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Full Length Research Paper

Potential activity of ethanolic extract of *Boesenbergia rotunda* (L.) rhizomes extract in accelerating wound healing in rats

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This study was conducted to evaluate the effects of topical application of ethanol extract of *Boesenbergia rotunda* rhizomes on the rate of wound healing closure and histology of healed wound. An area of uniform wound 2.00 cm in diameter using circular stamp, was excised from the nape of the dorsal neck of all rats with the aid of round seal. The animal groups were topically treated with 0.2 ml of each vehicle (gum acacia), Intrasite gel, 100 and 200 mg/ml of ethanol extract, respectively. Macroscopically, wound dressed with rhizomes extract and Intrasite gel significantly healed earlier than those treated with vehicle. Histological analysis of healed wounds with rhizomes extract showed comparatively less scar width at wound closure and healed wound contained less inflammatory cells and more collagen with angiogenesis compared to wounds dressed with vehicle. In conclusion, wounds dressed with rhizomes extract significantly enhanced the acceleration of wound healing enclosure in rats.

Key words: *Boesenbergia rotunda*, rhizomes, ethanol extract; wound healing, histology.

INTRODUCTION

Normal wound healing response begins the moment the tissue is injured. 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 (Souba and Wilmore, 1999). It is accepted that wound repair is an immune-mediated physiologic mechanism (Singer and Clark, 1999). Wound healing, or wound repair, is an intricate process in which the skin repairs itself after injury (Nguyen et al., 2009). Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine (Nayak et al., 2009). Finger root, *Boesenbergia rotunda* (L) formerly *Boesenbergia* or *Kaempferia pandurata* (Schult) is a member of the ginger family (Zingiberaceae) and distributed in Southeastern Asian countries such as Indonesia, Malaysia, and Thailand (Saralamp et al., 1996).

As regards its biological activities, the rhizomes of this plant have been used for the treatment of oral diseases, colic and gastrointestinal disorder, diuretic, dysentery, inflammation and aphrodisiac (Saralamp et al., 1996). Several animal studies suggested that this plant may have potent antioxidant activity and neuroprotective

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effects (Shindo et al., 2006), anti-inflammatory activity (Tuchinda et al., 2002), anti-mutagenic (Trakoontivakorn et al., 2001), anti-cancer activity (Kirana et al., 2007), anti-dermatophytic activity (Bhamarapravati et al., 2000), antibacterial activity (Voravuthikunchai et al., 2005), chemopreventive and anti-*Helicobacter pylori* activities (Bhamarapravati et al., 2003), anti-dengue 2 virus NS3 protease (Kiat et al., 2006), anti-feeding activity against larvae of *Spodoptera littoralis* (Stevenson et al., 2007), and inhibitory effect on tumor necrosis factor α -(TNF-α)-induced cytotoxicity in L929 cells (Morikawa et al., 2008). Several studies have shown that rhizomes of this plant may contain pharmacologically active compounds. Flavonoid compound named pinostrobin, three flavanones, pinostrobin, pinocembrin and alpinetin and two chalcones, cardamonin and boesenbergin A were isolated from the rhizomes of *B. rotunda.*

The chalcone, cardamonin, isolated from *B. rotunda* was reported to exhibit appreciable anti-HIV-1 protease inhibition. There were no data regarding the wound healing effect of *B. rotunda* rhizomes, and to confirm their traditional uses as a remedy for wound healing. This encourages us to assess the rate of wound healing enclosure of *B. rotunda* rhizomes macroscopically and microscopically in rats.

MATERIALS AND METHODS

Intrasite gel

Intrasite gel was purchased from University of Malaya Medical Centre Pharmacy. Intrasite gel is a colorless transparent aqueous gel, which contains 2.3% of a modified carboxymethylcellulose polymer together with propylene glycol (20%) as a humectants and preservative. When placed in contact with a wound, the dressing absorbs excess exudates and produces a moist environment at the surface of the wound, without causing tissue maceration. Intrasite gel is an amorphous hydrogel which gently re-hydrates necrotic tissue and facilitates autolytic debridement while loosening and absorbing slough and exudates, clearing the way for effective wound healing. It is also designed for wounds that are granulating and epithelialising. It can also be used to provide the optimum moist wound management environment during the later stages of wound closure. It is non-adherent and does not harm viable tissue or the skin surrounding the wound. This makes the use of Intrasite gel ideal for every stage in the wound management process. Intrasite gel is a trademark for Smith and Nephew Healthcare Limited (Williams, 1994).

