Trad. Med. J., January-April 2023
Vol. 28(1), p 16-21
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 29-11-2022
Revised : 24-02-2023
Accepted : 03-04-2023

Skin Histopathology of Diabetes Mellitus Rats Treated with Edible Nest Swiftlets (*Aerodhramus fuciphagus*) Ointment

Dita Ayulia Dwi Sandi[1*], Yaumi Musfirah[2]
[1] Lambung Mangkurat University, Banjarbaru, South Borneo, Indonesia
[2] Borneo Lestari University, Banjarbaru, South Borneo, Indonesia

ABSTRACT

The edible nest swiftlets are suspected to contain *sialic acid* that can increase the process of cell growth that can reduce the formation of scar tissue and accelerate wound healing. This study aimed to determine the effect and the optimal dosage of edible nest swiftlets ointment on skin histopathology of diabetes mellitus rats that suffered a scratch wound. This study used 25 male white rats, divided into 5 groups, all groups were treated with betadine and the intervention of group 1 was vaseline only (control group), group 2, 3, and 4 were edible nest swiftlets (ENS) ointment with 10%, 20%, and 30% concentration and group 5 were the sanoskin as control positive. The rats were injured in the back area and given the treatment according to the group once a day for 14 days. Skin tissue was taken to make histopathologic preparations for observation on the 0th, 7th, and 14th days. The parameters observed included the number of macrophages, neo-capillarization, and fibroblasts. The result on the 14th day showed that the number of macrophages in the control group, sanoskin group, and ENS 10%, 20%, and 30% group was 3.8±5.019; 3.2±2.489; 1.84±2.387; 1.8±2.049 and 1.2±1.095. The result of neocapillilization were 1.28±1.673; 1.4±1.673; 2.8±1.778; 5.4±4.159; and 15±30.773. The conclusion showed there was a significant difference and there was the effect of edible nest swiftlets ointment on the number of macrophages, the number of neocapillilization, and the density of fibroblasts on the rats with diabetes mellitus that suffered a scratch wound.

Keywords: Edible nest swiftlets*; Aerodhramus fuciphagus*; skin histopathology; Ointment; wound-healing

INTRODUCTION

Indonesia is a country rich in natural resources and the Indonesian people can get care from nature. Edible nest swiftlets are a very popular commodity that is used for food or drinks. Edible nest swiftlets in China are delicious food and have many health benefits, especially for the prevention of aging, treating various diseases, and strengthening the body's resistance to diseases related to low blood pressure, high temperature, and other diseases (Trade Consul, 2016). The Chemical Content of edible nest swiftlets (*Aerodramus fuchipagus*) is a glycoprotein, carbohydrates, amino acids, and mineral salts which are thought to have a wound-healing effect. The main carbohydrates found on edible nest swiftlets are 10.8% N-acetylneuraminic acid, 4.19% galactosamine, 5.3% glucosamine, 5.03% galactose, and 0.44% fucose. Edible nest swiftlets originating from Indonesia contain greater Glucosamine and N-acetylneuraminic acid compared to those from Thailand and Vietnam (Tung, *et al.*, 2008).

One of the precursors of glycosaminoglycan is Glucosamine (Nakagawa *et al*., 2007. Glycosaminoglycan has an important role in wound healing because can regulate growth factors and cytokines (Rolfe & Grobbelaar, 2012). Kyung (2012) showed edible nest swiftlets extract can increase the proliferation of stem cells taken from human adipose tissue. Irma (2014) showed the wound healing effect of 10% edible nest swiftlets cream on mice, it can increase epithelialization and doesn't increase neovascularization after the fourth day of treatment. Sandi and Musfirah (2019) also showed 30% of edible nest swiftlets ointment has the optimal wound-healing effect in Alloxan-induced male rats.

Patients with diabetes mellitus often experience slow wound healing due to poor blood circulation in the extremities. The slow healing process of wounds, especially on the feet, often develops into diabetic ulcers. Nearly 14%-24% of patients with diabetic ulcers require amputation, which means that every 30 seconds a person's lower limb is lost due to diabetes. Diabetic ulcers are a major cause of morbidity and mortality in diabetics (DepKes RI, 2015).

