The Effectiveness of Ethanolic Extract of Moringa Leaves (Moringa Oleifera Lam.) Gel on the Wound Healing Process of the Rat's Palate

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Abstract

Moringa leaves (Moringa Oleifera) have many medical uses with high nutritional content thus known as the miracle tree. Moringa leaves also having phytochemical properties related to the wound healing process. **The purpose** of this study was to investigate the effectiveness of the ethanolic extract of Moringa leaves gel on the wound healing process of the Sprague Dawley rat's palate, clinically assessed by the wound healing area.

As much as 60 rats were divided into four treatment groups; group I treated with 2% ethanolic extract of Moringa leaves gel; group II with 4% ethanolic extract of Moringa leaves gel; group III with 10% povidone-iodine; and group IV with 4% HPMC gel (as control). Wounds were made on the rat's palate with a punch biopsy with a diameter of 4 mm. Wound healing clinical assessment was observed from the palate's mucosal tissue wound closure on day 0, 3, 7, 10, and 14; with the wound closure area measured using the callipers and rulers. All data obtained were statistically analysed using a one-way analysis of variance (ANOVA), followed by the Mann-Whitney's difference test.

Significant difference was found on the wound closure of Moringa group on day 3 and 14 ($p < 0.05$). A decreasing wound area on the groups applied with the ethanolic extract of Moringa leaves gel was higher than the control groups, with the highest closure was found in group II (4% ethanolic extract of Moringa leaves gel concentration).

Extract of Moringa leaves gel (Moringa oleifera) promotes the wound healing process by enhancing the wound closure of the rat's palate.

Experimental article (J Int Dent Med Res 2019; 12(2): 504-509)

Keywords: Ethanolic extract of Moringa leaves, wound healing.

Received date: 24 August 2018 **Accept date:** 10 December 2018

Introduction

Moringa oleifera Lam is a substantial medicinal plant, which included to the *Moringaceae* family. This plant has been widely consumed in many rural and urban areas of developing countries, such as countries in the African and Asian continents, due to its benefits in medicine and nutritional contents.[1,2] The people called Moringa plants as the miracle tree because of its remarkable healing properties on various diseases and some chronic diseases.[3,4] The medicinal properties have been recognised long enough in traditional healing treatment, with very high nutrients, extracted from the plant's leaves, seeds, and roots, that are potential remedies of the inflammatory and infectious diseases treatment.[5,6]

Moringa leaves contain aspartic acid, glutamic acid, glycine, threonine, alanine, valine, leucine, isoleucine, histidine, lysine, phenylalanine, tryptophan, cysteine, and methionine.[2] Various studies claimed that Moringa plants could be used as an immune boosting, antimicrobial, and helps the wound healing process.[7,8] Caceres et al. reported the antimicrobial activity of the Moringa leaves and roots in an in-vitro study using the disc-diffusion method. *Moringa oleifera* as an antibiotic is identified by the pterygospermin content, with the

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bioactive phytochemical of glucosinolate 4 alpha-L-rhamnosyloxy benzyl isothiocyanate.[9] Also, the anti-inflammatory properties are identified by pterygospermin and moringinine alkaloid content which can cause blood vessels constriction. Moringa leaves also contains other substances such as proteins, fats, carbohydrates, minerals, vitamins, and essential amino acids.[3,10]

The wound is a loss of tissue continuity, which will be followed by the wound healing process begins with the scar tissue formation. Wound healing is a complex process which divided into three phases: the inflammatory phase, the proliferation, and the remodelling phase.[11] Wound healing is a form of response from the connective tissue. The initial phase of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules which then form scars or scar tissue.[12] Appropriate wound healing methods are essential for a sustained recovery of damaged anatomical.

Many factors are affecting the wound healing period, such as age, nutrition, immunosuppression status, systemic interference, and the extrinsic factors such as medication and treatment. Many ways can be done in the wound care management, for example by giving antiseptic substances. However, side effects often arise in the use of chemical substances, so finding the alternative herbal remedies with relatively small side effects is necessary. Research the effect of herbal ingredients on wound healing has been done, various herbal ingredients such as betel quid extract,[13] propolis,[14] turmeric liquid extract,[15] brotowali extract (Tinospura crispa).[16] Exploration, cultivation, and research of herbal remedies are developing, such as the Moringa plants (*Moringa Oleifera Lam.*).[17] This study was aimed to determine the effectiveness of Moringa leaves extract gel in 2% and 4% concentration on the wound healing of Sprague Dawley rat palate, through the clinical assessment.

