Gel from unripe *Musa sapientum* peel to repair surgical wounds in rats[1]

Gel da casca de *Musa sapientum* verde no reparo de lesões operatórias em ratos

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ABSTRACT

PURPOSE: To determine the optimum concentration of a gel obtained from unripe banana (*Musa sapientum*) peel for wound treatment in rats.

METHODS: A randomized triple blind study was conducted with 40 Wistar rats, which were divided into 4 groups: CG, control group; G2%, 2% gel concentration group; G4%, 4% gel concentration group; and G10%, 10 % gel concentration group. The banana peel gel was applied daily, for 7 days, to a 4-cm² wound created on the back of each animal of all groups. After this period, the wounds were biopsied. Statistical analysis was carried out using the Kruskal-Wallis test complemented by the Student-Newman-Keuls test.

RESULTS: Macroscopic examination revealed that partial epithelialization occurred in all groups. Wound contraction was also observed in all groups and ranged from 1.38 to 1.57 mm in the study groups, and from 1.03 to 1.10 mm in the control group, with significant differences ($p < 0.05$) between the groups: CG and G10%, G2% and G4%, G2% and G10%. The interquartile deviation was smaller between the groups CG and G4%.

CONCLUSION: The 4% gel obtained from unripe banana peel (G4%) resulted in better epithelialization of wounds healed by secondary intention compared with other gel concentrations.

Keywords: Musa sapientum. Wound Healing. Rats.

RESUMO

OBJETIVO: Avaliar a concentração ideal do gel da casca de *Musa sapientum* verde no tratamento de feridas em ratos.

MÉTODOS: Estudo randomizado, triplo cego, com 40 ratos da linhagem Wistar divididos em quatro grupos: GC controle, G2% gel a 2%, G4% gel a 4%, G10% gel a 10%. Realizou-se aplicação diária do gel nas diferentes concentrações, durante sete dias, em uma ferida de 4 cm² realizada no dorso de cada rato. Após este período, as lesões foram biopsiadas. Para analise dos dados utilizou-se o teste de Kruskal-Wallis complementado pelo teste de Student-Newman-Keuls.

RESULTADOS: Os achados macroscópicos demonstraram reepitelização parcial em todos os grupos. A contração da área da ferida variou entre 1,38 a 1,57 mm nos grupos de estudo, e entre 1,03 a 1,10 mm no grupo controle. Houve diferença significante ($p < 0,05$) entre os grupos: GC e G10%, G2% e G4%, G2% e G10%, sendo o desvio interquartílico menor entre os grupos GC e G4%.

CONCLUSÃO: O gel a 4% da casca de *M. sapientum* verde promoveu maior área de epitelização em feridas com cicatrização por segunda intenção, em relação ao gel nas outras concentrações testadas.

Descritores: Musa sapientum. Cicatrização. Ratos.

Introduction

Currently, it is estimated that about 3% of the Brazilian population have some type of wound. In absolute numbers, there are about four million persons with chronic wounds or complications of the healing process. This requires the search for new resources and technologies that can provide an effective wound treatment at low costs and more accessible to the Brazilian population[1]. The progress of accidental or surgical wounds depends on the tissue regeneration capacity; therefore, it is very important to understand and promote methods that help the healing process[2,3].

At present, wound care is focused on the study of proteolytic enzymes (a class of drugs with bactericidal action) in the form of gels or creams. These drugs are indicated in all stages of wound healing by secondary intention. Enzymes can provide chemical debridement, increase the tensile strength of the scar, and stimulate epithelialization and formation of granulation tissue[1]. Papain is the most used substance in the treatment of wounds; it is a proteolytic enzyme composed of sulfhydryl proteases extracted from the *Carica papaya* plant that can be found either alone or combined with urea or chlorophyl[1].

Unripe banana has been used as a new therapeutic alternative in the treatment of nipple fissures and peptic ulcers[3]. However, the use of unripe banana in wound healing by second intention is not well established[4]. Studies on this subject may corroborate or refute the effects of the treatment, and may also lead to different treatment approaches based on new findings[4].

Methods

This study was approved by the Research Ethics Committee of the College of Health Sciences, Vale do Sapucai University (UNIVÁS), Pouso Alegre-MG, under the process 105/08. The animals were obtained from the Laboratory Animal Facility of UNIVÁS. The use of laboratory animals followed the principles of the Brazilian College on Animal Experimentation (COBEA) and the Council for International Organization of Medical Sciences (CIOMS) ethical code for animal experimentation. The research was performed at the Division of Plastic Surgery, Department of Surgery, Federal University of Sao Paulo (UNIFESP).

