DOI: 10.1590/0100-6991e-20192245 Original Article

Comparative analysis of the effects of honey, copaiba oil-resin and a commercial product (fibrinolysin, deoxyribonuclease and chloramphenicol) on second intention healing, in rats.

Análise comparativa dos efeitos do mel, do óleo-serina de copaíba e de um produto comercial (fibrinolisina, desoxirribonuclease e cloranfenicol) na cicatrização por segunda intenção, em ratos.

Maria de Lourdes Pessole Biondo-Simões, ECBC-PR[1]; Lucélio Henning Júnior[2]; Bruno Russiano de Oliveira Boeno[2]; Julyana Leoni do Prado[2]; Luiza Rodrigues da Costa[2]; Rogério Ribeiro Robes[3]; Sérgio Ossamu Ioshii[4]

ABSTRACT

Objective: to compare the healing by second intention under the effects of topical application of honey, copaíba oil-resin and a commercial product (fibrinolysin, deoxyribonuclease and chloramphenicol) with a control group in rats. **Methods:** we carried out a skin resection, 1cm in diameter, on the back of 40 rats allocated to four groups of ten animals. All wounds were cleaned daily with 2ml of 0.9% NaCl solution. The first group (control - GC) was restricted to such procedure. In the wounds of the second (GM), third (GO) and fourth groups (GF), after cleaning, we respectively applied 1ml of honey, 1ml of copaíba oil-resin and 1ml of cream containing fibrinolysin, deoxyribonuclease and chloramphenicol. The wounds were occluded with sterile gauze. Immediately after the incision and on days three, seven and 14 of the experiment, the wounds were copied and contraction was analyzed using planimetry. After euthanasia, we histologically evaluated the inflammatory reaction and collagen in the scars. **Results:** the reduction of the wound area of GM (p=0.003), GO (p=0.011) and GF (p=0.002) were higher than the GC. The amount of type-I collagen present in GM and GO was higher than in GC and GF groups (p<0.05). There was a predominance of chronic inflammatory stage in GM (p=0.004), GO (p<0.001) and GF (p=0.003) when compared with GC. **Conclusion:** the topical use of honey and copaíba oil-resin increases wound contraction, the presence of type-I collagen and accelerates healing.

Keywords: Honey. Copaiba. Wound healing. Rats.

INTRODUCTION

Wound is the anatomical and physiological loss of skin continuity[1]. Wounds are responsible for increased morbidity and mortality and, therefore, have a significant impact on public health[2]. In Brazil, there are a large number of people with complex wounds, unable to perform their daily activities, and without access to medicines that facilitate the healing process. This leads to complications such as infections, limb amputation and difficulty in reintegration into the economically active class[3].

Healing can be didactically divided into four phases: hemostasis, inflammation, proliferation and remodeling. These phases do not occur individually in time, and there is interposition between them, with predominance of one or the other depending on the physiological state of the wound[1]. Increasing the speed of healing and the quality of the tissue formed are matters of great interest to the medical community, given not only the large number of injuries related to high morbidity and mortality, but also the high costs related to the stabilization, maintenance and improvement stages of this process. In this context, there is a growing interest in alternatives to conventional treatment, especially the use of natural products, which are easily accessible and inexpensive[1,4].

1 - Federal University of Paraná (UFPR), Center of Health Sciences, School of Medicine, Department of Surgery, Curitiba, PR, Brazil. 2 - Federal University of Paraná (UFPR), Center of Health Sciences, School of Medicine, Department of Surgery, Curitiba, PR, Brazil. 3 - Federal University of Paraná (UFPR), Center of Agrarian Sciences, School of Veterinary Medicine, Department of Veterinary Medicine, Curitiba, PR, Brazil. 4 - Federal University of Paraná (UFPR), Center of Health Sciences, School of Medicine, Department of Medical Pathology, Curitiba, PR, Brazil.

Throughout history, natural compounds have been the basis of medicines, often starting from popular knowledge and coming to the market purified and improved. In this context, honey is the oldest material used to accelerate the healing process known to man[5], presenting several properties that make it a potential element for dressing formulations. It can reduce edema, inflammation and exudation of a wound and increase the efficiency of the healing process[6].

This viscous solution, produced by several bee species, is predominantly composed of sugars (75% to 79%) and water (20%)[6]. Other components are: proteins (invertase enzymes, catalase and glucose oxidase), vitamins (nicotinic acid, pyridoxine and thiamine), minerals (potassium, iron, magnesium, phosphorus, copper, zinc and calcium) and antioxidants. The quantities of these components vary according to species, seasonality, local flora and storage[7].

Copaíba oil-resin is another compound that has shown potential in improving healing. It is an oil-resin extracted from trees of the genus *Copaifera*, which has more than 72 species. Among the highlights are its anti-inflammatory, analgesic, antitumor, antineoplastic and antinociceptive properties[8,9]. Because of these properties, honey and copaíba oil-resin are potential options to accelerate the healing process at a more affordable cost than current drugs, with easy access in the Brazilian territory.