Materials and methods

This study was an experimental study on Sprague Dawley rats, aged 2 - 3 months, weighing 250-300 grams. As much as sixteen rats were divided into four groups; group I was applied with 2% Moringa leaves extract gel; group II with 4% Moringa leaves extract gel; group III with 10% povidone-iodine; and group IV with 4% HPMC gel (vehicle only). Each group consisted of 15 rats, analysed on five periods, from day 0, 3, 7, 10, and 14 (with three rats in each analysing period). The protocol and treatment of the animals have been approved by the animal care and use committee of the Faculty of Medicine Universitas Padjadjaran, with registration number 33/UN6C10/2018.

Moringa leaves extract preparation

Moringa leaves were collected in April 2017 from Kefamenanu, East Nusa Tenggara, at 50 m above the sea level. It was authenticated with the collection number of 049/HB/02/2017, deposited in The Plant Herbarium of Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java, Indonesia. The fresh leaves were collected, surface sterilised, sun-dried for seven days and then grounded. The powdered material (1.5 kg) was exhaustively extracted (3 cycles/hour) with 95% ethanol in soxhlet apparatus by continuous hot extraction. After each extraction, the solvent was recovered using a flash evaporator, and the extract was concentrated under reduced pressure. Then the crude extract was dissolved in the solvent and stored in air-tight glass bottles at 4°C, and later re-dissolved in their respective solvents to the desired concentrations for the various experiments.

The examination was performed by dividing the preparation of Moringa leaves gel extract into two different concentration, 2% and 4%, by dissolving as much as 1 g and 2 g extract in 5 ml of aquadest with an ultrasonic instrument, and subsequently mixed with 50 g gel basis of 4% HPMC as vehicle until it became homogeneous. The ethanol extract gel was prepared in the Laboratory of Pharmacy, Jenderal Ahmad Yani University, Cimahi, West Java, Indonesia.

Surgical procedure

As much as 60 Sprague Dawley rats were obtained from a local vendor farm in Bandung. One week before the experimental procedures, all the rats were adapted to the air-conditioned animal laboratory room (22 ± 3°C) with 12h light and dark cycle. All rats were fed with a

commercial common rodent pellet and filtered water ad libitum. The anaesthetic action was performed using ketamine 75 mg/kg BW and xylazil 10 mg/kg BW, injected intra peritoneum. The wound was made on the palate using a 4 mm diameter biopsy punch (Mentok® Co., Ltd, India).[18] All rats were divided into four treatment groups; group I was applied with 2% Moringa leaves extract gel; group II with 4% Moringa leaves extract gel; group III with 10% povidone-iodine; and group IV with 4% HPMC gel. The gel application towards the wound area in the rat's palate once a day for 14 days. Each group was performed necropsies towards every three rats on day 0, 3, 7, 10, and 14, with exsanguination under the anaesthetic effect. Clinical assessment of wound healing was observed from the wound closure area in the palate mucosal tissue, using callipers and rulers.

Statistical analysis

All data were presented in the mean ± SD of every group. The average difference of the wound area in every group was statistically analysed using the one-way analysis of variance (ANOVA) followed by Mann-Whitney different test, with a significance value of $p < 0.05$.

Results

The measurement of the wound area diameter on day 0, 3, 7, 10, and 14, resulted in quantitative data analysed statistically. The fastest wound contraction was seen in the group with application of 4% Moringa leaves extract. In this group, decreasing of the wound area on day 3 reached 1 mm thus made the wound diameter became 3 mm wide. In the group with application of 2% Moringa leaves extract, decreasing of the wound area on day 3 reached 0.7 mm thus made the wound diameter became 3.33 mm wide; while in the group with application of povidone-iodine and HPMC, decreasing of the wound area on day 3 only reached 0.5 mm thus made the wound diameter became 3.5 mm. The highest decrease was seen in the group with application of 4% Moringa leaves extract, followed with the 2% Moringa leaves extract application group, povidone-iodine application, and HPMC application respectively (Figure 1).

