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CANTHARANTHUS ROSEUS LEAF EXTRACT ACCELERATES HEALING OF WOUND WISTAR RAT

[1]Anom-Dada, I K., [1]Berata, I K., [2]Putra-Manuaba, I. B., and [1]Damriyasa, I M.

[1]Faculty of Veterinary Medicine, Udayana University
[2]Faculty of Mathemathic and Natural Science Udayana University

ABSTRACT

Cantharantus roseus (C. roseus) is a medical plant that has been used to treat various diseases. This study was carried to determine *C. roseus* leaf extract able to acclerate wound healing in Wistar rat. Full skin-tickness round wounds were created on the back of the rats. Wound healing was evaluated after treated with 15% of a mixture of leaf extract of *C. roseus* and vaseline. The rats were divided into two groups, each group consist of 16 rats. Wound healing was assesed in five and fifteen days after treatment by measuring wound area, wound closure, and counting the new capillary vessel number (angiogenesis). The result of the study indicated that extract of *C. roseus* leaf accelerates the wound healing in Wistar rat. Coverage rate of wound is faster on treated group than control group (p<0.05). Wound closure and angiogenesis in early wound healing were higher in treated group than control group (p<0.05). These results suggested that the first phase of wound healing was shortened, and the proliferative and maturation phases were advanced by methanol extract of *C. roseus* leaf. Therefore, it can be concluded that *C. roseus* could be potential to help in topical management of wound healing.

Key words: *C. roseus, wound, healing, collagenesis, angiogenesis.*

INTRODUCTION

Many indigenous plants have been described for therapy of various diseases. They are a source of synthetic and traditional herbal medicine. In many countries such as India, China and also Indonesia the traditional medical plants are still the primary health care system in human and animal.[1] *C. roseus* is one of the tradiotional medical plants that empirically has been reported to have an effect on wound healing.[2] Recently, we observed that there was a considerable change in scientific investigation of the application of natural product, such as herb to be used for ethnopharmacology therapeutic applications. The presence of various active compounds in many indigenous plants has urged to evaluate these plants with a view to determine potential to accelerate the wound healing. Wound healing is the process of repair that follows injury to the skin and other soft tissues. To study wound repair, wound healing is often subdivided into three phases: an inflammatory phase, a proliferative phase, and a maturation phase or remodeling.[3-7] Along with inflammatory phase, an inflammatory response occurs and the cells below the dermis begin to increase collagen production, and followed by the epithelial tissue regeneration. This was then followed by the proliferative phase that is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Angiogenesis is the important process of generating new capillary blood vessels that a fundamental to wound healing, new blood vessels originate from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts exert collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells.[8-11]

C. roseus also known as *Tapakdara* in Indonesia, has empirically been used to treat a various of diseases. It has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat various diseases.[6] Alkaloids and tannins are the two important active compounds in *C. roseus*. Vincamine is the major alkaloid that is widely used as a medicinal agent. The compund is closely related semi-synthetic derivative known as ethyl-apovincaminate or vinpocetine. It has actions in vasodilating, blood thinning, hypoglycemic and memory-enhancing.[3] Extracts from the flower of C. roseus has wound-healing activity in Sparague Dawley rats.[8] There is no previous report on wound healing activities of the extract of the *C. roseus* leaf. In the study we evaluated

Correspondence: ***I K. Anom-Dada***
Faculty of Veterinary Medicine, Udayana University
Udayana University,Bali-Indonesia
Email: anomdada@yahoo.com

the wound healing of the Wistar rat after treated with the methanol extract of the *C. roseus* leaf.

MATERIAL AND METHODS

Male 2-month-old Wistar rats weighing of 240±3.85 g, were purchased from Balai Besar Veteriner (BBV) Denpasar Bali. The animals were maintained in individual cages during the experiment. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

Extract was obtained from the leaf of Chantaranthus roseus at UPT Laboratorium Analitik Universitas Udayana. The extract was diluted with ethanol. The leafes of *C. roseus* were collected locally in Bali and identified by the plant taxonomist and curator. The fresh leaves were shade dried and ground into a powder using an electric blender. The fine powder was suspended in methanol for 24 hours at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (GF227). The filtrate was placed in a water bath to dry at 40°C and the clear residue was used for the study.

Rats were anesthetized with ketamine, and their dorsal hair was shaved. A full-thickness round wound was prepared on the back of the rat with a skin biopsy punch (10 mm diameter). The animals were divided into two groups, of 16 each. Group 1 animals were topically treated with vaseline as a placebo control. The animals of group 2 were topically treated with the ethanol extract of *C. roseus* at a dose of 15% v/v per day till complete epithelization. The wound closure rate was assessed by tracing the wound on days 1, 5 and 15 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. Five and fifty days after the creation of the wound, the rats were killed and the wound area excised. The tissue was fixed in 10% phosphate buffered formalin solution. The formalin-fixed tissues were dehydrated, embedded in paraffin, and cut into 4mm ections. The sections were stained with hematoxylin and eosin (H-E). The number of capillaries was counted in the H-E stained sections, and the collagen area in the granulation tissue was measured. The number of capillaries was counted in three parts of the granulation tissue. The area of collagen synthesis was derived from the area stained blue by the Azan stain.

