Activity of Sea Cucumber and Spirulina in Wound Healing and Blood Glucose Decreasing in Streptozotocin- Induced Diabetic Rats.

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Abstract. Previous study showed that sea cucumber and spirulina had an antioxidant activity and antihyperglycemic activity. Sea cucumbers contain a cell growth factor that is useful in wound healing. This research aimed to determine the activity of sea cucumber and spirulina in decreasing the fasting blood glucose and wound healing process in streptozotocin-induced diabetic rats. The antihyperglycemic activity and wound healing test were done to experimental rats that have been conditioned with diabetic wound. 2840 mg/kg body weight of liquid preparation of sea cucumber methanol extract and 81 mg/kg body weight of spirulina extract were given through oral gavage and sea cucumber methanol extract was given topically once a day. The results showed that group of golden sea cucumber orally-topically had the best antihyperglycemic activity with the highest percentage reduction in blood glucose level while group of golden sea cucumber topically-spirulina orally had the best wound healing effect with the highest percentage of wound healing.

1 Introduction

Diabetes mellitus (DM) is a metabolic disease characterised by chronic hyperglycemia (postprandial glucose levels reach ≥200 mg/dL). There are three types of DM, one of the classification was DM type 1 which is generally experienced by children and adolescents because the pancreas is unable or only produces little insulin, so daily insulin therapy is required according to doctor's recommendations, while DM type 2 is more commonly experienced by adults and accounts for ± 90% of diabetes mellitus. The total cases of DM was caused by an unhealthy lifestyle so that the body is unable to properly use the insulin to metabolise the glucose intake [1]. Based on the results of Basic Health Research [2], the risk factors for type 2 diabetes mellitus are consumption patterns of sweet foods with a prevalence of 47.8% (1–6 times per week) and consumption of sweet drinks with a prevalence of 61.3%

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(> 1 time per day), in addition the prevalence of physical activity which only reaches 33.5% (less than 66.5%) can also cause blood sugar not to be controlled properly. The International Diabetes Federation predicts an increase in diabetes mellitus patients in the age range of 20–79 years in Indonesia from 19.5 million people (2021) to 28.6 million people (2045). Riskesdas results also show an increase in the number of patients aged ≥15 years with a peak of sufferers aged 55-64 years from 6.9% (2013) to 8.5% (2018). Diabetic wounds are the main complication of diabetes, 5-24% of patients with diabetic foot wounds lead to amputation [3]. There are three important components in the management of diabetic wounds, namely metabolic control, wound control and infection control in addition to vascular control, pressure control and counselling. Treatment of diabetic wounds with indications of contamination/infection was commonly treated with oral, parenteral and topical antibiotic therapy, according to the severity of the infection. Many natural ingredients are known to have the potential to be used as a medicine for diabetic wounds, including sea cucumbers and spirulina. Drug preparations or sea cucumber supplements are generally marketed in liquid and dry forms. *In vivo* research on experimental white rats for 14 days of treatment showed that liquid sea cucumber preparations resulting from heating sea cucumber meat which is commonly used in the community and powdered spirulina have antioxidant activity by significantly reducing liver malondialdehyde levels [4] can also reduce blood glucose levels although the percentage decrease was not significant, namely only 3.36% for the sea cucumber-feeding and spirulina-feeding treatment of 6.8% [5]. Therefore, it is necessary to conduct research on the activity of these two ingredients in reducing blood glucose levels in diabetic white rats by extending the treatment time to 21 days and healing the wounds of diabetic white rats using topical preparations derived from methanol extraction of liquid sea cucumber preparations resulting from heating sea cucumber meat.

2 Research method

2.1 Materials

The materials used in this study were golden sea cucumber (*Stichopus hermanni*) from Labuan Bajo, Flores waters, East Nusa Tenggara, gama sea cucumber (Stichopus variegatus) from Lampung waters, spirulina powder (*Spirulina platensis*) from Jepara, Central Java, all three were obtained from PKSPL-IPB, white rats Sprague Dawley strain (44-100g) 5 weeks old obtained from BPOM RI, streptozotocin (Sigma Aldrich), glibenclamide (Indofarma), SanoSkin OXY (Sanomed), citric acid p.a. (Merck), sodium citrate p.a. (Merck), formaldehyde 40% w/v (Merck), NaH2PO4.2H2O p.a. (Merck), anhydrous Na2HPO4 p.a. (Merck), hematoxylin and eosin staining reagents as well as Masson Trichrome, levofloxacin (Novell), Sagestam/Gentamicin sulphate cream (Sanbe), and NaCl p.a (Merck).

