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Evaluation of In-*Vivo* Wound Healing Activity of Chromolaena Odorata Leaf Extract on Excision Wounds Model in Rats

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Abstract: Four groups of adult male Sprague Dawley rats each consist of ten animals. All animals were experimentally wounded in the posterior neck area. Normal saline was applied topically to wounds of Group 1 as a negative control and pure un-boiled honey was applied topically to wounds of Group 2 animals. Wounds of Group 3 rats were treated with honey in combined with *Chromolaena odorata* aqueous leave extract. Group 4 rats act as positive control and treated with solcoseryl - Jelly. The effects of vehicles on the rate of wound healing and rate of infections were assessed. Wounds treated with honey alone or honey in combination with plant extracts and solcoseryl –Jelly showed clean and remain sterile and significantly healed earlier and much faster as compared to wounds treated with normal saline (Group 1). Wounds treated with solcosery – Jelly or honey in combination with plant extract also showed significantly earlier healing than honey alone There were no significant differences in wound healing rate between plant extract and solcoseryl - Jelly. This was indicated by improve rates of contraction and a decreased period of epithelialization and result in minimal scar formation. These results strongly document the beneficial effects of honey in combined with this extract for the acceleration of wound healing process, minimal scar formation, and the rates of wounds sterility.

Key words: Sorghum, Fermentation, Whey protien, Supplementation, Protien digestibility, Sensory evaluation

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. *Chromolaena odorata* was first identified in Antilles archipelago in Central America (Nghiem, 1992). The aqueous extract and decoction from the leaves of this plant have been used for the treatment of soft-tissue wounds, burns, and skin infections (Phan *et al.*, 1996; 1998; 2000; 2001 and Thang *et al.*, 2001). A number of studies carried out by investigators on clinical and experimental wounds have demonstrated that the extract of the leaves of *C. odorata* inhibits the growth of bacteria (*Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*) (Akah, 1990; Bamba *et al.*, 1993; Irobi, 1992 and Le, 1995). In clinical use, it has been noted that the aqueous extract of *C. odorata* enhances hemostasis (Triratana *et al.*, 1991; Bamba *et al.*, 1993 and Le, 1995).

Honey has long been used to accelerate wound healing (Subrahmanyam, 1991; Oryan and Zaker, 1998; Al-Waili and Saloom (1999); Dunford *et al.*, (2000); Kingsley (2001); Molan (2002) and Fox (2002). Existing literature attributes honey with a number of useful properties, such as a broad-spectrum antimicrobial activity, deodorization, debriding and anti-inflammatory actions and stimulation of new tissue growth (Rath, 1996; Al-Waili and Saloom, 1999 and Dunford *et al.*, 2000). Honey, for the most part, is made up of simple sugars and is an excellent source of energy. It is hypertonic and has been shown to be sterile and highly bactericidal (Cooper, 1998 and Dunford *et al.*, 2000). Honey has been found, when applied locally, to reduce infection and promote wound healing (Efem, 1988; Al-Waili and Saloom, 1999). Physiological properties of honey such as hypertonicity, low pH (3.6) and hygroscopicity were thought to augment the healing process. Antibacterial effect s were also attribute to these elements (Al-Waili and Saloom, 1999 and Dunford *et al.*, 2000).

Honey as an excellent adjuvant for acceleration of wound healing is widely accepted in folk medicine. Topical application of honey was observed to be effective in decubitus ulcers, infected wounds, and burns. 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 (Efem, 1988; Cooper, 1998; Al-Waili and Saloom, 1999 and Molan, 1999).

The aim of the present study was carried out to assess the effects of honey alone or in combined with aqueous extracts of *C. odorata* on the rate of wound-healing process, rate of wound enclosure and on the rate of infection.

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 4 groups of 10 rats each. Each rat that weighted between 180 - 200 gm was housed separately (one rat per cage). The animals were left for 48 hours 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 cm 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: Group 1 (negative control) wounds were rinsed with normal saline twice daily, a thin layer of pure, un-boiled commercial honey was applied topically twice daily to Group 2 animals; a thin layer honey in combined with aqueous leaf extract (10% of leaf extract ointment and 90% honey w/w) was applied topically twice as daily dressing to Group 3 wounds, whereas a thin layer of solcoseryl - Jelly was topically applied twice daily to Group 4 wounds as positive control animals. The diameter of wound enclosure were measured and recorded in all groups at interval one day until complete wound-healing process.

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 agar overnight at 37°C for any bacterial growth.

