*Original Article* *J. Clin. Biochem. Nutr.*, **45**, 304–308, November 2009

# Wound Healing Activities of *Rafflesia Hasseltii* Extract in Rats

**Mahmood A. Abdulla[1,*], Khaled A. Ahmed[1,2], Hapipah M. Ali[3], Suzita M. Noor[1], and Salmah Ismail[1]**

[1]*Department of Molecular Medicine, Faculty of Medicine, University Malaya 50603 Kuala Lumpur, Malaysia*
[2]*Faculty of Dentistry, Ibb University, P.O. Box 70270, Ibb, Yemen*
[3]*Department of Chemistry, Faculty of Science, University Malaya 50603 Kuala Lumpur, Malaysia*

Received 13 February, 2009; Accepted 7 May, 2009

***Summary*** **The effects of topical application of *Rafflesia hasseltii* buds and flowers extract on the rate of wound healing and histology of healed wound were assessed. Four groups of adult male *Sprague Dawley* rats were experimentally wounded in the posterior neck area. A thin layer of blank placebo was applied topically to wounds of Group 1 rats. Wounds of experimental animals (Group 2 and 3) were treated with placebo containing 5% and 10% *R. hasseltii* buds extract, respectively. A thin layer of Intrasite gel was applied topically to wounds of Group 4 animals as reference. Macroscopically, wounds treated with placebo containing 5% and 10% *R. hasseltii* buds extract or Intrasite gel have been significantly accelerated the rate of wound healing compared to placebo-treated wounds. Histological analysis of healed wounds has confirmed this effect. Wounds treated with placebo containing 5%, 10% *R. hasseltii* buds extract or Intrasite gel showed markedly less scar width at wound enclosure and granulating tissue contained markedly more collagen and proliferating fibroblasts, but with the absence of inflammatory cells compared to wounds treated with blank placebo. In conclusion, the findings of increased rate of wound closure and contraction together with the histological findings suggest that *Rafflesia hasseltii* buds extract is very effective in accelerating the wound healing process.**

***Key Words*****: Wounds healing, *Rafflesia hasseltii* extract, Intrasite gel, placebo**

## Introduction

Burn trauma and wounds are still a major problem in developing countries, often having severe complications and involving high costs for therapy. An important aspect of the use of traditional medicinal remedies and plants in the treatment of burns and wounds is an important mode to improve healing and the same time to reduce the financial burden. Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine [*1*, *2*].

*To whom correspondence should be addressed.
Tel: +603-7697-6604 Fax: +603-7967-6600
E-mail: khaaah@gmail.com

Rafflesia, among the world's largest flowers, belongs to the family Rafflesiaeae. The plant family Rafflesiaeae has eight genera which includes the genus Rafflesia. Rafflesia is a genus of parasitic flowering plants and it is found in tropical rainforest of Malaysia, Singapore, Indonesia and Philippines. It occurs only in certain habitats as a bud on the Tetrastigma species of woody veins. Rafflesia is a very rare flower, difficult to reproduce in the lab and its dried specimen is difficult to preserve [*3*]. In Peninsular Malaysia, Rafflesia buds are used by women to stop internal bleeding and shrink the womb after childbirth, as well as for the treatment of fever. The bud was once sought after as a traditional medicine. It is used by men as an energy drink or an aphrodisiac [*3*, *4*]. The buds and flowers have a high content of tannin and phenols which can be toxic when taken in large quantities [*3*, *4*] and also *R. hasseltii* extract had

anti-microbial activity [*5*]. The present study was undertaken to evaluate the rate of wound healing properties of *R. hasseltii* buds extract in experimental rats.

## Materials and Methods

*Placebo and Intrasite gel*

Blank placebo (an aqueous semisolid cream) and Intrasite gel were obtained from Department of Pharmacy, University Malaya Medical Centre (Sunward Pharmaceutical, Kuala Lumpur, Malaysia). Intrasite gel is an amorphous hydrogel which gently re-hydrates necrotic tissue, facilitate autolytic debridement, while being able to loosen and absorb slough and exudation, cleaning the way for effective wound healing. It is also designed for wounds that are granulating and reepithelialising. 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 [*6*]. Intrasite gel is a trademark for Smith and Nephew Ltd., UK.