The equipment used was a freeze dry tool (EYELA FDU 1200), rotary evaporator, analytical balance (Shimadzu), glucometer (GlukoDr®, Korea), scalpel, oral probe, glucometer (GlukoDr®, Korea), microscope, digital camera (Casio EX-ZS6), scaled paper (Cutimed), autoclave (All America 50x), vortex (Boeco), micropipette (Socorex), and glassware.

Experimental animals that were not induced by streptozotocin were grouped as a normal control group which would only be force-fed with distilled water for 21 days of treatment. The hyperglycemic experimental animals were grouped into 6 groups, namely the aquadest-feeding group as a diabetic wound control group, the commercial drug group namely Glibenclamide and SanoSkin OXY dressings, the gold-feeding and sea-cucumber dressing group, the group-feeding and gama sea cucumber dressing, the spirulina-feeding group and golden sea cucumber dressing and spirulina and gama sea cucumber dressing group. Wounds were made on the back of all experimental animals, the day after they were determined to be in diabetes. The feeding and topical treatment is carried out once a day. Feeding is carried out using an oral sonde according to a predetermined dose, while topical treatment is carried out by applying a thin layer of the drug to the entire surface of the wound that has been cleaned of scabs and necrotic tissue with 0.85% saline solution.

2.2 Research design

A total of 30 experimental animals with body weight of 215-221 g were conditioned by streptozotocin-induced diabetes following [6]. Streptozotocin was dissolved in a fresh citrate buffer pH 4.0 with a concentration of 1 mg/mL. Streptozotocin was injected intraperitoneally using a 1 mL syringe at a dose of 50 mg/kg BW. Before being induced by streptozotocin, the experimental animals' fasting blood glucose was measured using a glucometer. Furthermore, the experimental animals were left for 48 hours and still given food and drink with ad libitum [7]. Fasting blood glucose measurement is performed to determine the condition of hyperglycemia (fasting blood glucose ≥ 200 mg/dL).

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2.3 Data analysis

Data analysis was performed by ANOVA test using MINITAB 16 software to see comparisons between different groups or observation days, while to see significant differences between treatment groups or observation days an analysis was performed using *Tukey t-test*.

3 Results and discussion

3.1 Weight and feed consumption

The average body weight of the rats during the experiment is presented in Figure 1. Feed consumption during the experiment is presented in Figure 5. The increase in body weight was significant and the value was significantly different ($p<0.05$) for all groups during the three weeks of adaptation period indicating that the rats did not experience stress and were able to adjust with a new environment. Stress levels in experimental rats are one of the factors that influence body weight and food intake (Jeong 2013). As stated with [5], to avoid mortality, the body weight of experimental rats that could possibbly induced by streptozotocin must be above 200 g. Induction of streptozotocin at a dose of 45-75 mg/kg BW intraperitoneally was carried out on experimental rats weighing 140-300 g.

After the wound was made until the end of treatment, the body weight of the normal wound group was not significantly different ($p<0.05$) from the day before the wound was made, indicating that there was an influence of the wound on the body weight of the experimental animals. [6] and [5] reported that white mice that were not induced by streptozotocin and were only fed 2 mL of distilled water experienced an increase in body weight until the 14th day of treatment. Different things happened in the diabetic group that was injured, body weight decreased after streptozotocin induction and Making wounds until the 14th day showed streptozotocin induction which resulted in hyperglycemia causing a decrease in the body weight of experimental mice. According to [8] decreasing body weight is one of the classic symptoms of diabetes mellitus sufferers apart from polyuria, polydipsia and polyphagia.

