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Research Article

Influence of Honey on Energy Metabolism during Wound Healing in Rats

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The aim of this study is to find out how honey influences the activities of the enzymes involved in energy metabolism during wound healing. Carbohydrate metabolism is the main source of energy for skin cells during wound healing. Since honey is full of sugars, and hence a rich source of energy, we have investigated the efficacy of honey on energy metabolism during wound healing in rats. A total number of 48 animals were used. From these, 24 animals were divided into two groups, control and experimental. Full thickness excision wounds were made on the back of rats. The control rats were left untreated. The experimental rats received 500 μL of honey topically, once daily, for 12 days. Six animals from each group were sacrificed on different time point intervals like 4, 8, and 12 days. The remaining 12 animals were used to evaluate the contraction and epithelialization. The activities of hexokinase, phosphofructokinase glyceraldehyde-3-phosphate dehydrogense, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase were measured in the granulation tissue. A marked increase in the activities of all the glycolytic enzymes in the experimental wounds when compared to control suggests that honey could provide sufficient energy for cellular activity needed for the repair process.

1. Introduction

Wound healing represents a dynamic physiological process initiated and influenced by many factors. This complex cascade of event starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding. The process can be broadly categorized into three stages; inflammatory phase (consisting of the establishment of homeostasis and inflammation); proliferate phase (consisting of granulation, contraction, and epithelialization); finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue [1].

Honey is widely accepted as an excellent adjuvant for wound healing [2]. Honey has been used in the treatment of burns and wounds for many centuries, with documents describing this use dating back to 1700 BC [3, 4]. Honey acts mainly as a hyperosmolar medium and prevents bacterial growth [5–7]. Because of its high viscosity, it forms a physical barrier, and the presence of enzyme catalase gives honey an antioxidant property [8, 9]. Its high nutrient content improves substrate supply in local environment promoting epithelialization and angiogenesis [10, 11].

The skin cells undergo rapid cell division during the process of wound repair and this is accompanied by a greater demand of metabolic energy. The skin presents an active site for carbohydrate metabolism [12]. It has been demonstrated that the proliferating epithelial cells as well as wounded skin are primarily dependent on the glycolytic pathway for energy requirement [13–15]. Since honey has been used for wound healing, since time immemorial, and is a rich source of energy, we have investigated the role of honey on the glycolytic enzyme activities during wound healing in rats.

2. Materials and Methods

2.1. Animals. Male Wistar rats weighing 150–200 g were used for this study. The animals were maintained on clean, sterile, polyvinyl cages and fed with commercial rat feed from M/s Hindustan Unilever Limited, Mumbai, India (mixed with wheat flour in the ratio of 1 : 1 (w/w)). Food and water

were provided *ad libitum* to the animals. All procedures were carried out according to the stipulations of the Institutional Animal Care and Use Committee (IACUC).

2.2. Chemicals. Natural honey was procured from the Dabur India Limited, Uttar Pradesh, India. Remaining chemicals were purchased from Sigma Chemical Co., St Louis, Mo, USA.

2.3. Wound Creation, Animal Grouping, and Drug Administration. Rats weighing 150–200 g were used for the study. The fur on the back of the rats was shaved under light ether anaesthesia. Subsequently, full thickness open excision type of wounds of standard size (2 cm^2, square shaped) was made by removing the skin using a sterile blade [16]. A total of 36 animals were divided into two groups (control and experimental groups). The control rats were left untreated, whereas the experimental rats received 500 μL of unprocessed natural honey topically, once daily, for a period of 12 days. Six rats from each group were sacrificed at different time point intervals. The wound tissues from the center of the wounds were removed on the 4th, 8th, and 12th days postwounding and used for the enzyme assays.

To find out the rate of contraction and period of epithelialization of wounds, a separate group of animals (six animals per group) were maintained. 500 μL of honey was administered topically to the experimental group once daily and the control group was left untreated. The rate of contraction was measured planimetrically by tracing the wound area on a transparent graph paper. Period of epithelialization was measured as number of days taken for the complete healing of wounds so that no raw wound is left behind.

2.4. Preparation of Tissue Homogenate. Samples for the estimation of enzyme activities associated with energy metabolism were prepared by the method described by Gupta et al. [17]. All operations were performed at 4°C unless mentioned specifically. Briefly, a 20% homogenate was made in chilled 0.15 M KCl containing 5 mM EDTA using Polytron Homogenizer. The granulation tissues were homogenized by giving four strokes of 15 seconds duration for 2 minutes. Samples were centrifuged at 9000 × g in a refrigerated centrifuge for 20 minutes to collect the supernatant.

The assays of all the enzymes were performed at room temperature. The methodology was designed to ensure maximal enzyme activities in the homogenate.

