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Evaluation of Burns Wound Healing Properties of Different Varieties of Honey from Cameroun

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The use of local natural products, such as honey, is empirically prescribed in the treatment of burns and several medical conditions.
Aim: This was conducted to evaluate the burn wound healing activity of three types of local types of honey on rats.
Materials and Methods: This was a comparative experimental study held at the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. We used 3 varieties of honey

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designated by their cities of origin: Ngaoundal, Okuh, and Yaounde. Brulex® (Zinc Oxide) was our reference medicine against burns. Male rats of the Wistar strain have served as animal material on which the burns were performed. There were 36 individuals divided into 4 groups of 9 rats per batch for treatment with reference medicine and 3 honey samples. We realized experimental burns under general anesthesia by a heated mass. The surface areas were calculated using Autodesk AutoCAD 2014® software. The photos were taken using an XTIGI V10® telephone. At the various dates selected, the wound surfaces calculated for each batch were expressed in the form of the mean ± standard deviation.

Results: There wa no significant variation in pH and free acidity. Okuh honey is the most acidic (pH 5.73 ± 0.682). Concerning the Brix degree, the least sweet honey is that of Okuh (73.94 ± 0.115). There is a significant difference in the Brix degree between these three samples (p=0.00000009; Table 1). The richest honey in metabolites was that of Ngaoundal, with polyphenols of 323.79±53.57 mEq/kg, flavonoids of 47.45±3.84 mEq/kg and total flavonols of 21 .82±0.90 mEq/kg.

Concerning the evolution of the healing process, the group of rats treated with Brulex® showed the largest injured surfaces on day 0 (8.83 ± 2.34 cm^2), the least extensive being those of the Okuh group (6.83 ± 0.66 cm^2). On the 24th day, the smallest areas were in order those of the Ngaoundal, Yaounde, and Okuh lots. While the rats in the Brulex® and negative control lots were not yet completely cured. On histology sections, Okuh, Yaoundé, and Ngaoundal groups had slight fibrosis, hypervascularization and an abundance of immune cells.

Conclusion: The three honey type seem effective in burn wound care and can be suggested.

Keywords: Burns; wound healing; honey.

1. INTRODUCTION

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals [1]. According to the World Health Organisation (WHO) [1], the International Society for Burn Injuries (ISI) [2], and the WHO Emergency Medical Teams Technical Working Group on Burns (WHO TWGB) [3], burns are a global public problem. Burning is responsible for 180,000 deaths per year worldwide, the vast majority occurring in low and middle-income countries [1]. In Cameroon, the incidence is not known and a study by the major burns center in Douala showed that domestic accidents are the most frequent causes (63.13%), followed by work accidents (22.50%) [4]. Burns injuries gravity varies according to many factors: the dept, the surface burned, the etiology, the location, the tissues and organs involved [1]. The healing of the burn goes through the different phases of inflammation, including the crucial vascular-oxidative phase. During this last phase, reactive oxygen species (ROS) are released, and are responsible for oxidative stress [5]. In this sense, ROS blocking methods in the therapeutic management of burns have significantly improved their prognosis.

In the management of a burned patient is a multidisciplinary approach involving emergency care, anesthesia, surgery and wound care. For superficial limited burns, many anstiseptics are available locally and as published by Norman et al. [6], but their use is often limited by the high price, especially in developing countries. An empiric alternative is therefore the use of local natural products, that are available, safe and affordable if they are proven to be effective. Honey is one of these natural products. Honey is derived from plant and animal origin, obtained by the transformation of flower nectar or honeydew by the bee *Apis mellifera* [1]. Some studies have already been carried out globally on honey and its antimicrobial activity through its acidity (PH<7), activation of autolytic debridement(by increased the activity of the enzyme plasmin in macrophages, anti-inflammatory activity (decreased levels of malondialdehyde and lipid peroxide, antioxidant and histologically observation of reduced numbers of inflammatory cells present in biopsy samples), antioxidant activity (blocked by phenolic antioxidants), haility to increase the rate of healing (osmotic action increasing oxygen availability, vacum-like effect with increase vascularisation, lymphatic drainage and wound contraction , painless wound dressing removal) and an osmotic action (due to sugar content and that increases the availability of oxygen and nutrients for growth of repair tissues) [5]. Nicolaas and al. also insisted on antimicrobial activity [7]. Yan-Teng and al. [8] published also on wound contraction effects and antibacterial properties of Honey. In Greece, Vallianou et al. demonstrated the anti-inflammatory, healing and antioxidant properties of a variety of honey [9]. With its climatic diversity, Cameroon presents a diversity of kinds of honey for such scientific experiments. This is why it seemed important for us to evaluate the healing activities of three varieties of honey from Cameroon, harvested in three localities, different both by their climate and their vegetation: yaounde, ngaoundal and okuh. This variation may lead to content variation and variation in clinical wound healing process.

The general objective was to investigate determine phytochemical screening of honeys, study physico-chemical parameters and the healing properties of these three varieties of Cameroonian honey on experimental burns in rats. The aim of this study is to find out if these 3 varieties have the same composition or not and if they are equally effective and could be recommended or not.

2. MATERIALS AND METHODS

2.1 Nature, Type, and Location of the Study

This was a comparative experimental study related to the therapeutic efficacy of varieties of honey in Cameroon. It was held at the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I (Anatomopathology, and Animal Care Laboratories) and at the National Polytechnic School of the same university, where the manufacture of a small mass was done in the Mechanical Engineering Laboratory.

2.2 Equipment

For our study, we used 3 varieties of honey designated by their cities of origin: Ngaoundal, Okuh, and Yaounde. Brulex® was our reference medicine against burns as it is oftenly use for wound dressing in our hospitals. A 100g of this topical ointment contains 5g of Zinc Oxide; 1g of Peruvian Balsam; 0.5g of Phenazone, 30mg of phenol, 3.6mg of sodium salicylate and excipients (glycerol, sodium carbonate decahydrate, sodium benzoate, levomenthol, wool fat, and purified water, according to the 2020 version of the French Vidal reference drug book. Male rats have served as animal material on which the burns were performed.

2.3 Determination of Physico-chemical Parameters and Phytochemical Screening of Honeys

In the qualitative phase, the measurement of the pH of the honey solutions was done using a pH meter, as was the estimation of the free acidity; followed by the measurement of the sugar content by the Brix degree in 10g of honey dissolved in 75ml of distilled water. Expressed in content, the dosage of total polyphenols was carried out by the method of Singelton et al. [10]. That of total flavonoids was carried out according to Zhishen et al. [11]. And, for total flavonols, the method was that of Kumaran et al. [12].

