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Combined Anti-Diabetic and Wound Healing Effects of Binahung Leaf (*Basella rubra* L.) and Snakehead Fish (*Channa striata*) Extracts in Alloxan-Induced High-Fat Diet Rats

(Kesan Gabungan Anti-Kencing Manis dan Penyembuhan Luka Ekstrak Daun Binahung (*Basella rubra* L.) dan Ikan Haruan (*Channa atriata*) pada Tikus Diet Tinggi Lemak Teraruh Aloksan)

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ABSTRACT

Diabetes mellitus is a metabolic disease that continues to increase in the world. Lifestyle factors cause diabetes cases to increase uncontrollably. The development of medicines based on natural ingredients has become a subject of study for researchers throughout the world. Meanwhile, binahong leaf extract contains flavonoids and saponins, which can reduce the absorption of glucose. Snakehead fish extract containing albumin also influences the reduction of diabetic wounds. The combination of binahong and snakehead fish extract is expected to synergistically lower blood glucose levels and cure diabetic wounds. Therefore, this study aimed to determine the anti-diabetic and wound healing activity resulting from the combination of binahong leaf and snakehead fish extract in rat models of diabetes induced with alloxan and fat diet. The test animals were divided into six groups with five per group, namely normal, positive, and negative control, as well as dose 1 (snakehead fish 250 mg/kg and binahong extract 100 mg/kg), dose 2 (snakehead fish 250 mg/kg and binahong extract 300 mg/kg), and a single dose of snakehead fish extract 500 mg/kg. The experiment was conducted for 21 days by administering the test solution once a day. The results showed that administering a combination of binahong extract at a dose of 100 mg/kg and snakehead fish at a dose of 500 mg/kg significantly reduced blood glucose levels in diabetic rats ($p \leq 0.05$). Treatment using a combination of the two extracts was more effective in lowering blood sugar levels compared to a single dose of snakehead fish extract.

Keywords: Albumin; diabetes mellitus; diabetic wounds; flavonoid

ABSTRAK

Diabetes mellitus ialah penyakit metabolik yang terus meningkat di seluruh dunia. Faktor gaya hidup menyebabkan kes diabetes meningkat tanpa kawalan. Pembangunan ubat-ubatan berdasarkan bahan-bahan semula jadi telah menjadi subjek kajian bagi penyelidik di seluruh dunia. Sementara itu, ekstrak daun binahong mengandungi flavonoid dan saponin, yang boleh mengurangkan penyerapan glukosa. Ekstrak ikan toman yang mengandungi albumin juga memberi kesan kepada pengurangan luka diabetes. Gabungan ekstrak binahong dan ekstrak ikan toman dijangka secara sinergis mengurangkan tahap glukosa darah dan menyembuhkan luka diabetes. Oleh itu, kajian ini bertujuan untuk menentukan aktiviti anti-diabetes dan penyembuhan luka yang dihasilkan daripada gabungan binahong daun dan ekstrak ikan toman pada model tikus diabetes teraruh diet aloksan dan lemak. Haiwan ujian dibahagikan kepada enam kumpulan dengan lima setiap kumpulan, iaitu normal, positif, dan kawalan negatif, serta dos 1 (ekstrak ikan toman 250 mg/kg dan ekstrak binahong 100 mg/kg), dos 2 (ekstrak ikan toman 250 mg/kg dan ekstrak binahong 300 mg/kg) dan satu dos ekstrak ikan toman 500 mg/kg. Uji kaji ini dijalankan selama 21 hari dengan pemberian larutan ujian satu kali sehari. Hasilnya menunjukkan bahawa pengambilan gabungan ekstrak binahong pada dos 100 mg/kg dan ikan toman pada dos 500 mg/kg secara signifikan mengurangkan tahap glukosa darah pada tikus diabetes ($p \leq 0.05$). Rawatan menggunakan gabungan kedua-dua ekstrak adalah lebih berkesan dalam mengurangkan tahap gula darah berbanding dengan satu dos ekstrak ikan toman.

