Efficacy of Bidara Leaf (Ziziphus Mauritiana) Viscous Extract to Gingival Wound Healing in Wistar Rats

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Abstract

This study aims to determine the efficacy of bidara leaf (Ziziphus mauritiana) viscous extract to accelerate wound healing in the gingiva of Wistar rats (Rattus norvegicus). The method used is an in-vivo experimental laboratory with a post-test-only control group design. Three groups of bidara leaf extract were made with different viscosities (100%, 80% and 70%) in a gel form, and 10% Povidone-iodine as a control group. Forty male Wistar rats were applied gel extract on the gingival wound according to the group, twice a day. On the 8th day, the animal's study were decapitated and continued with a histological examination procedure by counting the number of fibroblasts in each group.

The results showed that the highest number of fibroblasts was found in Bidara leaf (Ziziphus mauritiana) viscous extract group compared to other groups. The difference number of fibroblast cells of different concentrations is due to the effect of active substances contained in bidara leaf, like alkaloids, terpenoids, saponins, phenolics, flavonoids, and tannins. The active substances in bidara leaf could improve wound healing due to antibacterial and anti-inflammatory properties.

It can be concluded that bidara leaf viscous extract gel is more effective to increase the number of fibroblast cells on incision wounds of the experimental animals.

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Introduction

In dentistry, the wound healing process is often encountered in tooth extractions, incisions, oral and facial surgery, gingival flaps, and biopsies. Post tooth extraction wound is one of the mediums that can allow pathogenic microbes to breed and infect the wound. Post extraction wounds will heal easily as body physiologic response but will sometimes develop some complications. During abnormal healing, an acute post-surgical lesion could lead to chronic injury and generates severe disruption to the patients. Wound management must be performed as soon as possible to restore the skin integrity thus prevent bacteria infection penetrates the body.

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Wound healing is a specific biologic process related to tissue growth and regeneration. Wound healing consists of several phases: hemostatic and inflammation phase, proliferation phase, and maturation phase. This process consists of granulation tissue formation containing inflammation cells, new blood vessels, and fibroblast bonded to the extracellular collagen matrix.[1] During the proliferation phase, fibroblast from undifferentiated mesenchyme will produce collagen fiber-based material to link the wound edge. Fibroblast will also create a new connective tissue providing strength and integrity resulting in a good wound-healing process.[2] Enhancement of the number of fibroblasts will increase the number of collagen fibers resulting in the acceleration of wound healing.[3]

Treatment and handling of wounds so far have used Povidone-iodine, because it is antiseptic so that it can kill microbes and prevent infection by bacteria. The previous studies[4] showed that 10% Povidone-iodine can inhibit the growth of fibroblasts in wound tissue, and using above 10% doses can inhibit the formation of

granulation tissue.[5] Recently, the demand for plants and their formulation products is increasing tremendously all over the world due to the safety of plant drugs. Herbal medicines derived from plants and pure natural ingredients have side effects, the level of danger and risk is much lower than chemical drugs.[6] Presently, plants and their formulation products constitute more than 35% of the total drugs in use.[7] One of the medicinal plants that have the potential to treat wounds is bidara leaf or Indian jujube (Ziziphus mauritiana).

Indonesia is known as a great biodervisity country. There are about 30,000 plants and around 9,600 species are known to have medicinal properties, and about 200 species of which are important medicinal plants for the traditional medicine industry, used for digestive disorders, urinary troubles, diabetes, skin infections, diarrhea, fever, bronchitis, liver complaints, anemia, etc.[8,9] Biologically, Ziziphus species are known to possess various important pharmacological activities including antimicrobial10,[11,12,13,14] antioxidant, and anti-inflammatory properties,[15,16,17] antidiabetic, anti-malarial and anthelmintic properties,[18,19,20] anticancer, antiulcer, analgesic, sedative and antipyretic effects,[21,22,23] hepatoprotective activity,[24,25] diuretic[26,27,28] amongst other important activities.

