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ORIGINAL ARTICLE

Optimum Dose of Sea Cucumber (*Stichopus Chloronotus*) Extract for Wound Healing

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ABSTRAK

Di Malaysia, timun laut lebih dikenali sebagai Gamat. Di kalangan kaum Melayu, gamat sering digunakan sebagai ubat tradisional untuk melegakan kesakitan, merawat luka dan kesan terbakar. Ianya juga digunakan sebagai tonik untuk memberi sumber tenaga tambahan. Stichopus chloronotus merupakan salah satu spesies timun laut yang boleh didapati di Malaysia. Kajian ini bertujuan untuk menentukan dos optimum ekstrak akues Stichopus chloronotus emulsi salap ke atas luka pada model tikus. Beberapa siri kepekatan iaitu 0.1%, 0.5% dan 1% ekstrak akues Stichopus chloronotus emulsi salap diberikan ke atas luka eksisi sekali sehari selama 10 hari. Perubahan pada kawasan luka diukur dengan menggunakan angkup dan gambar luka diambil pada hari pertama, ke-3, ke-6, ke-8 dan ke-10 selepas pembentukan luka. Keputusan daripada peratusan pengurangan luka dan pemerhatian makroskopik akan menentukan dos optimum Stichopus chloronotus. Hasil kajian menunjukkan, kumpulan tikus kajian yang menerima rawatan Stichopus chloronotus 0.5% mempunyai peratusan pengurangan luka yang lebih tinggi dan pemerhatian makroskopik yang lebih baik bermula dari hari ke-6 selepas pembentukan luka berbanding kumpulan yang lain. Kesimpulannya, dos 0.5% merupakan kepekatan optimum bagi Stichopus chloronotus memberikan kesan kepada penyembuhan luka dan akan digunakan pada kajian sebenar.

Kata kunci: Stichopus chloronotus, timun laut, luka, penyembuhan luka, gamat

ABSTRACT

In Malaysia, sea cucumber is also known as Gamat. Among the Malays, Gamat is frequently used as a traditional or folk medicine to relieve pain, to treat wounds and burns. It is also used as a tonic to provide extra energy. *Stichopus chloronotus* is one of the species of sea cucumber which is found in Malaysia. An experimental study was carried out to determine the optimum dose of aqueous extract of *Stichopus*

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chloronotus on the healing of wounds in rat model. A series of concentrations 0.1%, 0.5% and 1% aqueous extract emulsifying ointment mixed with *Stichopus chloronotus* was applied once a day for 10 days on the excision wound model. Changes in the wound area were measured using a caliper and photographs were taken on day 1, 3, 6, 8 and 10 after the wound creation. The wound reduction rate and macroscopic observation were evaluated to determine the optimum dose concentration. Results demonstrated that percentage of wound reduction and macroscopic observation of 0.5% aqueous extract *Stichopus chloronotus* emulsifying ointment mixed group was significantly higher than the other groups from day 6 following wound creation. In conclusion, 0.5% aqueous extract of *Stichopus chloronotus* emulsifying ointment mixture demonstrated the best dose for wound healing in a rat model.

Keywords: *Stichopus chloronotus*, sea cucumber, wound, wound healing, gamat

INTRODUCTION

Wound is one of the most common form of morbidity, afflicting millions of individuals daily. Normal wound healing process involves three phases which are inflammation phase, proliferation phase and remodeling phase. Each phase is continuous and overlapping (Witte & Barbul 1997). The objective in wound management is to heal the wound in the shortest time possible, with minimal scarring and to reduce discomfort to the patient.

Sea cucumbers, belonging to the class Holothuroidea, are marine invertebrates. Generally, sea cucumber has soft, slimy body, mouth, an anus and tube feet. Sea cucumber has long been appreciated in Asia and Middle Eastfor nutritional and pharmacological value (Bordbr et al. 2011). While in China, sea cucumbers are health products that are very valuable and popular based on the value of health and delicious taste (Kiew & Don 2012). Even without scientific proof, sea cucumbers have long been known, particularly in Malaysia as a traditional food that is effective in treating hypertension, asthma, rheumatism, cut and burns (Chen 2003; Wen et al. 2010). The effectiveness of sea cucumbers in the therapeutic effect can be attributed to the presence of many bioactive substances such as glycoprotein, phenolic peptides and essential fatty acid. These compound provide a variety of benefits in terms of pharmaceutical and therapeutic health and does not cause side effects (Bordbr et al. 2011).

