

Synergistic Effects of Alcoholic Extract of Sweet Basil (*Ocimum basilicum* L.) Leaves and Honey on Cutaneous Wound Healing in Rats

I. Salmah, A.A. Mahmood and K. Sidik

Department of Molecular Medicine, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur, Malaysia

Abstract: Wound healing activities of sweet basil was studied on cutaneous excision wounds in rats. Three groups of male *Sprague Dawley* rats each consist of 6 animals. Rats were placed individually in cages and all animals were experimentally wounded in the posterior neck area. Pure un-boiled honey was applied topically to wounds of Group 1 animals. Wounds of Group 2 rats were treated with honey in combination with *O. basilicum* L. alcoholic leaf extract and solcoseryl - jelly was applied topically to wounds of Group 3 animals. The effects of vehicles on the rate of wound infections and on the rate of wound healing were assessed. Wounds of all groups of animals showed clean and remain sterile throughout the experiment. Wounds treated with honey in combination with plant extract and those treated with solcoseryl - jelly significantly ($p < 0.05$) accelerates wound healing compared to wounds treated with honey alone. These results strongly document the beneficial effects of plant extract for the acceleration of wound healing process and the rates of wounds sterility.

Key words: Honey, wounds healing, rats, *Ocimum basilicum* extract, solcoseryl - jelly

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 the potential 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^[1-3].

Sweet basil (*Ocimum basilicum* L.) is a popular culinary herb and its essential oil has been used for many years to flavour foods, as an ingredient of dental and oral health care products and in fragrances^[4]. In addition, basil essential oils have been reported to have potent anti-HIV-1 activity^[5], anti-ulcer^[6], anti-inflammatory activity^[7], anti-microbial^[8], antioxidant activity^[9] and analgesic^[10] properties without any noticeable toxicity. It has also been demonstrated that the anti-microbial activities of essential oils of different species of *Ocimum* were predominantly associated with the main constituents of linalool and methyl chavicol (estragole)^[11,12]. Moreover, Basil leaf extract was highly effective in inhibiting carcinogen-induced tumor induced in both the tumor models at perinatal level^[9].

Honey has long been used to accelerate wound

healing^[13-15]. Existing literature attributes honey with a number of useful properties, such as a broad-spectrum anti-microbial activity, deodorization, debriding and anti-inflammatory actions and stimulation of new tissue growth^[16,17]. Honey, for the most part, is made up of simple sugars and is an excellent source of energy. It is hyper tonic and has been shown to be sterile and highly bactericidal^[18,19]. Honey has been found, when applied locally, to reduce infection and promote wound healing^[20]. Physiological properties of honey such as hyper tonicity, low pH (3.6) and hygroscopicity were thought to augment the healing process. Antibacterial effects were also attributed to these elements^[21,22]. The wound healing properties of honey have been cleansing, absorption of edema, antibacterial activity, deodorization, promotion of granulation, tissue formation and epithelialization and improvement of nutrition^[16,17,23].

Solcoseryl is a protein-free, standardized haemodialysate derived from calf blood, which has been shown to improve wound healing is well documented^[24-26]. Experimental studies of the mechanism of action^[26,27] have indicated that solcoseryl stimulates wound healing by enhancing the development of new capillaries, thereby improving the microcirculation and nutrition of ischaemic tissue. In biochemical study,^[28] were able to show a stimulatory effect on several aspects of granulation tissue formation, including vascularization,

cellularity and the subsequent accumulation of collagen. A positive effect on wound healing has been described in a number of clinical reports^[25,29].

The aim of the present study was carried out to assess the synergic effects of honey in combined with alcoholic extracts of *O. basilicum* L. on the rate of wound-healing process and on the rate of infection in rats.

MATERIALS AND METHODS

Honey: Pure, unprocessed, un-boiled commercial honey was obtained from Faculty of Agriculture, University Putra Malaysia, Serdang Selangor Malaysia, was used for the present study.

Experimental animals: *Sprague Dawley* rats were obtained from the animal house, Faculty of Medicine, University of Malaya. The rats were divided randomly into 3 groups of 6 rats each. Each rat that weighted between 180 - 200 gm was housed separately (one rat per cage). The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on standard pellet diet and tap water.

Experimentally induced wounds: An area of tissue 2 by 2 cm was excised from the nape of the neck, in previously shaved, disinfected with 70% alcohol and injected with 1 ml of Lignocaine HCl (2%, 100 mg/5 ml), to the depth of the muscle, avoiding incision of the muscle layer itself. A fresh surgical blade was used for the perpendicular cut in each animal and tension of skin was kept constant during the procedure.

