The Effects of Different Types of Honey on Tensile Strength Evaluation of Burn Wound Tissue Healing

M.Z. Rozaini, BSc[a]
A.B.Z. Zuki, PhD, DVM[a]
M. Noordin, PhD, MSc, DVM[a]
Y. Norimah, PhD[b]
A. Nazrul Hakim, PhD, DVM[c]

[a]*Faculty of Veterinary Medicine, University Putra Malaysia, Selangor Darul Ehsan, Malaysia*
[b]*Malaysian Institute for Nuclear Technology Research (MINT) Bangi, Selangor Darul Ehsan, Malaysia*
[c]*Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor Darul Ehsan, Malaysia*

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ABSTRACT

The effects of different types of honey on the tensile strength of burn wound tissue healing were evaluated in 105 male Sprague-Dawley rats. The rats were randomly divided into 7 groups of 15 rats each. Rats were anesthetized and burn wounds were created using cylindrical aluminum templates heated in a waterbath for 3 hours at a constant temperature of 85°C. Honey harvested from different vegetation (0.5 mL) was applied to wounds of rats in 5 of the groups approximately 30 minutes after the skin was burned. A positive control group was treated with silver sulfadiazine cream and one group served as untreated controls. The rats were euthanized 3, 7, 14, 21, and 28 days after burns were created. Test areas from the skin were removed in strips and a universal testing machine was used to measure the tensile strength. In general, the tensile strength values increased with time. Tensile strength of skin treated with Manuka honey was highest throughout the study except on Day 21. The present study showed that topical application of honey on burn wounds can improved healing with regards to the tensile strength property.

INTRODUCTION

One of the most crucial phases in dermal wound healing is the progressive increase in biomechanical strength of the tissue resulting from the formation and turnover of granulation tissue.[1] The mechanical properties of the skin are mainly attributed to the function of the dermis in relation to the structure of collagen and elastic fiber networks.[2]

Experimental and clinical trials designed to influence wound strength or healing rates have been conducted with mixed success. Cytokines or growth factors applied systemically, topically or more recently, by gene transfer are some such examples.[3] Honey has also been shown to accelerate wound healing by augmenting the rate of collagen synthesis when applied topically and administered systemically.[4] In 1993, Suguna and his colleagues demonstrated that honey accelerated the synthesis and maturation of collagen, thus resulting in increased tensile strength of the wound healing skin.[5] Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network.[6] According to

Jimenez and Rampy,[7] tensile strength is one of the necessary parameters for determining the pharmacologic effects of potential wound healing agents.

Consequently, the present study was designed to correlate the tensile properties of the burned skin with the healing process. The effects of various types of honey on the tensile strength property of the burn wound tissue healing were evaluated in the study.

MATERIALS AND METHODS

Animals

One hundred-five male Sprague-Dawley rats weighing 250 to 300 g each were used in this study. The rats were kept in the animal unit at least 1 week prior to initiation of the study. The rats were given commercial pellet and water *ad libitum* throughout the study. The protocol was approved by the Faculty's Ethics Committee.

Skin Preparation

Rats were anesthetized with an IM injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Under anesthesia, the back and flank of both sides of the body were shaved. Following this procedure, rats were returned to their cages for 24 hours to allow any edema caused by the shaving procedure to recede.

Thermal Source

A method described by Kaufman et al,[8] was used with modification. Cylindrical aluminum templates (2.5 cm diameter ? 3 cm length, a handle measuring 24 cm, and total weight 400 g) were heated in a waterbath at a constant temperature of 85˚C for 3 hours prior to inflicting burn areas on the skin of the rats. Five templates were heated simultaneously and used alternately and then were returned to the waterbath to ensure maintenance of the desired temperature of the template surface. Approximately 5 minutes elapsed between each use of a template.

