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Study of anti-inflammatory activity of Tibetan mushroom, a symbiotic culture of bacteria and fungi encapsulated into a polysaccharide matrix

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Abstract

Tibetan mushroom (TM) is a fermented beverage composed by a dozen of bacteria and yeasts living together into polysaccharide grains secreted by them. TM is similar to kefir, a probiotic beverage originated in the Caucasian mountains exhibiting some anti-bacterial, anti-mycotic, anti-neoplastic and immunomodulatory effects. Aiming to evaluate a plausible anti-inflammatory property of TM we conducted cotton-induced granuloma and paw edema assays in rats, the latter using carrageenin, dextran and histamine as stimuli. TM samples were thawed and continuously cultured during 15 days into molasses solutions (50 g/l). The experiments used TM suspensions after 24 h fermentation and TM grains mechanically disintegrated. The results showed a significant inhibition on the formation of granuloma tissue for the test group as compared to the negative control group. TM suspensions presented an inhibition of 43% for the inflammatory process. Rat paw edema also showed significant decreases with the mediators. The edema induced by carrageenin was inhibited 62% at the 3rd hour. The edema dextran-induced was completely inhibited at 1 h and antagonized the histamine edema 52% at 1 h.

Keywords: Anti-inflammatory; Tibetan mushroom; Granulomatous tissue; Rat paw edema; Probiotic

1. Introduction

Tibetan mushroom (TM) are gelatinous and irregular grains formed by a symbiotic association of yeasts and lactic acid bacteria which causes an acid-alcoholic fermentation in sugar and milk preparations. This association is sometimes mistaken with mushrooms originated from Tibet like *Camellia assamica* [1] and *Cordyceps sinensis* [2]. Furthermore TM is also misidentified with symbiotic associations of the same nature like kombucha [3] and kefir [4], both originated from Asian countries. Kombucha and kefir are popular health promoting beverages and natural folk remedies made by fermenting green or black tea (kombucha), milk or molasses (kefir). TM can be distinguished from kombucha and kefir samples from their products, microbiological content and morphological structure. Furthermore TM is the only symbiotic association able to produce a leaflike lamellae membrane around the culture grains. The microflora of TM is embedded in a resilent polysaccharide matrix similar to kefiran matrix presented in kefir [5]. Kefir can be considered as a probiotic resource because it can enjoy a variety of health claims besides their nutritional status. There are several studies investigating immunomodulatory, pathogenic barrier, anti-neoplastic and pro-digestive effects leading through kefir intake [6]. Although kefir and TM are very similar in structure, microbial content, cultivation procedures and fermentation products, only kefir is usually reported to lead health benefits of probiotic nature [7]. In this sense, the authors conducted induction of granulomatous tissue (cotton pellet test) and paw edema experiments in rats aiming to evaluate a plausible anti-inflammatory activity of TM cultured into molasses solutions.

2. Material and methods

2.1. TM culture

Tibetan mushroom samples were kindly given by Dominiq Anfiteatro, a TM producer from Australia (10B Harrow Avenue, Magil, 5072 Adelaide SA, Australia). Starter grains (5 g) were continuously cultured in 50 g/l of molasses during 15 days before experiments. The grains were placed into plastic bottles containing the nutrient media

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and allowed to grow up at room temperature during 48 h. Soured suspensions were withdrawn from the containers and discarded, the grains were gently washed in mineral water and settled again into a fresh nutrient preparation. After the 6th day fermentation TM was used for the inflammatory experiments. Disintegrated TM grains were obtained with a tissue grinder and resuspended in 0.9% NaCl (1 g/ml).

2.2. Anti-inflammatory activity assessment

2.2.1. Animals

Wistar rats (males and females) weighing between 180 and 200 g were used in the experiments for the assessment of the anti-inflammatory activity. The rats were kept in five-animal groups in polyethylene boxes at room temperature, and fed on water ad libitum during 24 h before the experiments.

