Volume 3(2), 2023

Open Access

33-HJPV3(2)-2023 ORIGINAL ARTICLE

WOUND HEALING EFFECTIVENESS OF *BERBERIS BALUCHISTANICA* AND *DAPHNE OLEOIDES* EXTRACTS NATIVE TO PAKISTAN

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ABSTRACT

The study investigated the traditional claim of *Berberis baluchistanica* and *Daphne oleoides* plant extracts' wound healing potential in healthy male albino rats. The extracts were evaluated using excision and incision wound models, wound contraction, epithelization period, tensile strength, and histopathological studies. *D. oleoides* roots crude methanolic extract (CME) encouraged pronounced wound repairing process in both adopted wound models. In this case, boosted wound retrenchment rate ($P < 0.001$), shrinkage of epithelialization period (10.13 ± 0.11), high skin disintegration strength (410.0 ± 1.8) and remarkable enhanced granulation tissue weight ($P < 0.001$) were experienced in albino rats. As well as the granulation tissue histological examination exhibited more collagen with reduced inflammatory cells number propagation versus control. In *B. baluchistanica* stems CME treated animals, wound contraction, and granulation tissue content were not much prevailed being ($P < 0.05$) moderately high. The histology of the granulation tissue disclosed greater number of inflamed cells with less collagen than the *D. oleoides* treated animals. However, it was more amplified compared to control group. The collective research data emphasized that *D. oleoides* possesses significant wound management activity versus *B. baluchistanica*, furthermore both extracts rationalize the traditional claim to cure wounds and can be utilized in different wound healing therapies in human beings as well.

Keywords: *Berberis baluchistanica*, *Daphne oleoides*, excision and incision wound models, wound healing.

INTRODUCTION

Wound is an injury to living tissue caused by physical, chemical, microbial or immunological disruption of skin and wound healing is a restoration process of injured or damaged tissue to its pre-wound conditions. In order to reinstate the normal functions and to avoid complications, proper and effective wound healing management plays an important role **[1]**. Traditionally various plants were used for treatment of wounds and up till now several medicinal plants are scientifically proven for wound healing management **[2]**. The process of wound healing occurs in well-organized and orderly manner. The process progresses through four different stages of wound healing that is hemostasis, inflammation, proliferation and remodeling. Overlapping of these phases and involvement of specialized cells like platelet, neutrophils, macrophages, fibroblasts, epithelial and endothelial cells make wound healing a complex process. Hemostasis phase includes aggregation of platelets at injury site releasing cytokines and growth factor that interact with collagen to release fibrin forming a clot at injury point. Inflammation is second phase, which occurs through release of histamine and other factor from mast cells to start inflammation process. Neutrophils, macrophages and monocytes remove microbial contaminants and cleanse the wound during inflammation process. Release of cytokines, interleukins and growth factors trigger the fibroblast and epithelial cells to progress to next stage of healing. Fibroblast interacts with collagen and other proteins to form extracellular matrix that is deposited at injury site (proliferative stage). Epithelial cells also proliferate like fibroblast to make a thick layer over granulated tissue to close the wound surface. As the healing process progress, the density scar tissue decreases initially, removed and replace by extracellular matrix that resembles to normal skin. Remodeling stage is dependent of proteases that perform a balanced and regulated activity in wound healing. Each stage of wound healing has characteristic attributes that must occur to progress normal wound healing **[3]**. In the mainstream of developing countries about 70 to 95% of residents practice traditional medicine to address their healthcare requirements and concerns **[4]**. Several traditional medicinal plants were evidenced scientifically to be claimed as management for wounds and are cited in the Asian folk remedies for wound treatment **[2]**. Berberis berries were traditionally used in the form of decoction as blood purifier. In Ayurveda berberis extracts are used for wound healing, piles, hemorrhoids and various infections of ear, eye and mouth and also antidote for snake bite **[5]**. *Berberis baluchistanica* belongs to genus *Berberis* family Berberidaceae. *B. baluchistanica* is an endemic species, found mainly Mastung and Quetta district of Baluchistan. In local languages, plant is known as karoskai, zrolg and korae **[6]**. Traditionally *B. baluchistanica* is used for gastrointestinal diseases (diarrhea, ulcers and piles), hepatitis, eye diseases, internal injuries and snake bite antidote **[6]**. *Daphne oleoides*, a multi-branched evergreen shrub belongs to genus *Daphne* of Thymelaeaceae family. Three species of genus *Daphne* were identified in Pakistan. *Daphne oleoides* is indigenous to North Africa, Europe, and South Asia at altitudes of 3000-9000. *D. oleoides* in Pakistan is mainly found in Northern and Hilly areas **[7]**. *D. oleoides* is used in traditional medicines to cure different ailments such as gonorrhea, rheumatism, fever, inflammatory disease, cutaneous infections and malaria. Traditionally, aerial parts of *D. oleoides* plant were used in Turkish medicines for rheumatic pains, fever and lumbago, leaves for gonorrhea and boils **[8]**. In some area roots as laxative and leaves for ulcers and wound healing **[9]**. *Daphne oleoides* sub species *kurdica* wound healing applications are well established. Its extracts and fractions were revealed to exhibit anti-inflammatory action by inhibiting of macrophage cytokines, interleukin and TNF alpha **[10]**. Ethyl acetate and methanolic extract of roots exhibited significant antioxidant ability **[11, 12]**. Anti-nociceptive activity of methanolic extract has been reported for this species **[13]**. Gut modulatory potential of *D. oleoides* was reported by inhibiting cholinergic receptor and calcium channel **[14]**. Urease enzyme inhibition was linked with di- and trimeric coumarins isolated from *D. oleoides* CME. Hepatic encephalopathy, urolithiasis and nephrotoxicity is speeded up with urease enzyme eventually providing a suitable pH condition for H. pylori to grow causing gastric and peptic ulcers **[15]**. In our earlier research antidiabetic, antioxidant, antifungal, anti-microbial potential of methanolic extracts of these both plants (*B. baluchistanica and D. oleoides*) have been explored in detail **[12]**. The present investigations were planned to evaluate scientifically the traditionally practiced wound healing activity of *Berberis baluchistanica* stem and *Daphne oleoides* roots extracts.