Lignocaine HCl (2%, 100 mg/5 ml)

Lignocaine is a local anesthesia and was purchased from the Experimental Animal House, Faculty of Medicine, University of Malaya (Delta Veterinary Laboratory PTY LTD, NSW 20011). 1 ml of Lignocaine was injected subcutaneously.

Boesenbergia rotunda rhizomes extract preparation

Fresh mature rhizomes of *B. rotunda* were obtained from Ethno Resources Sdn Bhd, Selangor Malaysia, and identified by comparison with the voucher specimen deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. The rhizomes were tap washed followed by washing with distilled water. The rhizomes were sliced and shade-dried for 7 - 10 days and were then finely powdered using electrical blender. 100 g of fine powder were soaked in 1000 ml of 95% ethanol in conical flask for 3 days at room temperature. After that the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The extract was placed in incubator to dry at 40°C and the clear semi solid extract was dissolved by using the vehicle, gum acacia in normal saline as described by Shetty et al. (2007) with slight modification. Two grams of gum acacia was dissolved in 100 ml of normal saline. From this, 10 ml of solution, which contains 200 mg of gum acacia, was used for dissolving one gram and two grams of ethanolic extract each. So one ml of each solution contains 100 and 200 mg of extract, respectively (100 mg/ml = 20 mg/0.2 ml and 200 mg/ml = 40 mg/0.2 ml).

Acute toxicity studies

The acute toxic study was used to determine a safe dose for *B. rotunda*. Thirty six healthy *Sprague Dawley* rats (18 males and 18 females) were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya, and were assigned equally into 3 groups labeled as vehicle (gum acacia in normal saline); 2 and 5 g/kg of *B. rotunda* in vehicle, respectively. The animals were made to fast overnight (food but not without water) prior dosing. Food was withheld for a further 3 to 4 h after dosing. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of two weeks.

The animals were sacrificed on the 15^{th} day. Hematological, serum biochemical and histological (liver and kidney) parameters were determined following standard methods (Bergmeyer, 1980; Tietz et al., 1983). The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia and the Ethic No. PM/07/05/2008/MMA (a) (R). Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health, Malaysia.

Experimental animals

S. Dawley healthy adult male rats were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya, and the rats were divided randomly into 4 groups of 8 rats each. Each rat that weighted between 220 - 250 g (8 weeks old) was housed separately (one rat per cage). The animals were maintained on standard pellet diet and tap water. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Ethic No. PM/27/07/2009/MAA (R). Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health, Malaysia.

Experimentally induced excision wounds

An area of uniform wound 2.00 cm in diameter (circular area = 3.14 cm^2) was excised from the nape of the dorsal neck of all rats with

Table 1. Time required for wound healing by *B. rotunda* rhizomes extract in rats*.

Animal groups	No. of animals	Type of dressings (twice daily) (0.2 ml/animal)	Healing time (days) (Mean ± S.E.M)
Group 1	8	Gum acacia in normal saline (20 mg/ml)	19.33 ± 0.62^{a}
Group 2	8	Intrasit gel (standard control)	13.50 ± 0.22^{b}
Group 3	8	*B. rotunda* rhizome extract (100 mg/ml)	14.83 ± 0.31^{b}
Group 4	8	*B. rotunda* rhizome extract (200 mg/ml)	13.33 ± 0.21^{b}

*All values were expressed as mean and ± standard error mean. Mean with different letters were significantly different ($P < 0.05$).

the aid of round seal under local and general anesthesia as described by Morton and Melone (1972). Avoiding incision of the muscle layer and tension of skin was kept constant during the procedure, the entire wound left open (Nayak et al., 2005). The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1 mm^2 graph sheet, and traced out. The squares were counted and the area was recorded, as described by Chah et al. (2006) with slight modification.

Topical wound application

Wounds of group 1 animals were topically treated with 0.2 ml of vehicle, gum acacia in normal saline (20 mg/ml), twice daily as placebo control group (Shetty et al., 2007). Wounds of Group 2 rats were topically treated with 0.2 ml of Intrasite gel twice daily as a reference standard control. Moreover, 0.2 ml of 100 mg/ml and 200 mg/ml of ethanol extract of *B. rotunda* in vehicle each were applied topically twice daily to the wound of groups 3 and 4, respectively. The wound was observed daily until complete wound-healing enclosure occurs.