Burn Lesions

Rats were again anesthetized with an IM injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The anesthetized rat was restrained and stretched on a metal stage, and location of the burn was marked between the last ribs and the horizontal line of the sacroiliac joints. The rat was positioned in sternal recumbency, and a burn was inflicted on the dorsum of the body between the last thoracic vertebra and the first sacrum by placing the heated and moistened template at right angles perpendicular to the dorsum of the rat on the pre-marked location for 5 seconds using an analogue stopwatch. Minimal and constant pressure was applied to ensure a perfect contact between the template surface and the skin. The shaved skin was smooth ensuring sufficient contact and uniform pressure over the entire lesion.

Treatments

The rats were randomly divided into seven groups of 15 animals each. Four selected monofloral *Apis Cerana* honeys which produced by bees kept under different plantation were used in this study.Their floral sources were from *Melaleuca spp.* (Gelam) trees, *Cocos Nucifera spp.* (Kelapa) trees, *Ananas Comosus spp.* (Nenas) trees and *Durio Zibethinus spp.* (Durian) trees.The honeys were named according to their floral sources.All the honeys were supplied by the Department of Agriculture, Malaysia.The Manuka honey was supplied by ZHR Technologies (Sdn. Bhd., Petaling Jaya, Selangor, Malaysia), while silver sulphadiazine (SSD) cream was purchased from the Hospital Kuala Lumpur.All of the honeys and the SSD cream were irradiated at 25kGy using a sinagama machine (Model JS8900; Nordion International Inc., Canada) for sterilization purposes.

Mode of Treatment

For all the treatment groups except in control group, the different type of honey in quantities of approximately 0.5 mL was applied topically onto the surface of each burn wound.A thin layer of SSD cream was applied on the burn wound in the positive control group.The first treatment was applied approximately half an hour after burn infliction. The treatment groups received topical application twice a day until

Table 1. Tensile Strength Measurement (MPa) of Healing Wounds*

Experimental Groups	Days Post Injury				
	3	7	14	21	28
Control	0.81±0.36^{a,x}	1.35±0.52^{a,xy}	1.47±0.20^{a,xy}	1.97±0.32^{a,y}	2.08±0.70^{a,y}
Ananas Comosus (Nenas) honey	0.95±0.43^{ab,x}	1.25±0.26^{a,x}	2.40±1.03^{a,xy}	2.61±0.67^{a,xy}	3.95±1.89^{a,y}
Melaleuca (Gelam) honey	0.93±0.14^{ab,x}	1.55±0.68^{a,x}	1.71±0.26^{a,xy}	2.47±0.31^{a,yz}	2.80±0.49^{a,z}
Durio Zibethinus (Durian) honey	1.28±0.31^{ab,x}	1.61±0.65^{a,x}	2.19±1.29^{a,x}	2.93±1.05^{a,x}	3.56±3.98^{a,x}
Cocos Nucifera (Kelapa) honey	1.12±0.46^{ab,x}	1.24±0.04^{a,x}	1.43±0.91^{a,x}	2.37±1.34^{a,x}	2.54±0.65^{a,x}
Manuka honey	1.53±0.24^{b,x}	1.74±0.20^{a,x}	2.73±0.46^{a,y}	2.81±0.31^{a,y}	4.68±0.39^{a,z}
SSD	0.86±0.04^{a,x}	1.22±0.33^{a,x}	1.67±1.01^{a,xy}	1.79±0.28^{a,xy}	2.54±0.37^{a,y}

*Data of tensile strength (MPa) expressed in mean ± S.D.

$^{a\text{-}b}$Values within the same column with different superscript is significant at 0.05 level (*P*<0.05) due to treatment group.

$^{x\text{-}z}$Values within the same row with different superscript is significant at 0.05 level (*P*<0.05) due to days of post injury.

they were euthanized. The negative control group received identical burn and environmental exposure but no treatment given.

Preparation of Strips

The rats were serially euthanized at 3, 7, 14, 21 and 28 days post burned.After euthanasia, the skin samples were cut into 6.0 cm x 1.0 cm strips parallel to the long axis of the body by using perplex template and sharp blades. The strips were then placed in a container soaked with saline solution to prevent drying of the skin.

