Unripe *Musa sapientum* peel in the healing of surgical wounds in rats[1]

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ABSTRACT

PURPOSE: To assess the effects of unripe *Musa sapientum* peel on the healing of surgical wounds in rats.

METHODS: One hundred and twenty Wistar rats were divided into two treatment groups of 60 animals each: the control group (gel without the active ingredient) and experimental group (4% *Musa sapientum* peel gel). A 4 x 4 cm surgical wound was created on the back of each animal. The wound was cleaned daily with 0.9% saline, treated with 4% gel or natrosol gel (control), and covered with gauze. Animals from both groups were sacrificed after seven, 14 and 21 days of treatment; the tissue from the wound site was removed together with a margin of normal skin for histological analysis.

RESULTS: No significant differences in wound contraction rates (p=0.982) were found between time points (seven, 14 and 21 days of treatment) in both groups. However, a significantly higher wound contraction rate was observed in the control group on day 21 compared with the experimental group (p=0.029). There were no significant differences in histomorphological features between groups. The experimental group showed an increased number of polymorphonuclear cells on day 7, with a significant reduction on day 21 (p=0.026).

CONCLUSION: The use of 4% unripe *Musa sapientum* peel gel on surgical wounds in rats resulted in an increased number of polymorphonuclear cells on day 7, reduced wound contraction, reduced vascular proliferation and increased concentration of collagen fibers on day 21.

Key words: Musa sapientum. Wound Healing. Phytotherapy. Rats.

Introduction

The prevalence of chronic wounds in the general population is relatively high. This condition is associated with several diseases and imposes a major social and economic burden on patients and healthcare systems. Thus, the search for alternative therapies for the treatment of chronic wounds is of fundamental importance[1,2].

There are many treatment options for chronic wounds in the market, but it has been difficult to properly determine the cost-benefit relationship and best treatment option for each type of ulceration[3]. Due to their antibacterial and anti-inflammatory properties, proteolytic enzymes have been used as gels or creams applied to necrotic or granular tissues in the treatment of different types of wounds. These enzymes promote the chemical debridement of the wound, increase the tensile strength of scar tissue, and stimulate re-epithelialization and formation of granulation tissue at all phases of wound healing by secondary intention. Collagenase and papain are the most commonly used debriding enzymes, and collagenase was observed to be more tolerant when used in combination with antimicrobial dressings than papain[4].

Despite the predominance of substances of synthetic origin in the therapeutic arsenal, including anti-inflammatory drugs, in recent years there has been a renewed interest in therapeutic practices considered by many health professionals as popular or unscientific. Hence, herbs and other natural remedies have been used as an alternative or complementary therapy. Many phytotherapeutics, including extracts of *Aloe vera*, passion fruit (*Passiflora edulis*), aroeira (*Schinus terebinthifolius*), and unripe banana (*Musa sapientum*) have been tested and used in the treatment of skin lesions[5,6].

The pseudo-stem of the banana plant and unripe banana peels contain copper, zinc, sodium, potassium, calcium, phosphorus, and iron[7], and the fruit contains antioxidants, including vitamins C and E, and beta carotene[8]. Unripe banana extract promotes increased incorporation of thymidine into cellular DNA, which enhances cell proliferation[2]. Unripe banana peel contains leucocyanidin, a flavonoid that induces cell proliferation, accelerating the healing of skin wounds[9]. The pulp and peel of unripe banana have been used in the treatment of cracked nipples and peptic ulcers in humans[2]. Studies with rats have shown the efficacy of unripe banana in the prevention and treatment of peptic ulcers. The active agent in unripe bananas is water soluble and becomes inactive in ripe bananas[10].

However, further studies on the use of unripe banana in wound healing by secondary intention are necessary. If its therapeutic efficacy could be demonstrated, unripe banana would be a new and inexpensive treatment option for skin wounds, accessible to the general population.

Methods

This experimental, longitudinal, prospective, analytic, randomized, triple blind study was approved by the Research Ethics Committee of the Federal University of Sao Paulo (UNIFESP), process number 0305/09. The animals were obtained from the Laboratory Animal Facility of UNIVAS. The use of laboratory animals followed the Federal Law number 11.794 (08/10/2008) and the decree 6.689 (15/07/2009). The study is classified as United States Department of Agriculture (USDA) Category C for laboratory animal usage. The sample consisted of 120 adult male Wistar rats (*Rattus norvegicus albinus*) aged about 120 days and weighing approximately 300 grams. All animals were marked for identification.

