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Nanoemulgel activity of Binahong (*Anredera cordifolia* (Ten.) Steenis) leaf extract againts wound healing of hyperglycemic rats

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ABSTRACT

Hyperglycemia is a condition where blood sugar levels exceed normal limits. This is related to damage throughout the body, causing ulcers on the legs or so-called diabetic wounds. The content of flavonoid compounds, steroids, and saponins in binahong leaves plays a role in healing diabetic wounds. This study aims to determine the wound healing activity of diabetes in nanoemulgel preparations of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) with its extract in male rats wistar hyperglycemia. The study group was divided into 4 groups got each negative control group, positive control group, N1 test group, and N2 test group. Measurements of the diameter of diabetic wounds are carried out on days 1, 5, 14, and 16. On the 16st day, or as one of the groups healed, animals were sacrificed to continue histopathological testing of wound tissue with Hematoxylin-Eosine staining and saw an increase in the number of fibroblasts and thickening of the epithelium. Diabetic wound healing results between the positive control group and the N1 treatment showed significant differences compared to the negative controls versus N2 treatment of diabetic wounds healing. The results of histological observations showed thickening of the epithelium and increase in the number of fibroblasts of the positive control group and significantly different N1 treatments to negative controls versus N2 treatment. Nanoemulgel is accelerating the healing process of rat diabetic wounds, as well as accelerating the reepitalization process by increasing the thickness of the epithelium and fibroblast tissue compared to the binahong leaf extract group.

Keywords: *Anredera cordifolia* (Ten), steenis, activity, diabetic wounds, Nanoemulgels

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INTRODUCTION

Hyperglycemia is conditions when the level of sugar in the blood exceeds normal limits. Hyperglycemia is associated with damage to some organs, such as narrowing of blood vessels, hardening of blood vessels as well as nerve damage throughout the body. This causes ulcers on the legs, called diabetic wounds. In general, the treatment of diabetic wounds in the community is carried out with a bandage accompanied by betadine compresses and normal saline. However, the long-term use of the dressing can lead to slow wound healing and can appear various infections (Mutiara et al., 2015).

The wound healing process involves several processes, including inflammatory, proliferation, maturation, and remodeling phases involving various cells, such as fibroblast cells, that are responsible for the formation and maintenance of connective. In the proliferation phase, there is an increase in the number of wound components, one of which occurs fibroblast proliferation.

The plant associated with improving the condition of diabetes mellitus through a healing diabetes wounds that have been widely used in Indonesia is binahong plant (Anredera cordifolia (Ten.) Steenis). Binahong contains the main compounds flavonoids, saponins, and terpenoids that work in lowering blood sugar, accelerating wound healing through an increased number of fibroblast cells and thickening of epithelium (Hu et al., 2014; Mutiara et al., 2015; Patra, 2012). However, treatment using natural ingredients has a drawback, namely the relatively small content of active substances so that large doses are needed, which can cause the administration of drugs not acceptable.

One of the uses of herbs developed in pharmaceutical preparations is through nanoemulgel. Nanoemulgel is an emulsion preparation with a droplet size of 1-100 nm suspended in a hydrogel. Oil components, surfactants, and cosurfactants can increase the penetration of active substances which will increase therapeutic activity. Based on research conducted by (Anwar et al., 2021) obtained the optimal formula of nanoemulgel binahong leaf extract (Anredera cordifolia (Ten.) Steenis) with formulas and formulations that support the transdermal activity of the drug, but the wound healing activity of the preparation is not yet known. The purpose of this study was to determine the activity and mechanism of wound healing of diabetic nanoemulgel binahong leaf extract (Anredera cordifolia (ten.) Steenis) in Wistar male rats.

MATERIALS AND METHODS

Materials

Fresh binahong leaves, aquadest (technical), carbopol 940 (technical), chloroform (technical), disposable (medical), ethanol 96% (technical), formalin 10% (technical), glucose (pharmaceuticals), hypafix, iodine 5% (technical), ketamine hydrochloride, madecassol 1%, methylparaben (pharmaceuticals), oral disposable, PEG 400 (pharmaceuticals), propylene glycol (pharmaceuticals), standard rat feed, triethanolamine (pharmaceuticals), tween 80 (pharmaceuticals), VCO (pharmaceuticals).

