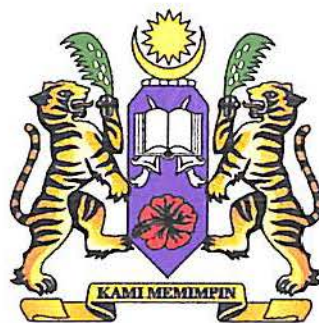


**IN VIVO EVALUATION OF THE ANGIOGENIC
AND ANTIMICROBIAL PROPERTIES OF
TUALANG HONEY USING A FULL-THICKNESS
BURN WOUNDS IN ANIMAL MODEL
IN COMPARISON TO HYDROFIBRE**

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III PREFACE

Wound management is tremendous scope in plastic surgery and its multitude of approaches for every clinical situation varied largely. Yet precisely these aspects of plastic surgery make its study especially challenging. The sophisticated development of modern dressing materials have been introduced in wound management, in order to obtain ideal wound healing, particularly in burn wounds. Both local and oversea literature studies have revealed usage of medicinal honey on wound management, for instance Manuka honey from New Zealand.

When preparing for the dissertation of my plastic and reconstructive surgery training, I was exposed to many types of honey in the market, including the medicinal honey, in the hope that this exposure would open my eyes to differentiate the medicinal honey on clinical usage. Honey is thought to promote wound healing. I found it difficult and time consuming to extract information from vast types of honey available in the market. One of the honeys available in Malaysia is Tualang honey, which is suitable for clinical study. Further study is needed to evaluate its topical application benefits on wound.

Although there are several excellent references that focus on the honey in vitro study, in vivo study regarding the efficacy of topical dressing for deep burn injury is limited. Hence, the purpose of my dissertation is to provide a preliminary study of local Tualang honey on deep burn wound in animal model to plastic surgery, as it can be implied and

practiced in academic medical centers. I hope that this dissertation will be useful to manage burn wounds in medical fields.

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VII LISTS OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
CFU	Colony Forming Unit
FAMA	Federal Agricultural Marketing Authority
H & E	Haematoxylin & Eosin stain
IL-1	Interleukin-1
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
PDGF	Platelet-derived growth factor
TBSA	Total body surface area
TGF- β	Transforming growth factor-beta
TNF- α	Tumour necrosis factor-alpha
TSA	Tryptic Soy Agar
USM	Universiti Sains Malaysia
VEGF	Vascular endothelial growth factor
γ -irradiation	Gamma-irradiation
kGy	KiloGray
ml	Millilitre
mm ²	Square millimeter
μ m	micrometer

VIII ABSTRAK

Luka akibat kebakaran di peringkat *full-thickness* memerlukan pembedahan untuk membuang kulit-kulit mati dan proses penampalan kulit diperlukan supaya tidak mengalami kecacatan fizikal yang kekal. Penyediaan luka kebakaran sebelum pembedahan adalah memainkan peranan yang penting dalam proses penyembuhan kulit. Proses tersebut membantu pencegahan jangkitan kuman dan mengakibatkan proses penyembuhan. Madu boleh digunakan untuk merawat luka kebakaran. Namun, bukan semua jenis madu mempunyai ciri-ciri yang sama. Objektif kajian ini adalah untuk menguji pengecutan luka kecederaan kebakaran dan sifat antimikrobial serta aspek histologi madu Tualang di dalam rawatan luka kebakaran *full-thickness* pada model tikus “*Sprague Dawley*” berbanding dengan Aquacel Ag and Aquacel biasa. Kajian ini melibatkan tiga puluh enam ekor tikus betina “*Sprague Dawley*”. Tikus dibahagi secara rawak ke dalam tiga kumpulan. Setelah tikus mendapati ubatan bius, tiga luka kebakaran *full thickness* diperlakukan pada belakang tersebut. Luka-luka ini diinokulasi dengan organisma-organisma tertentu, iaitu *Pseudomonas aeruginosa* (n=12), *Klebsiella pneumoniae* (n=12), or *Acinetobacter baumannii* (n=12). Ketiga-tiga luka ini diberi rawatan dengan madu Tualang, Aquacel biasa atau Aquacel Ag. Setiap tiga hari (hari ke 3, 6, 9, 12, 15, 18 dan 21), sample ‘swab’ diambil untuk menjalani kajian mikrobiologi secara kuantitatif dan semi-kuantitatif. Penilaian klinikal dan histopatologika diperlakukan. Keputusan kajian ini mendapati bahawa pengurangan saiz luka sebanyak 32.26% pada luka-luka yang dirawat dengan madu Tualang pada hari ke-6, 49.27% pada hari ke-15, dan luka tersebut lebih kecil pada hari ke-18 ($p < 0.032$). Tikus yang diberi

