UNIVERSITI PUTRA MALAYSIA

EFFICACY OF VARIOUS LOCAL HONEY FOR THE TREATMENT OF BURN WOUNDS

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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To my mum and dad for their love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Since time immemorial honey has been known to treat myriad of wounds and ailments. Recently, honey has been revived as an effective treatment for wounds and the interests that spark in approaching alternative treatments stem partly from the emergence of antibiotic-resistance pathogens. In addition burn care is an expensive proposition which requires significant duration of hospital stay as well as expensive medications. Since honey is produced from many sources of nectar, the chemical and physical activities vary greatly with origin of the nectar as well as environmental conditions. Thus, the present study was undertaken to assess the potential of various Malaysian honeys in treating burn wound.

The efficacy of topical application of Malaysian honeys on burn wound healing in Sprague-Dawley rats was investigated on the basis of biophysical and histological changes. A total of 210 Sprague-Dawley male rats weighing between 200 - 300 g were used in this study. Deep partial skin thickness burn wound was inflicted on the dorsal part of the

body. Imported Manuka honey as well as four selected local honeys collected from different plantations namely nenas, gelam, durian and kelapa were applied twice daily in a quantity of 0.5 ml for each application. Control animals received no treatment while silver sulphadiazine (SSD) cream served as a standard burn wound treatment. The rats were inspected daily and the general appearance as well as the rate of wound contraction was recorded at 3, 7, 14, 21 and 28 days post burned. Six rats from each experimental group were euthanized at each time interval and the skin samples taken were evaluated histologically and subjected to tensile strength test. Tissue sections were stained with haematoxylin and eosin (H&E) and Masson's trichrome staining, while tensile strength testing was done using an Instron™ tensiometer.

The results obtained from this study showed that Manuka honey and Gelam honey significantly stimulated the rate of burn wound healing as demonstrated by increased rate of wound contraction and from gross observations. Microscopic evaluation demonstrated that there was a significant acceleration of the dermal repair in wound healing treated with Manuka and Gelam honeys. Early attenuation of inflammatory reaction and early reparative activities were observed in wounds treated with the two types of honeys. Differential cells count showed a significant decrease in the number of inflammatory cells in the Manuka honey and Gelam honey treated wounds as early as 3 days post injury. In

addition, epithelial regeneration appeared to be quite advanced whereby re-epithelialization was observed as early as 7 days after burn treatment as compared to other experimental groups. Histological findings of this study also showed enhanced proliferation of fibroblasts and collagen synthesis in wounds treated with Manuka honey and Gelam honey. In addition, tensile strength of the wounds treated with these honeys was also enhanced during the course of study.

Thus, results obtained from the present study suggested that topical application of Manuka and Gelam honey may have favourable influence on the various phases of burn wound healing hence accelerating the healing process.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEGUNAAN PELBAGAI JENIS MADU TEMPATAN SEBAGAI RAWATAN ALTERNATIF UNTUK MERAWAT LUKA TERBAKAR

Oleh

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Madu telah digunakan untuk merawat pelbagai jenis luka dan penyakit sejak berabad tahun dahulu. Kebelakangan ini, madu telah dikenali semula sebagai rawatan yang efektif untuk luka dan minat dalam mengkaji rawatan alternatif terbit daripada masalah kewujudan patogen yang mempunyai ketahanan terhadap antibiotik. Tambahan pula, rawatan terhadap luka akibat terbakar memerlukan kos yang tinggi dan pesakit terpaksa tinggal di hospital dalam jangka masa yang lama serta ubat-ubatan yang mahal diperlukan. Oleh kerana madu dihasilkan daripada sumber nektar yang berlainan maka aktiviti kimia dan fizikalnya turut berbeza bergantung kepada sumber nektar dan keadaan kawasan persekitaran. Oleh itu, ujikaji ini dijalankan untuk mengesan keberkesanan madu Malaysia dalam merawat luka terbakar.

Keberkesanan madu Malaysia yang disapu secara topikal ke atas luka terbakar diuji melalui perubahan biofizikal dan histologikal menggunakan

tikus-tikus jenis Sprague-Dawley. Sejumlah 210 ekor tikus jantan jenis Sprague-Dawley yang beratnya antara 200 – 300 g digunakan untuk tujuan tersebut. Luka terbakar tahap kedua telah diwujudkan pada bahagian belakang badan tikus. Madu jenis Manuka yang diimport dan empat jenis madu tempatan terpilih yang diambil daripada dusun yang berbeza iaitu Nenas, Gelam, Durian dan Kelapa disapu 2 kali sehari ke atas luka tersebut dalam kuantiti 0.5 ml untuk setiap kali sapuan. Tikus dalam kumpulan kawalan tidak diberi apa-apa rawatan lanjutan manakala krim silver sulphadiazine (SSD) dijadikan sebagai rawatan kawalan untuk kesan luka terbakar. Tikus-tikus tersebut diawasi setiap hari untuk jangka masa 28 hari. Keadaan serta perubahan luka secara umum dicatat dan kontraksi luka yang berlaku direkodkan pada hari ke 3, 7, 14, 21 dan 28 hari selepas terbakar. Enam ekor tikus daripada setiap kumpulan ujikaji dimatikan mengikut tempoh masa yang ditetapkan dan sampel kulit diambil untuk ujian secara mikroskopik dan ujian kekenyalan. Pewarnaan haematoxylin & eosin (H&E) dan Masson's trichrome digunakan dalam ujian mikroskopik manakala ujian kekenyalan dijalankan menggunakan mesin Instron™ tensiometer.