In the formulation of medicines available on the market is fibrinolysin, a proteolytic enzyme that acts by favoring enzymatic debridement of injured tissues. This enzyme may have come associated with deoxyribonuclease, an enzyme that catalyzes the hydrolytic cleavage of phosphodiester bonds in the DNA structure. Many of these synthetic agents also come associated with an antibiotic, most commonly chloramphenicol and gentamicin. They are sold in the form of ointments, gel or adhesives to be applied to wounds.

The objective of this work is to evaluate the potential for secondary intention healing of topical application of honey and copaíba oil-resin by comparing them to the application of a commercial product containing fibrinolysin, deoxyribonuclease and chloramphenicol in rat skin wounds.

METHODS

This study was evaluated and approved by the Animal Use Ethics Committee of the Biological Sciences Sector of the Federal University of Paraná (UFPR). It complied with Federal Law No. 11,794 of October 8, 2008, which establishes the procedures for the scientific use of animals, and with the guidelines of the Brazilian College of Animal Experimentation (COBEA). The study was approved on April 17, 2018 and received the numbers RO03/2018, 1165 and 1169.

We used 40 male Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia)*, from Wistar strain, from the Central Biottery of UFPR, with an average weight of 486±18.47 grams and age between 120 and 140 days. Throughout the study period, the animals were housed in the Laboratory of the Discipline of Surgical Technique and Experimental Surgery, in species-specific polypropylene boxes with a white sawdust bed, with three animals per box. The room temperature was maintained at 22±2ºC, with a 12 hour light/dark cycle and the relative humidity and noise level of the environment. All the boxes were arranged on shelves at equal distance from the light source. The animals had free access to their own food and water. We randomly split the sample into four groups of ten animals.

Anesthesia was performed by a veterinarian, obtained by intramuscular injection (0.1ml/100g animal weight) of a 1:1 solution of 10% ketamine (50mg) and 2% xylazine (20mg), supplemented by inhalation with 1.5% isoflurane under 100% oxygen mask. Under anesthesia, we performed trichotomy of the dorsal region with a tricotome and identification of the animals. This was followed by antisepsis performed with polyvinylpyrrolidone iodine and the delimitation of the operative field with a fenestrated sterile field.

Afterwards, we made a 1cm diameter circular lesion in the central region of the dorsum and, with an 11 scalpel blade, produced a lesion that exposed the dorsal muscular fascia. This was the day zero of the experiment.

Subsequently, we evaluated the area of the surgical wound by means of a decal applied to the lesion. Control rats (GC) had their wounds cleaned with 2ml of 0.9% NaCl solution. Honey group (GM) rats had the wound bed covered by 1ml of honey after cleaning, those of the copaíba oil-resin group (GO), by 1ml of copaíba oil-resin, and those of the deoxyribonuclease and chloramphenicol fibrinolysin group (GF), by 1ml of this product. Treatment was completed with occlusion of the wounds with dry gauze dressing. We changed the dressing daily.

On days three, seven and 14, we recalculated the wound area by decal of the lesion. We performed area analysis using the MATWORKS® MATrix LABoratory (MatLab) v.R2018a software, which provided individual measurements in square millimeters. We defined wound contraction as the area on day zero subtracted from the area on the day of assessment.

After macroscopic analysis, on day 14 of the experiment we performed euthanasia of the animals. Once anesthetized according to the protocol described above, we carried out an intracardiac injection of 2ml of 19.1% potassium chloride solution. Once confirmed the death, we resected a square segment of skin, 3cm in the side that contained the scar in the central part. We extended the flap under filter paper and submerged it in formalin for fixation and further processing for histopathological study.

Paraffin blocks containing the material were cut 4µ thick and, after mounted on slides, were stained with hematoxylin-eosin (HE) and picrosirius (Sirius Supra Red F3BA).

In HE staining, we evaluated the inflammatory process by reading four 400x magnification fields. For classification and quantification, we assessed the type and quantity of predominant cells, poly and monomorphonuclear infiltrates. We used the following parameters: 0= absence, 1= one to ten cells per field, 2= 11 to 50 cells per field, and 3= 50 or more cells per field. With these data, it was possible to quantify the reaction in intense, moderate, discrete and absent. When there was a predominance of polymorphonuclear cells, we considered the reaction acute. When the monomorphonuclear cells were predominant, we called the reaction chronic, and when there was no predominance, we called it acute-chronic.

In the analysis of picrosirius-stained slides, the thicker and birefringent collagen fibers are orange-red (collagen I), and the thinner and more dispersed, weakly birefringent fibers are greenish (collagen III). The images were taken by a Sony CCD101 camera. We performed image analysis using the MediaCybernetics Image-Plus® 4.5 for Windows® application. We analyzed six fields located in the scar line with 100x magnification in each optical microscope with polarized light lens. In each one, we calculated the percentage of area occupied by the red and yellow (collagen I) and green (collagen III) fibers.