2.4 Study Subjets and Sampling

Quantitatively, the random sampling selected albino rats of the Wistar strain, from the animal laboratory of the Faculty of Medicine and Biomedical Sciences. There were 60 of them in the test phase: we selected 36 out of 60 rats weigning 150-200grammmes and they were divided into 4 groups of 9 rats per batch for treatment with Brulex, Yaounde, Ngaoundal and Okuh hpney samples. The rest of 24 rats were unburned and non treated. Before this distribution, a pre-test was carried out with five batches of two rats of the Wistar strain (2 non treated but observed and 2 for each of the 4 treatment groups). The realization of experimental burns, according to the method of Cai et al. [13], was performed under general anesthesia by intravenous administration of ketamine® and diazepam® for 15 to 20 minutes. It used a mass of 100g and a round contact surface of 22mm in diameter. The mass was heated in boiling water (100 ° C) until thermal equilibrium (reached at 5 min). After heating, it is removed from the water, quickly wiped off, and then applied without pressure for 20 seconds at the place of election. The photos were taken using an XTIGI V10 telephone; the surface areas were calculated using the Autodesk AutoCAD 2014® software. The evaluation of healing by digital planimetry proceeded from the calculation and the percentage of retraction, using the equation developed by Gopinath et al. in 2004 [14]. We then calculated the average of the areas of the six wounds of the same batch at time t, in comparison to the area of the initial burn.

2.5 Determination of Biochemical Parameters during the Evolution of the Burn

Estimation of oxidative stress was carried out in all rats in the timeline of 8 hours (blood collection only for negative control and non-burnt rats), 2 days (blood collection only for all batches), 10 days and 24 days (blood and skin for all batches), to take these samples. Blood collected in dry tubes was centrifuged at 3000 tours per minute for 20 minutes and then stored at -20°C. The determination of nitrites was done by the method of Sinha et al. in 1972, with Griess' reagent [15]. The dosage of reduced glutathione was carried out according to the method of Weckbecker and Cory [16]. In our study, malondialdehyde (MDA) levels were assessed using Ohkawa's method [17]. While that of the total antioxidant capacity of the serum was done by the FRAP method according to Benzie et al. [18]. To further appreciate the evolution of the wounds in relation to the type of treatment, we performed a microscopic analysis of the tissues by histology.

2.6 Statistical Analysis

At the various dates selected, the wound surfaces calculated for each batch were expressed in the form of the mean ± standard deviation. For their comparison, we used Fisher's ANOVA test. The data was saved as a Microsoft Office Excel® 2013 file and then analyzed using SPSS version 20.0 software.

3. RESULTS

Physico-chemical parameters and phytochemical screening of honeys (Tables 1 to 6). Values are expressed as mean ± standard deviation. In the same row, the values in bold are significantly different ($p<0.05$).

Table 1 presents the values of the pH, the Brix degree and the free acidity of the different varieties of honey. It does not show any significant variation in pH and free acidity. However, Okuh honey is the most acidic (pH 5.73 ± 0.682) while that of Ngaoundal is the most basic (pH 5.93 ± 1.659) and also has the most free acidity (12.47 ± 4.563 mEq/l). As for the Brix degree, the least sweet honey is that of Okuh (73.94 ± 0.115). There is a significant difference in the Brix degree between these three samples ($p=0.00000009$; Table 1). Our results show that the richest honey in metabolites was that of Ngaoundal, with polyphenols of 323.79±53.57 mEq/kg, flavonoids of 47.45±3.84 mEq/kg and total flavonols of 21 .82±0.90 mEq/kg. As shown in Table 2 for this purpose, Okuh honey presented the lowest values of polyphenols and total flavonols. Nitrites concentrations are shown on table 4, the malondiadehyd concentration on tabe V and the total oxidative capacity of serum of differents groups on Table 6. The difference noted are few and non persistant with time.

3.1 Morphological Parameters and Healing of Burns

The rats weght variation is shown in Fig. 1. Concerning the evolution of the healing process (Table 3, Figs. 2,3, 4, 5 and 6), the group of rats treated with Brulex® showed the largest injured surfaces on day 0 (8.83 ± 2.34 cm2), the least extensive being those of the Okuh group (6.83 ± 0.66 cm^2). On the 24th day, the smallest areas were respectively and crescendo of the Ngaoundal, Yaounde, and Okuh lots. While the rats in the Brulex® and negative control lots were not yet completely cured. Fig. 3 represents the variations in the rate of contraction of the surfaces of the wounds of the Okuh, Brulex®, and negative control groups. We observe that the rats of the Okuh batch have a faster surface contraction than that of the other two batches. Similarly, the same evolutionary pattern is observed in the comparison of two other batches with the Yaounde (Fig. 4) and Ngaoundal (Fig. 5) batches. For a comparison of the 3 therapeutic trials, it is observed (Fig. 6) that the rats of the Okuh batch were those which had a slower healing rate than that of the other two batches. But all of those rats were cured around the 22nd day. Fig. 2 shows the macroscopic characteristics observed on the burns on days 0, 10, and 22. The wounds treated with honey had scabs at the start of the experiment, which ended up falling off after. Also in the negative control group, the wounds tended to dry quickly and crust, but with very slow healing. Also, those treated with Brulex® had relatively more difficulty in drying. They always kept a moist character, while shrinking over time.

Analysis of the histological sections of the injured tissues (Figs. 7-9) revealed that the rats of the Brulex® and negative control groups presented dense fibrosis on day 10, which would be due to a massive loss of fluids and hypovascularization. Unlike those of the Okuh, Yaoundé, and Ngaoundal groups where slight fibrosis was observed, but hypervascularization and an abundance of immune cells.

4. DISCUSSION

The pH results of the tested honey samples vary from 3.3 to 6. They are comparatively similar to those found by Mbogning et al. [19]. Indeed, the studies of El Sohaimy et al. [20] indicate that honey is naturally acidic. This acidity contributes to its flavor, texture, stability and shelf life. Constituting an important criterion of its quality, its acidity gives very important indications of the state of the honey [9]. The natural acidity of honey increases when the honey ages, when it is extracted from the combs with propolis and especially when it deteriorates by fermentation [7]. This consequently prevents the growth of many species of bacteria [9]. The acidity of the 3 types of honey studied varies from 7 to 17 mEq/kg. These figures are consistent with the normal set by the Food Codex in 2001 of ≤50 mEq/kg. Only Okuh honey had values below 75 in Brix, thus meeting the Codex Foods standard, the maximum limit of which is 75.

