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Effect of seabuckthorn *(Hipphophae* L.) Leaf extract and seed oil on infected cutaneous wound healing process in rabbit model

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Plants have been used as remedies since time immemorial (Mahajan *et al.* 2005). The plants are effective (Varshney *et al.* 2005) in the treatment of fever, inflammation of the mucosa, cough or in indigestive problems. Different preparations of seabuckthorn such as decoctum, powder, pill, medicinal extract, shortbread, ash and tincture were used for the treatment of various disease conditions in ancient time (Mingyu *et al*. 2001).

Seabuckthorn (SBT) seed oil and leaves have an efficacy on wound healing area (Gupta *et al.* 2001), and hiporamin a tannin fraction from SBT possessing a wide spectrum of antiviral activities and a mild antimicrobial activities (Shipulina 2006). The paper reports efficacy of leaf extract and seed oil of seabuckthorn on healing process of experimentally created cutaneous wound in rabbit model.

The seabuckthorn leaves and seed were collected from the Lahaul and Spiti valley of the Himachal Pradesh. After drying of leaves at 37°C for 3 to 5 days, leaves were ground to fine powder without exposure to sunlight. About 100 g of seabuckthorn leaves powder was mixed with 400 ml of absolute methanol and left for 24 h in orbital shaker at 25 to 30°C at 120 rpm. Residues was filtered by using triple whatman filter paper no.1, and filtrate was dried by the rotator vaccume pressure for recovery of methanol. The semi-solid extract produced was kept in the deep freezer at –80°C overnight. Seed oil was extracted in an expeller. All the twigs and debris were removed manually from the seeds. Seeds were sun-dried for 2 days and the dehusking was done manually. The machine was washed thrice before the extraction procedure started. The initial seed oil was discarded and was not used for testing purpose because it contained extraneous material. The seed oil was kept at room temperature and pH of the oil was checked and found 7.0.

Standard strain of *S. aureus* was maintained on the stock culture media at 4°C. The growth after 24 h was reconstituted in the sterilized nutrient broth and the concentration was matched to 0.4 O.D with McFarland standard densitometer.The protective effect of seabuckthorn seed oil and leaf extract against infected wound was studied using rabbits as the experimental model. Rabbits (12) were procured from a local rabbit farm and maintained in the experimental animal house under standard hygienic conditions throughout the experiment. All the experiments were performed in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC). The animals were housed in single cage system and provided water and food *ad lib.*

Rabbits (12) were equally divided into 4 groups for conducting infected wound healing study. The group 1 was kept as negative control in which the wounds were dressed with only base material that include mixture of vaseline, 85 g; hard paraffin, 10 g and Lanolin, 5 g. In group 2, methanolic seabuckthorn leaf extract (5%) was used to dress the wounds by topical application. In group 3, seabuckthorn oil was used for wounds dressing. While the group 4 was taken as positive control in which the wounds were dressed with the 5% povidone iodine. For creation of wounds, thoracolumbar region of each animal was prepared for aseptic surgery. Two equi-dimensional (2 cm × 2 cm) full thickness excisional cutaneous wounds, one on either side of the vertebral column were created under local infiltration anesthesia using 2% xylocaine. Thereafter, 1 ml of *Staphylococcus aureus* suspension having 1×10^8 cfu/ml was swabbed onto the created wounds of all the animals of groups 1,2,3 and 4. The wounds in all groups were covered with sterile gauze piece soaked in normal saline solution and covered by a clean cloth. The infection was allowed to flare up for 48 h. The treatment in various groups was started after 48 h of bacterial suspension inoculation. Dressing of wounds was done daily

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for 14 days and on alternate days subsequently. Clinical parameters like heart rate, rectal temperature, respiration rate, degree of inflammation, extent of cicatrisation and epithelization were noted daily for 28 days. Percentage of wound contraction was measured using formula:

$$\% \text{ of wound contraction} = A\text{-}B/A \times 100$$

where, A is area in square cm of wound at zero day and B is square cm area of the wound at 3rd, 7th, 14th, 21th and 28th day.

