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Study of The Anti-Inflammatory Activity and Healing Power of The Hydroethanol Extract of Terminalia Ivorensis In Wistar Rats

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ABSTRACT

T. ivorensis is a plant used in traditional settings for the treatment of wounds and microbial infections. The lack of scientific study on the healing power of this plant is the basis of this study. The objective of this study is to evaluate the anti-inflammatory and healing potential of the hydroethanol extract of *Terminalia ivorensis* bark. The methodology consisted in the determination of the phenolic compounds present in the extract by the Folin-Ciocalteu reagent. The determination of antioxidant activity by the DPPH test was carried out using the method described by Molyneux. The anti-inflammatory effect was estimated from the measurement of the volume of the oedema of the paw of rats induced by carrageenan. In addition, a circular incision of 2 cm diameter was made in the dorsal region of each rat to evaluate the healing activity of the ethanolic extract against a reference ointment (L-Mesitran). The results showed that the phenolic compound content of the hydroethanolic extract is 62, 34±1.41 mg GSE per g powder. The IC50 values obtained were 9,67±1.12 µg/mL for vitamin C and 16,23±0.33 µg/mL. The inhibition of inflammation is 87% at the dose of 600mg/kg bw of *T. ivorensis* extract. This inhibition is greater than that of diclofenac, which resulted in 76,25% inhibition. At doses of 300 and 150mg/kg bw of the extract the inhibitions obtained were 76.43 and 72%. The reference ointment showed 50% healing at day 10 and 80% at the end of the experiment. For the ointments with concentrations of 15, 30, 60mg/ml the healing was 61,54%, 77,27% and 91,67% at the end of the 21 day study. For treatment with the extracts at the same concentrations, healing was 84,13%, 93,06% and 100% at concentrations 15, 30, 60mg/ml. The effect of the extract alone is greater than that of the ointment (extract + shea) at the same concentrations. The concentration of 60 mg/ml is the most active extract with or without shea butter.

Keywords: anti-oxydant; anti-inflammatory; healing; extract; *Terminalia Ivorensis*

INTRODUCTION

A wound is defined as an interruption in the continuity of body tissue, a break in the skin barrier [1]. Wounds are an extremely frequent reason for consultation in emergency departments. A wound is a seemingly trivial accident that can pose complex treatment problems [2]. Wound healing in the skin is a coordinated dynamic process involving active cell multiplication and migration, followed by the production of extracellular matrix [2]. Many wounds result from traffic accidents; they are also found among workers with sharp tools, children, medical personnel (surgical wounds), in rural areas and during field work [3]. In rural areas, many injured people use traditional medicine, especially medicinal plants, as an alternative to conventional treatment [4]. In this context several studies have shown the healing activity of some plants such as *Kalanchoe crenata*, *Tetrapleura tetraptera* [5] and *Baphia nitida* [6]. *Terminalia ivorensis* is an indigenous plant of the Combretaceae family. Numerous pharmacological studies conducted on the stem bark of T. ivorensis have revealed that the plant has antimicrobial [7], anti-arthritic properties [8]. In ethno-medicine, the pulverised leaves of the plant are used as a poultice to treat burns and bruises [9]. The decoction of the plant's bark is also used in traditional Ivorian medicine to treat wounds and other injuries. This therapy has not yet been subjected to scientific evaluation criteria as has been the case for other plants. The stem barks of *T. ivorensis* are known to be rich in bioactive substances [8].

The general objective is to contribute to the search for new substances capable of healing traumatic injuries. Specifically, this work consists of measuring phenolic compounds and evaluating the anti-inflammatory and healing activities of the hydroethanol extract from the bark of the trunk of *Terminalia ivorensis*.

MATERIAL AND METHODS

PLANT MATERIAL

Terminalia ivorensis barks were collected in the south of Côte d'Ivoire in the Mé region. They were then washed, cut into small pieces and dried in the sun for 21 days before being pulverised into a fine powder.

ANIMAL MATERIAL

The animal material consisted of 47 male and female albino rats of Wistar strain from the animal house of the Ecole Nationale Supérieure (ENS). Before being used, the animals were left to acclimatise for 7 days. The rats were fed a standard diet.

They had free access to water and food and were placed in room temperature conditions with normal day and night alternation.