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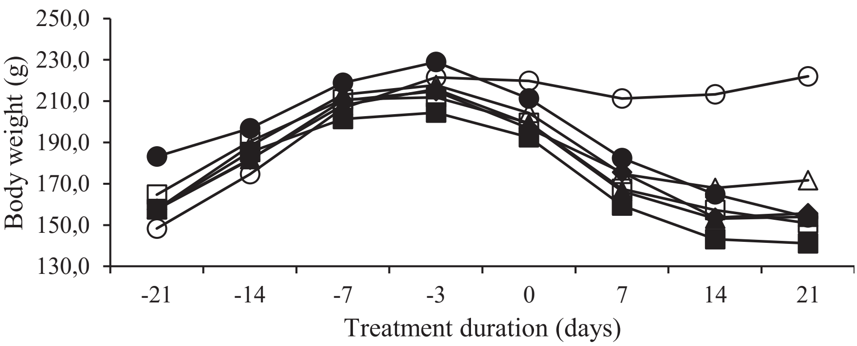

Fig. 1. Body weight of rats during the experiment. Normal wound control, Diabetic wound control, commercial drug rubs, golden sea cucumber bast, gama sea cucumber rubs, spirulina rubs gold sea cucumbers, spirulina gama sea cucumbers rubs. Day-(-3): the day before streptozotocin induction. Day 0: wound creation (day after confirmation of diabetes).

The differences in the body weight patterns of diabetic experimental rats on day 21 where the spirulina and commercial drug-feeding groups and gama sea cucumbers were not significantly different ($p <0.05$) from day 14, while the golden sea cucumbers fed group tended to decrease, presumably due to the effect of the force-feeding. The type of force-feeding given is also thought to result in a different response to body weight. This also happened along with the increase in the amount of feed consumption as well as changes in diabetes status in this group. The increase in feed consumption and fasting blood glucose levels <200 mg/dL indicated that there had been an improvement in the body metabolism of the experimental rats which resulted in an increase in the body weight of the experimental rats. In addition to the type of force-feeding given, the duration of the force-feeding treatment also affected the trend of increasing body weight in experimental rats. The increase in the body weight of the experimental rats from the adaptation period to the 14th day in the group that was fed gold sea cucumber and spirulina preparations had the same pattern as [5] who fed them with the same preparations and for the same duration of time. The addition of the feeding time to 21 days changed the trend of the body weight of the experimental rats.

The feed consumption of the commercial drug group, the gama sea cucumber-feeding group and the golden sea cucumber spirulina-feeding group had a different pattern from the control group (Figure 2). The commercial drug group experienced a decrease until the 14th day of treatment and the value of each observation was significantly different ($p<0.05$) from feed consumption 7 days before wounding, then on day 21 the value was not significantly different ($p<0.05$) from day 14th. The gama sea cucumber-feeding group experienced a decrease until the end of the study and all values were significantly different ($p<0.05$) with feed consumption 7 days before wounding. The group of golden sea cucumbers, the group of gold sea cucumbers and the group of gama spirulina and sea cucumbers experienced a decrease until the 14th day of treatment and the value of each observation was significantly different ($p <0.05$) with feed consumption 7 days before the wound was made. subsequently increased and the values were significantly different ($p<0.05$).

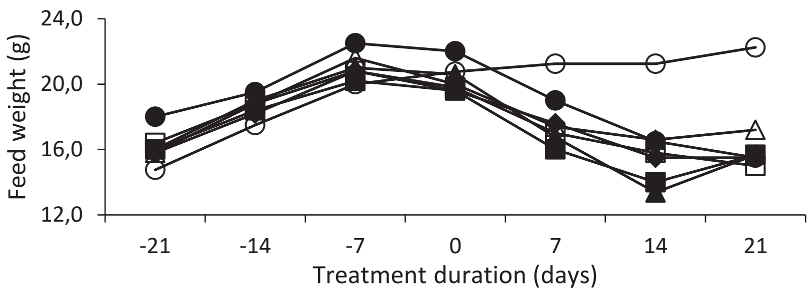

Fig. 2. rats feed consumption during the experiment. Normal control, Diabetic wound control, Commercial medicinal rubs, Gold sea cucumber rubs, Gama sea cucumber rubs, Spirulina rubs gold sea cucumbers, spirulina rubs Gama sea cucumbers. Day 0: wound creation (day after confirmation of diabetes)

