Research Article **ISSN:2321-2624**

International Journal of Medicine and Pharmaceutical Research
www.pharmaresearchlibrary.com/ijmpr

Wound Healing Efficacy of Leaf Extract of Sea Buckthorn Species (*Hippophae Rhaminoides*) in Sprague Dawley Rats

Namrata Sharma*, RD Patil, Ajay Thakur, VK Gupta

Department of Veterinary Pathology, College of Veterinary & Animal Sciences,
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062, H.P, India

Abstract

Wound healing is an important biological process involving tissue repair and regeneration that results in restoration of tissue integrity. The present study was undertaken in Sprague dawley male rats to evaluate the healing efficacy of leaf extract of *H. rhaminoides* (HR) and compared to reference control (Povidine iodine) and placebo control (Propylene glycol) using full thickness excised wound model. Animals were divided into three groups and were applied Propylene glycol, Povidine iodine solution, 1% (w/w) HR leaf extract once daily on the 4mm and 8mm full thickness excised wounds. Percentage wound contraction measured after every 2 days interval revealed that HR had significantly ($P<0.05$) faster reduction in wound on day 2, 4, 6, 8 in comparison to other groups. HR showed significant anti-inflammatory response, angiogenesis and proliferation of the fibroblast maturation. The histological examination confirmed superior healing efficacy of the HR. *H. rhaminoides* leaf extract was found to be superior for wound healing when compared to Povidine iodine.

Keywords: *H. rhaminoides,* wound healing, angiogenesis, epithelisation.

Contents

Corresponding author
Namrata Sharma
E-mail: rhyme2namu@gmail.com
MS. ID: PRL2014-IJMPR1976

PAPER-QR CODE

1. Introduction

The processes of wound healing involve a variety of biological responses, such as an acute inflammation, cellular proliferation and a contraction of the collagen lattice formed (1, 2). The healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events resulting in the formation of hypertrophic scars (3, 4). The sequence of events that repairs the damage is categorized into three overlapping phases: inflammation, proliferation and tissue remodelling. Several drug classes have been used in the management of wounds. Among these are the antibiotics Penicillin and streptomycin have been widely employed in combating post- operative infections in man and animals (5). The antibiotics are chosen based on their ability to destroy or inhibit the growth of pathogenic organisms, while the tissue is left unharmed. Recently, the use of antibiotics has got

lot of criticism due to adverse and unwanted results (6) such as undesired residue in animal products viz. meat, milk or eggs, residua in tissues, long withdrawal period, and development of resistance in microorganisms, allergies, genotoxicity and harmful effects on human health by development of microbial resistance to specific products. In the last few decades the use of herbal remedies has risen in the developed countries. In this connection, plants continue to be a rich source of therapeutic agents.

The active constituents of many drugs are found in plants or are produced as secondary metabolites (7). Traditional herbal medicines used by different communities in different region play an important role in alleviating different diseases (8). They are safe, effective and inexpensive Nature has a treasure of medicines to treat all kinds of ailments. But there is a lack of standardization or documentation of identification characters which is the major backslaps of herbal medicines. Therefore the correct and scientific identification of these plants is quite necessary to get the full therapeutic impact of the drugs (9). India is one of the world's mega biodiversity center having more than 45000 different plant species. However, only 7000-7500 species are used for their medicinal values by traditional communities (10). Seabuckthorn (*Hippophae sp.)* is one among the widely known important medicinal plants.

The plant has got tremendous potential for therapeutic utilization in human and veterinary medicine, as it is reported to possess many bioactive substances (11). Seabuckthorn leaves have been found to be effective in wound healing (12), a number of steroids, flavonoids and vitamins (particularly Vit. E and Vit. K) found in seabuckthorn were thought to be responsible for its versatile pharmacological activities as anti-inflammatory (12, 13), chemical and physical burn wound healing activity and antimicrobial activities (14). Present study was designed to compare *H.rhaminoides* (grown in India) for their potential in soft tissue healing using full thickness dermal excision wound model in rats with the objectives to find out the optimum concentration of Sea buckthorn leaf extract for wound healing and to evaluate for their wound healing potential in rats in comparison with reference control.