Data on wound closure, capillary number, and collagen in the granulation area were analyzed with SPSS and expressed as the mean ± S.D. or S.E.M. Significant differences were evaluated according to the Student's *t*-test.

RESULTS

The wound area were measured over a period of 15 days to determine whether the extract og *C roseus* affects the progress of wound healing of Wistar rat. The results of the present study indicated that the extract of *C roseus* leaf has an ability to accelerate wound healing in the rat (Figure 1). At five days, the decrease in wound area had been accelerated in treated group (Table 1).

Figure 1
Wound Healing in Treated Group and Control Group

Figure 1 shows the significant increase in the wound-healing activity was observed in the animals treated with the *C. roseus* extract compared with those who received the placebo control treatments.

Table 1
Wound healing effect of extract C. roseus leaf in Wistar Rat Wound Model

Parameter	Group	
	Treatment	Control
Wound area (mm^2)		
Day-5	12.57 ± 10.12*	30.31 ± 17.76
Day-15	0.002 ± 0.004	0.762 ± 1.271
Wound closure (mm^2)		
Day-5	78.94 ± 19.1*	60.42 ± 5.15
Day-15	99.99 ± 0.01	99.25 ± 1.69

*significant at $p < 0.05$

Table 1 shows the effects of methanolic extract *C. roseus* leafes administered topically at a dose of 15% v/v on wound area and wound clousure in Wistar rat. In the full skin-thickness round wound model, *C. Roseus* treated animals showed a significant reduction

in the wound area ($p < 0.05$) and a significant greater wound closure ($p < 0.05$) in five days after treatment, but in fifteen days after treatment showed no significant between treated and control group of the rats.

In contrast, control group delayed wound closure relative to treatment with vaselin. Complete wound healing also occurred earlier in treated group than in controls. There was a greater number of capillary vessels in 15% Leaf-extract *C. roseus*-treated than in controls, and the capillaries were distributed at the center of a wound rather than at the edge of wound in treated rat (Table 2).

Table 2
The Capillary Number of Treated and Control Group of The Rat

Group	Capillary Number	
	Day 5	Day 15
Control	4,00 ± 1,69	8,75 ± 4,92
Treatment	11,50 ± 2,30*	5,25 ± 4,46

* $p < 0.05$

In this study, the process of wound healing was also evaluated histologically. At the five and fifteen days, the amount of granulation tissue had increased remarkably in treated wounds.

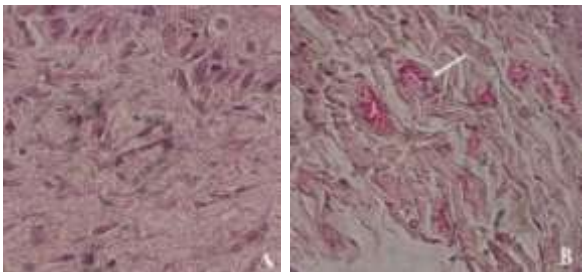

Figure 2
Histology of the Part of Wound (H-E Stain)
Control Group (A) and Treated Group (B), Capillary Vessels (arrow)

Figure 2 shows the neovascularization of the granulation tissue. The capillary vessels were counted in H-E-stained histological sections (Table 2). It was clear that the number of capillary vessels was significantly increased ($p < 0.05$) in the treated group than control group at five days after treatment. However, there was no difference in the number of capillary vessels between control and treated wounds at fifteen days after treatment.

DISCUSSION

C. roseu is known with various names in Indonesia; *sindapor* (Sulawesi), *kembang tembaga* (Sunda), *kembang tapak doro* (Jawa). the leafes and/or the whole plant is used as household remedy for various diseases. Traditionally, in India the flowers/leafes are used to control diabetes. The present study indicated that the leaf extract of C. roseus accelerates wound healing by evaluation of wound area and angiogenesis. Angiogenesis is the important process in wound healing that occured in the inflamatory phase. In these phase of wound healing is characterized by hemostasis and inflammation, followed by epithelization, angiogenesis. The next phase is the proliferative phase that characterized by collagen deposition. In the final phase or maturational phase that also called as remodeling phase, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. In the final phase characterized by granulation tissue formation that is primarily composed of collagen, edema, fibroblasts, and new small blood vessels. The methanol extract of the leave of *C. roseus* demonstrated a significant decrease of wound area and significant increase of wound clousure. The evidence indicates that the increasing granulation tissue formation. This formation indicating increased collagen turnover.

The results of this study indicate that leaf extract of *C. roseus* that administered topically can accelerate the wound healing process. Macroscopic observation showed that the wound healing was faster in treated group than control group. It is marked with a scab wound closure by a faster and higher percentage of wound closure of the wound area measurements. It is proved that the leaf extract Tapakdara can accelerate wound healing. Similar results were never proven by Nayak et al., (2006) that have properties tapakdara flower extract accelerates the wound healing process in Sparague Dawley rats.[8] Mice that were previously made a cut flower extract tapakdara then administered topically and orally at a dose of 100 mg / kg body weight recovery was faster when compared with control mice, as indicated by wound closure faster, faster epithelialization and granulation tissue dry weight higher in mice treated rats compared with controls.

