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Preliminary study on the Evaluation of Spirulina on TPA-induced Mouse Ear Inflammation

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Abstract

Spirulina, a water blue-green microalga, is considered a complex natural product that is widely used in treatment of chronic diseases including cancer, hypercholesterolemia, arterial hypertension, obesity and diabetes. Phycocyanin from spirulina is considered to be a strong radical scavenger (hydroxyl, peroxyl and alkoxyl radicals) providing significant antioxidant and anti-inflammatory effects. The aim of this study consists in the evaluation of the anti-inflammatory effect of SP in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in hairless SKH1 mice. The ears of mice treated with a higher concentration of SP (1000 µg/mL) showed a significant reduction of the inflammatory process than those treated with a smaller concentration of SP (200 µg/mL). Consequently, spirulina has proved dose-dependent anti-inflammatory effects in controlling and, also, in improving the acute inflammation process in mice, being a future alternative therapy for treating inflammation diseases.

Keywords: spirulina, inflammation, phycocyanin, transepidermal water loss

1. Introduction

Spirulina (SP) is a water blue-green microalga (Cyanobacterium) which belongs to the Oscillateriaceae family [1]. The most well-known species of SP are *Arthrospira platensis*, *Arthrospira maxima* and *Arthrospira fusiformis* [2, 3]. SP is a complex natural product that has been used for centuries [4] for its rich content in proteins (60–70%), essential amino acids, minerals (iron, nickel, calcium, potassium, iron, chromium, sodium, zinc, magnesium, manganese, zinc, copper, iron, selenium, lead), vitamins, carotenoids and essential fatty acids (3,6 gamma-linolenic acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid) [5-9]. The composition of SP depends both on the producing areas [10] and the mineral pollution [11].

Phycocyanin (PC) (Figure 1) is a water soluble, blue pigment found in cyanobacteria [12], that is used as a natural dye in food, pharmaceutical and cosmetics industry [13, 14]. PC is considered to be a strong radical scavenger (hydroxyl, peroxyl and alkoxyl

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radicals) providing significant antioxidant and anti-inflammatory effects [15].

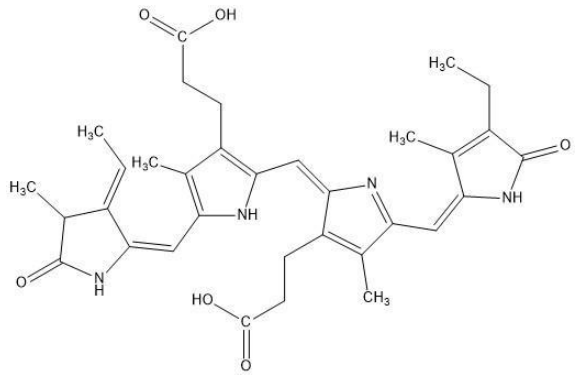

Figure 1. Phycocyanin – chemical structure

SP has *various* important *applications* in different areas such as medicine, pharmaceutics, nutraceuticals industry, perfumery, agriculture and new medicine models [16, 17]. For example, because of its antioxidant, anti-inflammatory and immunostimulant effects [18-20], SP is widely used in treatment of chronic diseases including cancer, hypercholesterolemia, arterial hypertension, obesity and sugar diabetes [21, 22]. In this regard, Health Food has recommended a daily dose of SP of 4 g for an adult with 50 kg of body weight [23]. Moreover, according World Health Organization projects SP is considered to be one of the most curative and prophylactic nutraceuticals of the 21st century [24].

The aim of this study consists in the evaluation of the anti-inflammatory effect of SP in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in hairless SKH1 mice.

2. Materials and Methods

The experimental study protocol was created according to the Universal Declaration of Animal Rights proclaimed in Paris in 1978 and to the Declaration of Helsinki, the European Convention (ETS No. 123) amended in 1998 (ETS No. 170) and Council Directive 86/609/EEC on the protection of vertebrate animals used for experimental and other scientific purposes. The animal study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy, Timisoara.

