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Potential effect of striatin (DLBS0333), a bioactive protein fraction isolated from *Channa striata* for wound treatment

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ABSTRACT

Objective: To characterize proteins and other nutrients in striatin (DLBS0333), a bioactive protein fraction isolated from snakehead fish (*Channa striata*) and to investigate its wound healing activity.

Methods: Proteins and other constituents in striatin were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, two dimension electrophoresis, immunoblotting assay, spectroscopy and high performance liquid chromatography. The wound healing activity of striatin was studied *in vitro* using 3T3 fibroblast cells and *in vivo* using wound-induced animal model. Various parameters related to wound healing process were evaluated.

Results: Striatin contained four major bioactive proteins with approximate molecular weight of 8.3, 10.9, 15.4 and 16.7 kDa. In addition to proteins, striatin also contained amino acids (10 essential and 7 non essential amino acids), fatty acids (palmitic acid, oleic acid, stearic acid, linoleic acid, arachidonic acid), vitamins (vitamin A, vitamin B_6) and other nutrients (carbohydrate, dietary fiber, biotin, choline, inositol, L-carnitine, selenium) which are potential for wound healing and increasing serum albumin level. Treatment with striatin both *in vitro* and *in vivo* indicated that striatin enhanced cell proliferation. Wound-induced animal model treated with striatin showed significantly faster wound healing process, as confirmed by wound size and faster serum albumin level recovery. Although striatin does not contain albumin, this bioactive protein fraction may lead to enhanced albumin synthesis in the liver thereby maintaining the blood albumin level. Thus, consuming striatin is a better option to improve albumin levels in diseased condition and the condition of being injured.

Conclusions: Striatin (DLBS0333) is a potential natural compound for accelerating wound healing in conditions such as post surgery and post partum, and increasing albumin level.

1. Introduction

Channa striata (*C. striata*) is a medicinal freshwater fish originated from India but also found in several Asian countries and some places in Indonesia. This fish is known with various names based on the region, such as “Kutuk” (Java), “Kocolan” (Betawi), “Licingan” (Banjarmasin), “Bogo” (Sunda), “Kabos” (Minahasa), “Haruan” (Melayu). The snakehead fish is usually consumed as a traditional food rich in proteins, amino acids, fatty acids and other essential nutrients for energy booster during illness. Mothers after giving birth normally or by caesarean delivery are recommended to consume meals containing the snakehead fish. In addition, post-

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operative patients who need nutritional support are encouraged to consume *C. striata* to reduce pain and to accelerate the healing process [1,2]. *C. striata* has been reported to induce cell proliferation, platelet aggregation and show anti-nociceptive effect [3]. Amino acid in snakehead fish has been reported to play important role in the process of wound healing [1]. Glycine, glutamine and arginine are examples of amino acids in *C. striata* that are essential for wound healing. Glycine is one of the primary amino acids required for synthesis of collagen, the major protein in the connective tissue. Glutamine plays a key role in the stage of inflammation and proliferation step of wound healing, and also serves as a source of energy. Meanwhile arginine is known to stimulate wound healing by modulating immune function and affecting endothelial function [4].

Wound healing is a complex and dynamic process to restore injured cellular structures and injured tissue layers. There are four steps of wound healing process: homeostasis (activation of platelets which initiate the coagulation cascade), inflammation (neutrophils, macrophages and lymphocytes activation), proliferation (formation of new extracellular matrix) and remodeling (maturation of new matrix) [5]. These steps were run in proper sequence and precisely [4]. Wound healing process is affected by both local and systemic factors. Local factors impact directly on the wound, such as level of oxygen or microorganism which infected the wound. Systemic factors are related to individual health conditions that affect the wound healing ability, such as age, stress, disease and nutrition. Nutrition has been known as an important factor in wound healing for more than 100 years.

Hypoalbuminemia is always associated with the condition of post-operative patients, post-partum mothers or patients with severe wounds. However, the influence of albumin administration on systemic protein metabolism and wound healing is still unclear [6]. In fact, consuming nutricious food with high protein content will increase the level of serum albumin in those patients. Nutrition deficiencies impede the normal processes that allow progression through stages of wound healing [7]. Malnutrition has negative effects on wound healing through prolonged inflammatory and impaired proliferative phases [8].

