Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

WOUND HEALING ACTIVITY OF *BRIDELIA RETUSA* BARK IN EXPERIMENTAL ANIMALS

UJWALDIP V. DEORE*, ANIL U. TATIYA, SANJAY J SURANA

Medicinal Plant Research Laboratory, Department of Pharmacognosy, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist. - Dhule, Maharashtra State, India

Email: ujwalvdeore@rediffmail.com, aniltatiya12171@gmail.com, sjsurana@yahoo.com

Received: 08 Nov 2013 Revised and Accepted: 16 Apr 2014

ABSTRACT

Objective: The present study was to evaluate the effect of methanol and aqueous extracts of *Bridelia retusa* (airyshawii) bark on different parameters related to wound healing in albino rats.

Methods: Wistar albino rats subjected to incision and excision wounds measuring approximately 6 cm and 500 mm² side of the vertebral column and on the back of each rat respectively, and randomized into six group (n=6): control group (I), reference standard povidone iodine group (II), topical application of 5 and 10 % methanolic extract ointment group III and IV respectively and group V and VI were treated topically with 5 and 10% aqueous extract ointment respectively. The increased tensile strength was measured for incision wound. The area of wound was measured on the days 4, 8, 12, 16 and 20 of the excision wound and total number of days required for complete epithelization wounds were noted in each group.

Results: The statistical study revealed that both methanolic and aqueous extracts formulation containing the bark of *Bridelia retusa* in the concentration of 5% and 10% have significant wound healing activity in both incision as well as excision models. However, the topical treatment with 10% methanolic extract was found to be superior to all other treatment as evidenced by increased tensile strength of incision wound and lesser number of days required for complete epithelization of excision wound.

Conclusion: The present study demonstrates the topical treatment of 10% methanol extract of *Bridelia retusa* bark for wound healing activity..

Keywords: *Bridelia retusa,* Methanolic extract, aqueous extract, Povidone Iodine, Wound healing activity.

INTRODUCTION

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells underneath the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated [1]. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling.

Bark of *Bridelia retusa,* (airyshawii) belonging to family Euphorbiaceae commonly known as Asana or Khaja, is a shrub or climbers found throughout the hotter parts of India. It is a valuable astringent and used in the form of a liniment in rheumatism [2]. Bark is well known for the presence of 16-40% of tannins. It is reported to be used traditionally in snake bite, wounds, and tonics for veterinary purposes [3]. It exhibited antiviral, hypoglycemic, hypotensive, antifertility activity and used for removal of urinary concretions in pharmacological trials [4]. It is reported to possess anti inflammatory activity in animal model [5]. Earlier work was reported for wound healing activity on leaves of *Bridelia retusa* [6]. Objective of the present study was to evaluate the effect of methanol and aqueous extracts of *Bridelia retusa* (airyshawii) bark on different parameters related to wound healing in albino rats.

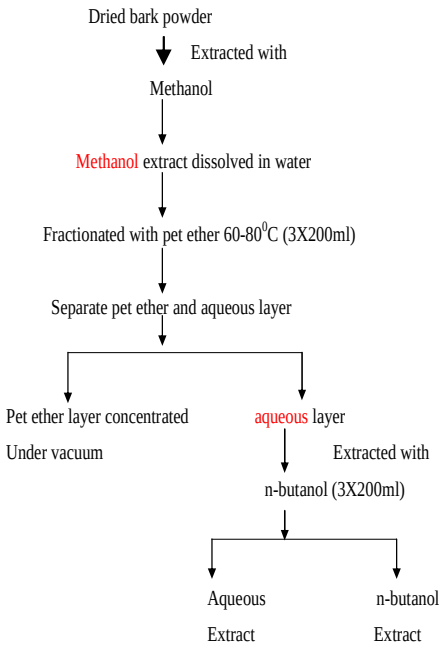
MATERIALS AND METHODS

Collection and authentication of plant material

The fresh bark of *Bridelia retusa s*preng was collected in the month of August (2008) from Ranipur (Toranmal) of Nandurbar District (MS), India. Plant species was authenticated by Dr. D. A. Patil, H.O.D. of Botany, Dr. P. R. Ghogrey College, Dhule. (MS). A voucher specimen of the bark is deposited in department for future reference.

