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# The Effect of Selected Herb Extracts on Superoxide Dismutase Activity in Jurkat Cells

## Wpływ ekstraktów wybranych roślin na aktywność dysmutazy ponadtlenkowej w komórkach Jurkat

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## Abstract

**Background.** Numerous dietary and medicinal herbs containing high levels of polyphenolic compounds exert antioxidative properties which are beneficial in the prophylaxis of many diseases, including cancer and atherosclerosis. Superoxide dismutase (SOD) is an enzyme scavenging superoxide radicals. It is hypothesized that some antioxidative effects of polyphenols may occur through interaction with this enzyme.
**Objective.** The aim of this study was to assess the influence of polyphenolic herb extracts on SOD activity in human leukemia cells.
**Material and Methods.** Aqueous extracts comtaining polyphenolic fractions were prepared from *Thymus serpyllum* (Ts), *Thymus vulgaris* (Tv), *Majorana hortensis* (Mh), and *Mentha piperita* (Mp). The experiments were conducted on human Jurkat cells, which were exposed to the Ts, Tv, Mh, or Mp polyphenolic fractions at concentrations of 10–500 μg/ml for 0.5, 1, or 2 hours. SOD activity was measured spectrophotometrically using a modified RANSOD kit protocol. The results were analyzed using the Repeated Measures Analysis of Variance (ANOVA) design in Statistica version 6 software. All effects were regarded as significant at a significance level of $p < 0.05$.
**Results.** The analysis of the results suggests a stimulatory effect of the extracts which was most evident at shorter incubation times (0.5 and 1 hour) and at concentrations of 50 and 500 μg/ml. However, in the case of Ts and Mh, 2-hour incubation at the highest concentration (500 μg/ml) also resulted in a significant increase in enzymatic activity. In contrast, the lowest concentration of the extracts (10 μg/ml) exhibited no significant effect on SOD.
**Conclusions.** The stimulation of SOD activity in Jurkat cells under the influence of *Lamiaceae* herb polyphenolic fractions suggests that some antioxidative effects of polyphenols may result from direct interaction with the free radical-scavenging enzyme (**Adv Clin Exp Med 2007, 16, 3, 361–364**).

**Key words:** superoxide dismutase, Jurkat cells, polyphenols, *Lamiaceae* herbs.

## Streszczenie

**Wprowadzenie.** Liczne rośliny lecznicze zawierające duże stężenie polifenoli wykazują właściwości antyoksydacyjne korzystne w profilaktyce chorób nowotworowych i miażdżycy. Wysunięto hipotezę, że niektóre antyoksydacyjne działania polifenoli mogą wynikać z interakcji z dysmutazą ponadtlenkową (SOD), enzymem zmiatającym rodnik ponadtlenkowy.
**Cel pracy.** Określenie wpływu frakcji polifenolowych na aktywność SOD w ludzkich komórkach chłoniaka.
**Materiał i metody.** Komórki Jurkat poddano działaniu frakcji polifenolowych otrzymanych z *Thymus serpyllum* (Ts), *Thymus vulgaris* (Tv)**,** *Majorana hortensis* (Mh) oraz *Mentha piperita* (Mp) w stężeniach 10–500 μg/ml przez 0,5; 1 i 2 godziny. Następnie spektrofotometrycznie mierzono aktywność SOD, posługując się zmodyfikowanym testem RANSOD. Wyniki opracowano statystycznie z użyciem analizy wariancji (ANOVA), przyjmując za istotne wartości o współczynniku $p < 0,05$.
**Wyniki.** Wykazano stymulację aktywności SOD pod wpływem badanych ekstraktów, najsilniejszą przy krótszym czasie inkubacji (0,5 i 1 godz.) i przy stężeniach 50 i 500 μg/ml. W przypadku Ts i Mh również 2-godzinna inkubacja przy najwyższym stężeniu 500 μg/ml spowodowała istotny wzrost aktywności enzymatycznej.
**Wnioski.** Stymulacja aktywności SOD w komórkach Jurkat pod wpływem roślin z rodziny *Lamiaceae* pozwala przypuszczać, że niektóre właściwości antyoksydacyjne polifenoli mogą wynikać z bezpośredniej interakcji z tym enzymem (**Adv Clin Exp Med 2007, 16, 3, 361–364**).