Optimal wound healing process requires adequate nutrition, and protein is one of important nutrients for proper wound healing. Protein helps capillary formation, fibroblast proliferation, proteoglycan synthesis, collagen synthesis and wound remodeling [4]. Thus, it is important to increase protein intake to optimize healing and immune function. An adequate protein intake from fish is essential for good wound healing because of its quality [9]. The activity of *C. striata* in wound healing may be associated with its biochemical components, such as amino acids, fatty acids, arachidonic acid, polyunsaturated fatty acids, and docosahexaenoic acid [2,10].

In the present study, we characterized striatin, a bioactive protein fraction of *C. striata*. Potential activities of striatin were analyzed *in vitro* using 3T3 fibroblast cell for proliferation phase in wound healing and *in vivo* using Wistar rats for wound healing function.

2. Materials and methods

2.1. Striatin bioactive fraction

Striatin (DLBS0333) was isolated in Dexa Laboratories Biomolecular Science, PT Dexa Medica, from *C. striata* that was collected from West Java, Indonesia. Fish fillets were extracted using water based solvent, under several steps of fractionation, concentrating and drying.

2.2. Assays of striatin

2.2.1. Protein content analysis

Protein content of striatin was analyzed using method of Bradford [11]. Bovine serum albumin was used as a reference standard and the absorbance was measured at 595 nm using Thermo Scientific spectrophotometer.

The protein profiles of *C. striata* crude extract, striatin and other *C. striata* commercial product were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% gels concentration using low molecular weight marker (14.4–97.0 kDa), ultra low molecular weight marker (1.02–26.60 kDa) and bovine serum albumin as markers. Proteins were visualized by Coomassie brilliant blue R-250 [12].

2.2.2. Albumin analysis

Albumin in *C. striata* crude extract and striatin was detected by 2D gel electrophoresis and immunoblotting assay. The crude extract of *C. striata* or striatin was diluted in rehydration buffer (urea 8 mol/L, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate 2%, dithiothreitol 50 mmol/L, Bio-lyte 0.2% and bromocresol blue). Immobilized pH gradient strip (pH 3–10, 7 cm) was rehydrated by diluting striatin under passive condition for 16 h at room temperature. The first dimension step was isoelectric focusing. This process was run with starting voltage of 0 V, end voltage of 4 000 V, 10 000 V-hours, rapid ramp, and temperature of 20 °C. After the first dimension of electrophoresis, immobilized pH gradient strip was run on SDS-PAGE using 12% polyacrylamide gel and stained using Coomassie brilliant blue R-250. Bovine serum albumin was used as a positive control in this analysis.

Identification with immunoblotting assay was carried out by subjecting *C. striata* crude extract, striatin, other *C. striata* commercial product or human serum albumin (as a positive control) in 12% SDS-PAGE. The gel was used for electroblotting onto polyvinylidenedifluoride membrane. Albumin was visualized by immunostaining using 1:2 000 dilution of goat anti-albumin antibody for primary antibody, horseradish peroxidase-conjugated anti goat immunoglobulin G antibody for secondary antibody and a chemiluminescent substrate.

2.2.3. Amino acid analysis

The amino acids profile was analyzed using high performance liquid chromatography (HPLC). Striatin was injected into Waters AccQ.Tag amino acid analysis column (3.9 mm × 150 mm) and eluted with a mixture of AccQ.Tag eluent A, acetonitrile and HPLC grade water, based on the instruction manual of Waters AccQ.Tag Chemistry Package.

2.2.4. Fatty acid and nutrition content analysis

The content of fatty acids (palmitic acid, oleic acid, stearic acid, linoleic acid, arachidonic acid) and other nutrients (vitamin A, vitamin B_6, total carbohydrate, dietary fiber, biotin, choline, inositol, L-carnitine, selenium) in striatin were analyzed by PT. Saraswanti Indo Genetech, Bogor Indonesia.

2.3. In vitro study of striatin

3T3 Fibroblast cell of Swiss albino mouse was purchased from American Type Culture Collection (CCL-92) and maintained in Eagle's minimum essential medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2. The cells were plated into 96-well plate (3×10^4 cells/well), cultured in medium containing 10% fetal calf serum, and incubated for 24 h at 37 °C with 5% CO_2. Afterward, the cell medium was changed with serum-free medium. Then, cells were treated with 0–50 μg/mL striatin, and incubated for 24 h under similar condition. The viable cells were counted by using MTT [13]. MTT assay was performed by adding MTT reagent to each well and incubated for 2 h under similar condition. Absorbance was measured at 490 nm using Thermo Scientific spectrophotometer.