Considering that the other types of collagen constitute very small fractions, for practical purposes we accepted the sum of collagens I and III as the total collagen of the scar.

For the contraction of the wound area, we statistically analyzed the data considering the mean, median, minimum value, maximum value and standard deviation. For the comparison of the groups in relation to the area, we used the non-parametric Kruskal-Wallis test, while for the comparison of the evaluation moments within each group, we used the non-parametric Friedman test and the Fischer exact test. For collagen analysis, we used the parametric ANOVA test: single factor. We first analyzed all the groups at the same time, and then by pairs. We verified the normality of the data with the Shapiro-Wilk test. We compared normal data with the t-test, and non-normal data, with the Mann-Whitney test. We chose the significance level $p \leq 0.05$, or 5%, for rejection of the null hypothesis.

Data were analyzed by the computer program IBM SPSS Statistics v.20.0 Armonk, NY: IBM Corp.

RESULTS

Two animals from the control group died due to anesthetic accidents on day zero.

The macroscopic evaluation of the wounds did not show the presence of hemorrhage or purulent discharge in the four groups throughout the study period.

The reduction in wound area, assessed by planimetry on days three, seven, and 14, showed no significant differences between groups (Table 1, Figure 1).

Table 1. *Wound areas in the four groups, at the three studied moments.*

	Wound area (cm^2)						
Variable/Group	n	Average	Median	Minimum	Maximum	Standard deviation	p-value*
3th dia							0.187
Control	8	0.722	0.669	0.566	1.011	0.161	
Copaíba	10	0.558	0.52	0.366	0.783	0.153	
Fib.+Deox.+Chlor.	10	0.644	0.628	0.481	0.814	0.118	
Honey	10	0.681	0.628	0.478	0.983	0.17	
7th dia							0.051
Control	8	0.3	0.272	0.181	0.484	0.111	
Copaíba	10	0.231	0.245	0.165	0.293	0.041	
Fib.+Deox.+Chlor.	10	0.245	0.249	0.16	0.385	0.065	
Honey	10	0.329	0.281	0.211	0.593	0.11	
14th dia							0.289
Control	8	0.002	0	0	0.012	0.004	
Copaíba	10	0	0	0	0	0	
Fib.+Deox.+Chlor.	10	0.002	0	0	0.009	0.004	
Honey	10	0	0	0	0	0	

** Kruskal-Wallis non-parametric test; Fib.+Deox.+Chlor.= fibrinolysin, deoxyribonuclease and chloramphenicol.*

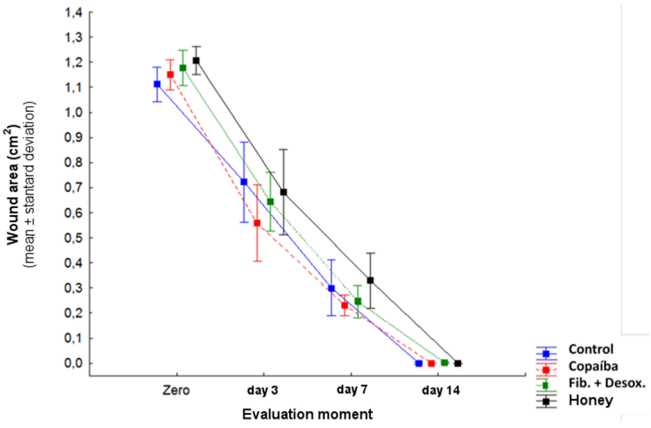

Figure 1. *Graphic demonstration of wound contraction.*

However, when comparing wound contractions by paired groups, the wounds treated with copaíba oil were smaller than those in controls (p=0.008) between day zero and day seven. The same was true for honey-treated (p=0.45) and for those treated with fibrinolysin with deoxyribonuclease and chloramphenicol compared with controls (p=0.001) (Table 2).

Wound contraction from day zero to day 14 was more expressive in the honey-treated group (p=0.003) when compared with the control one. We also observed a significant reduction in the group treated with copaíba oil (p=0.011). The group treated with fibrinolysin with deoxyribonuclease and chloramphenicol showed no significant difference compared with the control group (p=0.062) (Table 2).

At day 14, epithelization had occurred in seven out of eight GC (control) wounds, in eight of the ten GF (fibrinolysin with deoxyribonuclease and chloramphenicol) wounds, and in all GO (copaíba) and GM (honey) wounds (p>0,05). Figure 2 provides examples of wound closure.

The inflammatory reaction, present in the four groups, was of minimal intensity. There was a predominance of chronic inflammatory stage wounds in GM, GO and GF when compared with the control group (p=0.004, p<0.001 and p=0.003, respectively). There was no difference between GO, GM and GF (p=0.074).

In the quantitative, histological evaluation of collagen, although there was a higher average area in GC and GF, the difference was not significant in relation to the other groups (ANOVA p=0.612) (Table 3, Figure 3).

