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Der Pharmacia Lettre, 2010, 2 (2): 457-463
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of *Boswellia Serrata* oleo-gum resin for wound healing activity

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Abstract

In spite of tremendous advances in the chemical drug industry, the availability of substances capable of stimulating the process of wound repair is still limited. Moreover, the management of chronic wounds is another major problem due to the high cost of therapy and the presence of side effects. Since no studies were carried for wound healing activity of the oleo-gum resins obtained from Boswellia serrata an attempt has been made to evaluate the wound healing activity of the oleo-gum resins obtained from Boswellia serrata. For this purpose a cream was formulated using oleo-gum resins of Boswellia serrata and was applied topically on the excision wound surface as single dose in different percentages. Wound contraction and tensile strength measurements were used to evaluate the effect of Boswellia serrata oleo-gum resins on wound healing. The results obtained indicated that Boswellia serrata accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The greatest contraction was obtained at a single application of 15 % w/w of formulated cream. Measurements of tensile strength and healed area were in agreement. The results suggest that treatment with Boswellia serrata oleo-gum resin may have a beneficial influence on the various phases of wound healing like fibroplasias, collagen synthesis, and wound contraction, resulting in faster healing. In conclusion, the observations and results obtained in this study indicated that the Boswellia serrata oleo-gum resin significantly stimulated wound contraction. These findings could justify, role of this plant material in the management of wound healing.

Keywords: Boswellia serrata; Wound healing; Wound contraction; Tensile strength.

INTRODUCTION

Boswellia serrata Roxb (Bursaraceae) is a moderate to large branching tree found in India, Northern Africa, and the Middle East. Strips of bark are peeled away, yielding a gummy oleo-resin which contains oils, terpenoids, and gum. Up to 16 percent of the resin is essential oil, the majority being alpha thujene and p-cymene. Four pentacyclic triterpene acids are also present, with ß-Boswellic acid being the major constituent. Gummy exudates have been traditionally used in the Ayurvedic system of medicine. These gum resins are also known as guggals. Boswellia, or boswellic acids, exhibit potent anti-inflammatory properties, demonstrated both in vitro and in vivo. Triterpenes in boswellic acid reduce the synthesis of leukotrienes in intact neutrophils by inhibiting 5-lipoxygenase, the key enzyme involved in the biosynthesis of leukotrienes, which mediate inflammation[1,2]. 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 below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated [3]. Wound care and maintenance involve a number of measures such as dressing and administration of painkillers, anti-inflammatory agents, healing promoting drugs etc. It runs through a number of phases such as coagulation, inflammation, granulation, fibroplasias, collagenation, would contraction and epithelization [4]. Since inflammation precedes the process of healing, drugs affecting inflammation are known to slow down the healing. A treatment could influence the healing of wound by intervening into anyone of many phases of wound healing. Conventional treatment includes keeping the wound clean, dry and covered. The present study was planned to assess the efficacy of the indigenous drug Boswellia serrata on some parameters related to wound healing in rats.

MATERIALS AND METHODS

2.1 Plant Material

The *Boswellia serrata* oleo-gum resin was collected locally from Bhopal, India. It was cleaned and was used for the preparation of cream. The *Boswellia serrata* oleo-gum resin was authenticated and a voucher specimen was stored in the department of Pharmacognosy.

2.2 Animal

Inbred house Wistar rats of either sex were used in the study. The range of the weight of the animals was between 200–250 g. Animals were divided into five groups (control, standard test-1, 2 and 3) of 6 animals each. They were housed individually in standardized environmental condition. All the animals were provided with water food ad libitum during the whole period of study. The approval from animal ethical committee was obtained before the commencement of study and the research was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

2.3. Wound healing activity

2.3.1 Excision wound model

The animals were anaesthetized with intravenous ketamine hydrochloride of 2 ml/kg. The dorsal fur of the animals was shaved with electric clipper. The area of the wound to be created was marked on the back of the animals by methylene blue using circular stainless stencil. The full thickness excisional wound was created along markings using toothed forceps, surgical blade and pointed scissors. The area of the wound was recorded on transparency paper. The entire wound was kept open [5]. The wound area was measured graphically. This model was used to monitor the rate of wound contraction and tensile (breaking) strength.

2.3.2 Grouping of animals

After wound creation, experimental animals were divided into the following five groups, each group consisting of 6 Wistar rats of either sex.