2. Materials and Methods

The study was conducted in the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Palampur, HP, India**.**

Preparation of hydro-alcoholic extract

The leaves of seabuckthorn (HR) were collected from the hilly regions of Kukumseri Distt. Lahaul (HP). 100g dried leaves were ground to fine powder and soaked in 800ml water-ethanol mixture (1:1v/v) for 24 hrs at room temperature with intermittent shaking. The supernatant was filtered using fine muslin cloth followed by Whatman filter paper no.41.The filterate was concentrated into semisolid form using vacuum evaporation (Rota Vap, Bucchi). The contents were freeze dried using lyophilizer (Lyoplus) in a round bottom flask for 4-5 hrs until dark brown crystalline form was achieved. Percent dry weight recovery was estimated using the following formula.

% wt. recovery= (wt. of the extract /wt. of sample powder taken) X 100.

Experimental Design

Sprague Dawley male rats (200-250g weight) were procured from National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab. All the experimental procedures were performed according to the guidelines of Institutional Animal Ethical Committee (IAEC) after due approval from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were housed in polypropylene cages with autoclave sterilized rice husk and paper shredding as a bedding material. Full thickness aseptic excision wounds of 4mm size in the front and 8mm size in the rear were created on either side of the dorsal midline of the body (12, 15). The animals were anaesthised using intra peritoneal injection of combination of Ketamine@60 mg per kg and Xylazine @ 5 mg per kg body weight. Biopsy punch of 4mm and 8mm size applied to make wound on the skin on both sides. The animals were treated with 1.0% HR 20 microliter extract topically (12).

Wound healing potential of *H. rhaminoides*

The wound healing efficacy of seabuckthorn plant *H. rhaminoides* (HR) was evaluated and compared with the placebo control (PC) containing Propylene glycol and reference control (RC) Povidine iodine. 54 rats were used with 18 rats in each group. 6 rats were sacrificed on day 2, 6, 14 post surgery (15). The rats were examined for any clinical and behavioural changes including body temperatures after surgery until 2 consecutive readings were obtained. The wound area was measured grossly on alternate day that is day 0, 2, 4, 6, 8, 10, 12, 14 post excision.

Histopathological analysis

Histological assessment was done using semi-quantitative method (15) to evaluate following histolological processes and structures: reepithelization, polymorphonuclear leucocytes (PMNL), fibroblast, new vessels and new collagen and ratio of neutrophils and macrophages depending on their concentration in the granulation tissue and the surrounding tissue. Tissue sections were evaluated according to the scale: 0-absent, 1- mild, 2- moderate, 3- marked and 4- severe by two independent observations. The excised tissues were collected in 10% neutral buffered

formalin(NBF) for 72 hours and then processed by paraffin embedding technique for histological evaluation using routine Haematoxylin and Eosin stain as per the standard protocol (16).

Micrometry

Digital Olympus Camera (8.1 mega pixel) mounted on to a BX-40 Olympus microscope was used for microphotography. The exposure were done using LBD-IF (Natural) filter. The magnification bars were generated using micrometer scale.

Statistical Analyses

Data generated during the study are expressed as mean ± Standard error. For Wound healing efficacy statistical significant difference was analyzed using one way ANOVA followed by Duncan's test (SAS software). For Histological parameters statistical significant difference analyzed using non parametric ANOVA followed by Krush Kal Wallis test using Graph Pad Software, (San Diego, California, USA).

3. Results and Discussion

Wound Healing Efficacy

Gross observations:

At day 2 the borders of the wound were slightly raised and the wound cavity was completely covered by thick semidried scab with no exudation. The animals in HR group showed maximum and significant ($P<0.05$) wound contraction up to 36.16% wound contraction showing significant difference over that of RC and PC group (Table 1). At day 4 and 6 HR showed significant wound contraction over PC and RC treated groups. While at day 8 wound gap was filled up to the extent of 84.83% in HR group. The wound contraction in HR group was significantly superior to all other groups. By day 12 and 14 complete contraction of wound was noticed in all the groups. Grossly the PC and RC treated groups showed slow healing when compared with the HR. The leaf extract of seabuckthorn enhanced wound healing activity, which may be due to the increased antioxidant levels in the granulation tissue. The leaf extract of seabuckthorn enhanced wound healing activity, which may be due to the increased antioxidant levels in the granulation tissue. The presence of antioxidants such as beta-carotene; ascorbate and vitamin E (alpha-tocopherol) in seabuckthorn must have contributed to reduction in levels of LPO and increases enzymatic antioxidants.

