Research Article
DOI : 10.33751/jf.v12i2.4770

FITOFARMAKA: Jurnal Ilmiah Farmasi
Vol.12, No.2, December 2022 : 100-111
p-ISSN : 2087-9164 e-ISSN : 2622-755X

In Vivo Wound Healing Potential of Taro Leaf Extract (*Colocasia esculenta* L.) Emulgel on Diabetic Rat Models

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Submit: February 2nd, 2022 Revised: September 2nd, 2022 Accept: November 4th, 2022

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ABSTRACT

Diabetic wounds are one of the complications of Diabetes Mellitus (DM), which are at risk of infection and can end in amputation. Colocasia leaves contain metabolites of alkaloids, flavonoids, tannins, steroids and saponins, which have antibacterial and antioxidant activity and can accelerate wound healing by helping cell regeneration. This study aimed to determine the effectiveness of taro leaves extract emulgel worked for wound healing in diabetic rats. The test animals were 25 white male rats divided into five groups, namely positive control (applied with Cutimed® gel), negative control (applied with emulgel base), standard control (non-diabetic rats), Treatment-1 (applied emulgel once a day) and Treatment-2 (applied emulgel twice a day). Male white rats were injected with alloxan to make them diabetic and, additionally, given a grade 2 incision. Visual observations started from day 1 to day 16. The results of statistical analysis for measuring the diameter of the incision (Morton's method) and visual observation of the wound (method of scoring) obtained sig $\alpha < 0.05$, meaning that the treatment groups 1 and 2 were significantly different from the negative control. Treatment-2 produced nearly the same effect as the positive control. The conclusion was that 5% Taro Leaf extract emulgel applied twice daily was the most effective in healing cuts in diabetic rats for 13 days.

Keywords: emulgel; taro; diabetic; wound; rat

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occurs due to defects in insulin secretion, insulin action or both (American Diabetes Association, 2017). Diabetes has a reasonably high prevalence rate, and one of its complications is diabetic foot ulcers (International Diabetes Federation, 2017; Rasyid et al., 2018). The prevalence of DM in the world in 2014 was around 387 million and is estimated to be 592 million in 2035. In Indonesia, it is predicted that the number of people with diabetes will increase from 9,1 million in 2014 to 14.1 million in 2035 (International Diabetes Federation, 2015).

Diabetic wounds are one of the complications of diabetes with a risk of infection and end up in amputation of body parts (Ristanti *et al*., 2021). Diabetic wounds are experienced by 25% of diabetic patients and 85% of them are amputated. This can happen since 50% of DM sufferers experience neuropathy, namely loss of sensitivity in areas such as inability to feel heat, cold, or pain. The loss of sensitivity will cause a decrease in objects that can injure the feet; thus, it can trigger injuries and infections. On the other hand, an increase in blood sugar levels will also

inhibit the work of leukocytes, thus, the wound will become an ulcer and expand (Sari, 2015).

The prevalence of diabetes can increase in Indonesian society with increasing age and an unhealthy and balanced lifestyle. Thenceforth, treating diabetic wounds with topical antibiotics has side effects that cause skin irritation, redness, allergies, and edema. In addition, bacterial immunity to antibiotics causes the mortality rate to increase and the costs required for treatment (Khairany *et al*., 2015). This encourages the discovery of other sources of antibacterial drugs from natural ingredients that can act as safer and relatively cheaper antibacterials. Alternative treatment needs to be done to treat diabetic wounds, one of which is by using herbs.

Herbal ingredients that have the potential to be applied as diabetic wounds is taro leaf taro plants (*Colocasia esculenta* L.). Local people have often used this plant to heal minor wounds, burns, and wounds accompanied by bleeding. (Sangtam *et al*., 2012). Rahmi's research (2020) has proven that testing the effectiveness of taro leaf extract gel on wounds on the backs of rats with a diameter of ± 1.5 cm proved effective on 5% taro leaf extract gel preparations and could heal within ± 16 days and has the potential as anti-inflammatory and antibacterial. Has the potential to heal burns infected with Pseudomonas aeruginosa.