Table 2. *Paired-group analysis of wound area reduction.*

	p-values	
Comparative groups	Reduction (7th vs zero)	Reduction (14th vs zero)
Control x Copaíba	0.008	0.011
Control x Fib.+Deox.+Chlor.	0.001	0.062
Control x Honey	0.045	0.003
Copaíba x Fib.+Deox.+Chlor.	0.0479	0.002
Honey x Fib.+Deox.+Chlor.	0.164	0.151

Fib.+Deox.+Chlor.= fibrinolysin, deoxyribonuclease and chloramphenicol.

Table 3. *Average percentages area of histological sections represented by collagen.*

Variable	Group	n	Average	Median	Minimum	Maximum	Standard derivation
% area	Control	8	10.2	10.5	6.9	13.9	2.2
	Fib.+Deox.+Chlor.	10	10.4	10.0	7.4	13.7	2.8
	Honey	10	11.0	10.7	9.0	13.9	1.5
	Copaíba	10	9.8	9.6	8.7	11.1	0.9

One-way analysis of variance (ANOVA) and the least significant difference (LSD) post-hoc test; p=0.612; Fib.+Deox.+Chlor.= fibrinolysin, deoxyribonuclease and chloramphenicol.

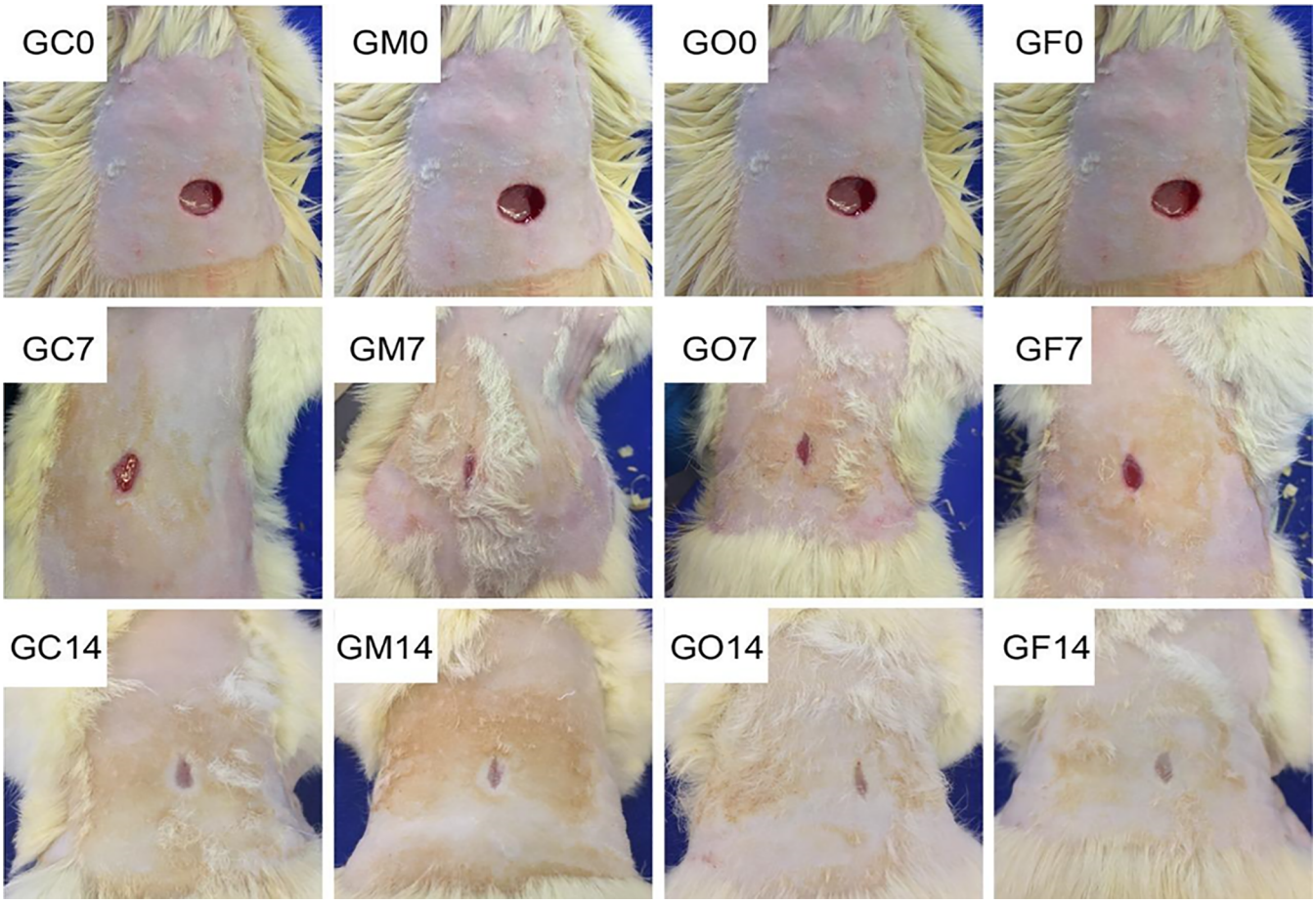

Figure 2. *Macroscopic evolution of wound healing. GC= control group, GM= honey group, GO= copaíba oil-resin group, and GF= fibrinolysin + deoxyribonuclease + chloramphenicol group; 0= moment zero; 7= moment seven days; and 14= moment 14 days.*