Table 1. Ph values, Brix levels and free acidity of differents varieties of honey

	Ngaoundal	Okuh	Yaounde	P value
pH	5,93±1,659	5,73±0,682	5,79±0,548	0,133
Free acidity	12,47± 4,563	5,77± 1,664	7,53± 3,765	0,133
Brix levels	78,83± 0,058	73,94± 0,115	81,73± 0,289	**0.00000009**

Table 2. Honey composition

	Ngaoundal	Okuh	Yaoundé	P value
Polyphenols	323,79± 53,57	**30,43± 16,42**	50,23± 10,05	**0.00000005**
Flavonoïds	47,45± 3,84	32,46± 13,01	**23,74± 10,72**	0,0684
Total Flavonoïds	21,82± 0,90	**2,09± 0,14**	6,04± 1,08	**0.000000002**

Table 3. Evolutoin of the burn surface size during experimentation for each group (in cm^2)

Day	Negative control	Brulex®	Ngaoundal	Okuh	Yaounde
0	7,69±0,89	8,83±2,34	7,76±1,3	6,83±0,66	7,80± 0,73
4	6,99± 1,515	8,14± 1,549	6,38±0,483	6,16± 0,483	6,34±0,906
8	5,95± 0,097	7,61±2,130	5,56±0,471	5,04±0,602	4,93±0,416
12	5,22± 0,964	6,14±0,850	3,34±0,103	3,62±1,805	3,54± 1,091
16	3,49± 0,082	3,98± 0,205	1,21± 0,131	1,95± 0,710	1,02±0,143
20	1,67± 0,777	2,51± 0,026	0,42± 0,204	0,79± 0,024	0,42±0,101
24	0,80± 0,062	0,74± 0,247	0,0± 0,000	0,0± 0,000	0,00± 0,000