Taro leaves are known to contain metabolites that can accelerate wound healing by helping skin cell regeneration. Taro leaves accelerate the healing of contaminated wounds, and taro leaves have antibacterial and antioxidant activities that have the potential to accelerate diabetic wound healing (Ristanti *et al*., 2021). The results of phytochemical screening indicated that the ethanolic extract of taro leaves contained alkaloids, flavonoids, tannins, steroids and saponins (Khairany *et al*., 2015). One of the functions of flavonoids and tannins is antibacterial, and these substances are active compounds in plants that are efficacious as drugs that can cure infectious diseases caused by microbes.

One of the topical drug dosage forms intended for wound treatment is emulgel, because it has advantages such as good dosage consistency, long shelf life, moisturizing, fast absorption process, easy to spread evenly, soluble in air and can be mixed with other drugs and other additives. (Haneefa *et al*., 2013). Emulgel preparations have the ability to penetrate damaged tissue layers caused by bacterial infections, and one of the factors that causes wounds in diabetics is bacterial infection. (Ulviani, 2016). Taro leaf extract with a concentration of 5% has been made into an emulgel preparation with carbopol 940 as the building block (Apriani, 2021). Furthermore, the emulgel will be tested preclinically for wound healing in diabetic rats.

MATERIALS AND METHODS

Instruments

The equipments use to formulate emulgel were a homogeniser (IKA®-Malaysia), refrigerator (LG®-south Korea), oven (Memmert®-Germany), pH meter (Ohaus®-Poland), furnace (Daihan®-Indonesia), analytical balance (LabPro), vacuum dryer, Brookfield viscometer (DV-I Prime®-India), analytical balance (Mettler Toledo®-United States), hair clipper (Gillette-United States), syringe (One Med®-Indonesia), Easy Touch glucometer, sterile scalpel (B BRAUN) and caliper (Trical Brand®-China).

Chemicals and Reagents

The material applied to make emulgel is fresh taro leaves (Cibalung-Bogor Village). The ingredients for carbopol 940, cetostearyl alcohol, virgin coconut oil, and sodium lauryl sulfate were obtained from (Palapa Muda Perkasa-Depok, Indonesia). Methyl paraben and propyl paraben were obtained from (Alfalab Chemika-Bogor, Indonesia), propylene glycol (SamirasChem®-Bogor, Indonesia), triethanolamine (Graha Chemical-Bogor, Indonesia), 70% ethanol (Brataco-Indonesia).

The pharmacological testing materials used were white male rats Spraque-Dawley strain (weight 150-250 grams aged 2-3 months), Ketamine HCL (Marcil Risty Shop-Indonesia), xylazine, 70% alcohol, Cutimed® Gel (BSN medical-Indonesia) and alloxan monohydrate (Rofa Laboratory Center-Indonesia).

Preparation of Plant Materials and Extraction

The material used in this study was taro leaves obtained from Cibalung village, Cijeruk sub-district, Bogor, West Java and identified at the Center for Biological Research Institute - LIPI Cibinong-Bogor.

The taro leaves were collected and separated from the impurities, washed thoroughly with running water, drained and dried; the dried taro leaves were sorted. Furthermore, the taro leaves were dried an oven at a temperature of 40-50ºC for ± 48 hours or until the leaves were dry. The dried taro leaves were crushed and sieved with a 40 mesh sieve.

The 500-gram taro leaf powder was extracted by maceration methods using 70% ethanol solvent in a ratio (1:10) for three times a day at a temperature of 25 degrees celsius in a place protected from direct sunlight. Stirring and changing the solvent is carried out every a day.

Determination of Total Flavonoid Content of Taro Leaf Extract

Total flavonoid levels were determined using quercetin analysis markers using a UV-Vis spectrophotometer. The classic series of quercetin made were 2, 4, 6, 8, and 10 ppm. The concentration was determined by weighing 50 mg of taro leaf extract and then dissolving it with methanol in a 50 ml volumetric flask to the limit. The solution was shaken for 10 minutes to dissolved the extract. Then 2 mL of the solution was pipetted, then put into a 50 mL volumetric flask, then 1 mL of 10% AlCL3, 1 mL of 1 M sodium acetate and distilled water was added to the limit. The solution was shaken until homogeneous and left for an optimum time of 20 minutes, and then the absorption was measured at a maximum wavelength of 434 nm.