The gel used in the study was prepared from unripe banana peels. The fruit was washed in running water and placed on a clean cloth to dry at room temperature for 20 minutes. Next, the unripe banana was washed again with 500 ml of distilled water, dried with white paper towel, and allowed to dry for another 20 minutes at room temperature. After, the peel of the fruit was removed and the pulp was discarded. The peel was diced and ground in a porcelain mortar for 60 minutes until complete homogenization. The ground material was placed on a watch glass and weighed on an electronic analytical balance (Gehakpara, São Paulo, Brazil). The peel of one unripe banana yield about 50g of ground peel. The banana peel gel was prepared using natrosol gel (hydroxyethyl cellulose, Botanik Kosmetics) as a base gel. The gel and ground material were mixed in a mortar for 5 minutes to assure 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.

Forty Wistar rats were used in this study. The rats were housed individually in standard polypropylene cages. The light-dark cycle, humidity, and temperature were the same as the environment. Alls cages were at the same distance from the light source. The animals were fed standard rat chow and water *ad libitum*. Dipyrone (500 mg/ml) was added to the water and the analgesic solution was renewed every 6 hours during the experiment. The rats were distributed randomly into groups using the software Biostat 5.0. The allocation of the rats was kept in numbered, closed, opaque envelopes. The envelopes were later opened by the researcher responsible for the preparation of the gel containers.

The animals were divided into four experimental groups of 10 rats each: CG, control; G2%, 2% banana peel gel; G4%, 4% banana peel gel; and G10%, 10% banana peel gel. Before the surgical procedure, the animals were anesthetized with a mixture of ketamine hydrochloride (50 mg, Konig do Brasil Ltda) and 2% xylazine hydrochloride (20 mg, Konig do Brasil Ltda) at 2 mL/kg body weight. The hair of the dorsal region of the animals was shaved in the cephalo-caudal direction, incision lines were marked on the skin with a thin felt tipped pen, and the skin was cleaned with 0.9% saline solution. A 2 x 2 cm surgical wound was created on the back of each animal in the interscapular region. The dorsal skin was resected together with the panniculus carnosus according to the incision lines previously marked; the incision was deepened until the dorsal muscular fascia, which was exposed. Hemostasis was achieved by digital pressure using sterile gauze.

The gel formulations were applied to the respective groups daily for seven days, including the day the surgery was performed. At the end of the 7-day period, the animals were euthanized by anesthesia overdose. Next, the tissue from the wound site was removed together with a 1-cm wide margin (Figure 1), fixed in 10% buffered formaldehyde solution, and sent to the

pathology laboratory. The specimen was embedded in paraffin and stained with hematoxylin and eosin. Slides were prepared and photographed with a camera coupled to a light microscope; the images were digitized with Winfast software, and measurements were performed using Image Tool software. The advancement of the granulation tissue from the margin to the center of the wound was measured.

Statistical analysis was performed using the Kruskal-Wallis test followed by the Student-Newman-Keuls test[4]. The Kruskal-Wallis test was used to compare quantitative variables. The Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc.) was used for the analysis. All statistical tests were performed at a significance level α of 0.05 ($p<0.05$).

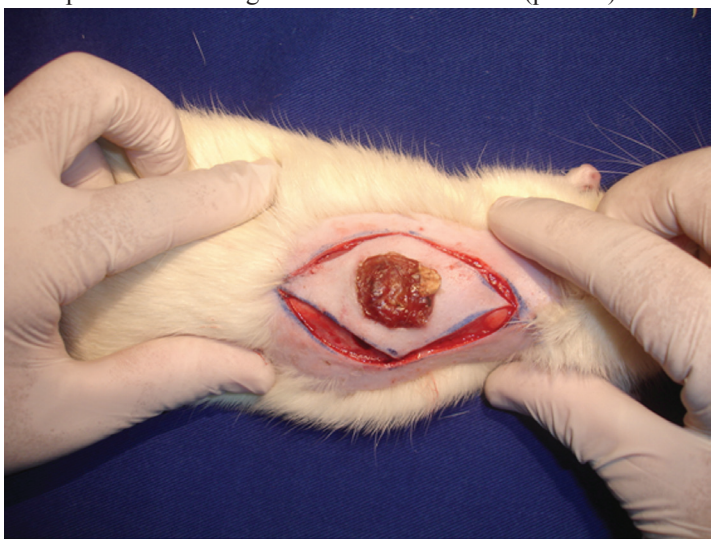

FIGURE 1 – Removal of tissue for histomorphometric analysis.

Results

During the study, three rats in the CG group, two rats in the G4% group, and three rats in the G10% group died. The statistical comparison showed that there were significant differences in the total area of epithelialization between the groups G2% and G10% ($p = 0.0329$), CG and G4% ($p = 0.0245$), and G4% and G10% ($p = 0.0153$). The smallest interquartile deviation occurred between the CG and G4% groups.