Kata kunci: Albumin; diabetes melitus; flavonoid; luka diabetes

INTRODUCTION

Diabetes Mellitus is a metabolic disease characterized by hyperglycemia caused by defects in insulin secretion, resistance, or both (Harahap, Elya & Bahtiar 2019). Hyperglycemia is defined by blood test results showing blood sugar levels ≥ 200 mg/dL, and fasting blood glucose levels ≥126 mg/dL (Sasongko et al. 2020). Elevated blood sugar levels in diabetics can lead to challenging wound complications with potential risks of amputation (Decroli et al. 2019). Furthermore, diabetic ulcers, stemming from hyperglycemia-induced angiopathy and neuropathy inhibit blood circulation, thereby limiting oxygen supply to nerve endings. The problem in curing this condition is worsened by hyperglycemia which potentially increases bacterial growth. In addition, people with diabetes mellitus often experience a decrease in their immune system resulting in prolonged wound inflammation (Ramadhanti, Sandhika & Widodo 2021).

Treatment of diabetes generally uses conventional medicines, but there is no permanent drug available for complete recovery (Tam et al. 2011). The treatment can also be supported by using plant and animal materials. Types of natural materials often used for the treatment of diabetes mellitus include binahong plants and snakehead fish. Binahong plant (*Basella rubra* L.) contains many active substances such as flavonoids, saponins, alkaloids, and tannins. These compounds have anti-cancer, anti-viral, anti-oxidant, anti-inflammatory, anti-cholesterol, anti-ulcer, anti-microbial, anti-hypoglycemic, wound healing, and androgenic properties (Eming, Martin & Tomic-Canic 2014).

Snakehead fish (*Channa striata*) has several healing properties including benefits for diabetes mellitus and wound (Đurašević et al. 2020), and the main nutritional content is albumin and fat (Liu et al. 2020). Albumin content is estimated at 6.22% and can be used to reduce blood glucose levels due to its high concentration in plasma (Đurašević et al. 2020). In addition, unsaturated fatty acids contained in snakehead fish play key roles as anti-inflammatory agents and regulate the synthesis of prostaglandins which influence vasodilation of blood vessels (Sakr et al. 2023).

This study was conducted on Wistar rats induced with diabetes using alloxan monohydrate. Aside from being given a drug solution, the rats also received a fat diet solution, containing a mixture of quail egg yolk and lard oil. The fat diet was implemented because a balanced calorie intake was essential for the stability of insulin sensitivity (Ahmad, He & Perrimon 2020). In this context, whole-body insulin sensitivity was expressed as glucose infusion rate per kilogram of body mass (Lundsgaard et al. 2017).

The treatment carried out in this study was a combination of binahong leaf and snakehead fish extract. The combination is expected to have a stronger effect compared to the use of only one type of material. Therefore, this study aimed to determine anti-diabetic and wound healing effects resulting from the combination of binahong leaf and snakehead fish extract.

MATERIALS AND METHODS

PREPARATION OF BINAHONG LEAF EXTRACT

Binahong leaf was washed twice with running water, then drained and dried in an oven at 50 °C until dry. Subsequently, maceration was carried out by soaking every 100 g of leaf with 1.5 L of 96% ethanol. This was continued for 7 days with daily stirring at the same time. The results obtained were evaporated using a rotary evaporator to make a thick binahong leaf extract (Harahap, Elya & Bahtiar 2019).

PREPARATION OF SNAKEHEAD FISH EXTRACT

Snakehead fish was cleaned to remove dirt from the body, followed by drying and boiling in water with a ratio of 1:1 by weight of fish to the cooking water. The cooked snakehead fish decoction was separated from the water filtrate and boiled until 1/3 of the initial filtrate water was obtained. The remaining filtrate water was mixed with Maltodextrin reaching 5% of fish weight and stirred over low heat until it crystallized. In this context, maltodextrin is a substance used to form granules or crystals of snakehead fish extract (Sasongko et al. 2023).