Phytochemical analysis of extracts of C. roseus showed that this plant contains tannins, triterpeneoida and various alkaloids. One of these components is responsible in accelerating of wound healing process. Previous studies reported that phytochemical components such as flavanoida and triterpenoide known to accelerate the wound healing process, especially due astrigen and antimicrobial properties.[11-13] The content of phytochemicals plays a role in wound contraction and accelerates epithelialization. Several other studies have also shown that triterpenoida wound healing properties such as those contained in any other herbal remedies such as Cecropia pellata, and Pentas lanceolata.[8]

The results of present study and previous study (Nayak et al., 2006) have shown that active

compounds in C. roseus may be responsible for the wound healing activity.[8] Tannins, triterpenoids and alkaloids are presence at the extract of whole plant of *C. roseus* by phytochemical analysis.

Microscopic examination of histological preparations of the formation of new blood vessels (angiogenesis), showed that the angiogenesis in the treated group at day 5 was significantly more compared with the control group. However, in this study found that angiogenesis in the day-to-15 for treatment group was less compared with the control group. Data angiogenesis in the treatment and control in day-to-15 this was not statistically significantly different. This is certainly due to the treatment group gave the wound healing process is faster than the control group, so that in the treated group did not form new blood vessels again. The research was supported by a study of Fujita et al., (2003), which proves that the seaweed extract Lithosporus erythrorhison which also contain tannins and tritrefenoid can accelerate wound healing characterized by increased number of new capillaries in the injured area.[5] Thus the findings in this study reinforce that angiogenesis plays an important role in wound healing process, as also noted by several previous investigators as well as role of the sympathetic nerves located in the skin also has a role in wound healing.[10-13]

CONCLUSION

The present study has demonstrated that an ethanol extract of *C. roseus* leaf has properties that render it capable of promoting accelerated wound healing activity compared with placebo controls. Wound contraction, increased tensile strength, increased hydroxyproline content and antimicrobial activity support further evaluation of *C. roseus* in the topical treatment and management of wounds.

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REFERENCES

1. Singh, A. and Singh, D. K. 2001: Molluscicidal activity of *Lawsonia inermis* and its binary and tertiary combinations with other plant derived molluscicides. *Indian J Exp Biol*, 39:263-268.
2. Nayak, B. S. 2006: *Cecropia peltata L* (*Cecropiaceae*) Has Wound Healing potential-A preclinical study in Sprague Dawley Rat model. *International Journal of Lower Extremity Wounds*, 5:20-26.
3. Chattopadhyay, R. R. 1999. A comparative evaluation of some blood glucose lowering agents of plant origin. *J Ethnopharmacol*, 67:367-372.
4. Eming, S. A., Brachvogel, B., Odorisio, T., and Koch, M. 2007. Regulation of angiogenesis: wound healing as a model. Prog Histochem Cytochem 42: 115–170.
5. Fujita, N., Ikuyo, S., Hiromi, K. I., Norikazu, I., Yoshiko, K., Miki, M., and Masamitsu, L. 2003. An Extract of the Root of *Lithospermun erythrorhison* Accelerates Wound Healing in Diabetic Mice, *Biol. Pharm. Bull.* 26(3): 329—335.
6. Hindmarch, I., Fuchs, H. H., and Erzigkeit, H. 1991. Efficacy and tolerance of vinpocetine in ambulant patients suffering from mild to moderate organic psychosyndromes. *Int Clin Psychopharmacol*, 6(1):31-43.
7. Kim, L. R., and Pomeranz, B. 1999. The sympathomimetic agent, 6-hydoxydopamine, accelerates cutaneous wound healing. Eur J Pharmacol 376: 257–264.
8. Nayak, B. S., Vinutha, B., Geetha, B., and Sudha, B. 2006. Experimental evaluation of Pentas *lanceolata* for Wound healing activity in rats. *Fitotherapia*, 76:671-675.
9. Pullar, C. E, Rizzo, A., and Isseroff, R. R. 2006. Beta-Adrenergic receptor antagonists accelerate skin wound healing: evidence for a catecholamine synthesis network in the epidermis. J Biol Chem 281: 21225–21235.
10. Seiffert, K. 2008. Regualtion of cutaneous immunity by catecholamines. In: Granstein RD, Luger TA, eds. Neuroimmunology of the Skin: Basic Science to Clinical Practice. Berlin Springer : 65–74.
11. Singer, A. J., and Clark, R. A. 1999. Cutaneous wound healing. N Engl J Med 341: 738–746.
12. Tonnesen, M. G., Feng, X., and Clark, R. A. 2000. Angiogenesis in wound healing. J Investig Dermatol Symp Proc 5: 40–46.
13. Harvey, C. 2005. Wound Healing. *Orthopaedic Nursing*. 24(2): 143-159.