Progress in Nutrition 2015; Vol. 17, N. 3: 245-249

Original article

Investigation of the wound healing potential of *Onosma hispidum* root extract in rabbit models

Asad Ali, Fatma Hussain, Muhammad Shahid

Department of Chemistry and Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad-38040, Pakistan.

Summary. *Onosma hispidum* Wall. (Boraginaceae) has historically been used to treat a wide assortment of diseases. Present study was conducted to investigate the wound healing activity of *Onosma hispidum* root extracts in animal models. Excision wound were inflicted upon four groups of five rabbits each. Group 1 assigned as vehicle (negative control) was treated with petroleum jelly. Group 2 and 3 were treated with *O. hispidum* MeOH and n-hexane extracts. Group 4 (positive control) was accorded standard drug polyfax. Healing was assessed by the percentage of wound contraction and histopathological analysis. Animal models treated with n-hexane and MeOH extracts ointments of *O. hispidum* had significantly ($p < 0.05$) positive healing effects as compared to the negative controls. Significant reduction (54-56%) in terms of percentage wound contractions were observed in plant treated groups as compared to the vehicle control. *O. hispidum* treatment resulted in partially developed dermal layer with stratified squmaous, stratum corneum and stratum germinatium development. Although major bioactive principles involved are yet to be determined, this investigation reveals the potential of *O. hispidum* for use as a natural wound curing agent.

Key words: *Onosma hispidum,* excision wounds, petroleum jelly, wound contraction, histopathology

Introduction

Wound is defined as the loss of breaking cellular and functional continuity of the living tissues. Wounds offer psychosocial issues and their management is frequently encountered with different problems such as drug resistance and toxicity (1).

The modern medicine faces the challenge to pursuit dynamic and low cost therapeutic approaches for wound healing. In this context, plants and their metabolites being great sources of novel biomolecules offer potent treatment options. Today a substantial number of drugs are developed from plants that are active against numerous diseases. About 450 plant species having wound curing properties have been identified (2-8). Majority of these are either the active ingredient or their subsequent modifications. Multiple phytochemicals concentrated and blended in optimal concentrations, are expected to be available in future to optimize wound healing as more curative properties of the key constituents are unveiled (9-11).

The genus *Onosma* L. (Boraginaceae) includes about 150 species distributed worldwide. The plants of this genus possess anticancer, antioxidant, antimicrobial, antipyretic, antidiabetic, antitussive and spasmolytic activities and traditionally used in rheumatism, bladder pain, kidney irritation, palpitation of heart. While roots are used as astringent, demulcent and diuretic agent and to treat hypertension, fever, pain and inflammatory disorders (12-14). *Onosma hispidum* Wall. possess antiseptic, antipyretic, antibacterial, anthelmintic, hypoglycemic and cholinesterase inhibitory activities. Its role in wound healing, foot ulcer, optical diseases, bronchitis and itch is well recognized (15-20).

O. hispidum Wall. is reported to be the source of ratanjot, a red dye yielding root, commonly used for col-

oring food stuffs, oils and medicinal preparations. Owing to its color, it has also been used as an adulterant in spices like chilli powder and food preparations (1).

Wounds are enormous socioeconomic encumber. Development of economical, effective agent with fewer side effects that could preclude hospitalization rate is the prerequisite. Pharmacological objectives of wound healing implicates the assessment of therapeutic options opted to endorse healing. In folklore medicine, *Onosma hispidum* has been used as a wound healer for thousands of years; however, there is a paucity of scientific data in support. With multiple medicinal mechanisms, the natural products demand further endorsement. Herein, we intend to investigate the wound healing activities of MeOH and *n*-hexane root extracts of *O. hispidum* in excision wound-induced rabbit models, so as to provide scientific evidence for the traditional application.

