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RESEARCH ARTICLE

REPAIR OF WOUNDS IN RATS: GEL WITH THE GREEN PEEL OF *MUSA SAPIENTUM* 10%

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ABSTRACT

In the world, it is estimated that the appearance of chronic wounds has increased and it is known that the bark of the green banana induces cellular proliferation, enhancing the healing of the skin. Objective: To evaluate the curative action of banana peel (Musa sapientum) at a concentration of 10% in the surgical wound of rats. Methods: Sixty adult male rats (Rattus norvegicus, variant albinus, wistar) were used. The animals were divided in two experimental groups: control group, using gel without active principle; Experiment group using natrosol gel with 10% green banana peel. Dressings were performed every three days, from the first postoperative day to the date of euthanasia. Results: In the present study we obtained results from the analysis of histological sections observed by light microscopy and noticed that there is vascular proliferation, mainly after twenty - one days of treatment with banana extract. In addition, there was an increase in the number of collagen fibers in relation to the control group. Conclusion: It is known that the number of people with chronic lower limb wound related to various diseases has increased and it is essential to seek new therapeutic alternatives to solve this problem. The use of banana extract in wounds, can help problematic.

INTRODUCTION

Currently there is a large proportion of the population affected by chronic skin wounds, and often these wounds are associated with several types of diseases. This fact implies a high social and financial cost for the patient and the health systems, in view of the above, it is essential to search for new therapeutic alternatives to solve this problem (Atzingen *et al.,* 2011; Novak *et al.,* 2003). There are many treatment options for chronic wounds, however, it has been difficult to properly determine the cost-benefit ratio and best treatment option for each type of ulceration (Hoppe and Granick, 2012). And even with the predominance of synthetic compounds in the therapeutic arsenal, including anti-inflammatory drugs, recent years have witnessed a renewed interest in therapeutic practices considered by many health professionals as popular or unscientific. Many phytotherapics, including extracts of Aloe vera, passion fruit (*Passiflora edulis*), aroeira (*Schinus terebinthifolius*) and green banana (*Musa sapientum*) have been tested and used in the treatment of skin lesions (Garros *et al.,* 2006; Branco Neto *et al.,* 2006). The banana (*Musa sapientum*) is a common tropical fruit, and originates from the

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Asian continent. In Brazil, its cultivation occurs throughout the continent, from the coastal strip to the interior of the country. Green banana extract has shown an increase in the incorporation of thymidine into the DNA of the cells, promoting a beneficial effect on cell multiplication (Novak *et al.,* 2003). It has been found that the green banana peel induces cell proliferation, potentiating wound healing of the skin because of its active component identified as flavonoid leucocianidina (Lewis *et al.,* 1999). The pulp and green banana peels have been used in the treatment of nipple fissures leading to the relief of these fissures, with possible healing (Novak *et al.,* 2003). And also in peptic ulcers. The active principle in green bananas is soluble in water and becomes inactive in mature bananas (Best *et al.,* 1984). If therapeutic efficacy is confirmed, green banana would be a new and inexpensive treatment option for skin wounds, accessible to the general population. This study aims to evaluate the healing action of banana green bark (*Musa sapientum*) in a concentration of 10% in surgical wound of rats.

MATERIALS AND METHODS

Study Design

The present study is experimental, longitudinal, prospective, analytical, randomized, triple-blind was approved by the Committee of Care and Animal Use of the University of Sapucaí Valley (UNIVÁS), approval number 161/12. The animals were obtained from the Univás Vivarium. The use of laboratory animals followed the principles of the Brazilian College of Animal Experimentation (COBEA), Federal Law 11,794 (08/10/2008) and Decree 6,689 (07/15/2009). The study is classified as a "C" category in the United States Department of Agriculture (USDA) for use in laboratory animals.

Sample

Sixty adult male mice (*Rattus norvegicus*, albinus variant, wistar) were used, with 120 days of age and body mass of approximately 400 grams, duly identified. Randomization of the rats was performed using random sequence generated by the Biostat 5.0 program, and allocation secrecy was guaranteed with the use of sealed and numbered opaque envelopes. The animals were distributed in two experimental groups: control group, in which natrosol gel (hydroxyethylcellulose) without active principle; Experiment group, using natrosol gel with banana peel (*Musa sapientum*) 10% green. Dressings were performed every three days, from the first postoperative day (day 0) to the date of euthanasia (day 35).