2.5. Assay of Enzyme Activity

2.5.1. Hexokinase. Hexokinase (HK, E.C.2.7.1.1) activity was measured by the method of Supowit and Harris [18], using glucose as a substrate. The sample was mixed with assay buffer consisting of 50 mM triethanolamine and 10 mM $MgCl_2$ (pH 7.4), 1 M glucose, 50 mM NADP, and 140 U/mL of glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of 200 mM ATP, and the rate of NADP reduction was recorded at 340 nm for 3 minutes. Calculation of enzyme activity was based on the reduction of NADP.

2.5.2. Phosphofructokinase. Phosphofructokinase (PFK, E.C.2.7.1.11) activity was measured by the method of Bergmeyer et al. [19], using fructose-6-phosphate as a substrate. The sample was added to 50 mM Tris-HCl buffer (pH 8.0), 500 mM $MgCl_2$, 1 M KCl, 100 mM ATP, 100 mM fructose-6-phosphate, 10 mM NADH, 50 mM phosphoenolpyruate, and 75 U/mL of pyruvate kinase with 40 U/mL of lactate dehydrogenase. The NADH metabolized was assessed at 340 nm. PFK activity was calculated based on 1 μmol fructose-6-phosphate = 2 μmol NADH consumed.

2.5.3. Glyceraldehyde-3-Phosphate Dehydrogenase. The activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, E.C.1.2.1.12) was measured by the method of Kirk and Ritz [20]. The enzyme sample was mixed with 15 mM pyrophosphate buffer (pH 8.4), 30 m M NAD, and 0.4 M sodium aresenate. The reaction was initiated by the addition of 0.42 M glyceraldehydes solution. The NAD metabolized was read at 340 nm for 5 minutes. The enzyme activity was expressed as units/mg protein under incubation conditions. Protein was estimated by the method of Lowry et al. [21].

2.5.4. Lactate Dehydrogenase. Lactate dehydrogenase (LDH, E.C.1.1.1.27) activity was estimated according to the method of King [22]. Lactate was used as the substrate. The enzyme sample was mixed with substrate and NAD to start the reaction. The reaction mixture was incubated at 37°C for 15 minutes. 2,4-dinitrophenyl hydrazine was added to arrest the reaction. The absorbance was measured at 420 nm. The enzyme activity was expressed as nmoles of pyruvate formed/mg protein/min.

2.5.5. Glucose-6-Phosphate Dehyrogenase. The activity of glucose-6-phosphate dehyrogenase (G6PD, E.C.1.1.1.49) was measured by the method of Lohr and Waller [23]. Glucose-6-phosphate was used as the substrate. The enzyme sample was mixed with 50 mM triethanolamine buffer, 5 mM EDTA, and 30 mM NADP. Reaction was initiated by the addition of 42.6 mM glucose-6-phosphate. The increase in O.D. at 340 nm was measured for 2 minutes at every 30 seconds interval.

2.6. Statistical Analysis. All results were expressed as mean ± SD. Statistical significance between two groups was determined by student's t-test and $P < .05$ was considered as significant.

3. Results

Table 1 depicts the HK activity at different time point intervals. There is a marked increase (more than 100%) in the enzyme activity in honey-treated wounds, particularly on

Table 1: Hexokinase activity of control and honey-treated wounds.

Day postwounding	Control	Experimental
4	890 ± 16.1	1200 ± 19.0***
8	1450 ± 24.2	3010 ± 29.5***
12	920 ± 10.1	1400 ± 13.6***

Enzyme activity is expressed as nmoles/min/g of wet tissue.
Values are expressed as mean ± SD from six rats in each individual experiment.
*** P <.001 as compared to control.

Table 2: Phosphofructokinase activity of control and honey-treated wounds.

Day postwounding	Control	Experimental
4	11.20 ± 0.93	25.21 ± 2.65***
8	13.20 ± 1.01	30.21 ± 3.12***
12	14.12 ± 1.93	30.32 ± 3.35***

Enzyme activity is expressed as nmoles/min/g of wet tissue.
Values are expressed as mean ± SD from six rats in each individual experiment.
*** P <.001 as compared to control.

Table 3: Glyeraldehyde-3-phosphate dehydrogenase activity of control and honey-treated wounds.

Day postwounding	Control	Experimental
4	6.41 ± 1.17	11.12 ± 1.42***
8	11.51 ± 0.92	20.12 ± 1.02***
12	9.41 ± 1.91	15.12 ± 1.52***

Enzyme activity is expressed as units/mg protein/g of wet tissue.
Values are expressed as mean ± SD from six rats in each individual experiment.
*** P <.001 as compared to control.

Table 4: Lactate dehydrogenase activity of control and honey-treated wounds.