ANIMAL EXPERIMENT

A total of 30 apparently healthy male Wistar rats weighing 140-240 g were purchased from the Kemuning Lab Science, Indonesia. The animals were kept in well-aerated cages, with access to animal feed and water *ad libitum*, and allowed to acclimatize for one week. Ethical clearance was obtained from the Dr. Moewardi General Hospital on Animal Use and Care (No: 1021/VII/HREC/2022). All institutional guidelines for experimental protocol were adhered to, alongside strict compliance with national and international laws and guidelines for the care and use of laboratory animals.

DIABETIC INDUCTION IN ANIMAL MODELS

Alloxan solution was prepared by dissolving in sterile normal saline (0.9% NaCl) and injected intraperitoneally (i.p) at 150 mg/kg dose (Sasongko et al. 2020). Diabetes was established after 7 days by measuring fasting blood glucose levels using a one-touch electronic glucometer (Easy Touch glucometer) and commercially available glucose strips. The study included rats with fasting plasma glucose levels exceeding 250 mg/dl.

ANIMAL GROUPING

Rat was divided into six groups with five members each, and the treatment was given for 21 days. Wound healing treatment was observed for 6 days.

The choosen concentration of the extracts were made based on the report from Rollando et al. (2022) that showed the first dose of binahong extract for rats was 100 mg/kg b.w. or the equivalent of 20 mg/200 g b.w. The second dose of binahong extracts for rat was 300 mg/kg b.w. or the equivalent of 60 mg/200 g b.w (Vita, Fatimah & Murtiwi 2019). The dose of snakehead fish extract used in this research was 500 mg/kg b.w, based on the study from Azemi et al. (2021).

FAT DIET ON ANIMAL OBJECT

A high-fat diet was administered to the test animals at a dosage of 15 g/kg b.w. consisting of 50% BR1 and 50% SPFA12 high-protein feed with a protein content of 32-34%. Rat was also provided with a test solution of pork oil and quail egg yolk with 1:1 ratio, both of which have high protein levels and could be used for dietary fat.

BLOOD BIOCHEMISTRY MEASUREMENT

Blood glucose levels were measured on days 0 (T0) and 21 (T21) after the rats had been diagnosed as diabetic. Meanwhile, other blood biochemical levels such as total protein, albumin, triglycerides, and total cholesterol were measured after 21 days of sample administration (end test). The measurement protocol followed the BioSystem kit reagent instructions.

MEASUREMENT OF CATALASE AND GLUTATHIONE ANTI-OXIDANTS

Measurement of endogenous anti-oxidant levels (CAT and GSH) in blood serum was conducted using the FineTest kit in line with the instructions of the manufacturer.

WOUND CREATION

The backs of test rats were cleaned of hair using a clipper and depilatory cream. After the outer skin became visible, sterilization was carried out using 70% alcohol to prevent interaction with bacteria. The scalpel used for slicing was also sterilized using 70% alcohol before its use. Wound made on the back of the test animal rat was 1.5 cm long and 2 mm deep (Ramadhanti, Sandhika & Widodo 2021).

WOUND HEALING PERCENTAGE

Wound measurements were carried out every 3 days using a caliper. The length data was recorded and the percentage of healing was calculated using the equation.

Group	Treatment
Normal control	Rat without alloxan induction
Positive control	Diabetic rat treated with Glibenclamide 5 mg/kg b.w
Negative control	Diabetic rat without sample treatment
Dose 1	Diabetic rat treated with 250 mg/kg b.w snakehead fish extract and 100 mg/kg b.w binahong leaf extract
Dose 2	Diabetic rat treated with 250 mg/kg b.w snakehead fish extract and 300 mg/kg b.w binahong leaf extract
Dose 3	Diabetic rat treated with 500 mg/kg b.w binahong leaf extract

$$\frac{T0 - Tn}{T0} \text{ x } 100\%$$

$$\text{Insulin sensitivity} = \frac{1}{Log\left\{Fasting\ serum\ insulin\left(\frac{u}{L}\right)\right\} X\ Log\ \{Fasting\ blood\ glucose\ \left(\frac{mmol}{L}\right)\}}$$

$$\text{HOMA-IR} = \frac{Fasting\ serum\ insulin\left(\frac{u}{L}\right) x\ Fasting\ blood\ glucose\left(\frac{mmol}{L}\right)}{22.5}$$

Conversion factor: Insulin (1 U/L = 7.174 pmol/L) and blood glucose (1 mmoL/L = 18 mg/dL).

