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Short communication

Immunomodulatory and antioxidants properties of fixed combination of fish oil with plant extracts

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ABSTRACT

The immunomodulatory and antioxidant properties of fixed combination of fish oil with *Schisandra chinensis* oil extract (CFS) and *Matricaria chamomilla* oil extract (CFM) (5 volumes of fish oil with 1 volume of plant extract) were evaluated *in vivo* and *in vitro*. The fixed combinations exhibited 27.8% (CFS) and 21.7% (CFM) inhibition of the delayed-type hypersensitivity in mice at 5460 mg/kg dose which was greater than that of fish oil (16.2%) along. Also CFM at 5460 mg/kg showed statistically significant ($p < 0.05$) stimulation of humoral immunity and increase of secondary antibody titer (5.3 ± 0.2) compared with the control (4.3 ± 0.2). The LD50 of both combinations was estimated to be greater than 5000 mg/kg since there were no lethality and signs of acute intoxication after 48 h observation. Fish oil showed weak efficacy toward scavenging of 1,1-diphenyl-2-picrylhydrazyl free radicals (IC50 = 250.2 ± 5.0 mg/kg), while combination of fish oil with plant extracts lead to significant synergistic antioxidant effect with IC50 = 119.1 ± 3.4 mg/kg for CFS and 49.4 ± 1.2 mg/kg for CFM. Overall, data presented here are important as a sign that fixed combinations of fish oil with chamomile and schisandra oil extracts substantially contributes to the immunomodulatory, as well as to the antioxidant activity.

1. Introduction

Fish oil and complementary medicines play a role of increasing importance in meeting daily requirements of essential nutrients such as long chain n-3 polyunsaturated fatty acids (PUFA), vitamins A, D, *etc*. A higher intake of n-3 PUFA may have a beneficial role in the prevention or treatment of diseases, such as cardiovascular disease [1], hypertension, autoimmune disorders [2,3], cancer [4], and diabetes [5].

Because of the numerous reports of the beneficial effects of fish oil intake, the Institute of Nutrition of Russian Academy of Science recently suggested consumption of n-3 PUFA intake of about 10 g per day [6]. Increasing fish intake is the most obvious way to increase n-3 PUFA intake, but many individuals would prefer to consume fewer servings of fish or supplements, leading to the need for the development of new n-3 PUFA products.

Natural compounds from medicinal plants having immunomodulation and antioxidant activities have great potential as therapeutic agent. The modulation of immune response by using medicinal plant product as a possible therapeutic measure have become a subject of active scientific investigation.

Schisandra chinensis (Turcz.) Bail. belongs to phytoadaptogen and exerts beneficial effects on the central nervous, sympathetic, endocrine, immune, respiratory, cardiovascular, gastrointestinal systems [7]. Potent antioxidant activity of schisandra extracts and individual lignans was shown both *in vitro* and *in vivo* [8,9].

Chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant often referred to as the “star among medicinal species” and is one of the important medicinal herb native to southern and eastern Europe. It’s multitherapeutic, nutritional, and cosmetic values have been established through years of traditional and scientific based application. Several laboratories have demonstrated high antioxidant potential of *M. chamomilla* essential oil and extracts [10,11].

However, to the best of our knowledge, no previous studies have ever investigated the effects of mixture of fish oil with herbal oil extracts on live organisms. We assume that combination of fish oil with oil extracts of schisandra and chamomile will result in strengthening of beneficial effects of such combinations.

In the present study we tested fixed combinations of fish oil with oil extracts of *S. chinensis* and *M. chamomilla* for determination of its immunomodulation and antioxidant effects in animal models.

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2. Materials and methods

2.1. Preparation of fixed combinations

The dried fruits of *S. chinensis* or dried flowers of *M. chamomilla* were extracted using corn oil (plant/oil 1:10, w/v) in a rotary-pulsation apparatus (JSC Progress, St.-Petersburg, Russia) in our modification [12]. Processes in the working zone of this apparatus involve a combination of various hydrodynamic phenomena, including high-gradient flow in the gap between rotating and immobile elements, intense turbulization, pump effects, complicated velocity fields, vortex formation, cavitation and pulsation due to variations of the by-pass flow cross section, resulted in deformation, cutting, and dispersion of plant material. After removal of the waste by filtering centrifugation, the oil extract was collected. Fish oil from salmon (JSC Baltic Coast, St.-Petersburg, Russia) was mixed with oil extract of schisandra (CFS) or chamomile (CFM), respectively (5 volumes of fish oil with 1 volume of plant extract).

2.2. Phytochemical characterization of fixed combination

2.2.1. Chromatographic fingerprint analysis

The liquid chromatographic apparatus Shimadzu (Kyoto, Japan) comprising two LC20AD pumps, a DGU-20 A3 degasser, and an SPD-M20 A diode-array detector. A Shimadzu LC Solution data-analysis system was used. Sample volume was 20 μl and each sample was analyzed in triplicate. The tocopherols, retinol, lignans, and coumarins were identified by comparison of their retention times to those of authentic standards under identical analysis conditions and the UV spectra with our in-house PDA library.