This experimental study was made on 70 healthy young adult SKH1 mice. Females should be non-pregnant. All the animals involved in the study were between 8 and 12 weeks old. The mice were housed in standard conditions: room temperature 22ºC (+ 3ºC), relative humidity between 30% and 70 % and artificial lighting characterized by 12 hours of light and 12 hours dark. During the experiment the mice have received food consisting in special pellets and water *ad libitum*.

The animals have been randomly selected and acclimated to the laboratory conditions for 5 days before to begin the experiment.

Randomization: The SKH1 mice were divided in seven groups (n=10 mice/group): group I (healthy mice), group 2 (mice that received acetone solution), group 3 (mice that received TPA in acetone – 2µg/20µL), group 4 (mice treated with indomethacin), group 5 (mice that received water – 20 µl), group 6 (mice treated with aqueous solution of SP 200 µg/mL) and group 7 (mice treated with aqueous solution of SP 1000 µg/mL).

TPA-induced ear inflammation: The ear inflammation was induced in both ears of each mouse by the topical application of TPA dissolved in acetone [25]. TPA was purchased from Sigma-Aldrich. It was locally used as a diluted solution of TPA in acetone 1µg/10µL. 20 µl of TPA solution in acetone was applied to the inner and outer surfaces of mouse ears 7 times in 14 days. After 1 hour, the mice from group 4 were topically treated with indomethacin in the same area, the mice from group 5 received the same quantity of water (20 µl) used as a solvent for SP. The mice divided in group 6 and group7 were locally treated with the same quantity of the aqueous solution of SP having the following concentrations 200 µg/mL and1000 µg/mL. Spirulina powder was purchased from FAVISAN Laboratories, Lugoj, Romania.

Transepidermal water loss (TEWL or TEWA) is usually used in dermatology for evaluation of skin damage produced by different chemical, physical or pathological factors. TEWL was measured using a Multiprobe Adapter System (MPA5) from Courage&Khazaka Electronics, Germany, equipped with a Tewameter®TM300 probe. This probe can record the following values depending on the skin

damage levels: 0–10 g/h/m^2 for very good skin condition, 10–15 g/h/m^2 for good skin condition, 15–25 g/h/m^2 - normal skin, 25–30 g/h/m^2 - bad skin condition and over 30 g/h/m^2 – very bad skin conditions [26].

After 14 days, mice were sacrificed and 5 mm-diameter ear biopsies were cut. It was measured the ear weight. The ear biopsies were fixed in 4% paraformaldehyde and after that were frozen and stored at -80°C, being sent for histological analysis.

One way Anova followed by Bonferonni–Dunn post-hoc test was used to determine the statistical difference between various experimental and control groups; *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$. The parameter measured on Oy is not absolute, but relative and is expressed as a DELTA difference between the values at some given time and the initial value (day 0) for the same mouse.

3. Results and Discussion

The inflammatory process induced by TPA was characterized by edema, erythema and polymorphonuclear leukocyte infiltration. Ear edema was observed in all TPA-treated animals. Moreover, ears weights were elevated from day 1 to the end of the study in TPA treated mice with vehicle control.

It was measured TEWL for the both 6 and 7 groups of mice treated with SP. The absolute TEWL measured in the first day of the experiment was of 4.3 units for the mice treated with aqueous solution of SP 200 µg/mL (lot S1) and 4.1 units for the mice that received aqueous solution of SP 1000 µg/mL (lot S2) (Figure 2). Consequently, in both cases it was observed an inflammatory process into the ear skin of mice. DELTA TEWL may be calculated as a permanent difference between a certain moment and the initial moment (the 1st day of experiment). So, the initial DELTA TEWL was 0 (DELTA TEWL = 4.3 - 4.3 = 0). During the experimental study it was observed a significant reduction of TEWL levels, especially in the first 6 – 7 days. In this regard, DELTA TEWL levels were negative which means a significant improvement of the skin treated with SP. Moreover, DELTA TEWL values have indicated a higher decreased of inflammatory process in mice treated with 1000 µg/mL of SP. For example, the ears treated with a higher concentration of SP (1000 µg/mL) showed a significant reduction of the inflammatory process than those treated with a smaller concentration of SP (200 µg/mL).