With regards to the above-mentioned facts, the present study was, therefore, undertaken to evaluate the wound-healing effect of edible nest swiftlets (*Aerodramus fuciphagus*) on Diabetes

***Corresponding author : Dita Ayulia Dwi Sandi**
Email : dita.sandi@ulm.ac.id

Mellitus Rat skin. This research was a continued study, that aimed to know histopathology treated by Edible nest swiftlets (*Aerodramus fuciphagus*) Oinment to Diabetes Mellitus Rat's skin.

METHODOLOGY

Materials

This study used test animals namely *Sprague Dawley* Rats (150-250 g, ± 2,5 – 3 months). The materials were used edible nest swiftlets, Alcohol 70 % (Brataco®), Formalin solution (Brataco®), Vaseline alba (Brataco®), Hematoxylin-Eosin dye (Brataco®), Sanoskin Meladerm® (Interbet), Light microscope (Olympus SZ61), and Betadine® (PT. Mahakam Beta Farma).

Methods

Ointment Preparation

Edible nest swiftlets were made in absorption bases ointment. Absorption basic ointment and The edible nest swiftlets were prepared by Formula and standard method following Sandi and Musfirah (2018).

Histopathology studies

This research was approved by The Ethical Committee of Medical Research, Medical Faculty of Lambung Mangkurat University by Ethical Clearance Nu. 614/KEPK-FK UNLAM/EC/III/2018.

We utilized male SD rats weighing between 150 and 250 g. The rats were taken from the Islamic University of Indonesia. Five groups were created from the 10 rats. The rats were acclimated for a week and allowed unlimited access to pellets and water. Alloxan monohydrate (150 mg/Kgbw) was administered intraperitoneally to the rats after their blood glucose level had been determined by a glucometer after one week (normal blood glucose level). To determine whether the rats had diabetes, the blood glucose level was checked using a glucometer after two days. If the blood glucose level was more than 200 mg/dL, the rats developed diabetes (Tuhin et al., 2017). Rats have a typical blood sugar range of 60 to 150 mg/dl (Butler, 1995).

A wound was then formed on the rat's back skin after diabetes mellitus was established in the animals. The rear of the rat's hair was first shaved to a diameter of 3 cm. All test animals received the same course of treatment. The wound was created by cutting a 2 cm long, 2 mm deep incision using a sterile scalpel number 11. Thereafter, mice that have been hurt are handled following (Table I).

After creating the wound (day 0), the treatment was administered twice daily until the 14th day. 200 mg of the medication was administered by putting it on the wound. On days 0, 7, and 14, histopathological observations were made.

Skin tissue sampling was carried out on days 0, 7th, and 14th after being sacrificed by euthanasia. After that, The dorsal area was removed from the hair, the skin tissue was cut with a thickness of ± 3 mm to the subcutaneous layer and about ± 2 cm from the edge of the wound. The skin tissue was soaked with 10% formalin solution and was stored in a preparation pot. Histopathological preparations of skin tissue by Hematoxyllin-Eosin dye. Histopathological observations were made on skin tissue preparations using a light microscope (Olympus SZ61) at 400x magnification. These observations include parameters in wound healing such as the formation of new blood vessels (neo-capillarization), growth in connective tissue (fibroblasts), and the presence of inflammatory cells (macrophages) (Fitriani, 2016).

Data Analysis

The values are represented as mean ± SE and statistical sigSnificance between treated and control groups was analyzed using one-way analysis of variance (ANOVA).

RESULT AND DISCUSSION

This study was a follow-up study to determine the skin histopathological of diabetic rats with wounds by treating edible nest swiftlets ointment. Sandi & Musfirah (2019) proved that 30% edible nest swiftlets ointment was the most optimal for wound-healing in Alloxan-induced male rats when compared to Sanoskin Melladerm (positive control). Observation of histopathological preparations was carried out on day 0 when the wound was fresh or in the inflammatory stage. Inflammation is a defense reaction of living tissue against all forms of injury by involving the function of blood and blood vessels around the wound. Inflammatory reactions are usually followed by pain, heat, redness, swelling, and impaired function in the area around the wound. One of the stages of the inflammatory reaction is the movement of phagocytes, namely Chemotaxin, which is produced by complement around the wound, which will guide leukocytes, especially neutrophils and monocytes, go to the wound area. At the beginning of the inflammatory process, neutrophils will phagocytize rapidly and then die due to the presence of several microorganisms. Neutrophils are also capable of killing bacteria, fungi, and viruses. In the next stage, monocytes that have reached the wound area will turn into macrophages and replace the

Table I. Animal Groups

Kelompok + Betadine®	Konsentrasi (%)
Control	-
EBN	10
	20
	30
Sanoskin Melladerm Plus	20

Table II. Parameter assessment on day 0

Groups	Neocapilerization	Fibroblasts*	Macrophages
Control	0±0	+	71,2±29,320
EBN Cream 10%	0±0	+	70,3±28,320
EBN Cream 20%	0±0	+	72,6±29,220
EBN Cream 30%	0±0	+	70,9±27,310
Sanoskin Melladerm Cream	0±0	+	71,1±27,720

*** =** (+) low tight; (++) moderate tigh; (+++) tight; (++++) very tight

position of neutrophils to carry out phagocytosis (Asep, 2014).