Emulgel dosage formulation

Gel formulation ingredients: Carbopol 940 2%, Methyl Paraben 0,18%, Propyl Paraben 0,02% Triethanolamine (TEA) 1%, Aquadest 21,8%. Emulsion formulation ingredients: Taro Leaf Extract 5%, sodium Lauryl Sulfate 0,5%, Propylene Glycol 10%, Setostearyl Alcohol 4,5%, Virgin Coconut Oil (VCO) 20%, Aquadest 35%.

The formulation emulgel began with making an emulsion base by mixing all the ingredients. The aqueous phase contained a mixture of sodium lauryl sulfate, cetostearyl, propylene glycol and aquadest, placed in a cup, whereas the oil phase contained virgin coconut oil. Furthermore, the water and oil phases were placed in a water bath at a temperature of 60 to 70 degrees celsius until dissolved. Taro leaf extract was added into the oil phase and stirred with a homogeniser at 200 rpm for 10 minutes. Then, the oil phase was mixed with the water phase, and the two phases were stirred at 500 rpm for 20 minutes until mixed homogeneously.

The stage of making a gel base was developing a gelling agent carbopol 940 by dispersing carbopol in hot aquadest at a temperature of around 80 to 100 degrees Celsius. The mixture was allowed to stand for 24 hours until colloidal dispersion acid was formed, then stirred with a homogeniser at a speed of 500 rpm. Furthermore, the mixture was neutralised by adding 1% TEA little by little until a thick transparent gel was formed for 1 hour. After the gel base was transparent, methylparaben and propylparaben were added, which had been dissolved in ethanol. The mixture was stirred with a homogeniser speed of 500 rpm for 5 minutes until mixed homogeneously. The final stage was mixing the emulsion into the gel base and then mixing it using a homogenizer at 500 rpm for 10 minutes.

Evaluation of Emulgel Preparations

Organoleptic testing was carried out by direct observation of the colour, odour and shape of the emulgel preparations made.

The pH test of the emulgel preparation was carried out using a pH meter. The safe pH range for the skin is between 4.5-6.5. The pH test of the emulgel preparation was repeated three times (Tranggono *et al*., 2013).

Viscosity testing using the emulgel method was weighed as much as 100 grams in a beaker, and then the viscosity was determined using a Brookfield viscometer (Brookfield, 2014). Viscosity values for semisolid preparations are 2000-50000 cps.

The total flavonoid content of emulgel was determined by weighing 10 grams of emulgel (equivalent to 500 mg of extract) and then dissolved in a 50 ml volumetric flask using 25 ml of methanol. The mixture was sonicated for 15 minutes to allow the extract to be dispersed into the solvent. Next, the solution was added to methanol to the limit of the 50 ml volumetric flask (stock solution concentration was 10000 g/ml).

Diabetic Wound Healing Test Method

Experimental Animal Preparation

Ethics approval for test animal was obtained from the Animal Ethics Committee of the Faculty of Mathematics and Natural Sciences, Pakuan University to ensure whether the procedures to be carried out comply with the animal ethical review. The test animals used in this study were 25 healthy male Sprague-Dawley rats. The characteristic of the rats were white, red eyes, elongated head and tail that exceed the body's length (Larasati, 2013).

A total of 25 rats were prepared, homogeneity of rat's body weight was determined by calculating the Coefficient of Variation (CV). Experimental animals were declared homogeneous if the CV ranged from 10-15%. The rats were placed in separate plastic tubs of 30 cm x 20 cm x 12 cm, fed ad libitum and acclimatized for seven days to accustom the animals to the experimental condition (Harahap et al., 2015). Acclimatization of rats aims to minimize the effects of stress on test rats in a new environment which can affect the body's metabolism and interfere with the results of the research.

