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Inhibitory effect of a propolis on Di-n-Propyl Disulfide or n-Hexyl salycilate-induced skin irritation, oxidative stress and inflammatory responses in mice

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ABSTRACT

Purpose: Thermal imaging has been utilised, both preclinically and clinically, as a tool for assessing inflammation. Psoriasis is a chronic inflammatory skin disease characterised by hyperkeratosis, dermal inflammatory infiltrate and increased angiogenesis. The aim of the present study was to assess the usefulness of thermography in psoriatic lesion regression after topical treatment with bee propolis, recognised as potent antioxidants and anti-inflammatory agents.
Methods: We monitored the inflammation process induced by irritants such as n-Hexyl salycilate (HXS) or Di-n-Propyl Disulfide (PPD) by histopatological assessment of the skin, thermographic scanning, total number of inflammatory cells in the peritoneal cavity, differential analysis of cells in the peritoneal cavity, macrophage spreading index, haematological and biochemical parameters, frequencies of micronucleated reticulocytes, lipid peroxidation and glutathione assay in the skin.
Results: Topically applied ethanolic extract of propolis (EEP) with HXS or PPD reduced the lipid peroxidation in the skin and total number of inflammatory cells in the skin and peritoneal cavity, functional activity of macrophages, the number of micronuclei in mouse peripheral blood reticulocytes and enzymatic activity of ALP and AST.
Conclusion: These results demonstrate that topical application of EEP may improve psoriatic-like skin lesions by suppressing functional activity of macrophages and ROS production. Taken together, it is suggested that EEP can safely be utilised in the prevention of psoriasis-related inflammatory changes without causing any toxic effect.

1. Introduction

Psoriasis is a chronic, recurrent, inflammatory skin disease which manifests itself by multiple erythematosquamous plaques, and 1–3% of the general population has it [1,2]. It is understood today that the disease incurs as a result of interplay between genetic and surrounding factors [3–5]. It is associated with a number of biochemical and immunological disturbances. Recently, it has been suggested that increased reactive oxygen species (ROS) production and compromised function of antioxidant system may be involved in the pathogenesis of this disease. Scientific information also supports the view that an insufficient antioxidant system contributes to the psoriasis pathogenesis [6–8]. In psoriatic skin context, reactive species are generated by keratinocytes and activated leukocytes, mostly neutrophils [9]. Lactoferrin released by specific neutrophil granules can promote neutrophil–endothelial cell adhesion and, as a source of iron, may promote the Fenton reaction with the generation of the hydroxyl radical (OH•) [6]. The increased levels of other reactive species such as nitric oxide (NO•) have been determined in the skin of psoriatic patients

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[7–14]. Hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet -}$) can be generated by the action of the enzyme xanthine oxidase, which displays a higher activity in psoriatic epidermis [10]. Additionally, cytokines such as tumour necrosis factor alpha can contribute to H_2O_2 production [11]. Psoriatic skin is also characterised by an advanced state of lipid peroxidation [7]. Thus, it has been suggested that the antioxidant treatment could be part of a more specific and effective therapy for the management of this skin disease [11].

Recent literature data continue to support the fact that polyphenolic compounds, found in most plants and bee products such as propolis, can have a positive effect on many chronic diseases [13,15–18]. Natural polyphenols, recognised as potent antioxidants, are multifunctional molecules that can act as anti-inflammatory [15–18] and antiproliferative agents [19–21] through the modulation of multiple signalling pathways [20]. This characteristic could be advantageous for the treatment of multi-causal diseases, such as psoriasis. There is increasing interest in exploring the anti-inflammatory activity of medicinal plant extracts because these are cost effective, easily available form of indigenous resources and are comparable to synthetics. Polyphenols present in propolis are ubiquitous constituents of plants and possess a broad spectrum of biological activities such as immune system activities [16,19,22–24], oxygen radical scavenging [25–29], and antimicrobial, anti-inflammatory [29,30] and antitumor activities [16,19–21].

However, the immunopathogenic mechanisms of psoriasis, as well as optimal therapeutical approach, are yet to be fully resolved [4,5]. That is why, despite the development in new drug groups, traditional therapy is still used although its toxicity and negative side effects are well known [5].