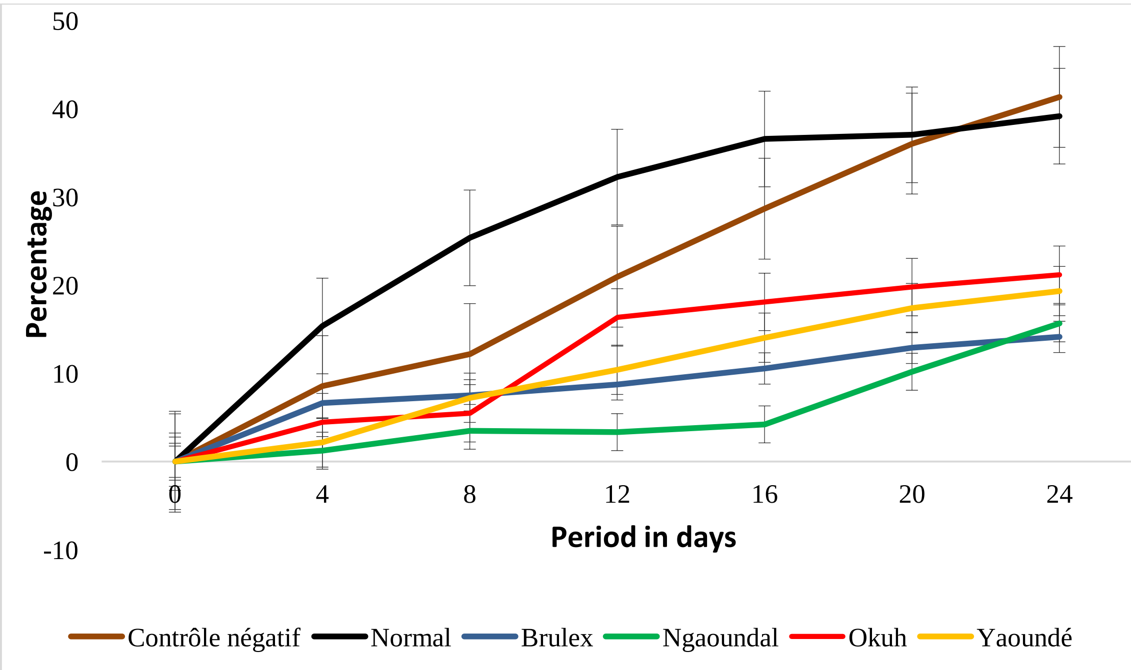


Fig. 1. Evolution of rats weight during experimentation

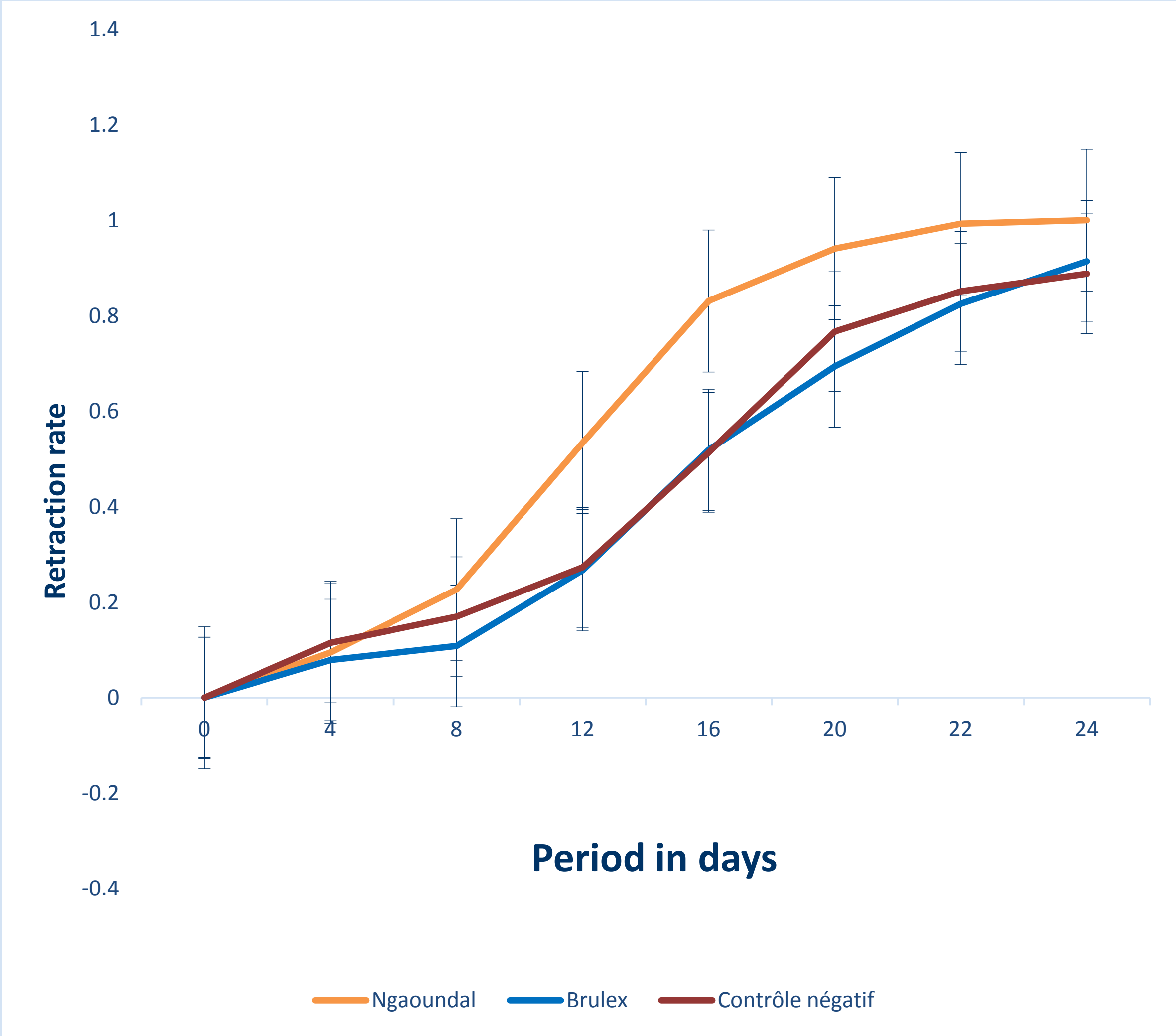


Fig. 2. Retraction rate evolution for the Nganoudal honey

Among the contents of polyphenols, flavonoids and total flavonols of the three samples of honey, we noticed that that of Ngaoundal is richer in these three metabolites. In comparison with Manuka honey, such results are correlated with the biological activities of honey experienced, namely antibacterial and antioxidant in the process of chemical cleansing of wounds [5]. Also, the polyphenol composition of honey and its biochemical properties are related to the composition of the soil, as well as the geographical and climatic situation [19]. Furthermore, most of the phenolic compounds in honey are flavonoids, although generally different in terms of their distribution [19]. In general, darker honeys (from Acacia, Manuka, and the Malaysian Tualang tree) contain higher amounts of flavonoids than lighter honeys, as well as greater antioxidant capacity [21].

We observed a rapid and variable real healing effect depending on the origin of the honey, but also in the three cases significantly better than that of Brulex®. This property of honey constitutes clinical justification for its addition as an important excipient, which can be used in cosmetology and pharmaceutical products for dermatological use [5]. The effectiveness of honey for the treatment of burns, seems effective which was already mentioned by other autors [5]. Because it is biocompatible and associated with established antimicrobial and antibacterial activity, the honey dressing of burns, wounds and loss of skin substances is even mentioned as a future solution to the growing problem of microbial antibiotic resistance [5]. Furthermore, we observed a significant decrease in nitrites in honey-treated rats over time. With their derivatives, these nitrites would be produced in the skin from the start of the inflammatory process by catabolism; having the property of accelerating the process of angiogenesis in order to allow good blood circulation [5].