Table.1 Wound Contraction Percentage along various groups at different time intervals

Group	Day 2 (N=18)	Day 4 (N=12)	Day 6 (N=12)	Day 8 (N=6)	Day 10 (N=6)
PC	21.22^{c} ±1.61	37.75^{c} ±3.38	55.08^{b} ±3.21	77.00^{b} ±4.21	94.33^{a} ±1.47
RC	25.50^{c} ±1.71	40.16^{c} ±2.38	60.50^{b} ±2.89	79.83^{b} ±3.17	93.5^{a} ±2.01
HR	36.16^{b} ±2.09	48.75^{b} ±2.20	68.08^{a} ±1.99	84.83^{a} ±2.21	95.66^{a} ±1.17

Values are Mean ±standard error, Values having superscripts in common do not differ significantly ($P\leq0.05$

Histopathological and Histochemical analysis

2nd day post treatment/excision

There was heavy infiltration of polynuclearleukocytes(PMNL) in PC group. A few fibroblasts were evident migrating from the surrounding tissue and neo angiogenesis was evident in the deeper dermis in wound gap. The epithelium started proliferating from the wound edge and covered upto a quarter of gap (23.25%). In RC group, epithelium also proliferated in all the cases but the extent remained more or less same (26%) as that of PC group. HR treated wounds revealed significant inhibition of inflammation as evident by low level of PMNL infiltration as compared to PC group. There was significantly higher proportion of infiltration with macrophages at the wound edges and new vessels in the deeper wound tissue than PC group.

At 6th day post treatment/excision

In PC group, the epithelization completely bridged all the wounds except one. There was mild PMNL infiltration in the granulation tissue. The granulation tissue consisted of mild to moderate proliferating fibroblasts and new blood vessels. There were minimal unorganised collagen fibres. The average thickness of the proliferating epithelium was 41.81µm. In RC group the epithelization was delayed as compared to PC group though the difference was not significant. The epithelium was not able to bridge three of the wounds and its average thickness was 36.67µm.The level of inflammation, fibroblast proliferation, collage fibres, new blood vessels formation was comparable to PC group. In HR treated wounds the level of epithelization was comparable to PC group in terms of bridging the wound gap though thickness of epithelium 45.56µm was more than RC group. The level of inflammatory reaction was mild and significantly lower than PC group.

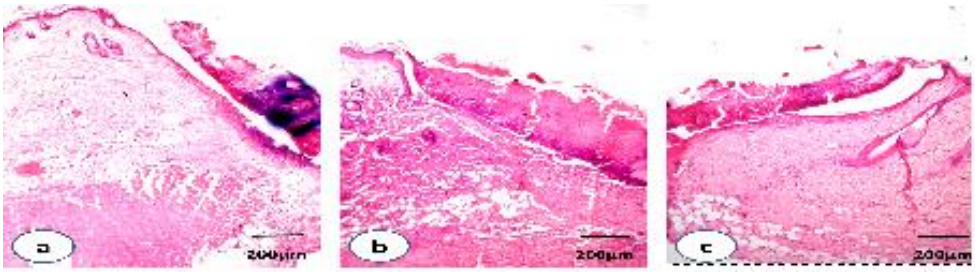

Fig.1 Histological section of healing wound after application of various treatments showing extent of epithelization (arrows) at day 2 post excision. (H&E stain) a: PC group; b: RC group; c: HR group

14th day post treatment/excision

At day 14 post excision the epithelium was fully mature with keratinization in all the treatment groups. However it was more thickened in RC group as compared to other treatments suggesting delayed maturation in Povidine iodine treatment. There was no trace of inflammation in any of the treatments including control groups. There was complete epithelial maturation in the HR group compared to other groups. The wound healing process is though complex, but follows a definite pattern of cellular and molecular events. 1.0% (w/w) HR lyophilized leaf extract is the most effective lowest concentration for excision wound healing activity in rats (12). Seabuckthorn is known to contain many bioactive molecules including 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 (17).