3.2 Blood glucose levels

Blood glucose level during fasting is one of the criterias for diagnosing diabetes mellitus in addition to plasma glucose levels 2 hours after the OGTT (Oral Glucose Tolerance Test) and HbA1c levels. The normal value of fasting blood glucose levels in humans is < 100 mg/dL and diabetic conditions are if you have fasting blood glucose levels ≥ 126 mg/dL [8], whereas in rodents, normal values fasting blood glucose < 199 mg/dL, prediabetes 200-249 mg/dL and diabetes criteria are established if fasting blood glucose levels ≥ 240 mg/dL (Fajardo et al. 2014).

The results of observations of fasting blood glucose are presented in Figure 3. On the 2nd day of measurement after streptozotocin induction, all diabetic groups had fasting blood glucose levels above 335 mg/dL, increased significantly and significantly different ($p<0.05$) from before streptozotocin induction who have fasting blood glucose levels below 100 mg/dL. The normal wound control group had relatively stable fasting blood glucose levels during the treatment, namely 88.5-116.0 mg/dL although there was a slight decrease on days 3, 7 and 14 and an increase on day 21 with significantly different values ($p < 0.05$) by day 2 after streptozotocin induction. The diabetic wound control group had fasting blood glucose during treatment above 270 mg/dL, which ranged from 273.0 to 349.0 mg/dL and the value was not significantly different ($p<0.05$) with that after streptozotocin induction.

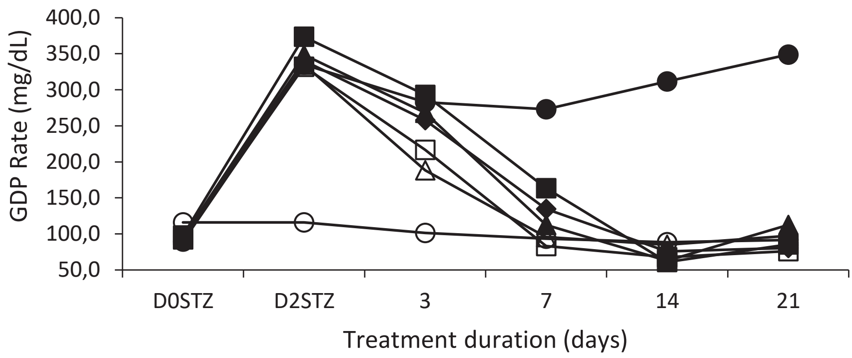

Fig. 3. Blood glucose levels of experimental rats. Profile of rat blood glucose levels. Normal wound control, Diabetic wound control, commercial drug rubs, golden sea cucumber rubs, gama sea cucumber rubs, spirulina rubs gold sea cucumbers, spirulina gama sea cucumbers rubs. spirulina gama sea cucumbers rubs. (D0STZ: before streptozotocin injection, D2STZ: 48 hours after streptozotocin injection; D0STZ: sebelum injeksi streptozotosin, D2STZ: 48 jam sesudah injeksi streptozotosin).

The commercial drug group had a trend of fasting blood glucose levels during the experiment falling significantly on the 3rd day of treatment although the value was still significantly different ($p<0.05$) from before streptozotocin induction, dropped sharply on the 7th day and the value was no longer significantly different ($p<0.05$). 0.05) before streptozotocin induction and until the end of the study stable fasting blood glucose levels were not significantly different ($p<0.05$) from before streptozotocin induction. The group that was fed sea cucumbers had a nearly the same percentage of decreased fasting blood glucose levels from the 2nd day after being induced by streptozotocin to the 21st day of treatment, which was almost the same, namely 77.16% in the golden sea cucumber-feeding group and 77.05% in the gamma-sea cucumber-feeding group. The percentage reduction was higher than the group that was force-fed spirulina powder and almost the same as the commercial drug group (Figure 4).