Venous blood from the ear vein (approximately 1 ml) was collected in EDTA at different time intervals (0, 3rd, 7th, 14th, 21th and 28th day) as mentioned for estimation of various haematological parameters like haemoglobin (Sahil's method), PCV (microhaematocrit), TLC and TEC (haemocytometer) and DLC (Wright's stain) as per Benjamin (1985).

The artificially-created wounds (2 × 2 cm) on the back of rabbits were examined daily for progression of healing. At the end of the experiment (28 days), the tissue from wound sites including skin, dermis and the part of muscles were dissected out and preserved in 10% formalin. After fixation, thin pieces of 5 m thickness were cut and processed for paraffin embedded sections. The sections were stained with routine haematoxylin and eosin (H and E) staining as per the standard protocol (Luna 1981). Swabs from the wounds were taken for microbiological examination on 3rd, 7th, 14th, 21st and 28th day. The culture were streaked on blood agar and nutrient agar for growth and further identified by gram staining.The data were analyzed statistically using analysis of variance (Anova).

The effect of seed oil and leaf extract of an Indian variety of seabuckthorn plant (*Hippophae rhamnoides*) on full thickness excisional-infected-cutaneous wound healing in rabbits was evaluated. After 48 h of bacterial suspension inoculation, the wound site in all the 4 groups was found to be filled with yellow exudates, indication of *S. aureus* infection. It was further confirmed by isolation and characterization of the invading microorganism. After 48 h, wound margin showed marked swelling and dilated blood vessels in the wound gap. The animals of all the groups exhibited sign of pain on palpation around the wound. All these symptoms are indicative of an acute localized wound infection. The rectal temperature and respiration rate showed transient increase in all the groups at 48 h. The animals remained active, alert and healthy throughout entire study period. The topical application of seabuckthorn seed oil and seabuckthorn leaf extract on wounds was non irritating and clinically safe.

The efficacy of different treatments was monitored by clinical, haematological, histopathological and microbial examination at 0, 3rd, 7th, 14th, 21st and 28th day. The gross finding revealed that wound dressed with 5% povidone iodine showed better healing in early stage of wound healing i.e. up to 7 days. However, afterward healing was better in seabuckthorn leaves extract followed with seabuckthorn seed oil as compared with the other groups. This was characterized by early shedding of scab, greater amount of granulation tissue formation and comparatively more % wound contraction at every subsequent observation intervals. Microbial examination of wounds on 21st day and onwards, revealed absence of inoculated organism from the wound site. Histopathological evaluation of wound healing process revealed that negative (G1) and positive (G44) control groups and the treatment groups (G1 and G3) showed marked differences in epithelization, amount of granulation tissue, collagen formation and infiltration of inflammatory cells (Table 1).

The comparative histopathological scores in treatment groups showed that seabuckthorn leaf extract group performed better as compared to seabuckthorn seed oil and even positive control with povidone iodine. Microscopically examination of healing tissue in negative control showed complete epithelization with moderately thick epithelium forming rete pegs. The upper and deeper dermis was filled with granulation tissue, characterized by parallely arranged fibrocytes/fibroblasts and perpendicularly arranged capillaries (angiogenesis). The granulation tissue showed

Table 1. Histopathological score in different treatment groups of rabbits

Parameters/ no. of animals	Group 1 (Negative control)			Group 2 (SBT leaf extract group)			Group 3 (SBT seed oil group)			Group 4 (Positive control)		
	I	II	III	I	II	III	I	II	III	I	II	III
Epithelization	++	++	++	++	++	++	+++	+++	+++	+++	+	++
Granulation tissue	++	++	++	++	++	++	+++	++	++	+++	+++	+++
Collagen formation	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++
Infiltration	+	+	+	+	+	+	+++	+++	+++	+	+	+
Total Score	7	7	7	7	7	8	12	11	11	10	8	9
HP Score	7	11.33	9	7.33								

Each parameter was evaluated on the scale of 1 (+) to 3 (+++): epithelization (thin epithelization, 1; moderately thick, 2 and very thick, 3); granulation tissue (mild, 1; moderate, 2 and thick, 3); collagen formation (mild,1; moderate,2 and thick,3); and infiltration (mild,1; moderately,2 and heavy,3). The grading score of each animal of the same group was added and subtracted by the number of animals in each group to obtain the histopathological score. The group showing higher HP score was considered for better healing than the lower score.