The rats were randomly divided into two groups (control and experimental groups) of 60 animals each, using the BioEstat 5.0 software. The allocation sequence was concealed in sequentially numbered, opaque, sealed envelopes. Animals in the control group were treated with natrosol gel (hydroxyethyl cellulose) without the active ingredient and those in the experimental group were treated with natrosol gel with extract from unripe banana peel at 4% concentration. The gel concentration was chosen based on a previous study, in which the 4% gel resulted in better epithelialization of wounds healed by secondary intention compared with gels at different concentrations (2% and 10% gel)[1]. Each group was subdivided into three subgroups of 20 animals each, according to the time of euthanasia.

A 4 x 4 cm wound was created on the back of each animal. The wound was cleaned daily (from the immediate postoperative period to the day of euthanasia) with 0.9% saline. The size of the wound was then measured with a caliper. Next, the wound was treated with 4% gel or natrosol gel (control) and covered with gauze. Samples for histological analysis were collected on postoperative days 7, 14, and 21, immediately after the animals were euthanized.

Gel preparation

The gel used in the study was prepared from unripe banana (*Musa sapientum*) peels. Unripeness was determined according to the scale devised by Loeseck[11], which relates ripeness

to the color of the peel. Only completely unripe fruits were selected to assure homogeneity. The fruits were purchased directly from a producer in Pouso Alegre-MG, Brazil, in the same conditions as sold to consumers.

First, the fruits were washed with running water and rubbed with a luffa sponge. After the first wash, the fruits were allowed to dry at room temperature for 20 minutes. The unripe bananas were then washed with 500 ml of distilled water, dried with paper towel, and allowed to dry for another 20 minutes at room temperature. Next, the fruits were peeled and the pulp was discarded; only the peels were used. The peels were diced into cubes of approximately 1 mm^3, transferred to a ceramic mortar, and manually ground with a pestle for 60 minutes until complete homogenization. The peel of one unripe banana yields about 50g of ground peel. The ground peels were placed in a watch glass and weighed on a precision balance model Gehaka BG2000. Following, 100g of gel (4% gel) was prepared by mixing 4g of ground peel with 96 g of natrosol gel for five minutes to ensure homogenization. The prepared formulation was placed in a previously sterilized, white 120g plastic container having a false bottom, stored at refrigeration temperature, and used within 30 days.

Surgical procedures

Before the surgical procedure, the animals were anesthetized with a mixture of ketamine hydrochloride (50mg) and 2% xylazine hydrochloride (20mg) at 2ml/kg body weight.

The hair of a 6x6cm area ($36cm^2$) in the dorsal region of the animals was manually removed in the cephalo-caudal direction. The incision lines were marked on the skin of the interscapular region between the anterior limbs with a thin felt tipped pen. The skin was cleaned with 0.9% saline and a 4x4cm surgical wound was created on the back of each animal. The incision was deepened to the dorsal muscular fascia and the dorsal skin was resected together with the panniculus carnosus.

Treatment of surgical wounds

All animals in both groups were treated daily from the immediate postoperative period to the day of euthanasia. Before daily treatment, the rats were anesthetized and the wounds were cleaned with 0.9% saline. Then, natrosol gel (control group) or 4% unripe banana peel gel (experimental group) was applied to the surgical wound using a disposable wooden spatula, which was discarded after each application. The same amount of gel (0.1g) was used in each application. The wound was covered with Transpore medical tape and a secondary dressing of microporous adhesive tape, prepared specifically for this experiment, was also applied.

Macroscopic analysis

The following formula was used to determine the rate of wound contraction (%):

$$Wound\ contraction\ rate = \frac{initial\ wound\ area - final wound\ area}{initial\ wound\ area} \times 100$$

The wound area was determined by tracing straight and perpendicular horizontal and vertical lines connecting the most external incision points; measurements were recorded in centimeters. Changes in wound area over time were determined by tracing the contour of the wound on sterilized clear acetate sheets and calculating its area. The comparison of both measurements provided the real size of the wound.

Photographs of the wound area were taken with a digital camera model PowerShot A100 mounted on a tripod at a distance of 20 cm from the wound. The photographs were utilized to evaluate the evolution of wound healing by digital planimetry using AutoCAD 14 software. Macroscopic analysis was performed by a researcher blinded to group allocation.