Methods

Nanoemulgel Formulation

The BLE nanoemulgel formula to be tested for activity can be seen in Table 1. The manufacture of nanoemulgel begins with mixing tween 80 and PEG 400 (mixture of 1). Mixture 1 was added into VCO and stirred using a magnetic stirrer for 2 hours (mixture 2). The binahong extract is dissolved into the aqueous and put into mixture 2 while stirring using a magnetic stirrer hotplate for 1 hour. The mixture was further sonicated for 1 h until a nanoemulsion formed (Sanaji & Liananda, 2019). The nanoemulsions that have been made are then tested for percent transmitter to determine the clarity of the nanoemulsions formed as an illustration of the formation of nanometer-sized droplets.

The addition of a gel base is carried out by first developing Carbopol 940 in a hot aqueous for 24 hours. The expanded carbopol is added with TEA until a gel mass is formed. Methyl parabens are dissolved in propyllenglikol and added to the gel base. The nanoemulsions are then added to the gel base and homogenized using a mixer until a mass of nanoemulgel is formed (Handayani et al., 2015).

Nanoemulgel preparations are then characterized as including organoleptic, dispersal, adhesion, pH, and viscosity (*viscometer* Rheosys Merlin VR).

Table 1. BLE nanoemulgel formula

Materials	Consentration (%)
Binahong leaf extract	5
Tween 80	50
PEG 400	26.25
VCO	6.25
Aquadest	12.5
Nanoemulsions	25
Carbopol 940	2.5
TEA	2
Metilparaben	0.05
Propilenglikol	10
Aquadest	60,45

Diabetic wound healing activity assay

The number of experimental animals used is based on the calculation of the freed formula, namely (n-1) (t-1) ≥ 15. The test animals used in this study were white rats (Rattus norvegicus), as many as 24 male sex that weighed 150-250 g which were divided into 4 groups, namely:

a. Negative control group (glucose + nanoemulgel base)
b. Positive control group (glucose + madecassol 1%)
c. N1 treatment group (glucose + EDB nanoemulgel preparation)
d. N2 treatment group (glucose + binahong leaf extract)

The test animals were adapted to a new environment for 7 days before testing, then satisfied the first 12 hours before being given glucose and checked blood sugar levels using a glucometer. Glucose administration is carried out orally at a dose of 4.5 g/KgBB for 8 days. Glucose is dissolved with 3 mL of aquaades and made in fresh condition for use within 10-15 minutes. Checking the blood sugar level (KGD) of the test animal is carried out after glucose administration (Tanuwijaya et al., 2019).

Hyperglycemia wound creation if blood sugar levels ≥135 mg/dL (Wolfensohn & Lloyd, 1994). Previously, rat hair was shaved to the back and marked with a size of 3x5 cm. Test animals were anesthetized first using ketamine hydrochloride 0.1 mL at a dose of 3-5 mg/KgBB intramuscularly. The test animal was put in a cage and waited for 5 minutes until the test animal lost consciousness. The back of the test animal was then disinfected using 5% povidone-iodine in the area to be injured. The back was wound using a 5 mm biopsy punch with a depth of 1 mm using scalpel handle no.3 and surgical blade no.10.

Topical application of BLE nanoemulgel preparations in rat wounds was carried out two times every 12 hours for 16 days (Tanuwijaya et al., 2019). The measurement of the wound diameter begins on the first day the rat is excised, measured using calipers on four sides of the wound diameter so that a percentage of wound closure is obtained. Observation of diabetic wound healing is carried out on the 1st, 5th, 14th, and 16th days.

Histopathological assay

On the 16th day, the test animal was sacrificed to continue histopathological testing of wound tissue. Skin tissue was taken, and made histopathological preparations with Hematoxylin-Eosin staining according to standards carried out at the Pathology Laboratory of the Faculty of Medicine UGM, Yogyakarta. The slides that were created were observed with a light microscope magnified 400 times and saw an increase in the number of fibroblasts and the thickness of the epithelium.

Data Analysis

Data on wound closure diameter, increase in fibroblast count, and epithelial thickening was each statistically analyzed using the SPSS 16.0 program with the Shapiro-Wilk test, followed by the Kruskal-Wallis test. The statistical test is continued with Mann-Whitney if the value is asymp. Sig. <0.05 Signification is established if $p<0.05$.

RESULT AND DISCUSSION

Diabetic wound making

Hyperglycemia rats were then anesthetized using ketamine hydrochloride at a dose of 125 mg/KgBB intramuscularly. The wound is made using a 5 mm biopsy punch with a depth of 1 mm at the moment when the rat loses consciousness at the 10-15th minute.