Grouping of Animal

After acclimatization, the CV values of test rats were recalculated, the rats that meet CV values requirements the divided into five treatment groups consisting of 5 rats. Grouping of test animal was divided into positive control (Diabetic mice treated with Cutimed® gel), negative control (Diabetic rats treated with emulgel base), normal control (Non diabetic rats treated with emulgel base), Treatment-1 (Diabetic mice treated with taro leaf extract once a day) and Treatment-2 (Diabetic mice treated with taro leaf extract once a day). Testing the effectiveness of emulgel as an antidiabetic includes:

a. Diabetes Induction Stage with Alloxan
   Prior to alloxan induction, white male rats were fasted for 12 hours. The initial blood glucose level of rast were measured to ensure normal blood glucose levels. The rats then intraperitoneally induced with alloxan at a dose of 150 mg/kg BW except the group of control rats (Nugroho, 2006).
b. Giving Treatment
   The rats' blood glucose levels were measured on the 4^{th} day or 72 hours after induction of alloxan. The blood glucose levels of rats was measured using a glucometer. The rats tail was pierced using a lancet or syringe to take rat blood (Harijanto *et al*., 2017), The rat's blood was pasted on glucometer stick to record the glucose blood glucose level. Rats were declared diabetes if their blood glucose levels were ≥ 200 mg/dL (ADA, 2017). The blood glucose levels in experimental animals were measured once a week to determined whether the mice were in a hyperglycemic condition or not (Candra *et al*., 2019).
c. Making incisions in diabetic mice

Experimental animals with diabetes were shaved on the back where the wound would be made. All experimental mice were anaesthetised with a combination of ketamine 0,05 mg/gBW and xylazine 0,005 mg/gBW via the intramuscular route applied (Rahmi, 2020). After being anaesthetised, the back of the shaved rat was applied with 70% alcohol and marked by drawing a circle with a diameter of 1 cm, by lifting the skin of the mouse (which has been marked) using tweezers and then carefully making a wound by cutting the part of the skin (which has been marked).) using a sterile scalpel (Cahaya *et al*., 2017) or reaching the dermis, characterised by bleeding (Eriadi *et al*., 2015).

d. Healing Cuts in Diabetic Rats
The treatment of the incision wound was started after the visual difference between the wound, and normal controls were performed topically and divided into five treatment groups. Emulgel applied to the wound as much as 200 mg or equal to 0,2 mL using a syringe. Applying was done evenly and equally to cover the surface of the wound. Appying done once a day (Muharty, 2019) and twice a day, and wound development was observed for 16 days (Rahmi, 2020).

e. Observation and Data Collection
Wound observation in diabetic rats was carried out visually from the development of wound healing on the rat's back. Observations started from day 1 in a row until day 16. Observations were made, including narrowing of the wound diameter and visual observation.

Observation parameters were:

1. Wound Diameter Measurement
The diabetic wound diameter were measure was done every three days by comparing the healing process of diabetic wounds. Measurements were carried out in various directions using the Morton method. Measurements started from the first day of treatment until the 16th day (Sumoza & Rahayu, 2014). Observation of the wound diameter started on the first day the rats were excised, measured using a calliper on four sides of the wound diameter and averaged; thus, the percentage of wound closure was obtained (Elfasyari *et al*., 2018).
2. Percentage of Diabetic Wound Healing Area
Using the percentage conversion formula, the wound diameter measurement was then converted into the percentage of healing (%).
3. Visual observation using a scoring
Parameters of visual observation using scoring are as follows:
1 = Wet wound, red
2 = The wound is slightly dry; a scab is formed
3 = Scab comes off, red and wet
4 = Redness, dryness and shrinking
5 = Wound heals without hair

Data Analysis

Data analysis applied SPSS software version 24. The experimental design used the Randomized Block Design method, arranged by experimental grouping units into several groups. The Randomized Block Design was used since two treatment factors were tested in the study. The first treatment factor was the factor once and twice the emulgel was applied and the second treatment factor was the duration. Furthermore, to test the difference in the mean of three or more groups, ANOVA (analysis of variance) was used. This facilitates the analysis of several different sample groups with minimal risk of error. If the results of the variance/variety test indicate a significant difference, further tests such as Duncan's multiple-area test or Duncan's Multi Range Test (DMRT) are carried out. The goal was to find out where the difference was or the location of the difference.