Materials and methods

Plant material and preparation of extract

Onosma hispidum roots were authenticated by the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Shade-dried, powdered material was homogenized with methanol (MeOH) and *n*-hexane. The final yields of MeOH (5.7%) and *n*-hexane (3.6%) extracts were prepared in 0.5% w/v carboxymethylcellulose.

Animal models

Male rabbits (*Oryctolagus cuniculus*) were housed in the metal cages (three per cage) with free access to standard feed, tap water *ad libitum*. The study protocol was approved by the Bioethical Committee, University of Agriculture, Faisalabad, Pakistan.

Determination of Wound Healing Activity

Animals were divided into the four groups (five per group): Group 1 (negative control; vehicle): treated with petroleum jelly without *O. hispidum* extracts; Group 2: treated with petroleum jelly ointment containing *O. hispidum* MeOH extract; Group 3: treated with petroleum jelly ointment containing *O. hispidum n*-hexane extract; Group 4 (positive control): treated with standard drug polyfax (GlaxoSmithKline). Animals were anaesthetized prior to and during excision wound induction. The plant extracts and the standard reference drug (polyfax) were applied once daily till complete healing of the wound. Specimens of skin were isolated from the healed skin of each group for the microanatomy (20-22). Data was analyzed by t-test with significance level set at $P<0.05$ (SPSS; version 12.0, 2003 ® SPSS Inc., Chicago, IL, USA).

Results

The effect of two different preparations, MeOH and *n*-hexane extracts, from *O. hispidum* roots on wound curing were investigated under the established conditions. The measurements of the progression of wound healing induced by the extracts, reference drug, negative and vehicle groups are shown in figure 1. MeOH extracted ointment had highest curing potential after 3 days applications. However, *n*-hexane ointment dominated MeOH in terms of percent wound contraction and it showed competitive potency after 7 and 9 days as compared to the standard ointment. The groups treated with *n*-hexane and MeOH extracts ointments had sig-

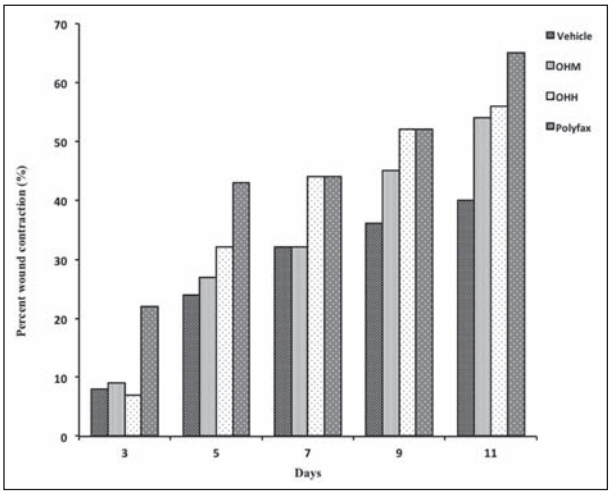

Figure 1. Comparative percent wound contraction of control and treatment groups. Values are averaged percent wound contraction. Vehicle: negative control; OHM: group given *O. hispidum* methanol extract; OHH: group given *O. hispidum* n-hexane extract; Polyfax: group given polyfax skin ointment

nificantly ($p < 0.05$) positive healing impact as compared to the negative controls. At the end of experimental period, measurements of the progression of wound healing induced by the extracts, reference drug and negative groups were done on per diem bases. Wound healing is a natural physiological process. Hence, for untreated animals, 40% contraction was observed. Whereas, in MeOH and *n*-hexane treated models, it was 54 and 56% respectively. Such a remarkable wound restoration by plant extracts were comparable with the results of reference drug (65%).

