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The effect of natural extracts on laser burn wound healing

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ABSTRACT

Background: Despite the achievements in modern medicine, there is no effective natural treatment of second-degree burns till now. Therefore, the purpose of this study was to assess the wound-healing efficiency of three extracted oils, from the prickly pear, pumpkin and linseed on partial-thickness burns induced by fractional CO_2 laser (an innovative up-to-date technique) in rats.

Materials and methods: Thirty rats completing partial-thickness burns by fractional CO_2 laser were randomly divided into five groups. The burns were measured, photographed, and topically treated daily with saline solution, "CYTOL BASIC," pumpkin-, linseed-, and prickly pear–extracted oils (0.52 $\mu L/mm^2$ of oil) until day 7. The response to treatments was assessed by macroscopic, histologic, and biochemical parameters.

Results: Due to their chemical composition, the extracted oils showed significant improvements over the control and reference groups during the trial for both general wound appearance and crusting. On day 7, the prickly pear, linseed, and pumpkin oils showed a significant decrease in the healing time (0 cm^2, 0.15 cm^2, and 0.18 cm^2, respectively) when compared to the control and reference groups (1.49 cm^2 and 0.85 cm^2). Histologic assessment of the prickly pear oil–treated group revealed good healing proprieties compared with the other groups. The collagen content in prickly pear oil–treated group was found to be significantly greater (270.67 $\pm$ 7.48) than that in all other groups.

Conclusions: Our experiment has shown, for the first time, a scientific evidence of the efficiency of extracted oils of prickly pear, pumpkin, and linseed on partial-thickness burns.

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1. Introduction

Burns are commonly considered as the most destructive type of traumatic injuries. Indeed, the improper healing in burn injury is manifested by high levels of free radical–mediated damage, delayed granulation tissue development, decreasing angiogenesis, and collagen remodeling. Therefore, the quality of the wound regeneration depends mainly on the efficiency of wound care [1]. Nevertheless, biomaterials of tissue engineering are extremely expensive, and burn charge entails heavy expenses outside the reach of most of the patients in developing countries. Therefore, medicinal plants could be a useful supply for primary health care providing cheap, safe, and effective natural product as compared with synthetic drugs [2,3]. As a matter of fact, the *Opuntia ficus-indica* (L.; prickly pear), *Cucurbita pepo* (pumpkin), and *Linum usitatissimum* (linseed), which have very prominent extracted oils and widespread pharmacologic properties including anti-inflammatory [4–6] and antimicrobial activities [4,7,8], are investigated as a possible burn skin ointment treatment. Accordingly, the aim of this study was to highlight the use of the extracted oils of *O ficus-indica* (L.), *C pepo*, and *L usitatissimum* by appraising their healing effect on fractional CO_2 laser wound second-degree burns on rats.

2. Material and methods

2.1. Plant material

The O ficus-indica L. plant seeds were harvested (September 2014) in Sbeitla (region of Kasserine, center west of Tunisia). However, the *C. pepo* L. and brown *L usitatissimum* seeds were harvested in August 2014 in Sidi Bouzid and Beja regions, respectively.

The seeds were authenticated by Dr Hamadi Ben Salah, and the voucher sample was deposited at The National Botanical Research Institute Tunisia (INRAT). The fixed oil was extracted by the first cold pressure from seeds using a mechanical oil press (SMIR, MUV1 65).

The topical emulsion "CYTOL BASIC" was purchased from a local pharmacy. "CYTOL BASIC" is a cream emulsion (oil in water) based on *Vitis vinifera* seed oil (grape seed oil). It is a medical prescription that restores the skin's hydrolipidic film creating a natural protective shield. This restorative emulsion promotes epithelial repair in superficial, partial, and full-thickness burns. The ingredients of "CYTOL BASIC" include mainly aqua (water), glyceryl stearate, *V vinifera* (grape) seed oil, propylene glycol, stearic acid, squalane, mineral oil, triethanolamine, allantoin, cetyl palmitate, tocopheryl acetate, disodium EDTA, retinyl palmitate, and fragrance. The remaining chemicals used were of analytical grade. This product is manufactured in Tunisia under the supervision of the French laboratory "Cytolnat" and tested under dermatologic control.

2.1.1. Animals

The experiment was conducted on adult male Wistar rats, weighing 298.46 ± 35.1 g, which were obtained from the local Central Pharmacy, Tunisia. All rats were kept in a controlled breeding room (temperature: $22 \pm 2°C$; humidity: $60 \pm 5\%$; 12 h dark/light cycle) where they had standard diets and free access to tap water.

The experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by the University of Sfax, Tunisia and approved by the Committee of Animal Ethics. The animals were kept in separate cages to avoid licking or biting of wound areas by other animals.

2.2. Fractional CO_2 laser burn creation

The laser typically applies fractional pulsed carbon dioxide (CO_2) creating microscopic areas of thermal necrosis in the epidermis and the dermis in a grid pattern with surrounding areas of undamaged viable tissue [9]. Previous findings have shown that the induction of the laser burn resulted in a microvascular damage [10].

The rats were anesthetized by intramuscularly injecting 50 mg/kg of ketamine, along with 5 mg/kg of midazolam. The animals' backs were shaved with an electrical clipper. Then, the rats were exposed to partial-thickness skin burns (wound area = 2.2 cm^2) using a CO_2 Fractional Laser System (DSE, Korea) with the following setting parameters: density: (level: 20; line: 29×29; dot: 0841); energy level: 25 MJ; depth level: 4.

2.3. Experimental design

After creating the burns, the 30 animals were randomly divided into five groups of six animals each:

Group 1: rats were treated with a saline solution (control group).

Group 2: rats were treated with a standard drug "CYTOL BASIC" cream (reference group).

Groups 3, 4, and 5: wounds were treated with *C pepo L.*–, *L usitatissimum*–, and *O ficus-indica*–extracted oils, respectively. A dosage of 0.52 $\mu L/mm^2$ of oil was topically administrated on burns.

All the treatments were topically administrated and carried out once daily using sterilized compresses immediately after burning until the first group completely healed.

2.4. Measurement of wound area and burn contraction rate

The wound area was measured by tracing manually the wound boundaries on a transparent paper everyday. The shapes of the wounds were scanned, uploaded to the computer, and the wound surface areas were measured using Autodesk AutoCAD 2015 software application for design and drafting.

The burn contraction rate was calculated according to the following equation:

$$\text{Burn contraction rate} = [(\text{Initial burn size} - \text{specific day burn size}) / \text{Initial burn size}] \times 100.$$

2.5. Wound healing assessment

To determine the efficiency of wound healing, burns were photographed and documented for colorimetric analysis; two