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Evaluation of the healing activity of therapeutic clay in rat skin wounds

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ABSTRACT

The use of clays for therapeutic practice is widespread in almost all regions of the world. In this study the physicochemical and microbiological healing characteristics of a clay from Ocara, Brazil, popularly used for therapeutic uses, were analyzed. The presence of Ca, Mg, Al, Fe, and Si was observed, which initially indicated that the clay had potential for therapeutic use. The average particle size of the clay (26.3 μm) can induce the microcirculation of the skin and the XRD analysis shows that the clay is formed by kaolinite and illite, a swelling clay. During the microbiological evaluation there was the need to sterilize the clay for later incorporation into the pharmaceutical formula. The accelerated stability test at 50 °C for 3 months has showed that the pharmaceutical formula remained stable with a shelf life of two years. After the stability test the wound-healing capacity of the formulation in rats was evaluated. It was observed that the treatment made with the formulation containing the Ocara clay showed the best results since the formula allowed greater formation of collagen fibers and consequent regeneration of the deep dermis after seven days of treatment and reepithelialization and continuous formation of granulation tissue at the 14th day.

1. Introduction

The use of clay with medicinal purpose is an ancient and widespread practice. However, the modern use of clay in pharmaceuticals or cosmetics needs a rigorous study regarding the preparation processes and evaluation techniques [1–6]. Substances for pharmaceutical use are organic or inorganic and they can be used as active ingredients for the production of medicines, which may be obtained from natural sources or not. These substances can also be used as such or as part of a drug formulation [7–9].

About half the medicines used today are of natural origin and many are still unexplored. However, before becoming an active ingredient in a pharmaceutical formula, it is essential to check the medicines' purity and to characterize them biologically, chemically and physically [7,10,11].

Among the products of dermatological interest those with topical action incorporated into pharmaceutical formulas deserve particular attention because they can restore the skin integrity after possible aggressions. Therefore, they must restore normal conditions of the skin and for that the healing process is of fundamental importance.

The healing process involves the migration of inflammatory cells, the synthesis of granulation tissue, deposition of collagen and proteoglycans and maturation of the scar, being associated with intense refurbishment. Complementary medicine has been used as an alternative to their treatment [4,12–15].

Many natural materials have been used in dermatological products; within this group are the so called medicinal clays [2,16]. Clays are composed of aluminosilicate particles and several trace elements that promote the absorbent, healing and antiseptic action [5]. The use of clays for therapeutic and cosmetic purposes must comply with certain physical, physicochemical and microbiological requirements [7].

The study of the stability of pharmaceutical and cosmetic formulas is of fundamental importance for the safety of those who consume them; therefore, in order to these formulations fit the quality standards imposed by regulators, it is necessary to evaluate the same through stability tests [3,7,8,17]. Considering the importance that medicinal clays have acquired and the complexity of the different components present in cosmetic preparations – that can interfere with the effectiveness of the active ingredients and these in vehicle stability – it is necessary to evaluate the real activity that the incorporated principle will have on cutaneous wound-healing, therefore the proposed formulation could be viable for commercialization and use.

The use of geological nanomaterials to heal skin infections has been evident since the earliest recorded history and specific clay minerals may prove valuable in the treatment of bacterial diseases, including

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infections for which there are no effective antibiotics. Clay minerals can affect bacterial metabolism indirectly by altering the physicochemical properties of a specific environment or directly through surface interactions. Thus, physicochemical properties of hydrated clays, mainly illite or montmorillonite indirectly kill bacteria by generating an unfavorable environment to them [18].

Therefore, the main objective of this study was to evaluate the therapeutic effect of natural clay from the Northeast region of Brazil. The clay was prepared as an emulsion and its therapeutic effect was studied on the healing of rat wounds.

2. Experimental

2.1. Characterization

The sample used was a black clay coming from a lake in the municipality of Ocara, Ceará State, Brazil. The sample was obtained from a batch of 200 kg extracted manually from the lake and the sample undergone a sieving step (100 μm sieve) to eliminate the non-clay fraction before characterization. After characterization and after the initial microbiological analysis – where the sample showed 300 CFU/g for bacteria and 100 CFU/g for fungi – the sample was sterilized. All samples were kept in a dry controlled environment for at least 48 h before testing. After the microbiological evaluation there was the need to sterilize the clay for later incorporation into the pharmaceutical formula. The chemical composition was determined by X-ray fluorescence (XRF, Philips PW2400, sample melted with lithium tetraborate). The particle size distribution was determined by laser diffraction (Cilas 1064, 20 s sonication). The mineralogical composition was determined by X-ray diffraction (Shimadzu XRD-6000, CuK_α radiation, scan rate of 2°/min, from 0° to 90°). The transmission spectroscopy analysis was performed by Fourier Transform Infrared (FTIR) analysis (Shimadzu IR Prestige 21, KBr pellets, from 400 to 4000 cm^{-1}). The microbiological tests were: agar diffusion and determination of bacteria and fungi by total score [19].