2.4. In vivo study of striatin

2.4.1. Animals

Six female Wistar albino rats weighing 250–350 g were used for *in vivo* study. Rats were caged individually in polysulfone cage and housed under standard condition (18–25 °C, relative humidity < 70%, light–dark cycle of 12 h:12 h). All procedures in this study have been reviewed and approved by Animal Care and Use Committee of Dexa Laboratories of Biomolecular Sciences with protocol number DOC-DLBS-BIOL-VVR-APF-008 and carried out in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.4.2. Excision of wound

The *in vivo* wound model was induced by excising the skin of rat [14,15]. Rats were anesthetized using ketamine (75 mg/kg, *i.m.*) and acepromazine (2.5 mg/kg, *i.m.*) before wound induction. The hairs on dorsal region were shaved, then the skin was excised up to the subcutaneous layer to obtain a wound area of about 150 mm^2 (3 cm × 0.5 cm). The rats were given flunixin (1.1 mg/kg, *s.c.*) as analgesic, two times a day for 5 days after the wound induction.

2.4.3. Effect of striatin on wound healing

Wounded rats were divided into two groups: control and treated group (striatin 5 mL/kg) with three rats in each group. The protein concentration of striatin was 20 mg/mL. The drugs were given two times a day orally for 15 days. Purified water was used as a vehicle. Then 1.7% (v/w) blood of each rat was taken every three days and the wound area was measured everyday by tracing the wound size using a millimeter block paper. The serum albumin level was measured by bromocresol green method. Data were expressed as mean ± SD. The differences between treated and control group were determined by student *t*-test using SPSS version 20 statistics software. All statistical tests were at 5% significance level.

3. Results

3.1. Assay results of striatin

3.1.1. Protein content analysis

Figure 1 shows the protein profile of *C. striata* crude extract which contained a lot of proteins, while striatin contained 4 major bio-active proteins with approximate molecular weight of 8.3, 10.9, 15.4 and 16.7 kDa, respectively. The total protein content of striatin was 214.81 mg/g. The figure also shows that the molecular weight of bovine serum albumin marker was around 66.0 kDa. However, there was no spot around 66.0 kDa in *C. striata* crude extract, striatin and other commercial product of *C. striata*.

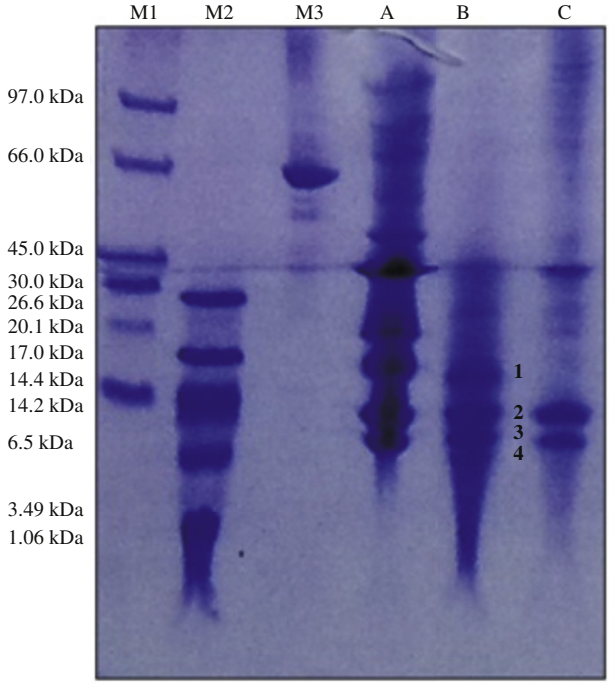

Figure 1. Proteins profile by SDS-PAGE.
M1: LMW marker; M2: ULMW marker; M3: Bovine serum albumin; A: *C. striata* crude extract; B: Striatin; C: Other *C. striata* commercial product.