Topical application of vehicles: A thin layer of pure, un-boiled commercial honey was applied topically twice daily to Group 1 animals. Group 2 rats were treated with a thin layer honey in combined with alcoholic leaves extract (10% of leaves extract in honey w/w) applied topically twice daily dressing, whereas a thin layer of solcoseryl - jelly was topically applied twice daily to Group 3 wounds as positive control animals (reference).

Bacterial isolation: Bacterial culture determination was performed in all wounds on day 3 after experimentally induced wounds. The swabs were taken from the surface of wound for culture before starting treatment. Later on day 7 another wound swab was taken for culture from all wounds. The swabs were cultured on Brain Heat Infusion (BHI) agar overnight at 37°C for any bacterial growth.

Statistical analysis of data: Results were expressed as mean + M.S.E. The statistical difference between the groups in the term of the mean rate of wound healing and rate of infection was calculated by using Student's *t*-test

RESULTS

Rate of infection: No bacteria were isolated from swabs cultured (BHI) agar in experimental animals wounds on day 3 and day 7 before dressing with honey alone, honey in combined with plant extract and solcoseryl - jelly treated animals (Table 1).

Rate of healing: The results obtained indicate the existence of anti-microbial compounds in the honey alone, honey in combined with plant extract and solcoseryl - jelly. Wounds treated with honey in combined with plant extract and wounds treated with solcoseryl – jelly showed considerable signs of dermal healing and significantly ($p<0.05$) healed earlier than those treated with honey alone (Group 1) (Table 1). There were no significant differences between honey in combination with plant extract and solcoseryl – jelly in the term of wound healing duration. In addition dermal wounds treated with honey in combined with plant extract and wounds treated with solcoseryl - jelly were rapidly replaced by granulation tissue and advancing epithelialization and the diameters of wounds become narrow gradually.

Table 1: Time required for wound healing rate and bacterial isolation in experimental animals.

Animal groups	No of animals	Type of dressings	Healing time (days) (Mean + M.S.E)	Bacterial isolated	
				Day 3	Day 7
Group 1	6	Honey alone	16.5 + 0.43	-	-
Group 2	6	Honey + extract	13.17 + 0.31*	-	-
Group 3	6	Solcoseryl - jelly	12.83 + 0.31*	-	-

* $p<0$ significant from control (Group 1)

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. The results of the current study showed that the usage of honey in combination with *O basilicum* plant extract and the solcoseryl - jelly as topical applicants significantly ($p<0.05$) accelerated wound healing compared to honey alone. Wounds pretreated with honey alone, honey in combination with plant extract and solcoseryl – jelly, as a topical application maintained sterility of wounds until complete healing in whole animals. *O. basilicum* L. and other *Ocimum* species were generally had anti-microbial activity^[30,31].

The anti-microbial activity of essential oils from *Ocimum* species was predominantly associated with the main constituents linalool and methyl chavicol, the anti-microbial effect of basil oil against Gram-positive and Gram-negative is bactericidal^[32,33].

Results in the present study also showed that wounds treated with *O. basilicum* extract in combination with honey accelerate the rate of wound healing. Flavonoids from leaves of sweet basil have shown significant protection against radiation lethality and chromosomal aberrations *in vivo*. The radioprotection seems to be associated with antioxidant activity^[34]. Rosmarinic acid, natural phenolic compound in sweet basil, would suppress the proliferation of mesangial cells and glomerular matrix expansion *in vivo* by its fibrinolytic and antioxidant activity. These results suggest that basil essential oil could be beneficial component of preventing infection and enhanced wound healing.

The wound healing properties of honey had been well documented^[23,14,15]. Topical application of honey has been recognized for a long time to be effective in controlling infection and producing a clean granulating wound bed. Honey acts mainly as a hyperosmolar medium and prevents bacterial growth. The high sugar content of honey renders the honey hyperosmolar. Due to this effect, it causes rapid absorption of edema fluid from the soggy weeping wounds. The viscosity of honey is high and it forms a physical barrier that prevents bacterial colonization of wounds and creating a moist environment, which appears to be a helpful and accelerates wound healing^[14,15,17]. The nutrient contents of the honey such as laevulose and fructose improve local substrate supply and may help promote epithelialization^[35-37]. Honey seems to cause more rapid epithelialization, presumably because of antibacterial properties as compared to control. The enzyme catalase present in honey has an antioxidant property^[38,39] and thus honey may have a role as an antioxidant in thermal injury^[40-41].