Preparation of plant extracts

In extraction process; dried and powdered material was extracted with Methanol. Then the soft mass obtained as methanol extract was dissolved in sufficient water and fractioned into petroleum ether and n-butanol. All extracts were dried in vacuum dryer and stored in desiccator.



Drug formulations

Two types of drug formulations were prepared from methanol and aqueous (5 & 10 % w/w ointment) extracts in 2% ointment base [7], to be used in the preparation of topical ointment.

In vivo experiments

Selection of animals

Albino Wistar rats of either sex weighing 200-250 g were procured from department of Pharmacology, R.C.Patel Institute of Pharmaceutical Education and, Shirpur. All rats were housed in polypropylene cages and maintained under standard conditions (12 hrs light and dark cycles, at 25±27°C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The study was conducted in accordance with Institutional Animal Ethical Committee of R.C.Patel Institute of Pharmaceutical Education and, Shirpur, India, registered no (RCPCOP/IAEC2007-8/9) under CPCSEA.

Phytochemical screening

Preliminary phytochemical screening was done for phytoconstituents like steroids, triterpenoids, flavonoids, tannins, and carbohydrates according to Evans W C, 1996 and Kokate C.K. *et al*., 2005 [8, 9].

Selection of dose

5 % methanol and aqueous extract ointment of *Bridelia retusa* as low and 10% as high dose were applied topically in excision and incision wound model. The treatment period was considered 10 days for incision wound model and the treatment period was considered till scar falling of wound in case of excision model.

Wound models

Wound healing activity was studied using two models viz. Resutured incisional wound model and Excision wound model.

Resutured incisional wound model

Wistar albino rat weighing between 200-250 gm b.w were divided into six groups, each group consisting of 6 rats and each animal kept separately under laboratory condition. They had free access to commercial pellet diet and *ad libitum*. Group I was used as control and group II received reference standard povidone iodine. Group III & IV received topical application of 5 & 10 % methanolic extract ointment respectively whereas group V & VI were treated topically with 5 & 10% aqueous extract ointment respectively. Two paravertebral incisions (6 cm long) were made through the full thickness of the skin on either side of the vertebral column of each rat. Wounds were resutured with silk thread at 1 cm interval. The sutures were removed on the 9th post wounding day. On10th day wound breaking strength (tensile strength) was measured for each animal, by continuous water flow technique [10].

Excision wounds

Wistar albino rat weighing between 200-250 gm body weigh were divided into six groups, as followed in Incision wound model. A circular piece of full thickness (approximately 500mm^2) was cut off from a predetermined area on the back of the rat. Wounds were measured with Digital Verneire caliper at every fourth day interval till the wound was healed. Changes in wound area were calculated giving an indication of the rate of wound contraction. Number of days required for falling of the Escher without any residual raw wound gives the period of epithelization[10]. The Percentage wound closure and epithelization period were calculated on day 20 [11] and hydroxyproline content measured on day 10.

Statistical analysis

Results were analyzed by one-way ANOVA. Followed by Tukey-Kramur Multiple comparison post hoc-test. P<0.05 were considered as significant.

RESULTS AND DISSCUSSION

Resutured incisional wound study

In incision wound model, methanol and aqueous extract (in both 5 and 10 % concentrations) treated animals showed significant (*P<0.001) increase in breaking strength (859.06±2.978 and 1019.67±4.19), (804.18±7.10 and 827.25±2.49), respectively when compared to the control (624.43±3.36). The mean breaking strength was also significant in animals treated with standard drug Povidone iodine (1208.43±5.06), Fig.1

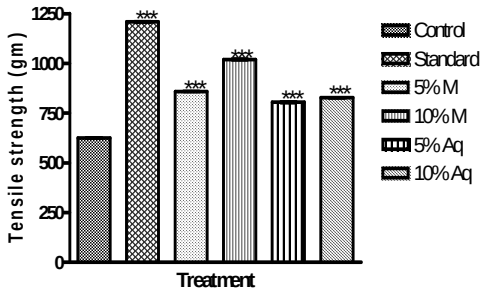
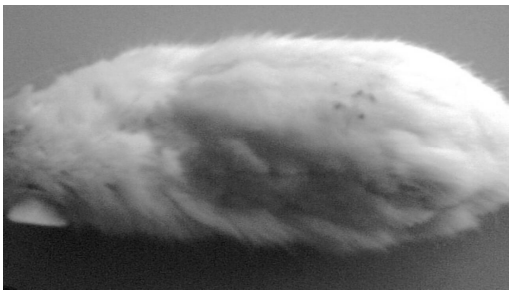