3.1.2. Albumin content analysis

Identification of albumin content in *C. striata* crude extract and striatin was then carried out by 2D electrophoresis and western blot analysis. Based on 2D electrophoresis analysis, there was no spot of albumin in both *C. striata* crude extract or striatin gel around 66 kDa (Figure 2A,B), while the albumin marker showed positive spot at around 66 kDa (Figure 2C).

Identification of albumin content was also performed by human albumin immunoblotting using albumin antibody which reacted with human serum albumin. Figure 3 shows that there was no spot of albumin in *C. striata* crude extract, striatin and other *C. striata* commercial product.

3.1.3. Amino acid analysis

Striatin contained 21.84% (w/w) of total amino acids, namely, 10 essential and 7 non essential amino acids (Table 1). The profile of these amino acids is shown in Figure 4. L-cysteine, L-leucine, tryptophan, glutamin and L-arginine were five major amino acids with the highest level.

3.1.4. Fatty acid and nutrition content analysis

In addition to proteins and amino acids, striatin also contained unsaturated fatty acids [palmitic acid (124.31 mg/100 g), oleic acid (0.8 mg/100 g), stearic acid (33.95 mg/100 g), linoleic acid (5.24 mg/100 g), arachidonic acid (0.7 mg/100 g), docosahexaenoic acid (0.23–0.76 mg/100 g) and eicosapentaenoic

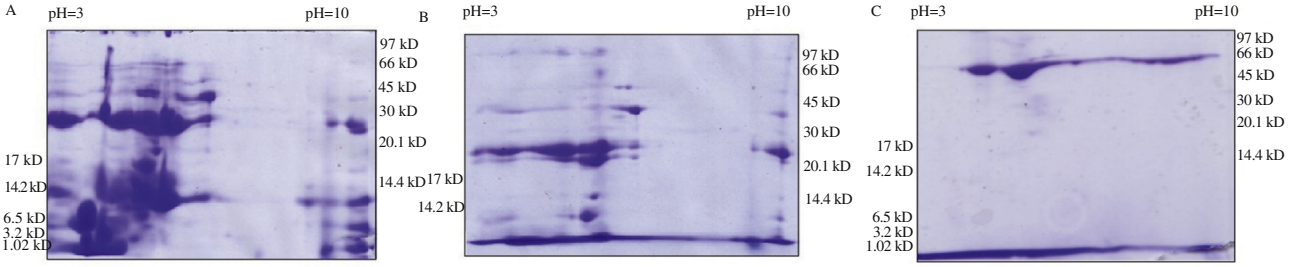

Figure 2. Coomassie blue stain of *C. striata* crude extract (A), striatin (B) and bovine serum albumin (C) from 2D gel electrophoresis.

Figure 3. Albumin immunoblotting.
1: *C. striata* crude extract; 2: Striatin; 3: Other *C. striata* commercial product; 4: Human serum albumin.

Table 1
Amino acid composition of striatin.

Amino acids		Amino acid content (%)
Essential amino acid	L-arginine	1.74
	L-histidine	0.78
	L-isoleucine	0.99
	L-leucine	2.01
	L-lysine	1.16
	L-methionine	1.07
	L-phenylalanine	1.64
	L-threonine	0.90
	Tryptophan	1.91
	L-valine	0.96
Non essential amino acid	L-alanine	0.81
	L-cysteine	2.26
	Glutamine	1.90
	Glycine	1.29
	L-proline	1.05
	L-serine	0.37
	L-tyrosine	1.00

acid (0.41–0.72 mg/100 g)], vitamins [vitamin A (27 μg/100 g), vitamin B_6 (72.18 μg/100 g)] and other nutrients [carbohydrate (36.88%), dietary fiber (33.2%), biotin (18.35 μg/100 g), choline (69.25 mg/100 g), inositol (38.69 mg/100 g), L-carnitine (27.96 mg/100 g) and selenium (20.85 μg/100 g)].