**Słowa kluczowe:** dysmutaza ponadtlenkowa, Jurkat, polifenole, *Lamiaceae*.

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Oxidative stress, during which an excessive amount of reactive oxygen species (ROS) is generated in the human organism, is thought to be involved in the pathogenesis of a variety of diseases, including cancer and atherosclerosis. Therefore, numerous scientific investigations have been conducted in search of antioxidative agents that could be applied in the prophylaxis of these and other conditions. Due to the well-documented association between high dietary intake of fruits, vegetables, and herbs and a reduction in cancer and heart disease risk, especially naturally occurring antioxidants derived from plant sources are of growing interest. Hence an array of medicinal and culinary herbs have been under investigation with respect to their beneficial effects on human health. Phytochemicals demonstrating high antioxidative properties comprise mainly phenolic compounds characterized by one or more hydroxyl groups bound by aromatic ring(s) [1]. A variety of substances belong to this group, from simple phenolic compounds, such as pyrogallol and caffeic acid, to more complicated flavonoids, consisting of two phenolic benzene rings linked to a heterocyclic pyran or pyrone, such as catechin [2]. The antioxidative properties of plant extracts are the result of the qualitative and quantitative content of phenolic compounds. Numerous substances of this type have been detected in herbs belonging to the *Lamiaceae* family, and for many of them strong antioxidative activity has been demonstrated both in extracts and in purified polyphenols [3–6].

Superoxide dismutase (SOD) is an enzyme forming the first line of defense against reactive oxygen species. It catalyzes the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen peroxide. Some experiments suggest a stimulatory effect of plant extracts on enzymes belonging to the ROS scavenging system, including SOD *in vivo* [7]. This allows the assumption that the antioxidative action of phytochemicals may be a resultant of direct interaction with ROS as well as SOD stimulation in living cells. The aim of this study was to assess the influence of polyphenolic herb extracts on SOD activity in human leukemia cells.

# Material and Methods

Aqueous extracts containing polyphenolic fractions were prepared from the following herbs purchased from KAWON (Gostyn, Poland): wild thyme (*Thymus serpyllum L.*) (Ts), garden thyme (*Thymus vulgaris L.*) (Tv), marjoram (*Majorana hortensis*) (Mh), and peppermint leaves (*Mentha piperita L.*) (Mp). The preparations were performed using column chromatography and semi-preparative HPLC at the Department of Pharmacognosy of Silesian Piasts University of Medicine in Wrocław.

The experiments were conducted on a human Jurkat T leukemia cell line stored at the Department of Medical Biochemistry of Silesian Piasts University of Medicine in Wrocław and grown in cell medium fluid to reach a concentration of $10^6$ cells/ml. The cells were cultured in RPMI-1640 (R8758) medium (Sigma-Aldrich) supplemented with 10% FBS (fetal bovine serum, Cambrex Corp.) at 37°C in an atmosphere of 5% $CO_2$ until a monolayer was formed (the second phase of the growth curve), avoiding the phase of overlapping cells. Then the cells were placed in a 12-well plate and supplemented with Ts, Tv, Mh, or Mp polyphenolic extracts whose final concentrations ranged from 10–500 μg/ml. The Jurkat cells were incubated with the extracts for 0.5, 1, or 2 hours in the same culture medium and conditions. Untreated Jurkat cells incubated for the same periods were regarded as controls. Then the cells were washed twice in PBS, centrifuged, and sonicated.

Superoxide dismutase activity of the samples was measured spectrophotometrically using a modified RANSOD kit protocol (RANDOX Laboratories Ltd., UK). The assay is based on the xanthine oxidase reaction, whose superoxide anion product reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye detected at a wavelength of 492 nm. The strength of inhibition of the formazan dye formation reflects the activity of SOD in the tested sample. The assay mixture, with a final volume of 0.2 ml, included xanthine oxidase, xanthine, INT, and the sample. Absorbance was monitored at 492 nm for 150 s after the addition of xanthine oxidase. On the basis of the absorbance increase after 1 min. and comparison with the calibration curve, the percentage of reaction inhibition and hence SOD activity was calculated according to the kit's procedure.

