control

### Table 3: Effect of drug on % wound contraction and epithelialization of excision wound

<table>
<thead>
<tr>
<th>Wound area (cm) and % wound contraction</th>
<th>04 Days</th>
<th>08 Days</th>
<th>12 Days</th>
<th>16 Days</th>
<th>Epithelialization period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>2.91±0.07 (19.45%)</td>
<td>2.48±0.07 (45.68%)</td>
<td>1.79±0.07 (65.53%)</td>
<td>0.99±0.09 (82.45%)</td>
<td>15.45±0.45</td>
</tr>
<tr>
<td>SC</td>
<td>2.84±0.04 (28.16%)</td>
<td>2.39±0.05 (51.18%)</td>
<td>1.56±0.05 (72.25%)</td>
<td>0.65±0.09 (91.39%)</td>
<td>14.95±0.38</td>
</tr>
<tr>
<td>CPS-5</td>
<td>2.49±0.05 (24.29%)</td>
<td>2.16±0.03 (68.57%)</td>
<td>1.33±0.04 (87.48%)</td>
<td>0.05±0.02 (99.96%)</td>
<td>09.48±0.27**</td>
</tr>
<tr>
<td>CPS-10</td>
<td>2.57±0.06 (25.57%)</td>
<td>2.23±0.05 (62.45%)</td>
<td>1.50±0.03 (83.21%)</td>
<td>0.10±0.03 (96.35%)</td>
<td>12.75±0.30**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=6). The **p<0.05 shows statistically significant against disease control and *p<0.05 against standard control. CPS = *C. paniculatus* Seed, DC = Disease Control and SC = Standard Control

### Table 4: Effect of drug on % burn wound contraction and tissue epithelialization

<table>
<thead>
<tr>
<th>Wound area (cm) and % wound contraction</th>
<th>04 Days</th>
<th>08 Days</th>
<th>12 Days</th>
<th>16 Days</th>
<th>Epithelialization period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>1.60±0.04 (16.75%)</td>
<td>1.26±0.06 (42.47%)</td>
<td>0.88±0.05 (67.21%)</td>
<td>0.57±0.05 (76.17%)</td>
<td>15.00±0.70</td>
</tr>
<tr>
<td>SC</td>
<td>1.59±0.04 (26.14%)</td>
<td>1.25±0.03 (49.71%)</td>
<td>0.81±0.04 (70.14%)</td>
<td>0.38±0.06 (89.01%)</td>
<td>12.30±0.28</td>
</tr>
<tr>
<td>CPS-5</td>
<td>1.55±0.02 (21.24%)</td>
<td>1.15±0.03 (57.41%)</td>
<td>0.56±0.03 (78.24%)</td>
<td>0.06±0.02 (99.98%)</td>
<td>09.61±0.35**</td>
</tr>
<tr>
<td>CPS-10</td>
<td>1.58±0.05 (24.49%)</td>
<td>1.19±0.03 (52.79%)</td>
<td>0.65±0.03 (73.16%)</td>
<td>0.18±0.04 (95.47%)</td>
<td>11.89±0.32**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=6). The **p<0.05 shows statistically significant against disease control and *p<0.05 against standard control, CPS = *C. paniculatus* Seed, DC = Disease Control and SC = Standard Control

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Fig. 1: Excision wound contraction and tissue epithelization in experimental animals, CPS = *C. paniculatus* Seed, DC = Disease Control and SC = Standard Control

Fig. 2: Burn wound contraction and tissue epithelization at different days, CPS = *C. paniculatus* Seed, DC = Disease Control and SC = Standard Control

The burn healing area was observed in the different treatment groups for 16 days study confirms that the test drug (CPS gel) produced considerable burn wound contraction and significant tissue epithelization at the end of the study. Furthermore, it was also observed that the lower dose of CPS (5% gel) produced considerable closure of burn wound and superior tissue epithelization as compare to other groups (Fig. 2).
Fig. 3: Histopathological changes in excision wound shows as; (A) Disease control, (B) Standard control, (C) Treatment with CPS 5% gel and (D) Treatment with CPS 10% gel. C-Collagen Fibers, B-Blood Vessels, F-Fibroblast Cells, HF-Hair Follicle, SG-Stratum Granulosum, G-Granulosum

Fig. 4: Histopathological changes in burn wound of experimental rats shows as; (A) Disease control, (B) Standard control, (C) Treatment with CPS 5% gel and (D) Treatment with CPS 10% gel, C-Collagen Fibers, B-Blood Vessels, F-Fibroblast Cells, HF-Hair Follicle, SG-Stratum Granulosum, G-Granulosum, EP-Epidermis Layer

It was observed that both excision and burn wound graphical representations visualize the wound contraction and tissue epithelization in the experimental rats. Further, it was envisioned that the CPS-5% gel shows better response in wound contraction and tissue epithelization against disease control and the standard treatment group.

**Histopathological analysis:** The histopathological changes indicate that the improvement in wound tissue progression in the animal treated with CPS 5% and CPS 10% showed significant improvement against disease control and the revolutionize structures were almost comparable with standard control (Fig. 3, 4).
It was observed that the reduced or absent capillary fibroblast cells, collagen fibers and blood present in the disease group, whereas the test drug (CPS 5% and 10% gel) shows high levels of fibroblast cells, collagen fibers and blood capillaries but less in standard control (Betadine). Disease group showed a lesion in fiber cells, collagen fibers and damaged blood vessels. The standard drug-treated rats showed decreased fibroblast cells, collagen fibers and new blood vessels in excision wound models. The CPS treated groups established more than standard control as improved fibroblast cells, collagen fibers and blood vessels (Fig. 3).

The reduced capillary fibroblast cells, collagen fibers and blood cells present in the disease group, whereas the test drug (CPS 5% and 10% gel) treated rat shows high levels of fibroblast cells, collagen fibers and blood capillaries but it was less in standard control (silver nitrate). Disease group showed a lesion in fiber cells, collagen fibers and damaged blood vessels. The standard drug-treated rats showed decreased fibroblast cells, collagen fibers and new blood vessels in burn wound models. The CPS treated groups established more than standard control as improved fibroblast cells, collagen fibers and blood vessels (Fig. 4).

CONCLUSION
The results from the study suggested that the CPS 5% gel has shown better wound healing property as compared to CPS 10% gel and standard drugs (betadine and silver nitrate). The improvement in wound contraction with CPS Gel 5% was highly significant (p<0.001) in comparison to disease control and statistically significant (p<0.05) against standard drugs (betadine and silver nitrate). The histopathological changes in the rat treated with CPS 5% gel were observed as considerable improvement in fibroblast cells, collagen fibers and generation of new blood vessels significantly present treatment groups and also coordinated standard group. Finally, it was concluded that the CPS gel significantly reduced the time for wound healing and highly improved the closer of the wound, hence the herbal medicine may be preferred as conventional medicines due to their high availability, improved efficacy and free from adverse effect so that it may be useful for the medicinal purpose.

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