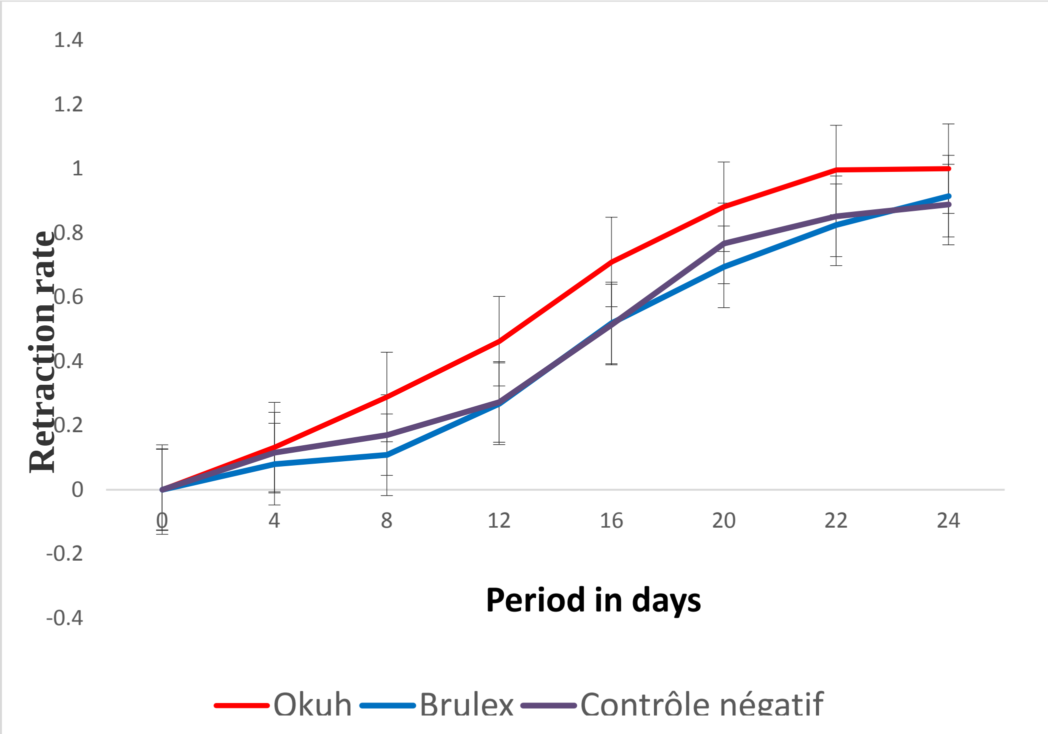


Fig. 3. Retraction rate evolution for the Okuh honey

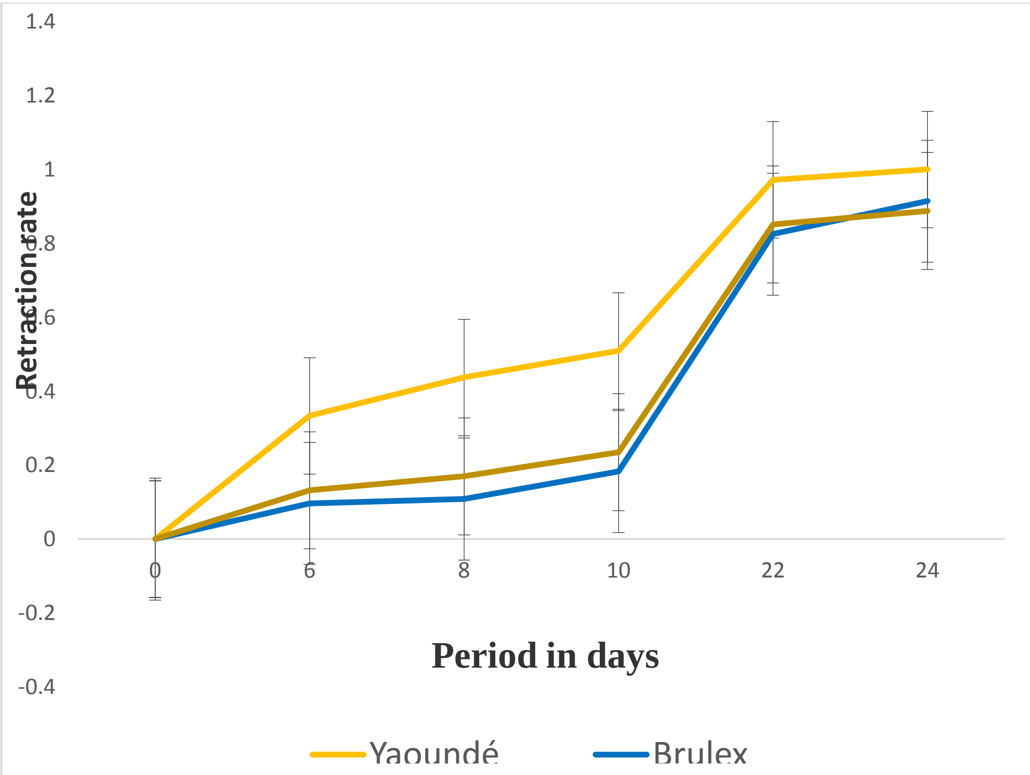


Fig. 4. Retraction rate evolution for the Yaounde honey

Table 4. Variation de of nitrates (NO_2) concentration during treatment

Day	Negative control	Normal	Brulex®	Ngaoundal	Okuh	Yaounde
0	0,306± 0,0734	0,321± 0,371	0,306± 0,0734	0,306± 0,0734	0,306± 0,0734	0,306± 0,0734
2	**0,724±0,069**	**0,181±0,034**	**0,339±0,1629**	0,9327± 0,441	**0,340±0,167**	**0,262±0,071(p<0,05)**
10	**0,754±0,0534**	0,165± 0,0152	0,1214± 0,032	**0,2191±0,133**	**0,2423±0,037**	**0,235±0,028 (p<0,05)**
24	0,1127± 0,0310	0,146±0,0365	0,1292±0,0662	0,06±0,0644	0,0521± 0,0519	0,0558± 0,0415

Table 5. Variation of the malondialdéhyd level during treatment

Day	Negative control	Normal	Brulex®	Ngaoundal	Okuh	Yaounde
0	26,79± 2,315	31,03±8,940	26,79± 4,315	26,79± 4,315	26,79± 4,315	26,79± 4,315
2	22,35± 2,439	12,52±8,426	20,63±3,327	20,97± 7,837	26,49± 9,999	31,98± 4,918
10	**40,65±5,243**	**18,01± 3,026**	38,67± 6,650	32,01± 9,366	55,37± 7,4721	31,06± 8,993
24	18,91± 6,396	8,72± 3,148	23,36± 4,499	24,13± 1,105	30,99± 6,735	28,84± 2,046

Table 6. Variation of the serum total antioxydant capacity

Day	Negative control	Normal	Brulex®	Ngaoundal	Okuh	Yaounde
0	943,65±16,774	789,88± 38,179	943,65±14,774	943,65±16,774	943,65±14,774	943,65±16,774
2	963,18±42,395	671,96± 63,213	970,65± 17,175	1004,13± 19,204	1030,72± 10,697	1058,89±19,248
10	987,04±16,433	813,46± 43,176	743,19± 18,473	620,13± 27,021	821,54± 28,240	768,91± 30,441
24	852,69± 26,670	814,84± 20,865	958,88± 15,478	874,21± 23,312	797,07± 41,773	742,71±16,814