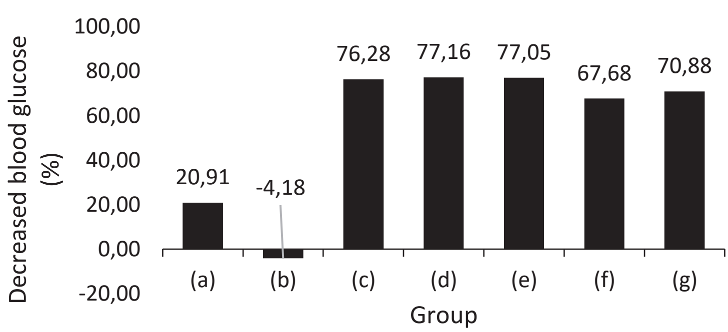

Fig. 4. Decreased blood glucose on the 2nd day of fasting after streptozotocin induction until the 21st day of treatment. (a) Normal wound control, (b) Diabetic wound control, (c) Commercial drug rubs, (d) Gold sea cucumber rubs, (e) Gama sea cucumbers rubs, (f) Spirulina rubs gold sea cucumbers , (h) Chek spirulina-oles gama sea cucumbers.

The normal wound control group had fasting blood glucose levels <199 mg/dL during treatment, with values ranging from 88.5 to 116.0 mg/dL indicating that the group had non-diabetic status. These results are relatively the same as [5] who reported fasting blood glucose levels of Sprague Dawley rats that were not induced by streptozotocin, respectively, of 77.0-95.0 mg/dL and 96.0-104.0 mg/dL. Instability of fasting blood glucose levels in the form of a decrease on days 3 and 7 with significantly different values ($p < 0.05$) is thought to be due to the level of stress experienced by the experimental animals due to the force-feeding and topical treatment.

In the diabetic wound control group, the diabetic condition lasted until the end of the treatment which was characterised by fasting blood glucose levels >240 mg/dL, namely 273.0-349.0 mg/dL. [5] also reported relatively similar values, namely 278.2-312.5 mg/dL and 334.4-417.0 mg/dL in diabetic white rats that were only fed aquadest. The absence of force-feeding in this group was the cause of the fasting blood glucose level. The diabetic status of the Glibenclamide-administered commercial drug group ended on the 7th day of treatment marked by a fasting blood glucose level of 134.6 mg/dL. The fasting blood glucose level then decreased to below the normal group fasting blood glucose level of 75.8 mg/dL on day 14 and then on day 21 the value was not significantly different ($p<0.05$) from before streptozotocin induction. Glibenclamide is an oral antihyperglycemic drug in the sulfonylurea class which works to increase insulin secretion by pancreatic beta cells (insulin secretagogue) and has the side effect of hypoglycemia [8]. This side effect is thought to be the cause of the low fasting blood glucose level on the 14th day. The percentage decrease in blood glucose levels on days 14 and 21 was 77.75% and 76.28%, respectively, higher than a decrease of 31.39% in diabetic rats that were given the same dose of glibenclamide, namely 3.22 mg/kg BW. This is thought to be due to the use of different individual rats.

Blood glucose levels while fasting at the end of the study in the sea cucumber and spirulina-feeding group with values not significantly different from the normal wound control group with a significant decrease, namely the golden sea-cucumber-feeding group of 77.16% and the gama-feeding group of 77.05%, which is suspected to be related to the content of the active substance in these preparations. According to [9] and [10] golden sea cucumber extract contains steroids, saponins, alkaloids and triterpenoids and has antioxidant activity through inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl). Likewise with the spirulina-feeding group which had a decrease of 67.68% (the spirulina-feeding group of golden sea cucumbers) and 70.88% (the spirulina-feeding group of gama sea cucumbers), spirulina ethyl acetate extract has antioxidant activity through inhibition of DPPH and contains active substances of phenolic compounds, triterpenoids, steroids, flavonoids and saponins. The active substances present in the golden sea cucumber and spirulina preparations are thought to increase insulin levels in the blood so that blood glucose levels can be controlled. According to research [5] there were insulin levels in the blood of 32.46% and 13.98% in spirulina doses of 81 mg/kg BW and sea cucumbers 284 mg/kg BW for 14 days. According to research [7] giving a combination of red betel extracts for 14 days (dose 9 mL/kg BW) reduced blood glucose levels in rats by up to 55.42%, significantly different ($p> 0.05$) compared to glucose levels on day 3. combination extract (dose of 9 ml/kg BW and 13.5 mL/kg 88) increased the number of Langerhans islands in rats up to 10.9% -30.6%. Levels of high-density lipoprotein (HDL) and blood triglyceride levels of rats in the control group with diabetes were significantly different ($p < 0.05$) compared to diabetics with red betel extract and the normal group. Giving red betel extract combination orally (various doses) for 14 days can suppress the weight loss of rats by 10% -11%.