CONCLUSIONS

In conclusion, the synergic effects of honey in combined with alcoholic extract of *O. basilicum* appeared to have several important properties that make it useful ideal as a dressing agent for wounds. We can suggest that it may be possible to use honey in combination with *O. basilicum* alcoholic extract as topical application for the treatment of wounds. However, further investigations are required to elucidate their exact mechanism (s) of wound healing activity.

ACKNOWLEDGEMENTS

This study was financially supported by the University of Malaya through the grand 06-02-03-1026 (Oracle 8361026).

REFERENCES

1. Suguna, L., S. Singh, P. Sivakumar, P. Sampath and G. Chandrakasan, 2002. Influence of *Terminalia chebula* on dermal wound healing in rats. *Phytotherapy Research*. 16: 227-231.
2. Rane, M.M. and S.A. Mengi, 2003. Comparative effect of oral administration and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats. *Fitoterapia*. 74: 553-558.
3. Shirwaikar, A., A.P. Somashekar, A.L. Udupa, S.I. Udupa and S. Somashekar, 2003. Wound healing studies of *Aristolochia bracteolata* Lam. With supportive action of antioxidant enzymes. *Phytomedicine*. 10:558-562.
4. Guenther, E. 1952. The essential oils. New York: D. Van Nostrand Co., Inc, 3: 399-433.
5. Yamasaki, K., M. Nakano, T. Kawahata, H. Mori, T. Otake and N. Ueba, *et al.*, 1998. Anti-HIV-1 activity of herbs in Labiatae. *Biological and Pharmacological Bulletin*. 21:829-833.
6. Singh, S., 1999. Evaluation of gastric anti-ulcer activity of fixed oil of *O. basilicum* L. and its possible mechanism of action. *Indian J. Experimental Biology*. 36: 253-257.
7. Singh, S. and D.K. Majumdar, 1997. Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J. Experimental Biol.*, 35: 380-383.
8. Suppakul, P., J. Miltz, K. Sonneveld and S.W. Bigger, 2003. Anti-microbial properties of basil and its possible application in food packaging. *J. Agricultural and Food Chem.* 51: 3197-3207.
9. Dasgupta, T., A.R. Rao and P.K. Yadava, 2004. Chemomodulatory efficacy of basil leaf (*O. basilicum*) on drug metabolizing and antioxidant enzymes and on the carcinogen-induced skin and fore-stomach papillomagenesis. *Phytomedicine*. 11:139-151.
10. Aziba, P.I., D. Bass and Y. Elegbe, 1999. Pharmacological investigation of *O. gratissimum* in rodents. *Phytotherapy Research*. 13:427-429.
11. Reuveni, R., A. Fleischer and E. Putievsky, 1984. Fungi static activity of essential oil from *O. basilicum* chemotypes. *Phytopathologische Zeitschrift*, 110: 20-22.