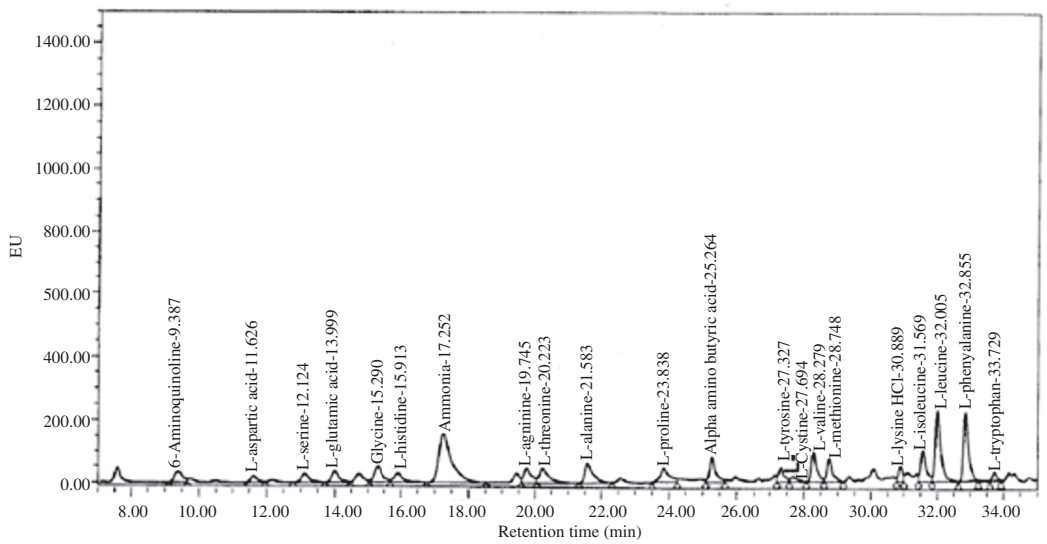

Figure 4. Amino acids profile of striatin by HPLC.

3.2. Activity of striatin

3.2.1. Striatin effect on the growth of 3T3 fibroblast cells

Figure 5 shows that striatin increased 3T3 cells growth in a dose dependent manner. At maximum dose of 50 μg/mL, the growth of 3T3 cells was almost double, compared to the control cells.

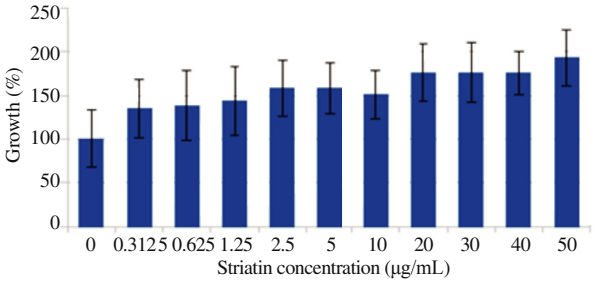

Figure 5. The effect of striatin on 3T3 cells growth as compared to untreated cell.

3.2.2. Striatin effect on wound healing

The potential effect of striatin was also evaluated in wound-induced animal model. Figure 6 shows the acceleration of wound area recovery indicated by wound area reduction.

Figure 8 shows the albumin level of both groups with 30% wound decreased. At the sixth day, the level of albumin in striatin treated group was significantly higher ($P < 0.05$) than the control group. Then at the ninth day, the level of albumin in this group increased significantly ($P < 0.05$). The decreased albumin content was completely recovered after 15 days.

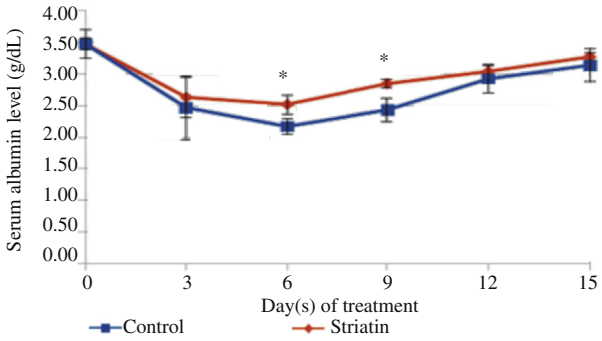

Figure 8. The effect of striatin on albumin level of wounded rats.