Tensile Strength Measurement

The Instron mechanical testing machine (Model 4301, Instron Corporation, Canton, Mass) was used to apply tensile stresses on the samples. Instructions provided for calibrating and balancing the Instron machine prior to each trial were used, that is, the load and gauge length were set to zero before each trial was performed. The tensile machine has fixed or essentially stationary member, carrying one grip and a movable member carrying a second grip. Statistical calculations were performed automatically and presented on a visual display and hard copy printout at the end of the test.

The strips of skin were held into place on the machine with pneumatic clamps.The samples were positioned vertically into top clamps first and then the bottom clamp, with the distance between the two clamps being 30 mm.The Instron machine was set to a constant crosshead speed of 100 mm/min and a data collection speed of 100 data points per second.The program was started and allowed to run until the sample tore.As the specimen elongated, the resistance of the specimen increased and detected by a load cell.This load value (Force) was recorded by the instrument.The elongation of the specimen was continued until a rupture of the specimen was observed.

Statistical Analysis

The results were analyzed statistically using two-way and one-way ANOVA methods to identify the differences between experimental groups within the period of study. The data were considered significant at *P*<0.05.

RESULTS

The results of the tensile strength measurement is shown in Table 1 and expressed graphically in Figure 1. In general, tensile strength values increased with time in all the experimental groups.Tensile strength of the animals treated with Manuka honey demonstrated the highest value throughout the study except at day 21 post burned, and followed by *Durio Zibethinus* (Durian) and *Ananas Comosus* (Nenas)-treated groups as opposed to other experimental groups. However, the difference between all experimental groups was statistically not significant except at day 3 post injury

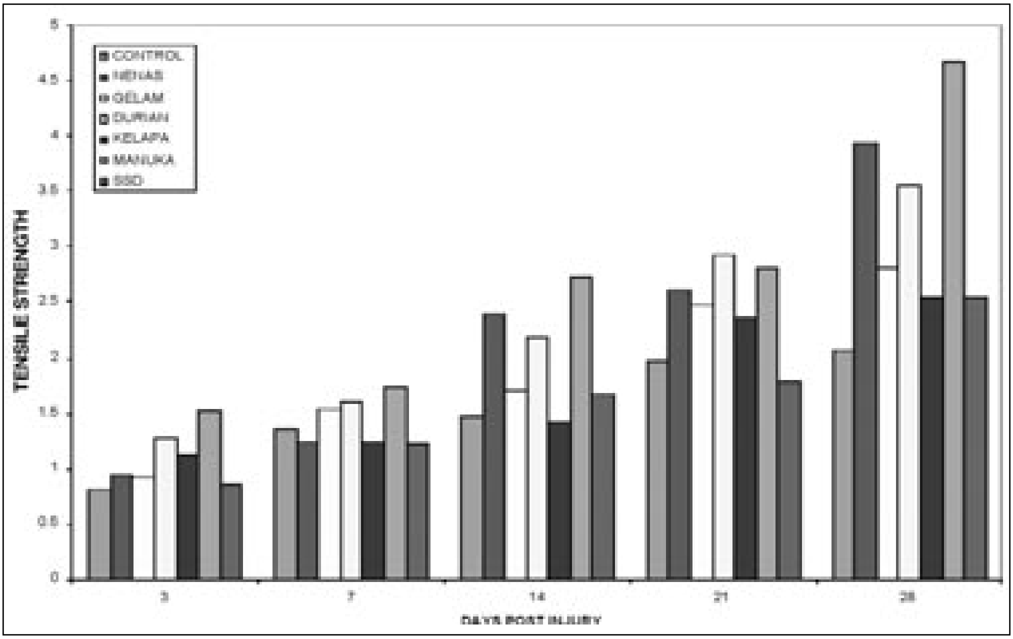

Figure 1. Tensile strength of healing burn wounds for all treatment groups.

where the tensile strength of Manuka-treated wounds was significantly higher ($P<0.05$) (1.5393 MPa) compared to untreated control group (0.8177 MPa) and SSD-treated group (0.8623 MPa) with the untreated control group being the lowest value.