RESULTS AND DISCUSSION

Preparation of Taro Leaf Extract Emulgel

Emulgel preparations have a thick form, brownish-green colour, and a characteristic odour of taro leaf extract. The emulgel preparation of taro leaf extract has a pH of 5.8 and was safe for the skin (pH between 4.5 - 6.5) (Anief, 2007). The viscosity value of the taro leaf extract emulgel preparation is 42367 cP and met the standard requirements of 6000-50000 cPs (Handayani *et al*., 2015). The total flavonoid content of emulgel taro leaf extract with quercetin analysis marker was 9.30% (96.77%). The taro leaf extract emulgel is shown in Figure 1.

Figure 1. Taro Leaf Extract Emulgel

Experimental Animal Grouping

This research has met the ethical review by the research ethics committee of the Faculty of Mathematics and Natural Sciences, Pakuan University, with a decree No.037/KEPHP-UNPAK/11-2021 dated November 19, 2021.

The test animals used in this study were 25 healthy 4 -5 months male Sprague Dawley rats, weighing 150-250 grams and then divided into five groups. The test animals were then acclimatised for one week to adjust to the new environment, and the experimental animals were given BR-512 type feed and drinking ad libitum.

During acclimatization, the physical condition of each experimental animal was healthy, and the body weight of each experimental animal was increasing, indicating that the rats could adapt well to their environment. The results of the acclimatisation of experimental animals that lasted for one week obtained a coefficient of variation of 10,18% of the weight of the rats declared homogeneous since they met the requirements of no more than 15% (Nasution, 1992).

Examination of Blood Glucose Levels in Rats

The results of the examination of blood glucose levels during the study were carried out once every week using a glucometer by taking rat blood by piercing the rat's tail vein using a lancet or syringe (Harijanto *et al*., 2017). Rats were declared diabetes if their blood glucose levels were ≥ 200 mg/dL (ADA, 2017).

In the first and the second week, blood glucose levels during the study were measured to determine whether the mice were still in a hyperglycemic state or not (Candra *et al*., 2019). Based on the results of the study, the rats were still in a state of hyperglycemia. Alloxan could damage pancreatic β-cells with different damage; thus, not all pancreatic β-cells are damaged, and there was the ability of β-cells to regenerate. Consequently, β-cell could still produce insulin; hence blood glucose decreased (Kurnawan *et al*., 2014).

Giving alloxan could increase rats' blood glucose levels, disrupting insulin production caused by damage to pancreatic β-cells. Alloxan can enter pancreatic β-cells and is reduced to dialuric acid, which will then be oxidized back to alloxan, resulting in a redox cycle with peroxide radicals as the final product. This peroxide radical will undergo a dismutase process to become hydrogen peroxide. Hydrogen peroxide and Fe^{2+} would form reactive hydroxyl radical compounds (OH^-) that could cause damage to pancreatic β-cells; thus, glucose levels in the blood are high (Khoiri, 2021). The increase in blood glucose levels is shown in Table 1.

Table 1. The Results of Blood Glucose Levels in Rats

Group	Blood Glucose Level (mg/dL)			
	Before Alloxan Induction	After Alloxan Induction	1st week	2nd week
Positive control	106,8± 23,56	231,8± 13,10	227,0± 14,83	226,8± 9,93
Negative control	105,8± 41,26	228,8± 27,01	229,8± 23,78	228,8± 22,97
Normal control	115,4±28,18	122,0±10,93	126,0±11,40	121,8±10,56
Treatment-1	105,4± 20,45	227,0± 11,51	224,0± 12,94	223,8± 12,11
Treatment-2	112,6± 18,07	228,0± 16,26	223,8± 15,25	223,2± 13,10

Observation and Measurement of Wound Diameter

Wound observations were carried out every day for 16 days, whereas wound diameter measurements were carried out every three days until the wound diameter was equal to zero. Before treatment, all rats had to be diabetic except for standard controls, and all rats were shaved to make it easier to observe the diameter of the wound on the rat's back.

