Seabuckthorn (*Hippophae rhamnoides*) seed oil as local dressing agent for infected cutaneous wounds in bovine

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Abstract

The study was conducted on 9 male calves of 10-12 months by creating full thickness cutaneous wounds (3 cm x 3 cm) on their thoracolumbar region. The wounds were inoculated with *Staphylococcus aureus* suspension having 2.1 x 10⁹ organisms/ml. The infection was allowed to flare up upto 48 hours. After that, the wound dressing was started with liquid paraffin in group I, 5% povidone iodine ointment in group II and seabuckthorn seed oil in group III. The acute inflammatory signs were less pronounced in group II up to day 7th, whereas later these were comparable in group II and III. The group III treated wound exhibited better healing response after 7th day as evidenced by greater wound contraction, early shedding of scab. Angiogenesis was more extensive in group III on 3rd day. Similarly, epithelialization was also slightly more in group III on day 7th. Whereas neutrophilic infiltration was least in group II by this stage indicating better antimicrobial activity of 5% povidone iodine. Epithelialization was still incomplete by day 21 in group III along with group I whereas it was complete in group II. On 28th day, epithelialization was complete in all the groups except two animals of group I.

Key Words: Seabuckthorn seed oil, infected cutaneous wounds, healing activity

Introduction

Healing is a fundamental process in nature to restore normal functions of the body. The ability of the wound to heal is a complex biological process which follows a definite pattern of cellular and molecular events, ultimately leading to complete repair of the injured tissue. Wound healing may be hastened by providing ideal environment which result in optimal body response to healing process. However, presence of infection cause delay in natural healing. The healing in such wounds may be hastened by application of some exogenous material. The wound healing properties of seabuckthorn oil are well established (Vlasov, 2001; Khirugia, 1995; Gupta *et al.*, 2001). The different parts of seabuckthorn plant have been used for the treatment of skin wounds and various ailments of cardiopulmonary and gastrointestinal system. Major natural bioactive substances in seabuckthorn seed oil are flavone, vitamin E, vitamin C, carotenoids and unsaturated fatty acid viz linoleic and linolenic acids. The versatile pharmacological activities of seabuckthorn oil were hence attributed to these bioactive substances. The present study was undertaken to establish the wound healing properties of this plant’s seed oil on infected cutaneous wounds in male cow calves.

Materials and Methods

Isolation and standardization of *Staphylococcus aureus* for producing infection: A clinical case of sever dermatitis selected for isolation and standardization of *Staphylococcus aureus* microorganisms. The swab was taken under sterile conditions and was streaked on nutrient agar and incubated at 37°C for 24 hrs. After 24 hrs, a colony which was round, glistening, convex,
smooth and opaque and possessing white/golden/lemon pigmentation was streaked on blood agar and incubated at 37°C for 24 hrs. The colony from nutrient agar was also streaked on mannitol salt agar which is selective for Staphylococci. Gram staining was done by using standard procedure (Carter and Cole, 1990) for identification of microorganisms. Microscopic examination of the stained slide revealed the presence of gram positive cocci, 3-4 microns in diameter arranged in grape like clusters. The colonies on blood agar also had the similar characteristics as on nutrient agar. Beta haemolysis was present and frequently a double zone was apparent in which the central clear zone was surrounded by a zone of partial haemolysis. Biochemical tests were performed using standard procedures (Carter and Cole, 1990). After confirmation, the colonies of Staphylococcus aureus were stored in stock culture media and kept in refrigeration.

A day before inoculation, the culture from stock culture media was streaked on mannitol salt agar. The growth after 24 hrs was reconstituted in lukewarm NSS and the concentration was matched with Mac Farland standard tube No.7 (concentration equivalent to 2.1x10⁹).

**Extraction of seabuckthorn oil:** The seabuckthorn seeds were dried under sun and later in oven. The dried seeds were grinded in a cyclotone mill to form fine powder. The grinded seabuckthorn powder was taken in thimble and extracted for 8 hours with petroleum ether (BP 60-80°C) in soxhlet extraction apparatus as described by Association of Official Analytical Chemists (AOAC, 1990). Oil was collected subsequently after evaporation of ether from collection flask.

