Evaluation of Wound Healing Property of Karanja Patra Ghana - Water Extract of Pongamia pinnata Linn Leaf

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ABSTRACT
Karanja, Pongamia pinnata Linn is traditionally used to treat various skin disorders, including wounds. It is widely used in Ayurveda as an effective and readily available treatment of various wounds. This study was aimed at investigating the healing efficacy of Karanja Patra Ghana - water extract of Pongamia pinnata Linn leaf. Swiss albino rats were divided into four groups, two control groups and two treatment groups consisting of excision wound healing model and incision wound healing model. In excision wound, percentage of wound contraction and period of epithelization were the parameters for the study and this was achieved by using planimetry (Morton and melone method). In incision wound tensile strength was the parameter and this was achieved by tensiometry (Hunts et al. method). Statistically treatment group showed better wound healing in terms of epithelization and tensile strength. It may be concluded that Karanja Patra Ghana - water extract of Pongamia pinnata Linn leaf formulated is effective in the treatment of wounds and thus supports its traditional use.

Key words: Karanja, Pongamia pinnata, Ayurveda, wounds, Morton and melone method.

INTRODUCTION
Wound is a major case of physical disability and is disturbed state of tissue caused by physical, chemical, microbial or immunological insults or typically associated with loss of function. According to the wound healing society wounds are physical injuries that results in an opening or break of the skin that cause disturbance in the normal skin anatomy and function.[1] Wound healing is an interaction of complex cascade of cellular and bio chemical actions healing to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell – cell interaction and cell matrix interactions that allows the process to proceed in different overlapping phases and process including inflammation , wound contraction, re-epithelialization tissue, re-modeling , & formation of granulation tissue with angiogenesis.[2] Several factors delay (or) reduce the wound healing process including bacterial infection, necrotic tissue, & interference with blood supply, lymphatic blockage & diabetes mellitus, generally if the above factors could be altered by any agent, an increased healing rate could be achieved.[3] Many Ayurvedic plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way.[4]

The use of traditional medicinal remedies and plants in the treatment of wounds is an important aspect of health treatment and at the same time a way to reduce the financial burden. Several plants are used as traditional healing remedies and have been reported to treat skin disorders, including wounds.[5,6,7] Various herbs have been screened out for their efficacy and were found to be effective as mentioned by Ayurvedic classical texts. Karanja is one important plant amongst them. The different parts of a drug have different kinds of activity. Thus the Karanja leaves have been selected for the present experimental study to
evaluate its *Vrana ropana* (wound healing) property.

**MATERIALS AND METHODS**

**Plant material and extraction:**

*Karanja* (*Pongamia pinnata* Pierre) tree was identified by a Botanist and its leaves were collected from Koppa taluk, Chikmagalur district, Karnataka. *Swarasa* (fresh juice) was extracted from *Karanja* leaves by Putapaka method (hot extraction method).\(^8\) Extracted *Putapaka swarasa* of *Karanja* was heated in a vessel on moderate fire till it solidifies (*ghana*) and rolled into *Varti* (wick). The *Ghana Sattva Vati* was made into a paste by adding distilled water and this paste was applied over the wound under all the hygienic precautions (Fig 1).

**Experimental animals:**

Male Swiss albino rats (total twenty four) weighing 150-250 g were fed in the animal house of ALN Rao memorial Ayurvedic Medical College. They were individually housed in clean polyethylene cages under standard experimental conditions of temperature 12 h light/dark cycle and fed on normal pellet diet and water *ad libitum*. The Albino rats were used for the experiment after one week of acclimatization period. The experiment was conducted after prior permission from the institutional animal ethics committee. Experiment was carried out according to the Ethical Guidelines for Care and Use of Animals in Health Research.

Experimental study was done with both Excision and Incision wound model.

