IN VIVO EVALUATIONS OF WOUND HEALING AND ANTI MICROBIAL PROPERTIES OF TUALANG HONEY USING A FULL THICKNESS BURN WOUND IN SPRAGUE DAWLEY RATS

BY

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IV ABBREVIATIONS

- MIC	minimum inhibitory concentration
- SSD	silver sulfadiazine
-CFU	colony forming unit
-TNTC	To numerous to count

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VII ABSTRAK

Secara amnya, luka kelecuran adalah merupakan portal untuk kemasukan microorganisma dan boleh menjejaskan penyembuhan luka. Kesan madu Tualang terhadap penyembuhan luka kelecuran yang dalam telah diuji dalam kajian ini. Objektif kajian ini adalah untuk menguji pengecutan luka kelecuran, sifat antimikrobial dan aspek histologi di dalam rawatan luka kelecuran *full-thickness* pada model tikus dengan membandingkan penggunaan antara madu Tualang, Chitosan gel atau Aquacel-Ag®. Kesan madu Tualang terhadap penyembuhan luka kelecuran yang dalam diuji ke atas 36 ekor tikus. Tikus dibahagikan kepada 3 kumpulan (12 ekor setiap kumpulan). Selepas tikus dibius, 3 luka kelecuran yang dalam dibuat menggunakan pemutar skru yang dipanaskan dengan penunu bagas. Luka setiap ekor tikus telah diinokulasi dengan bacteria yang berbeza: Kumpulan A telah diinokulasi dengan *Pseudomonas aeruginosa*, Kumpulan B telah diinokulasi dengan *Klebsiella pneumoniae* dan Kumpulan C telah diinokulasi dengan *Acinetobacter baumanii.* Setiap luka pada tikus telah dibalut dengan menggunakan samaada Madu tualang, Chitosan gel atau Aquacel-Ag®. Kemudian, tikus-tikus telah dinilai dari segi saiz luka, mikrobiologi dan histologi pada hari ke 3, 6, 7, 9, 12, 14, 15, 18 dan 21. Keputusan kajian ini mendapati bahawa tiada pembezaan dalam keputusan saiz luka apabila dirawat menggunakan samaada madu Tualang, Chitosan gel atau Aquacel-Ag® disepanjang keseluruhan kajian ($P > 0.05$). Tetapi saiz luka pada hari yang ke 21 telah menunjukkan bahawa luka yang telah dirawat oleh madu Tualang mempunyai saiz luka yang lebih kecil. Kajian mikrobiologi menunjukkan penurunan dalam pembiakan bakteria di dalam luka yang telah dirawat menggunakan madu Tualang jika dibandingkan dengan luka yang telah menerima rawatan menggunakan Chitosan gel atau Aquacel-Ag® di dalam kumpulan A dan C. Kajian ini juga

menunjukkan bahawa tiada pembezaan secara statistik dalam proses baikpulih tisu apabila dirawat menggunakan samaada madu Tualang, Chitosan gel atau Aquacel-Ag® disepanjang keseluruhan kajian. Namun, proses baikpulih tisu pada hari yang ke 14 menunjukkan bahawa luka yang telah dijangkiti oleh *Pseudomonas aeruginosa* mempunyai penyembuhan yang lebih awal apabila dirawat menggunakan madu Tualang. Kesimpulannya, penilaian madu Tualang terhadap saiz luka dan histologi telah menunjukkan penyembuhan luka yang cepat, inflammasi yang sedikit dan pembentukan salur darah baru yang cepat berbanding dengan balutan yang lain. Madu Tualang juga menunjukkan keberkesanan pada luka kelecuran yang diinfeksi dengan *Pseudomonas aeruginosa* dan *Acinetobacter baumanii.*

VIII ABSTRACT

The burn wound serves as a portal of entry for colonizing opportunistic microorganisms which can affect wound healing. The effect of Tualang honey on wound healing in the bacterial contaminated full-thickness burn wounds was evaluated in this study. The objectives of this study is to evaluate the wound contraction, antimicrobial properties and histological aspects of Tualang honey in treating full thickness burn wounds in a rat model in comparison to Chitosan gel or Aquacel-Ag®. The effect of Tualang honey on wound healing in the full-thickness burn wounds was evaluated in 36 male Sprague Dawley rats. The rats were randomly divided into 3 groups (n=12/group). Rat were anesthetized, and three full thickness burn wounds were created on each rat using a modified metal screwdriver heated using flame from blow torch. Each group of rats was inoculated with a different organism in the burn wounds: Group A was inoculated with *Pseudomonas aeruginosa,* Group B was inoculated with *Klebsiella pneumoniae* and Group C was inoculated with *Acinetobacter baumanii.* One wound on each rat was dressed with either Tualang honey, Chitosan gel or Aquacel-Ag®. The rats were subjected to the evaluation period of 3, 6, 7, 9, 12, 14, 15, 18 and 21 days, where the wound size, microbiological and histological findings were assessed. The results of this study revealed that the mean wound size of the Tualang honey-treated wounds was not statistically different than those of the Chitosan gel or Aquacel-Ag® treated wounds when the wounds were compared throughout the entire experiment ($P > 0.05$). However, comparing the mean wound size on day 21 alone revealed that the Tualang honey-treated wounds were smaller in comparison to that of the Chitosan gel and Aquacel-Ag® treated groups. The quantitative and semi-quantitative methods showed that there was a significant reduction in