Group A (control): received base devoid of Boswellia serrata oleo-gum resin.
Group B (Test 1): wounds treated topically by single application of 5 % of the preparation.
Group C (Test 2): wounds treated topically by single application of 10 % of the preparation.
Group D (Test 3): wounds treated topically by single application of 15 % of the preparation.

Group E (Standard): wounds treated topically by twice application of Povidone-iodine.
Control rats received tropically the base which was devoid of Boswellia serrata oleo-gum resin while the test group received different percentages of Boswellia serrata oleo-gum resin (5 %, 10 % and 15 %) respectively. Povidone-iodine was used as a standard. On the 16th post-operative day, the granulation tissue formed on the dead space wound was excised. Weight was recorded and tensile strength was determined [6].

2.3.3 Wound area measurement
The measurement of the wound area was taken on the 4th, 8th, 12th and 16th day for all groups over a transparency paper with marker pen, wound area was measured and recorded.

2.3.4 Determination of wound contraction
After surgery the excision wound margins were traced at 4-day intervals on transparent graph paper having a millimeter scale that was measured by a caliber with an accuracy of 1/20 mm. Measurements were continued up to 16 days. On each control day the wound of the animals was photographically documented. Wound contraction was expressed as percentage of wound area that had healed.

The wound contraction percentage was determined from the measurements using the following formula:

Percent wound contraction =healed area /total area × 100.

To apply this equation, at 4-day intervals, the wound margins were traced and measured to calculate the non-healed area which was then subtracted from the original wound area to obtain the healed area.

2.3.5 Measurement of tensile strength
Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of the repaired tissue. For this purpose the newly repaired tissue including scar was excised to measure the tensile strength measurement is called a tensiometer,. The instrument used was designed according to the method of [7]. For the quantitation one of the edges of the wound was fixed while applying a measurable force to the other one. The load (weight) in grams required to disrupt the wound is determined after complete healing of the wound, and that was on day 16th after surgery.

Statistical analysis
All treated groups were compared with the control and standard group. All the groups were used for the statistical analysis. The results were analyzed statistically using one-way and two-way ANOVA methods to identify the differences between treated groups, control and standard. The data were considered significant at $P < 0.05$.

RESULTS

Wound contraction on different days is shown in Fig 1 and 2 and in Table 1. The wound contraction percentage was determined from the measurements for the first time on the 4 th day after the application of oleo-gum resin of Boswellia serrata and carried out at 4-day intervals for the duration of 16 days. On 16 th day, all treated animal groups exhibited significant increase in the percentage of wound contraction as compared to control and standard groups. The percentage of wound contraction in test 2 group (94.33) and test 3 group (98.02) showed good wound healing activity as compared to that of control (64.13) and standard groups (84.37) which indicates complete healing of wounds.

Fig. 1: Control, Test 1, Test 2, Test 3 and Standard groups respectively on zero day of excision wound model

Fig. 2: Control, Test 1, Test 2, Test 3 and Standard groups respectively on 16 th day of excision wound model

Table 1: Effect of different percentages of *Boswellia serrata* Oleo-gum resin on % wound contraction at various time intervals

Sr. No.	Groups	% Contraction			
		4 th day	8 th day	12 th day	16 th day
1.	Control	10.84	28.01	51.57	64.13
2.	Treated (5 % w/w)	15.63	54.59	65.30	81.24
3.	Treated (10 % w/w)	26.89	78.16	86.20	94.33
4.	Treated (15 % w/w)	27.20	83.53	92.83	98.02
5.	Standard	19.46	51.25	78.81	84.37

Values are mean ± S.D. (standard deviation), n = 6 mice (in each group), All values of test groups are significant compared to the control and standard ($P \leq 0.05$).

The results of the measurement of the tensile strength, on day 16, are shown in Table 2, and in Fig. 3. Tensile strength of the animals treated with 15 % of oleo-gum resin of Boswellia serrata (test 3 group) was significantly greater than that of the untreated group (control), while the other test groups, (test 1 and test 2), showed the satisfactory results. The significant difference (P <