**Preparation of animal and creation of full thickness infected wounds:** Thoracolumbar region of each animal was prepared for aseptic surgery for creation of wounds. Equidimensional (3 cm x 3 cm) six full thickness cutaneous wounds, 3 on either side of the vertebral column and on the dorsal aspect of the thoracolumbar region were created under local infiltration anaesthesia (Xylocaine® 2%). Thereafter, 1ml of Staphylococcus aureus suspension having (2.1 x 10⁹ organism/ml) was injected subcutaneously into the created wounds. The wounds were covered by normal saline solution soaked sterile gauze piece and further overlapped by a clean cloth. The infection was allowed to flare up for 48 hours. After that, wound dressing was started with liquid paraffin in animals of group I, 5 % povidone iodine in group II and seabuckthorn seed oil in group III. Dressing of the wounds was done daily for 7 days and on alternate days for the next three weeks (till 28th day). The efficacy of various treatments on wound healing was monitored by clinical, haematological and histopathological parameters at 0, 3rd, 7th, 14th, 21st and 28th day after the start of experiment. Clinical observations include rectal temperature, respiration rate and heart rate, degree of inflammation, extent of cicatrisation, epithelization and percentage wound contraction. The percentage wound contraction was measured using following formula:

\[
\text{% Wound Contraction} = \frac{A - B}{A} \times 100
\]

Where,

A is area (sq cm) of the wound at day 0.
B is area (sq cm) of the wound at day 3rd, 7th, 14th, 21st and 28th.

**Haematological parameter** include haemoglobin (Sahli’s method), Packed Cell Volume (microhaematocrit), Total Leucocytes Count and Total Erythrocyte Count (haemocytometer) and Differential Leucocytes Count (Wright’s stain) as described by (Benjamin, 1995).

**Statistical analysis** of the data was carried out using analysis of variance method (ANOVA) and comparison between the treatments and days was done at 5% level of significance wherever applicable. Necessary permission for the experimentation was taken from Institutional Animal Ethic Committee.

**Results and Discussion**

In all the animals, there was increase in rectal temperature and respiration rate after 48 hours of bacterial inoculation, otherwise rectal temperature, respiration rate and heart rate remained in with physiological range throughout
the period of observation in all the three groups. There was transient increase in rectal temperature and respiration rate after bacterial inoculation after creating infected wounds in bovine calves (Mahajan et al., 2004). A rise in rectal temperature, respiration rate and heart rate are the normal responses of body to bacteremia as well as trauma (Radositis, 1994).

The wounds in all the three groups were filled with yellowish exudates with marked swelling of wound margin and dilated blood vessels (Fig. 1). The exudate was quite extensive and the wound dressing gauze had to be changed three times a day. The animal exhibited sign of pain even on slight palpation around the wound. In all the animals, there were mild increase in neutrophils and total leukocyte count. However, the values of haemoglobin, haematocrit and total erythrocyte count were decreased.

After the start of the treatment with respective dressing in different groups, on 3rd day of treatment the wound of all the groups were covered with layer of scab. The wound in group I (Liquid Paraffin) were characterized by accumulation of yellow purulent exudates underneath relatively transparent scab. Whereas the amount of exudates was relatively lesser in group II (5% Povidone Iodine) and group III (SBT Seed Oil). On 7th day, the animals had exhibited lesser pain reactions to palpation in group II and III as compared to group I. On day 3 and 7, the wounds in group II (5% povidone iodine) exhibited least exudation and inflammation as compared with other two groups. This indicated that 5% povidone iodine ointment dressing was able to control the infection and thereby more effective at early stage. Although, the wounds of group III showed greater exudation and inflammation than group II but their intensity of inflammation was lesser than group I at this stage. This indicated a possible mild antimicrobial and anti-inflammatory activity of seabuckthorn seed oil. Likewise (Varshney et al., 2004) also reported mild anti-inflammatory response of seabuckthorn oil against acute cutaneous inflammation in experimental dogs. Gupta et al. (2001) also documented that there was no visual signs of inflammation in wounds treated for 7 days with seabuckthorn seed oil and flavonoids of seabuckthorn in albino rats. After 7 days, the wound healing progressed fastest in group III as evidenced by relatively drier wound and the development of greatest amount of granulation tissue in this group. In general the other healing response, were almost similar in group II and group III. The seabuckthorn (SBT) seed oil which has been subjected to a number of wound healing studies (Vlasov, 2001; Khirugia, 1995; Gupta et al., 2001) in animals as well as humans has been reported to possess vitamin A, vitamin E, many trace elements, flavonoids etc (Yaonian et al., 1995). Vitamin A helps in collagen synthesis and cross-linking of collagen fibres. In addition to this it also helps in proper epithelization. Vitamin E has anti-oxidant properties.

By the 14th day, all the wounds of group I were still moist and covered with little amount of purulent exudates. Although the pinkish granulation tissue was evident but the wound contraction was less. The inflammation and swelling at the wound margin subsided in this group. The wounds in group II and group III were devoid of any gross exudates and the development of pinkish granulation tissue was obvious in all the wounds. Seabuckthorn oil also contains terpenoids and flavonoids, which might be responsible for anti-inflammatory activity as they have marked inhibitory effect on the platelet activating factor (PAF) responsible for acute inflammation (Zhang, 1988). Seabuckthorn has also shown to inhibit artificially induced acute inflammation of mouse subcutaneous tissue (Lebedeva, 1989).