bacterial growth in Tualang honey treated wounds compared to Chitosan gel and Aquacel-Ag® treated wounds in Group A and C. There was no statistically significant difference in the granulation tissue formation and epidermal thickness between the Tualang honey, Chitosan gel or Aquacel-Ag® treated wound when they were compared throughout the study. Nevertheless, early granulation tissue formation and epithelialization was seen in Tualang honey treated wounds by day 14 in burn wounds infected with *Pseudomonas aeruginosa.* In conclusions, clinical examination of the wounds and histological evaluation showed that Tualang honey gave the fastest rate of healing, the least inflammatory reaction and has most rapid neovascularisation compared with the other treatments. Tualang honey also provide good evidence of the effectiveness of the antibacterial activity of honey on burn wounds infected with *Pseudomonas aeruginosa* and *Acinetobacter baumanii.*

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Honey, an ancient remedy for the treatment of infected wounds, has recently been "rediscovered" by the medical profession, particularly in cases where conventional, modern therapeutic agents have failed. Honey has been used to treat infected wounds for 2000 years and was in use before bacteria were discovered to cause infection. More recently, honey has been reported to inhibit the growth of approximately 60 diverse species of bacteria, including both gram-positives and gram-negatives aerobes and anaerobes (Molan, 1992). Antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium,* as well as all the common dermatophytes. The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of ancient therapeutic remedies such as honey.

There are now many published reports describing the effectiveness of honey in rapidly clearing infection from wounds, with no adverse effects to slow the healing process (Molan, 1992). Besides being well-known for its antibacterial effects, honey has been demonstrated to hasten granulation and re-epithelialization (Efem, 1993, Efem, 1988, Subrahmanyam, 1998) and to reduce inflammation (Subrahmanyam, 1998) in wounds.

In light of the enormous potential for application of honey within a clinical environment, it is important that research continues to evaluate not only honeys already

recognized for their antibacterial properties, but also other locally-produced honeys, such as Tualang honey.

Tualang honey is extracted from honeycombs found atop Malaysia's tallest tree, Tualang tree (Koompassia excelsa). Tualang tree can grows to an astonishing height of more than 250m (about 30 storeys). It is found in East Asian rainforests, mostly in Peninsular Malaysia, southern Thailand, northeastern Sumatra and Borneo. Honey from hives found in tualang tree is believed of the highest quality. Tualang honey is used commonly as a medicinal product and as food in Malaysia (Ainul Hafiza *et al.,* 2005, Ghazali, 2009). However, little scientific information about its therapeutic potential, including wound healing properties and antimicrobial activity had been published to date. An attempt to evaluate the efficacy and usefulness of Tualang honey dressing on full-thickness burn wounds in an animal model was performed in this study.

1.2 Wound Healing Activity of Honey

The healing of acute wounds involves a complex and dynamic series of events leading to the repair of injured tissues. These events, triggered by tissue injury, involve four overlapping but well defined phases: haemostasis, inflammation, proliferation and remodelling.

Haemostasis is secured by platelet aggregation and clot formation. The inflammatory phase begins with the arrival of phagocytic neutrophils and, later, macrophages at the wound site; they are important sources of and substrates for growth

factors. The proliferative phase is characterised by the formation of new blood vessels (angiogenesis), synthesis of extracellular matrix components such as collagen and re-epithelialisation. The extracellular matrix is continually remodelled during the final phase; an avascular scar is the end result of the healing process.

In full thickness burn, all regenerative elements have been destroyed in these injuries, and healing only occurs from the edges and is associated with considerable contraction. All such injuries should therefore be excised and grafted unless they are less than 1 cm in diameter in an area where function would not be compromised (Remo, 2004).

Honey used as a wound dressing has been reported to promote the formation of clean healthy granulation tissue. It has also been reported to promote epithelialisation of the wound. Honey induces a fast rate of tissue regeneration and suppresses inflammation, edema, exudation and malodour in wounds, as observed in both animal studies and clinical trials (Efem, 1993, Efem, 1988, Subrahmanyam, 1998). A quickened clearance of infection could account for these effects by preventing the production of the products of bacterial metabolism.

The benefits of a moist wound environment are well established: it protects the wound, reduces infection rates, reduces pain, debrides necrotic tissue, and promotes granulation tissue formation. Moist wound dressings enable epithelialization to occur along the top surface of the wound, rather than underneath the scab, as occurs in dry wounds, resulting in a pitted scar. The physical properties of honey make it an ideal moist wound

dressing. The high viscosity of honey (which varies from floral source to floral sources) provides a protective barrier to prevent wounds from becoming infected, effectively sealing the wound (Bello & Phillips, 2000).