3.3 Wounded skin histopathology

The results of histopathic observations of wound skin are presented in Table 1. Neutrophil cells were found in all groups with varying amounts except for the commercial drug group where no cells were found at all. The normal wound control group and the diabetic wound control group had very few neutrophil cells, namely 3.7 and 2.8. This value was not significantly different ($p<0.05$) with the commercial drug group. Neutrophil cells in all treatment groups were relatively numerous and significantly different ($p<0.05$) from the diabetic wound control group, except for the spirulina-rubbed golden sea cucumber treatment group which had an average that was not significantly different ($p<0.05$) but the value was greater.

Tabel 1. Wound skin histopathic profile

Criteria[a]	Groups[b]						
	KN	KD	OK	Sh-Sh	Sv-Sv	Sp-Sh	Sp-Sv
Neutrophil Cells	3.7±9,2^{b}	2.8±8,2^{b}	0.0±0,0^{b}	112.7±260,4^{ab}	154.2±253,4^{ab}	51.7±88,2^{b}	249.0±301,9^{a}
Neovascularization	18.3±21.0^{abc}	37.2±25,4^{a}	11.1±5,9^{c}	11.00±7,4^{c}	31.95±21,8^{ab}	17.40±12,7^{bc}	23.7±21,3^{ab}
Repitelization (%)	100.00^{a}	100.00^{a}	100.00±0.00^{a}	82.58±24,63^{a}	9.31±3.07^{a}	50.00±70,71^{a}	66.13±47,90^{a}
Collagen Area (%)	92.42^{abcd}	87.00^{a}	64.33^{abcd}	17.69±13.33^{bcd}	18.96±1,90^{cd}	51.97±11,39^{abcd}	19.61±27,73^{d}

[a]Values are expressed in means ± SD. The same letter in the same row indicates no significant difference at the 95% level of significance ($p<0.05$)
[b]KN: normal wound control, KD: diabetic wound control, OK: vibrating commercial medicine, Sh-Sh: golden sea cucumber basting, Sv-Sv: gama sea cucumber basting, Sp-Sh: golden sea cucumber spirulina vibrating, Sp-Sv: Fortify spirulina and gama sea cucumbers.

The finding of neutrophil cells in all groups except the commercial drug group indicated that inflammation was still occurring in that group. The level of severe inflammation that occurred in the group that was smeared with gama sea cucumber was indicated by the number of neutrophil cells of 249.0 in the group that was rubbed on gama-chopped spirulina and in the group that was rubbed on with gama sea cucumber as much as 154.2. Inflammation also still occurred in the group that was smeared with golden sea cucumbers, although not as intense as that which occurred in the group that was smeared with gama sea cucumbers. The number of neutrophil cells was 112.7 and 51.7 in the golden sea cucumber basting group and the golden sea cucumber basting group and spirulina showed this. The normal wound control group and the diabetic wound control group had only a small amount of inflammation with a very small number of neutrophil cells and even the values were not significantly different ($p<0.05$) from the commercial drug group which did not find any neutrophil cells at all. The number of neutrophil cells also correlated with the rate of wound healing, the normal wound control group, the diabetic wound group and the commercial drug group whose wounds had closed completely almost did not find any neutrophil cells, on the other hand the groups that were smeared with golden sea cucumber and gama sea cucumber had wounds that had not completely closed even widened, as in the gold sea cucumber basking group and the gama sea cucumber basking group. Research by [11] healed wounds in diabetic rats treated with turmeric rhizome extract ointment, histopath of the 21st day wound skin was not found in the presence of neutrophils.