Stichopus chloronotus (Black Knobby or green fish) is one of the common species that can be found around indo-Pacific Ocean such as Taiwan, Singapore, Thailand and Malaysia. It is the second most common species found in Malaysia (Choo 2008). It has been reported that *Stichopus chloronotus* has composition of fatty acid which is important and play a key role in wound healing process (Fredalina et al. 1999). Therefore, we conducted a scientific evaluation of optimum dose

concentration of *Stichopus chlronotus* aqueous extract mixed with emulsifying ointment for wound healing.

MATERIALS AND METHODS

AQUEOUS EXTRACTION OF *STICHOPUS CHLORONOTUS*

The sea cucumber (*Stichopus chloronotus*) used in this study was collected from Bidong Island, Merang, Terengganu, Malaysia. The visceral organs of the animals were removed and kept in -70°C. Before extraction, the sea cucumber was thawed and washed using distilled water. The sea cucumber body was then dried in oven at 60°C until no change of mass. The method used is according to Fredalina et al. (1999) with minor modification. The dried sea cucumber was cut into smaller pieces and homogenized without water. Then, distilled water was added and soaked overnight. The filtered water was collected (flask A), and sea cucumber soak again at 4 hrs and followed by centrifugation at 3000 rpm for 20 mins. The resulted supernatant was collected (flask B). Both water in the flask A and B were mixed and stored at -20°C. After 24 hrs, the sample was lyophilized by freeze dryer to produce a powdery-like extract.

PREPARATION OF AQUEOUS EXTRACT EMULSIFYING OINTMENT OF STICHOPUS CHLORONOTUS

Total of 0.5 g of aqueous extract of *Stichopus chloronotus* powder was added on 99.5 g Cetamacrogol emulsifying ointment (paraffin ointment). The mixture was mixed well until homogenous. The mixture was applied topically to the wound area once daily.

ANIMAL HANDLING

Total of 12 male Sprague-Dawley rats weighing between 250-300 gm were used as experimental animals, supply from Laboratory Animals Resource Unit, Universiti Kebangsaan Malaysia. The rats were then divided randomly into 3 groups of control: Normal control group (NO), Positive control group (PC) and Negative control group (NC) and 3 groups of aqueous extract emulsifying ointment of *Stichopus chloronotus* (SC): SC 0.1%, SC 0.5% and SC 1.0%. NO group comprised non treated animals, PC group was animals treated with Flavine, NC group was animals treated with Cetamacrogol emulsifying ointment only.

After general anaesthesia, four round 6 mm diameter, full-thickness wound was made on the dorsal of each rat using disposable punch biopsy. Wounds were then treated daily with emulsifying ointment mixture and measurement of wound size were done on day 1, 3, 6, 8 and 10.

MACROSCOPIC OBSERVATION

Wound area reduction was measured using a calliper and photographs were taken. The percentage changes in wounds area was calculated using the following equation:

$$\text{Wound size reduction (\%)} = (W_0 - W_t) / W_0 \times 100$$

Where, W_0 =index wound area, W_t = wound area after time interval

Data were analysed using SPSS version 20. Results were presented as mean ± SEM. Mixed ANOVA and one-way ANOVA were used to analyse and compare data with $p < 0.05$ as the limit of significant.

This experiment was approved by the Animal Ethics Committee of the Faculty of Medicine of Universiti Kebangsaan Malaysia (FP/FAR/2013/ISA/20-MARCH/503-MARCH-2013-MAY-2014).

RESULTS

WOUND REDUCTION ANALYSIS

All treatments demonstrated a reduction in the wound at each time interval (Figure 1). However, the SC group demonstrated a reduction in wound more rapidly compared to other groups starting on day 6.