Rats were anaesthetised with a combination of ketamine 0,05 mg/gBW and xylazine 0,005 mg/gBW via the intramuscular route applied (Rahmi, 2020). Using ketamine and xylazine as an anaesthetic is to reduce pain due to injury. Ketamine as an analgesic and xylazine would cause muscle relaxation (Yudaniyanti *et al*., 2010).

Treatment of the cut was started after the visual difference between the wound, and normal controls were four days after the formation of infection, which was indicated by the presence of wet wounds or pus (pus) in the wound. There were five treatment groups; two groups were given taro leaf extract emulgel, treatment-1 and treatment-2 with a concentration of 5%, one group was given positive control (Cutemed gel), and two groups were negative control, and standard control was given emulgel basis. Applying on the wounds of each treatment group was carried out every day.

Applying the emulgel preparation on the incision wound as much as 0,2 mL using a syringe evenly and equally to cover the wound surface. Applying the emulgel was performed daily, at 09.00 am for positive control, negative control, normal control and treatment-1. Meanwhile, the two emulgel preparations were applied twice a day, namely at 09.00 am and 3.00 pm—for observation of wound development for 16 days (Rahmi, 2020).

Wound observations in rats were carried out visually every day from the development of wound healing on the rat's back, starting from day 1 to day 16. Observation of wound diameter was measured using a calliper on four sides of the wound diameter and averaged to obtain the percentage of wound closure (Elfasyari *et al*., 2018).

The method used in this study was to use the Morton method. This method measures wound diameters taken from different sides to determine the accurate narrowing of the incision in diabetic rats (Sumoza & Rahayu, 2014).

The data from the wound diameter study were analysed using parametric statistical tests; SPSS version 24 ANOVA analysis indicated a significant effect on treatment factors, days, and interactions between treatments and days on the results of the diameter of the incision with the value of Sig. 0,000, which is smaller than the value of the significance level (α) of 0,05.

Based on the results of Duncan's further test to determine whether there was a significant difference or not for the parameter of decreasing wound diameter, subsequently treatment-1 and treatment-2 gave significantly different results from the negative control. This indicated that applying the emulgel preparations of taro leaf extract in treatment-1 and treatment-2 had effective healing cuts in diabetic rats. The results of observing the diameter of the wound are in Table 2.

Table 2. Wound Diameter Results in Animals Test

Treatment	Length in days (cm)					
	1	4	7	10	13	16
Positive control	$1,01^{hi}$± 0,00	$1,02^{hi}$± 0,01	$0,89^{gh}$± 0,11	$0,67^{efg}$± 0,20	$0,27^{bc}$± 0,16	$0,05^{ab}$± 0,12
Negative control	$1,02^{hi}$±0,01	$1,03^{hi}$±0,01	$1,01^{i}$±0,01	$0,95^{h}$±0,06	$0,70^{fgh}$±0,13	$0,61^{ef}$±0,16
Normal control	$1,02^{hi}$±0,00	$1,01^{hi}$±0,00	$0,96^{hi}$±0,04	$0,91^{gh}$±0,08	$0,62^{ef}$±0,26	$0,27^{bc}$±0,37
Treatment-1	$1,02^{hi}$±0,00	$1,03^{hi}$±0,00	1^{hi}±0,03	$0,89^{gh}$±0,16	$0,39^{cd}$±0,29	$0,14^{ab}$±0,25
Treatment-2	$1,02^{hi}$±0,00	$1,02^{hi}$±0,01	$0,85^{gh}$±0,12	$0,50^{de}$±0,14	$0,17^{ab}$±0,16	0^{a}±0

Note: Figures followed by the same superscript indicate no significant difference between Treatment and the mean diameter of the incisions in P <α 0.05 mice based on Duncan's further test.