2.2. Development of the formulation containing the Ocara clay

The emulsion preparation process and the incorporation of the active principle – the Ocara clay – were performed according to the precepts of the Good Handling Practices RDC n. 67 [20]. The emulsions were prepared by heating in a water bath at 75 °C plates of aqueous and oily phases separately, then joined and kept under manual stirring and heating for 15 min. After this time, the emulsions were removed from the water bath, keeping the manual agitation for 5 min at room temperature. The emulsions prepared were kept at rest for 24 h at room temperature after sealing the containers in which they were potted. After 24 h, the samples were observed. Table 1 shows the neutral emulsion and the emulsion containing the Ocara clay. Polawax was used as the emulsion base, whose formulation and preparation process are described by Eccleston [21]. The production of a neutral emulsion without the addition of the Ocara clay was performed in order to evaluate possible interferences related to the emulsion base.

2.3. Accelerated stability test and determination of the validity

The preliminary/accelerated stability test (T = 50 °C) was used, and the samples were collected and evaluated at 0, 5, 10, 15, 30, 60 and 90 days. The organoleptic, centrifugation, rheological, density, pH and microbiological characteristics were analyzed [22]. In order to determine the organoleptic characteristics, the appearance of a small amount of the homogenized sample was checked in a transparent container and observed against a white background for the presence of residues, degree of turbidity and phase separation. The color was determined by visual identification and the odor by the sense of smell.

The relative density of the sample was determined using a calibrated pycnometer (25 mL, 20 °C). The rheological analysis was performed in a viscometer (Bohlin Visco 88). The centrifugation test was performed at 3000 rpm for 30 min with a small sample of the emulsion. The pH was determined in a calibrated pH meter (Quimis Q400, 1:10 dilutions).

For the microbiological evaluation, the Ocara clay and the prepared emulsion samples were diluted in BHI (Brain Heart Infusion) at a ratio of 1:9 and three dilutions were prepared. For each dilution, inoculations were made in duplicate using PCA (Plate Count Agar) culture media for bacteria and Sabouraud dextrose agar for fungi. In samples containing preservatives 1% Tween 80 (polyoxyethylene sorbitan monooleate) was used for inactivation by adding it to the BHI. Petri dishes were used with an adequate amount of medium to achieve a thickness of 4 mm. 0.1 mL of each dilution was spread with the aid of the Drigalski handle. The plates were incubated for 24 h at 35 °C for bacteria and for seven days at 25 °C for fungi, and after the specific time the colonies were counted [23].

The count of colonies was performed as described by the Farmacopeia Brasileira [24] based on the United States Pharmacopeial Convention [25]. The results were expressed as colony forming units (CFU) per gram of sample, divided into two categories: type I within a limit of 5×10^2 CFU/g and type II within a limit of 5×10^3 CFU/g. For both categories, the absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and total fecal coliforms was also observed in 1 g of product.

Finally, the provisional validity of the products was determined using Eq. (1) [24]:

$$T_{90}(T_2) = {}^{t_{90}(T_1)}\Big/_{Q_{10}^{\Delta T}/_{10}} \quad (1)$$

where $T_{90}(T_2)$ is the estimated shelf life, $t_{90}(T_1)$ is the expiration date at a certain temperature, Q is the activation energy and ΔT is the difference between temperatures T_1 and T_2. The commonly used values for Q are 2, 3 and 4 regarding the activation energies for temperatures close to room temperature (25 °C). In general, there is a reasonable estimate using the value 3 (activation energy: $E_a = 19.4$ kcal/mol). In this study 25 °C was used for T_2, which represents the temperature for the estimated shelf life [23].

2.4. Evaluation of the healing activity

The research was conducted in accordance with international standards for biomedical research on animals, according to the Brazilian Federal Law N. 6638 (1979). A total of 45 male Wistar rats of adult age and average body weight of 300 g (ranging between 250 and 350 g) were used. The animals were kept in proper cages with food and water ad libitum, in a semi-controlled macro-environment at room temperature (21 °C), while the brightness, noise and humidity were that of the general environment [23]. The animals underwent a burning.

After intramuscular anesthesia – consisting of 0.04 mL/100 g Rompun dose (which has a relaxing, sedative and anesthetic effect)

Table 1
Composition of the neutral emulsion and the emulsion containing the Ocara clay.

Emulsion	Neutral talc (g)	Glycerin (g)	Polawax cream (g)	Ocara clay (g)
Neutral emulsion	40	112.5	500	–
Emulsion/clay	40	112.5	500	75