Using SPSS Mixed ANOVA analysis, there were significant differences between the percentage in wound reduction and time. This interaction was directly proportional to the percentage reduction of wound increased with increasing time intervals.

On day 6 after wound creation, wounds treated with 0.5% aqueous extract *Stichopus chloronotus* emulsifying ointment (SC 0.5%) demonstrated a significant wound reduction advantage when compared to all other groups ($p<0.05$). Figure 2, SC 0.5% shown significant difference in percentage of wound reduction on day 3 and day 6 when compared to normal control group (NO) and also on day 3 until day 8 after wound creation when compare to positive control group (PC).

Macroscopic observation also demonstrated the effects of SC 0.5% was better than the other groups starting on day 6 after wound creation (Figure 3).

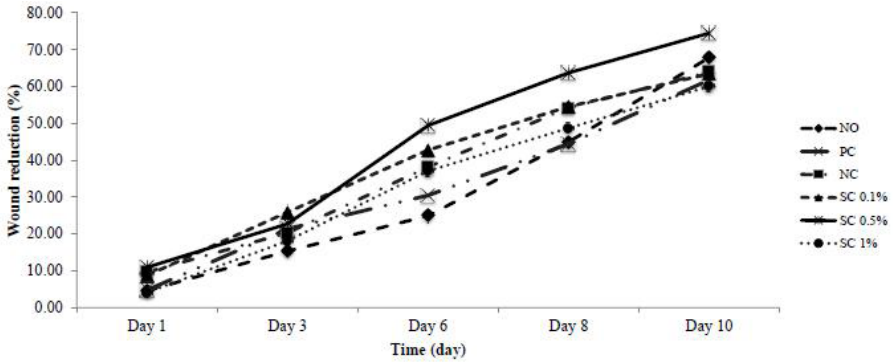

Figure 1: Figure showing the percentage in wound reduction versus time in day groups Normal (NO) - without treatment, Positive Control (PC) - Flavine, Negative Control (NC) - cetamacrogol emulsifying ointment, 0.1% aqueous extract *Stichopus chloronotus* emulsifying ointment. (SC 0.1%), 0.5% aqueous extract *Stichopus chloronotus* emulsifying ointment. (SC 0.5%) and 1.0% aqueous extract *Stichopus chloronotus* emulsifying ointment (SC 1.0%).

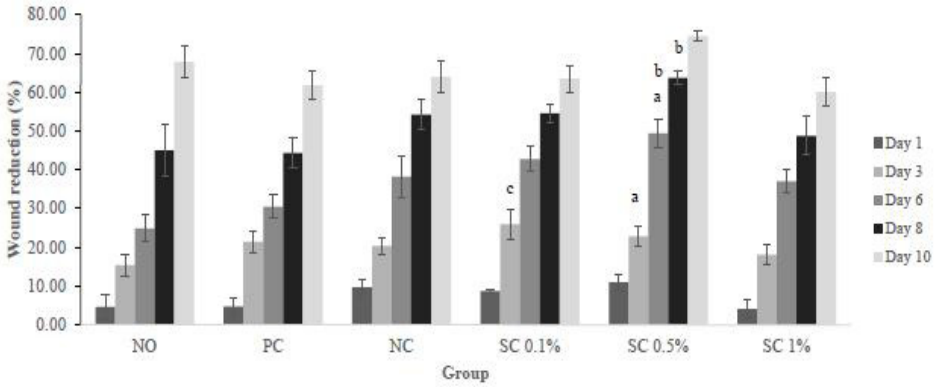

Figure 2: Figure showing the percentage in wound reduction versus time in day groups Normal (NO) - without treatment, Positive Control (PC) - Flavine, Negative Control (NC) - cetamacrogol emulsifying ointment, 0.1% aqueous extract *Stichopus chloronotus* emulsifying ointment. (SC 0.1%), 0.5% aqueous extract *stichopus chloronotus* emulsifying ointment. (SC 0.5%) and 1.0% aqueous extract *Stichopus chloronotus* emulsifying ointment. (SC 1.0%). Data are expressed as mean ± SEM (n = 8). a: indicate SC 0.5% significantly difference between NO group on day 3 andday 6 ($p < 0.05$), b-: indicate SC 0.5% significantly difference between PC group on day 6 and day 8 ($p < 0.05$), c: indicate SC 0.1% significantly difference between NO group on day 6 ($p < 0.05$)