Fig. 1: Skin breaking strength measurement in resutured incisional wound model

Before treatment



After treatment

Fig. 2: Resutured incision wound model

Excision wound study

The excision wound model was employed to assess the potency of crude extract to promote the wound healing in trauma types of wound which is assessed by the wound area measurement, rate of wound contraction, tensile strength, hydroxy proline content and number of days required for complete epithelialization of the wound area. Results of wound area measurement and % wound contraction of both methanolic & aqueous extract ointment,(5 and 10 %) and control groups (i.e. simple ointment) are shown in Table 1. It is observed that the wound contracting ability of the *B. retusa* bark extract either in the form of 5 or 10 % ointment was significantly (*P<0.001) greater than that of the control. The 10% methanolic extract treated group of animals showed significant contraction of wound area 218.96±0.6, 86.72±0.7, 5.42±0.5 mm^2 at 4, 8 and 12 days respectively as compared to control. Excision wound studies also demonstrated that there was a significant (*p<0.001) increase in tensile strength on the 10th post wounding day, treated with all tested group as compared to control. The mean hydroxyproline (OH-

P) content, was higher in groups treated with 5&10% MO and 5&10% AO treated group, it was found to be 35.41, 46.29, 25.65, 35.58 µg/ml respectively as compared to control group (11.2 µg/ml), Fig.3.Mean time taken for complete epithelialization of the excision wound in 10 % methanolic extract ointment treated group as observed on day 12 while in control animals it was delayed up to the day 20.,

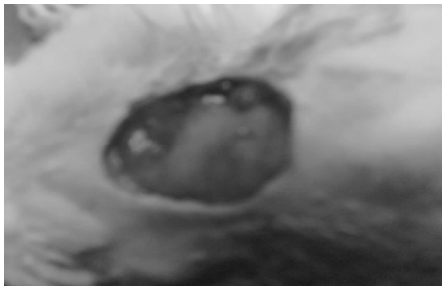
Table1.Significant increase was also observed in tensile strength and hydroxyproline content which was a reflection of increased collagen levels.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen composed of amino acid (hydroxyproline), is a major protein of the extracellular matrix and the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline and its peptides therefore measurement of the hydroxyproline has been used as an index of collagen turnover. The increased hydroxyproline content of the excision wounds has indicated faster collagen turnover leading to rapid healing with concurrent increase in the tensile strength of the treated wounds [12].

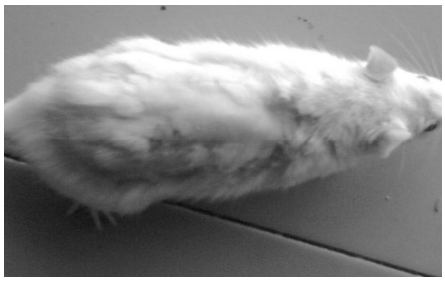
Table 1: Excision wound study of topical ointment Formulation

Treatment	Epithelization period (Days)	Wound Area Measurement (mm^2) (% of wound contraction by day)				
		4	8	12	16	20
Control	20.4±0.50	445.31±0.8 (13.20±0.76)	388.24±0.7 (24.32±0.66)	141.07±1.7 (72.51±0.86)	68.66±0.9 (86.21±0.58)	15.77±0.5 (96.92±0.40)
5 % M	16.0±0.31	297.35±1.7* (42.68±1.18)	224.84±1.7* (56.67±2.25)	20.2±0.7* (96.10±1.18)	0.54±0.1* (99.90±0.96)	_
10 %M	12.4±0.24	218.96±0.6* (56.84±0.78)	86.72±0.7* (82.91±0.55)	5.42±0.5* (98.93±0.82)	_	_
5 % Aq	19.8±0.37	395.52±0.9* (21.64±0.73)	254.56±0.6* (49.56±0.97)	126.47±0.9* (74.95±0.50)	31.54±0.5* (93.76±1.08)	1.94±0.1* (99.62±0.78)
10% Aq	16.0±0.31	279.48±0.6* (45.43±0.65)	128.85±0.9* (74.85±0.97)	57.20±0.5* (88.83±0.83)	7.27±0.4* (98.59±0.79)	_
Std.	11±0.441	301.75±1.4* (42.31±0.75	87.13±1.5* (83.35±0.81)	0.49±0.1* (99.90±1.14)	_	_