Wound histology was performed to verify the wound healing activity of the *O. hispidum*. Wound repairing involves regeneration of dermal and epidermal tissue. Therefore, in the first group; epidermis which is composed of stratified squamous epithelium is thin and consists of four layers of stratum corneum and stratum germinatium. The dermal layer which is composed of dense singular connective tissue is partially developed. Papillary layer (subepithelial layer) of dermis has very thin projection in the dermal connective tissue (fig. 2a). In second group (methanol extract treated), proliferation of cells of epidermal layer is quite prominent. Layer of stratified squmaous, stratum corneum and stratum germinatium are developed. Subepithelial layer is prominent. Dermal layer of dense connective tissue is normal in thickness (fig. 2b). In third (*n*-hexane extract treated) group, there is high proliferation of cells of epidermal layer. Layer of stratified squmaous epithelium is fully grown. Layers of stratum corneum and stratum germinatium are fully developed. Subepithelial layer is quite prominent. Dermal layer of dense connective tis-

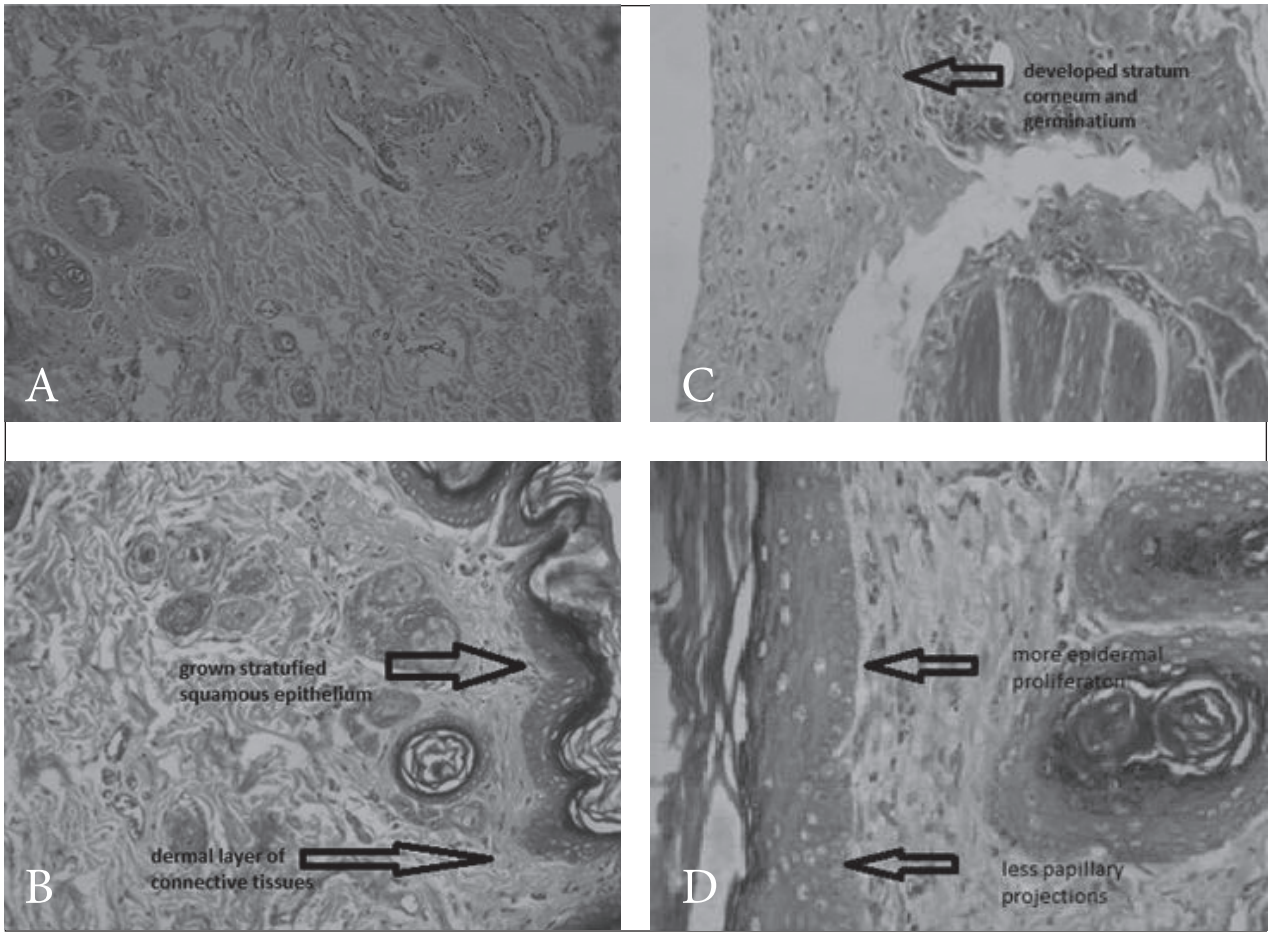

Figure 2. Microscopic view of wound healing. a- wound treated with petroleum jelly with partially developed dermal layer; b & c- methanol and n-hexane extracts treated wounds with stratified squmaous, stratum corneum and stratum germinatium development and dermal layer of connective tissues; d- Polyfax treated wound with epidermal proliferation and fewer papillary projections

sue is fully grown (fig. 2c). While in fourth group (Polyfax treated), proliferation of epidermal layer is relatively more as compared to the rabbits of the control group. Papillary projections into the dermal layer are less as that of control group (fig. 2d). Microanatomy revealed that all the extract treated models showed almost similar regeneration patterns in dermal and epidermal layers as compared to those treated with reference drug.

Discussion

The search for natural remedies has drawn attention to plants. Flavonoids, alkaloids, saponins and phenolic compounds are active constituents that facilitate wound closure. Plants of Boraginaceae family contain naphthoquinones, shikonin and alkannin that contribute wound curing properties. Naphthoquinones are major chemical constituent of *O. hispidum* roots (19).