*Preparation of plants extracts*

*Rafflesia* flowers (*R. hasseltii*) were purchased from Orang Asli in Perak state, Malaysia. The flowers were cut into small pieces, labelled, washed with distilled water and stood at 50°C for 5–7 days until fully dried. The flower samples were ground to a fine texture form using a grinder and stored at −20°C until use. The dried flower powder was extracted by being macerated in methanol (100 g/1500 ml, w/v) in a conical flask for 5 days at room temperature. Afterwards, the residue was removed by filtration using a mesh and filter funnel. Rotator evaporator was used to concentrate the extracted filtered material.

*Preparation of the treatment mixture*

The semisolid mass of *R. hasseltii* extract (methanol free) was homogeneously mixed with placebo in a concentration of 5% and 10% (w/w) each as described by Mukherjee *et al.* (2000) with little modification [*7*]. The mixtures were kept at 4°C, and brought to a room temperature before application.

*Acute toxicity test*

A standard acute toxic method was used to determine the safe dose for the extracts. Thirty six animals (18 males and 18 females) were assigned equally into 3 groups labeled as vehicle (distilled water); low (2 g/kg) and high (5 g/kg) dose of leaf extract preparation, respectively. The animals were fasted overnight (food but not water) prior dosing. Food was withheld for a further 3 to 4 h after dosing. Observations were done on mortality and behavioral changes of the rats following treatment for 24 h. The acute toxicity $LD_{50}$ was calculated at the geometric mean of the dose that resulted in 100% lethality and that cause no lethality at all.

*Experimentally induced wounds*

*Sprague Dawley* adult male rats were randomly divided into 4 groups of 6 rats each. Each rat that weighed between 180–200 g was housed separately (one rat per cage). The animals were maintained on standard pellet diet and tap water. The animals were anesthetized by diethyl ether. The skin shaved by electrical shaver, disinfected with 70% alcohol and injected with 1 ml of Lignocaine HCl (2%, 100 mg/5 ml). An area of uniform wound 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Suguna *et al.* (2002) with few modifications [*8*]. Avoid incision of the muscle layer and tension of skin was kept constant during the procedure.

*Topical application of vehicles*

A thin layer of blank placebo was applied topically to the wounds of Group 1 rats twice a day. The semisolid mass of *R. hasseltii* extract was homogeneously mixed with blank placebo aqueous cream in a concentration of 5% and 10% (w/w), as described by Mukherjee *et al.* [*7*] and thin layers of the mixture were applied topically twice a day to the wounds of Groups 2 and 3 animals, respectively. Wounds of Group 4 rats were treated with a thin layer of Intrasite gel twice daily. The wound was observed daily until complete wound enclosure occurs.

*Histological evaluation of healed wounds*

The skin specimens 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 μm section followed by staining with hematoxylin and eosin.

*Ethics*

The experimental protocol for animal work was approved by the local ethics committee for animal experimentation in the Faculty of Medicine, University of Malaya, Ethic No. PM 28/-9/2007 MAA (R).

*Statistical analysis*

All values were expressed as mean ± SEM and the statistical significance of differences among groups in term of rate of wound healing were evaluated using one-way analysis of variance ANOVA. A value of $p<0.05$ was considered significant. Statistical computations were calculated using SPSS 11.5 for Windows software (SPSS Inc, Chicago, IL).

## Results

*Acute toxicity study*

No mortality occurred amongst the *Sprague-Dawley* rats

Table 1. Time required for wound healing in experimental animals

| Animal groups | No. of animals | Type of treatment | Healing time (days) (Mean ± SEM) |
|---|---|---|---|
| Group 1 | 6 | Blank placebo | 21.67 + 0.48* |
| Group 2 | 6 | Placebo containing 5% *R. hasseltii* | 15.83 + 0.33** |
| Group 3 | 6 | Placebo containing 10% *R. hasseltii* | 14.67 + 0.56** |
| Group 4 | 6 | Intrasite gel | 12.33 + 0.31 |

One-way ANOVA was used for statistical analysis. All values are expressed as mean ± SEM. *$p$<0.01 vs Group 2, Group 3, and Group 4. **The difference between these two groups is not statistically significant.