The presence of hemorrhage, desquamation, and secretion was assessed by macroscopic examination. During the study period, hemorrhage was not observed; the animals had only a normal bleeding inherent to the procedure, which was controlled by application of digital pressure with gauze. Desquamation was observed to a greater extent on the third day of gel treatment in all groups. Surgical wounds in the CG group showed delayed reepithelialization and contraction compared to those in the G2%, G4% and G10% groups (Figure 2).

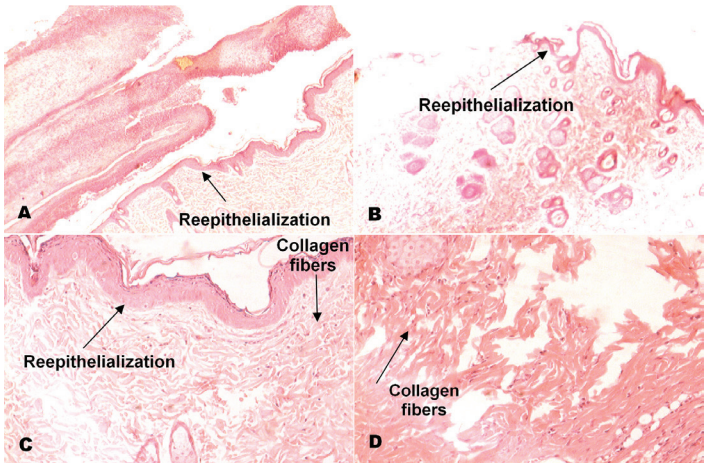

FIGURE 2 – Reepithelialization and collagen fibers in the CG group (**A**); G2% group (**B**); G4% group (**C**); and G10% group (**D**). Hematoxylin and Eosin stain, magnification x40.

Discussion

Brazil has a large and unexplored pharmacopeia in the form of plants and herbs with medicinal properties. This privileged botanical diversity opens up possibilities for the development of new herbal drugs that could be part of a therapeutic arsenal[5]. Herbal therapy is widely used in several countries as an alternative to synthetic drugs due to a number of factors, such as local culture and lower costs (no royalties), because herbal products are typically not patentable. In many countries, herbal therapy is an important form of access to medication. In Africa, for example, it accounts for up to 80% of the medicine used by the population. However, the use of herbal medicine has its risks since there are many adverse reactions to the herbal compounds. There is a tendency for herbal products in their many different forms and applications to be labeled by the popular culture as natural and risk-free. The use of medicinal plants and herbal medicines to restore health is a common practice in many ethnical groups and a cumulative result of hundreds of years of empirical knowledge on the action of plants[6].

The attention given to the use of medicinal plants by governments and health officials has increased significantly in the past years[7]. As a consequence, studies on the properties and uses of fruits and their pulps and peels have been carried out in India and China to determine their nutritional and medicinal value[8,9]. Studies on the properties of the banana, especially of its pulp, have shown that it is rich in flavonoids and leucocyanidin, which are

compounds known to have anti-inflammatory and anti-neoplastic properties and liver-protective activity; in the peel it showed potent antihypertensive activity in renal hypertensive rats[8,9]. There are also studies in the literature reporting on the use of the peel and leaves of the banana plant to improve epithelialization and alleviate pain in the treatment of chronic wounds[10].

Musa sapientum var. paradisiaca has been studied intensively for the past 30 years. A multicenter study showed that banana was useful in the treatment of ulcer dyspepsia; however, its medicinal use is still incipient[10]. *Musa sapientum* also promotes cellular alteration of the mucosa and increases the synthesis of DNA without carcinogenic or mutagenic effects[11-14].

A study on the use of *M. sapientum* var. *paradisiaca* extracts was based on the premise that, since the plant has a healing action when used to treat gastric ulcers, it could also be used to treat skin wounds. The authors used techniques that allowed the assessment of the contraction of the scar surface area, epithelialization time, and presence of antioxidants. Rats were treated with aqueous and alcoholic extracts of *M. sapientum* var. *paradisiaca for a period of 21 days. The results were satisfactory regarding the antioxidative properties of the extracts[14].* However, the use of the gel of unripe banana peel in wound healing by secondary intention is not well documented. The high economic and social costs for both the government and patients associated with the treatment of wounds are an important motivation for the search of new therapeutic alternatives. Therefore, if the effectiveness of unripe banana in the treatment of surgical wounds is confirmed, it will be an important step towards the development of a new alternative wound treatment. The identification of an extract prepared from either the peel or pulp of unripe bananas gives rise to new therapeutic possibilities.

Conclusion

The gel of unripe *M. sapientum* (banana) peel in the concentration of 4% improved the wound healing process in rats compared to the other gel concentrations as evidenced by the smaller residual wound area.

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