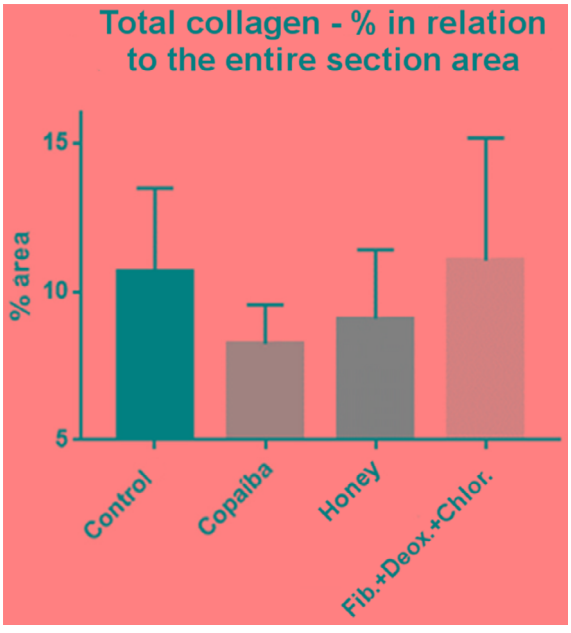

Figure 3. *Average percentage area of the histological sections occupied by collagen in each group.*

In the qualitative evaluation of type-I collagen, we found that the groups that used honey and copaíba oil reached higher values when compared with the groups treated with fibrinolysin with deoxyribonuclease and chloramphenicol and control ($p<0.001$) (Table 4, Figure 4). In the paired comparison, we observed that wounds treated with copaíba oil showed more collagen type I than those in the control group ($p=0.0475$), as well as those treated with honey compared with the control ($p=0.0031$). Copaíba oil was superior to fibrinolysin with deoxyribonuclease and chloramphenicol ($p=0.0423$). The same occurred with the use of honey ($p=0.0299$) (Table 5). Figure 5 shows images of collagen marking.

Table 4. *Average percentage of collagen I in relation to total collagen.*

Variable	Group	n	Average	Median	Minimum	Maximum	Standard derivation
% col. I	Control	8	13.4	13.8	10.1	15.8	1.9
	Fib.+Deox.+Chlor.	10	13.6	14.0	11.0	15.6	1.7
	Honey	10	16.6	16.5	13.0	18.5	1.6
	Copaíba	10	16.4	16.1	14.9	18.4	1.2

One-way analysis of variance (ANOVA) and the least significant difference (LSD) post-hoc test; p<0.001; Fib.+Deox.+Chlor.= fibrinolysin, deoxyribonuclease and chloramphenicol.

Table 5. *Paired-group comparison of mean collagen I in relation to total collagen.*

Comparison	p-value
Control x Copaíba	0.0475
Control x Honeyel	0.0031
Control x Fib.+Deox.+Chlor.	0.6494
Copaíba x Honey	0.9886
Copaíba x Fib.+Deox.+Chlor.	0.0423
Honey x Fib.+Deox.+Chlor.	0.0288

Student's t test for normal data and Mann-Whitney test for non-normal data; Fib.+Deox.+Chlor.= fibrinolysin, deoxyribonuclease and chloramphenicol.

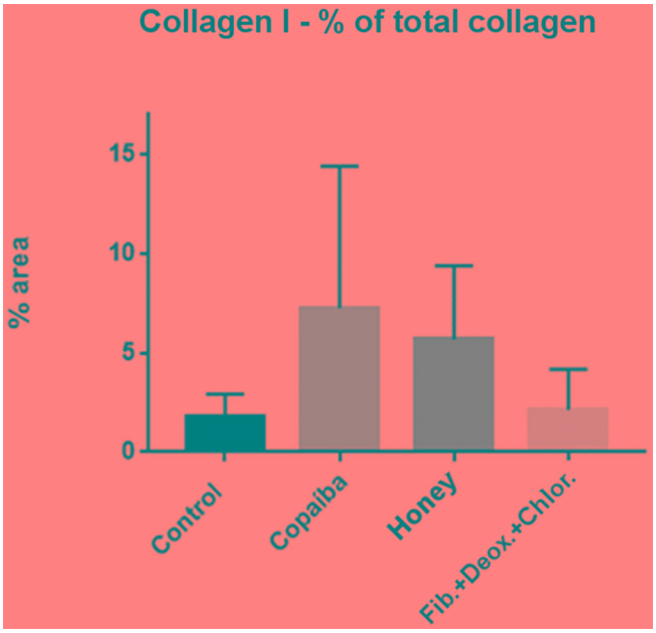

Figure 4. *Average type-I collagen area in each group.*

DISCUSSION

Complete epithelialization of the wounds occurred at the end of the study time in 35 of the 38 lesions followed. However, the speed with which the process happens and the quality of the scar formed are of great interest. The present study evaluated the influence of different treatments by quantifying the contraction of the wound area, the inflammatory reaction and the amount and quality of collagen at the end of the healing process.