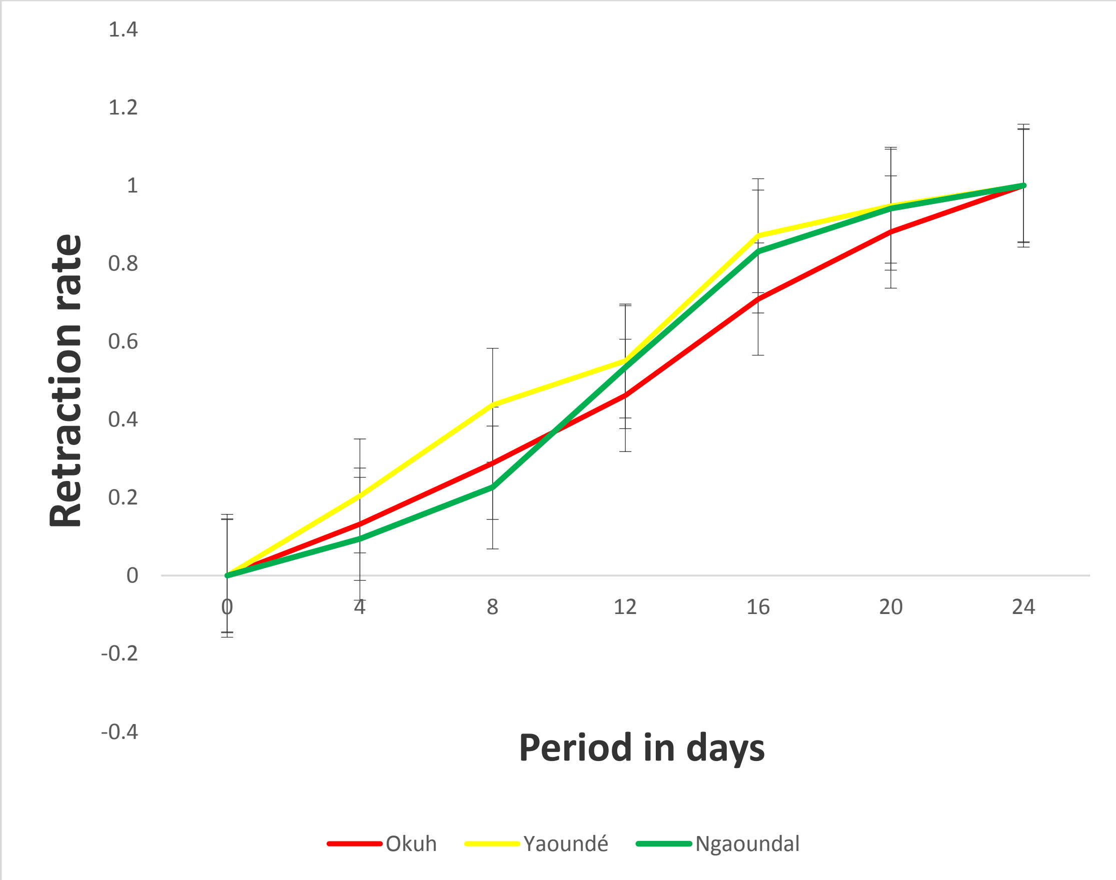


Fig. 5. Retraction rate evolution with Ngaoundal, Okuh and honeys

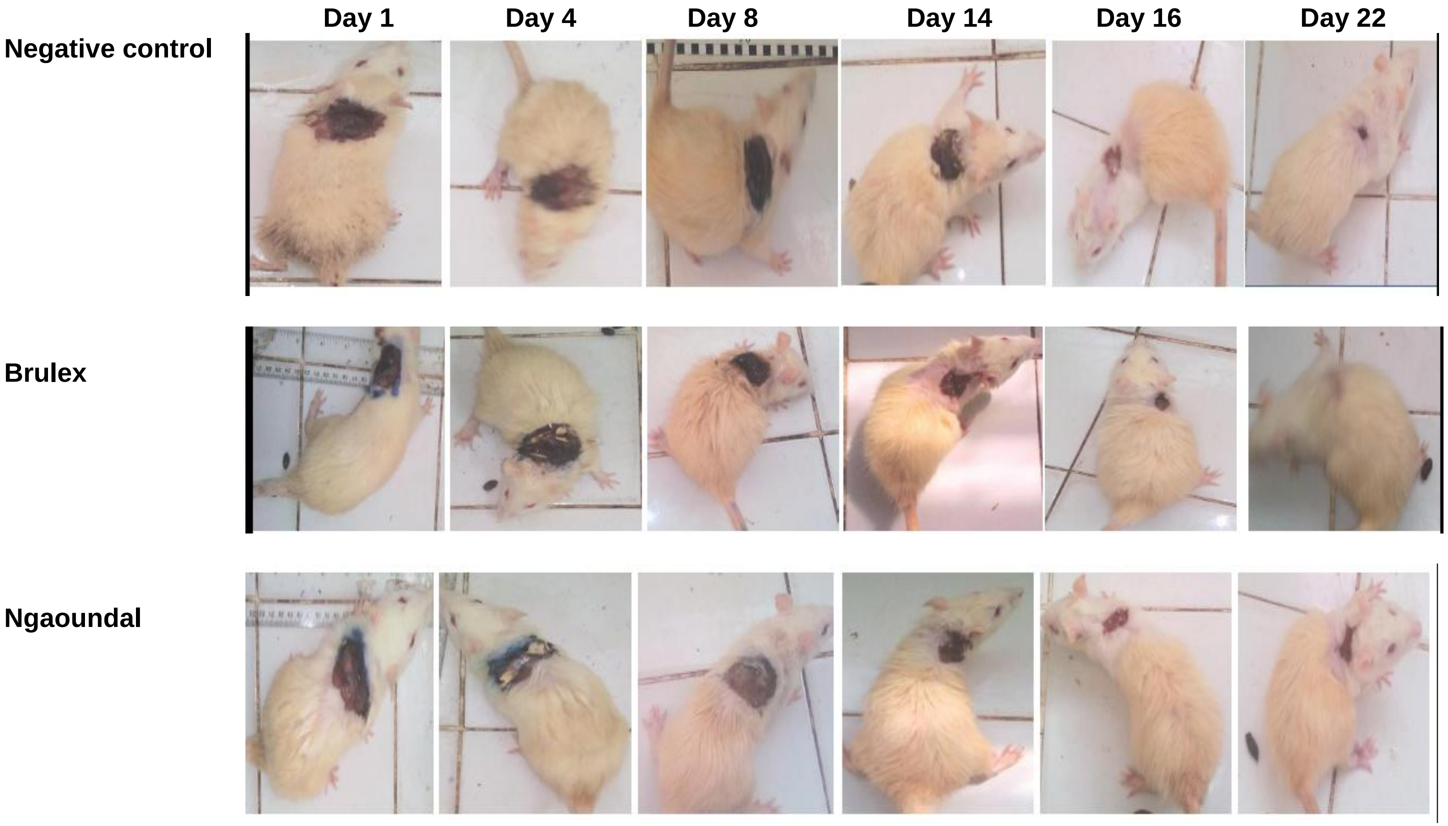
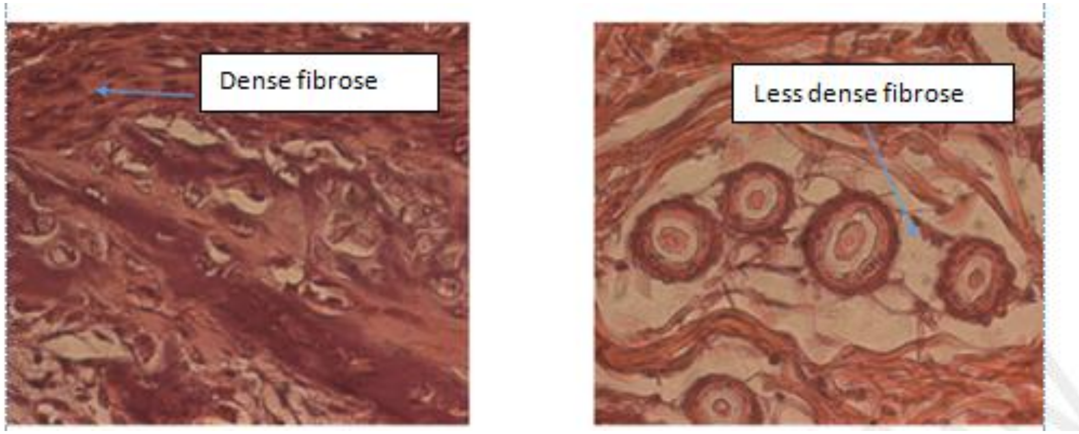
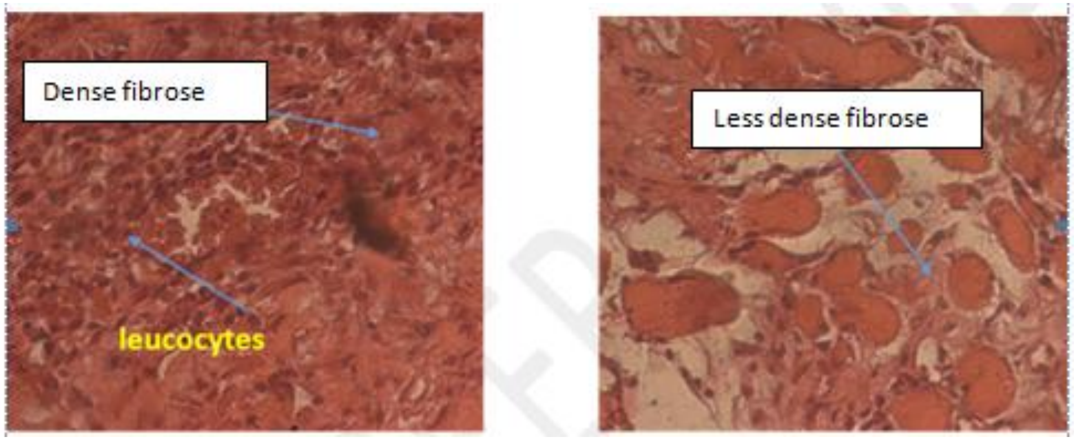