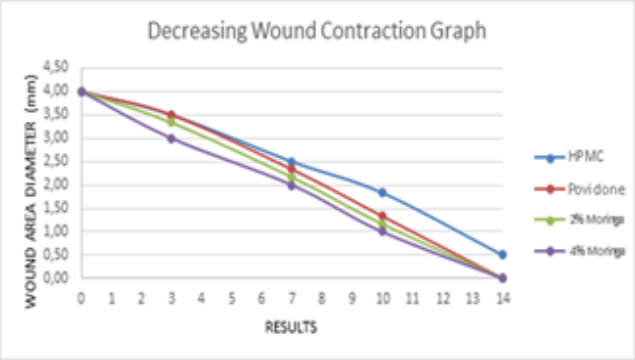

Figure 1. Wound area diameter of groups with the application of HPMC, povidone-iodine, 2% moringa leaves extract, and 4% moringa leaves extract, based on the wound healing time.

On day 14, only the HPMC application group still had a wound diameter with an average of 0.5 mm (Table 1). The difference in the average of the wound area between all four treatment groups was statistically analysed using the one-way analysis of variance (ANOVA) test. The test results showed that there was a significant difference between the average of the wound area on day 3 and day 14 ($p = 0.014$; $p = 0.01$). This result showed that there was an effect of Moringa leaves application towards the wound healing, observed from the decrease of the wound area on day 3 and day 14. While on day 0, 7, and 10, no significant differences between all treatment groups found.

Group/Day	D0	D3	D7	D10	D14
I (2% Moringa)	4.00	3.33	2.17	1.17	0.00
II (4% Moringa)	4.00	3.00	2.00	1.00	0.00
III (Povidone-Iodine)	4.00	3.50	2.33	1.33	0.00
IV (HPMC)	4.00	3.50	2.50	1.83	0.50
p-value	**1.00**	**0.041***	**0.11**	**0.16**	**0.01***

Table 1. Wound area average results on day 0, 3, 7, 10, and 14.

Advanced statistical test with the Mann-Whitney test was performed to analyse differences between all treatment groups on day 3 and 14. The result showed that on day 3, there was a significant difference of the wound area between the group with 2% Moringa leaves extract application with the group with HPMC application ($p = 0.025$); and also between the group with 4% Moringa leaves extract application with the group with povidone-iodine application ($p = 0.025$) (Table 2). On the 14th day of the wound healing, significant differences can be seen between the group with 2% Moringa leaves extract application with the group with HPMC application; and between the group with

povidone-iodine application with the group with HPMC application (p = 0.025) (Table 2). The wound closure in the group with 4% Moringa leaves extract application was faster than the groups with HPMC and povidone-iodine application.

Day	GI-GII	GI-GIII	GI-GIV	GII-GIII	GII-GIV	GIII-GIV
Day 3	0.114	0.317	0.317	0.025*	0.025*	1.00
Day 14	1.00	1.00	0.025*	1.00	0.025*	0.025*

Notes: *showed significant differences (p < 0.05)
I = 2% Moringa leaves extract application
II = 4% Moringa leaves extract application
III = Povidone-iodine application
IV = HPMC application

Table 2. Wound area differences between all treatment groups on day 3 and 14.

Discussion

The wound healing process consists of different phases such as granulation, collagenization, and collagen maturation. It is an orderly progression phase that establishes the tissue integrity. In the groups with the application of povidone-iodine, 2% Moringa leaves extract, and 4% Moringa leaves extract, the wounds were started to closed until 100% on the 14th day, while in the group with HPMC application the wound did not close until 100% until the 14th day. The results of this research showed that the ethanolic extract of Moringa leaves increased the percentage of wound closure by epithelization. This enhanced epithelization may be caused by the effect of Moringa leaves extract on enhancing the collagen synthesis.[19] Deposition of newly synthesised collagens at the wound area increases the collagen concentration per unit area, hence will also increase the tissue tensile strength.[20]

From the observed value of the wound healing process, it was assumed that the groups with Moringa leaves extract application showed better and faster healing than the control groups. Moringa plants have been practically used in the medicinal field for decades to heal various acute and chronic conditions. The properties of its phytochemicals, such as flavonoid and phenolic acids were related to the anti-inflammatory, antioxidant and antibacterial activities.[21]