Consequently, SP has proved dose-dependent anti-inflammatory effects.

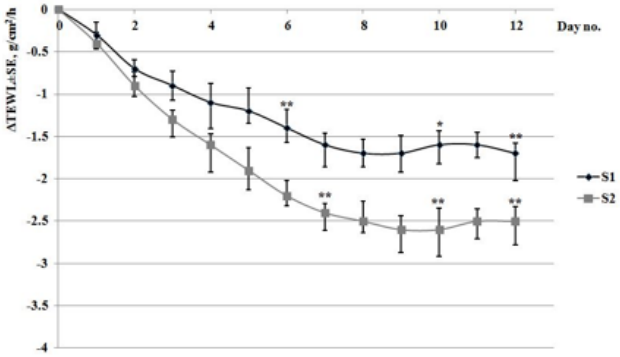

Figure 2. TEWL evolution ± SEM

According to other experimental studies, SP *maxima* has proved its anti-inflammatory effect through increasing the leukocytes, lymphocytes and monocytes levels [27].

The anti-inflammatory effect of SP may be attributed to PC that inhibits proinflammatory cytokine (TNFα) and leukotriene B4 formation, suppresses cyclooxygeanase-2 (COX-2) expression and decreases prostaglandin E(2) production. PC has been reported to suppress the activation of nuclear factor-κB (NF-κB) through preventing degradation of cytosolic IκB-α [28] [13] [29]. A novel mechanism to explain the anti-inflammatory activity of PC consists in suppressing nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression, which may be associated with the attenuation of TNF-α formation and nuclear NF-κB activation in LPS-stimulated RAW 264.7 macrophages [30]. The pro-inflammatory cytokine TNF- α plays a fundamental role in vascular disease development by promoting atherogenesis and inflammation through vascular cell adhesion molecule -1 and Intercellular Adhesion Molecule-1 expression, vascular remodeling through Matrix Metalloproteinases (MMPs), and blunted vascular

function and oxidative stress through endothelial Nitric Oxide Synthase (eNOS) and iNOS activity.

According to an *in vitro* study on BV-2 microglial cells, SP water extract and C-PC have significantly decreased lipopolysaccharide-induced lactat dehydrogense release and up-regulated the expression of COX-2, iNOS, TNF-α, and interleukin – 6 (IL-6) expression [31].

Moreover, another evidence showed that β-carotene from SP may be responsible for the inhibition of NO and prostaglandin E(2) synthesis and for the suppression of iNOS, COX-2, TNF-α, and IL-1 β expressions [29].

Alpha-tocopherol from SP has also proved to inhibit nuclear transcription factor kappa B (NF-kB) [20].

Nevertheless, SP water extract has improved salicylate-induced tinnitus in mice through inhibiting the nitrate reductase gene expression, COX-2 and proinflammatory genes [32] although more experimental and clinical evidence are required for using SP to treat tinnitus.

4. Conclusions

This study proved the beneficial effect of SP in controlling and, also, in improving the acute inflammation process in mice, being an alternative therapy for treating inflammation diseases. Moreover, this experimental study showed that the anti-inflammatory activity of SP is a dose-dependent effect. In this regard, SP may be an important anti-inflammatory agent for treating different acute and chronic skin diseases, although further experimental and clinical studies are required.

Abbreviations

COX – 2 = cyclooxygenase-2
IL = interleukin
iNOS = inducible nitric oxide synthase
NF-κB = nuclear factor-κB
NO = nitric oxide
PC = phycocyanin
SP = spirulina
TEWL = transepidermal water loss
TNF-α = tumor necrosis factor - α
TPA = 12-O-tetradecanoylphorbol-13-acetate

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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