STATISTICAL ANALYSIS

Data were presented in the form of mean + standard deviation, while the analyses were conducted using normality and homogeneity tests followed by One-way ANOVA and a Post Hoc LSD test to determine the level of differences in each treatment (p≤0.05).

RESULTS

Table 1 shows the blood glucose levels after 21 days of administration snakehead fish extract and binahong leaf extract. The statistical test at T0 between the normal control and negative control groups showed a significance value (p ≤ 0.05), which means there is a significant difference. This shows that streptozotocin induction succeeded in increasing blood glucose levels significantly. In addition, at T0, all sample dose groups also showed significant differences when compared with the negative group. In this study, it was proven that at T0, the blood glucose levels measured in the negative control, positive control, dose 1, dose 2, and dose 3 groups reached more than 200 mg/dL. There was a decrease in blood glucose levels at T21 to below 200 mg/dL in the following groups: dose 1, dose 2, and dose 3. The negative group and normal group did not experience a significant decrease in blood glucose levels because they were not given the medication. Meanwhile, the positive group experienced a decrease of 164 mg/dL. Dose group 1, 2 and 3 experienced a decrease of blood glucose to 247, 278, and 170 mg/dL, respectively. Statistical tests at T21 showed that all treatment groups experienced a significant reduction when compared with the negative control group. The group 2 (250 mg/kg b.w. snakehead fish extract + 300 mg/kg b.w. binahong leaf extract) showed the greatest reduction in blood glucose levels when compared with the other groups. This shows that administration of binahong leaf extract at a dose of 300 mg/kg b.w. have increased in activity when compared to dose 3 (500 mg/kg b.w. snakehead fish extract).

Table 2 shows the effect of snakehead fish extract and binahong leaf extract on insulin concentration, insulin sensitivity and HOMA-IR values. The data shows that the insulin concentration between the negative control group and the other groups shows a significance difference (p ≤ 0.05). The insulin sensitivity parameter shows no significance difference. Meanwhile, in the HOMA-IR parameters, there was a significant difference between the negative control group compared to the normal and positive control groups. Table 3 shows that the triglyceride values between the negative control group and the other groups showed a significance difference (p ≤ 0.05). Meanwhile, the parameters cholesterol, albumin and protein did not show significant differences.

Table 4 demonstrates that the influence of snakehead fish extract and binahong leaves on antioxidant enzymes (GSH and Catalase) results in a reduction in GSH levels in diabetic rats that do not receive samples (negative control). Meanwhile, catalase levels showed no significant decrease when compared to other groups of rats. When compared to the negative control group, administration of samples at doses 1-3 demonstrated significant ability to maintain GSH levels (p ≤ 0.05). Administration of a combination of snakehead fish extract 250 mg/kg b.w + binahong extract 100 mg/kg b.w showed significant changes in GSH levels when compared to doses 2 and 3. This shows that increasing the dose of binahong extract in combination administration does not show the same changes in endogenous antioxidant parameters.

TABLE 1. Blood glucose levels after 21 days of administering snakehead fish and binahong leaf extract

Groups	Blood glucose level (mg/dL)	
	T0	T21
Negative control	390.80 ± 45.24	361.40 ± 38.75[abc]
Positive control	286.20 ± 78.48*	122.20±12.75*
Normal control	107.80±10.33*	91.82± 6.90*[abc]
Dose 1	398.20 ± 32.18*	150.40±24.30*
Dose 2	441.20±20.87*	163.00± 25.65*
Dose 3	299.80±15.84*	129.20± 20.82*

*, p≤0.05 showed significantly different results from the negative group

[a], p≤0.05 showed significantly different results with the dose 1 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 100 mg/kg b.w

[b], p≤0.05 showed significantly different results with the dose 2 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 300 mg/kg b.w

[c], p≤0.05 showed significantly different results with the single dose group of snakehead fish 500 mg/kg b.w