Topical application of Manuka honey on the burns wound demonstrated a significant increase in wound tensile strength at day 14 (2.7333 MPa) and day 28 (4.6860 MPa) post burned as compared to other groups. The increment of tensile strength value of the burn wound tissue for all the groups from day 3 post-burned to day 28 post-burned was significantly different except in *Durio Zibethinus* (Durian) and *Cocos Nucifera* (Kelapa)-treated groups.

DISCUSSION

One of the most important factors in the healing of wounds is the stimulation of wound strength.[7] Wound strength is determined by the amount and quality of newly synthesized and deposited collagen, as well as degradation of preformed collagen.[3] Tensile strength, which demonstrates the force per unit of cross-sectional area needed to break the wound, is an important measure since it reflects the subdermal organization of the collagen fibers in the newly deposited collage.[7] Tensile strength indicates how much the repaired tissue resists to break under tension and may indicate in part the quality of the repaired tissue.[9]

Collagen is one of the major components that is mainly responsible for the mechanical properties of the skin.[10] The net amount of wound collagen deposition depends on collagen turnover and is a reflection of collagen synthesis minus collagen breakdown.[3] The changes in the diameter of collagen fibrils have also been related to mechanical strength of the skin. Apparently thick collagen fibrils can resist greater tensile strength as opposed to thin ones.[11] Once the skin is injured, the normal collagen will be replaced by scar collagen and the connective tissue will not regain the original highly organized structure of collagen.Thus, the healing skin is weaker and results in lower tensile strength as opposed to the normal skin.[12]

In this study, the initial phase of wound healing was characterized by low tensile strength, which resulted from the severing of collagen bundles and fibrils at the wound site. Heat denaturation, such as in thermal burn, destroys the helical structure of the collagen molecule and if the temperature is sufficiently high, cleaves the Schiff-base bond thus solubilizing a fraction of intact collagen as a high molecular weight gelatin.[13] Newly formed collagen does not possess any substantial mechanical strength until cross-links of the Schiff-base type have been formed between collagen molecules and this process is catalyzed by lysyl-oxidase.[11] Although the mechanical strength of soft connective tissues is mediated by the collagen, there are no substantial amounts of collagen present in a wound until after 2 to 3 days of healing.[14] This is due to the fibroblast proliferation and collagen synthesis, which usually begins 2 to 3 days post wounding.[7]

A slow increase in wound tensile strength corresponds to the increase in fibroblasts, which begin to produce immature collagen during the proliferative phase of wound healing.[7] Fibroblasts play an important role in producing the collagen necessary to restore the tensile strength of wounded skin.[15] Suguna et al[4] have proved that honey enhanced collagen synthesis, particularly by stimulating the activity of fibroblasts due to the sugars present in honeys. Al-Jady et al[16] have demonstrated that honey treatment significantly increased cell proliferation in the newly formed granulation tissues, which reflected the high activity of fibroblasia. Honey contains sugars, amino acids, minerals, vitamins, and low level of hydrogen peroxide, which help in enhancing cell proliferation, as well as in stimulating fibroblast proliferation.[16] Since fibroblasts are necessary for collagen production, it may be inferred that application of Manuka, *Durio Zibethinus* (Durian) and *Ananas Comosus* (Nenas) honeys could play a role in increasing the amount of granulation tissue, by attracting fibroblasts to the wound site and facilitating and accelerating the wound healing process, thus augmenting the tensile strength.