Another way in which honey may promote healing is by supplying glucose to the epithelial cells, as these have to build up an internal store of carbohydrate to provide the energy they need to migrate across the surface of a wound to restore skin cover (Molan, 2002).

The pH of honey may help to create and maintain optimal conditions for fibroblast activity (migration, proliferation, and organization of collagen), which requires mildly acidic wound conditions (Calvin, 1998).

Although hydrogen peroxide can be harmful to wounds when added as a rinse solution, honey continuously provides hydrogen peroxide at a consistent level that is antibacterial and physiologically nontoxic. Levels produced by diluted honey are approximately 1000 times lower than in rinse solutions. At this concentration hydrogen peroxide may act as a novel intracellular and intercellular "messenger" capable of promoting growth responses and stimulating expression of early growth genes important in wound healing. Hydrogen peroxide generated by immune cells is likely to be physiologically important in contributing to the growth modulation of adjacent non inflammatory cells such as fibroblasts at the site of inflammation (Burdon, 1995).

Excessive inflammation prevents healing and the attraction of inflammatory leukocytes gives rise to high levels of proteolytic enzyme activity at the site of the inflammation. The potent anti-inflammatory action of honey would resolve such a situation and prevent excessive proteolytic activity. Although the mechanism for the anti-inflammatory action of honey is not entirely understood, there is histological evidence for the anti-inflammatory activity of honey from some of the studies on experimental animals (Kumar *et al.*, 1993, Postmes & Vandeputte, 1999). In some of the experimentally induced burns, there was no infection evident, yet honey still brought about a decrease in inflammation. This indicates that the anti-inflammatory activity of honey is a direct action and not a secondary consequence of removal of infection through its antibacterial activity (Molan, 2006).

The potential benefits of honey's anti-inflammatory effect include alleviation of the pain associated with inflammation. A reduction in edema provides other positive effects. The pressure from edema restricts the blood flow of oxygen and nutrients, which leukocytes need to fight infection and fibroblasts need for connective tissue synthesis. Thus, reducing edema not only alleviates associated pain but also improves microcirculation and increases the availability of dissolved oxygen and nutrients needed for tissue repair and regeneration (Molan, 2002).

The anti-inflammatory effects of honey reduce hypertrophic scarring during the maturation phase of wound healing. The free radicals formed when excessive or prolonged inflammation is present stimulate the fibroblasts that produce the collagen fibers of a scar. The anti-inflammatory effects of honey reduce formation of reactive oxygen species, thus

decreasing the fibroblast and collagen production needed to create a hypertrophic scar (Pieper, 2009).

The clearance of infection may not only be the result of the antibacterial action of honey. Recent research indicates that honey may work by stimulating the activity of the immune system. Honey at concentrations as low as 0.1% has been found to stimulate proliferation of peripheral blood β-lymphocytes and T-lymphocytes in cell culture and activate phagocytes from blood. Also, honey at a concentration of 1% has been reported to stimulate monocytes in cell culture to release the cytokines TNF-1, IL-1, and IL-6, which are intermediates in the immune response. In addition to the reported stimulation of leukocytes, honey has the potential to augment further the immune response by supplying glucose. This is essential for the 'respiratory burst' in macrophages that generates hydrogen peroxide, the dominant component of the bacteria-destroying activity of these cells (Molan, 2002).

These studies conclude that honey may have a number of effects on the molecular mechanisms of wound healing.

1.3 Antimicrobial Activity of Honey

The burn wound serves as a dangerous portal of entry for colonizing opportunistic microorganisms, which invade thermally damaged tissue and potentially lead to serious sequelae, such as invasive wound infection, septicemia, and death. Microorganisms commonly implicated in invasive burn wound infections include, but are not limited to,

Pseudomonas aeruginosa, ***Acinetobacter*** species, members of the family *Enterobacteriaceae* (eg, *Escherichia coli*, ***Klebsiella pneumoniae***, *Serratia marcescens*, *Enterobacter cloacae*, and *Proteus mirabilis*), *Staphylococcus aureus* (including methicillin-resistant strains), *Streptococcus pyogenes*, other [beta]-hemolytic *Streptococcus* species, *Enterococcus* species (including vancomycin-resistant strains), anaerobes, *Candida* species, *Aspergillus* species, and viral agents such as herpes simplex virus and cytomegalovirus (Karyoute, 1989, Mousa, 1997, Smith & Thomson, 1992)

Bacterial load greater than 100 000 organisms or colony forming units per gram of tissue is a predictor of wound infection. However, some wounds that are more heavily colonised will heal spontaneously, and, conversely, some organisms are able to cause serious infection at much lower levels of colonisation. Infection depends on the pathogenicity of the organism, the type of wound, and the host response (Healy & Freedman, 2006)

The antibacterial property of honey has been recognized since 1892, and there has been a large amount of laboratory research carried out investigating this in the intervening period (Molan, 2002).

It has been observed that the high osmolarity of honey causes rapid absorption of edema fluid from weeping burns. The low water activity of the honey could be expected to dry out the wound; however, the osmotic pressure draws out fluid from the plasma or lymph in the underlying tissues. This mechanism dilutes the honey, activating glucose

oxidase to produce continuous low levels of hydrogen peroxide, which inhibits bacterial growth.