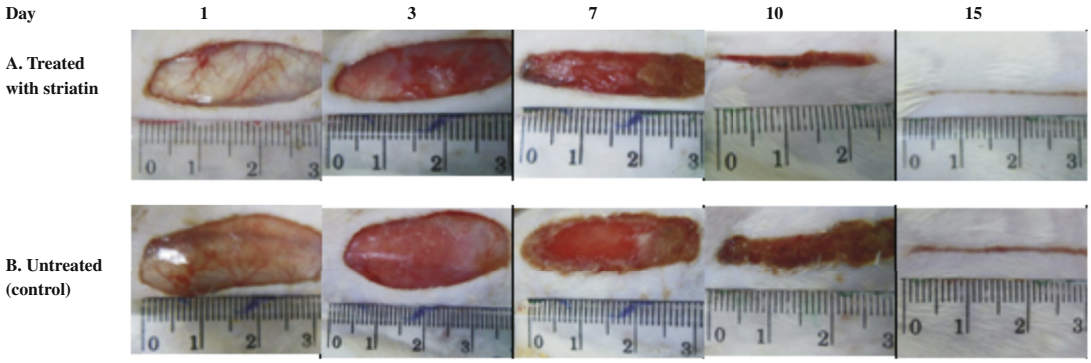

Figure 6. Macroscopic analysis of wound appearance on rat treated with striatin (A) and untreated/control (B).

This qualitative data was then confirmed with quantitative parameter by tracing the wound on a millimeter block paper (Figure 7). The wound area treated with striatin was reduced significantly after 8 days. The wide wound of treated group was significantly different from control group ($P < 0.05$).

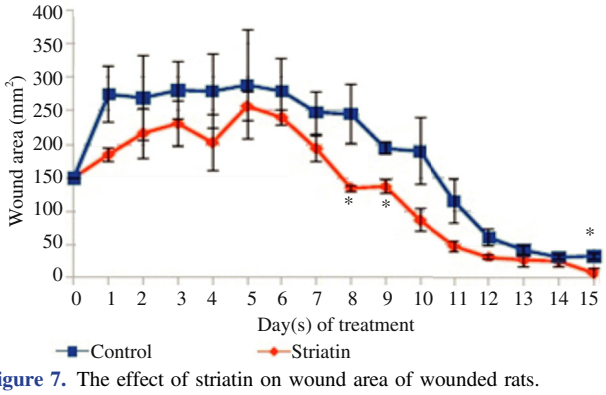

Figure 7. The effect of striatin on wound area of wounded rats.

4. Discussion

In the present study, adult *C. striata* was obtained from Indramayu, West Java (Indonesia) and the biological identity was confirmed at Research Center for Biology, Indonesia Institute of Science, Bogor, Indonesia. Fish fillet was frozen at −20 °C in plastic bag before transported to the laboratory. Extraction method of *C. striata* was selected to produce striatin (DLBS0333) fraction containing proteins, amino acids and other nutrients.

Striatin contained 214.81 mg/g protein that is important in the process of wound healing. Protein deficiency can cause impairment or delay in all stages of wound healing process by reducing immune function and lost of energy [4,16]. Wound healing process consumes energy, and during the stress response, proteins are also broken down to provide energy and to prevent loss of lean body mass [17]. Patients with low protein level indicate that they have high risk of delayed wound healing [6]. One of the vital components in collagen synthesis is also protein. Proteins in striatin are suitable for

fibroblast proliferation. Some proteins are associated with energy-producing metabolism. The presence of energy compound such as adenosine triphosphate in *C. striata* can decrease the healing time [10].

The presence of specific amino acids in striatin (21.84%) is also important in wound healing process. Arginine and glutamine are specific amino acids that are important in wound healing. Arginine plays a role in wound repair and recovery from stress. This amino acid is associated with more rapid wound healing and greater collagen synthesis [18]. In addition, arginine increases the cellular immune response and protein synthesis at the wound site, and has anti-bacterial activity. In the study on healthy people and surgical or intensive care unit patients, Kirk reported that arginine increased lymphocyte and monocyte proliferation and enhanced the formation of T-cell [19]. This substance also increases intestinal calcium absorption. In the case of trauma, the need of arginine increased five times from normal daily condition [20]. This is due to diminution of various growth factors. Arginine is one of various supplements that have been studied for the ability to enhance growth factors [21]. Glutamine is needed for fast growing and multiplying cells including white blood cell. This amino acid stimulates fibroblast proliferation for wound closure. Glutamine is also involved in the stage of inflammatory process and suggested via enhancement of immune function in individuals who are critically ill and immune suppressed; it can prevent infection in post-surgical patients as well. Glutamine supplement may speed up skin healing in severe burns or pressure ulcers due to its role in the inflammatory response, cell proliferation, and collagen formation [22].