Present study explored the wound healing activity of *O. hispidum* by *in vivo* models. Wound healing is a complex phenomenon involves various phases e.g. coagulation, inflammation, collagenation, wound contraction and epithelization. Both the extracts showed remedial potency. Current inferences are in accordance with that of previous studies. It was observed previously that *O. hispidum* root methanol extract exhibited significant increase in mean percentage wound contraction and tensile strength in excision and incision wound models in both normal and diabetic rats (20). Moyer et al. (23) stated that *O. hispidum* might provoke faster maturation of granulation tissues. Perhaps it is able to promote the granulation tissue, myofiroblast proliferation and contraction for the faster closure of wound. These pharmaceutical properties of *O. hispidum* may engross experiential wound healing potential. They illustrated that the faster wound contraction rate from the *O. hispidum* treatment may be due to antioxidant, antimicrobial, and antiseptic properties of the plant extract and to stimulation of interleukin-8, an inflammatory *a*-chemokine that affects the function and recruitment of various inflammatory cells, fibroblasts, and keratinocytes. *O. hispidum* may increase the gap junctional intracellular communication in cultured fibroblasts and induce a more rapid maturation of granulation tissue. Contrary to that, negligible restorative effects of *Onosma dichroanthum* roots extracts on the burn wound in animal models were documented (24). Previously, some species of Boraginaceae have shown accelerative effects on granuloma tissues proliferation owing to the presence of naphthoquinone derivatives (25, 26).

Survey of literature has revealed that several Boraginaceae plants *viz. Alkanna tinctoria, Cordia dichotoma, Onosma argentatum, Symphytum x uplandicum, Arnebia densiflora, Heliotropium indicum, Symphytum officinale, Echium amaenum, Helichrysum graveolens* have demonstrated their potent role in wound healing (27-35).

The exact mechanism of current healing by plant extracts is uncertain. Though, contribution of phytoconstituents in wound curing can be narrated in terms of their antimicrobial, antioxidant, mitogenic activities, as free radical scavengers and angiogenesis enhancers. These chemicals especially, naphthoquinones not only affect wound restoration phases positively but also constrain the factors that may dwindle the curative process (10). The antioxidant activity of plants extract may be ascribed to biologically active polyphenolic bioflavonoids that facilitate wound closure. *O. hispidum* extracts demonstrated considerable wound healing activity. Although, which phytochemical(s) of the extracts are responsible for this effect, is not investigated in detail yet (19, 36).