The hydrogen peroxide concentration produced in honey activated by dilution is typically around 1 mmol/l and it is about 1000 times less than in the 3% solution commonly used as an antiseptic. A study with *Escherichia coli* exposed to a constantly replenished stream of hydrogen peroxide, showed that bacterial growth was inhibited by 0.02-0.05 mmol/l hydrogen peroxide, a concentration that was not damaging to fibroblast cells from human skin (Hyslop *et al.*, 1995).

Honey's antibacterial properties and viscosity also provide a barrier to cross-infection of wounds. It also provides a supply of glucose for leucocytes, which are essential for the 'respiratory burst' that produces hydrogen peroxide, the dominant component of the antibacterial activity of macrophages. Furthermore, it provides substrates for glycolysis, which is the major mechanism for energy production in the macrophages, and thus allows macrophages to function in damaged tissues and exudates where the oxygen supply is often poor (Molan, 1998).

In manuka honey (*Leptospermum scoparium*) and honey from some other *Leptospermum* species, the phytochemical activity can be high, with a broad spectrum of antimicrobial activity. Manuka honey with a median level of phytochemical activity are equally as potent as antibacterial agents *in vitro*, although manuka honey is about twice as effective against enterococci (Cooper *et al.*, 2002*b*). In wound care, the catalase in serum and tissues decomposes to some extent the hydrogen peroxide produced by many honeys,

whereas the phytochemical component of *Leptospermum* honey maintains its activity. Besides that, the phytochemical factor in *Leptospermum* honey is active in full-strength honey, giving a more potent antibacterial action that diffuses into the depth of infected tissues, making it the honey chosen for sale for wound care.

The antimicrobial activity of honey has been measured most commonly by the use of agar well diffusion techniques. A study of type culture collection specimens of seven common wound-infecting species of bacteria found the MIC (minimum inhibitory concentration) of honey ranged from 1.8% to 10.8% (v/v), *i.e.* the honey was still able to stop bacterial growth if diluted nine to fifty-six times (Willix *et al.*, 1992). A study of 58 clinical isolates of *Staphylococcus aureus* found the MIC of honey to range from 2% to 4% (Cooper *et a*l., 1999), and a study of 20 isolates of *Pseudomonas spp.* from infected wounds found the MIC of honey to range from 5.5% to 9.0% (Cooper and Molan, 1999). Therefore, honey differs from other antiseptics because it retains its bactericidal activity in vitro even after dilution.

Research has shown that honey exerts antibacterial activity against clinical isolates of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, hemolytic streptococci, and vancomycin-sensitive enterococci (Molan, 2002, Blaser *et al.*, 2007).

Overall, honey has been demonstrated in many studies to have antibacterial effects, attributed to its high osmolarity, low pH, hydrogen peroxide content and content of other, phytochemical activity.

1.4 Safety of Honey

Honey sometimes contains spores of clostridia, which poses a small risk of wound botulism. If spores germinated, any vegetative cells of clostridia, being obligate anaerobes, would be unlikely to survive in the presence of the hydrogen peroxide that is generated in diluted honey. But the use of honey as a wound dressing has been argued against, however, on the grounds that the risk of it possibly causing wound botulism is unacceptable. This objection can be overcome by the use of honey that has been treated by gamma-irradiation, which kills clostridial spores in honey without loss of any of the antibacterial activity (Molan & Allen, 1996)).

The problem of attraction of flies and ants to honey dressings not commonly noted, can be overcome by using effective secondary dressings so that the honey is prevented from leaking out or being exposed to insects.

1.5 Chitosan gel and Aquacel-Ag® Dressings

Chitosan is a biopolymer derived from chitin and has been employed in a variety of applications in the biomedical field such as bone reconstruction, cell encapsulation, drug delivery and tissue engineering. In wound healing evaluation, histopathological examination confirmed increased epithelialization rate as well as well organized deposition of collagen in the dermis in Chitosan based wound dressing treated group (Harish Prashanth & Tharanathan (2007). This similar findings was confirmed in another study by Okamoto *et al.*, where they have reported that chitosan influenced all stages of wound

repair in experimental animal models. In the inflammatory phase, chitosan has unique hemostatic properties that are independent of the normal clotting cascades. In vivo these polymers can also stimulate the proliferation of fibroblasts and modulate the migration behavior of neutrophils and macrophages modifying subsequent repair processes such as fibroplasias and reepithelialization. These studies have added further to the body of evidence that chitosan are suitable as wound healing materials (Okamoto *et al.*, 1995))

Aquacel-Ag® dressings is a silver-impregnated hydrofiber wound dressing and has been employed in both acute and chronic wounds, including partial thickness burn, skin grafts (and donor sites), surgical wounds, diabetic foot ulcers, leg and pressure ulcers. It provides the broad-spectrum antimicrobial properties of ionic silver, which kills a broad range of pathogens in the dressing, including MRSA and vancomycin-resistant enterococci. It is well known that Aquacel-Ag® dressings is safe and beneficial in the management of partial-thickness burn in promoting faster healing (Ziedenhuis R.K., 2001).