Histological analysis

Samples for histological analysis were collected on postoperative days 7, 14, and 21, immediately after the animals were sacrificed by anesthesia overdose. The tissue from the wound site was removed together with a 1cm wide margin of normal skin, fixed in 10% buffered formalin, pH 7.5, and sent to the pathology laboratory. Paraffin-embedded specimens were cut into 3-µm sections, mounted onto slides, and stained with hematoxylin and eosin (HE). The slides were photographed with a camera (model Canon PowerShot SX 100 IS) coupled to an optical microscope.

Histomorphological analysis was performed on the images to determine fibroblast proliferation, quantification of polymorphonuclear and mononuclear cells, vascular proliferation, and quantification of collagen fiber content. These parameters were graded as absent, mild, moderate, or marked. All histological examinations were performed by the same pathologist, who was blinded to group allocation. Results were recorded for subsequent analysis.

Statistical analysis

Wound contraction rates at different time points (seven, 14 and 21 days of treatment) were compared between and within groups using Student's t-test, and multiple comparisons between groups were performed with analysis of variance (ANOVA) followed by post hoc Dunnett's test.

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 13.0. All statistical tests were performed at a significance level α of 0.05 ($p<0.05$).

Results

Six animals in the control group and four in the experimental group died during the study due to adverse conditions, but the loss of these animals did not affect the statistical analysis of data.

Wound contraction rates measured by digital planimetry (macroscopic analysis) at the end of treatment on days seven, 14 and 21 for both groups are shown in Table 1.

TABLE 1 – Rate of wound contraction in the experimental and control groups at the end of treatment.

Wound contraction rate (%)	Groups	
	Control (n = 54)	**Experimental** (n = 56)
Mean	55.37	55.21
SD	35.01	38.58
Median	70.63	70.94
Minimum	-27.50	-95.63
Maximum	91.88	94.38

SD=standard deviation. Student's t test (p=0.982)

Comparisons of wound contraction rates between groups at the three time points are listed in Table 2. The experimental group showed significantly lower wound contraction rates compared with the control group on day 21 (p=0.029).

TABLE 2 – Comparison of wound contraction rates between groups at the three time points.

Time points	Comparison	p-value
Day 7	Control x Experimental	0. 094
Day 14	Control x Experimental	0. 746
Day 21	Control x Experimental	0. 029*

Asterisks (*) indicate statistical significance. Student's t test

The quantification of polymorphonuclear cells in the control and experimental groups at the three time points are depicted in Table 3. A significant difference in polymorphonuclear cell content (p=0.026) was found in the experimental group after seven days of treatment with 4% gel.

TABLE 3 – Quantification of polymorphonuclear cells in the wounds of animals in the control and experimental groups at the three time points.

Polymorphonuclear cell content		Day 7		Day 14		Day 21		p-value
Groups		n	%	n	%	n	%	
Control	Absent	0	0.0	0	0.0	0	0.0	0.057
	Mild	2	10.5	5	27.8	9	52.9	
	Moderate	7	36.8	8	44.4	4	23.5	
	Marked	10	52.6	5	27.8	4	23.5	
	Total	19	100.0	18	100.0	17	100.0	
Experimental	Absent	0	0.0	0	0.0	0	0.0	0.026*
	Mild	1	5.0	2	11.1	4	22.2	
	Moderate	4	20.0	8	44.4	10	55.6	
	Marked	15	75.0	8	44.4	4	22.2	
	Total	20	100.0	18	100.0	18	100.0	

Asterisks (*) indicate statistical significance.

No significant differences in fibroblast proliferation and mononuclear cell content were observed between groups at the three time points.

Vascular proliferation was significantly higher in the control group compared with the control group on day 21 (p = 0.033).

Quantification of collagen fibers in the control and experimental groups at the three time points is shown in Table 4. No significant difference in collagen fiber content was found between groups at the different time points. However, a significant increase in collagen fiber content was observed in both groups on day 21.

TABLE 4 – Quantification of collagen fibers in the wounds of animals of the control and experimental groups at the three time points.

