UNIVERSITI SAINS MALAYSIA

EVALUATION OF THE WOUND HEALING IN FULL THICKNESS SKIN AUTOGRAFT WITH DIFFERENT DOSES OF TOPICAL *STICHOPUS SP1* EXTRACT IN SPRAGUE DAWLEY RATS

BY

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DECLARATION

This is to certify to the best of my knowledge, this dissertation is entirely the work of the candidate, Husnaida Abdul Manan@Sulong.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
°C	Degree Celsius
µm	micrometer
cm	centimeter
et al.	And others (Latin: *et alii)*
g	gram
H&E	Hematoxylin and Eosin
kg	kilogram
mg	milligram
ml	milliliter
mm	millimeter
w/w	weight per weight
rpm	revolution per minute
Gy	Gray
MV	Megavolts
°	degree
<	less than
>	More than
IQR	interquartile range
T/CM	Traditional and Complementary Medicine
WHO	World Health Organization

SCE	*Stichopus sp1* Extract
sp	*species*
PPSP	Pusat Pengajian Sains Perubatan
PPSK	Pusat Pengajian Sains Kesihatan
LARUSM	Laboratory Animal Research Unit Universiti Sains Malaysia
AEC	Animal Ethic Committee
IACUC	Institutional Animal Care and Use Committee
CRL	Central Research Lab
SPSS	Statistical Package for Social Sciences
PS	Power and Sample Size Software
FTSG	Full Thickness Skin Graf
STSG	Split Thickness Skin Graft
MOH	Ministry of Health

ABSTRAK

EVALUASI KE ATAS PEMULIHAN LUKA TERARUH AUTOGRAF KULIT KETEBALAN PENUH DENGAN DOS-DOS BERBEZA EKSTRAK TOPIKAL *STICHOPUS SP1* KE ATAS TIKUS SPRAGUE DAWLEY.

Pengenalan: Gamat merujuk kepada beberapa jenis timun laut yang seringkali diambil oleh rakyat di Malaysia sebagai ubatan tradisional. Dengan keupayaan gamat dalam penyembuhan luka yang telah diketahui setakat ini kesan topikal ekstrak gamat jenis *Stichopus sp1* ke atas pemulihan autograf boleh dibuktikan dan dosnya yang efektif boleh ditentukan. ***Objektif:*** Kesan topikal ekstrak gamat jenis *Stichopus sp1* pada kepekatan yang berbeza (iaitu 5, 10 dan 20 peratus berat per berat) diperhatikan ke atas pemulihan luka teraruh autograf kulit ketebalan penuh tikus betina Sprague Dawley secara makroskopik dan mikroskopik. ***Kaedah kajian:*** Kajian ini adalah uji klinik secara rawak (*randomized control trial*) dengan kerangka selari (*parallel design*). Haiwan-haiwan telah dibahagikan secara rawak kepada kumpulan kawalan, kumpulan rawat dos rendah (5 %), kumpulan rawat dos tengah (10 %) dan kumpulan rawat dos tinggi (20 %). Graf kulit berukuran 2 cm x 2 cm diambil dan disimpan pada suhu antara 6-8°Celcius selama lima hari. Sebanyak 0.5 ml ekstrak gamat dan/atau bes control diratakan pada lantai luka graf pada hari kosong dan hari kelima. Graf kulit kemudian ditransplantasikan pada lantai luka graf pada hari ke lima. Tujuh hari selepas transplantasi, graf diperhatikan secara makroskopik dan mikroskopik. ***Keputusan:*** Pemerhatian makroskopik menunjukkan tiada perbezaan ketara antara parameter yang

diuji (perlekatan graf, warna graf dan kelenturan graf: $p>0.05$ masing-masing) antara kumpulan rawat gamat dengan kumpulan kawalan. Pemerhatian mikroskopik semikuantitatif menunjukkan tiada perbezaan ketara dalam infiltrasi sel-sel inflamasi (neutrofil, makrofaj) dan proliferasi sel fibroblas dan salur darah baru graf antara kesemua kumpulan rawat tikus dengan kumpulan kawalan: $p>0.05$). Walaubagaimanapun, terdapat perbezaan yang ketara terhadap anjakan satah graf (*plane separation*) antara kumpulan kawalan dengan kumpulan tikus rawat dos tengah: $p<0.005$. Dalam setiap kumpulan tikus, ukuran berat badan menunjukkan perbezaan yang ketara dari hari kosong ke hari kelima. Tiada perbezaan yang ketara dalam setiap kumpulan tikus dilihat pada hari keenam ke hari kedua belas. ***Kesimpulan:*** Aplikasi ekstak gamat jenis *Stichopus sp1* (10 %) dilihat mempercepatkan penerimaan graf kulit oleh lantai graf seperti yang boleh dilihat pada pengurangan anjakan satah berikutan aplikasi ekstrak tersebut dalam kajian ini. Maka, ekstrak gamat *Stichopus sp1* didapati ada mempunyai kesan terhadap penyembuhan luka graf kulit tikus betina Sprague Dawley.

ABSTRACT

EVALUATION OF THE WOUND HEALING IN FULL THICKNESS SKIN AUTOGRAFT WITH DIFFERENT DOSES OF TOPICAL *STICHOPUS SP1* EXTRACT IN SPRAGUE DAWLEY RATS

Introduction: Gamat refers to a collection of sea cucumbers most commonly consumed as traditional remedies by Malaysians. With its known healing properties to date, the effect of topical gamat extract could be beneficial in the healing process of the skin grafts and the effective dose could be determined. ***Objectives:*** The aim of this study was to investigate the effect of topical gamat from the species *Stichopus sp1* at different concentrations (5 %, 10 % and 20 % w/w) on the healing process of skin autografts in female Sprague Dawley rats using macroscopic and microscopic parameters respectively. ***Method:*** This study was a randomized control trial with parallel design. Animals were randomly divided into control, low dose treated (5 %), medium dose treated (10 %) and high dose treated (20 %) groups. Dorsal full thickness skin sheets (2cm x 2cm) were harvested and were preserved between 6° to 8° Celsius for 5 days. 0.5 ml of topical substances (control and gamat extracts) was uniformly applied onto the graft wound beds at Day zero and Day five post-harvesting. Skin grafts were auto-transplanted at Day five and fixed with 5-0 silk interrupted sutures. Seven days post graft transplantation, the grafts were assessed macroscopically and microscopically. ***Results:*** The animal mortality rate observed in this study is 27.6 %. Macroscopic assessment showed that there were no significant difference, in term of graft adherence,

graft color and graft pliability between all gamat-treated groups and the controls. Semiquantitative microscopic assessment revealed that there were no significant difference in the infiltration of inflammatory cells (neutrophils, macrophages) and proliferation of fibroblasts and new blood vessels of the grafts between gamat-treated animals and the controls. However, there was a significant difference in term of graft plane separation between the controls and the medium dose treated group (10 %) ($P<0.005$). In all groups, the body weight showed significantly difference with declining pattern from Day zero to Day fivr post-harvesting ($P<0.05$). There were no significant differences within groups seen from Day six to Day twelve. ***Conclusion:*** Application of *Stichopus sp1* extract (10 % w/w) seemed to hasten the acceptance of the skin graft by the wound bed, as demonstrated by the reduction of the plane separation following the application of the extract. Therefore, *Stichopus sp1* extract does have an effect on the healing process of skin autografts in Sprague Dawley rats.