Day postwounding	Control	Experimental
4	120 ± 23	300 ± 38***
8	200 ± 12	429 ± 29***
12	160 ± 12	380 ± 42***

Enzyme activity is expressed as nmoles of pyruvate formed/min/mg protein.
Values are expressed as mean ± SD from six rats in each individual experiment.
*** P <.001 as compared to control.

the 8th day wherein the fibroblast proliferation and collagen synthesis are actively taking place.

The differences in the PFK activity are depicted in Table 2. There is more than twofold increase in this enzyme activity in honey-treated wounds.

Table 3 shows the GAPDH activity in the control and experimental wounds. There is about 75% increase in this enzyme activity in honey-treated wounds when compared to the control.

Tables 4 and 5 depict the LDH and G6PDH activities on the control and experimental wounds, respectively. There is a 114% and 68% increase in LDH and G6PDH, respectively, in honey-treated wounds as compared to control. The enzyme

Table 5: Glucose-6-phosphate dehydrogenase activity of control and honey-treated wounds.

Day postwounding	Control	Experimental
4	67.2 ± 6.5	101.4 ± 7.9***
8	89.2 ± 4.5	150.4 ± 9.9***
12	86.2 ± 6.5	141.9 ± 3.2***

Enzyme activity is expressed as nmoles/min/g of wet tissue.
Values are expressed as mean ± SD from six rats in each individual experiment.
*** P <.001 as compared to control.

Table 6: Rate of contraction as percent of original wound size of control and honey-treated wounds at different days.

Groups	Experimental days		
	4	8	12
Control	16 ± 2	40 ± 3	75 ± 5
Honey treated	38 ± 3***	72 ± 5***	99 ± 1***

Values are expressed in mean ± SD from six animals.
*** P < .001 as compared to control.

levels start increasing on the 4th day, reach the maximum on the 8th day and decline on the 12th day.

Figure 1 shows the photographical representation of the contraction rate of wounds at different time point intervals. From the pictures, we could observe that the contraction is much faster in honey-treated wounds (data also given as Table 6). Figure 2 shows the period of epithelialization wherein we could observe that the experimental wounds took 12 ± 1 day for complete epithelialization when compared to control wounds which took 22 ± 2 days.

4. Discussion

In normal skin, glucose utilization provides energy equally through glycolysis and the mitochondrial system. But in wounded regenerating skin, especially in the migrating epithelium, glucose utilization provides energy mainly through glycolytic pathway [12]. Hence, measuring the glycolytic enzyme activities may be a useful indicator of the degree of wound repair.

Honey is a natural product which contains almost 80% sugars. The energy needed for the collagen synthesis may be provided by these sugars, which enter the glycolytic pathway. The glycolytic pathway is the main source of energy for fibroblasts, which synthesize collagen [12, 24]. Lactate, the end product of glycolysis, increases prolyl hydroxylase activity so as to prepare the fibroblasts for an increased rate of hydroxylation of proline and collagen extrusion after the fibroblasts are exposed to oxygen [25].

Before the new capillary network forms, there is a marked gradient of oxygen within the wound [26] with the center of the wound being the most deficient in oxygen [27, 28]. Therefore, the increased activities of the glycolytic enzymes indicate that the connective tissue cells, probably fibroblasts, in the early stages of wound healing have metabolically adopted for the survival in a hypoxic environment. The