The pharmaceutical industry is always developing or improving products to aid healing. As an example, there is the drug used in this study, composed of fibrinolysin, deoxyribonuclease and chloramphenicol. The enzymes fibrinolysin and deoxyribonuclease act favoring the enzymatic debridement of exudate present in the injured tissues, accelerating the removal of damaged and non-viable cells. Chloramphenicol has bactericidal and bacteriostatic action, limiting bacterial colonization in the wound bed. Thus, the combination of compounds allows healing to occur at an accelerated rate, with less bacterial colonization and thus less inflammation, promoting earlier granulation and epithelialization. However, there is controversy regarding the ability of this compound to repair the wound and its cost[8].

The use of natural products in healing is a practice with records since prehistory. Honey is one of the oldest compounds used to accelerate the healing process known to man. It is cited in Edwin Smith's Papyrus, circa the XX Century b.C[10]. In addition, its properties have been studied in numerous works, which have evidenced its ability to accelerate the healing process and improve the quality of the scar formed[7,11-13].

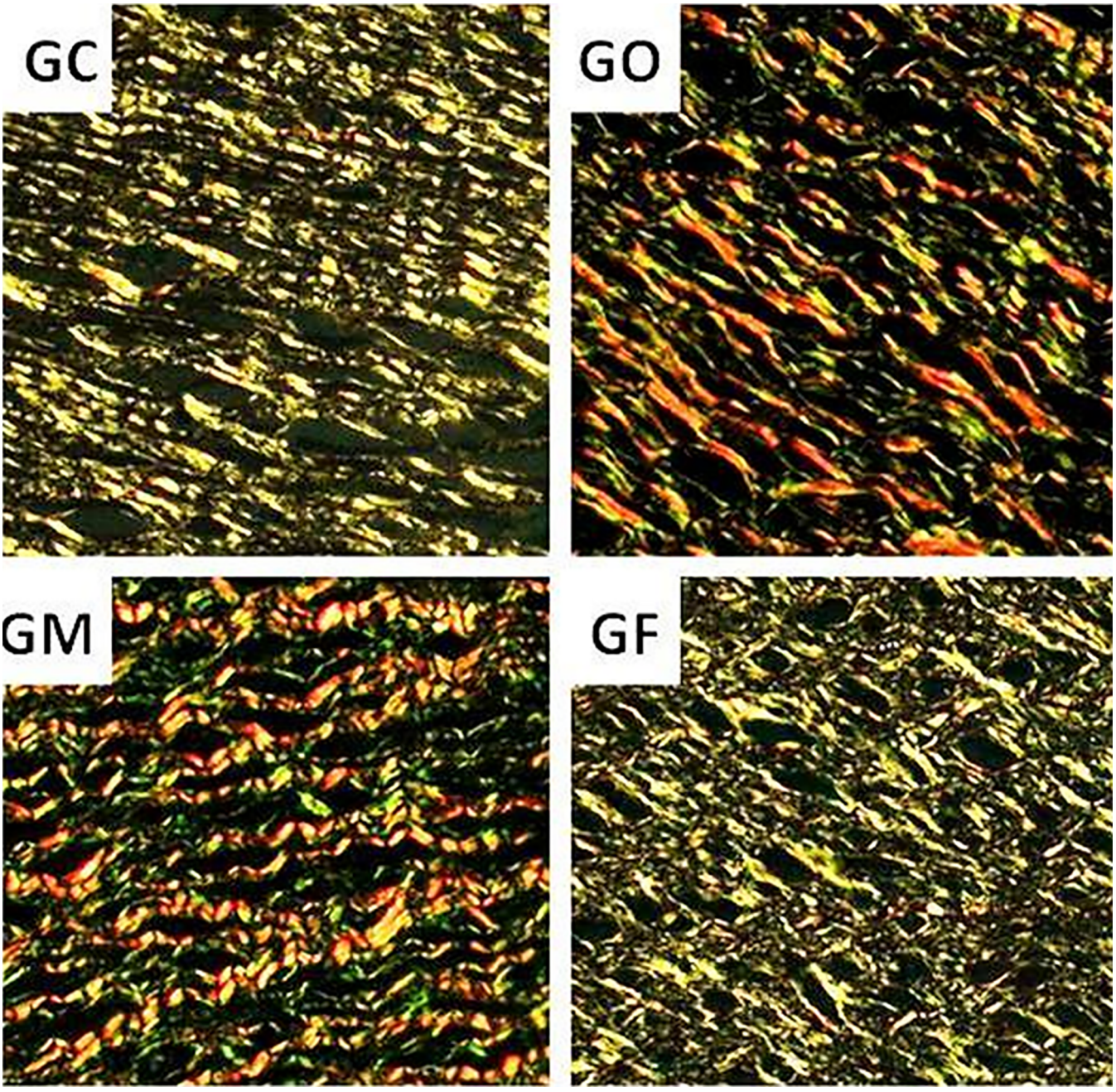

Figure 5. *Photomicrographs of histological sections stained by picosirius (Sirius supra red F3BA), polarized light, 100x. Type-I collagen: fibers stained in shades of yellow to red; Type III collagen: green-stained fibers.*

Copaíba oil-resin is also very old in use, being the first known citation in a letter from Petrus Martius to Pope Leo X, in 1534, that reported a drug used by the Indians, called "Copei"[10]. Like honey, the ability of copaíba oil-resin to improve the healing process is also known[8].

Considering that both honey and copaíba oil-resin are natural products, available practically all over the national territory, their use could be more accessible to the needy population.

The increase in contraction velocity in the first seven days of the study, observed in the three

treated groups when compared with the control one, demonstrates that the use of the compounds is more effective at the beginning of the healing process. Other studies have already concluded this higher efficiency, but found a higher speed at the end of the healing process, contrary to this work[8].

Such speed increases can be explained by characteristics common to the studied elements: the three compounds compared with the control have direct antibacterial activity, if not bactericidal, at least bacteriostatic. The difficulty of infection promoted by the non-immunological means facilitates reepithelization, since it allows a scenario closer to the ideal for the healing process[14,15].