To our knowledge, there is no published report of animal study that test specifically on the efficacy of Tualang honey in treating full thickness burn wound comparing to Chitosan gel or Aquacel-Ag®. Therefore, in this experimental study, we compared the wound healing and antimicrobial properties of locally-produced honey, Tualang honey with Chitosan gel and Aquacel-Ag® dressing on full thickness burn wound in Sprague Dawley rats.

2.0 OBJECTIVES OF STUDY

2.1 General objective

To evaluate the wound healing and anti-microbial properties of Tualang honey in full thickness burn wound in Sprague Dawley rats model.

2.2 Specific objectives

1. To determine the antimicrobial properties of Tualang honey on burn wound in comparison with Chitosan gel and Aquacel-Ag® dressings.

2. To assess the wound healing properties of Tualang honey in comparison with Chitosan gel and Aquacel-Ag® dressings.

3. To compare the angiogenesis of Tualang honey, Chitosan gel and Aquacel-Ag® dressings in burn wound healing.

4. To compare the inflammatory cells infiltration in burn wound healing of Tualang honey, Chitosan gel and Aquacel-Ag® dressings.

2.3 Research hypothesis

Tualang honey possesses better wound healing and antimicrobial properties in comparison with Chitosan gel and Aquacel-Ag® dressings in burn wound animal model.

2.4 Null hypothesis

Tualang honey does not possesses better wound healing and antimicrobial properties in comparison with Chitosan gel and Aquacel-Ag® dressings in burn wound animal model.

3.0 MATERIALS AND METHODS

3.1 The animals

Male Sprague Dawley rats weighting 350 to 400 grams were obtained from the animal centre of the School of Medical Sciences, Universiti Sains Malaysia, USM, Kota Bharu, Kelantan. All parts of this project were performed in the animal house, School of Medical Sciences, Universiti Sains Malaysia, USM.

This study complied with the 'Principles of Laboratory Animal Care' and 'Guide for the Care and Use of Laboratory Animals' and was approved by the university's ethical committee. (No. of Animal Ethics Approval: USM/Animal Ethics Approval/2007/(34) (108).

3.2 Study Design

Thirty six male Sprague-Dawley rats weighing between 350 to 400 grams were used throughout the study. The rats were housed individually in a cage, and feed with free access to standard commercial rat diet and water throughout the study.

The animals were distributed at random into three groups of twelve animals each and submitted to the following experimental protocols. The rats were subjected to the evaluation period of 3, 6, 7, 9, 12, 14, 15, 18 and 21 days.

3.3 Anaesthesia and Skin preparation

On the day of wounding, the rats were placed in ventral position and immobilized on their abdomen for the surgery. The dorsum of the rats were shaved. Immediately before operation the rats were anaesthetized with intramuscular injection of Ketamine 35.0 mg/kg and Xylazine 5.0 mg/kg on gluteal area.

When fully anaesthetized, the shaved areas were cleaned with povidone iodine, alcohol 70 % and Hibiscrub®. The operation site was isolated with sterile towel.

3.4 Full Thickness Burn Wound Creation

Burn wounds were created using a modified metal screwdriver heated using flame from blow torch for 30 seconds (Figure 2). Under sterile technique, full thicknesss burn wounds were created on the dorsum of the rats between the thoracic vertebra and the sacrum by placing hot metal at right angles perpendicular to the dorsum of the rat for 30 seconds using an stopwatch. This method was adopted for the study as it was confirmed to produce full thickness burn wound after performing series of test.

The area of the wound was approximately 1.0 cm x 1 .0 cm in size. A total of 3 wounds were created on each rat (Figure 1). The distance between the wounds were 2 cm apart (Figure 3).