2.2.2. Fatty acids analysis

Fatty acid methyl esters were analyzed on an Agilent 7890A GC combined with an Agilent 5975C mass selective detector (Agilent Technologies, Inc., USA). The silica capillary column was Agilent FFAP (25 m × 0.2 mm × 0.3 μm). Helium was used as carrier gas with a split ratio of 15:1. The oven temperature program was from 70 °C (2 min) to 235 °C at a rate of 10 °C/min, total run time was 30 min. The temperatures of the injector and MS source were 220 and 230 °C, respectively. The samples (2 μl) were injected by a Gerstel MPS injection system and the data were collected in EI mode (70 eV) at a mass range of m/z 40-600.

2.2.3. Cholecalciferol

Cholecalciferol was analyzed by thin-layer chromatography (TLC) on Kieselgel 60 F 254 glass plates (Merck, Darmstadt, Germany). The solvent system chloroform/ diethyl ether (9:1, v/v.) was used as the mobile phase for the separation and quantification [13]. The component was visualized and quantified by direct densitometric scanning of the developed plate at 280 nm using a Camag TLC Scanner (Camag TLC scanner 3 under software control of WinCats v. 1.3.2, Muttenz, Switzerland).

2.3. Animals and doses used

Male Balb/c mice weighting 18–20 g (Rappolovo, Russia) were used in this study. The animals were housed in standard cages and were maintained under standard laboratory conditions (temperature 20–24 °C, relative humidity 45–55%, 12 h light/12 h dark cycle) with free access to a solid pellet (Volosovo, Russia) diet and water ad libitum throughout the study. All procedures used in the present study were approved by the Institutional Ethics Committee on the Use of Animals, complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and follows the Principles of Good Laboratory Practice (GOST R 53434-2009, identical to OECD GMP). The minimum number of animals required to obtain consistent experimental data was used.

Doses were calculated based on our previous studies and Guideline by Institute of Nutrition of Russian Academy of Science [6].

2.4. Acute toxicity study

The acute lethal dose (LD_{50}) of CFS and CFM were ascertained by the method described by Lorke [14]. Briefly, the study was performed in two phases. In the first phase, 18 mice were divided into 6 groups of 3 mice per group, and treated with the CFS and CFM at the doses of 10, 100 and 1000 mg/kg (*p.o.*) respectively. The animals were observed for 24 h for signs of toxicity. In the second phase, two groups of four mice were used. Three mice of each group were treated separately with CFS and CFM respectively at 1600, 2900 and 5000 mg/kg body weight, while the fourth (the control) received 10 ml/kg of distilled water. The animals were observed for 24 h period.

2.5. Delayed-type hypersensitivity (DTH)

Eighty mice were randomly divided into eight groups consisting of ten animals per group. Delayed type hypersensitivity was induced in mice using dinitrochlorobenzene (DNCB, Aldrich, USA) [15]. The fixed combinations were administered by oral gavage to the animals for 21 consecutive days.

Groups 3–5 and 6–8 received CFS and CFM respectively at dose 1365, 2730 and 5460 mg/kg body weight, while group 1 was control and group 2 received 1365 mg/kg of fish oil. Mice were primed on day 14 by a subcutaneous injection in the interscapular area of 100 μl of 2% DNCB dissolved in 50% dimethyl sulfoxide (DMSO; Sigma, USA). Six days after priming, mice were injected with the challenge dose of 50 μl DNCB (0.1 g/100 ml in 50% DMSO) into the right footpad and with 25 μl of 50% DMSO into the left. Footpad thickness was measured 24 h later by a digital micrometer.

2.6. Determination of humoral immune response

Fresh sheep blood (10 ml) was aseptically taken from the jugular vein of a healthy male sheep and transferred to heparinized tube. The blood samples were washed thrice in about 5–10 ml of pyrogen-free sterile normal saline by centrifugation at 3000 rpm for 10 min. The washed sheep erythrocytes (SRBCs) was adjusted to a concentration of 1 × 109 cells/ml with sterile normal saline and used for immunization.

Eighty mice were randomly selected and divided into eight groups of ten animals in each group. The fixed combinations and fish oil were administered by oral gavage to the animals. Groups 3–5 and 6–8 received CFS and CFM respectively at 1365, 2730 and 5460 mg/kg body weight, while group 1 was control and group 2 received 1365 mg/kg of fish oil. The method of Nelson & Mildenhall [16] was used to determine the effect of fixed combinations on the antibody level in animals after sensitization with SRBCs. Antibody titers were expressed in graded manner, the minimum dilution (1/2) being ranked as 1 (calculated as log 2 of the dilution factor).

2.7. DPPH free radical-scavenging activity

The capacity of samples to scavenge DPPH was assessed as previously reported [17]. Samples were assayed at concentrations in the range of 10–400 mg/ml. The DPPH-scavenging effect was evaluated spectrophotometrically at 517 nm (Shimadzu UV–Visible Recording Spectrophotometer PharmaSpec 1700). The values are reported as means ± SD of three determinations.