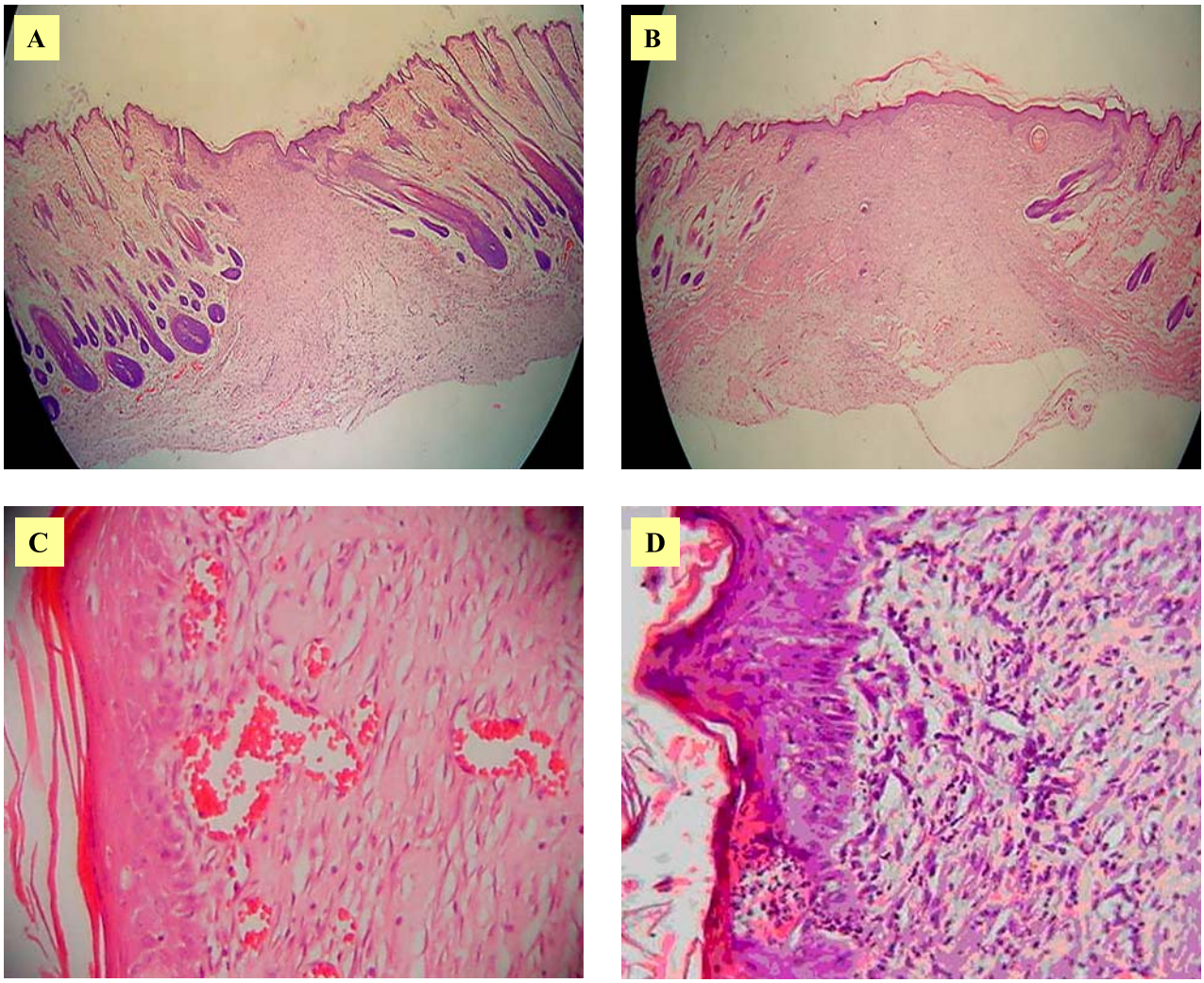


Fig. 1. Histological sections of healed wounds treated with placebo containing 10% *Rafflesia. hasseltii* showing narrow scar at the wound closure (A), dressed with blank placebo revealed wide scar at the wound closure (B), treated with placebo containing 10% *R. hasseltii* extract showing granulation tissue with more collagen, fibroblast and blood capillaries as well as absence of inflammatory cells (C), and after treatment with blank placebo where granulation tissue contains less collagen, fibroblast, and blood capillaries as well as more inflammatory cells (D). (H & E stains, 80× magnification).

