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Evaluation of wound healing potential of *Ageratum conyzoides* leaf extract in combination with honey in rats as animal model

[1]Mustafa, M.R., [2]A.A. Mahmood, [2]K. Sidik and [2]S.M. Noor
[1]Department of Pharmacology, [2]Department of Molecular Medicine,
Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: The aqueous extract of *Ageratum conyzoides* leaves in combination with honey were investigated for evaluation of their wound healing potential 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 honey was applied topically to wounds of Group 1 animals. Wounds of Group 2 rats were treated with *Ageratum conyzoides* leaves extract in combination with honey (100 mg leaf extract/ml of honey w/v), and solcoseryl ointment was applied topically to wounds of Group 3 animals as a reference. The effects of vehicles on the rate of wound infection 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 plant extract in combination with honey, and wounds treated with solcosery ointment significantly ($p<0.05$) accelerate wound healing potential 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, solcoseryl ointment, *Ageratum conyzoides*

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].

Honey has long been used to accelerate wound healing[4, 5]. 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[6]. 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[7, 8]. Honey has been found, when applied locally, to reduce infection and promote wound healing[9]. 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 attribute to these elements[10]. 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[6, 11].

A. conyzoides (Family: Asteraceae) is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions[12]. The plant has been used for treatment of several diseases. Githens[13] listed the uses of the plant in folk remedies to include, the use as purgative, febrifuge, treatment of ulcers and wound dressing. In addition to its popular use for skin diseases and wound healing, a decoction of the plant is taken internally to treat diarrhea and to relieve pain associated with navel in children[14]. It is used in the treatment of leprosy and as an oil lotion for purulent ophthalmia[15], treats wounds and fractures[16, 17] and its leaves are used in cases of parasitic infection, rheumatism, headache and colic[14, 18]. In Folk medicine, medicinal teas of *A. conyzoides* are used as anti-inflammatory, analgesic and anti-diarrheic[14, 19, 20]. Its juice is commonly used for peptic ulcer[21]; gasroprotective and anti-oxidant activity[3]; analgesic[22]; and its oil are reported to possess antibacterial and antifungal activities[7, 23, 24] and spasmolytic[25]. Realizing the potential use of this plant in wound healing, the present study was undertaken to study the effect of aqueous leaves extract

Corresponding Author: Mustafa, M.R., Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

of *A. conyzoides* in combination with honey on the rate of wound-healing and on the rate of infection in rats.

MATERIALS AND METHODS

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

Collection of plant materials: The plant was collected from different localities of Malaysia. Plant sample was identified and deposited at the department of Botany, Science School, University Malaya. Specimen was labelled, numbered, annotated with the date of collection, the locality and the medicinal use. The plant leaves were cut into small pieces, wash with distilled water and dried in oven 50°C for 5-7 days until fully dried. The leaves were ground to a fine texture or become powder form using a grinder and stored at 4°C.

Preparation of Plants Extracts: Weighing 40 g of plant powder and mixing it with 800 ml of sterile distilled water in a conical flask using a ratio of 1:20. It was then heated and stirred on a hotplate for 3 hours. After being left to cool, the residue was removed by filtration using a mesh and filter funnel. Rotatory evaporator then extracted the filtered material.

Aqueous extract was then submitted to lyophilization by a freeze-dryer, to produce powdered forms of the extract. Lyophilization removes the water from the solutes and stabilizes the formulation so that it can retain satisfactory pharmacological activity during long-term storage.

Preparation of a mixture (honey with plant extract): The freeze-dried products were homogeneously mixed with honey in a concentration of 100 mg of extract/ ml honey (w/v). The mixtures were kept at 4°C, and brought to a room temperature before application.

Solcoseryl-jelly: Solcoseryl ointment (Jelly) manufactured by Solco Basle Ltd. CH-4127 Birsfelden, Switerzerland were bought from pharmacy.

Experimental animals: *Sprague Dawley* adult male 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 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: A thin layer of honey was applied topically twice daily to Group 1 animals. Group 2 rats were treated with a thin layer of aqueous leaves extract in combined with honey applied topically twice daily dressing, whereas a thin layer of solcoseryl jelly was topically applied twice daily to Group 3 wounds as 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 6 another wound swab was taken for culture from all wounds before dressing. 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 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 6 before dressing with honey alone, plant extract combined with honey or solcoseryl jelly treated animals (Table 1).

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

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 6
Group 1	6	Honey alone	16.00±0.89	-	-
Group 2	6	Honey + extract	13.00±0.63*	-	-
Group 3	6	Solcoseryl-jelly	12.67±0.82*	-	-

*p<0.05 significant from control (Group 1)

advancing epithelialization, and the diameters of wounds become narrow gradually compared to wound treated with honey alone.

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 *A. conyzoides* leaf extract in combination with honey or solcoseryl jelly as topical applicants significantly ($p<0.05$) accelerated wound healing compared to wounds treated with honey alone. Also wounds pretreated with honey alone, honey in combination with leaf extract or solcoseryl jelly, as a topical application maintained sterility of wounds until complete healing in whole animals. The aqueous leaf extract of *A. conyzoides* at a dose level of 10% in honey exhibited marked wound healing activity. The aqueous extract of *A. conyzoides* was found to possess free radical scavenging effect, thereby showing antioxidant property. The flavonoids kaempferol and quercetin, in addition to several other flavonoids earlier isolated from *A. conyzoides*[3, 26, 27] are known to possess antioxidant activity, and significant wound healing activity could be mediated by its antioxidant activity[3, 28-31]. *A. conyzoides* was generally had anti-microbial activity[24] and thus remain the wound sterile and enhance healing. These results suggest that *A. conyzoides* leaf extract could be beneficial component of preventing infection and enhanced wound healing.

The wound healing properties of honey had been well documented[4, 5, 11]. 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.[4-6]. The nutrient contents of the honey such as laevulose and fructose improve local substrate supply and may help promote epithelialization[32, 33]. 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[34, 35] and thus honey may have a role as an anti-oxidanat in thermal injury[36, 37].

In conclusion, the effects of aqueous extract of *A. conyzoides* in combination with honey 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 plant leaf extract in combination with honey as topical application for the treatment of wounds. However, further investigations are required to elucidate their exact mechanism (s) of wound healing activity.

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