TABLE 2. Effect of snakehead fish and binahong leaf extracts on the insulin sensitivity and HOMA-IR values

Groups	Insulin concentration (U/L)	Insulin sensitivity	HOMA-IR
Normal control	3257.83 ± 362.46*	0.05± 0.008	355.6± 88.05*
Negative control	7641.41 ± 603.30	0.04 ± 0.004	771.6 ± 69.53
Positive control	949.31 ± 104.91*	0.05±0.001	294 ±73.06*
Dose 1	3211.37 ± 241.75*	0.04±0.005	1267.6 ±51.12
Dose 2	2744.09 ± 242.60*	0.04±0.004	1077.6 ±80.97
Dose 3	2641.74 ± 270.73*	0.05± 0.008	690.2 ± 32.96

*, $p \leq 0.05$ showed significantly different results from the negative group
[a], $p \leq 0.05$ showed significantly different results with the dose 1 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 100 mg/kg b.w
[b], $p \leq 0.05$ showed significantly different results with the dose 2 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 300 mg/kg b.w
[c], $p \leq 0.05$ showed significantly different results with the single dose group of snakehead fish 500 mg/kg b.w

TABLE 3. Effect of snakehead fish and binahong leaf extracts on the blood profile

Profile	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Proteins (mg/dL)	Albumin (mg/dL)
Normal control	123.03± 20.52*	96.69± 7.23	79.05 ± 35.69	28.26 ± 1.49 *
Negative control	140.36± 30.73	105.68± 11.45	96.07 ± 24.80	51.91 ± 24.86
Positive control	71.89±19.19*	121.07±16.11	93.28 ± 14.58	65.65 ± 10.52
Dose 1	77.39±8.06*[c]	100.46±17.75	88.44 ± 7.84	53.82 ± 16.52
Dose 2	89.74±23.69*[c]	116.65±7.21	87.91 ± 6.20	53.92 ± 10.31
Dose 3	108.29± 28.76*	128.56±4.94	82.27 ± 7.11	68.12 ± 14.35

*, $p \leq 0.05$ showed significantly different results from the negative group
[a], $p \leq 0.05$ showed significantly different results with the dose 1 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 100 mg/kg b.w
[b], $p \leq 0.05$ showed significantly different results with the dose 2 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 300 mg/kg b.w
[c], $p \leq 0.05$ showed significantly different results with the single dose group of snakehead fish 500 mg/kg b.w

TABLE 4. Effect of snakehead fish and binahong leaf extracts on the GSH and catalase reagents

Groups	GSH	Catalase
Negative Control	191.87± 52.43[abc]	652.27± 176.39
Positive Control	297.14±99.09*[bc]	597.61±41.89
Dose 1	329.60±207.50*[bc]	664.11±81.83
Dose 2	220.12±93.14*	563.42±100.92
Dose 3	231.57± 86.44*	497.05±44.84

*, $p \leq 0.05$ showed significantly different results from the negative group
[a], $p \leq 0.05$ showed significantly different results with the dose 1 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 100 mg/kg b.w
[b], $p \leq 0.05$ showed significantly different results with the dose 2 group of snakehead fish extract 250 mg/kg b.w + binahong leaf extract 300 mg/kg b.w
[c], $p \leq 0.05$ showed significantly different results with the single dose group of snakehead fish 500 mg/kg b.w

Table 5 shows wound healing activity with the wound length parameter. The LSD post hoc test in the negative group against the positive and treatment groups showed significant difference ($p \leq 0.05$). This means that there was a significant difference between the negative control with the positive and the treatment group in wound healing. The positive control and the treatment group showed a significance ($p \geq 0.05$) indicating that there was no significant difference in wound healing. Similarly, in the treatment group, the results of the LSD post hoc test showed no significant difference. The percentage of wound healing can be seen in Figures 1 and 2.

Based on the Figures 1 and 2, on the third day, the negative group had a result of -12.667%, meaning that there was no wound healing. This decline was attributed to increasingly severe wound tissue damage. The experimental animals in the negative group experienced deteriorating wound due to skin tissue tears, leading to an increase in wound length. The high activity of the test animals further contributed to the worsening of wound length. Furthermore, the negative group was induced by a 0.5% CMC Na solution which did not affect wound healing process on the third day.