OTHER MATERIALS

- Unrefined raw shea butter was used as an excipient because of its strong consistency, its composition in vitamin A, D, E and F and its nourishing character.
- L-Mesitran (reference ointment)
- Ether was used to anaesthetise the rats prior to wound induction.
- A digital caliper was used to measure the diameters and paws of the rats.
- Scalpel was used to create the wound in the rats. Carrageenan was used to induce inflammation in the rats' paws.

HYDROETHANOL EXTRACTION

The extract was made using the extraction method described by zirihi et al (2003). For this purpose, one hundred grams of plant powder were dissolved in one litre of 70% ethanol. The mixture was then vigorously homogenised in a blender. The resulting homogenate was wrung out in a square of percale cloth and then filtered three times on cotton wool and then on wattman paper. The filtrate was evaporated at 45°C using a ventilated oven of the Venticell® type for 24 hours.

DETERMINATION OF POLYPHENOLS

One millilitre of a methanolic solution of plant extract with a concentration of 1 mg/mL was introduced into a test tube. 1 mL of Folin-Ciocalteu reagent is added. The tube is then left to stand for 3 min and 1 mL of 20% (w/v) sodium carbonate solution is added. The contents of the tube are made up to 10 mL with distilled water and the tube is placed in the dark for 60 min. The Optical Density (OD) was then read at 745 nm against a blank with a spectrophotometer (DRAWELL DU-8200). A calibration line established from a concentration range of a stock solution of gallic acid, at 1 mg/mL, treated under the same conditions as the assay, made it possible to determine the quantity of phenolic compound in the sample [11, 12].

ANTIOXIDANT ACTIVITY

The determination of antioxidant activity by the DPPH test was carried out using the method described by Molyneux (2003). For this purpose, a methanolic solution of DPPH was prepared by dissolving 4 mg of DPPH in 100 ml of methanol. To 950 µl of this solution, 50 µl of methanolic solvent was added in order to obtain a blank. At the same time, a concentration range from 200 µg/ml to 0 µg/ml was performed from the hydroethanol extract. 50 µl of each of the previously prepared extract concentrations was added to 950 µl of the methanolic solution of DPPH. The absorbances of the different solutions were measured at 517 nm after 30 minutes of standing in the dark at room temperature. The antioxidant activity related to the $DPPH^{\cdot}$ radical scavenging effect is expressed as percentage inhibition (PI) using the following formula:

$$PI = (A0-A1)/A0\times10$$

concentration of antioxidants required to reduce 50% of the DPPH radical [13].

ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity was assessed in vivo, using the carrageenan-induced rat paw oedema method [14]. Rats weighing between 120 g and 150 g were fasted 24 hours before the experiment. These rats were divided into 5 batches of 4 rats.

For each rat, the initial volume (V0) of the right hind leg was measured with a caliper before the treatments were administered. The different treatments specific to each batch were administered by gavage as defined by the following schedule:

- Batch 1: physiological water at a rate of 10 mL/kg/pc (control)
- Batch 2: T. ivorensis hydroethanol extract solution at a dose of 150 mg/kg/pc
- Batch 3: solution of hydroethanolic extract of T. ivorensis at a dose of 300 mg/kg/pc
- Batch 4: T. ivorensis hydroethanol extract solution at a dose of 600 mg/kg/pc
- Batch 5: DICLOFENAC solution at a dose of 10 mg/kg/pc.

One hour after gavage, 0.1 mL of 1% (w/v) carrageenan solution was injected subcutaneously into the plantar fascia of the right hind leg of each rat. The diameter of the right hind paw oedema was determined at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h [15].

The extent of the oedema was assessed by determining the average percentage increase (%AUG) in the volume of the rat paw according to the formula:

$$\%AUG = \frac{Vt - V0}{V0} x100$$

Vt: Volume of the paw at time t; V0: Initial volume of the paw. Anti-inflammatory activity was assessed by calculating the percentage inhibition (% INH) of oedema according to the formula:

$$\%INH = \frac{\%AUGcontrol - \%AUGtreated}{\%AUG\ control} x\ 100$$

ASSESSMENT OF HEALING ACTIVITY

The animals used in this test were 12 weeks old and weighed between 180 and 230 g. The animals were randomly divided into nine groups of three rats before induction of the different wounds. The animals were placed individually in boxes to avoid further wounds or other aggressive reactions that could interfere with the study. The healing activity was evaluated in an experimental incision model in rats as described by Sagliyan et al., (2010) [16].