2.2.2. Induction of granulomatous tissue

Pellets weighing approximately 40 mg each were made with 5 mm of dental cotton tampon. The pellets were sterilized and then impregnated with 0.4 ml 5% ampicillin aqueous solution at the moment of implantation. Having the animals anesthetized, the pellets were subcutaneously introduced through abdominal skin incisions, in accordance with Meier's method [8]. The following was administered daily, 4 ml/kg of 5% Tween solution (orally), and 0.2 mg/kg dexamethasone (topically). Test groups were carried out using 1 ml TM suspension 24 h fermented. The treatment was initiated 2 h following the implantation of the pellets and continued until the 6th day. On the 7th day, the animals were killed with ether overdose. The granulomas were removed, left to dry for 24 h at 60 °C temperature and the weight were then determined. The difference between the initial weight and the final weight was the weight of the granulomatous tissue sample thus produced.

2.2.3. Rat paw edema

An amount of 1 ml TM suspension and 1 g disintegrated grains resuspended in 0.9% NaCl (1 g/ml) were administered to the animals 30 min before the experiments. The right rear plantar region of the rats were injected with 1 mg per paw (0.1 ml) carrageenin (Iota-Fluka Biochemika), dextran (T-70; MW 70,000; Pharmacia) 50 μg per paw (0.1 ml), and histamine 50 μg per paw (0.1 ml). The left rear paw of each animal receiving any of the three drugs listed above was also injected with an equal volume of 0.9% saline solution. The edema produced in each paw was determined by measuring the paw diameter using an analogic pakimeter (Vernier, Beaverton, OR) after stimulations [9].

2.2.4. Statistical analysis

The statistical analysis were done using one way ANOVA. The data are expressed as mean ± S.E.M. Differences between the controls and the treated groups of animals in theses experiments were tested for statistical significance by Student's *t*-test for non-parametric data.

3. Results and discussion

The biomass production of a typical TM culture fermenting into molasses is presented in Fig. 1. After a 6-day period, TM grains underwent a linear profile in biomass production assured a media replacement at 48 h intervals. After the 6th day fermentation, TM was used for the inflammatory experiments.

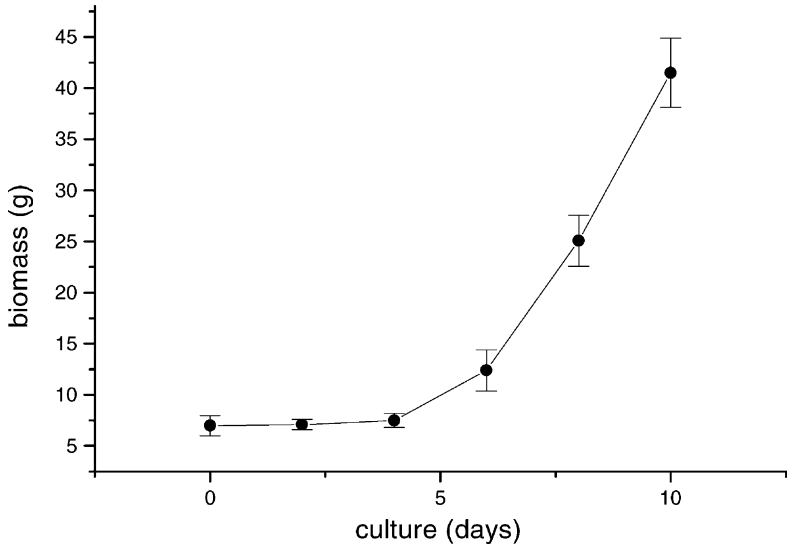

Fig. 1. Growth curve of TM fermented in aqueous solution (5 g starter grains) containing 50 g/l molasses. Numbers are represented by mean ± S.E.M. of biomass produced.