The in vitro study[29] showed that 100% bidara leaf extract showed the most sensitive zone of inhibition to Staphylococcus aureus bacteria compared to other concentrations. Although many studies on the effectiveness of bidara leaf extract on wound healing have been carried out, there are still few studies on the effect of the viscosity of bidara leaf extract on the effectiveness of wound healing. This study aims to determine the efficacy of bidara leaf (Ziziphus mauritiana) viscous extract to accelerate wound healing in the gingiva of Wistar rats (Rattus norvegicus).

Materials and methods

This study was cleared for ethics by the Faculty of Dentistry, Mahasaraswati Denpasar University Review Board. The study procedures were the preparation of bidara leaf extract, phytochemical studies, and preparation of bidara leaf extract gel. The next procedures were in vivo study and histological examination.

Preparation of bidara leaf extract

The green bidara leaves were washed and dried at room temperature for 3 weeks and then crushed to a size of 60-70 mesh (simplicia). The extraction process was carried out by immersing the simplicia into 70% methanol solvent at a ratio of 1:10 for 24 hours. The maceration results were filtered 3 times with a butcher funnel lined with filter paper and stored in an Erlenmeyer. The filtered filtrate was evaporated with a vacuum rotary evaporator and heated with a water bath at 40ºC to obtain a viscous extract of bidara leaf.[30,31]

Preparation of bidara leaf extract gel

The bidara leaf extract is dissolved in some water that has been heated in a water bath, added Na-CMC, stirred until a homogeneous gel is formed and packaged in a gel container.[32]

Phytochemical study

A phytochemical study of bidara leaf extract was carried out at the Laboratory of Analytical Chemistry, Udayana University, Denpasar, Bali, Indonesia to determine the active substance content using Gas Chromatography-Mass Spectroscopy (GCMS).

To determine the flavonoid content, magnesium and hydrochloric acid reduction tests were used. The extract (50 mg) was dissolved in 5 ml of alcohol and some fragments of magnesium band and concentrated hydrochloric acid were added. If a pink to dark red color (reddish precipitated) is present, the presence of flavonoid content.[33] To determine saponin content, a foam test was used. The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a measuring cup for 15 minutes. A layer of foam of two cm indicates the presence of saponins.[34] To determine tannin content, a Ferric chloride test was used. The extract (50 mg) was dissolved in 5 ml of distilled water. A few drops of a 5% neutral solution of ferric chloride are added. Bluish precipitated indicates the presence of tannin content.[35]

The Mayer test was used to determine the alkaloids. For several ml of plant sample extract, two drops of Mayer's reagent are added along the side of the test tube. The appearance of a creamy white precipitate indicates the presence of alkaloids.[36] To determine steroids and terpenoids using the Libermann-Burchard method. The extract (50 mg) was dissolved in 2 ml of acetic anhydride. Two drops of

concentrated sulfuric acid are slowly added along the sides of the test tube. A series of color changes indicate the presence of steroids and terpenoids.[37] To determine the phenol content, a Ferric chloride test was used by adding FeCl3. A greenish precipitate indicates the presence of phenolic compounds.[36]

In vivo study

Forty male Wistar rats were divided into 4 groups. Wistar rats were anesthetized with ketamine (40 mg/kg BW) and xylazine (5 mg/kg BW), then an incision was made on the gingiva with a length of 15 mm to touch the alveolar bone. G1 is a group of animals that was applied 70% bidara leaf extract gel, G2 is a group of animals that was applied 80% bidara leaf extract gel, G3 is a group of animals that was applied 100% of bidara leaf extract gel, and G4 is a positive control group, that was applied with 10% Povidone-iodine. Applying the materials was carried out twice a day, in the morning and evening.

On the 8th day, the animal's studies were decapitated and followed by a histological examination procedure using Harris Hematoxylin – Eosin staining. Fibroblasts were seen using an electric microscope (Olympus type CX 21) with a magnification of 400 times using five fields of view. The fibroblasts counted were fibroblasts that had large cytoplasm, fine chromatin, ovoid nucleus and were visible. Furthermore, data analysis was carried out.