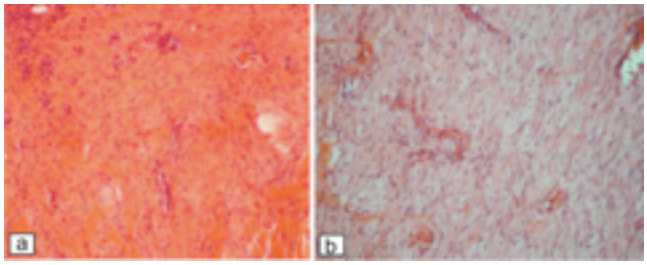

Fig. 1. H and E stained paraffin embedded skin sections of rabbits showing epithelization and granulation tissue formation at the wound site. The SBT leaf extract treated group (a) showed dense granulation tissue and its re-modelling than SBT oil group (b).

mild to moderate amount of collagen tissue indicating remodelling. In positive control group, the healing process was slightly better as compared with negative control group. In SBT seed oil group, the healing was even better than the controls, characterized by complete epithelization and presence of fibrocytes of mature phenotype in granulation tissue along with mild infiltration with mononuclear cells. The SBT leaf extract group showed better epithelization, and formation of more granulation tissue having more mature collagen fibers and more infiltration with chronic cells (plasma cells, lymphocytes, macrophages and eosinophils) around the vessels (Fig 1) compared to controls and the SBT seed oil group.

SUMMARY

In-vivo (wound healing) activity of methanolic extract of SBT leaves and seed oil was studied by excisional-cutaneous wound model in 12 rabbits which were divided into 4 groups. Group 1 was dressed with base, group 2 was dressed with 5% SBT leaf extract, group 3 was dressed with seed oil and group 4 was dressed with 5% povidone iodine. The effect of topical application was judged by clinical observations, haematological parameters and % wound contraction. These findings were also confirmed by histopathological examination. Results from these findings suggested that SBT leaf extract and seed oil may be used as natural antimicrobial agents and significantly enhanced wound contraction in rabbits.

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REFERENCES

Benjamin M M. 1995. *Outline of Veterinary Clinical Pathology.* Kalyani Publishers.

Gupta A, Pal K, Singh V and Sawhney R C. 2001. Healing potential of seabuckthorn (*Hippophae rhamnoides*) on cutaneous wounds in albino rats. *Proceedings of International workshop on Seabuckthorn* Feb.18–21, New Delhi.

Luna L G. 1986. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*. 3rd edn. Mc.Graw hill.

Mahajan A, Sharma S K, Asrani R K, Varshney A C, Tyagi S P and Amit K. 2005. Efficacy of seabuckthorn (*Hippophae rhamnoides*) in the healing of infected cutaneous wounds in calves. *Indian Journal of Veterinary Surgery* **26**: 31–33.

Mingyu X, Xiaoxuan S and Jinhua C. 2001. The medicinal research on seabuckthron. *Proceedings of International Workshop on Seabuckthron.* 18th- 21st Feb. 2001. New Delhi, India.

Shipulina L D, Tolkachev O N, Krepkova L V, Bortnikova V V and Shkarenkov A A. 2006. Anti-viral, anti microbial and toxicological studies on seabuckthorn (*Hippophae rhamnoides* L.). Seabuckthorn (*Hippophae* L.)-A multipurpose wonder plant. Vol-II. *Biochemistry and Pharmacology*. 471–83.

Varshney A C, Tyagi S P, Kumar A and Singh V. 2005. Studies for the evaluation of anti-inflammatory activities of seabuckthorn (*Hippophae rhamnoides*) oil in canine. *Global Seabuckthron Research* and *Development* **2**: 7–10.