



Research Article

A New Topical Application for Hydrogel Composite Based on Sodium Carboxymethyl Cellulose with Bee Pollen for Second Degree Burns in Adult Rabbits

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ABSTRACT

Numerous previous studies have demonstrated improved wound healing associated with natural-based formulations. Therefore, in the present work, a hydrogel composed of biopolymer gelatin incorporated with bee pollen was chosen to develop BPHG of healing in second degree burn wounds in adult rabbits. A total of 09 rabbits with superficial and partial thickness burns were divided into three equal groups randomly by consecutive sampling method, one group were dressed with BPG while the other was treated with paraffin-impregnated tulle gras (PTG). Adult rabbits were anesthetized and dorsum shaved. Cylindrical stainless-steel rod (2x4 cm diameter) was heated to 100°C. Additionally the bee pollen powder used has been characterized by FTIR spectroscopy. On Day 25, Only in the BPHG-treated groups, was degree of wound healing increased up to normal condition. Besides, the healing area of the BPHG-treated wound was better than that of the untreated and PTG- treated groups during 2, 14 and 14 days after burn. In this first approach with BPGH our results provide the first demonstration that bee pollen gel can be considered as a possible alternative for the treatment of burns.

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INTRODUCTION

In recent years, the nutritional and therapeutic properties of bee pollen (BP) have been the subject of intense scientific studies [1]. The nutritional properties of BP including proteins, amino acids, carbohydrates, vitamins, minerals and 11 enzymes or coenzymes have been determined and reported [2-3]. In addition, BP is constituted mainly by flavonoids, phytosterols and polyphenolic substances [4]. Also, BP is a natural bee product with a great potential for use in medicine and recently in animal [5]. As a natural resource, BP has attracted the attention of researchers for its wide range of biological properties, among which, the antibacterial [6-7], anti-inflammatory [8], Immunostimulatory [9] and antioxidant, properties [10]. A large number of topical agents and dressings are available to treat burns.

Many topical antimicrobial agents are cytotoxic to cells essential for second-degree burns such as keratinocytes and fibroblasts [11]. Studies suggest that a number of natural remedies may aid in the treatment of second-degree burns.

Therefore, the aim of the present study was to compare the therapeutic efficiency of another natural agent based on bee pollen gel which has not been studied before with a commonly used pharmaceutical paraffin tulle grass in treatment of thermal burns in adult in rabbits.

MATERIALS AND METHODS

Preparation of Bee Pollen Powder (BPP)

BPP (80 wt %) and sodium carboxymethyl cellulose (CMC) powder (5 wt %) were mixed and dissolved in distilled water at room temperature. The gel was allowed to swell in a sterile tube at room temperature.

FTIR Profile

The identification of functional groups of our compound by infrared spectroscopy was recorded using Shimadzu 1800 spectrophotometer. Samples were prepared

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using KBr (spectroscopic grade) disks by means of hydraulic pellet press with a pressure of 35-40 bar. The samples were scanned from 4000 to 450 cm^{-1} .

In Vivo Wound Healing Experiment

Skin Irritation Test

Skin tolerance tests of BPG were performed by the following method. Twenty-four hours before the experiment, the fur of the back of three rabbits was clipped. A few millimeters of fresh gel were applied evenly and gently to a test site, while untreated skin areas served as a control. The test sites were then examined critically at 24hrs after removing the test dressing for dermal reaction.

Animals

Nine (09) adult rabbits weighing between 1.735 and 2.430 kg aged 100 days were used in this preliminary study. All the animals were maintained under standard conditions.

The animals were divided into three groups with 3 adult rabbits in each group.

- Group CG (I):** was considered as the control group treated daily with 9% saline solution
- Group PTG (II):** animals treated with a Paraffin tulle gras (PTG)
- Group BPG (III):** animals treated with a bee pollen hydrogel under the dressing.

Induction and Treatment of Second-Degree Burn

Adult rabbits were anesthetized with an i.m. injection of ketamine (1.05 ml/kg) and xylazine (0.22 mg/kg). The backs of the animals were shaved and, under aseptic conditions, a cutaneous wound of approximately 4 cm x 2cm was performed at the dorsum using a cylindrical stainless-steel rod was heated to 100°C.

The treatment was initiated 30 min after the burn induction. The wounds of all the animals were covered with gauze and the bandages were removed after two days [12].

Assessment of the Degree of Healing of Burn Wounds

Every two days post-burn, the wound dressing was removed, and the wound was photographed. The burnt area was measured immediately after

the burn and on days 2, 7, 14 and 25 after burn injury using millimeter-scale.

The degree of wound healing was calculated using the following formula [13]:

$$\text{Degree of Wound Healing (\%)} = 1 - [\text{wound area on the corresponding day (cm}^2\text{)} / \text{wound area on day zero (cm}^2\text{)}] \times 100$$

RESULTATS AND DISCUSSION

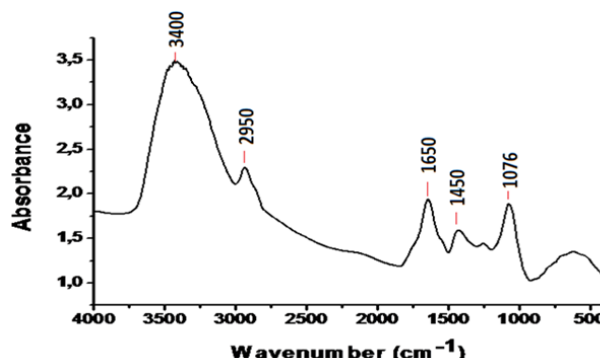


Figure 1: Fourier transform infrared (FTIR) spectra of Bee Pollen Powder (BPP) sample investigated in the region from 4000 to 500 cm^{-1}

Fig. 1 shows the Fourier transform infrared spectra of the sample. The different peaks observed confirm the presence of the following functional groups: The relatively broad band recorded at about 3400 cm^{-1} indicates the presence of hydroxyl groups [14]. The presence of aliphatic C—H (stretching vibration) was confirmed by the characteristic peak at 2950 cm^{-1} [15]. The peak at 1650 cm^{-1} is attributed to the stretching vibration of C = C [16]. The peak whose position is located around 1450 cm^{-1} is characteristic of the bending band of C—H [17], and finally the C-O bending modes of sugars can be detected by the peak recorded at 1070 cm^{-1} [18].