Procedures

For this study, all-green bananas, selected according to the scale of[8] (Figure 1)

Preparation of gel

First the fruits were washed with running water, then removed from the bark. This washing was carried out with vegetable bush. After washing, the bananas were allowed to dry at room temperature over clean tissue for 20 minutes. After this period, they were again washed, now with 500 ml of distilled water, dried with white paper towel and left for another 20 minutes at room temperature. The pulp of the fruit was then removed and discarded for the use of the bark alone. After this process, the green banana peel was cut into cubes of approximately 1mm x 1mm and placed in a porcelain grate, where the manual grinding was done with the aid of pistil, until complete homogenization, for 60 minutes timed, obtaining or macerated. The macerate was then weighed in a watch glass on the BG 2000-Gehakpara® scale (São Paulo, SP). To prepare 100 grams of the 10% gel, natrosol gel (90 grams) and the maceration of the green banana peel (10 grams) were used. The prepared formulation was placed in pre-sterilized 120g capacity white plastic container, which will then be stored in a refrigerator, valid for up to 40 days.

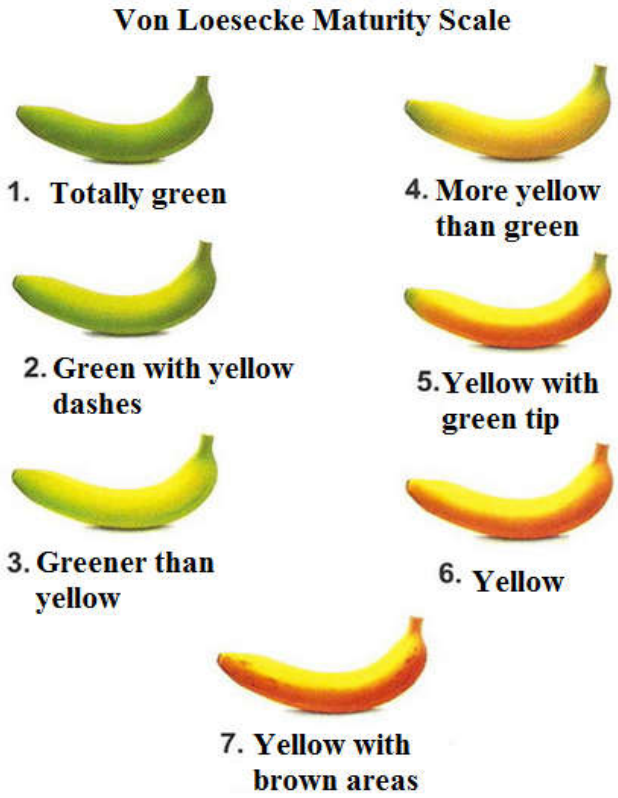


Figure 1. Von Loesecke maturation scale

Operative Procedure

For the operative procedure, the animals were anesthetized with a dose of 0.2ml / 0.1kg of anesthetic association of ketamine (50mg) with 2% xylazine hydrochloride (20mg) intramuscularly. The anesthetic induction takes on average 100 seconds and guarantees postural loss of the animal in a period of less than one minute. This anesthetic step is to avoid hurting the animals or causing them stress during the wounds. After anesthesia, the animals were submitted to manual epilation in the dorsal region with an area of 36cm² (6cm long x 6cm wide), in the cephalocaudal direction, the area being marked with an ultrafine dermatographic pen before epilation for precise epilation. The skin was cleaned with 0.9% saline. Hemostasis was done by digital compression with sterile gauze. The design for the incision was performed after epilation, located caudally to an imaginary line passing through the anterior limbs. The size of the wound area (day 0) was 4cm x 4cm (16 cm²) and the depth should reach the muscular layer, with the removal of the supematant fleshy paniculus (Figure 2).