MATERIAL AND METHODS

Plant material and preparation of the extract

B. baluchistanica stems and *D. oleoides* roots (Ref. No. DOF-MBD-0024 and DOF-MBD-0025 respectively) were collected from Quetta city of Baluchistan province (Pakistan), identified by Mr. Irshadullah (DOF) District Officer Forest in Mandi-Bahauddin Pakistan and authenticated by Professor Dr. Ghulam Abbas Miana, Riphah International University Islamabad, Pakistan. The plants were washed shade dried, turned into to fine powder and stored in closed vessel at room temperature. Powdered *B. baluchistanica* stems and *D. oleoides* roots (2.0 Kg each) were placed in percolators separately and submerged in methanol (10.0 liters). Intermittent stirring in percolator was carried out for two weeks before collection of methanolic extracts. The collected extracts were concentrated using rotary vacuum evaporator **[16]**. Percentage recovery of crude methanolic extracts (CMEs) from *B. baluchistanica* and *D. oleoides* was 65% and 56% respectively.

Animals

Healthy inbreed male albino rats of weight 220–235g were picked out from Amsons vaccines and Pharma Islamabad, Pakistan to conduct the experiment; reference number (AHC06/07/1). Animals were approved by Ethics Committee as described for diabetic section by Muddassir et al., 2022 and supplied with food accordingly **[12]**.

Wound healing Activity

Different models have been adopted for *in vivo* evaluation of wound healing activity. These models include excisional and incisional. Standard drug, samples (extracts) and controls were applied topically on daily basis till healing of wound. Wound healing process was determined by measuring wound contraction period, epithelization period and/or measurement of tensile strength of healing skin **[17]**.