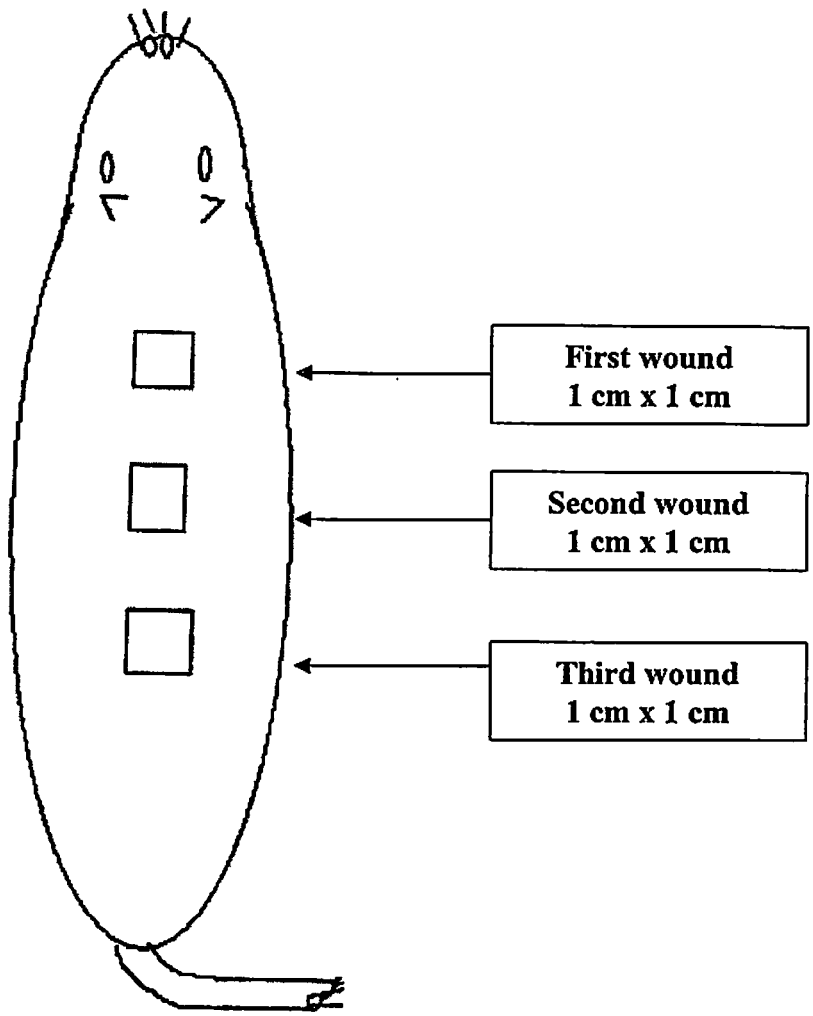

Figure 1: Illustrations of the burn wound

3.5 Treatments

The rats were randomly divided into three groups of twelve animals each. In the first group (Group A), the 3 created burn wounds were inoculated with 10^4 CFU of *Pseudomonas aeruginosa* , in the second group (Group B) , the wounds were inoculated with 10^4 CFU of *Klebsiella pneumoniae* and lastly in the third group (Group C), the wounds were inoculated with 10^4 CFU of *Acinetobacter baumanii* . *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* were used in this study as they were the common pathogens that can cause nosocomial infections in a hospital setting. Inoculation was performed by pipeting 10^4 CFU of each microorganism directly onto the wound using automatic pipet and was spread evenly.

In each rat from each group, the wound was then treated with Tualang honey (on the first wound), Chitosan gel (on the second wound) and Aquacel-Ag® dressing (on the third wound). The Tualang honeys were provided by FAMA (Figure 4), the Chitosan gel were provided by Nuclear Malaysia and Aquacel-Ag® dressing was purchased from ConvaTec. Approximately 0.2 ml of either Tualang honey or 0.5ml of Chitosan gel was applied topically onto the surface of each burn wound and Aquacel-Ag® dressing measuring 2.5 cm x 2.5 cm in size was used on the wound (Figure 5).

The first treatment was applied approximately 10 minutes after burn infliction and the wound was covered with a sterile gauze and bandage. The dressing was changed every three days until they were euthanized and the wound was cleaned with saline during dressing change (Figure 6).

3.6 Evaluation of Wounds

At day 3, 6, 9, 12, 15, 18 and 21 post-operations, the condition of each wound was examined and photographs were taken after burn wound creation until complete healing. For wound size measurement, the wounds were traced on a transparency paper and the tracings were measured.

Swab for culture was taken during each dressing change at day 3, 6, 9, 12, 15, 18 and 21 post-operations by cotton swab and consistency was maintained for all swab sampling and was transported in the transport media.

Three rats of each group were euthanized at day 3, 7, 14 and 21 post-operations and the tissue biopsies from the wounds were retrieved. The samples were cut into 2.0 cm X 2.0 cm strips parallel to long axis of the body by using sharp blades. Biopsies from all rats were randomly chosen and sectioned. These sections were stained with Hematoxylin and Eosin (H&E) and examined using a light microscope.

3.7 After surgery care and follow up

The animals were monitored immediately postoperatively for spontaneous breathing effort and movement. After surgery, each animal was housed in an individual cage in a room and feed with standard rat diet and water.

The rats were humanely euthanized with an intraperitoneal injection of 5 mg phenobarbitone sodium.

Flow-chart of experiment protocol:

