International Journal of Molecular Medicine and Advance Sciences 2 (2) : 231-235, 2006


# Acceleration of Wound Healing by Aqueous Extract of *Allium sativum* in Combination with Honey on Cutaneous Wound Healing in Rats

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**Abstract:** The aqueous extract of garlic (*Allium sativum*) in combination with honey was evaluated for wound healing activity in rats. Three groups of male *Sprague dawley* rats each consist of 6 animals. Rats were experimentally wounded in the posterior neck area. A thin layer of pure unboiled honey was applied topically to wounds of Group 1 animals. *A. sativum* L. in combination with honey was applied topically to wounds of Group 2 rats and a thin layer of solcoseryl - jelly was applied topically to wounds of Group 3 animals. The effects of these topical applicants on the rate of wound infections and on the rate of wound healing were assessed. Wounds of all animals showed clean and remain sterile throughout the experiment until complete healing. Wounds treated with garlic extract in combination with honey significantly accelerate wound healing compared to wounds treated with honey alone. In addition, wounds treated with solcoseryl-jelly also significantly accelerates wound healing compared to wounds treated with honey alone. These results strongly document the beneficial effects of garlic extract in combination with honey for the acceleration of wound healing process and the rates of wounds sterility.

**Key words:** Honey, *Allium sativum* aqueous extract, wound healing, rats

## INTRODUCTION

The wound healing properties of honey had been well documented[1-3]. Honey has been used topically for centuries to accelerate wound healing. It has been reported to be helpful in treating burns, decubitus ulcers and infected wounds[4]. *In vitro* it has been shown to have anti-bacterial and anti-fungal activity to organisms commonly infected surgical wounds[5,6]. The wound healing properties of honey are thought to result from the debriding properties of the enzyme catalase, absorption of edema because of honey's hygroscopic properties, its ability to promote granulation and re-epithelialization from wound edges and its anti-microbial properties[7,8,3].

*Allium sativum* (garlic), a member of the lily family, is most commonly used worldwide for flavorful cooking; much of the clinical literature on garlic has focused on its potential antioxidant activity[9], anti-platelet and fibrinolytic effects[10] and microcirculatory effects (e.g., allicin and ajoene for use in hypertension and hyperlipidemia)[11]. Garlic derivatives are also frequently used for antibacterial[12]; antifungal activity[13], anti-inflammatory[14], antiparasitic activity[15] hypoglycemic properties[16] and may have anticancer activity[17]. The use of herbal medicine is widespread and growing with the increasing number of herbal products available in conjunction with, or as replacement for, conventional medicine. To our knowledge, nothing is known about the effect of garlic on the rate of wound healing. Therefore, the purpose of the present study was to determine the synergistic effects of garlic extract in combination with honey on the rate of wound healing in an experimental excision wound in rats.

## MATERIALS AND METHODS

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

***A. sativum*:** Garlic bulbs (*A. sativum* L.) were purchased directly from shop. It was fully grinded by blender and then 50 g of blended garlic were weighted and placed into 1000 ml flask. The water was added in ratio 1:20. After that, it was stirred for 3 hrs. Next, using filter paper and filter funnel filtered it. The step was followed by rotor vaporised to remove the water. Then, it was mixed with honey (10% w/w).

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

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**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 150 - 180 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 pure, un-boiled commercial honey was applied topically twice daily to Group 1 animals; a thin layer aqueous extracts of *A. sativum* in combined with honey (10% of extract w/w) was applied topically twice as daily dressing to Group 2 rats, whereas a thin layer of solcoseryl - jelly was topically applied twice daily to Group 3 animals. The wound enclosure was observed daily 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 (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 of wound enclosure, rate of wound healing was calculated by using Student's t-test

## RESULTS

No bacteria were isolated from swabs cultured on BHI agar in all experimentally wounded animals on day 3 and day 7 before dressing with honey alone, honey in combined with plant extract and solcoseryl-Jelly treated animals. The results obtained indicate the existence of anti-microbial compounds in the honey alone, honey in combined with plant extract and solcoseryl-Jelly (Table 1).