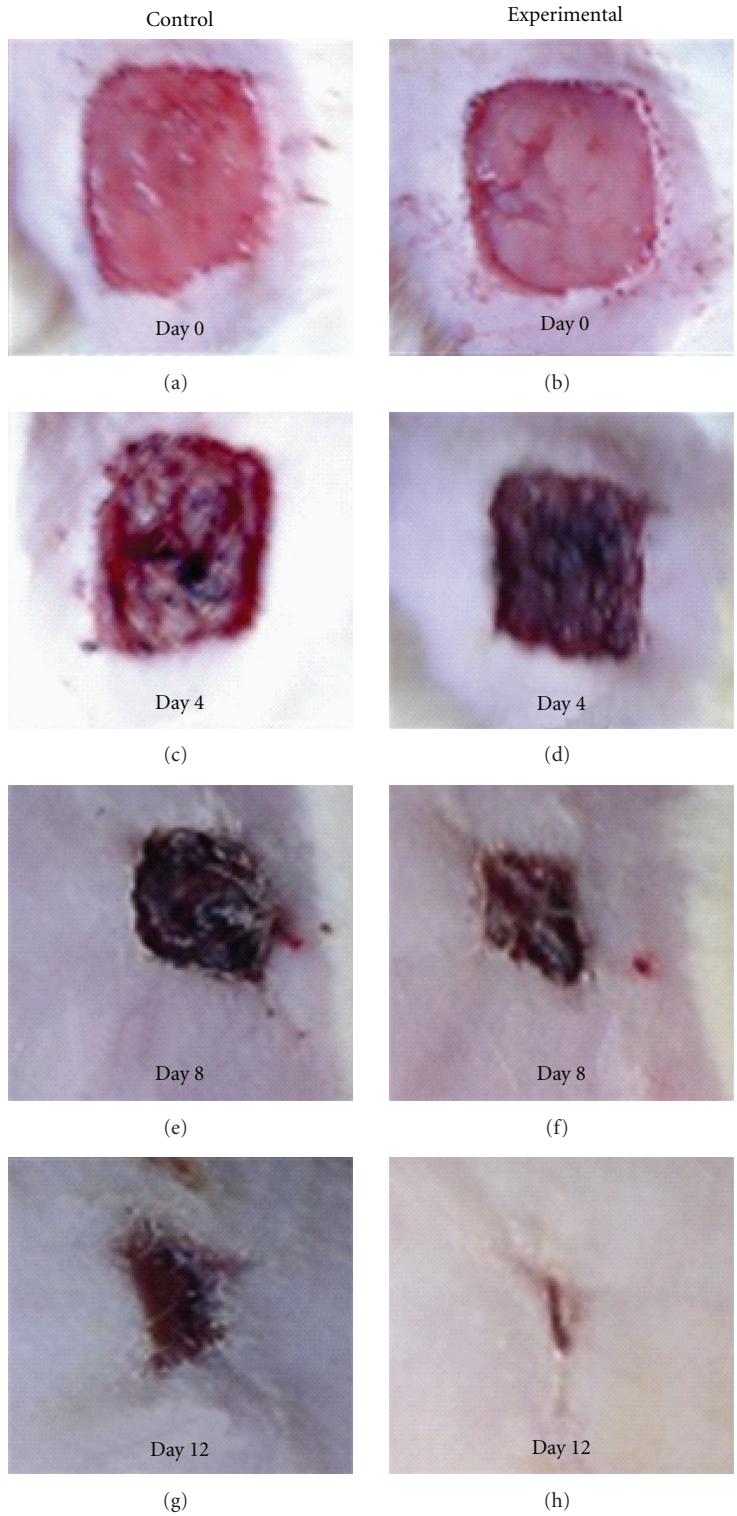

Figure 1: Rate of contraction as percentage of original wound size of control and experimental wounds—scale 1 cm.

increase in G6PDH and glycolytic enzymes may indicate that glucose is utilized by the repairing tissue to provide both energy in the form of ATP and certain basic substances for the increased cellular activities of biosynthesis, mitosis, and locomotion [12].

The significant increase in lactate content, the end product of glycolysis, increases the prolyl hydroxylase activity so as to prepare the fibroblasts for an increased rate of hydroxylation of proline and collagen extrusion after the fibroblasts are exposed to oxygen [25]. The increased rate of

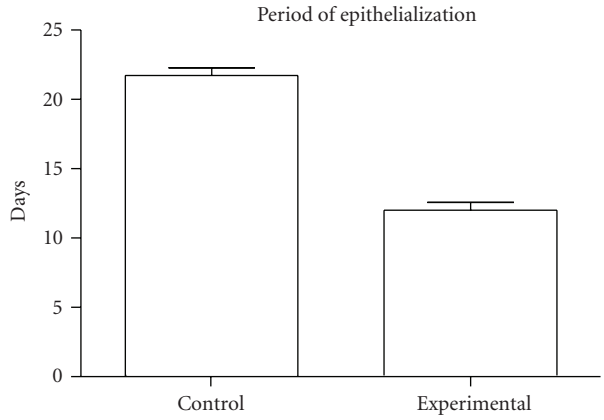

Figure 2: Values are expressed in mean ± SD for six animals. $^*P < .01$ as compared to control.

contraction and epithelialization in honey-treated wounds may be due to its hygroscopic property which enables it to dehydrate bacteria, rendering them inactive to dehydrate edematous wounds, reducing their surface area and making them more clearly defined. Dehydration of unwanted fluids around the wound might also decrease tissue turgor and improve tissue oxygenation and hence wound healing [29].

The acidic pH of honey [30] and its hypertonicity also helps the wounds to heal fast. In a double blind study, Kaufman et al. [31] applied buffered solutions to experimental second degree wound burns in guinea pigs and noted significantly increased reepithelialization in wounds treated with a solution of pH 3.5 compared with wounds treated with neutral and alkaline solutions. Bergman et al. [32] showed that honey increases the thickness of the granulation tissue and the area of reepithelialization, when applied topically.

Gupta et al. [17, 33] have reported the importance of energy metabolism during wound healing in diabetic and immunocompromised and aged conditions. The results of the present investigation suggest that honey could enhance wound healing by accelerating the glycolytic enzyme activities, making sufficient energy available for cellular activity needed for the repair process.

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