The application of gamma sea cucumber topical accelerated the formation of new blood vessels compared to the golden sea cucumber topical preparation, even the amount was higher than commercial drugs and normal controls, although it was still less than the diabetic wound control group. Neovascularization is needed in the wound healing process to bring oxygen and nutrients that are very necessary for cell metabolism and tissue regeneration [11]. In the group that was rubbed on golden sea cucumbers and gamma sea cucumbers, the percentage of reepithelialization had not reached 100%, as well as the percentage of wound healing. According to [11], the faster the re-epithelialization process occurs, the faster the skin reaches normal conditions.

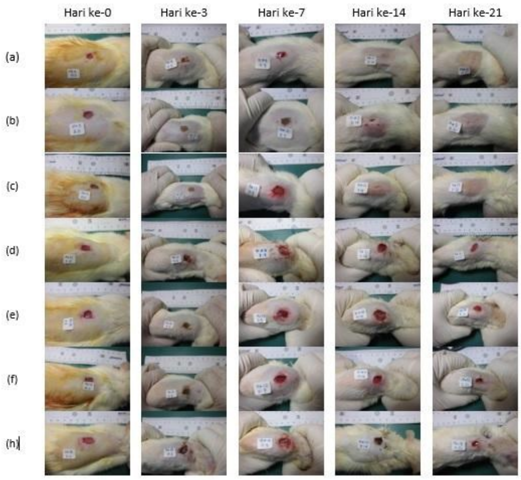

Fig. 5. Macroscopic observation of wound healing. (a) Normal wound control, (b) Diabetic wound control, (c) Commercial drug rubs, (d) Gold sea cucumber rubs, (e) Gama sea cucumbers rubs, (f) Spirulina rubs gold sea cucumbers, (h) Chek spirulina-oles gama sea cucumbers.

In general, the percentage of wound healing in all groups decreased until the 3rd, 7th or 14th day, then until the end of the study, except for the gold sea cucumber bashing group and the gama sea cucumbers bashing group, the percentage value of wound healing was stagnant, that is, all values were not different. significant ($p<0.05$) on each observation day (Figure 9). The percentage of wound healing in the normal wound control group decreased significantly from day 0 to day 3 and the values of the two were significantly different ($p <0.05$) then rose slightly on day 7 and then increased significantly to 100% on the observation day 14th. The diabetic wound control group had a pattern of wound healing percentages that decreased slightly from day 0 to days 3 and 7, rose significantly on day 14 and then rose to 100% on day 21. The four values were significantly different ($p<0.05$) except that the values on day 3 were not significantly different ($p<0.05$) from day 7.

Wound healing is not just closing the wound skin, but the formation of a matrix, one of which is collagen connective tissue. In the normal wound group, the diabetic wound group and the commercial drug group, the percentage of collagen tissue area did not reach 100% even though the percentage of reepithelialization and the percentage of wound healing had a value of 100%. This value was higher than the group that was smeared with sea cucumbers. The highest percentage value of collagen area was shown by the group of golden sea cucumbers rubbed with spirulina with a value of 51.97%. In general, in this study, in the treatment group that was treated with golden sea cucumbers and gama sea cucumbers, histopathological wound skin on the 21st day still found neutrophil cells and had neovascularization, the average percentage of reepithelialization and collagen area was less than that of diabetic wound controls. These results are different from the results of a study [11] which reported histopathic observations of the wound skin of diabetic rats treated with turmeric rhizome extract ointment on day 21 which had returned to normal, found no neutrophil cells and had neovascularization, the average percentage of reepithelialization and collagen area. more than control diabetic wounds. Differences in active compounds that play a role in the wound healing process contained in topical preparations are the cause of these differences in results.

4 Conclusion

During the 21 days of treatment, feeding sea cucumber and spirulina preparations could significantly reduce blood glucose levels in diabetic rats with the highest reduction percentage of 77.16% in the golden sea cucumber basting group. Application of methanol extract of sea cucumbers to diabetic rats did not have better wound healing power than control diabetic wounds with the highest percentage of wound healing of 52.36% in the spirulina-feeding group of golden sea cucumbers.

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