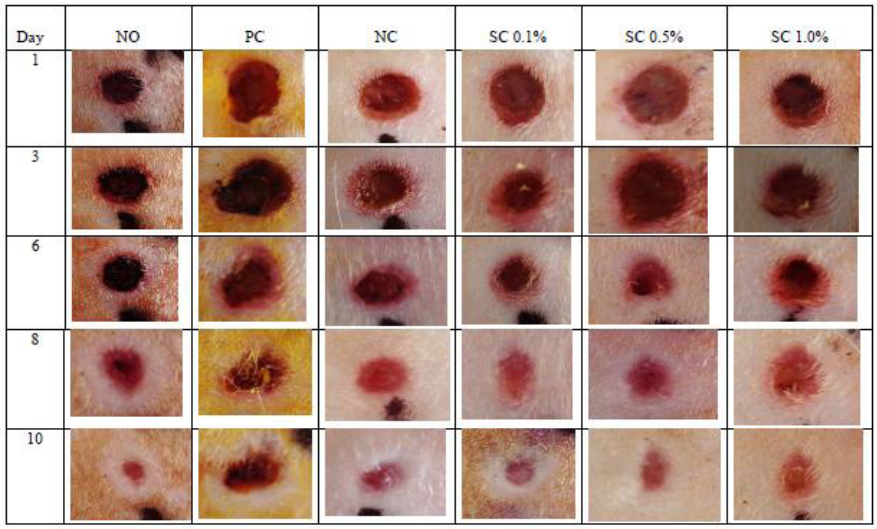

Figure 3: Photograph of the wound after day 1, day 3, day 6, day 8 and day 10 following wound creation.

DISCUSSION

Gamat has been used for many decades to heal minor wounds by local Malay population and this study attempts to validate that use. There are three basic mechanisms for wound healing: connective tissue matrix deposition, contraction and epithelialization (Diegelmann & Evans 2004).

Our results demonstrated clear wound healing advantage with wounds treated with aqueous extract

*Stichopus chloronotus*e emulsifying ointment mixture. Wound healing was significantly better when compared to gold standard for minor wound treatment like flavine. This effect could be due to the large number of therapeutic properties of sea cucumber, which includes antioxidant (Althunibat et al. 2009), antimicrobial (Kumar et al. 2007), anti-inflammatory (Bordbr et al. 2011), antiviral and other wound healing properties (Aydin et al. 2011; Fredalina et al. 1999).

In this study, we used aqueous extract of *Stichopus chloronotus*. According to Althunibat et al. (2009), aqueous extract of *Stichopus chloronotus* exhibited superior antioxidant activity compared to its organic extract by about 80%. The majority of sea cucumber antioxidant property was contributed by its hydrophilic components. According to Fredalina et al. (1999), fatty acid compound in *Stichopus chloronotus* plays an important role in wound healing process. The study also demonstrated that the fatty acids contained in the aqueous extract of *Stichopus chloronotus* are palmitic acid 2.2%, stearic acid 9.71%, linolenic acid 12.59%, oleic acid 7.50%, eicosapentaenoic acid 27.84, decosahexaenoic acid 57.55% & Arachidonic acid 1.46%.

Furthermore, Ridzwan et al. (2001) reported that methanol, ethanol and aqueous extract of *Stichopus chloronotus* showed positive anti-fungal effects. In another study using a different species of sea cucumber, *Holothuria polii*, both the methanolic and aqueous extracts showed anti-fungal activities. Based on these studies, it was concluded that both the aqueous and methanolic extracts of sea cucumber contained antimicrobial components (Fredalina et al. 1999; Ismail et al. 2008). The antimicrobial potential of sea cucumber extract can also be ascribed to the presence of steroidal sapogenins antimicrobial agents (Abraham et al. 2002). These antimicrobial effects of sea cucumber could reduced inflammation and infection to promote wound healing.