Treatment-1 and regular control indicated the same superscript letters to decrease wound diameter, although the rats were not diabetic in standard control. This means that the healing rate can match this standard control since the body is homeostatically capable of protecting and restoring itself and restoring damaged tissue components by forming new and functional structures similar to the previous state (Maryunani, 2013).

Treatment-2 can reduce the diameter of the wound to be smaller than the positive control on decreasing the diameter of the wound, meaning that two times the emulgel has the most effective healing effect. In addition, treatment-1 and treatment-2 indicated significantly different superscript results in reducing wound diameter, meaning that applying emulgel twice (treatment-2) was more effective than applying one time (treatment-1).

Healing Time for Cuts in Diabetic Rats

Based on the length of wound healing, it could be seen that there was a significant difference in the decrease in the diameter of the incision, and the 13th day of wound healing is the best. The other interaction test result between treatment and applied duration indicated that positive control, negative control and Treatment-2 had significantly different effects on the 10th, 13th and 16th days. On the 7th day, treatment-2 had decreased wound diameter, the same as the positive control. On the 10th, 13th and 16th days, Treatment-2 had the best reduction in diameter compared to the other treatment groups since treatment-2 rats were given treatment-2 times to accelerate the healing of cuts in diabetic rats.

Percentage of Healing of Cuts in Diabetic Rats

The measurement of wound diameter is then carried out by calculating the percentage of wound healing using the percentage conversion equation (1).

$$P_x = \frac{d_1 - d_x}{d_1} \times 100\% \quad (1)$$

Information :
Px : Percentage of wound healing on day x (in %)
d1 : First-day wound diameter (cm)
dx : wound diameter x day (cm)

Observations on the 4th day gave a negative value in all treatment groups except the standard control group (non-diabetic mice) since all treatments were in a diabetic state; thus, the wounds formed were infected, which was indicated by wet or festering wounds. This condition indicates that wounds in people with diabetes who do not get treatment and care immediately will be prone to bacterial infections that quickly spread and, in other circumstances, cause gangrene (Prameswari, 2017). On the 7th, 10th, 13th and 16th days, the wound began to shrink since the wound had been given treatment.

Visual Observation of Cuts in Diabetic Rats

Observing the condition of the wound visually is an additional parameter that is observed after observing the diameter of the wound and is made based on the scoring table. Observational data are shown in Table 3.

Table 3. Percentage of Cut Wound Healing in Diabetic Rats

Treatment	Percentage of Cut Wound Healing (%)					
	1	4	7	10	13	16
Positive control	0^{ab}	$-1{,}19^{a}\pm 1{,}75$	$21{,}40^{bcde}\pm 18{,}30$	$53{,}46^{fg}\pm 22{,}71$	$90{,}79^{jkl}\pm 6{,}62$	$98{,}49^{kl}\pm 3{,}37$
Negative control	0^{ab}	$-2{,}38^{a}\pm 2{,}93$	$1{,}53^{ab}\pm 3{,}49$	$12{,}27^{abcd}\pm 10{,}85$	$36{,}90^{ef}\pm 24{,}89$	$61{,}91^{ghi}\pm 20{,}13$
Normal control	0^{ab}	$1{,}94^{abc}\pm 0{,}01$	$10{,}45^{abcd}\pm 8{,}64$	$22{,}36^{cde}\pm 11{,}95$	$57{,}41^{gh}\pm 29{,}36$	$81{,}91^{jkl}\pm 24{,}98$
Treatment-1	0^{ab}	$-1{,}95^{a}\pm 0{,}01$	$4{,}19^{abc}\pm 5{,}06$	$22{,}71^{cde}\pm 25{,}17$	$78{,}86^{ijk}\pm 31{,}10$	$92{,}85^{jkl}\pm 14{,}99$
Treatment-2	0^{ab}	$-1{,}18^{a}\pm 1{,}75$	$28{,}54^{de}\pm 19{,}33$	$73{,}96^{hij}\pm 12{,}94$	$95{,}03^{kl}\pm 5{,}97$	$100^{l}\pm 0{,}00$

Note: numbers followed by the same superscript in the same column indicate that there is the same effect on the percentage of wound healing in rats ($P < 0.05$)

The results of visual observation indicated that treatment-2 had almost the same score as the positive control; Treatment-2 had almost the same effect as the visual control from day 0 to day 16.