The Observation was carried out descriptively using a light microscope (Olympus CX41) at a magnification of 40x to 400x to assess histopathological parameters, i.e formation of new blood vessels [neocapilerization], growth in connective tissue [fibroblasts] and presence of inflammatory cells [macrophages]) which play a role in wound healing. Neo-capillarization shows that many new blood vessels will develop into new branches in the wound tissue. Blood vessels have an important role in tissue repair to provide nutrition for regenerating tissues (Prasetyo *et a*l., 2010). Fibroblasts are cells in connective tissue that are influential in the wound-healing process. Fibroblasts will cause the edges of the wound to pull and come closer so that the two edges of the wound will stick together. As the wound-healing process continues, fibroblasts also increase (Napanggala *et al.,* 2014).

Based on the results of Table II, it was shown that the freshly injured rat skin was then viewed microscopically using a light microscope (Olympus CX41) showing macrophages spread with moderate density. This shows that on day 0 it has entered the inflammatory stage. The inflammatory stage occurs from day 0 to day 5 (Prabakti, 2005). Macrophages appeared after injury and reached their peak on the 3rd day. Macrophages are long-lived cellular elements and remain in the wound until the healing process is complete. Macrophages, like neutrophils, phagocytize and digest pathological organisms and tissue debris. Macrophages also release growth factors and cytokines that initiate and accelerate the formation of granulation tissue (Novriasyah, 2008).

On the 7th day of observation like Table III, there were still macrophages indicating that the inflammatory phase was still ongoing. However, on the 7th day, fibroblasts and neo-capillarization have started to form but are still not solid and perfect, this indicates that on the 7th day, they have entered the proliferative phase. The proliferative phase is marked by the formation of granulation tissue in the wound (Prabakti, 2005). Based on the results (Table III), showed that the wounds treated in the control group and the treatment group were still in the inflammatory stage but neo-capillarization and fibroblasts had already formed. The control group was still in the inflammatory stage where the inflammatory cells (macrophages) had a higher number (50.2 ± 31.507) than the sanoskin group (11.4 ± 7.162) and the EBN group, while the neo-capillarization (4.6 ± 3.847) and fibroblasts had fewer numbers than the sanoskin group (5.4 ± 6.65) and the EBN group. The sanoskin group, although there were still macrophages indicating that they were still in the inflammatory phase, were fewer in number (11.4 ± 7.162) than the control group (50.2 ± 31.507). Two other parameters, i.e neo-capillarization and fibroblasts in the sanoskin and EBN groups, there were more in number than the control group so they had entered the proliferative phase which was marked by the formation of neo-capillary and fibroblasts.

Based on Table IV, the results of observations on the 14th day, the injured tissue had entered the maturation stage, which was

Table III. Parameter assessment on day 7th

Groups	Neocapilerization	Macrophage	Fibroblasts*
Control	4,6±3,847	50,2±31,507	+
EBN Cream 10%	5,2±5,449	13±19,987	++
EBN Cream 20%	5,4±6,148	6±3,391	++
EBN Cream 30%	9±7,176	4,6±3,049	+
Sanoskin Melladerm Cream	5,4±6,65	11,4±7,162	++

*** =** (+) low tight; (++) moderate tigh; (+++) tight; (++++) very tight

Table IV. Parameter assessment on day 14th

Groups	Neocapilerization	Macrophage	Fibroblasts*
Control	1,28±1,673	3,8±5,019	+
EBN Cream 10%	2,8±1,778	1,84±2,387	++
EBN Cream 20%	5,4±4,159	1,8±2,049	++
EBN Cream 30%	15±30,773	1,2±1,095	+++
Sanoskin Melladerm Cream	1,4±1,673	2,2±2,489	++

*** =** (+) low tight; (++) moderate tigh; (+++) tight; (++++) very tight

characterized by the perfectly formed neo-capillary formation and a dense number of fibroblasts. The evaluation of the preparation parameters on the 14th day showed that the number of macrophages in the control group decreased (3.8 ± 5.019) compared to the 7th day of observation but still appeared to be more numerous than the sanoskin group (3.2 ± 2.489), the EBN group 10% (1.84±2.387), EBN 20% (1.8±2.049) and EBN 30% (1.2±1.095) on day 14.