Both honey and copaíba oil-resin have the capacity to promote angiogenesis[11,16], which facilitates oxygen delivery to the wound bed, the arrival of inflammatory cells and the early establishment of granulation tissue. In addition, both compounds exhibit direct, already determined anti-inflammatory activities[5,9].

In this study, the inflammatory reaction was minimal. In GM, GO and GF, however, it was in the chronic phase (predominance of monomorphonuclear cells), suggesting that they were able to modulate the inflammatory response pattern in the scar bed, reaching more advanced stages in a shorter period of time, when compared with GC. The same inflammatory pattern has been shown in other studies[17,18]. By increasing the contraction capacity of the wound bed while improving the inflammatory pattern, the compounds provide a more conducive environment for the maturation of scar elements such as collagen.

There was therefore a greater amount of type-I collagen fibers in GM and GO compared with the other groups, and these data are already present in the literature[7,8]. Thus, it can be inferred that honey and copaíba oil-resin induce fibroblast proliferation and extracellular matrix formation. As the type-I collagen is more resistant, a higher concentration of it indicates a better quality wound when compared with GF and GC.

In addition, for the group treated with copaíba oil-resin, we observed a smaller total amount of collagen in the wound bed. Since copaíba oil-resin is able to increase the activity of metalloproteinase type 2[18], an enzyme responsible for the collagen remodeling and homeostasis phase, it is believed that the topical application of the oil-resin leads to the earlier occurrence of the later healing phases.

A major advantage of honey and copaíba oil-resin over the commercial product is their easy access. In this study, we used commercialized products, sold in common retail. The commercial product is sold only in drugstores and requires a prescription, which makes it difficult to use for populations with low access to medical services. In addition, both honey and copaíba oil-resin can cost 60 times less than the commercial product, depending on where in the country they are purchased. The use of these natural products makes treatment more affordable and more accessible, increasing patient compliance, especially for minor injuries that would not receive any kind of medical care but are subject to the same complications as others.

Therefore, there is evidence to say that honey and copaíba oil-resin are as effective as the commercial wound care product, with the advantage of forming a better quality scar, having greater accessibility and reduced treatment cost. However, there was no significant difference in the action of honey and copaíba oil-resin compounds when compared with each other. Other studies are still needed to compare the performance of the different types of honey and copaíba oil-resin and to show their mechanisms of action, thus providing the evidence for their clinical recommendation.