Collagen fiber content		Day 7		Day 14		Day 21		p-value
Groups		n	%	n	%	n	%	
Control	Absent	0	0.0	0	0.0	1	5.9	0.035*
	Mild	16	84.2	8	44.4	7	41.2	
	Moderate	3	15.8	10	55.6	9	52.9	
	Marked	0	0.0	0	0.0	0	0.0	
	Total	19	100.0	18	100.0	17	100.0	
Experimental	Absent	0	0.0	0	0.0	0	0.0	0.044*
	Mild	15	75.0	11	61.1	6	33.3	
	Moderate	4	20.0	7	38.9	12	66.7	
	Marked	1	5.0	0.0	0.0	0.0	0.0	
	Total	20	100.0	18	100.0	18	100.0	

Asterisks (*) indicate statistical significance.

Micrographs showing collagen fiber content in samples obtained on day 21 from animals in both groups are presented in Figure 1. Re-epithelialization was not observed in either the treatment or control group.

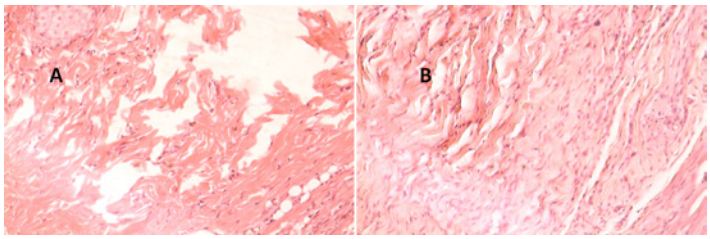

FIGURE 1 – Micrographs of collagen fibers in wounds from animals in the control (**A**) and experimental (**B**) groups after 21 days of treatment (original magnification 10x).

Discussion

Wound healing consists of an orderly sequence of events, which includes cellular and molecular processes that interact to restore tissue integrity. Medicinal plants play an important role in maintaining the health of the general the population. The World Health Organization (WHO) estimates that the use of medicinal plants is the first therapeutic option for about 80% of the world's population. It is estimated that US$ 22 billion are spent annually in the world with herbal medicine, and the annual market is approximately US$ 400 million in Brazil[12].

Unripe banana peels are used as a food source and in research as well. The chemical composition of unripe banana peels was first described by Selema and Farago[7]. Agrawal *et al.*[13], related that extract of plantain banana was given by gavage to rats for the treatment of skin wounds and favoured wound healing which could be due to its antioxidant effect and on various wound healing biochemical parameters. Best, Lewis, Nasser reported on the ulcer-healing and anti-ulcerogenic properties of unripe plantain banana[10]. The efficacy of the topical use of unripe banana peel in the treatment of skin wounds needs further confirmation. Many studies have been conducted to determine the effects of chemical compounds present in unripe bananas, as well as their optimal concentrations for therapeutic use[9,10].

In this study, no significant difference in wound contraction was observed in the experimental group after either seven, 14 or 21 days of treatment with 4% unripe banana peel gel. However, a significant difference in wound contraction rate was observed on day 21 between the control and experimental group (p=0.029). Atzingen *et al.*[1] conducted an experimental study in which unripe banana peel gel was used at different concentrations (2%, 4% and 10% gel) in the treatment of surgical wounds in rats, and reported wound contraction rates similar to our findings in animals treated with 4% gel for 21 days.

Histological analysis performed in a placebo-controlled study on the effects of sweet briar (*Rosa rubiginosa*) oil on the treatment of open wounds created on the back of rats revealed that animals treated with sweet briar oil showed accelerated wound healing and reduced inflammatory activity compared with those in the placebo group[14].

D'Acampora *et al.*[15] found that, in a clean surgical incision, neutrophils appear within 24 hours and epidermal thickening is observed, and that granulation tissue fills the incision on day 5, and fibroblast proliferation and collagen synthesis are observed on day 7. Granulation tissue facilitates epithelial migration and helps to close the incision wound by contraction, reducing the area requiring epithelial coverage. In the present study, the number of polymorphonuclear cells was significantly higher in the experimental group on day 7, suggesting the formation of granulation tissue. Moreover, animals treated with 4% gel had also reduced wound contraction and vascular proliferation combined with increased concentration of collagen fibers on day 21, indicating an acute inflammatory response.

Further studies are necessary to search for the existence of other effects of unripe banana peel on wound healing, such as toxicity, and routes and forms of administration. It is also important to isolate active components of unripe banana peel extracts and confirm their pharmacological activity in improving tissue repair.

Conclusion

Gel from unripe *Musa sapientum* peel at 4% concentration applied to surgical wounds in rats resulted in increased number of polymorphonuclear cells on day 7, and reduced wound contraction and vascular proliferation, as well as increased concentration of collagen fibers on day 21.

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