Estimation of wound healing (wound closure)

Wound areas were traced manually and calculated in square millimeters. The wound closure area of each animal was assessed by tracing the wound on days 1, 5, 10, 15 and 20 post-wounding surgery and the wound closure rate was expressed as the percentage of wound area compared with that on post-operative day by using a transparent paper and a permanent marker under light diethyl ether anesthesia as described by Nayak and Pinto-Pereira (2006) with slight modification. The wound areas recorded were measured using a graph paper. The percent of wounds healing on these days were determined (Chah et al., 2006). Number of days required for falling of scar without any residual raw wound gave the period of epithelization.

Histological evaluation of healed wounds

The skin specimen from wounds healed areas were fixed in 10% buffered formalin and processed by paraffin tissue processing machine. The healed skin was assessed by taking a 5 $\propto$ section, stained with hematoxylin and eosin.

Statistical analysis

All values are reported as mean $\pm$ S.E.M. and the statistical significance of differences among groups were assessed using one-way ANOVA. Value of $p < 0.05$ was considered significant.

RESULTS

Acute toxicity

Acute toxicity study is a study in which the animals were treated with the *B. rotunda* extract at a dose of 2 and 5 g/kg respectively and were kept under observation for 14 days. All the animals remained alive and did not manifest any significant visible toxicity at these doses. Thus, clinical observations, blood biochemistry, hematology, and histopathology data did not show any significant differences between control and treated groups. We conclude that *B. rotunda* orally administered to rats was safe and that no drug-related toxicity was detected even at the highest dose investigated.

Wound healing activity

Wounds dressed with *B. rotunda* -treated groups or reference standard control wounds showed considerable signs of dermal healing and significantly healed faster compared to group that received the placebo control treatment (gum acacia in normal saline) (Table 1 and Figures 1). Table 2 showed the effects of *B. rotunda* rhizomes extract on the percentage of wound healed on days post surgery. Throughout the experiment, the percentage of healing in placebo control group wounds was significantly lower than those of *B. rotunda* rhizomes extract-treated groups and reference standard control wounds. Histologically, *B. rotunda* rhizomes extract-treated groups contained comparatively less scar at wound closure than the placebo-treated group (Figure 2), and the granulation tissue in healed wound contained comparatively few inflammatory cells, and more collagen and proliferating blood capillarie (angio-genesis) com-pared with placebo-treated group (Figures 3).

DISCUSSION

It is important to note that throughout the period of wound treatment, the *B. rotunda* rhizomes extract did not cause irritation or pain to the animals as the rats neither show

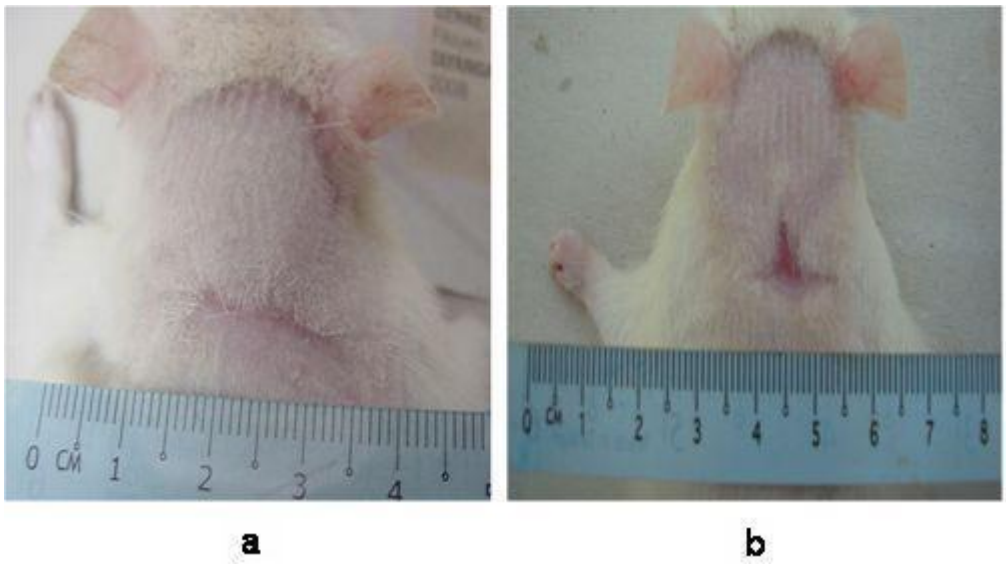

Figure 1. Complete wound healing in (a) 40 mg/ml of *B. rotunda* rhizomes extract-treated group on day 13 (b) Gum acacia-treated group on day 19.