Subsequent measurements on the sixth day in the normal group showed that wound healing occurred, with an increase of 9.067%. Despite using a 0.5% CMC Na solution, rat bodies experienced a biological process of wound healing attributed to cell regeneration and repair of tissue damage. Wound healing is characterized by tissue closure, a fast epithelialization process, and the formation of several collagen and connective tissues (Tam et al. 2011). As the inflammatory phase ends, angiogenesis occurs, comprising endothelial cell proliferation, migration, and branching to form new blood vessels (Rodrigues et al. 2019). On day 0, wound was made on the back of rats with a length of 1.5 cm and a depth of 2 mm resulting in bleeding from cut blood vessels. When blood vessels are severed, the body initiates vasoconstriction, retraction, and hemostatic reactions to halt bleeding. The back of the rat had a reddish and swollen wound due to an inflammatory reaction (Ramadhanti, Sandhika & Widodo 2021). Therefore, on day 0, a long wound-healing process did not occur. Modeling and establishment of new blood vessels is critical in wound healing. This takes place concurrently during all phases of the reparative process (Velnar, Bailey & Smrkolj 2009).

On the third day, there was no inflammation and the animals underwent a proliferative phase. In this phase, the formation of fibrin nets and granulation tissue results in an increase in wound resistance due to the process of collagen fibrogenesis. On the sixth day, the wound was almost covered by new tissue due to the action of macrophages which stimulated proliferation, migration, and the formation of an extracellular matrix, resulting in epithelialization to cover wound (Eming, Martin & Tomic-Canic 2014).

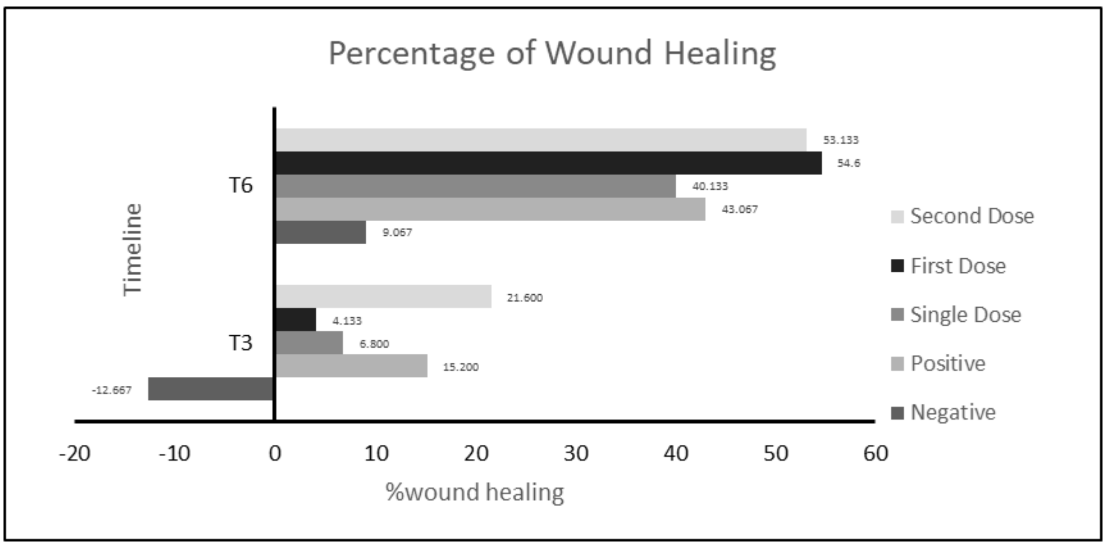

FIGURE 1. Percentage of wound healing

TABLE 5. Wound healing effect of snakehead fish and binahong leaf extracts on the sixth day

Groups	Wound length (Mean±SD)
Negative control	1.364±0.162
Positive control	0.854±0.224*
Dose 1	0.681±0.238*
Dose 2	0.703±0.175*
Dose 3	0.898±0.238*