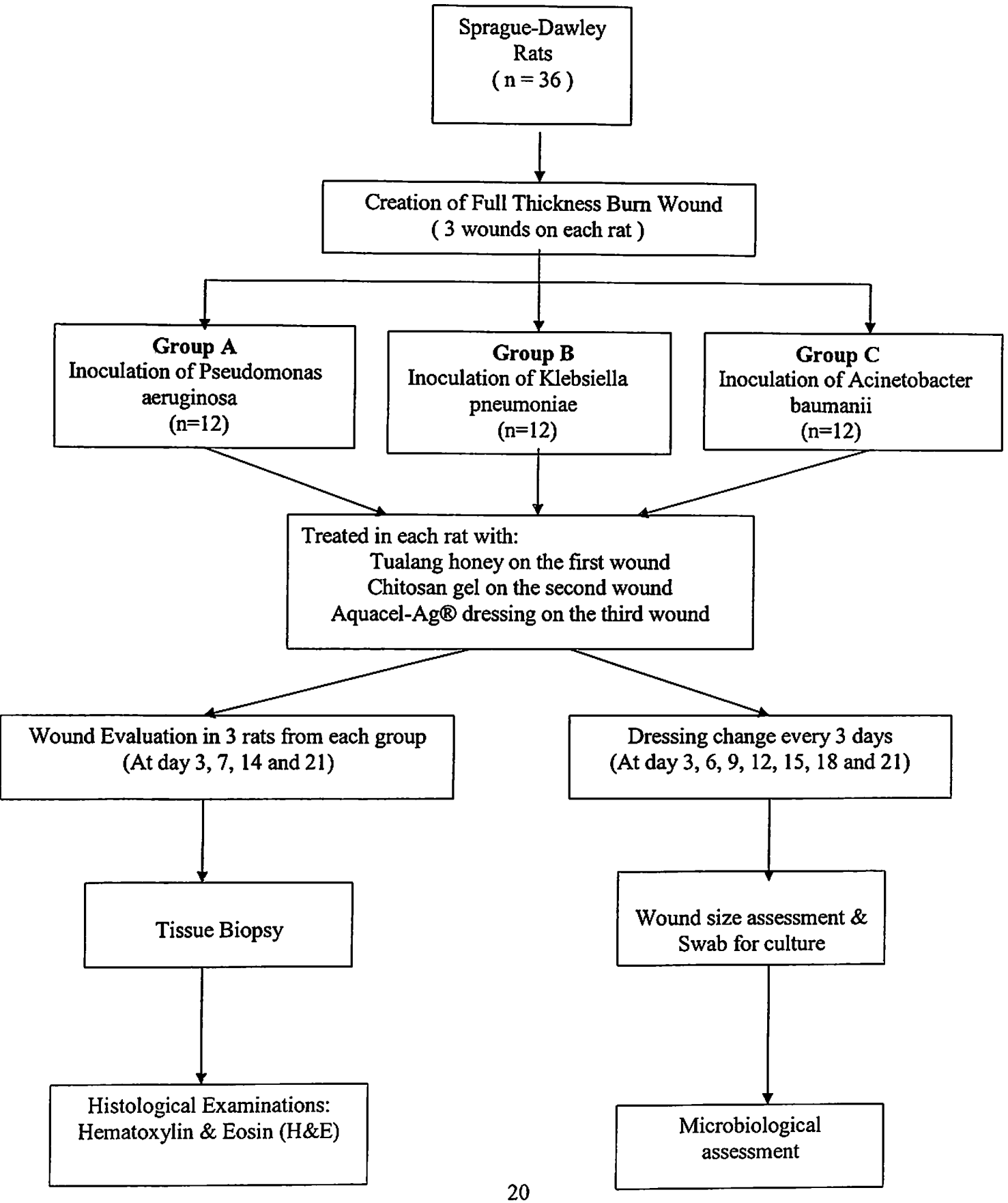

Figure 2: Modified metal screwdriver and blow torch used to create the burn wound.

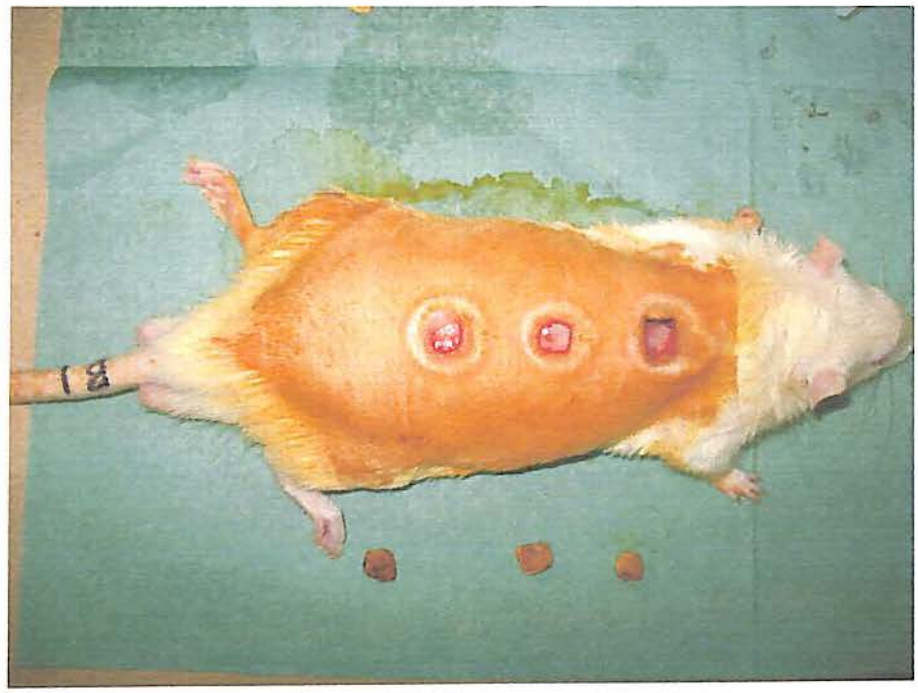

Figure 3: Creation of the full thickness burn wound.