Results

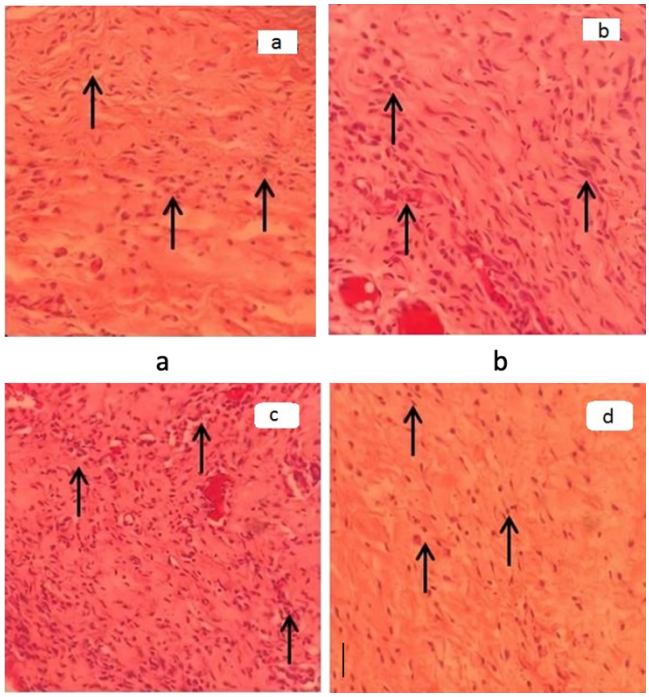
Phytochemical tests (see Table 1)

Fibroblast cell appearance

Histopathological appearance of fibroblast cells in wound healing was observed with 400x magnification, using an Olympus Type CX21 microscope taken from the gingival wound area of Wistar rats on the 8th day after incision (Figure 1).

No	Type	Sign	Results	Methode
1	Alkaloids	creamy white precipitate	++	The Mayer test
2	Steroids	greenish precipitated	-	Liebermann Buchard method
3	Terpenoids	reddish precipitated	++	Liebermaan Buchard method
4	Phenolic	greenish precipitated	+	Ferric chloride test
5	Saponins	foam	++	The foam test
6	Flavonoids	reddish precipitated	+	Mg and HCl reduction tests
7	Tannins	bluish precipitated	+	Ferric Chloride test

Table 1. Phytochemical tests of bidara leaf extract.



a b

Figure 1. Histopathological appearance of fibroblast cells. a)70% bidara leaf extract, b)80% bidara leaf extract, c)100% bidara leaf extract, d)10% Povidone-iodine

Groups	Mean of fibroblast	F	*p*
G1	118.7 ± 6.06	4.682	0.03*
G2	120.1 ± 5.87		
G3	159.7 ± 7.43		
C	66.8 ± 1.15		

Table 2. The difference means of fibroblasts number.

Note: G1: 70% bidara leaf extract, G2: 80% bidara leaf extract, G3: 100% bidara leaf extract, C: 10% Povidone-iodine.

The average number of fibroblast

Analysis of treatment effect was performed based on the average number of fibroblasts between the groups after treatment. The significance analysis using One-way ANOVA was presented in Table 2.

Discussions

Gas Chromatography-Mass Spectroscopy (GCMS) can be applied to solid, liquid, and gaseous samples. First, the samples are converted into a gaseous state then the analysis

is carried out base on the mass to charge ratio. In this method, samples distribute between a gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. The rate of migration depends on how much chemical substance is distributed into a liquid phase.[38] Phytochemical test (Table 1) showed that bidara leaf extract contained alkaloids, terpenoids, phenolic substances, saponins, flavonoids, and tannins, but on the contrary did not contain steroid substances. According to previous studies,[39] namely this extract contains large amounts of cyclopeptide alkaloids, flavonoids, tannins, saponins, terpenoids, fatty acids, and various phenolic compounds.