**Excision wound model:**

This was conducted according to the technique developed by Morton and Malone. The animals were anaesthetized using ketamin intra-peritoneal injection. After the animals were sufficiently anaesthetized, they were secured to the dissection plate in prone position. The hairs were removed using fem hair removing cream from the part to be operated and subsequently the area was cleaned with betadine. A round seal of 2.5 cm in diameter was impressed on the dorsal thoracic central
region 5 cm away from the ears of the anaesthetized rats. Full skin thickness from the marked area was excised in circular fashion with the help of forceps, surgical blade and scissors. The approximate area thus formed was 500 squares of mm. After achieving full haemostasis, the animals were placed in individual cages (Fig 2). [9]

**Incision wound model:**
This was conducted according to the technique mentioned by Hunts et al. The animals were anesthetized by using ketamin as intra-peritoneal injection; after the animals were sufficiently anaesthetized they were secured to the dissection plate in prone position. The hairs were removed using fem hair removing cream from the part to be operated and subsequently the area was cleaned with betadine. Two para-vertebral incisions measuring 6 cm in length of full skin thickness was made, then the incision was closed by interrupted sutures at an interval of 1 cm. After achieving full haemostasis, the animals were placed in individual cages (Fig 3). [10]

**RESULTS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>post wounding days</th>
<th>4th day</th>
<th>8th day</th>
<th>12th day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Natural Recovery)</td>
<td>29.2±1.406%*</td>
<td>50.2±1.41%*</td>
<td>76.6±0.74%*</td>
<td>88.0±0.54%*</td>
<td></td>
</tr>
<tr>
<td>Treatment (Karanja Ghanasatva)</td>
<td>49.6±1.46%*#</td>
<td>81.6±0.47%*#</td>
<td>97.8±0.36%*#</td>
<td>99.5±0.09%*#</td>
<td></td>
</tr>
</tbody>
</table>

*= P<0.001 in same group comparison
#=P< 0.001 in inter group comparison

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**Fig 3:** Incision wound model of Treatment group

**Fig 4:** Bar diagram showing the Percentage of wound closure
Table 2: Tensile strength in gm of incision wound on 10th day of the wound:

<table>
<thead>
<tr>
<th></th>
<th>Control/Natural Recovery</th>
<th>Treatment(Karanja Patra Ghanasatva)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>367.3 ± 5.76</td>
<td>513.8 ± 7.77</td>
</tr>
<tr>
<td>t-value</td>
<td>54.37</td>
<td>66.42</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>comparative tensile strength of incision wound</td>
<td>t-value=14.409 p-value= P=0.001</td>
<td>Remarks=Highly significant</td>
</tr>
</tbody>
</table>

DISCUSSION

Classics of Ayurveda have emphasized at various places to take care of wounds which occur due to vitiated Doshas or any trauma. Acharya Sushruta, an eminent surgeon, was much ahead of his time in expounding and practicing the beautiful concept of Vrana Ropana (wound healing). Healing of Vrana (wound) is either primary or secondary by nature. Secondary healing requires more attention than primary healing. Basically, two things, Shodhana (making free from undesirable healing factors) and Ropana (closure of wound), are desirable for proper healing. Exposure to infection and prolongation of inflammatory phase plays the prime role to delay the healing process, whereas creating the favorable conditions that allow the wound to heal properly is the ultimate aim of any surgeon. Acharya Sushruta has explained much elaborately about accidental wounds under the heading Sadhyo vrana which shows that Vrana is not new to men it is a very old concept in Ayurveda. In Sushruta Samhitā, Vrana is classified into Nīja (wounds occurring due to vitiation of doshas) and Agantuja vrana (traumatic wounds). Agantuja or Sadhyo Vrana is defined as Vrana with several margins and surfaces caused by different types of instruments at different sites and with various shapes which occur as a consequence of external injury. Various herbs have been screened out for their efficacy and were found to be effective as mentioned by Ayurvedic Samhitās and Acharyas. The different parts of a drug have different kinds of activity. Thus the Karanja leaves have been selected for the present experimental study to evaluate and compare its Vrana Ropana property (wound healing property). Various references reveal that the soldiers have been treated very effectively with herbs available in the vicinity.