In conclusion, it can be stated that *O. hispidum* roots exhibited promising wound healing properties and data obtained may contribute towards validation of its traditional use for the healing of wounds.

References

1. Khan AA, Kumar V, Singh BK, Singh R. Evaluation of wound healing property of Terminalia catappa on excision wound models in wistar rats. Drug Res (Stuttg) 2014 64: 225-8.
2. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing-exploring medicinal plants in India. J Ethnopharmacol 2007; 114: 103-13
3. Ayyanar M, Ignacimuthu.S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. Int J App Res Nat Prod. 2009; 2: 29-42.
4. Bhaskar, Anusha, Nithya V. Evaluation of the wound-healing activity of Hibiscus rosa sinensis L (Malvaceae) in Wistar albino rats. Indian J Pharmacol. 2012; 44: 694.
5. Chen WC, Liou SS, Tzeng TF, Lee SL, Liu IM. Wound repair and anti-inflammatory potential of Lonicera japonica in excision wound-induced rats BMC Complement Altern Med. 2012; 12: 226.

6. Asadi SY, Parsaei P, Karimi M, et al. Effect of green tea (Camellia sinensis) extract on healing process of surgical wounds in rat. Int J Surg. 2013; 11: 332-37
7. Khan AA, Kumar V, Singh BK, Singh R. Evaluation of Wound Healing Property of Terminalia catappa on Excision Wound Models in Wistar Rats. Drug Res. (Stuttg) 2014; 64: 225-28
8. Lodhi S, Singhai AK. Wound healing effect of flavonoid rich fraction and luteolin isolated from Martynia annua Linn. on streptozotocin induced diabetic rats. Asian Pac J Trop Med. 2013; 6: 253-59.
9. Wang R, Lechtenberg M, Sendker J, Petereit F, Deters A, Hensel A. Wound-healing plants from TCM: in vitro investigations on selected TCM plants and their influence on human dermal fibroblasts and keratinocytes. Fitoterapia. 2013; 84: 308-17
10. Ghosh PK, Gaba A. Phyto-extracts in wound healing. J Pharm Pharm Sci 2013; 16: 760-820.
11. Pazyar N, Yaghoobi R, Rafiee E, Mehrabian A, Feily A. Skin wound healing and phytomedicine: a review. Skin Pharmacol Physiol 2014; 27: 303-10.
12. El-Shazly A, El-Domiaty M, Witte L, Wink M. Pyrrolizidine alkaloids in members of the Boraginaceae from Sinai (Egypt). Biochem Systems Ecol. 1998; 26: 619-36
13. Ahmad I, Anis I, Malik A, Nawaz SA, Choudary MI. Cholinesterase inhibitory constituents from Onosma hispida. Chem Pharma Bull. 2003; 51: 412-14
14. Papageorgiou VP, Assimopoulou AN. Lipids of the hexane extract from the roots of medicinal boraginaceous species. Phytochem Anal. 2003; 14: 251-58
15. Naz S, Ahmad S, Rasool SA, Sayeed SA, Siddiqi R. Antibacterial activity directed isolation of compounds from Onosma hispidum. Microbiol Res. 2006; 161: 43-8
16. Arora A, Gulrajani ML, Gupta D. Identification and characterization of Ratanjot (Arnebia nobilis Reichb. F.). Nat Prod Rad. 2009; 8: 142-45
17. Kumar N, Gupta AK, Parkash D, Kumar P. Hypoglycemic activity of Onosma hispidum (Ratanjot). Int J Diab Dev Ctries 2010; 30: 213
18. Fahad S, Bano A. Ethnobotanical and physiological studies of some endangered plant species collected from two different altitudes in Gilgit Baltistan. Pak J Bot 2012;44: 165-70