PREPARATION OF THE OINTMENT

The ointment was prepared from 70% ethanolic extract, distilled water and shea butter. The preparation was done manually: the quantities of extract corresponding to the concentrations 15, 30, 60 mg/ml were triturated until total homogeneity in a porcelain mortar. The necessary quantities of shea butter were added gradually during the preparation of each sample.

WOUND INDUCTION

The animals were anaesthetised using ether. After shaving and disinfecting the dorsal area of all rats, a circular incision of 2 cm diameter was made in the dorso-omoplate region of each anaesthetised rat. Each wound was cleaned and the animals were individually placed in cages 24 hours before the start of treatment. Treatment was performed daily and wound diameter measurements were taken every four days for 21 days using a digital caliper. All animals were monitored regularly until the wounds were completely healed and had free access to food and water.

The percentage of wound shrinkage or contraction was calculated using the following formula:

$$\text{Wound contraction (\%)} = \frac{(wound\ surface\ D1 - wound\ surface\ D1) \times 100}{wound\ surface\ D1}$$

TREATMENT OF THE ANIMALS

- Batch 1: Negative control: physiological water: cleaning the wound with physiological water
- Batch 2: Excipient alone (placebo): approximately one nut of shea butter on the wound surface.
- Batch 3 positive control: L-Mesitran, topical application, approximately one nut.
- Batch 4: Ointment (15 mg/ml) topical application (about one nut of ointment)
- Batch 5: Ointment (30 mg/ml) topical application (about one walnut of ointment)
- Batch 6: Ointment (60 mg/ml) topical application (about one walnut of ointment)
- Batch 7: Extract (15 mg/ml) topical application (about 30 microliters)
- Batch 8: Extract (30 mg/ml) topical application (about 30 microliters)
- Batch 9: Extract (60 mg/ml) topical application (about 30 microliters).

STATISTICAL ANALYSIS OF RESULTS

The results are expressed as means plus or minus standard errors on the mean (m ± esm). The comparison of the means between the treated and control batches was done by analysis of variance (ANOVA) and Dunnet multiple comparison test. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

The phenolic compound content of the hydroethanol extract was 62, 34±1.41 mg gallic acid equivalent per g of powder. The phenolic compound content is higher than the total polyphenol content in dry extract of Kalanchoe crenata which is 1 .73 mg GAE/gMS [5]. This high value of total phenol could explain the results of antioxidant activity of the hydroethanol extract of T. ivorensis. These results were obtained from the curve analysis of the evolution of free radical reduction by T. ivorensis extract and vitamin C. The IC50 obtained are 9.67 ±1.12 µg/mL for vitamin C and 16.23±0.33 µg/mL for T. ivorensis extract. The IC50 of the extract is higher than that of the vitamin but, lower than that of Millettia zechiana (IC50 = 96 ± 0.577 µg/mL) Ageratum conyzoides (IC50 =76 ± 0.577 µg/mL), Parquettina. nigrescens (IC50 = 75.5 ± 0.166 µg/mL) and Sherbournia bignoniifolia (IC50 = 49.5 ± 0.288 µg/mL) [17].