Excision wound model

Crude extracts of *B. baluchistanica* and *D. oleoides* were evaluated for wound-healing profiling by excision wound model. Ketamine (25 mg/kg) intraperitoneally was used to anaesthetize male albino rats in four groups (I, II, III and IV, n=4) prior to the induction of wounds. For wound infliction in albino rats standard procedure of Morton and Malone, 1972 was adopted. Electric clipper was used to shave the dorsal fur of the rats. On the back of the animals' wound area was marked by using methylene blue dye with stencil. An excision wound of 25 x 2 mm (length x depth) (circular area = 300 mm^2) was formed around the anticipated markings with the help of blade, forceps and sharp scissors. Wounds were kept undressed **[18]**. Animals of group I (negative control) were medicated with placebo CMC, whereas group II and III with the *B. baluchistanica* and *D. oleoides* CMEs topically (100 mg/kg b.w /day) respectively till entire epithelization. Group IV was treated with topical pyodine solution (1% solution of PVP-Iodine) which served as positive control (standard). Negative control, positive control and CMEs were applied once daily for 15 days. Wound intact proportions was measured through inspecting wound diameter on 1st, 5th and 15th days post-wounding by using permanent marker and transparent paper. The anticipated wound areas were assessed and measured with a graph paper. Duration of epithelization was assured and achieved by the days utilized for falling of eschar with no lasting raw wound (Table 1).

Histopathological study

The healing tissues recovered from all four groups of the incision wound model on the 15th day were set for histological study. The amount of collagen was measured using Vangeison stain (Figure 1).

Incision wound model

Animals' anesthesia procedure was carried out highly likely to that of excision wound model. Following shaving of dorsal fur in each mouse, three centimeters long longitudinal incision was made at paravertebral position subcutaneously in the skin. The parted portion of skin was sutured about one centimeter apart by using a surgical needle and thread **[19]**. After 24 h of wound formation animal groups were made as in excision model and topically (100 mg/kg b.w) treated with negative control, plants extract and standard drug once in a day for 8 days. The suture was removed on 9th day of wound followed by the measurement of tensile strength on 10th day using continuous water flow procedure **[20, 21]** (Table 2).

STATISTICAL ANALYSIS

The groups for wound healing assays were compared with positive control for respective time point using independent t-test. p-value less than 0.05 was considered significant. All values are provided as Mean ± S.D.

RESULTS

Wound healing activity

Following In-vivo study, in the both wound models, *B. baluchistanica and D. oleoides* extracts treated animals demonstrated remarkable improved wound healing potential compared to negative controls and standard (Tables 1 and 2). *B. baluchistanica and Daphne oleoides* extracts were observed to reduce the wound area ($p < 0.001$) and epithelization time (day) 11.30 ± 0.23 and 10.13 ± 0.11 respectively as well as increase in the dry granulation weight ($P < .001$) compared to negative controls (table 1). During the treatment of animals with *D. oleoides* significant ($P < .001$) skin tensile strength (410.0 ± 1.8) was apparent in comparison to negative control group (342.31 ± 2.2) as well as the significant collagen content observations as shown in Figure 1 (A and C respectively). On the other hand, while comparing both plant extracts, there was significant increase in the wound-healing activity in the albino rats treated with the *D. oleoides* than *B. baluchistanica* extract and the placebo control receiving groups but the activity was not superior to standard pyodine solution treatment as shown in figure 1 (B).

Table 1: Wound healing effect of *B. baluchistanica and D. oleoides* in excision wound model

Groups	Wound area (mm^2)			Epithelization period (day)
	Day 1	Day 5	Day 15	
I (negative control)	222.31± 21.80	174.4 ± 20.7	133.6 ± 22.71	14.43 ± 0.40
II (*B. baluchistanica* CME)	222.40 ± 13.7	161.14 ± 21.28	66.43 ± 41.7 **	11.30 ± 0.23**
III (*D. oleoides* CME)	222.56 ± 11.2	156.15 ± 31.28	57.40 ± 21.8 **	10.13 ± 0.11**
IV (pyodine standard)	221.10 ± 11.2	67.14 ± 21.23	7.10 ± 11.3	8.34± 0.11

n = 4, Values are given as mean ± SD, ** P < 0.001 vs. positive control. Independent *t*-test