R E S U M O

Objetivo: *comparar a cicatrização, por segunda intenção, sob os efeitos da aplicação tópica de mel, óleo-resina de copaíba e um produto comercial (fibrinolisina, desoxirribonuclease e cloranfenicol) a um grupo controle, em ratos.* **Métodos:** *ressecção de pele, com 1cm de diâmetro, foi realizada no dorso de 40 ratos alocados em quatro grupos de dez animais. Todas as feridas foram limpas, diariamente, com 2ml de solução de NaCl 0,9%. O primeiro grupo (controle - GC) ficou restrito a tal procedimento. Nas feridas do segundo (GM), terceiro (GO) e quarto grupos (GF), após limpeza, aplicou-se, respectivamente, 1ml de mel, 1ml de óleo-resina de copaíba e 1ml de creme contendo fibrinolisina, desoxirribonuclease e cloranfenicol. Ocluíram-se as feridas com gaze estéril. Imediatamente após a incisão e nos dias três, sete e 14 do experimento, as feridas foram copiadas e, usando planimetria,analisou-se a contração. Após a eutanásia, a histologia foi utilizada para avaliação da reação inflamatória e do colágeno nas cicatrizes.* **Resultados:** *a redução da área da ferida do GM (p=0,003), GO (p=0,011) e GF (p=0,002) foram superiores ao do GC. A quantidade de colágeno tipo I presente no GM e no GO foi superior aos grupos GC e GF (p<0,05). Houve predominância do estágio inflamatório crônico no GM (p=0,004), GO (p<0,001) e GF (p=0,003) quando comparados ao GC.* **Conclusão:** *o uso tópico do mel e do óleo-resina de copaíba aumenta a contração da ferida, a presença de colágeno tipo I e acelera a cicatrização.*

Descritores: *Mel. Copaíba. Cicatrização de feridas. Ratos.*

REFERENCES

1. Morton LM, Phillips TJ. Wound healing and treating wounds: differential diagnosis and evaluation of chronic wounds. J Am Acad Dermatol. 2016;74(4):589-605.
2. Drew P, Posnett J, Rusling L; Wound Care Audit Team. The cost of wound care for a local population in England. Int Wound J. 2007;4(2):149-55.
3. Jeffcoate WJ, Harding KG. Diabetic foot ulcers. Lancet. 2003;361(9368):1545-51.
4. Lam TK, Lowe C, Johnson R, Marquart JD. Secondary intention healing and purse-string closures. Dermatol Surg. 2015;41 Suppl 10:S178-86.
5. Saikaly SK, Khachemoune A. Honey and wound healing: an update. Am J Clin Dermatol. 2017;18(2):237-51.
6. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci. 2013;16(6):731-42.
7. Alves DFS, Cabral Júnior FC, Cabral PPAC, Oliveira Junior RM, Rego ACM, Medeiros AC. Efeitos da aplicação tópica do mel de Melipona subnitida em feridas infectadas de ratos. Rev Col Bras Cir. 2008;35(3):188-93.
8. Collaço LM, Scapini JG, Collaço LM, Matsubara A, Veiga Júnior VF. Comparative analysis of the effects of Copaifera multijuga oil-resin and nitrofurazona in the cutaneous wound healing process. Rev Col Bras Cir. 2016;43(6):445-51.
9. Gomes Nde M, Rezende CM, Fontes SP, Matheus ME, Pinto Ada C, Fernandes PD. Characterization of the antinociceptive and anti-inflammatory activities of fractions obtained from Copaifera multijuga Hayne. J Ethnopharmacol. 2010;128(1):177-83.
10. Veiga Junior VF, Pinto AC. O gênero copaifera L. genus. Quím Nova. 2002;25(2):273-86.
11. Goharshenasan P, Amini S, Atria A, Abtahi H, Khorasani G. Topical application of honey on surgical wounds: a randomized clinical trial. Forsch Komplementmed. 2016;23(1):12-5.
12. Javadi SMR, Hashemi M, Mohammadi Y, MamMohammadi A, Sharifi A, Makarchian HE. Synergistic effect of honey and Nigella sativa on wound healing in rats. Acta Cir Bras. 2018;33(6):518-23.
13. Al-Waili N, Salom K, Al-Ghamdi AA. Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. Scientific World J. 2011;11:766-87.
14. Simões CA, Conde NC, Venâncio GN, Milério PS, Bandeira MF, da Veiga Júnior VF. Antibacterial activity of copaiba oil gel on dental biofilm. Open Dent J. 2016;10:188-95.
15. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed. 2011;1(2):154-60.
16. Estevão LRM, Medeiros JP, Scognamillo-Szabó MVR, Baratella-Evênciuo L, Guimarães EC, Câmara CAG, et al. Neoangiogênese de retalhos cutâneos em ratos tratados com óleo de copaíba. Pesq Agropec Bras. 2009;44(4):406-12.

17. Estevão LRM, Medeiros JP, Baratella-Evêncio L, Simões RS, Mendonça FS, Evêncio-Neto J. Effects of the topical administration of copaiba oil ointment (Copaifera langsdorffii) in skin flaps viability of rats. Acta Cir Bras. 2013;28(12):863-9.
18. Masson-Meyer D, Enwemeka CS, Bumah V, Andrade T, Frade MA. Topical treatment with Copaifera langsdorffii oleoresin improves wound healing in rats. Int J Phytomedicine. 2013;5(3):378-86.

Received in: 05/21/2019
Accepted for publication: 08/10/2019
Conflict of interest: none.
Source of funding: none.

Mailing address:
Maria de Lourdes Pessole Biondo-Simões
E-mail: malubiondo@gmail.com