A comparatively less pronounced inflammatory reaction in HR group might have been due to the anti-inflammatory and bacteriostatic properties of seabuckthorn (13) also due to biochemical constituents (flavonoids, tri-terpines, betaine, vitamins and mineral elements) which enhance the healing process mainly due to high contents of flavonoids in the leaves (3.8-4.0 %), (18). The stronger inflammatory reaction in the cutaneous wounds of RC group is due to the fact that betadine destroys fibroblasts and healing tissues (19). It has been reported that the use of betadine in wounds slows healing and causes injury and death to the tissues (20) besides cytotoxic damage (21) and thus delays healing process. Anti-inflammatory effect, faster and better epithelization, increased vascularisation and more deposition of collagen fibres is indicative of better wound healing potential of seabuckthorn particularly *H.rhaminoides*. Ianev *et al.* (22) reported that in seabuckthorn group the epithelization is more intensive and occurs earlier, and granulation tissue differentiation (collagen fibers, profuse vascularity) is quicker, by comparison with other groups. Seabuckthorn fruit and seed oil preparation used in experimental healing of excised wounds in different species of animals were found to be leading to lesser inflammatory reaction, faster wound contraction, earlier granulation tissue response and remodeling of the repair tissue (12).

4. Conclusion

H. rhaminoides leaf extract was found to be superior for wound healing when compared to Povidine iodine. *H.rhaminoides* showed anti-inflammatory properties besides early induction of fibroblasts, angiogenesis and epithelization. Sea buckthorn has antioxidant properties promoting wound healing properties.

5. References

1. G Bodeker; MA Hughes. Plants for Food and Medicine, 1998, 1(1), 345-359.
2. RJ Suresh; PR Rao. J. Ethnopharmacol., 2002, 79(5), 249- 251.
3. RAF Clark. Wound Healing, 1993, 11(2), 647-666.
4. M Sumitra; P Manikandana. Int. J. Biochem and Cell Biol., 2005, 37(3), 566- 573.
5. EO Gyang. Introduction to Animal Surgery, Agitab Publishers. Nigeria. 1986.
6. NA Botsoglou; DJ Fletouris. Drug Residues in Foods. Pharmacology, Food Safety and Analysis. New York, Marcel Dekker. 2001; 541-548.
7. MR Remya; PS Oommen. Int. J. Current Trends in Pharmaceutical Research, Pharma Research Library. 2013, Vol. 1(1), 12-18.
8. Girendra Kumar Gautam; Devendra Pratap Singh; Umesh Dhakad; Ankit Saini; Ravi Krishna. Int. J. Chemistry and Pharmaceutical Sciences, Pharma Research Library. 2014, Vol. 2(1), 576-580.
9. Chowdhury Saikat; K Nishteswar; CR Harisha. International Journal of Current Trends in Pharmaceutical Research, Pharma Research Library. 2013, Vol. 1(1): 45-53.
10. AS Burungale; BS Shinde; SV Thite; BA Kore; VT Aparadh. Int. J. Chemistry and Pharmaceutical Sciences, Pharma Research Library. 2013, Vol.1 (2): 117-121.

11. E Risto; Y Baoru. Agro Food Industry Hi-tech., 2003, 32, 53-57.
12. A Gupta; R Kumar; K Pal; V Singh; PK Banerjee; RC Sawhney. Molecular Cell Biochemistry, 2006, 290, 193-198.
13. W Zang. Seabuckthorn, 1988, 1, 27-30.
14. AA Nikulin; EN Yukusheva; NM Zakhorova. Experimental Nayal Klinicheskaya Farnakologiya, 1992, 55, 64-66.
15. P Gal; R Kilik; M Mokry; B Vidinsky; T Vasilenko; S Mozes; N Bobrov; Z Tomori; Z Bober; L Lenhardt. Veterinarni Medicina, 2008, 53(12), 652–659.
16. L Luna. Manual of Histologic Staining Methods of the AFIP, 3rd Ed, pp 94-95, McGraw-Hill, NY. 1968.
17. P Zhang; X Ding; L Mao; D Li; L Li. Seabuckthorn, 1989, 2(3), 31-34
18. DB Rasputina; NF Kommissarenko; DT Tsybikova. Khim. Priroden. Soedin, 1976, 1(5), 96-97.
19. G Lynne. Wound management forum-Archive, 1999, 8(2), 0-52.
20. Ollie. Wound management forum-Archive, 1999, 9, 4-8.
21. G Rodeheaver. Wounds: A compendium of clinical research and practice, 1989, 1, 99-105.
22. E Ianev; S Radev; M Balutsov. Khirurgiia (Sofiia), 1995; 48:30-33.