Wounds treated with aqueous extracts of garlic in combined with honey (Group2) and wounds treated with solcoseryl-jelly (Group 3) showed considerable signs of dermal healing and significantly ($p<0.05$) healed earlier than those treated with honey alone (Group 1) (Table 1). In addition dermal wounds of Group 2 and Group 3 animals were rapidly replaced by granulation tissue and advancing epithelialization and the diameters of wounds become narrow gradually and enclosed faster compared to wounds treated with honey alone (Group 1). There were no significant differences between wounds of Group 2 and Group 3 in term of duration of wound healing.

## 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 garlic aqueous extracts in combination with honey as topical applicants of wounds dressing significantly ($p<0.05$) accelerated wound healing compared to wounds treated with honey alone and the wounds were maintained sterility until complete healing in all animals. Similarly, topical application of honey has been recognized for a long time to be effective in controlling infection and producing a clean granulating wound bed[18,1,3]. The wound healing properties claimed for honey include stimulating new tissue growth, moist wound healing, fluid handling and promoting epithelialization[19,3]. Honey acts mainly as 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[2,20].

The use of honey resulted in rapid and complete healing of wound. In addition, the antibacterial activity of honey had a deodorizing effect on the wounds and its anti-inflammatory action helped reduce the level of pain.[6,1]. Anti-microbial activity of honey has been attributed to hydrogen peroxide, which is produced by naturally occurring glucose oxidase and phenolic compounds[21]. The enzyme catalase present in honey has an antioxidant property[22] and thus honey may have a role as an anti-oxidant in thermal injury[23]. The accelerative effect of honey in the wound healing process is related to its specific physical properties of hygroscopicity,

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±M.S.E) | Bacterial isolated Day 3 | Bacterial isolated Day 7 |
|---|---|---|---|---|---|
| Group 1 | 6 | Honey alone | 16.67±0.33 | - | - |
| Group 2 | 6 | Honey±extract | 13.5.00±0.43* | - | - |
| Group 3 | 6 | Solcoseryl-Jelly | 13.00±0.37* | - | - |

*p<0.05 significant from control (Group 1)

hypertonicity, lower pH and complex chemical composition[24]. The nutrient contents of the honey such as laevulose improve local substrate supply and may help promote epithelialization[25]. Honey seems to cause more rapid epithelialization, presumably because of antibacterial properties[26,3].

Garlic has been used for medicinal purpose for centuries. The most active ingredient in garlic is allicin (contain sulfur and combined with breakdown products, give garlic its characteristic smell). Crushing the garlic clove activates the enzyme allinase, which converts allin to allicin[27]. It has been found by Tsao and Yin [28,12]that garlic has a strong antibacterial activity and the active principle of garlic is named Allicin and it has been confirmed by Ness *et al.*[27] that allicin is derived from alliin-alliinase system. The antimicrobial activity of garlic has been attributed to the presence of sulphides in garlic oil[29]. Garlic oil, with higher concentration of diallyl sulphides, showed greater antimicrobial activity[28] and antifungal activity[13]. These authors indicated that the disulphide bound of diallyl disulphide was important for its antimicrobial and antifungal activity, suggesting that they may be useful in the prevention or treatment of variety of infection.

The antioxidant properties of garlic was well documented[30,9], garlic extract can prevent oxidized LDL-induced injury such as membrane damage and loss of cell viability in vascular endothelial cells[31]. Garlic extract and its constituents exert an antioxidative effect by protecting membranes and enhancing antioxidative enzyme activity[9]. Garlic administration experienced a significant reduction in lipid peroxidation with simultaneous elevation in antioxidant levels. The presence of organosulphur compounds could decrease lipid peroxidation and increased antioxidant level[9].

Garlic caused an increase in fibrinolytic activity, inhibited platelet aggregation and lowered cholesterol[32]. It has been used historically to enhance circulation, fit stress and fatigue and stimulate immune function. Bordia *et al.*,[33] reported that a garlic oil preparation rich in vinyl dithiins, sulfides and ajoene could inhibit platelet aggregation and result in increased fibrinolytic activity. Bordia *et al.*,[34] showed that their garlic oil preparation inhibits platelet function in both healthy subjects and in patients with coronary heart disease. Garlic may increase bleeding when taken with blood-thinning drugs, such as aspirin and warfarin. It reduces blood sugar and may therefore affect glucose control[35,36].

## ACKNOWLEDGEMENTS

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

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