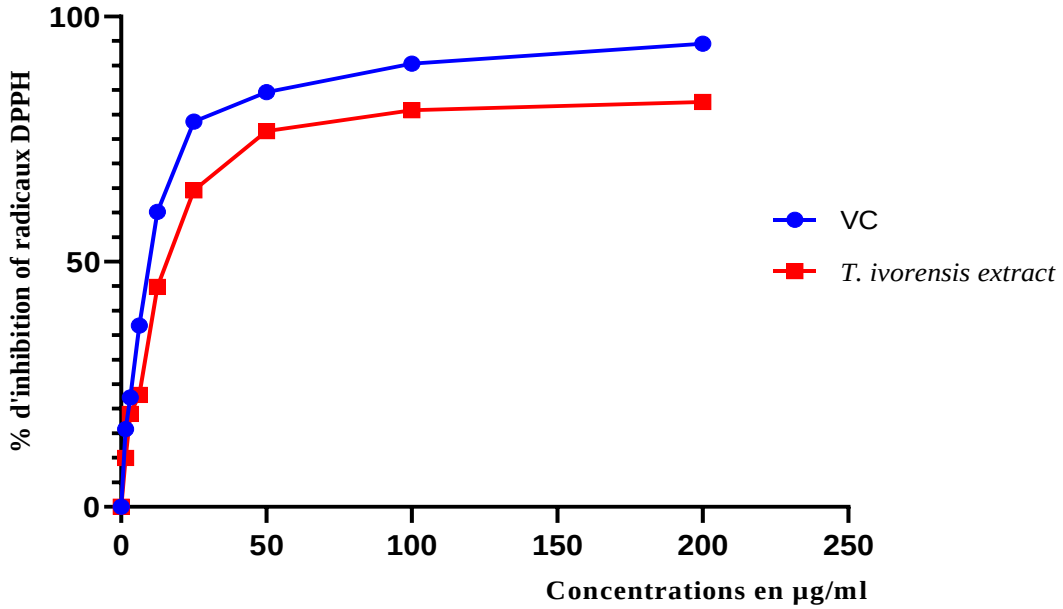

FIGURE 1: Percentage of DPPH inhibition

ANTI-INFLAMMATORY ACTIVITIES

The anti-inflammatory activity of the hydroethanol extract of T. ivorensis was evaluated at doses of 150, 300, 600 mg/kg bw. Injection of carrageenan caused a significant progressive increase in edema volume in rats treated with physiological saline during the six-hour experiment. However, in rats treated with hydroethanol extract of T. ivorensis and diclofenac, an opposition to the increase of the paw diameter of each rat was observed. The increase in the paw of the rats was 67.55% and 46.94% respectively from the first hour for the animals that received NaCL and diclofenac.

For the animals that received the three doses of extract 150, 300, and 600 mg/Kg Pc the increase in paw was 49.91%, 43.58% and 40.98% respectively. After four hours this increase increased to 85.15% with NaCL, 20.22% with diclofenac, and to 23.53%, 16.71% and 10.20% for the extract at the dose of 150, 300, and 600 mg/Kg Pc. Statistical analysis of the results indicate that all substances tested showed a less significant increase than that of the physiological fluid. These results suggest that the extract counteracts the inflammatory effect of carrageenan.