Table 2: Wound healing effect of *B. baluchistanica* and *D. oleoides* in incision wound model

Groups	parameters		
	Breaking strength (g)	Granulation tissue wet (mg)	Granulation tissue dry (mg)
I (Negative control)	342.31± 2.2	79.32 ± 7.1	9.35 ± 2.1
II (*B. baluchistanica* CME)	394.41 ± 3.7	112.46 ± 1.2	17.39 ± 7.7 **
III (*D. oleoides* CME)	410.60 ± 1.8**	124.52 ± 3.2**	23.40 ± 1.3 **
IV (pyodine standard)	467.11 ± 3.2	131.24 ± 1.3	22.20 ± .54

n = 4, Values are given as mean ± SD, ** P < 0.001 vs. positive control. Independent *t*-test

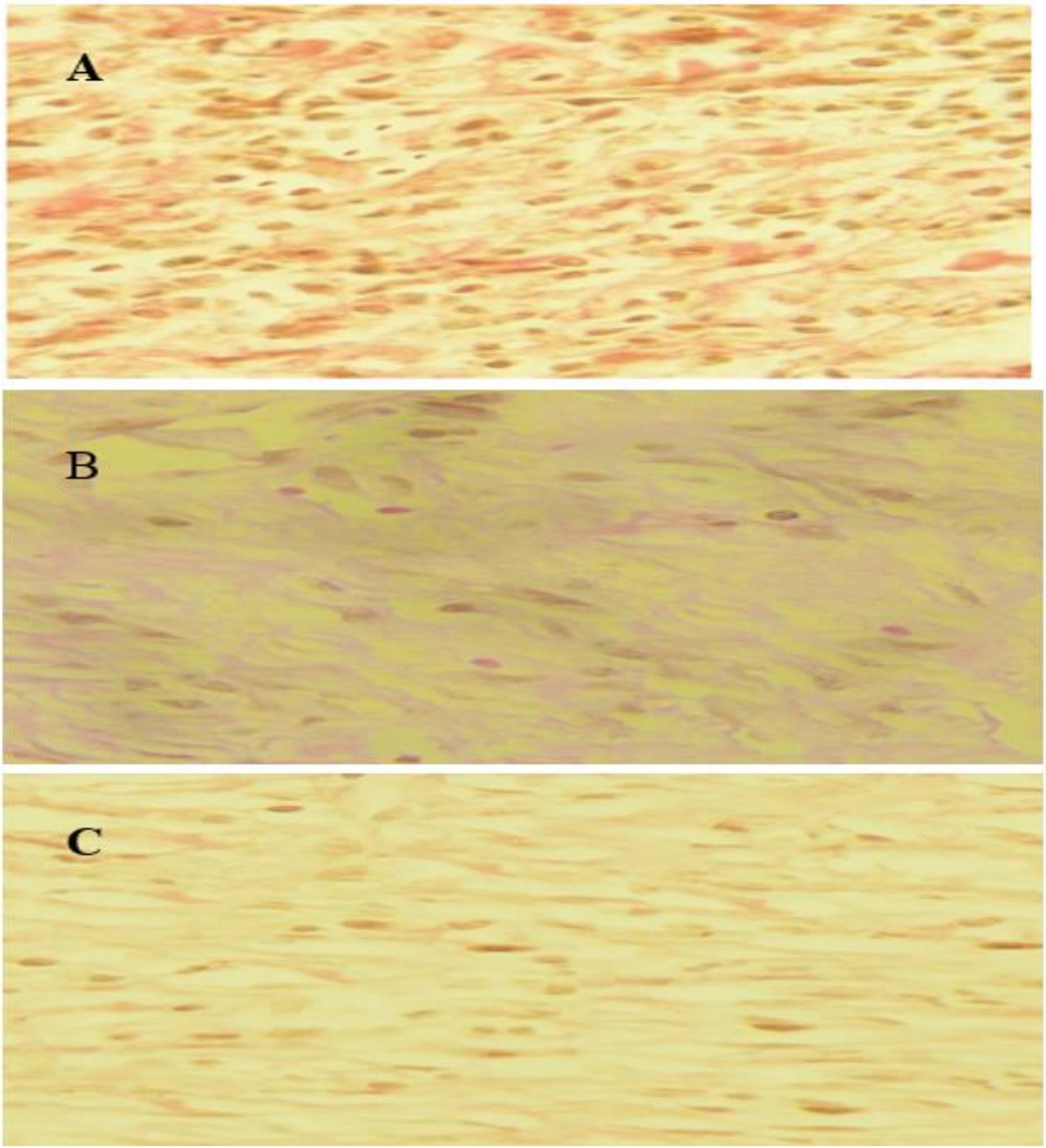

Figure 1: Granulation tissue of group 1 (controls) with less collagen but more macrophages (A), Granulation tissue of group 2 (standard) animals with moderate deposition of collagen (B), Granulation tissue of group 3 (*D. oleoides*) animals showing more collagen with less macrophages (C).

DISCUSSION

The results in excision and incision models revealed that the wounds treated with *B. baluchistanica* and *D. oleoides* extracts were observed as to epithelialize rapidly. For both plants, the rate of wound reduction was significantly improved in comparison to both models control wounds ($P < 0.001$). Furthermore, the duration of epithelialization was appreciably condensed from 14 days (negative control) to 10 and 11 days for *D. oleoides* roots and

B. baluchistanica stems extract treated groups respectively. However, the wound healing activity of *D. oleoides* extract was found as more augmented (10.13 ± 0.11) as compared to *B. baluchistanica* extract (11.30 ± 0.23). This supports the efficiency of medication having faster rate of wound contraction and epithelization. The wound contraction rate amplification in both studied plants extract may be attributed to either their antibacterial profile or instigation of macrophage cell proliferation and collagen synthesis **[22]**. Moreover, in the previous part of this research **[12]** *B. baluchistanica* stems and *D. oleoides* roots extracts were proven as an outstanding antioxidants. During the management of inflammation, the prime focus relies on the elimination of the wreck, injured tissues and microbial engulfment by phagocytic cells i.e., macrophages and neutrophils. phagocytic cells function to provide defense against microbes by destroying the devitalized tissue, generating proteolytic enzymes and free radicals / reactive oxygen species (ROS**)** in higher amounts at the injured site **[23].