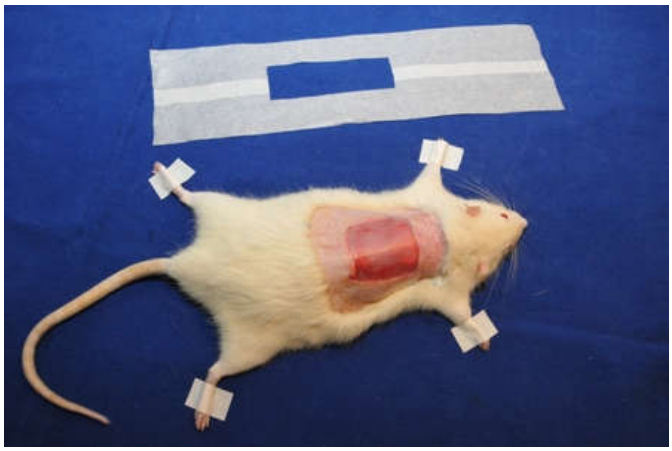


Figure 2. Demarcation of the surgical wound area 4x4cm and secondary dressing of porous tape

Injury Treatment

After wound production the first dressing was performed (day 0); In the experimental group, the gel was applied to 10% of the green banana peel and in the control group the gel was applied without active principle. Both gel applications from both the experimental and control groups were made with disposable wooden spatulas that were discarded after each application. The same amount of gel was also used in each application (0.1g). During dressing the rats were anesthetized and the wounds were cleaned with 0.9% saline prior to the application of the 10% green banana peel gel. The same procedure was performed in the control group during the given period. The wounds were treated in the immediate postoperative period and every three days for 7, 14, 21, 28, 35 days, with 10% green banana gel in the experiment group, and with no active principle gel in the control group. The wound on the rat was occluded after application of the gel with sterile gauze and a porous tape as a secondary dressing, made specifically for the experiment (Figure 2). All animals received sodium dipyrone at 1 mg / kg body weight three times daily for postoperative and post-dressing analgesia during the study period.

Euthanasia

Injection with anesthetic overdosage was used in euthanasia with 7, 14, 21, 28 and 35 days after the start of the experiment. Histological analysis:

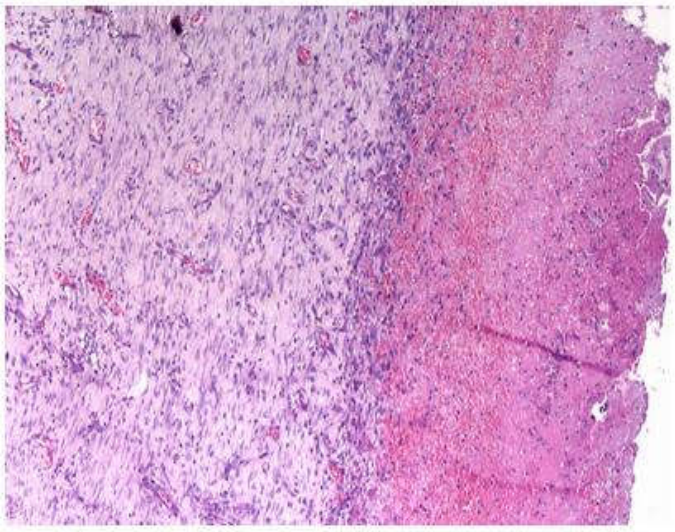
Samples for histological analysis were collected on days 7, 14, 21, 28 and 35 postoperative days with anesthetized and living animals. The wound was resected with 1cm margin with normal tissue and fixed in 10% buffered formalin solution, pH 7.5, and sent for histological analysis. The specimens were embedded in paraffin and cut into 3 µm slices and then stained with hematoxylin eosin (HE). The cuts were photographed with a camera (Canon PowerShot SX 100 IS model) attached to the microscope. Histomorphological analysis was performed by micrograph to analyze fibroblast proliferation, vascular proliferation and reepithelialization, and quantify mononuclear and polymorphonuclear cells and the content of collagen fibers. Ten fields of high magnification (40x) were analyzed. The occurrence of cells was graded in a few (<10 mononuclear or polymorphonuclear cells), moderate (10 to 50 cells), marked (> 50 cells). All histological analysis was done by a pathologist, who was unaware of the allocation of groups. The results were stored for further analysis.