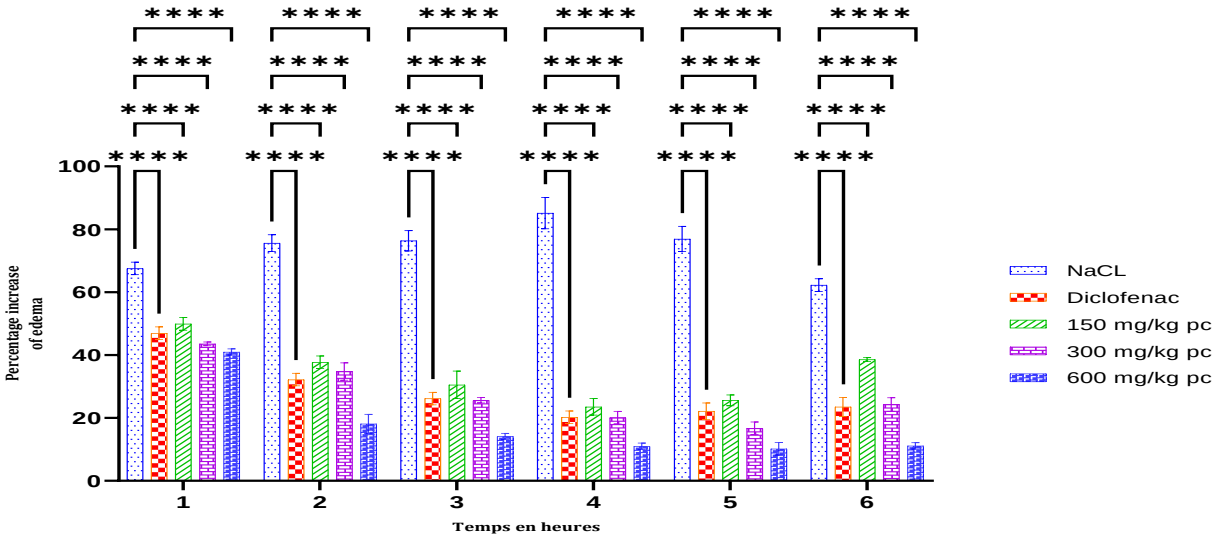

figure 2: % increase in rat paw as a function of time

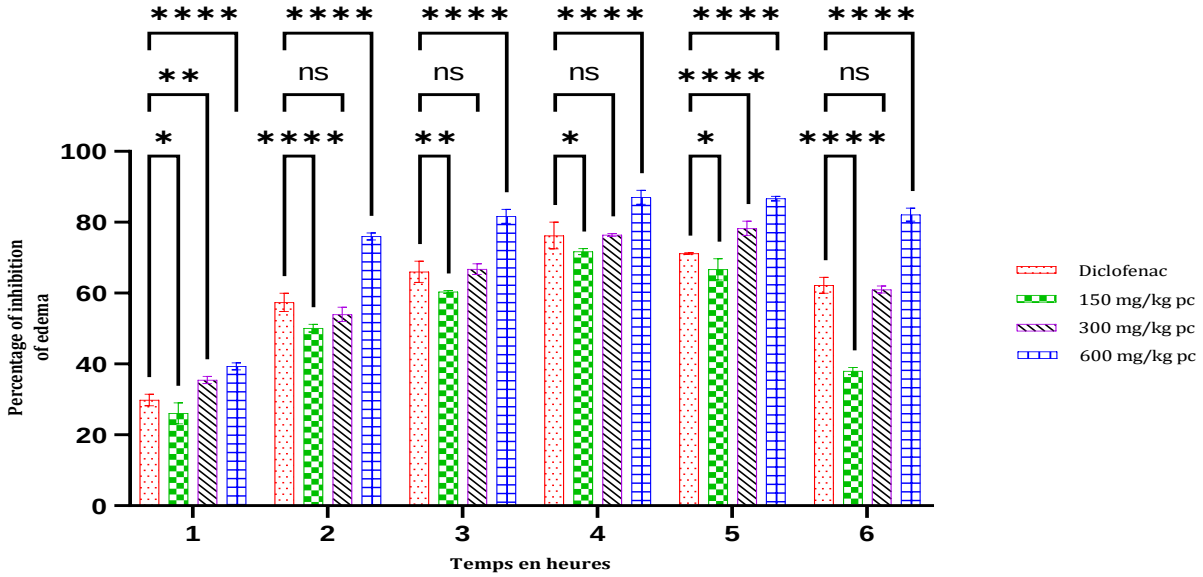

figure 3: % inhibition of rat paw edema versus time

Inhibition of paw edema was observed at the different doses tested (Figure 3). These results show that the extract opposes the installation of inflammation in the tested animals. The amplitude of the inhibition is reached after 4h of time for all the tested substances. The inhibition of inflammation is 87% at the dose of 600mg/kg bw of the extract of T ivorensis. This inhibition is greater than that of the reference molecule (diclofenac), which caused an inhibition of 76.25%. At the doses of 300 and 150mg/kg bw of the extract the inhibitions obtained are 76.43 and 72%. The tested plant extract has a similar activity to diclofenac at the dose 300 mg/kg bw. These results show that T. ivorensis has a dose response effect on inflammation. This plant appears more active at high doses than diclofenac.

The richness of T. ivorensis in phenolic constituents (62, 34±1,418) is able to prevent the formation of prostaglandins which cause inflammation. In addition, some works indicate that flavonoids contained in plant extracts, possess anti-inflammatory properties able to modulate the functioning of the immune system by inhibiting the activity of enzymes that may be responsible for inflammation. In addition, Kim and colleagues, demonstrated that flavonoids are able to inhibit histamine, flavones and flavonols in glycosylated or free form such as quercetin, have a Cyclooxygenase inhibitory activity.

The anti-inflammatory effects of polyphenols, which can be exerted at the molecular level, depend on the structural specificity of the polyphenolic compounds. Macrophage functions, including cytokine production, can also be affected by certain flavonoids through modulation of inducible cyclooxygenase (COX-2) and inducible oxide nitrite synthase (INOS). Several experimental studies have reported the immunomodulatory effects of polyphenolic compounds on humoral and cellular immunity [18]. Our results indicate that the ethanolic crude extract of T. ivorensis shows remarkable anti-inflammatory activity.

WOUND HEALING ACTIVITY

The evaluation of the in vivo wound healing activity of the extract was performed on albino rats. Its purpose is to evaluate the acceleration of the potential of the neoformation of dermal tissues. The comparison was made with a group of animals receiving a reference cream L-Mesitran, a control group treated with shea butter only and a group treated with physiological water.

The healing process went through several phases; a gradual disappearance of inflammation (wounds becoming less red), a contraction phase (wounds became hard and covered with slightly blackish crusts) and finally the healing phase. The treatment resulted in complete healing of the wounds (Figure 4).