Table 2. Effect of *B. rotunda* rhizomes extract on percentage (%) wound healing in experimental rats*.

Animal groups	Vehicles (twice daily) (0.2 ml)	Percentage of wound healing (Mean ± S.E.M) on day post surgery			
		5	10	15	20
Group 1 (N = 8)	Gum acacia in normal saline	32.17 ± 0.6^{a}	75.17 ± 0.7^{a}	83.5 ± 0.4^{a}	100.00 ± 0.00 ^{a}
Group 2 (N = 8)	Intrasite gel	75.17 ± 1.4^{b}	96.17 ± 1.17^{b}	100 00 ± 0.00^{b}	100.00 ± 0.00 ^{a}
Group 3 (N = 8)	*B. rotunda* (100 mg/ml)	60.00 ± 0.95^{c}	88.33 ± 0.99^{c}	100.00 ± 0.00^{b}	100.00 ± 0.00 ^{a}
Group 4 (N = 8)	*B. rotunda* (200 mg/ml)	72.00 ± 0.6^{u}	92.83 ± 0.83^{u}	100 00 ± 0.00^{u}	100 ± 0.00 ^{a}

*All values were expressed as mean and ± standard error mean. Mean in columns with different letters were significantly different (P < 0.05).

any signs of restlessness nor scratching/biting of wound site when the extract were applied. In the authors laboratory, all the surgical interventions were carried out under sterile conditions and animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. This is very important and researchers proved that the control microbial infection is necessary for better healing and its management (Muhammad and Muhammad, 2005).

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithetlialization, and wound contraction resulting in a smaller amount of apparent scar tissue (Midwood et al., 2004). In the current study, topical application of *B. rotunda* rhizomes extract significantly accelerated the rate of wound healing, and histology, healed wound contain comparatively less inflammatory, more collagen and angiogenesis. Wound healing effects may be due to regulation of collagen expression (Bonte et al., 1993) and increase in tensile strength of the wounds (Suguna et al., 1996). Similarly, enhanced healing activity has been attributed to collagen formation and angiogenesis (Trabucchi et al., 1986; Shukla et al., 1999). Collagen played a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue (Cohen et al., 1992). Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and nutrients essential for the healing process (Szabo et al., 1995) that include re-epithelization.

Stimulate epithelial cell proliferation and angiogenesis are important for wound healing process (Buntrock et al., 1982). Habibipour et al. (2003) showed that histological analysis of the treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing

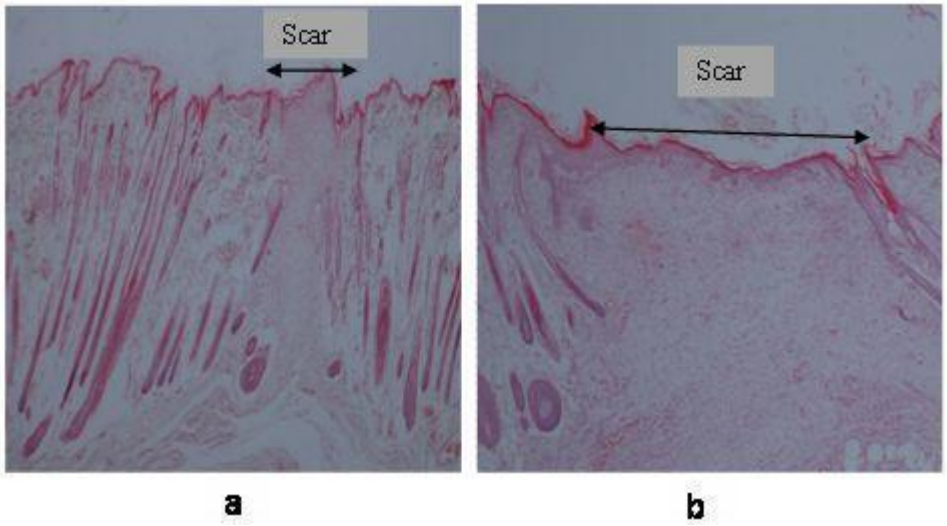

Figure 2. Histological section of healed wound in (a) 200 mg/kg of *B. rotunda*-treated group showing narrow scar region of wound closure, (b) in Gum acacia-treated group showing wide scar region of wound enclosure (H & E stain 4×).