rawatan dengan madu Tualang menunjukkan pengurangan di dalam pembiakan bakteria di dalam kumpulan yang diinokulasi dengan *Pseudomonas aeruginosa*. ($p = 0.005$). Namun demikian, Aquacel Ag dan Aquacel biasa lebih berkesan daripada madu Tualang di dalam rawatan luka-luka yang diinokulasi dengan *Acinetobacter baumannii* ($p = 0.035$). Di dalam segi histologi, bilangan neutrofil berbeza di madu Tualang pada hari ke-7 dalam kedua-dua kumpulan *Acinetobacter baumannii* dan *Klebsiella pneumoniae* ($p < 0.05$). Di dalam kumpulan *Pseudomonas aeruginosa*, ketebalan tisu *granulation* dengan pembalut madu Tualang menunjukkan $50.42\mu\text{m}$ berbanding dengan pembalut Aquacel ($24.87\mu\text{m}$) pada hari ke-14 ($p = 0.045$). Pada hari yang ke-21, di dalam kumpulan *Klebsiella pneumoniae*, penyembuhan luka kebakaran sepenuhnya dengan meliputi empat lapisan *epidermis*. Dari segi histology dalam proses pembentukan kapilari yang baru, tiada pembezaan di antara ketiga-tiga kumpulan. Kesimpulannya madu Tualang memberi keputusan yang lebih baik berbanding dengan rawatan-rawatan lain dari segi pengecilan luka kebakaran serta mengawal jangkitan *Pseudomonas aeruginosa*. Madu tersebut menggalakan proses penyembuhan luka kebakaran yang mengandungi *Pseudomonas aeruginosa* ataupun *Klebsiella pneumoniae*.

IX ABSTRACT

Tangential excision and skin grafting are inevitable in managing full-thickness burns. Wound bed preparations prior to surgery are necessary in order to prevent wound infection and promote wound healing. Honey can be used to treat burn wounds. However, not all the honey is the same. The objective of this study is to evaluate the effect on the wound contraction, antimicrobial properties and histopathological aspects of Tualang honey in full-thickness burn wounds in a rat model, in comparison to Aquacel dressings. Thirty-six female Sprague Dawley rats were randomly divided into three groups. Under anesthesia, three full-thickness burn wounds were created on the dorsum of the rats. The full-thickness burn wounds were inoculated with a specific organism (10^4), namely *Pseudomonas aeruginosa* (n=12), *Klebsiella pneumoniae* (n=12), or *Acinetobacter baumannii* (n=12). The three burn wounds were dressed with Tualang honey, plain Aquacel or Aquacel Ag. Swab samples were obtained every 3 days (day 3, 6, 9, 12, 15, 18 and 21) for microbiological analyses. Clinical assessments were performed. At day 7, 14 and 21 days of burn, tissue samples were sectioned and histopathological examination was performed. The result of this study reveals a rapid 32.26% reduction in wound size by day 6 ($p = 0.008$) in the Tualang honey-treated wounds, and 49.27% by day 15 ($p = 0.005$). The wounds remained smaller by day 18 ($p < 0.032$). Tualang honey-treated rats demonstrated a reduction in bacterial growth in *Pseudomonas aeruginosa* inoculated wounds ($p = 0.005$). However, Aquacel-Ag® and plain Aquacel-treated wounds are superior to honey-treated wounds with *Acinetobacter baumannii* ($p = 0.035$). Neutrophil count was reduced in honey-treated wound on day 7 in both *Acinetobacter baumannii*

and *Klebsiella pneumoniae*-inoculated wounds ($p < 0.05$) and similar findings on day 14 in *Pseudomonas aeruginosa*-inoculated wounds ($p = 0.003$). Granulation thickness of honey treated wound was 50.42 μm compared to Aquacel-treated wound (24.87 μm) on day 14 in *Pseudomonas aeruginosa* group ($p = 0.045$). On day 21, in *Klebsiella pneumoniae* group, there was complete epidermis coverage of the wound ($p = 0.002$) and increased thickness of granulation tissue ($p = 0.001$) in honey treated group. In histologic analysis of new capillary formation, there was no statistical significance between all the dressings. In conclusion, this experiment shows the positive effect of Tualang honey as a topical dressing for full-thickness burn wounds in an animal model. Tualang honey has better results with regard to the eradication of *Pseudomonas aeruginosa*. It also promotes burn wound healing process on wound inoculated with *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*.