Statistical analysis

For all procedures, the software application Statistical Package for Social Sciences (SPSS) 13.0 was used, setting the possibility of rejection of nullity in 5% ($p < 0.05$). Rates of wound contraction at different time points (7, 14, 21, 28, and 35 days) were compared within and between the groups using the chi-square test and Fisher's exact test. Comparisons of animal characteristics (eg, age and body weight) between groups were made before and after study intervention using Student's t-test.

RESULTS

No significant differences in age and body weight of the animals were found between the study groups and the control group before and after the study intervention.



Histological analysis

On day 7, wound healing was more advanced in the study group, which showed extensive vascular proliferation ($p = 0.01$) when compared to the control group (Figure 3), which guarantees the wound a greater blood supply necessary for an effective Healing (Table 1). At day 14, the healing process was even more advanced in the study group, with more extensive vascular proliferation ($p = 0.002$), mononuclear cells ($p = 0.01$), collagen ($p = 0.02$) compared to the control group. However, no significant differences were observed between groups for the amount of fibroblasts in that period. At 21 days, there was persistence of greater vascular proliferation ($p = 0.03$) in the study group, accompanied by intense reepithelialization ($p = 0.01$) in the same group. With 28 days of evolution, intense reepithelialization ($p = 0.001$) of the operative wounds of the study group was evidenced in relation to the control group, associated to the fact that the study group had a marked collagen production ($p = 0.04$), indicating a remodeling phase of the (Table 1). The presence of polymorphonuclear cells ($p = 0.002$), mononuclear cells ($p = 0.001$), fibroblasts ($p = 0.04$), collagen ($p = 0.002$) and reepithelialization ($p = 0.001$) were still significantly more intense in the study group. Than in the control group.

Table 1. Histological results for groups in five periods

Variables	Groups	Postoperative days									
		Day 7		Day 14		Day 21		Day 28		Day 35	
		Study	Control	Study	Control	Study	Control	Study	Control	Study	Control
Vascular proliferation											
Discreet		1	0	0	1	0	2	0	1	0	0
Moderate		1	6	0	5	2	4	3	5	2	6
Accented		4	0	6	0	4	0	3	0	4	0
P value		0.01*		0.002*		0.03*		0.10		0.14	
Polymorphonuclear											
Discreet		1	0	1	4	5	1	6	2	6	0
Moderate		5	3	5	1	1	3	0	3	0	5
Accented		0	3	0	1	0	2	0	1	0	1
P value		0.10		0.06		0.05		0.05		0.002*	
Mononuclear											
Discreet		1	0	0	0	0	0	0	0	0	0
Moderate		5	6	2	6	3	6	0	6	0	6
Accented		0	0	4	0	3	0	6	0	6	0
P value		0.29		0.01*		0.04*		0.001*		0.001*	
Fibroblasts											
Discreet		0	0	2	0	3	1	1	0	0	0
Moderate		4	6	4	6	3	5	5	6	3	6
Accented		2	0	0	0	0	0	0	0	3	0
P value		0.12		0.12		0.22		0.29		0.04*	
Collagen											
Discreet		1	6	1	5	1	1	0	4	0	6
Moderate		4	0	5	1	2	5	5	2	3	0
Accented		1	0	0	0	3	0	1	0	3	0
P value		0.01*		0.02*		0.11		0.04*		0.002*	
Reepithetization											
Discreet		6	6	5	6	2	6	0	6	0	6
Moderate		0	0	1	0	4	0	6	0	6	0
Accented		0	0	0	0	0	0	0	0	0	0
P value		0	0	0	0	0	0	0	0	0	0
Discreet		0		0.29		0.01*		0.001*		0.001*	

* Statistical Significance (p<0.05)