Toxic effects caused by synthetic antioxidants and the increasing resistance to antimicrobials have made the use of natural antioxidants derived from plants the focus of current scientific research. The use of various parts of medicinal plants has long been practiced to cure certain diseases due to the presence of several bioactive compounds such as alkaloids, flavonoids, essential oils, glycosides, tannins, terpenoids, steroids, and others.[40,41] The presence of these bioactive compounds has been widely used in pharmacology research and drug development. Antioxidants are effective compounds that can delay, interrupt or inhibit the process of oxidative reactions by neutralizing free radicals via donation of hydrogen atom or electron, quenching singlet and triplet oxygen, and chelating metals and thus play a proactive role towards improving the shelf-life of food products as well as reducing the incidence of different ailments such as cancer, aging, and inflammation.[42,43,44]

Group	G2	G3	C
G1	0.286	0.026*	0.045*
G2		0.015*	0.015*
G3			0.014*

Table 3. The least significant difference test of the number of fibroblasts between groups.
Note: G1: 70% bidara leaf extract, G2: 80% bidara leaf extract, G3: 100% bidara leaf extract, C: 10% Povidone-iodine.

One way Anova test reveals that the average of fibroblast number among the four groups was significantly different (p<0.05) after treatment. Table 3 shows that the 70% bidara leaf extract group was not significantly different from the 80% bidara leaf extract. On the contrary, the 80% bidara leaf extract group was significantly different from the 100% bidara leaf extract and the control group. In addition, the 100% bidara leaf extract group was significantly different (p<0.05) from all other groups. This shows that the administration of bidara leaf viscous extract has been shown to accelerate healing compared to other groups. Difference number of fibroblast cells found in the administration of bidara leaf extract gel caused by concentrations difference that affect active substances content in bidara leaf, like alkaloid, terpenoid, saponin, phenolic, flavonoid, and tannin. The higher the viscosity of the bidara leaf extract, the higher the active substance content, so that it can improve wound healing due to its antibacterial and anti-inflammatory properties.

Increased number of fibroblasts presence will accelerate the healing process. The content of compounds that contribute greatly to the anti-inflammatory effect of bidara leaf extract is alkaloids, polyphenols, and saponins. Alkaloid compounds could initiate fibroblasts towards the lesion area.[45] Saponin compounds could support wound healing from their abilities as a cleanser and stimulate collagen formation. Saponins have the ability as cleansers and antiseptics that function to kill germs or prevent the growth of microorganisms that usually arise in wounds so that wounds do not experience severe infections.[46,47] In addition to saponins, terpenoids are also known to play an important role in improving the wound healing process because they have antimicrobial effects, and strong antioxidants are responsible for wound contraction and increasing the speed of epithelialization. Flavonoid compounds can stimulate the induction of vascular endothelial cell growth factor (VEGF) in the angiogenesis process which is very important in the wound healing process because it has a function to facilitate growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) which play a role in the healing process.[48]

In this study, fibroblast cell has been used as the main indicator during the wound-healing process, because proliferation and migration of fibroblast cell into granulation tissue were needed

during wound closure, followed by extracellular matrix component deposition, wound contraction and remodeling. Fibroblasts are a wound healing component widely spread in connective tissue, producing collagen precursors substance, elastic fibers, and reticular fibers.[49] Fibroplasia is a wound repair process involving the connective tissue consist of four components, which are the formation of new blood vessels, fibroblast migration and proliferation, extracellular matrix deposition (ECM), and maturation and organization of fibrous tissue (remodeling). Among those four components, fibroblasts act in the process of fibrosis including two components, the migration, and proliferation of fibroblasts, and the deposition of ECM by fibroblasts. [49]

During the wound-healing proliferation phase, fibroblast cells produce cytokine and growth factors as TGF-β, CTGF, IL-6, and IL-8, which was a pleiotropic growth factor synthesize by different cell types in three isoforms. During wound healing, platelet and macrophage have a role as a trigger to the formation of TGF-β. TGF-β stimulates the migration and proliferation of fibroblast cells, supporting metalloproteinase's matrix to produce collagen and increase angiogenesis with Vascular Endothelial Growth Factor (VEGF). Furthermore, TGF-β stimulates reepithelialization and fibroblast transformation into myofibroblast.[1,50] Macrophage wills later developed granulation tissue which will attract fibroblast as the biggest number component in granulation tissue to the injury site and starting the collagen synthesis.

Conclusions

It can be concluded that bidara leaf viscous extract gel is more effective to increase the number of fibroblast cells on incision wounds of animals study, due to the higher content of active substance compared to other concentrations groups.

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Declaration of Interest

All authors have none to declare. All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

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