The 10% EBN group had the same number of macrophages (1.84±2.387) as same as the 20% EBN group (1.8±2.049) and had a lower number of neo-capillarization (2.8±1.778) than the other EBN groups. In the 10% and 20% EBN groups, neo-capillarization was also seen which almost filled the blood vessel cell walls, whereas, in the 30% EBN group, there was a higher number of neo-capillarization (15 ± 30,773) and was fully formed, the number of densely packed fibroblasts and a small number of macrophages (1.2±1.095). This is the 30% EBN group indicated that wound-healing had entered the maturation phase. The maturation phase is marked by the formation of perfect neo-capillarization and the number of fibroblasts with dense density which can be seen in Figure 1.

Macrophages appear after the injury and reach a peak on the 3rd day. Macrophages are long-lived cellular elements and remain on the wound until the healing process. Macrophages, like neutrophils and phagocytes, are phagocytosis pathological organisms and tissue. Macrophages also release growth factors and cytokines that initiate and accelerate the formation of granulation tissue (Novriasyah, 2008). Neo-capillarization is new blood vessels that will develop on wound tissue. Blood vessels have an important role in tissue repair to provide nutritional intake for regenerating tissue (Prasetyo et al., 2010). Fibroblasts are cells in the connective tissue that influence the wound-healing process. Fibroblasts cause the wound edges will be attracted and closer, then both of the wound edges will stick. As the wound-healing process progresses, fibroblasts increase (Napanggala et al., 2014).

Histopathological evaluation of rat's skin wounds showed that edible nest swiftlets ointment had good effectiveness compared to a positive control (Sanoskin Melladerm). On day 14th, 30% EBN Ointment showed more neo-capillarization (15±30,773) and fully formed, tight fibroblast and fewer macrophages (1,2 ± 1,095). Tung *et. al.* (2008) showed that edible nest swiftlets (*Aerodramus fuciphagus*) contain sialic acid. Components of sialic acid in edible nest swiftlets consist of N-acetylneuraminic acid, galactosamine, galactose, and glucose. Sialic acid can accelerate wound healing which has a role in cell division or the formation of new cells (Irma, 2014). Edible nest swiftlets (*Aerodramus fuciphagus*) also contain growth factors. Gope (2007) showed that growth factor like EGF (Epidermal Growth Factor) plays an important role in wound healing in the process of re-epithelialization. Sanoskin Melladerm contains eco honey, glycerin, propylene glycol, and PEG 4000 which can stimulate the wound-healing process so that healing is faster and can increase the proliferation of fibroblast cells.

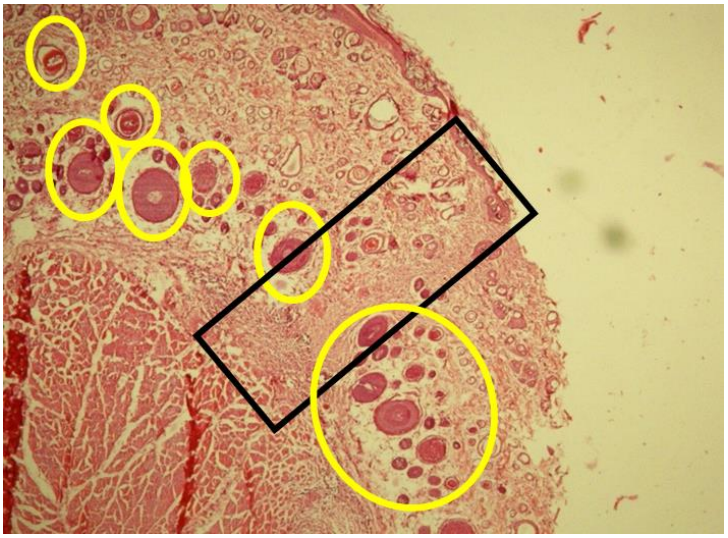

Figure 1. The perfect neo-capillarization and tight fibroblast

CONCLUSION

The conclusion of this research showed there was a significant difference (sig. <0.005) and there was the effect of edible nest swiftlets ointment giving on the number of macrophages, the number of neo-capillarization and density of fibroblasts on the Sprague Dawley rats with diabetes mellitus that suffered a scratch wound. Edible nest swiftlets ointment 30% can be a wound-healing alternative because had good effectiveness compared to the positive control and showed more neocapillarization and fully formed, tight fibroblast and fewer macrophages on day 14th.