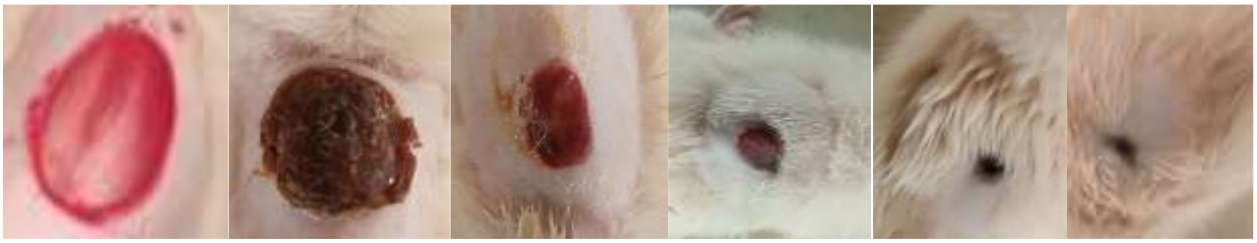

FIGURE 4: Evolution of the wound over time

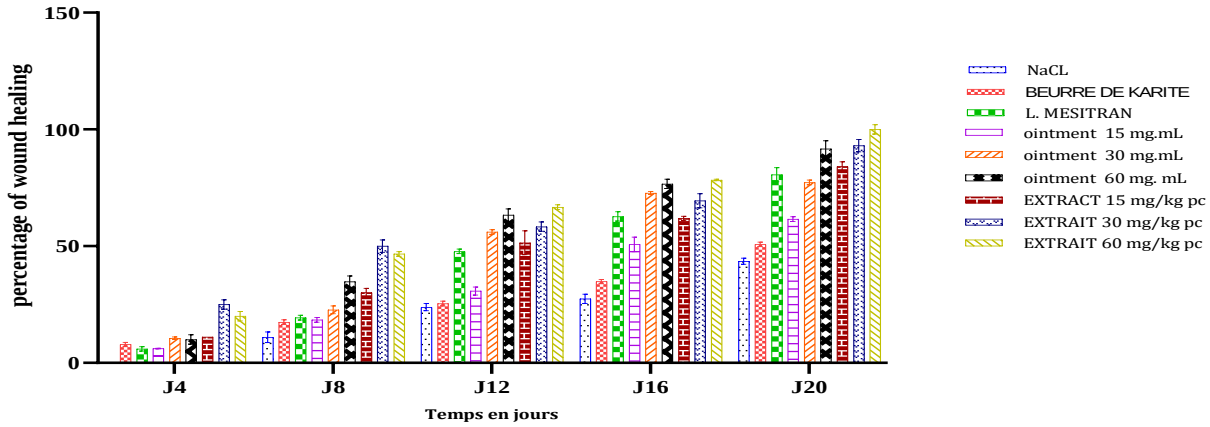

figure 5: wound healing as a function of time

The wounds were odorless throughout the treatment. Wound areas were measured on days 4, 8, 12, 16, and 20 after excision. The healing process was seen in all animals (Figure 5). Healing was less than 50% for animals treated with physiological water and 50, 79% for the vehicle at the end of the experiment. The reference ointment showed 50% healing at day 10 and 80% at the end of the experiment. For the ointments with concentrations of 15, 30, 60mg/ml the healing was 61.54%, 77.27% and 91.67% at the end of the 21 days study. However, it should be noted that at the 60mg/ml concentration, there was a total absence of wound even if the epidermis had not completely covered the wound. Regarding the treatment with the extracts at the same concentrations, the healing was 84.13%, 93.06% and 100% at concentrations 15, 30, 60mg/ml. The effect of the extract alone is greater than that of the ointment (extract + shea) at the same concentrations. The concentration of 60 mg/ml is the most active extract with or without shea butter. Complete healing was obtained at this concentration and a reappearance of the rat hair was also observed (figure 5). Healing is characterized by significant changes in the extracellular matrix in which fibronectin, fibrinogen and integrins occur. Topical application of the crude extract induces a decrease in wound diameter, this decrease is significant than that of L-Mesitran used as standard. The healing effect of the extract can be attributed to the presence of polyphenols and flavonoids with the ability to accelerate the tissue regeneration process by stimulating the production of collagen and fibronectin [19].

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