Hasil ujian menunjukkan bahawa madu jenis Manuka dan Gelam merangsang kadar penyembuhan luka terbakar secara signifikan melalui peningkatan kontraksi luka dan daripada perubahan secara umum keadaan fizikal luka-luka tersebut. Ujian mikroskopik juga menunjukkan bahawa proses penyembuhan derma meningkat secara signifikan apabila dirawat dengan madu jenis Manuka dan Gelam. Kawalan awal terhadap reaksi

inflamasi dan proses penyembuhan luka yang awal dapat diperhatikan daripada luka-luka yang dirawat dengan 2 jenis madu berkenaan. Tambahan pula, lapisan epitelium berproliferasi dengan cepat iaitu seawal 7 hari selepas dibakar jika dibandingkan dengan kumpulan-kumpulan eksperimen yang lain. Ujikaji secara mikroskopik juga menunjukkan peningkatan proliferasi sel fibroblast dan sintesis awal kolagen baru dalam luka yang dirawat dengan madu jenis Manuka dan Gelam. Ujian kekenyalan pula menunjukkan peningkatan kekenyalan kulit secara beransur sepanjang eksperimen dijalankan.

Hasil kajian menyarankan keberkesanan madu jenis Manuka dan Gelam yang disapu secara topikal ke atas kesan luka terbakar dapat merangsang pelbagai peringkat dalam proses penyembuhan luka.

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LIST OF ABBREVIATIONS

%	percent
<	less than
>	more than
°C	degree Celsius
µm	micrometer
cm	centimeter
et al.	and others (Latin: et alii)
g	gram
H&E	haematoxylin and eosin
kg	kilogram
kGy	kilo Gray
mg	milligram
MINT	Malaysians Institute for Nuclear Technology Research
ml	milliliter
mm	millimeter
Mpa	megapascal
P	probability
SSD	silver sulphadiazine

CHAPTER ONE

INTRODUCTION

General Introduction

In the Al-Quran, Verse 16 of Surah Al-Nahl (No. 68-69) is quoted as saying "And the Lord inspired the bee, saying: Take your habitations in the mountains and in the trees and in what they erect. There comes forth from their bellies a drink of varying color wherein is healing for men. Verily in this is indeed a sign for people who think."

During the past decade there has been a global interest in the use of traditional and complementary medicine. Most scientific research has focused on herbal as well as aromatherapy products. In addition, a number of other naturally occurring substances have been proven to show therapeutic promise. One such resource that was claimed to have curative value is honey.

Ironically, honey has been used as a medicine for thousand of years and its healing properties are well documented (Molan, 1999a). Honey has been used to treat a wide range of wounds of various aetiology including abscess, surgical wounds, ulcers and burns (Molan, 1999b). It was claimed that early Egyptians were the first to use honey as a component in the topical treatment of wounds as evidence from their writing in the Smith papyrus (1650BC)

(Forrest, 1982). Thus, Zumla and Lulat (1989) referred honey as 'a remedy rediscovered' due to the resurgence of its usage in modern professional medicine. Perhaps the rising interest in the use of alternative therapies is mainly due to the expanding problem of antibiotic resistance in bacteria or because some people are experiencing the possible side effects of many pharmaceuticals products (Sai & Babu, 1998).

Honey is a mixture of sugars prepared by the bees from the natural sugar solutions called nectar obtained from flowers (Subrahmanyam, 1996). It is produced from many sources, and its antimicrobial activity varies greatly with origin and processing (Molan, 2001). Therapeutic honeys offer considerable benefits in wound care, particularly for the treatment of chronic and infected wounds and for the treatment of burns (Lusby *et al.*, 2002). Its efficacy in wound healing remains largely anecdotal with claims that it reduces inflammation, debrides necrotic tissue, reduces oedema and promotes angiogenesis, granulation and epithelialization (Molan, 1998a). When used as a topical application, honey was found to accelerate wound healing and its antibacterial properties reduced bacterial growth (Bergman *et al.*, 1983). Therapeutic effects of honey were also found to be useful in the treatment of burn, by helping the rapid healing of wounds with less scarring (Subrahmanyam, 1991). It gives rapid deodorization of offensively smelling wounds, which is an unpleasant characteristic feature of burn treatment unit (Molan, 1998b). Its antibacterial effect caused the wounds to heal earlier by