Based on the picture of wound healing, on day 0, the wound looks red, and bleeding occurs after being injured. Treatment of cuts started on the fourth day after the infection, which was marked by the presence of wet or purulent wounds in all treatment groups except normal controls (non-diabetic rats). The formation of pus characterizes the occurrence of infection. Visual observations for wound healing are shown in Table 4 and the scoring score is in Table 5.

Table 4. Visual Observation of the Wound

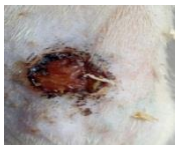
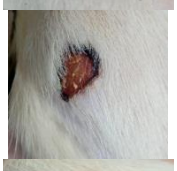
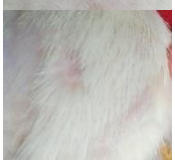
Day	Control Positive	Control Negative	Control Normal (Normal Control (Without Diabetes)	Treatment-1	Treatment-2
0					
4					
7					
10					
13					
16					

Table 5. Visual Scoring Observation of Cuts in Diabetic Rats

Treatment	Score on Day-					
	1	**4**	**7**	**10**	**13**	**16**
Positive control	1^{a}±0	2^{bc}±0	$2,6^{de}$±0,54	$3,6^{fg}$ ±0,54	$4,2^{ghi}$±0,83	$4,8^{ij}$±0,44
Negative control	1^{a}±0	1^{a}±0	$1,4^{ab}$±0	$2,2^{bcd}$±0,44	$2,8^{de}$±0,44	$3,4^{ef}$±0,54
Normal control	1^{a}±0	$1,4^{ab}$±0,54	2^{bc} ±0	3^{ef}±0	$3,6^{fg}$±0,54	$4,2^{ghi}$±1,09
Treatment-1	1^{a}± 0	$1,6^{ab}$±0,54	$2,6^{cd}$±0,54	$3,4^{ef}$±0,54	$3,6^{fg}$±0,54	$4,4^{hij}$±0,89
Treatment-2	1^{a}±0	$1,8^{b}$±0,44	$2,8^{de}$±0,44	$3,8^{fgh}$±0,44	$4,4^{hij}$±0,54	5^{j}±0

Note: numbers followed by the same letter in the same column state that there is no significant difference based on Duncan's further test at a significance level of 0.05

Mechanism of Flavonoids in Taro Leaf Extract Emulgel for Healing Cuts in Diabetic Rats

Wound healing in diabetic conditions is different from normal conditions due to the presence of blood flow and oxygenation in diabetic wounds due to impaired blood glucose, decreased synthesis of collagen and fibronectin and decreased insulin levels. The purpose of wound healing in diabetic conditions is to accelerate wound closure by stimulating growth factors; thus, they can generally work (Kurnawan *et al*., 2014).

The mechanism of wound healing with taro leaf extract can occur since taro leaf extract contains flavonoid and phenolic compounds that can accelerate the wound healing process. Flavonoids work as anti-inflammatory and antibacterial, regenerating skin tissue; thus, the skin will be covered by new skin faster (Ristanti *et al*., 2021). In comparison, hence, polyphenol compounds contain antioxidants that increase anti-inflammatory and immune capabilities (Larissa *et al.,* 2017). The mechanism of action of all flavonoids is blood circulation to the body and preventing events in the blood vessels, as an anti-inflammatory and antioxidants that help reduce pain if bleeding or swelling occurs (Handayani *et al.,* 2015).

CONCLUSION

The emulgel preparation of taro leaf extract at a concentration of 5% (Treatment-2) was effective for wound healing in diabetic rats. Emulgel preparations applied twice a day were most effective for wound healing. The length of time for wound healing in diabetic rats was 13 days. There was an interaction between treatment and the length of time for wound healing in diabetic rats given taro leaf extract emulgel.

ACKNOWLEDGMENT

The writer would like to thank LPPM and the Pakuan Siliwangi Foundation for providing funding. Thus, this research can run smoothly.

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