** Massive amounts of ROS and neutrophils at the site of wound lead to the destruction of the pathogens and ultimately bacteriostatic action **[24]**, however this higher concentration results in impaired wound healing process. Antioxidants impede these adverse effects of wounds healing process by removing mediators of inflammation. They pledge the excessive proteases and dominantly participate in scavenging ROS. Reactive oxygen species secure protease inhibitors to experience oxidative damage **[25]**. The predominant protection prospective of antioxidant is highly likely to its direct integration with free radical or ROS in contrast to modify the cell membrane integrity and restraining the damage **[26]**, therefore; the compounds possessing high radical-scavenging potential facilitate the orchestration of wound-healing response.

CONCLUSION

The results of both models demonstrate that, the various wound repair phases, wound contraction, epithelialization and skin breaking strength, were promoted by *Daphne oleoides* roots and *Berberis baluchistanica* stem extracts in comparison to the negative control groups. The research collectively emphasizes that both methanolic extract possess wound healing activity that rationalize the literature traditionally claimed utilization of *Daphne oleoides* roots and *Berberis baluchistanica* stem to cure wounds. This study also showed that the both plant crude extracts were endowed with significant antibacterial and antioxidant activities that explain at least in part its wound healing activity as was proved in previous part of this research.

ACKNOWLEDGEMENTS: The authors are thankful to Amson vaccines and pharma Islamabad, Pakistan for the provision of animals and Riphah institute of pharmaceutical sciences, Riphah international University Islamabad, Pakistan to conduct histological examination.

CONFLICTS OF INTEREST: No conflicts of interest to declare

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