DISCUSSION

Brazil's biodiversity and socioeconomic situation have led to an intense search for effective and less expensive solutions to the most prevalent diseases. Skin wounds affect a large part of the population, especially the poorest. Brazil has the greatest biodiversity in the world. The use of this vast biodiversity associated with a low cost of drug production can provide a solution to several public health problems in Brazil. The *Musa sapientum* var. Paradise has been studied intensely in the last 30 years. Green banana was also effective in treating peptic ulcer (Best *et al.,* 1984; Goel and Sairam, 2002). Further research has been carried out in view of its healing properties, especially studies on skin wounds, which are very common and require prolonged treatments, overloading and burdening the health system. No study was found in the literature identifying and specifying the components of *Musa sapientum* var. Paradise responsible for its healing properties. However, studies on banana properties have shown that especially its pulp is rich in flavonoids such as leucocidin, which has anti-inflammatory and anticancer activity (Gupta *et al.,* 2004; Liu *et al.,* 2009).

Histological analysis

At the beginning of the inflammation phase of wound healing there is an increase in vascular permeability, extravasation of plasma, erythrocytes, platelets and leukocytes, especially neutrophils, monocytes and macrophages (Modolin and Bevilacqua, 1985). The inflammatory response is an important step in wound healing that prepares the wound environment for the repair process. In this study, the use of the 10% gel of unripe banana peel increased significantly the inflammatory response at day 14 postoperatively in the study group, compared to the control group. This increase in the inflammatory response may contribute to a reduction in healing time. However, this phase should not be too intense, because an excessive inflammatory response may cause delay in wound healing and favor an imbalance between synthesis and degradation of the collagen, promoting matrix degradation (Ashcroft *et al.,* 2002). Re-epithelialization begins a few hours after injury with the migration of the epithelial cells from the margins and the epidermal appendages located at the center of the wound. Fibroplasia and vascular proliferation are of fundamental importance in wound healing because they are

involved in the formation of granulation tissue, which gradually fills the wound cavity four days after injury. Although total re-epithelization was not achieved with the use of unripe banana peel gel, re-epithelization was significantly higher in the study group compared to the control group on days 21, 28 and 35 of the postoperative period, Showing that the gel stimulated contraction of the wound. Chronic wound healing began on day 28 with reduction of the inflammatory process and a significant increase in fibroblast proliferation in the study group compared to the control group. Fibroblasts produce new extracellular matrix necessary for cell growth, whereas new blood vessels carry oxygen and nutrients necessary for cellular metabolism (Singer and Clark, 1999). After neutrophils and mononuclear cells migrate to the wound area in response to chemotactic agents. Macrophages produce various growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), which Are the main cytokines required to stimulate the formation of granulation tissue (Singer and Clark, 1999). In the present study, the use of gel at 10% of unripe banana peel positively affected wound healing, which is in agreement with other studies in the literature.

The gel concentration was chosen based on an earlier study, in which the 10% gel resulted in the epithelialization of healed wounds by second intension (Atzingen *et al.,* 2011). Samples for histological analysis were collected on days 7, 14, 21, 28 and 35 postoperatively. In previous studies, no beneficial effects of the use of unripe banana peel gel were observed before day 14 (Atzingen *et al.,* 2011), but in this study already on day 7 significant differences were observed in the study parameters between the group Of study and control. An extended analysis of the samples was performed until day 35, unlike other studies that evaluated up to 28 postoperative days.[1] In the analysis of postoperative day 35, it was clear the persistence of the chronicity of the lesion already found as of day 28, with a reduction of the inflammatory process and a significant increase of the proliferation of fibroblasts in the study group in comparison with the control group. Although the present study was performed on acute wounds, the 10% gel can be used in chronic wounds. This can be inferred by comparing the evolution of the wounds at different time points (ie at 14, 21, 28 and 35 days of treatment). Another study evaluated the effect of unripe banana peel gel in the time interval[1]suggesting that our findings may also be relevant for the treatment of chronic wounds. Further studies are needed to confirm the beneficial effects of this gel and extend our results.

Conclusion

The 10% gel of unripe banana peel showed anti-inflammatory action and stimulated wound healing in rat skin when compared to a gel containing no active ingredient for both acute and chronic wounds.

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