We therefore tried to investigate the possible positive effects of natural antioxidants, propolis on animal model psoriasis, induced by the Di-n-Propyl Disulfide (PPD) or n-Hexyl salycilate (HXS) irritants during 5 days [31]. Taking into account that the basic pathohistologic properties of psoriasis, such as hyperkeratosis, inflammatory infiltrates and cardiovascular changes, can induce significant changes in skin temperature, we used thermographic imaging to monitor the inflammatory reactions and the effectivity of tested compounds [32–34]. In fact, many studies have shown that blood flow in psoriatic lesions is up to 10 times greater than in clinically normal skin, which leads to skin-temperature increase [35]. Thermography is an efficient and simple method which successfully and reproductively records thermal images of tested areas, and is widely used in diagnostics and treatment of diseases (e.g. malignant diseases like breast cancer and melanoma, scleroderma, osteoarthritis, rheumatoid arthritis, psoriasis and psoriatic arthritis, and Reynaud phenomenon), as well as different experimental studies, including the possible effects and application of new compounds [34,36–42].

Given the widespread use of propolis and its performance in a number of human diseases, we wanted to investigate its effectiveness in the treatment of psoriasis. We aimed to study (a) its effect on the pathohistologic properties of psoriasis, such as hyperkeratosis and inflammatory infiltrates in normal and psoriasis-like skin and possible application of thermography in the assessment of inflammatory skin changes after propolis treatment, (b) its anti-inflammatory capacity in the peritoneal cavity, (c) its genotoxic properties on normal blood cells, (d) its effect on the functional activity of macrophage and haematological and biochemical properties and (e) propolis antioxidative properties to inhibit lipid peroxidation in PPD or HXS-induced psoriasis like lesions in the skin as well as the effects of propolis on GSH level in normal and psoriasis-like skin.

2. Material and methods

2.1. Animals

The present study was approved by the ethical committee (Faculty of Science, University of Zagreb, Croatia). Male Swiss albino mice 2 to 3 months old, weighing 20 to 25 g, obtained from the Department of Animal Physiology, Faculty of Science, University of Zagreb, were used in this study. The animals were kept in individual cages during the experiment and at 12 h of light per day. They were fed a standard laboratory diet (4 RF 21, Mucedola, Settimo Milanese, Italy) and tap water ad libitum. Maintenance and care of all experimental animals were carried out according to the guidelines enforced in the Republic of Croatia (Law on the Welfare of Animals, N.N. #19, 1999) and carried out in compliance with the Guide for the Care and Use of Laboratory Animals, DHHS Publ. # (NIH) 86-123.

2.2. Irritants

Psoriasiform lesions were induced by topical application of irritant n-Hexyl salycilate ($C_{13}H_{18}O_3$) or Di-n-Propyl Disulfide (PPD, $C_6H_{14}S_2$) in the area of ~3 cm shaved abdomen of mice. Di-n-Propyl Disulfide (98%) and n-Hexyl salycilate (99%) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Fig. 1 shows the chemical structures of selected irritants.

2.3. Ethanolic extract of propolis (EEP)

Raw Croatian propolis was collected by scraping it off from hive frames. The collected propolis samples were kept desiccated in the dark until analysis at room temperature. Ethanolic propolis extract (EEP) was prepared by the method described elsewhere [43,44]. Briefly, propolis (10 g) was crushed into small pieces in a mortar and mixed vigorously with 34.85 mL 80% (V/V) ethanol during 48 h at 37 ± 1 °C. After extraction, the ethanolic extract of propolis was filtered through Whatman no.4 paper and then the extract was lyophilised.

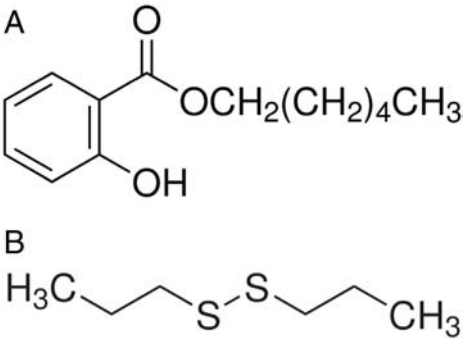

Fig. 1. Chemical structures of selected irritants: (A) n-Hexyl salycilate (HXS, $C_{13}H_{18}O_3$). (B) Di-n-Propyl Disulfide (PPD, $C_6H_{14}S_2$).

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