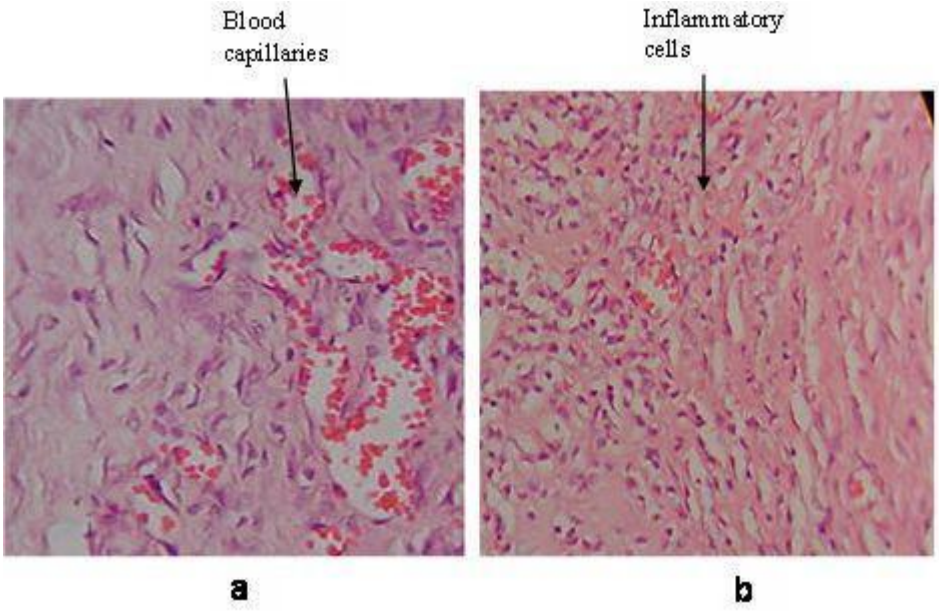

Figure 3. Histological section of healed wound in (a) 200 mg/kg of *B. rotunda* rhizome extract-treated group. Granulation tissue contains comparatively more collagen, fibroblast and blood capillaries, and few inflammatory cells. (b) Gum acacia-treated group. Granulation tissue contains comparatively less collagen, fibroblast and blood capillaries, and more inflammatory cells (H & E stain 40×).

of wound.

Phytochemical constituents present in *B. rotunda* rhizomes extract may be responsible for wound-healing activity and studies with plant extracts have shown that constituent like flavonoids (Tsuchiya et al., 1996) are known to promote the wound-healing process mainly due to their antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialization. Flavonoids are known to reduce lipid per- oxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibers by increasing the strength of collagen fibers, increasing the circulation,

preventing the cell damage and by promoting the DNA synthesis (Getie et al., 2002).

Mechanisms of wound healing may be due to stimulating the production of antioxidants in wound site and provides a favorable environment for tissue healing (Shukla et al., 1999). *B. rotunda* extract has shown antioxidant activity (Shindo et al., 2006). It have been reported that antioxidants may play a significant role in the wound healing process and may be important contributory factor in the wound healing property (Shukla et al., 1999). Antioxidants have been reported to play a significant role in the wound healing process and significantly improve wound healing and protect tissues from oxidative damage (Martin, 1996). *B. rotunda* contain a wide array of free radical scavenging molecules and flavonoids were the major naturally occurring antioxidant components in this plant (Shindo et al., 2006). The higher flavonoids content, the stronger the antioxidant activity. Flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol. These reactive intermediates are potentially implicated in delayed wound healing (Lewis and Hanson, 1991).

To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans. The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal, and cardiovascular adverse effects (Olson et al., 2000). Liver and kidney of the treated rats showed no significant change as compared to the control group. Hematology and clinical biochemistry values were within the range of the control animals tested and similar to some of the control reference values published by other researchers (Ringler and Dabich, 1979; Witthawaskul et al., 2003).

The highest dose of *B. rotunda* rhizomes extract which did not cause any toxicity was 5 g/kg body weight, suggesting that the *B. rotunda* extract is relatively non-toxic since in acute toxicity studies, the product is considered non-toxic if no deaths are registered after 14 days of observation and no clinical signs of toxicity are observed at doses at or below 5 g/kg (Brock et al., 1995).

In conclusion, *B. rotunda* rhizomes extract showed remarkable wound healing activity and it may be suggested for treating various types of wounds in human beings. The acute toxicity profile of *B. rotunda* rhizome extract could be considered favorable judging from the absence of adverse clinical manifestations. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of *B. rotunda* rhizome extract.

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