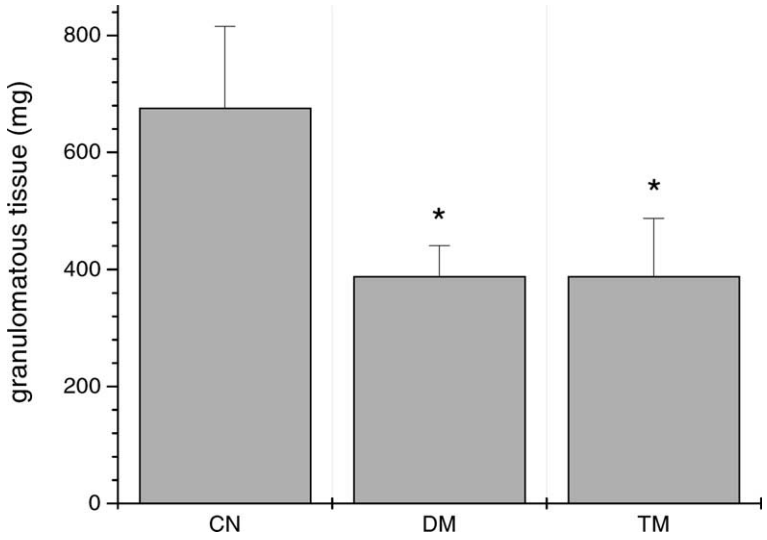

Fig. 2. Effect of administration of TM suspension and dexamethasone during 6 days on the formation of granulomatous tissue. Numbers are represented by mean ± S.E.M. of granuloma weight ($^*P < 0.05$; Student's *t*-test). CN—negative control (0.9% NaCl, 1 ml), DM—dexamethasone, positive control (0.2 mg/kg, topical application), TM—Tibetan mushroom suspension (50 g/l, 1 ml).

TM suspensions were able to reduce the inflammatory process of granuloma formation in rats after the 6th day treatment at 42 ± 5% as related to negative control samples (Fig. 2). Although the inhibition with the positive control dexamethasone was at the same degree as TM inhibition, this substance is well-known to induce immunosuppressive effects [10].

The anti-edematogenic activity of TM suspensions and disrupted TM grains was evaluated by the rat paw edema test. The administration of carrageenin (1 mg per paw, 0.1 ml), dextran (50 μg per paw, 0.1 ml) and histamine (50 μg per paw, 0.1 ml) showed significant edema in the rat paws (Figs. 3 and 4, $P < 0.05$). The inhibition of rat paw edema induced by the inflammatory agents was significantly different between the negative control and test groups ($P < 0.05$). TM suspensions in aqueous molasses (50 g/l) as well as TM grains mechanically disintegrated (1 g/ml, 0.1 ml) presented meaningful decreases in the inflammation response induced by those compounds. TM suspensions orally administered were found to be more effective than TM grains mechanically

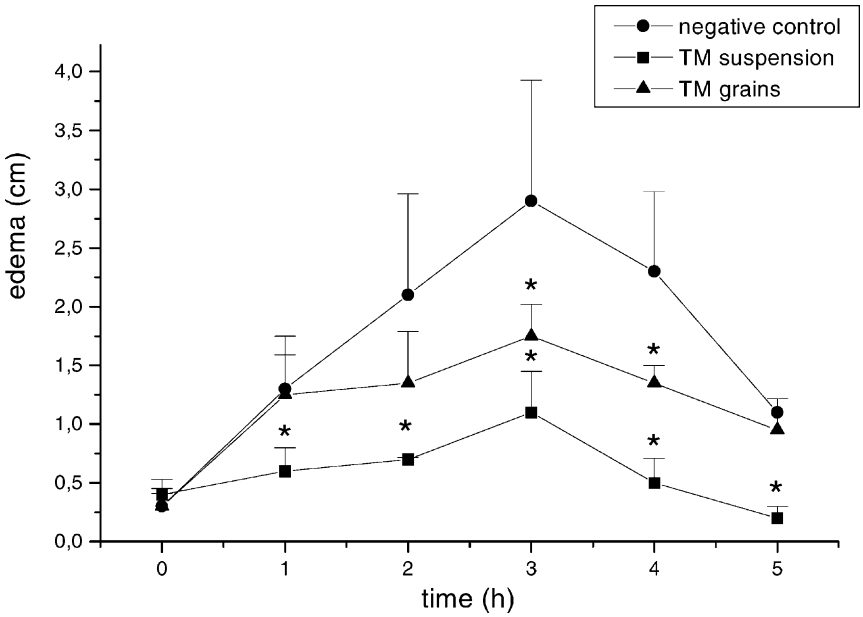

Fig. 3. Effect of p.o. administration of TM suspension with molasses (50 g/l) and disintegrated grains cultured in the same media (1 g/ml, 0.1 ml), on the rat paw edema induced by intraplantar carrageenin injection (1 mg per paw, 0.1 ml). Numbers are represented by mean ± S.E.M. of edema lengthiness. $^*P < 0.05$ (Students's *t*-test), $n = 8$ per group.