Values are Mean ± S.E. (n=6); ANOVA: P<0.0001, F=5890, d.f=29, R^2 =0.9992 Tukey-Kramur Multiple comparison post hoc-test: *p<0.001 as compare with control. M= formulation containing 5 and 10 % methanol extracts; Aq= formulation containing 5 and 10 % aqueous extracts



Before treatment



After treatment

Fig. 3: Excision wound model

The repair of wounds involves different phases including contraction, the formation of epithelization and fibrosis. The biological response regulating the body's own cellular defense mechanisms contributes to the wound and its repair [13]. Phytochemical screening of extracts revealed the presence of steroids, triterpenoids, tannins, saponins and flavonoids. Tannins possess wound healing activity mainly due to astringent and antimicrobial property [14]. It also promotes the wound healing through several cellular mechanisms, chelating of the free radicals and reactive species of oxygen, promoting contraction of wound and increasing the formation of capillary vessels and fibroblasts. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing. Thus, the enhanced wound healing may be due to free radical scavenging action of the plant, and enhanced level of antioxidant enzymes in granuloma tissue. Better collagenation seen under the influence of *Bridelia retusa* extract may be because of improved antioxidant status.

CONCLUSION

The present study revealed that both the methanolic and aqueous extracts of the bark of *B.retusa* in concentration (5% and 10%) have significant wound healing activity exhibited in incision as well as excision wound models. However, the topical treatment with 10% methanol extract was found to be superior to all other treatments as evidenced by lesser number of days required for complete epithelization of excision wounds and increased tensile strength of incision wounds. Our results indicate that the plant *Bridelia retusa* is capable with significant wound-healing activity with complete epithelization of excision wounds and increased tensile strength of incision wounds, thereby justifying its use in the indigenous system of medicine.

ACKNOWLEDGEMENT

The authors are sincerely thank to the dept. of pharmacology for their providing necessary facilities for carrying out animal study.

REFERENCES

1. Nayak BS, Anderson M, Pinto Pereira LM. Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia 2007; 78: 540–544.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, International Book Distributors, Dehradun;1935; 3:2212-14.
3. Joshi MC, Patel MB, Mehta PJ. Some medicines of Dangs, Gujarat State. Bull Medicoethanobt. Res 1980; 18-24.
4. Anonymous. The wealth of India, Raw materials. New Delhi, CSIR; 1958; 2(B): 295-296.

5. Mehare ID, Hatapakki BC. Anti-inflammatory activity of Ethanolic extract of bark of *Bridelia retusa*. Indian J. Pharm Sci 2003; 65-68.
6. Bagad YM. Evaluation of Pharmacognostical, Phytochemical and Pharmacological Screening of Leaves of Bridelia airyshawii. (Euphorbiaceae). M.Pharm. Thesis, Faculty of Pharmacognosy, North Maharashtra University, Jalgaon, India. (2007).
7. Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ, Singh SD. Jagadeesh. Evaluation of wound-healing potency of *Vernonia arborea*. Indian J Pharmacol 2005; 37 (4): 223-226.
8. Evans WC. Trease and Evans. Pharmacognosy. 15th ed. Toronto, Saunder's Publication; 1996. 93-321,336.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 33rd ed. Pune, Nirali Prakashan; 2005.115-121,446-450, 593-597.
10. Sourav KR, Pratyush KM, Subhangkar N, RD, Bodhisattwa C. wound healing activity of the different extract of *Typhonium trilobatum* in albino rats. Asian Pac J Trop Biomed 2012; 2, 3: 1477-1486.
11. Awol M, Temesgen S, Kaleab A, Ephrem E. *In vivo* wound healing activity and phytochemical screening of the crude extract and various fractions of *Kalanchoe petitiana A.Rich*(Crassulaceae) leaves in mice. J Ethnopharmacol *2013*; 145; 2, 30: 638-646.
12. Gupta S, Gupta SK. Quarterly medical review, Mumbai, India: Raptakos; 1985.
13. Charles VM, Rusell RCG, Williams NS. Short Practice of Surgery, 20th edn. Champan and Hall, London;1995, 9–11.
14. Rane MM, Mengi SA. Comparative effect of oral administration and topical application
15. of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats.
16. Fitoterapia 2003, 74: 553-558.