Statistical Analysis of Data: Results were expressed as mean $\pm$ M.S.E. The statistical difference between the groups in the term of the mean of wound enclosure, rate of wound healing and rate of infection was calculated by using Student's *t*-test

Results

No bacteria were isolated from swabs cultured (Brain Heat Infusion 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. *Staphylococcus aureus* was isolated on day 3 from negative control wounds (Group 1) before dressing with normal saline. (Table)

The results obtained indicate the existence of antimicrobial compounds in the honey alone, honey in combined with plant extract and Solcoseryl - Jelly. Wounds treated with honey in combined with plant extract (Group 3) or wounds treated with Solcoseryl – Jelly showed considerable signs of dermal healing and significantly ($P<0.05$) healed earlier than that treated with honey alone (Group 2) or negative control wounds (Group 1) (Table). In Group 2 animals, the wounds healed significantly ($P<0.05$) earlier than that of Group 1 rats. 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 healing of wounds in experimental animals and bacterial isolation.

Animal groups	No of animals	Type of dressings	Healing time (days) (Mean $\pm$ M.S.E)	Bacterial isolated	
				Day 3	Day 7
Group 1	10	Normal saline	20.00 $\pm$ 0.42.		S. aureus
Group 2	10	Honey alone	17.10 $\pm$ 0.84*		-
Group 3	10	Honey + extract	15.50 $\pm$ 0.56**		-
Group 4	10	Solcoseryl - Jelly	15.00 $\pm$ 0.42**		-

*$P<0.001$ significant from control (Group 1)
**$P<0.0001$ significant from control (Group 1)
*$P<0.001$ significant from (Group 4)

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 present study showed that the usage of honey in combination with plant extract or Solcoseryl - Jelly (positive control) as topical applicants significantly ($P<0.05$) accelerated wound healing, maintained sterility of wounds until complete healing compared to honey alone or negative control animals, also wounds treated with honey alone showed significantly ($P<0.05$) earlier wound healing compared to wound treated with normal saline only (negative control).

The wound healing properties of honey had been well documented (Bergamn *et al.*, 1983; Efem, 1988; Subrahmanyam, 1996 and Molan, 1999). 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 ahyperosmolar 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. (Bergamn *et al.*, 1983; Efem, 1988 and Subrahmanyam, 1991). The nutrient contents of the honey such as laevulose and fructose, improve local substrate supply and may help promote epithelialization (Efem, 1988). Honey seems to cause more rapid epithelialization, presumably because of antibacterial properties as compared to control.

The antibacterial activity of honey is due mainly to an inhibine factor, which is hydrogen peroxide, an end product of the enzymatic reaction of glucose oxidase (from the bee) with glucose in diluted honey. This help in debridement of the wound due the Fenton reaction, it can easily produce free hydrogen radicals with a bactericidal effect (White *et al.*, 1963). The enzyme catalase present in honey has an antioxidant property (Molan, 1992) and thus honey may have a role as an anti-oxidanat in thermal injury (Subrahmanyam, 1996). The antibacterial activity of honey may also be due to the low pH (3.6) and hygroscopic properties of honey. The hydroscopic property of honey enables it to dehydrate bacteria rendering them inactive and to dehydrate edematous and soggy wound reducing their surface area and making them more clearly defined. Dehydration of unwanted tissue fluids around the wound might also decrease tissue turgor and improve tissue oxygenation and hence wound healing. Presumably the sterility of honey treated wounds because of the antibacterial properties of honey as mentioned above. The bactericidal activity of honey was well documented by the current study. The wounds treated with honey showed control of infection and thus wounds healing process was accelerated.

The studies conducted so far by various workers have shown that honey has accelerated wounds healing properties and can be used effectively for topical application (Efem, 1988; Subrahmanyam, 1991and Molan, 1999). In this study we have aimed to show the antibacterial properties of honey in an experimental model the results obtained. We can suggest that it may be possible to use honey in combination with *C. odorata* aqueous extract as topical application for the treatment of wounds.

The fresh leaves and extract of the plant *C. odorata* are a traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infection. Extract from the leaves of *C odorata* have been shown to be beneficial for treatment of wounds. The fact that the extract produced antibacterial activities against both Gram-positive and Gram-negative bacteria suggests that there may be a scientific basis for their utility in traditional medicine for the treatment of skin infections (Irobi, 1992). The extract from *C. odorata* contains a mixture of powerful antioxidant compounds that may be one of potential mechanism contributing to enhance wound healing (Phan *et al.*, 2000). Protection of cells against destruction by inflammatory mediators may be one of the ways in which the extracts from the plant, *C. odorata*, contribute to wound healing (Thang *et al.*, 2001). A number of clinical studies have demonstrated the efficacy of *C. odorata* on the wound healing process. Phan *et al.* (2000) have shown that Eupolin stimulated the expression of many proteins of the adhesion complex and fibronectin by human keratinocytes. The adhesion complex proteins are essential to stabilize epithelium and this effect could contribute to the clinical efficacy of Eupolin in healing.

In conclusion, honey in combined with aqueous extract of *C. odorata* appeared to have several important properties that make it useful ideal as a dressing agent for wounds.

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