*, p≤0.05 showed significantly different results from the negative control group

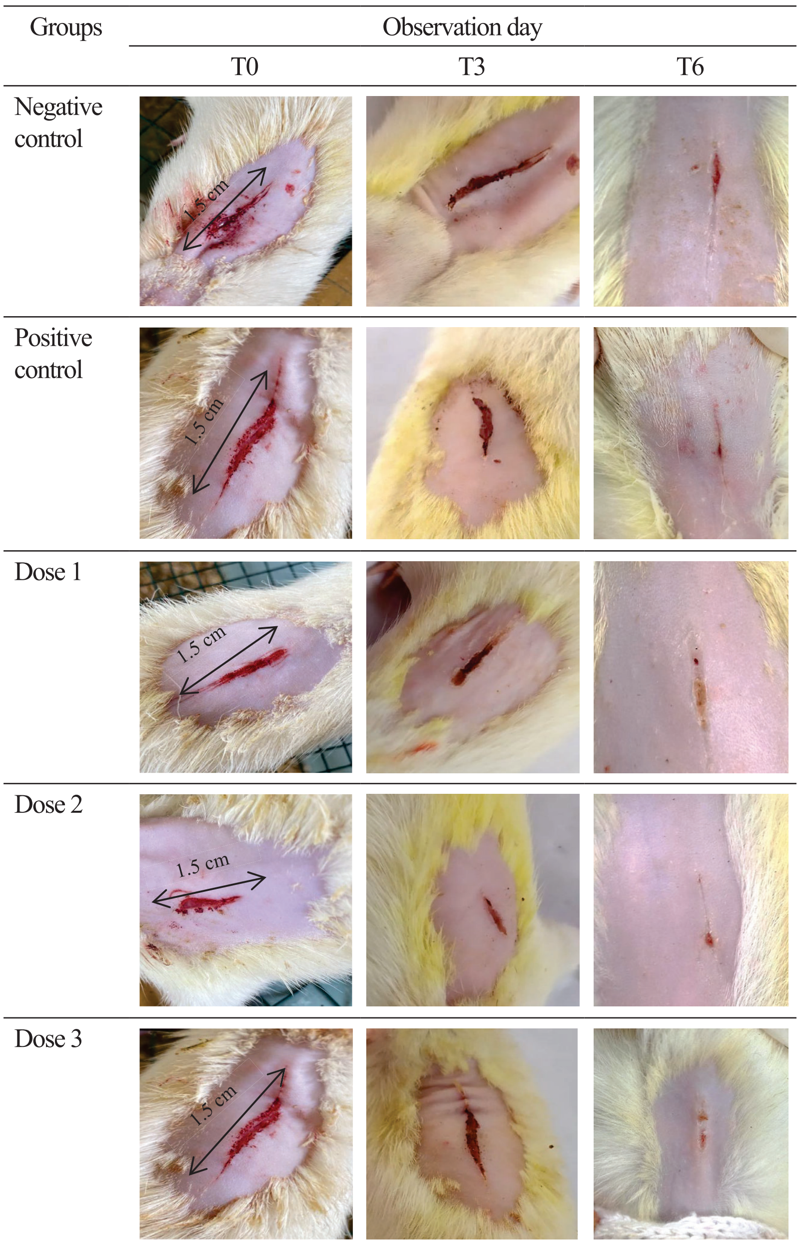

FIGURE 2. Documentation of wound healing process in rats as experimental animals on Day (T) 0, 3, and 6

DISCUSSION

In the first week, all treatments other than the normal group started with the aim of achieving diabetic blood glucose levels. One factor contributing to the elevated blood glucose levels was insulin resistance induced by dietary fat and alloxan (Đurašević et al. 2020). Insulin resistance causes glycogenolysis which is the process of breaking down glycogen to form energy (Liu et al. 2020). This process produces glucose, eventually excreted through the urine due to insulin resistance, leading to long-term shrinkage of muscle and adipose tissues (Sakr et al. 2023). Furthermore, the blood glucose levels of non-diabetic rats were influenced by the hormones of each subject, namely insulin and glucagon. Under normal circumstances without diabetes, pancreatic beta cells increase insulin production proportionately to elevate blood glucose levels. Conversely, when blood glucose levels are low, a significant amount of glucagon is produced (Ahmad, He & Perrimon 2020). As stated in a previous study, to improve insulin performance in the body, a healthy lifestyle is needed (Hansen et al. 2020).