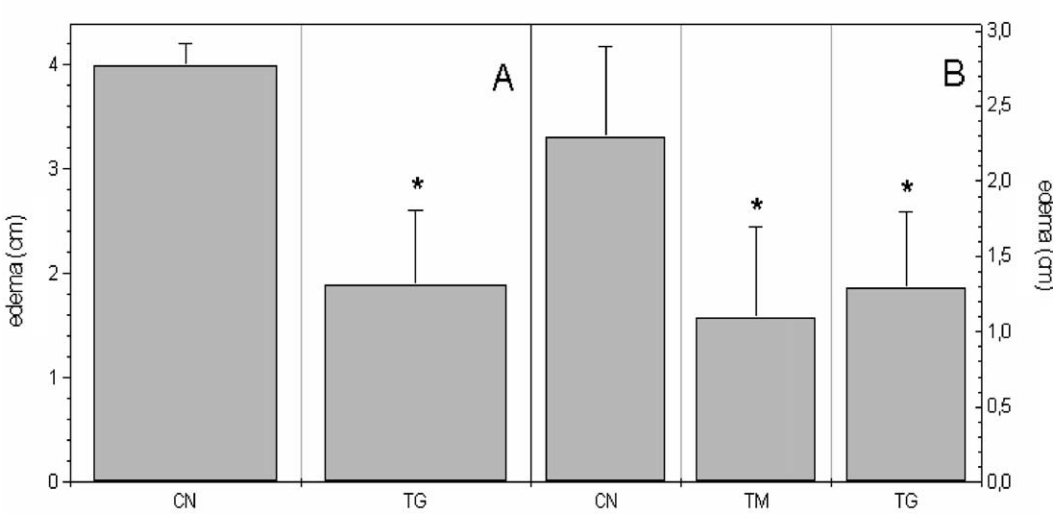

Fig. 4. Effect of p.o. administration of TM suspension in molasses (50 g/l) and disintegrated grains cultured in the same media (1 g/ml, 0.1 ml), on the rat paw edema (50 μg per paw 0.1 ml) after 1 h of intraplantar injection of dextran (panel A) and histamine (panel B). Numbers are represented by mean ± S.E.M. of edema lengthiness. $^{*}P < 0.05$ (Students's *t*-test), $n = 8$ per group. CN—negative control, TM—Tibetan mushroom suspension, TG—disintegrated TM.

disintegrated. Edema induced by carrageenin was gradually observed after 30 min administration (Fig. 3), with an inhibition of 62% using the suspension and 40% with TM grains at the 3rd hour. The inflammatory process carrageenin-induced is referred in the literature as comprising three phases [11] with the presence of histamine, serotonin, kinin system, leucotriens (LTC_4 and LTD_4), and prostaglandins [12–14]. Therefore the data presented in Fig. 3 suggested a participation of prostaglandins mediators more than histamine and serotonin, though these latter are released only in the initial phase after inflammatory challenges [11]. TM suspensions administered 30 min before dextran stimulation, however, showed 100% inhibition for the inflammation at the 1st hour. Nonetheless ground grains were found to inhibit 53 ± 18% with dextran at the same period (Fig. 4A). The dextran-induced edema is considered as a consequence of histamine and serotonin release from mast cells [15]. Even though orally administered TM suspension showed the same significant reduction for the histamine-induced edema (Fig. 4B). The antagonized histaminic effect of TM was observed with small changes during the 1st hours after stimuli, with 52±26% inhibition using TM suspension and 43±22% with disintegrated grains at the same period (Fig. 4B). Intriguing there was no inhibitory activity of TM grains after this period. Although the suggested mechanisms need to be sustained, this work presented the ancient culture of TM as a potential resource for anti-inflammatory therapeutics.

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