

Antioxidant and anti-inflammatory efficacy of Symbio-Complete

A dietary supplement with a high content of premium bioactive ingredients

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Our body constantly reacts with oxygen as part of the energy producing processes of cells¹. As a consequence, most reactive oxygen species (ROS) come from endogenous sources as by-products of normal and necessary metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome P450 enzyme system^{2, 3}. Free radicals have a very important role in the origin of life, biological evolution and possess beneficial effects on the organisms⁴. Oxygen radicals are involved in many biochemical activities of cells such as signal transduction, gene transcription and many others. ROS are either free radicals, reactive anions containing oxygen atoms, or molecules that can either produce free radicals or are chemically activated by them. The most common reported cellular free radicals are hydroxyl (OH·), superoxide (O₂⁻) and nitric monoxide (NO·).

Oxidative stress is caused by an excess of ROS which the cell is unable to counterbalance. The result is damage to one or more biomolecules including DNA, RNA, proteins and lipids. Oxidative stress has been implicated in the natural aging process as well as a variety of human diseases including cancer, neurodegenerative processes and (chronic) inflammation⁵⁻⁸. An antioxidant is a molecule that inhibits the oxidation of other molecules and terminates chain reactions by removing free radical intermediates. They do this by being oxidized themselves, so antioxidants are often reducing agents⁹.

Prompted by this background, the present experimental study investigated the potential of Symbio-Complete, a novel dietary supplement with a high amount of premium bioactive ingredients, which has been developed to improve and main-

tain the body's health, function and performance. The interest of the experiments was focused on its antioxidant and anti-inflammatory efficacy.

Symbio-Complete

Symbio-Complete is a dietary supplement with a high content of premium bioactive ingredients, especially of plant origin. The ingredients are filled into capsules of 900 mg which are packed in purple coloured glass to ensure maximum protection of the contents.

When considering (i) a recommended daily uptake up to 3 capsules of Symbio-Complete (equivalent to 3 x 900 mg = 2,700 g), (ii) a theoretical bioavailability of 100% and (iii) a distribution within the blood fluid of about 3.3 liters, a concentration of 820 µg/ml of the ingredients is achieved in the blood fluid. Thus, the primary stock solutions (10x) were prepared by mixing the appropriate amount of Symbio-Complete with phosphate-buffered saline with calcium and magnesium. By adding the stock solutions to the reaction mixture, they were diluted 1:10 in the test assays yielding test concentrations ranging from 0 (= untreated control) to 2 and 5 mg/ml, respectively.

Antioxidant effect against free exogenous oxygen radicals

By using a cell-free test system, the efficacy of Symbio-Complete to inactivate exogenous free oxygen radicals (superoxide anion radicals) was examined. Potassium superoxide (stock solution: 1 mg/ml in distilled water) served as a donor for superoxide anion radicals present in the reaction mixture. The reaction was started by the addition of the appropriate Symbio-Complete concentration and WST-1, a red-coloured tetrazolium salt (Roche Diagnostics, Mannheim, Germany). The

course of superoxide anion radical inactivation was monitored by cleavage of the tetrazolium dye to yellow-coloured water-soluble formazan¹⁰⁻¹². This colour change was monitored continuously for 30 min at 37°C as a differential measurement of the optical density at 450 nm minus 690 nm using a BioTek ELx 808 ELISA reader (BioTek Germany, Bad Friedrichshall). The reading interval was 60 seconds with a vigorous shaking for 4 seconds prior to each reading. It was also assured that incubation of WST-1 with the different concentrations of Symbio-Complete in the absence of superoxide anion radicals did not cause a time-dependent alteration in optical density due to unwanted interactions or direct cleavage of the dye.

Anti-inflammatory effect against endogenously generated oxygen radicals

The basic principle of the test assay has been already described in detail elsewhere¹³. This cell-based test assay uses the formation of intracellular superoxide anion radicals of human phagocytic cells (functional neutrophils) as a model to investigate the efficacy of biologically active substances to inactivate endogenously generated oxygen radicals. An overload of endogenous radicals might be the result of oxidative stress or occurs during complicated wound healing or inflammatory processes^{14, 15}.