Day 1
Day 4
Day 8
Day 14
Day 16
Day 22
Negative control
Brulex
Ngaoundal

Fig. 6. General clinical wound evolution for each group

A. Photo of a bruned an non treated rat

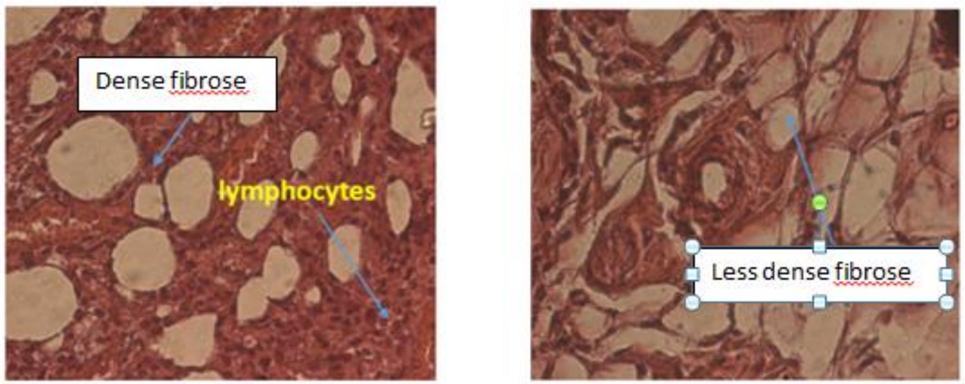


B. Photo of a bruned rat treated with Brulex®



Day 10 **Day 10**

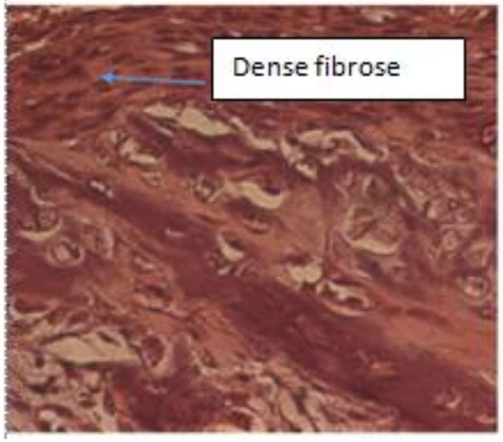
C. Photo of a bruned rat treated with Ngaoundal honey

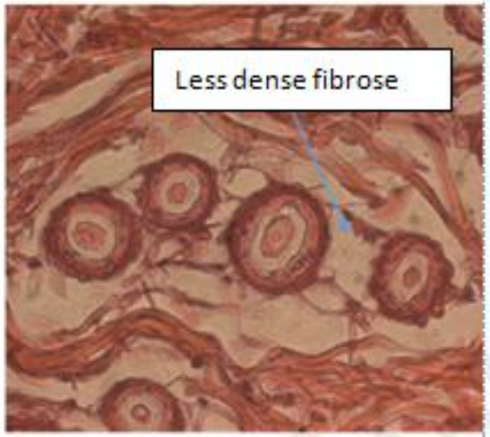


Day 10 **Day 10**

Fig. 7. Comparative images of histological sections of rat skins: A) untreated, B) treated with Brulex® and C) treated with Ngaoundal honey.