After a period of 24 hours of observation the Rabbits showed no irritation signs or skin edema after treatment with BPG. The rabbits were found to be normal and active with no mortality. These formed the basis for considering the formulation safe for cutaneous applications.

BP is an insect-derived substance that has been used for treatment of many disorders. Its therapeutics effects depend on its composition, and its composition depends on the botanical and geographical origin [19]. The antibacterial, anti-edematous, anti-inflammatory, and antioxidant properties of BP were found to contribute to wound healing, especially in burns.

BP also probably has its nutritional action in the wound, directly by a contribution of easily metabolized carbohydrates, vitamins amino acids and minerals [20]. Nevertheless, the mechanism of BP biological action has not been elucidated in a sufficient manner.

In this presented research, we evaluated a curative effect of bee pollen for healing of second-degree thermal burn wounds. According to Olczyk et al. [21] All other parameters of the wound and its surroundings were assessed with a standard physical examination. The granulation formation process was assessed as well as the course of scar organization taking place on the wound surface. Macroscopic examination showed the presence of swelling around the burn wound with fibrous exudate could be observed indicated that, in the first days of the experiment, all injuries for each group were the same.

The effects of BPG on the degree of healing of burn wounds are shown in Fig. 2.

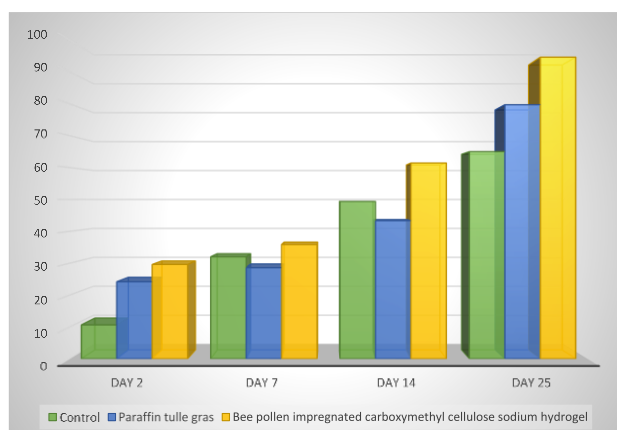


Figure 2: Comparisons of the degree of wound healing changes between CG, PBG and PTG

On Day 2, the wound in BPG-treated and PTG-treated groups had begun to contract from the wound edge. In contrast, the wounds in all in the untreated groups showed a mild degree of swelling. The degree of wound healing was 29.6; 24.17 and 10.62 % respectively.

On Day 7, all wounds in the untreated, PBG-treated and PTG-treated groups were dark red, showed thickening of the skin at the wound site. The degree of wound healing was 32; 28.6 and 35.85 % respectively.

On Day 14, all wounds in the untreated, PTG-treated and BPG-treated groups showed moderate exudation and no hair growth. The

degree of wound healing was 49.37; 43.35 and 61.45 % respectively.

On Day 25, the last day of the experiment, all wounds in the untreated, PTG -treated and BPG -treated groups showed moderate exudation and scabs starting to separate from the wound surface and hair growth. The degree of wound healing was 65; 79.73 and 94.65 % respectively.

Wound healing activities of physiological serum (PS), PTG and BPG were also observed, but at different degree. Animals treated with BPG had higher degree of healing on Days 2, 7, 14 and 25 compared to the PS and PTG-treated groups. Acceleration of wound healing could be due to intrinsic characteristics of BP. Selenium, Copper, zinc, and vitamins C, A and E are antioxidant micronutrients that are present in BP [22]. Differences in the major constituents of the BPG may account for the results of the study. These micronutrients that is beneficial to burn patients when intakes are adequate [23]. Previous studies have shown that topical administration of a vitamin C solution could be effective in promoting the healing of second-degree burn wounds [24] Also, Vitamin C influences all phases of wound healing such as the process—homeostasis inflammatory phase, proliferative phase, and maturation, synthesis, and degradation of collagen [25-26].

In a 2019 Review article published in *Int. J. Mol. Sci.* Barchitta, et al. [27] noted that Vitamin A deficiency impairs B cell and T cell function and antibody production during the inflammatory phase. Also, MacKay et al. [28] noted that Vitamin A is required for epithelial and bone formation, cellular differentiation, and immune function. In addition, Vitamin A increases the number of monocytes and macrophages at the wound site early in the inflammatory phase, facilitating epithelial cell differentiation [29].

In a 2016 study published in *Evid Based Complement Alternat Med.* Olczyk, et al. [21] noted that on the applied bee pollen ointment may affect the wound healing process of burn wounds, preventing infection of the newly formed tissue.

The results of this study support the notion that BPG can promote wound healing by reducing wound oxidative stress (Antioxidant effect), inhibiting inflammation (inflammatory effect), inducing collagen synthesis.

The results in this study confirmed the notion that BPG could be considered as a burns healing

agent, especially if topically administered. In addition, the further studies are needed to clarify the possible mechanism of bee pollen and their constituents to improve its effects in second-degree thermal burn patients.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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