with dose levels of 2 g/kg and 5 g/kg of Rafflesia bud and flower extracts during the study period. Behavioral observation did not show evidence indicative of significant drug toxicity. The results suggested that the oral $LD_{50}$ of Rafflesia *hasseltii* extracts was greater than 5 g/kg.

*Wound healing observasions*

Grossly, wounds treated with 5%, 10% *R. hasseltii* extracts, or with Intrasite gel showed considerable signs of dermal healing and significantly ($p<0.05$) healed faster than wounds treated with blank placebo (Table 1). There were no significant differences between wounds treated with 5%, 10% *R. hasseltii* extract or Intrasite gel in terms of rate of accelerating the wound healing process (Table 1). Histologically, wounds treated with *R. hasseltii* extracts or Intrasite gel showed markedly less scar at wound enclosure (Fig. 1A, B) and granulation tissue contained markedly increased collagen fibres, fibroblasts and proliferating blood capillaries, and absence of inflammatory cells, while wounds treated with blank placebo showed less collagen fibre, fibroblasts and blood capillaries, and more inflammatory cells (Fig. 1C, D).

There were no differences in histology of Intrasite gel and Rafflesia extract treated wounds; both contain less scar at wound enclosure and same granulation tissue which contained markedly more collagen fibers, fibroblasts and proliferating blood capillaries, and absence of inflammatory cells (data not shown).

## Discussion

The majority of the world's population relies on traditional medicine for their health care. This is also the case in the treatment of wounds. In developing countries, remedies prepared from herbal plants have been widely used for the treatment of soft tissue wounds and burns by medical personnel trained in western medicine as well as by traditional practitioners.

In the present study, topical application of *R. hasseltii* extract significantly enhanced the rate of wound healing and granulation contains more collagen, fibroblasts and blood capillaries, but without inflammatory cells. Wound healing effects may be due to up-regulation of human collagen I expression [*9*] and an increase in tensile strength of the wounds [*10*]. Enhanced healing activity has been attributed to increased collagen formation and angiogenesis [*11*, *12*]. Collagen plays 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 [*13*]. Angiogenesis in granulation tissues improves circulation in the wound site thus providing oxygen and nutrients essential for the healing process [*14*]. Stimulation of epithelial cell proliferation and angiogenesis are important processes for the increased wound healing [*15*]. Similarly, Habibipour *et al.* (2003) showed that histological analysis of the treated healed wound contained a large amount of fibroblasts proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing of wounds [*16*].

The extract of *R. hasseltii* posses significant antioxidant activity [*3*, *4*]. It is likely that the antioxidant property of *R. hasseltii* extract could be linked to its wound healing acceleration. Topical applications of compounds with antioxidant properties significantly improve wound healing and protect tissues from oxidative damage [*17*]. Kanchanapoom *et al.* (2007) isolated four hydrolysable tannins from the flowers of *Rafflesia. Kerrii*. The tannins are known to possess antioxidant activity [*4*]. It could be conceivable that the *R. hasseltii* extract exert their wound healing activity through the tannins since tannins are reported to improve wound healing and protect tissues from oxidative damage [*18*]. *R. hasseltii* extract had anti-microbial activity [*5*] which may be contributed to promote wound healing. In conclusion, the current study revealed that wounds treated with *R. hasseltii* extracts, as topical application of wounds, significantly accelerate the wound healing process.

## Acknowledgment

This study was financially supported by Ministry of Science, Technology, and Innovation, Malaysia (grant No.: 12-02-03-2051 Science Fund RMK-9 Cycle 1/2008).