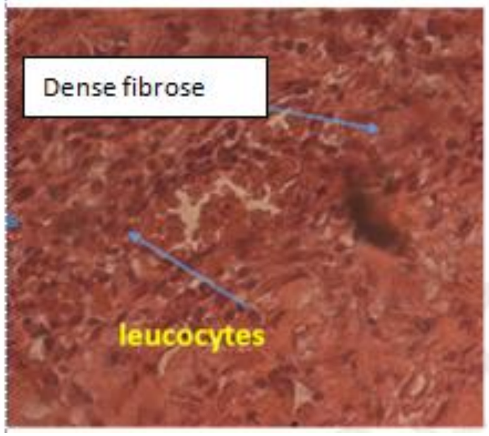
A. Photo of a bruned an non treated rat

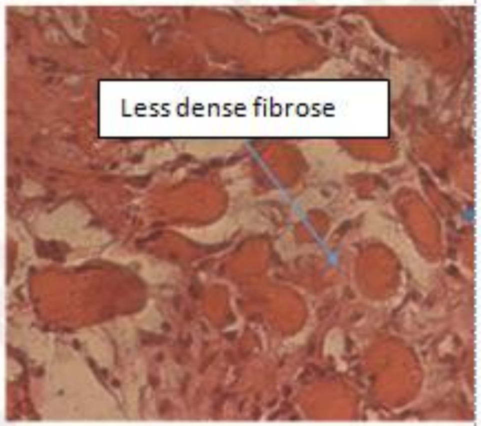




Day 10 **Day 10**

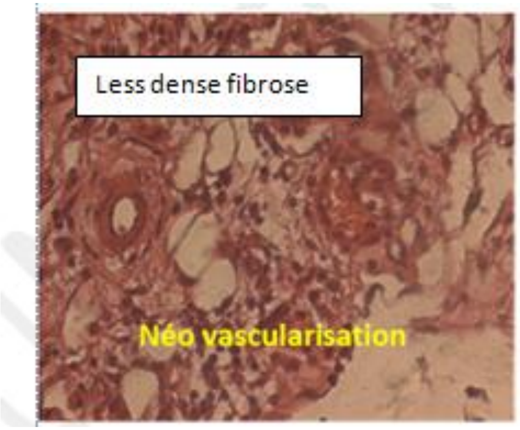
B. Photo of a bruned rat treated with Brulex®



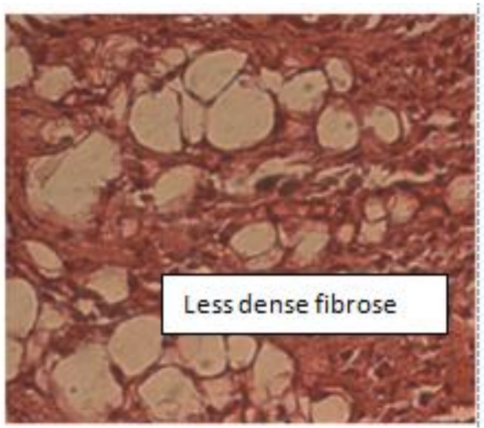


Day 10 **Day 10**

C. **Photo of a bruned rat treated with Okuh Honey**



Day 10

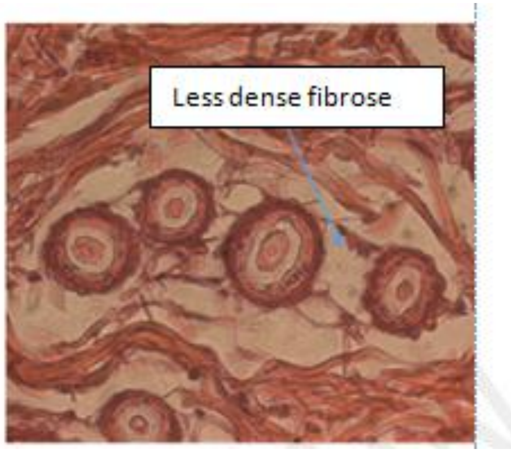


Day 10

Fig. 8. Comparative images of histological sections of rat skins: A) untreated, B) treated with Brulex® and C) treated with Okuh honey

A. Photo of a bruned an non treated rat

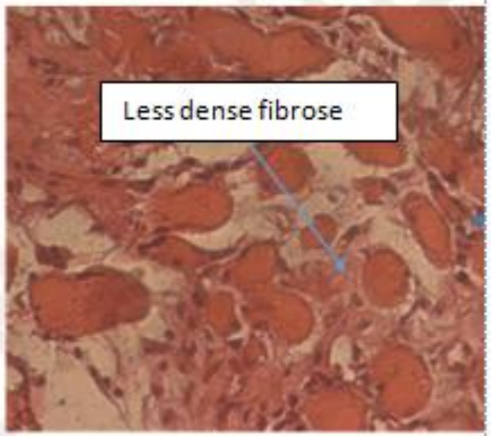
Day 10



Day 10

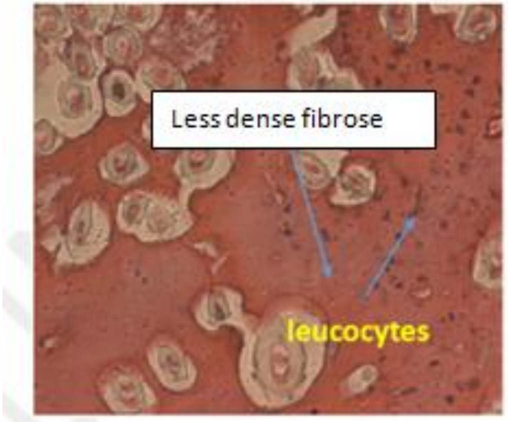
B. Photo of a bruned rat treated with Brulex®

Day 10



Day 10

C. Photo of a bruned rat treated with Yaounde Honey



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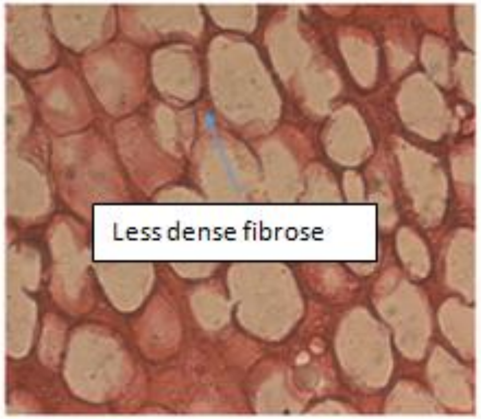

Day 10

Fig. 9. Comparative images of histological sections of rat skins: A) untreated, B) treated with Brulex® and C) treated with Yaounde honey.

Analysis of the histological sections (Figs. 7-9) of the injured tissues revealed that the rats of the Brulex® and negative control groups presented dense fibrosis on day 10, which would be due to a massive loss of fluids and hypovascularization. Unlike those of the Okuh, Yaoundé, and Ngaoundal groups where slight fibrosis was observed, but hypervascularization and an abundance of immune cells. These images thus show that honey would have the property of regenerating new blood vessels. This suggests that revascularization, mentioned in other studies [5], will accentuate good diffusion of immune cells and nutrients.

5. CONCLUSION

We can suggest these 3 honeys as topical treatment of superficial limited burns. The 3 varieties have as superimposable parameters their acidity, then their richness in sugars and poly-phenolic compounds. The evaluation the healing time is shorter, with a higher rate of contraction when the batches of rats are treated with honey, compared to those treated with the reference drug.

ETHICAL APPROVAL

From a bioethical point of view, the experimental protocol used in this study complies with the recommendations for animal care used in research and teaching of the NRC of 1996. In addition, this work has been validated by the institutional research ethics committee of the Faculty of Medicine and Biomedical Sciences, located in Yaounde.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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