12. Sinha, G.K. and B.C. Gulati, 1990. Anti-bacterial and anti-fungal study of some essential oils and some of their constituents. *Indian Perfumer*. 34: 126-129.
13. Molan, P.C., 2002. Re-introducing honey in the management of wounds and ulcers-theory and practice. *Ostomy Wound Management* 48: 28-40.
14. Ahmed, A.K.J., M.J. Hoekstra, J.J. Hage and R.B. Karim, 2003. Honey-mediated dressing: Transformation of an ancient remedy into modern therapy. *Annals of Plastic Surgery*. 50: 143-148.
15. Ghaderi, R. and M. Afshar, 2004. The effect of topical application of honey on wound healing in mice: An experimental study. *J. Investigative Dermatol.* 123: A7.
16. Al-Waili, N.S. and K. Y. Saloom, 1999. Effects of topical honey on post-operative wound infections due to Gram-positive and Gram-negative bacteria following Caesarean sections and hysterectomies. *Euro. J. Med. Res.* 4: 126-130.
17. Lusby, P.E., A.B. Coombes, J.M. Wilkinson and F.E.T. GradDip 2002. Honey: A potent agent for wound healing? *J. Wound, Ostomy and Continence Nursing*. 29: 295-300.
18. Shamala, T.R., Y.P. Shrijiyothi and P. Saibaba, 2002. Antibacterial effect of honey on the *in vitro* and *in vivo* growth of *E. coli*. *World J. Microbiology and Biotechnol.*, 18:863-865.
19. Miorin, P.L. N.C. Junior, A.R. Custodio, W.A. Bretz and M.C. Marcucci, 2003. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *staphylococcus aureus*. *J. Applied Microbiology*. 95: 913-920.
20. Osuagwu, F.C., O.W. Oladejo, I.O. Imosemi, A. Aiku, O.E. Ekpos, A.A. Salami and O.O. Oyedele, 2004. Enhanced wound contraction in fresh wounds dressed with honey in Wistar rats. *West African J. Medicine*. 23:114-118.
21. Dunford, C., R.A. Cooper, P.C. Molan, and R. White 2000. The use of honey in wound management. *Nurses Standard* 15: 63-68.
22. Aysan, E., E. Ayar, A. Aren. and C.C. Cifter, 2002. The role of intra-peritoneal honey administration in preventing post-operative peritoneal adhesion. *Euro. J. Obstetrics, Gynecology and Reproduction Biology*. 140: 152-155.
23. Molan, P.C., 1999. The role of honey in the management of wounds. *J Wound Care* 8: 415-418.
24. Henze, U., A. Lennartz, B. Hafemann, C. Goldmann and C.J. Kirkpatrick, 1997. The influence of the C1-inhibitor berinert and the protein-free haemodialysate actihaemyl 20% on the evaluation of the depth of scald burns in a porcine model. *Burns*. 23: 473-477.
25. Ibishov, K.G. 1999. Transdrainage closed ultrasonic cleansing of the abdominal cavity in the prevention and treatment of infectious complications in abdominal gunshot wounds. *Vestnik Khirurgii Imeni-Grekova*. 158: 40-42.
26. Wilmink, J.M., P.W. Stolk, P.R. van Weeren and A. Barneveld, 2000. The effectiveness of the haemodialysate solcoseryl for second-intention wound healing in horse and ponies. *J. Vet. Med.* 47: 311-320.
27. Brzozowski, T., T. Radecki, R. Sendur, P. Gustaw and S.J. Konturek, 1987. Prevention of acute gastric mucosal lesions by solcoseryl. *Hepato-Gastroenterology*. 34:86-89.
28. Niimikoski, J. and S. Renwall, [Effect of deproteinized blood extract on experimental granulation tissue. *Acta Chir. Scand.* 145:287.
29. Kubanova, A.A. M.A. Gomberg and O.A. Liapon 1999. Solcoseryl-dental adhesive past in the treatment of stomatitis. *Stomatologiya*. 78:2023.
30. Prasad, G., A. Kumar, A.K. Singh, A.K. Bhattacharya, K. Singh and V.D. Sharma, 1986. Anti-microbial activity of essential oils of some *Ocimum* species and clove oil. *Fitoterapia*. 57: 429-432.
31. Farag, R.S., Z.Y. Daw, F.M. Hewed and G.S.A. El-Baroty, 1989. Anti-microbial activity of some Egyptian spice essential oil. *J. Food Production*. 52:665-667.
32. Simon, J.E., J. Quinn and R.G. Murray, 1990. Basil: A Source of Essential Oils. In *Advances in New Crops* Ed. Janik, J. and Simon, J.E. Portland, Oregon: Timber Press, pp: 484-489.
33. Lachowicz, K.J., G.P. Jones, D.R. Briggs, F.E. Bienvenu, J. Wan, A. Wilcock and M.J. Coventry, 1998. The synergistic preservative effects of the essential oils of sweet basil (*O. basilicum* L.) against acid-tolerant food microflora. *Litters in Applied Microbiology*. 26:209-214.
34. Virinda, B. and P. Uma Devi, 2001. Radiation protection of human lymphocyte chromosomes *in vitro* by orientin and vicenin. *Mutation Research*. 498:39-46.
35. Subrahmanyam, M., 1998. A prospective randomized clinical and histological study of superficial burn wound healing with honey and silver-sulfadiazine. *Burns*. 24:157-161.
36. Molan, P.C., 2001. Potential of honey in the treatment of wounds and burns (Review). *Am. J. Clinical Dermatology*. 2:13-19.
37. Stephen-Haynes, J., 2004. Evaluation of a honey-impregnated tulle dressing in primary care. *Br J. Community Nursing*. Suppl:S21-27.

38. Mahgoub, A.A., A.H. el-Medany, H.H. Hagar, D.M. Sabah, 2002. Protective effect of natural honey against acetic acid-induced colitis in rats. *Tropical Gastroenterology*. 23:82-87.
39. Schramm, D.D., M. Karim, H.R. Schrader, R.R. Holt, M. Cardetti and C.L. Keen, 2003. Honey with high levels of antioxidants can provide protection to healthy human subjects. *J. Agricultural and Food Chem*, 15:1732-1735.
40. Al-Waili, N.S., 2003. Effects of daily consumption of honey solution on hematological indices and blood levels of mineral and enzymes in normal individuals. *J. Medicinal Food*. 6:135-140.
41. Facino, R.M., 2004. Honey in Tumor Surgery. *Archives of Surgery*. 139:802.