In a study conducted by Singh et al. in 2012, the antimicrobial activity of *Moringa oleifera* was examined using the main model of Kirby-Bauer disc diffusion method, in which as much as 50% ethanolic extract of Moringa leaves successfully showed low antibacterial activity. Even at higher concentrations, the extract displayed only mild inhibitory activity, and no activity at all towards the *Pseudomonas*.[22] Peixoto et al. in 2011 reported that the aqueous and ethanolic extract of Moringa leaves indicated a promising potential as a treatment for certain bacterial infection.[23] The aqueous and ethanolic extracts of Moringa leaves have anti-bacterial potential properties, with higher inhibitory effects on gram-positive species (*Staphylococcus aureus* and *Enterococcus faecalis*) over gram-negative species (*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Aeromonas caviae*).[24] Also, the ethanolic extract of Moringa leaves has demonstrated the highest inhibitory zone average on the growth of both *S. aureus,* and *Streptococcus mutans* during the comparison between the experimental toothpaste contained the extract from different parts of the Moringa plants and the mouthwash solutions.[24]

Usually, natural compounds with polyphenols have strong antioxidant properties and able to decrease any oxidative damage in the tissues by scavenging the free radicals. The ethanolic extract of Moringa leaves contains chlorogenic acid, rutin, quercetin glucoside, and kaempferol rhamnoglucoside.[25] Similarly, the Moringa genus has high antioxidant activity, mainly due to its high content of bioactive polyphenols. The extract of Moringa leaves also contains tannins, saponins, flavonoids, terpenoids and glycosides, which also have medicinal properties. These compounds have been shown to be an effective antioxidants, antimicrobial, and anti-carcinogenic agents.[26] Phenolic compounds are known to act as primary antioxidants, due to their properties for inactivating of the lipid-free radicals, or prevention of the decomposition of hydroperoxides into free radicals by their redox properties. These properties play a key role in neutralising free radicals, quenching singlet or triplet oxygen, or decomposing peroxides. Fresh Moringa leaves are a good vitamin A source. It is well known that vitamin A has important functions in vision, reproduction, embryonic growth and development, immune competence, and cell differentiation.[27] Moringa leaves also contain 200 mg/100 g of vitamin C, a higher concentration than the vitamin C concentration found in oranges. Moringa leaves also protect the body from various deleterious effects of free radicals,

pollutants, and toxins through its antioxidant properties.[28]

The Moringa leaves extract inhibited the human macrophage cytokine production (tumor necrosis factor alpha, interleukin-6 (IL-6), and IL-8), which are induced by cigarette smoke and lipopolysaccharide (LPS).[29] Further, Waterman et al. reported that both Moringa leaves concentration and isothiocyanates decreased the gene expression and production of inflammatory markers in RAW macrophages. The Moringa leaves extract stimulated both cellular and humoral immune responses in cyclophosphamide-induced immunodeficient rats, by increasing the white blood cells, the percentage of neutrophils, and serum immunoglobulins.[30] In addition, quercetin may have been involved in the reduction of the inflammatory process by inhibiting the action of neutral factor kappa-beta (NF-k_) and subsequent NF-kB-dependent downstream events and inflammation.[31]

The wound area humidity is an important factor affecting the growth of fibroblast cells. Appropriate moisture can accelerate formation of the growth factors to increase its amount. The gel base is a fat-based HPMC that can keep the right humidity of the wound area. In the ointment with a concentration of 4% Moringa leaves extract, the basic composition of the ointment and extract are in accordance with the required moisture, thus increasing the cell growth. In the ointment with a concentration of 4% Moringa leaves extract, the salve's basic content is higher, thus increasing the moisture, and the cell growth will be more excessive compared to the HPMC, povidone iodine and 2% Moringa leaves extract salves.

The results of this study showed that the ethanolic extract of Moringa leaves helps to accelerate the wound healing on the rat's palate. It is hoped that the ethanolic extract of Moringa leaves gel can be used as a herbal remedy that can be used safely for the wound healing. This gel may be used to accelerate the healing of the palatal lesions in the procedure of obtaining a donor of connective tissue graft from the palate as an action in the periodontal surgery treatment.

Conclusions

Moringa leaves (Moringa oleifera) extract gel promotes and accelerates the wound healing of the rat's palate. At the concentration of 4%, the Moringa leaves extract gel tends to show better and faster wound closure.

Declaration of Interest

None declared.

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