19. Khajuria RK, Jain SM. Two new naphthoquinones from the roots of Onosma hispidum. Indian J Chem B 1993; 32: 390-91
20. Kumar N, Gupta AK. Wound-healing activity of Onosma hispidum (Ratanjot) in normal and diabetic rats. J Herbs Spices Med Plants. 2010; 15: 342-51
21. Morton JJP, Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. Arch Int Pharmacodyn. 1972; 196:117-26.
22. Sadaf F, Saleem R, Ahmed M, Ahmad SI, Navaid-ul Z. Healing potential of cream containing extract of Sphaeranthus indicius on dermal wounds in Guinea pigs. J Ethnopharmacol. 2006; 107:161-63
23. Moyer KE, Saggers GC, Allison DM, Mackay DR, Ehrlich HP. Effects of interleukin-8 on granulation tissue maturation. J Cell Physiol. 2002; 193: 173-79.
24. Moghaddam PZ, Zolfaghari MR, Ghaemi EA, Mazandarani M, Mansourian AR, Taheri SA. Negative performance of root extract of Onosma dichroanthum Boiss. on the burn wound healing in an animal model. Arch Clin Microbiol. 2011; 2: 3823/238.
25. Ozaki Y, Xing L, Satake M. Accelerative effect of nanshikon and its constituents on the proliferation of granulation tissue in rats. Biol Pharma Bull 1996; 19: 233-36
26. Shen CC, Syu WJ, Li SY, Lin CH, Lee GH, Sun CM. Antimicrobial activities of naphthazarins from Arnebia euchroma. J Nat Prod 2002; 65: 1857-62
27. Ogurtan Z, Hatipoglu F, Ceylan C. The effect of Alkanna tinctoria Tausch on burn wound healing in rabbits. Dtsch Tierarztl Wochenschr 2002; 109: 481-85
28. Kuppast IJ, Vesudeva N. Wound healing activity of Cordia dichotoma Forst. f. fruits. Nat Prod Rad 2006; 5: 99-102
29. Ozgen U, Ikbal M, Hacimuftuoglu A, et al. Fibroblast growth stimulation by extracts and compounds of Onosma argentatum roots. J Ethnopharmacol 2006; 104: 100-103
30. Barna M, Kucera A, Hladícova M, Kucera M. Wound healing effects of a Symphytum herb extract cream (Symphytum x uplandicum NYMAN: results of a randomized, controlled double-blind study. Wien Med Wochenschr 2007; 157: 569-74
31. Kosger HH, Ozturk M, Sokmen A, Bulut E. Wound healing effects of Arnebia densiflora root extracts on rat palatal mucosa. European J Dent 2009; 3: 96-9
32. Dodehe Y, Barthelemy A, Calixte B, Jean DN, Allico JD, Nelly F. In vitro wound healing effect of n-butanol fractions from Heliotropium indicum. JITPS 2011; 2: 1-7
33. Araújo LU, Reis PG, Barbosa LC, et al. In vivo wound healing effects of Symphytum officinale L. leaves extract in different topical formulations. Pharmazie 2012; 67: 355-60
34. Farahpour MR, Mavaddati AH. Effects of borage extract in rat skin wound healing model histopathological study. J Med Plants Res 2012; 6: 651-56
35. Süntar I, Küpeli Akkol E, Keles H, Yesilada E, Sarker SD. Exploration of the wound healing potential of Helichrysum graveolens (Bieb.) Sweet: Isolation of apigenin as an active component. J Ethnopharmacol 2013; 149: 103-10
36. Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M, Roy S. Oxygen, oxidants, and antioxidants in wound healing. Ann N Y Acad Sci 2002; 957: 239-49.

Correspondence:
Fatma Hussain,
Department of Chemistry and Biochemistry,
Faculty of Sciences, University of Agriculture,
Faisalabad-38040, Pakistan.
E-mail: fatmauaf@yahoo.com