In the latter stage of healing also known as the remodeling phase, the tensile strength increased as the collagen reorganized and matured into larger bundles and thus increased the strength of the wound.[6] Subsequently, an increase in the extent of intermolecular covalent cross-linking within a fibril and establishment of collagen-ground substance interaction within the wound, such as proteoglycan or glycoprotein, may be additional factors responsible for greater tensile strength.[6] The increase in tensile strength in the Manuka, *Durio Zibethinus* (Durian) and *Ananas Comosus* (Nenas) honey-treated wounds might be due to the increase in collagen concentration per unit area and stabilization of the fibrils.[5]
Maturation of collagen fibrils resulted in stable cross links between several chains, and these cross links are responsible for the gain in strength. In addition, honey was claimed to contain high levels of glycine, methionine, arginine, and proline, which play definite roles in collagen formation and deposition.[17]

According to Jimenez and Rampy[7] substantial amounts of collagen undergo fibril maturation in an intricate cross-linking system. Collagen intermolecular cross-links were essential for providing the stability and tensile strength of the skin. Cross-linking formation took place as the triple helical collagen molecules lined up and began to form fibrils and then fibres.[12] Cross-linking was influenced by many factors and the cross-linking pattern may, therefore, reflect the structural status of the collagen fibrils.[18] The increased in tensile strength of the 3 different types of honey-treated wounds namely Manuka, *Durio Zibethinus* (Durian) and *Ananas Comosus* (Nenas) honeys may be due to the increased in collagen maturation and crosslinking. Since wounds treated with these 3 types of honey showed greater tensile strength, it may be inferred that it not only increases collagen synthesis per cell, but also aids in the crosslinking of the protein. The gain in tensile strength may also be related to collagen interaction with other macromolecules, such as proteoglycan or glycoprotein, or possibly by a slower process of cross-linking between scar tissue collagen and the mature collagen of the skin adjacent to the wound.[19] Honey also has been proven to enhance the synthesis of glycosaminoglycans.[16] Glycosaminoglycans are one of the major components synthesized by fibroblasts in the wound area, as a provisional matrix on which collagen fibers are embedded.[20]

Honey could be an important cellular mediator responsible for the initiation and acceleration of wound healing and may enhance the healing of burn wounds. Based on our findings, Manuka honey and the two honeys, namely *Durio Zibethinus* (Durian) and *Ananas Comosus* (Nenas), may be potent stimulators of wound healing, as demonstrated by increased mechanical strength. Manuka Honey derived from the manuka tree (*Leptospermum scoparium*), a native of New Zealand is currently one of the honeys approved for therapeutic use.[21] Manuka is thought to have additional therapeutic properties, which are derived from the floral source.[21] Manuka honey has a uniquely high level of herbal antibacterial component and is particularly effective against some of the important wound-infecting bacteria.The increased in tensile strength of honey (Manuka, *Durio Zibethinus* [Durian] and *Ananas Comosus* [Nenas]) treated wounds could reflect not only the increased collagen content but also acceleration of collagen maturation resulting from the cross-linking of the collagen deposited by the fibroblasts. In addition, the pH of honey was claimed to create and maintain optimal conditions for fibroblast activity.[21]

The recovery of tensile strength of healing skin related to several factors, including healing time and the nutritional status of the healing tissues.[14] Conditions such as scurvy and lathyrism may impair the synthesis or stability of fibrillar collagen and thus have a detrimental effect on wound strength.[22] Honey contains a wide range of amino acids, vitamins, and trace elements, in addition to large qualities of readily assimilable sugars, which help stimulate tissue growth.[4]

The present study shows that topical application of honey on burn wounds can improve healing through increased mechanical strength. Since wounds treated with *Durio Zibethinus* (Durian) honey and *Ananas Comosus* (Nenas) honey showed greater tensile strength compared to wounds treated with two other honeys (*Melaleuca* [Gelam] and *Cocos Nucifera* [Kelapa]), it may inferred that the former honey groups increased collagen concentration and stabilization of the fibers. However, in the future, further studies need to be carried out to confirm it.

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