Figure 4: Tualang Honey used for dressing.

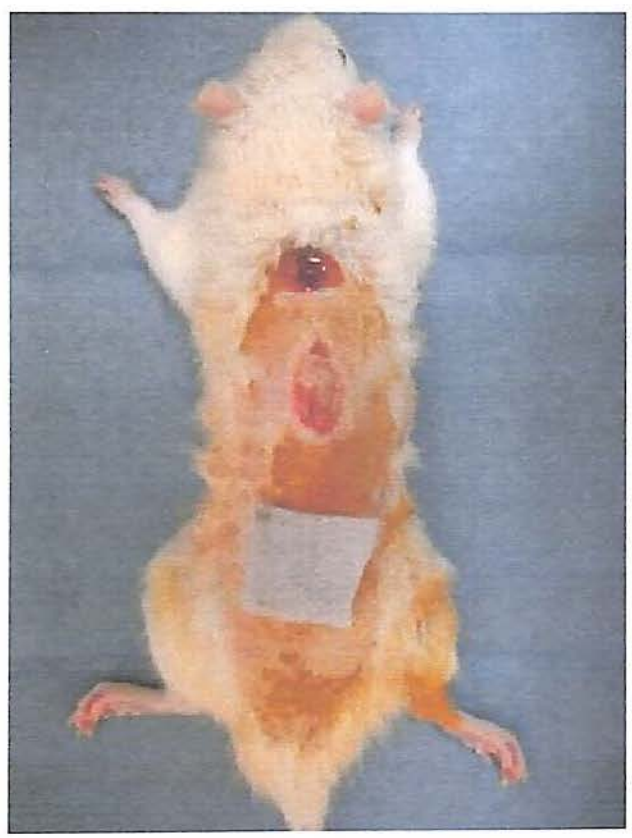

Figure 5: Application of treatments on burn wound.

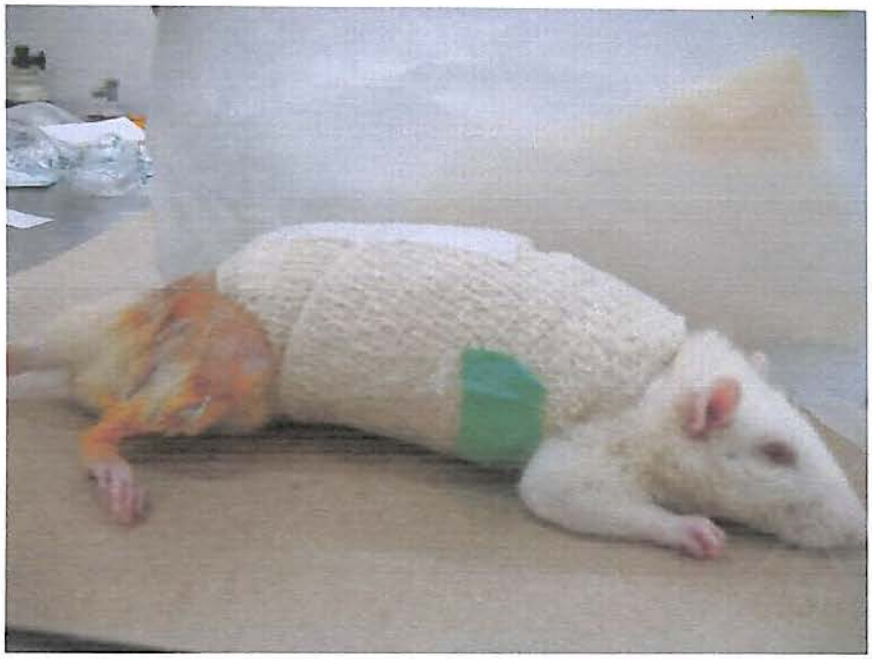

Figure 6: Complete dressing of the wound.

3.8 Outcomes measured

3.8.1 Evaluation of Wound Size

Evaluation of wound size is to assess the burn wound healing using Tualang honey in comparison with Chitosan gel and Aquacel-Ag® dressings. For wound size measurement, the wounds were traced on a transparency paper and the tracings were measured in mm^2 on day 3, 6, 9, 12, 15, 18 and 21 post-operations. The reduction in wound size was evaluated, reflecting the degree of wound contraction and expressed as a percentage of the created burn wound size ($100mm^2$).

3.8.2 Evaluation of microbiological assessment

Evaluation of microbiological assessment are important to determine the antimicrobial properties of Tualang honey on burn wound in comparison with Chitosan gel and Aquacel-Ag® dressings.

Swab for culture were taken during each dressing change on day 3, 6, 9, 12, 15 ,18 and 21 by cotton swab and consistency were maintained for all swab sampling and were transported in a suitable transport media to the Department of Medical Microbiology & Parasitology immediately after completion of study on that day.