Administration of Nanoemulgel BLE to cover diabetic wounds

Topical application of BLE nanoemulgel preparations is carried out every 2 times a day from the 1st to the 16th day. Observations on diabetic wound closure obtained data as in Table 2.

Table 2. The mean diameter of ± SD wounds of rats at various treatments and times

Group	Diabetic wound healing (mm)			
	Day-1st	Day-5th	Day-14th	Day-16th
Negative Control	4,5 ± 0	4,28 ± 0,06	2,49 ± 0,54	2,1 ± 0,27
Positive Control	4,5 ± 0	3,6 ± 0,28*	1,25 ± 0,68*	0*
N1 Treatment	4,5 ± 0	3,7 ± 0,31*	0,76 ± 0,36*	0*
N2 Treatment	4,5 ± 0	4,24 ± 0,07	2,42 ± 1,50	1,7 ± 1,32

* = significantly different ($p<0.05$) compared to negative control

Based on the data obtained in Table 2, the diabetic wounds of the positive control group and the N1 treatment group experienced a faster closure compared to the negative control group. On the 1st day, all groups did not show any wound healing. Significantly different wound healing ($p<0.05$) was demonstrated by the positive control group and the N1 treatment on the 5th, 14th, and 16th days compared to the negative control group. In contrast, the N2 treatment group showed no significantly different wound closure ($p>0.05$) compared to negative controls on days 5, 14th and 16th. A graph of the diabetic wound closure of rat hyperglycemia of each group can be seen in Figure 1.

The closure of this diabetic wound proves that the secondary metabolite compounds in the extract are flavonoids that act as anti-inflammatory, the formation of prostaglandins, and the release of histamine in inflammations, and as antimicrobials (Landen et al., 2016; Wang et al., 2016), Antioxidant (Munhoz et al., 2014), Saponin compounds are antiseptic and influential in spurring the proliferation of fibroblasts which plays a role in healing diabetic wounds (Melguizo et al., 2021; Sukandar et al., 2011), Polyphenol compounds act as antimicrobials (PI et al., 2015).

Wound closure in the N1 treatment group was faster compared to the N2 treatment group. This is because binahong leaf extract formulated in the form of nanoemulgel will more easily penetrate the membrane. The active substances in binahong leaf extract are difficult to penetrate the lipid membrane because it has a larger molecular size and low solubility in water, so the absorption of binahong leaf extract becomes low.

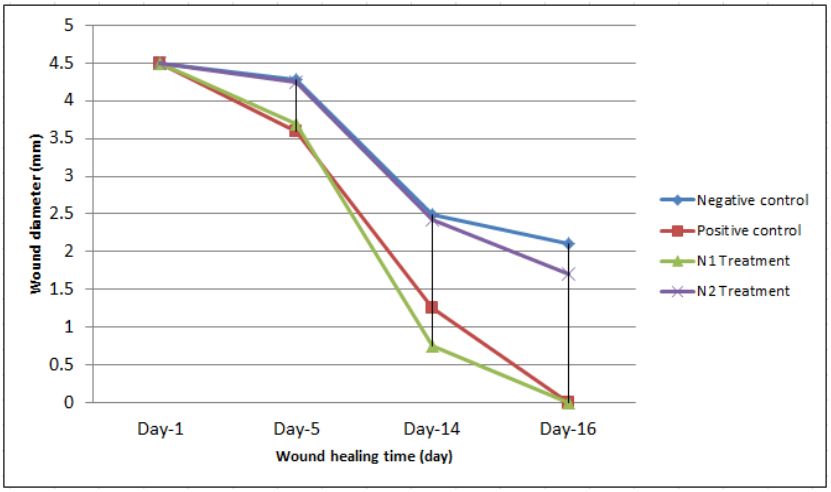

Figure 1. Graph of diabetic wound closure in hyperglycemia rats at various treatments and times

Histopathological observations of wounds

The results of testing using a microscope can be seen in Figures 2 and 3. As well as the average epithelial thickening and fibroblast scoring can be seen in Tables 3 and 4.