## References

[1] Lodhi, S., Pawar, R.S., Jain, A.P., and Singhai, A.K.: Wound healing potential of Tephrosia purpurea (Linn.) Pers. in rats. *J. Ethnopharmacol.*, **108**, 204–210, 2006.

[2] Majumdar, M., Nayeem, N., Kamath, J.V., and Asad, M.: Evaluation of Tectona grandis leaves for wound healing activity. *Pak. J. Pharm. Sci.*, **20**, 120–124, 2007.

[3] Thulaja, N.R.: *Rafflesia*, National Library Board Singapore, pp. 2–25, 2003.

[4] Kanchanapoom, T., Kamel, M.S., Picheansoonthon, C., Luecha, P., Kasai, R., and Yamasaki, K.: Hydrolyzable tannins and phenylpropanoid from Rafflesia kerrii Meijer (Rafflesiaceae). *J. Nat. Med.*, **61**, 478–479, 2007.

[5] Wiart, C., Mogana, S., Khalifa, S., Mohan, M., Ismail, S., Buckle, M., Narayana, K., and Sulaiman, M.: Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia*, **75**, 68–73, 2004.

[6] Williams, C.: Intrasite Gel: a hydrogel dressing. *Br. J. Nurs.*, **3**, 843–846, 1994.

[7] Mukherjee, P.K., Verpoorte, R., and Suresh, B.: Evaluation of *in-vivo* wound healing activity of Hypericum patulum (Family: hypericaceae) leaf extract on different wound model in rats. *J. Ethnopharmacol.*, **70**, 315–321, 2000.

[8] Suguna, L., Singh, S., Sivakumar, P., Sampath, P., and Chandrakasan, G.: Influence of Terminalia chebula on dermal wound healing in rats. *Phytother. Res.*, **16**, 227–231, 2002.
[9] Bonte, F., Dumas, M., Chaudagne, C., and Meybeck, A.: Influence of asiatic acid, madecassic acid, and asiaticoside on human collagen I synthesis. *Planta Med.*, **60**, 133–135, 1994.
[10] Suguna, L., Sivakumar, P., and Chandrakasan, G.: Effects of Centella asiatica extract on dermal wound healing in rats. *Indian J. Exp. Biol.*, **34**, 1208–1211, 1996.
[11] Trabucchi, E., Preis Baruffaldi, F., Baratti, C., and Montorsi, W.: Topical treatment of experimental skin lesions in rats: macroscopic, microscopic and scanning electron-microscopic evaluation of the healing process. *Int. J. Tissue React.*, **8**, 533–544, 1986.
[12] Shukla, A., Rasik, A.M., and Dhawan, B.N.: Asiaticoside-induced elevation of antioxidant levels in healing wounds. *Phytother. Res.*, **13**, 50–54, 1999.
[13] Cohen, I.K., Diegelmann, R.F., and Lindblad, W.J.: *Wound healing. Biochemical and Clinical Aspects*, W.B. Saunders Co., Philadelphia, 1992.
[14] Szabo, S., Kusstatscher, S., Sakoulas, G., Sandor, Z., Vincze, A., and Jadus, M.: Growth factors: New endogeneous drug for ulcer healing. *Scand. J. Gastroenterol.*, **210**, 15–18, 1995.
[15] Buntrock, P., Jentzsch, K.D., and Heder, G.: Stimulation of wound healing. Using brain extract with fibroblast growth factor (FGF) activity. II. Histological and morphometric examination of cells and capillaries. *Exp. Pathol.*, **21**, 62–67, 1982.
[16] Habibipour, S., Oswald, T.M., Zhang, F., Joshi, P., Zhou, X.C., Dorsett-Martin, W., and Lineaweaver, W.C.: Effect of sodium diphenylhydantoin on skin wound healing in rats. *Plast. Reconstr. Surg.*, **112**, 1620–1627, 2003.
[17] Martin, A.: The use of antioxidants in healing. *Dermatol. Surg.*, **22**, 156–160, 1996.
[18] Saurez, J., Herreta, M.D., and Marhuenda, E.: Hesperidine and neohesperidine dihydrochalcone on different experimental models of induced gastric ulcer. *Phytother. Res.*, **10**, 616–618, 1996.