Fatty acids play the chief role in cell membrane structure and function, including wound healing process, and implicate the synthesis of new cells. Twenty percent of calories are obtained from fat, especially from saturated and monounsaturated fatty acids such as palmitic acid and oleic acid. Striatin provides 235 mg/100 g unsaturated fatty acids to accelerate wound healing.

Carbohydrate is a rich source of cellular energy. It helps to meet the body's heightened energy requirements, aids in fibroblast movement, and enhances white blood cell activity to strengthen the immune response. The wound healing process requires substantial energy for repairment of cells and tissue disruptions. Thirty-three percent carbohydrate were contained in striatin.

The contents of vitamin A and B_6 in striatin were 27 and 72.18 μg/100 g, respectively. In wound healing process, vitamin A increases the strength of scar tissue, and it is required for adequate inflammatory response and used to counteract the catabolic effect of glucocorticosteroid [23]. Rabess reported that vitamin A supplementation contributed to enhancing immune response, promoting collagen synthesis and epithelialisation, and had antioxidant properties [24]. Vitamin B_6 is involved in the inflammatory response and participated in the conversion of tryptophan into niacin which helps the wound healing process. Vitamin B can affect wound healing in a number of ways for collagen linkage, ensuring a healthy immune system that is able to fight against infection. In addition, Vitamin B is required for protein synthesis and formation of red blood cells which supply the wound with oxygen and nutrients [25–27].

Due to active components present in striatin as described previously, striatin can improve the healing of wound. To confirm this statement, fibroblast cells (3T3 cells) were used to check the effect of striatin in the proliferation phase of the healing process.

We used 3T3 cells (mouse fibroblast cells) to evaluate the activity of striatin especially in cell proliferation phase. During wound healing process, much more energy is needed to repair cell and tissue disruptions. Based on assay results, striatin promotes cell growth in a dose dependent manner, which is a critical process in patient's recovery. The ability of striatin in inducing wound healing is suggested through ability to increase the growth of fibroblast cells. Fibroblast proliferation is important in formation of granulation tissue needed for wound closure [28,29]. Fibroblast growth is related to neo-angiogenesis and extracellular matrix secretion needed for cell ingrowth and tissue development, and production of some cytokines and growth factors [29–32].

To confirm *in vitro* data, the effect of striatin was also evaluated in wound-induced animal model. The present study revealed the acceleration of wound recovery indicated by wound area reduction. Based on assay results, the recovery progress of wound in group treated with striatin was faster than untreated group. Ten days after the treatment, wound of treated group was smaller than that of control. This rapid wound recovery was suggested mainly via cell proliferation enhancement which further facilitated the formation of granulation tissue [28]. This effect was clearly observed and related to previous report that the granulation tissue formation in wound occurred around four days after the injury [33].

In addition to visual observation of wound healing area, we also measured serum albumin level by bromocresol green method. The level of albumin during the wound healing process in the group treated with striatin increased significantly compared to the control group. It might be due to the potency of striatin, a non-albumin protein from *C. striata*, contributing to acceleration of albumin synthesis. Actually, if the level of albumin decreased, normally, the rate of albumin synthesis will increase automatically [34]; however protein administration will help to accelerate serum albumin level [35]. In addition to hormones synthesis, stress, colloid osmotic pressure and presence of tumor, nutrition is also contributing to improving albumin synthesis [36]. Striatin contains good quality proteins due to the presence of essential amino acids and other nutrients which balanced the hypoalbuminemia condition induced by the wound. The constituents like non-albumin proteins, amino acids and other nutrients seem to contribute to the albumin level enhancement in the hypoalbuminemia condition which in turn accelerates the process of wound healing. Our data indicated that when the wound was healed, shown by wound closure (on Day 15), the serum albumin was remained constant at normal level. Our result is in line with Kaysen *et al.* who described the mechanism of action of protein to improve the albumin synthesis [37]. In hypoalbuminemia patients or when protein intake is too low or in bad quality, muscles and fat break down to supply amino acids needed for the body. In this case, striatin functions as proteins, amino acids and other nutrients which maintain the albumin level in the body.

Striatin (DLBS0333) is a potential natural compound for accelerating wound healing in conditions such as post surgery and post partum, and increasing albumin level.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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