On 21st day, no wound in any of the group was grossly moist and there was no exudation in any of the wounds. All the wounds in group I were covered with a thick scab and yellowish crusty layer. The wounds in group II and group III were also covered with a firm scab. At the end of study i.e. 28th day, in group III wounds were characterized by peeling of scab where as none of the wound in group I showed falling of scab (Fig. 1). However, in group II pealing of scab was seen in one animal and partial detachment of scab was seen in two of the animals. The pain response to palpation in all the groups was almost negligible. Mahajan et al. (2004) and Kumar et al. (2010) also noticed that seabuckthorn oil is superior to 5%...
Figure: 1 An early shedding of scar tissue and faster wound contraction and epithelization in the Seabuckthorn seed oil treated wounds.

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<th>A1</th>
<th>B1</th>
<th>C1</th>
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<tbody>
<tr>
<td></td>
<td>Liquid Paraffin</td>
<td>5% Povidone Iodine</td>
<td>Seabuckthorn Seed Oil</td>
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<td><img src="image8" alt="Image" /></td>
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Table1: Effect on wound contraction (%) following different treatments of infected cutaneous wound in cow calves (mean ±S.E.)

<table>
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<tr>
<th>Treatments Groups</th>
<th>Time interval (Days)</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
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<tr>
<td>Liquid Paraffin</td>
<td>(Group I)</td>
<td>8.77</td>
<td>±3.64</td>
<td>26.59</td>
<td>±3.88</td>
<td>63.57</td>
</tr>
<tr>
<td>5% Povidone-iodine</td>
<td>(Group II)</td>
<td>12.23</td>
<td>±4.61</td>
<td>26.04</td>
<td>±4.93</td>
<td>66.76</td>
</tr>
<tr>
<td>Seabuckthorn</td>
<td>(Group III)</td>
<td>11.14</td>
<td>±3.36</td>
<td>23.0</td>
<td>±7.93</td>
<td>72.63</td>
</tr>
</tbody>
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povidone iodine ointment and paraffin as evidenced by early subsiding of inflammation, greater contraction of the wounds and early shedding of burn eschar.

The percent wound contraction gradually increased in all the groups. On day 3 and 7 the wounds of group II showed the greatest contraction but thereafter percent wound contraction was greater in group III on 14, 21 and 28 days as compared to other two groups (Table 1). In group III, per cent wound contraction on day 3rd was greater than group I but lesser than group II.

However in group III, percentage wound contraction on day 14th, on day 21st and on day 28th was found to be greater than group I and group II (Table 1). The percent wound contraction when compared from day 3 value was found to be significant on day’s intervals. However when compared statistically within each groups on different intervals i.e. 3rd, 7th, 14th, 21st, 28th day was found to be non significant (P>0.05). Wound contraction was greatest in group III after 14th day of treatment in comparison to other groups which indicate faster healing. Gupta et al. (2005) also reported that seabuckthorn ointment and 5 % povidone iodine treated wounds in dogs showed greater wound contraction up to 36 % and 32 % respectively on 7th day of treatment as compared to control group (liquid paraffin) which showed only 17 %. The faster wound contraction in group III was comparable to group II might be due to early formation of collagen which is exerted by rich contents of vitamin A, vitamin C and vitamin E etc. and microelements (S, Se, Zn, Cu etc.) along with the triterpene components in the seabuckthorn which have regenerative and epitheliotrophic properties (Xu, 1993). The seabuckthorn oil had vitamin C as a one of its bioactive components (Zhemin, 1990). The vitamin C is required for formation of hydroxylsine and hydroxyproline which are constituent of collagen.

The variation noticed in the haematological parameters i.e. haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, and differential leukocyte count in all the animals of group-I, II and III when compared between the groups was non-significant on different days intervals. Total leukocyte count increased in all the groups from day 0 to 48 hrs and decreased gradually afterward toward the base value. It remained within the normal physiological range throughout the period of study. This favoured the topical application of seabuckthorn seed oil on wounds was non-irritating and clinically safe. These observations are in conformity with the findings of (Mahajan et al., 2004; Kumar et al., 2010) used seabuckthorn ointment /oil for different kinds of wound healing studies in animals.

Microbial examination of wounds on 21st day and onwards, revealed absence of inoculated organism from the wound site except in group I where organism was isolated from one animal. The animals remained active, alert and healthy throughout entire study period without exhibiting any clinically manifested complication.

Conclusion

In seabuckthorn oil treated group, wound healing was better after 7th day as evidenced by greater wound contraction, early shedding of scab, however it accelerates in later stages in seabuckthorn oil treated would compare to other treatments. No adverse effect or complication was not observed during this study indicated that seabuckthorn oil can be used as mild antimicrobial and anti-inflammatory agent in wound healing.

References

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