Human promyelocytic HL 60 cells were differentiated to functional neutrophils capable of undergoing an oxidative or respiratory burst upon phorbol ester stimulation after addition of 1.5 % dimethylsulfoxide to the culture medium for 6 days¹⁶. The cells were collected by centrifugation (5 min at 200 x g), washed twice with phosphate-buffered saline by resuspending and centrifugation and were finally resuspended in phosphate-buffered saline with calcium and magnesium

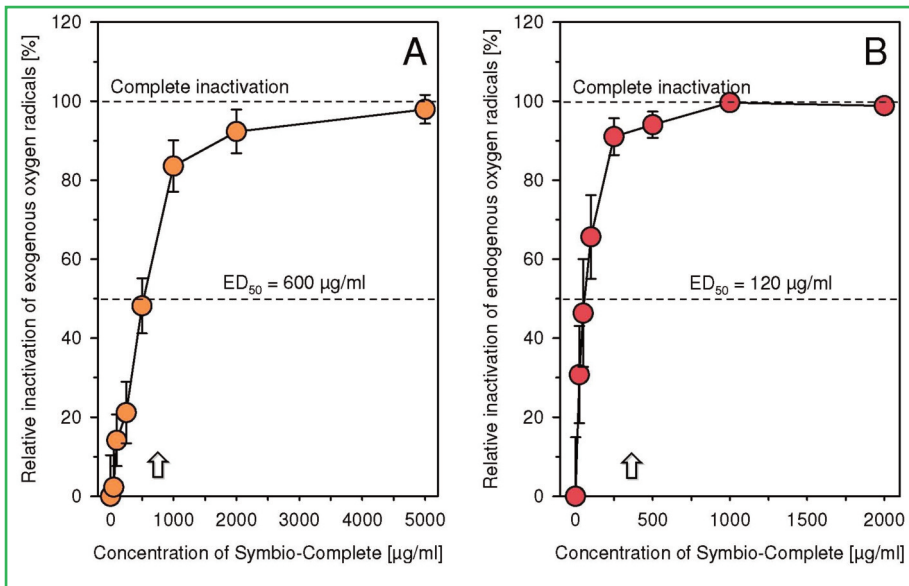


Figure 1: Dose-dependent antioxidant (A) and anti-inflammatory effect (B) of Symbio-Complete. Note the different ED50 representing the effective dosage which causes a radical inactivation by 50 %. A complete oxygen radical inactivation is achieved at 100 % and marked by the dashed line. Arrows = Calculated blood fluid concentration of 820 µg/ml at 100 % ingredient absorption. Data represent mean value ± standard deviation of three experiments.

containing 10 mM glucose. Cell suspension was added to the reaction mixture consisting of phosphate-buffered saline with glucose, phorbol ester, WST-1 as tetrazolium dye and increasing concentrations of Symbio-Complete. The course of superoxide anion radical inactivation as produced by the stimulated functional neutrophils was monitored by cleavage of the dye as a differential measurement of the optical density at 450 nm minus 690 nm using a BioTek ELx 808 ELISA reader.

Results and discussion

As depicted in Figure 1A, Symbio-Complete showed a dose-dependent antioxidant effect which reached a nearly complete oxygen radical inactivation at a concentration of 5,000 µg/ml. At the calculated blood fluid concentration of 820 µg/ml for complete ingredient absorption, a radical inactivation of about 60 % was achieved. The curve progression with its steep increase also demonstrates a good efficacy of 30 % radical inactivation at a more realistic blood fluid concentration of about 300 µg/ml representing an ingredient absorption of about one third of the recommended daily dosage. The ED50, i.e. the effective dosage of Symbio-Complete causing a 50 % inactivation of exogenous oxygen radicals was calculated to be 600 µg/ml.

In comparison to the antioxidant efficacy of Symbio-Complete, its anti-inflammatory efficacy by inactivating endogenously generated oxygen radicals was even stronger (Figure 1B). A complete radical inactivation was achieved at concentrations of 1,000 µg/ml and higher, whereas a radical inactivation of approximately 90 % was achieved for the calculated blood fluid concentration of 820 µg/ml. The ED50 was calculated to be 120 µg/ml and, thus, demonstrates its 5x higher anti-inflammatory efficacy in comparison to its antioxidant potential. Additional experiments have shown that the radical inactivation by Symbio-Complete was mainly related to a reduced production of superoxide anion radicals (data not shown).

Taken together, the results demonstrate a marked antioxidant and anti-inflammatory efficacy of Symbio-Complete which helps to minimise oxidative stress from exogenous or endogenously

generated oxygen radicals. Therefore, the frequent use of Symbio-Complete can be strongly recommended to improve and maintain the body's health, function and performance.

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