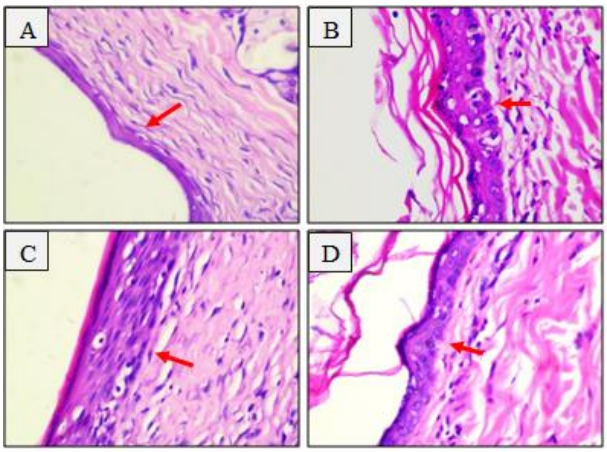

Figure 2. Epithelial thickening with HE staining using Olympus Microservice microscope with 400x magnification

Description: A: negative control, B: positive control, C: N1 treatment, and D: N2 treatment

Table 3. Average epithelial thickening of each group after treatment for 16 days

Group	Epithelial thickness (µm ± SD)
Negative Control	33,52 ± 9,86
Positive Control	109,69 ± 45,13*
N1 Treatment	89,44 ± 10,36*
N2 Treatment	49,31 ± 16,14

* = significantly different ($p<0.05$) compared to the negative control group

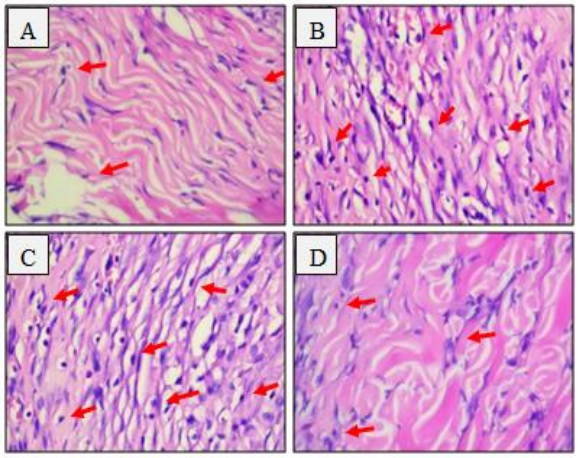

Figure 3. Increased number of fibroblasts (arrows) with HE staining using Olympus Microservice microscope with 400x magnification

Description A: negative control, B: positive control, C: N1 treatment, and D: N2 treatment

Table 4. Scoring the average number of fibroblasts of each group after treatment for 16 days

Group	Number of fibroblasts (Score ± SD)
Negative Control	10,66 ± 9,64
Positive Control	32 ± 34.42*
N1 Treatment	32.77 ± 33.40*
N2 Treatment	14.55 ± 14.67

* = significantly different ($p<0.05$) compared to the negative control group

Based on the results of histopathological observations of epithelial thickening and fibroblast cell enhancement, diabetic wounds of the positive control group and N1 treatment showed a significantly thicker epithelial layer ($p<0.05$) compared to the negative control group. Meanwhile, diabetic wounds in the N2 treatment group showed a thick epithelial layer that did not differ significantly ($p>0.05$) compared to the negative control group. Likewise, with the increase in the number of fibroblast cells, where the negative control group and the N1 treatment showed a significantly different increase in the number of fibroblasts ($p<0.05$) compared to the negative control group. Whereas the N1 treatment group showed a significantly different number of fibroblast cells ($p>0.05$) compared to the negative control group. This is due to the presence of active compounds of binahong leaf ethanol extract, including flavonoids that act as anti-inflammatories that can stimulate the proliferation of fibroblasts, support the epithelialization process, the formation of prostaglandins, and the release of histamine in inflammations so as to accelerate the wound healing phase (Mutiara et al., 2015). The content of saponins and terpenoids can spur the proliferation of fibroblasts and collagen formation and accelerate the epithelialization of wounds that play a role in healing diabetic wounds (Patra, 2012;Sukandar et al., 2011). In addition, it is influenced by binahong leaf extract formulated in the form of nanoemugel. Nanoemulgel easily penetrates the membrane. It helps the permeability of drugs on the surface of the membrane because the skin membrane is lipophilic and can maintain the oxidative stability of antioxidant compounds by accumulating oxygen molecules in the oil-water interphase (Rahman & Susianti, 2018).

CONCLUSION

Based on the research conducted, it was concluded that the administration of nanoemulgel preparations of binahong leaf extract is more effective in accelerating the healing of diabetic wounds in

hyperglycemia rats compared to binahong leaf extract. The wound healing mechanism of hyperglycemia rats given nanoemulgel preparations of binahong leaf extract through an epithelialization process with an increase in epithelial thickness and fibroblast cell count when compared to binahong leaf extract.

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