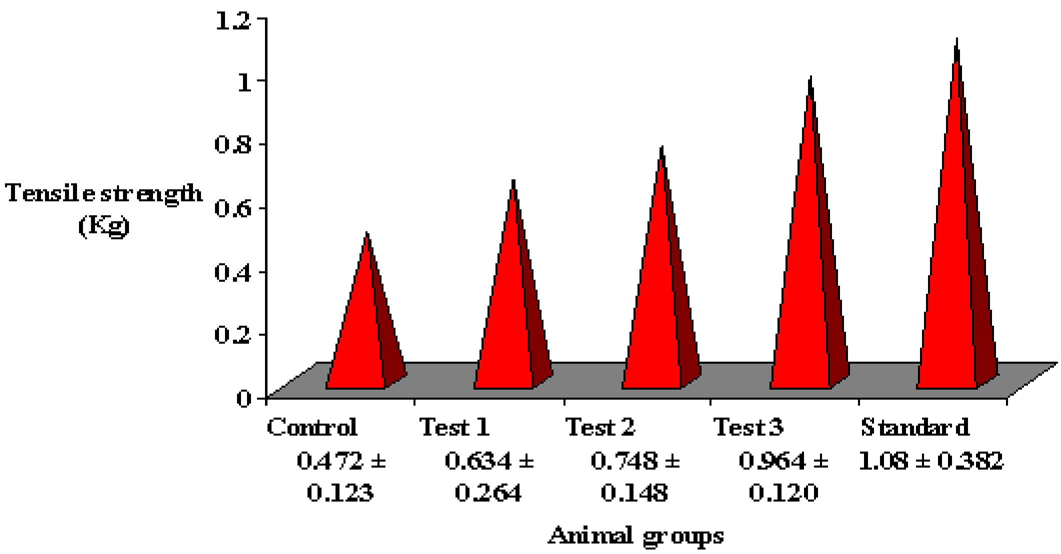
0.05) was observed with the test 3, (0.964 kg) and standard group (1.08 kg) while the control animals showed only 0.472 kg.

Table 2: Tensile (Breaking) Strength of healed wounds (day 16)

Groups	Breaking Strength (kg)
Control	0.472 ± 0.123
Test 1	0.634 ± 0.264
Test 2	0.748 ± 0.148
Test 3	0.964 ± 0.120
Standard	1.08 ± 0.382

Values are mean ±S.D., Significant difference from control and standard (P ≤ 0.05).

Fig. 3: Tensile strength of healing wounds treated with different percentages of *Boswellia serrata*



DISCUSSION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is a product of the integrated response of several cell types to injury. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue[8]. In spite of tremendous advances in the chemical drug industry, the availability of substances capable of stimulating the process of wound repair is still limited [9].Moreover, the management of chronic wounds is another major problem due to the high cost of therapy and the presence of side effects [10]. Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. This sequence of physiologic events occurs by a process of connective tissue repair. These events involve four phases [11]:

(i) Coagulation, which prevents blood loss.
(ii) Inflammation and debridement of wound.
(iii) Epithelial repair, including proliferation, mobilization, migration and differentiation.

(iv) Tissue remodeling and collagen deposition.

Any agent which accelerates the above processes is a promoter of wound healing. The application of medicinal concoctions from plants to treat skin lesions, in particular, burns and wounds, has had a long tradition. Plants with wound healing activity have been reported and experimentally studied on various animal models to reveal the most active promising compounds [12]. Results obtained in the present study suggest that treatment of rat excision wounds with Boswellia serrata oleo-gum resin has accelerated the wound healing process. Treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. Tensile strength was measured to confirm the wound healing activity of *Boswellia serrata* oleo-gum resin. The increase in tensile strength of treated wounds may be due to increase in collagen concentration and stabilization of the fibers [13]. The results obtained in this study were similar to the influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. It was observed that Aloe vera increased the collagen content of the skin ultimately and contributed to wound strength [14]. Also, a similar effect has been observed with the ethanolic extract of Centella asiatica on the rat dermal wound healing, increasing the tensile strength and enhancing the wound healing process [15] studied the effect of Anchusa strigosa, Artemisia herba-alba, Nigella sativa, Punica granatum, and Trigonella foenum-graecum, in wound healing, and found that there was a strong correlation between the collagen fiber formation and acceleration of wound healing. The results suggest that treatment with Boswellia serrata oleo-gum resin may have a beneficial influence on the various phases of wound healing like fibroplasias, collagen synthesis, and wound contraction, resulting in faster healing. In conclusion, the observations and results obtained in this study indicated that the Boswellia serrata oleo-gum resin significantly stimulated wound contraction. The breaking strength of the treated excision wounds increased in the treated groups compared with the control and standard groups. These findings could justify, role of this plant material in the management of wound healing. Further experiments are needed to test the effect of this plant in treatment of chronic wounds.

Acknowledgement

The author wants to thank the principal, Bansal college of Pharmacy, for his valuable suggestions and support.

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