ACKNOWLEDGEMENT

This research was funded by research grants from the Ministry of Research and Technology and Higher Education Republic of Indonesia (2018).

REFERENCES

Butler, L.K. 1995. Regulation of Blood Glucose Levels in Normal and Diabetic Rats. *Texas:* Division of Biological Sciences. 181-202.

Depkes RI. 2005. *Pharmaceutical Care untuk penyakit Diabetes Mellitus*. Departemen Kesehatan Republik Indonesia. Jakarta.

Fitriani, N. 2016. Uji Aktivitas Gel Etil P-Metoksinamat terhadap Penyembuhan Luka Terbuka pada Tikus Putih (*Rattus novegicus)* Jantan. *Skripsi*. UIN Syarif Hidayatullah, Jakarta.

Gope, M.L. & Gope R. 2007. Topically applied EGF and PDGFs affect positively the co-ordinate expression of EGF and PDGF receptor genes during the acute cutaneous wound-healing process. *Current Sciences.* Vol. 92, No. 5.

Irma. 2014. Pemberian krim ekstrak sarang burung walet 10% meningkatkan epitelisasi pada penyembuhan luka kulit mencit (*Mus musculus*). *Tesis*. Universitas Udayana Denpasar.

Kyung, B.R., *et al*. 2012. Mechanisms of Edible nest swiftlets Extract Induced Proliferation of Human Adipose-Derived Stem Cells. Evidence-Based Complementary and Alternative Medicine. Vol. 2012.

Konsul Perdangan. 2016. *Laporan Market Brief : edisi Januari 2016 Sarang Burung Walet di Pasar Hongkong.* Konsulat Jenderan Republik Indonesia Hongkong SAR. Republik Rakyat Tiongkok.

Nakagawa, H., Hama, Y., Sumi, T., Li, S., Maskos, K., Kalayanamitra, K., Mizumoto, S., Sugahara, K. & Teh, L. 2007. Occurrence of a nonsulfated chondroitin proteoglycan in the dried saliva of Collocalia swiftlets (edible bird's-nest). *Glycobiology.* 17 (2): 157–164.

Napanggala, Susianti, Apriliana, A. 2014. Pengaruh pemberian getah tanaman jarak pagar (*Jatropa curcas* L.) secara topikal terhadap tingkat kesembuhan luka iris pada tikus putih jantan galur *Sparague dawley*. ISSN 2337-3776.

Novriansyah, R. 2008. Perbedaan kepadatan

kolagen di sekitar luka insisi tikus wistar yang dibalut kasa konvensional dan penutup oklusif hidrokoloid selama 2 dan 14 hari. Semarang : Universita Diponegoro.

Prasetyo, B.F., Ietje, W., Bambang, P.P. 2010. Aktivitas Sediaan Gel Ekstrak Batang Pohon Pisang ambon dalam Proses penyembuhan luka pada mencit. *Jurnal Veteriner.* ISSN : 1411-8327. Vo. 11 No. 2:70-73.

Rolf, K.J. & Grobbelaar, A.O. 2012. A Review of fetal scarless healing. *ISRN Dermatology*. 2012: 1-9.

Sandi, D.A.D and Musfirah, Y. 2018. Pengaruh Basis Salep Hidrokarbon dan Basis Salep Serap terhadap Formulasi Salep Sarang Burung Walet Putih *(Aerodramus fuciphagus).* Jurnal Ilmiah Manuntung. 4 (2) : 149-155.

Sandi, D.A.D and Musfirah, Y. 2019. Wound-healing Effects of Edible nest swiftlets Oinment (Aerodramus fuciphagus) in Alloxan-Induced Male Rats. Traditional Medicine Journal. 24 (1): 33-39.

Tung, C.H., Pan, J.Q., Chang, H.M. & Chou, S.H. 2008. Authentic determination of Birds' nests by saccharides profile. *Journal of Food and Drug Analysis.* 16 (4): 86-91.