For the treatment of Vrana, main treatment principles are vrana shodhana (cleansing of wound) and Vrana ropana (wound healing). Karanja possesses Katu (Pungent), Tikta (bitter) and Kashaya rasas (astringent tastes) , Laghu (light), Ushna (hot) and Teeksha gunas (penetrating property), Ushna virya (hot in potency), Katu vipaka (Pungent post digestion effect). Katu rasa (Pungent taste), is mentioned as Vranam Avasadayati (eschars the wound) in the major ayurvedic classics along with the properties like Mamsa Lekhana (scraping of the wound), Shodana (cleansing of the wound), Chedana (cuts off the unhealthy tissue). Katu rasa (pungent taste) removes the unhealthy tissue formed at the site of Vrana (wound) and removes the sluff formation.

Tikta rasa (bitter taste) does the shoshana (drying up) of Kapha, Puya srava (pus) & Kleda (moisture). Twak mamsa Sthirikarana (brings stability in the tissue) property mentioned for Tikta rasa (bitter taste) helps in providing strength to the tissues. Kashaya rasa (Astringent taste) helps in Sthambhana (styptic action) & both Tikta (bitter) and Kashaya rasas (astringent taste) does Shoshana (dries up) thus maintaining a dry locality at the site of Vrana (wound), which prevents the invasion of Krimis (microbes). Sandhaniya karma (holding together of the tissue) of Kashaya rasa (Astringent taste) enhances the process of contraction and Ropana karma (healing process) helps in formation of healthy granulation tissue thus facilitating the Wound healing Process. The predominant gunas (qualities) present in the drugs are Laghu (light), Ruksa (dry), Teekshna (penetrating). Laghu guṇa (light) by nature is Kaphaghna (alleviates kapha) and sroto shodhaka (cleanses the channels), this quality helps in repairing all the blocked channels and aid in the proliferation of surrounding connective tissue elements and capillaries, which migrate into site to be repaired. Ruksa guna (dry) helps in drying up the raw area and helps in wound contraction.

Present study was planned to evaluate the wound healing property of the trial drug Karanjapatra ghanasatva. While comparing the result of the experiment, Karanja the trial drug showed highly significant wound healing property in comparison with the control group. In the excision wound model two parameters were assessed, Percent contraction of original wound area and Period of epithelization. Whenever a breach occurs in the continuity of tissue the surrounding connective tissue and capillaries grows to cover up the area damaged, to achieve the contraction of wound. In the trial group wound closure was achieved on 14th day, but in the control group it has been achieved by 18 -19 days.

The Period of epithelization is calculated by the scar formation and by falling off leaving no raw
area behind. Once there is a break in the epithelium, it will proliferate and grow from the surrounding tissue before that due to the clotting and other factors a scar tissue will be formed on the wound, initially this will be covering the whole area of the wound, gradually when the new epithelium grows this will reduce in size and finally will fall off. The earlier the falling of the scar the faster the healing.

The mean period of epithelization in the treatment group was 14.33 days and that of control group was 17.16 days. This clearly shows that epithelization was achieved early in the trial group than the control group. In the incision wound model, the strength of the granulation tissue was measured. Once a breach or gap has occurred in the tissue, it is closed by the granulation tissue. Here the strength of the granulation tissue is assessed by using tensiometer in grams. The mean of the treatment group was 515.8gm and that of control group was 367.5 gm. This clearly shows that the trial group was having better tensile strength than the control group.

CONCLUSIONS

Therefore, from these findings it could be concluded that application of *Karanja Patra Ghana* to an open wound induces significant wound contraction and accelerates wound healing and this herbal aqueous extract may be a promising medication for open wounds. Macroscopic, *i.e.* and biomechanical results indicated that topically administered *Karanja Patra Ghana* accelerated epithelialization, wound contraction, tissue alignment and tissue strength at the later stage of wound healing.

REFERENCES