The results from the second week showed an increasingly visible decrease in blood glucose levels across all treatments. One contributing factor to this decrease was the performance of albumin contained in snakehead fish extract. Albumin affects blood glucose levels because the concentration in plasma is directly related to disorders such as diabetes mellitus and metabolic syndrome (Abdulgani et al. 2020).

A significant decrease in blood glucose levels for weeks was influenced by the performance of saponins and flavonoids in binahong extract. These compounds reduce blood glucose absorption by damaging the arrangement of cell membranes (Calderón Guzmán et al. 2020). The positive group was given the glibenclamide test solution. This group served as a benchmark for comparing formulas made with conventional medicines used for diabetes therapy. Glibenclamide, a second-generation sulfonylurea used in the treatment of non-insulin-dependent diabetes (NIDDM) can maintain a longer increase in insulin levels by inhibiting its degradation. This inhibition significantly affects the blood glucose-lowering effect of glibenclamide (Laxmi et al. 2009). Moreover, the positive group was used to compare conventional diabetes drugs with anti-diabetic activity in binahong leaf and snakehead fish extract.

Based on the results of statistical analysis and the reduction observed in weeks 0 to 21, it was concluded that binahong leaf and snakehead fish extracts had anti-diabetic effects. This was characterized by a decrease in blood glucose levels across the control, as well as first, second, and single doses. The best dose for lowering blood glucose levels was dose 2 with a decrease amounting to 278 mg/dL. This dose consisted of binahong leaf of 300 mg/kg b.w and snakehead fish extract of 250 mg/kg b.w.

Binahong leaf and snakehead fish extracts also impacted long wound healing in diabetic rats. The principle of moisture in wound care includes preventing wound from becoming dry and hard, increasing the rate of epithelialization, preventing the formation of eschar tissue, enhancing the formation of dermis tissue, controlling inflammation, providing a more cosmetic appearance, and accelerating the autolysis debridement process. These processes can reduce the incidence of infection, are cost-effective, can maintain normal voltage gradient, maintain neutrophil activity, reduce pain, provide psychological benefits, and are easy to use (Rahmasari et al. 2022). Binahong leaf and snakehead fish extracts affected wound healing process in both the inflammatory and proliferation phases, facilitating proper maturation. Anti-oxidant properties of betel leaf as observed in correlation studies, play a role in biological activity during the inflammatory process (Darmawan et al. 2021). According to a previous study, one of the factors for diabetic wound healing is controlled blood glucose levels (Ramzy et al. 2020). Elevated blood glucose levels potentially cause an increase in blood viscosity, fostering microorganism development (Daiber et al. 2019). Another factor hindering the healing of diabetic wound is the obstruction of blood and oxygen circulation due to increased blood viscosity which causes a decrease in the synthesis of collagen and fibronectin (Abas, El Masry & Elgharably 2020), ultimately diminishing the immune system (Kim et al. 2021). Although no significant difference was observed between the positive control, dose one, dose two, and single dose, the first dose group had the best healing effect with a percentage of 54.6% on the sixth day, attributed to a decrease in blood glucose levels.

CONCLUSIONS

In conclusion, the combination of binahong leaf and snakehead fish extracts effectively reduced blood glucose levels and cured diabetic wound in rats induced with alloxan and fat diet. Binahong leaf extracts at a dose of 300 mg/kg b.w. and snakehead fish extract at a dose of 250 mg/kg b.w showcased a better ability to lower blood glucose levels compared to a single dose of snakehead fish extract at 500 mg/kg b.w. In the context of wound healing, the best dose was binahong leaf (*Basella rubra*) at 100 mg/kg b.w and snakehead fish extract at 250 mg/kg b.w.

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