Another factor affecting wound healing is nutritional status. Carbohydrates and lipids are the primary source of energy in wound healing process. Glucose, the basic form of sea cucumber is rich in polyunsaturated fatty acids (PUFA) especially arachidonic acid (AA), the precursor of eicosanoids, which supports the growth and blood clotting processes to expedite wound healing. These nutrients are found in fresh sea cucumber, which contains moisture, protein, lipids, ash and carbohydrates. The compositions of these nutrients vary from 82.0% to 92.6%, 2.5% to 13.8%, 0.1% to 0.9%, 1.5% to 4.3% and 0.2% to 2.0%, respectively (Aydin et al. 2011). However, Chen (2003) demonstrated that fully dried sea cucumber may still contain protein as high as 83%. This all can contribute to the effectiveness of the *Stichopus chloronotus* in the wound healing process.

CONCLUSION

In conclusion, 0.5% aqueous extract of *Stichopus chloronotus* emulsifying ointment mixture demonstrated the

best concentration for wound healing in a rat model.

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REFERENCES

Abraham, T.J., Nagarajan, J., Shanmugam, S.A. 2002. Antimicrobial substances of potential biomedical importance from holothurian species. *Indian J Mar Sci* **31**: 161-4.

Althunibat, O.Y., Hashim, R.B., Taher, M., Daud, J.M., Ikeda, M.A., Zali, B.I. 2009. In Vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. *Eur J Sci Res* **37**(3): 376-87.

Aydın, M., Sevgili, H., Tufan, B., Emre, Y., Köse, S. 2011. Proximat composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *Int J Food Sci Technol* **46**(3): 500-8.

Bordbr, S., Anwar, F., Saari, N. 2011. High-value components and bioactives from sea cucumbers for functional food- A Review. *Mar Drugs* **9**(10): 1761-805.

Chen, J. 2003. Overview of sea cucumber farming and sea ranching practices in China. *SPC beche-de-mer Information Bulletin* **18:** 18-23.

Choo, P.S. 2008. Population status, fisheries and trade of sea cucumbers in Asia. In *Sea Cucumbers, a global review of fisheries and trade*. Edited by Toral-Granda, V. et al. FAO Fisheries and Aquaculture Technical Paper, 516; 81-118.

Diegelmann, R.F., Evans, M.C. 2004. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci* **9**(1): 283-9.

Fredalina, B.D., Ridzwan, B.H., Abidin, A.A., Kaswandi, M.A., Zaiton, H., Zali, I., Kittakoop, P., Jais, A.M. 1999. Fatty acid compositions in local sea cucumber. *Gen Pharmacol* **33**(4): 337-40.

Ismail, H., Lemriss, S., Aoun, Z.B., Mhadhebi, L., Dellai, A., Kacem, Y., Boirom, P., Bouraoui, A. 2008. Antifungal activity of aqueous and methanolic extracts from the Mediterranean sea cucumber, Holothuria polii. *J Mycol Med* **18**(1): 23-6.

Kiew, P.L., Don, M.M. 2012. Jewel of the seabed: Sea cucumbers as nutritional and drug candidates. *Int J Food Sci Nutr* **63**(5): 616-36.

Kumar, R., Chaturvedi, A.K., Shukla, P.K., Lakshmi, V. 2007. Antifungal activity in triterpene glycosides from the sea cucumber *Actinopyga lecanora*. *Bioorg Med Chem Lett* **17**(15): 4387-91.

Ridzwan, B.H., Zarina, M.Z., Kaswandi, M.A., Nadirah, M., Shamsuddin, A.F. 2001. The antinociceptive effects of extracts from Stichopus chloronotus Brandt. *Pak J Biol Sci* **4**: 244-6.

Wen, J., Hu, C., Fan, S. 2010. Chemical composition and nutritional quality of sea cucumbers. *J Sci Food Agric* **90**(14): 2469-74.

Witte, M.B., Barbul, A. 1997. General principles of wound healing. *Surg Clin North Am* **77**(3): 509-28.

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