The protein concentration was assessed according to the Bradford reagent protocol using bovine serum albumin (BSA) as a standard. Bradford reagent and the chemicals for buffer preparations were from Sigma-Aldrich, Inc. (USA).

The results were analyzed using the Repeated Measures Analysis of Variance (ANOVA) design in Statistica version 6 software. All effects were regarded as significant at a significance level of $p < 0.05$.

# Results

The analysis of the effect of Ts on SOD activity in Jurkat cells demonstrated that a 0.5-hour expo-

sure of the cells to extract concentrations of 100 and 500 μg/ml caused over 27-fold and 17-fold increases in SOD activity compared with the control ($p < 0.05$), whereas a 2-hour incubation with 500 μg/ml of the extract resulted in an over 8-fold increase in comparison with the control ($p = 0.001$). Generally, the shortest incubation time resulted in the greatest increase in SOD activity, observed mainly at the 100-μg/ml concentration, when compared with the 1- and 2-hour incubation times at the same concentration ($p = 0.002$) (Fig. 1).

Unlike Ts, the Tv extract affected SOD activity most after 1 hour of incubation, where at 500 μg/ml it enhanced the enzymatic activity 37-fold ($p < 0.001$) and at 50 μg/ml it caused a 10-fold SOD increase ($p = 0.002$) compared with the control. These experimental conditions caused the greatest SOD activity increase also when compared with the remaining exposure times at the same concentrations of Tv extract (Fig. 2).

Exposure for 0.5 hour to the Mh extract at a 50-μg/ml concentration increased SOD activity 50-fold in comparison with the control ($p < 0.05$) and 2 hours of incubation with the 500 μg/ml concentration exhibited a 14-fold increase ($p < 0.001$) (Fig. 3). The Mp extract caused the greatest increase in enzymatic activity also at the 50-μg/ml concentration after 1 hour of incubation ($p < 0.05$), whereas the 500 μg/ml concentration caused the greatest activity augmentation (12-fold) after the shortest incubation time (0.5 h) ($p < 0.001$) (Fig. 4).

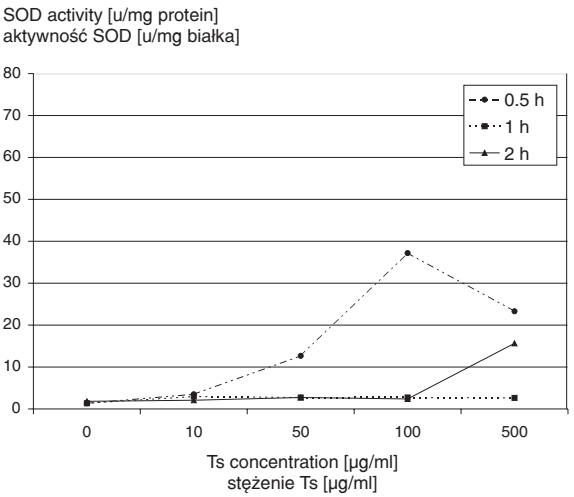


**Fig. 1.** Effect of *Thymus serpyllum* on SOD activity in Jurkat cells

**Ryc. 1.** Wpływ *Thymus serpyllum* na aktywność SOD w komórkach Jurkat

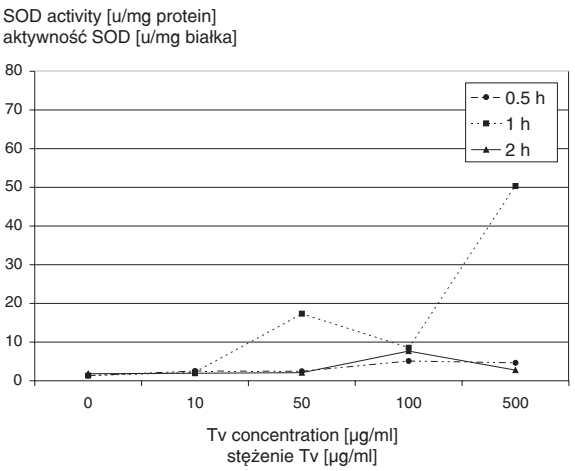


**Fig. 2.** Effect of *Thymus vulgaris* on SOD activity in Jurkat cells

**Ryc. 2.** Wpływ *Thymus vulgaris* na aktywność SOD w komórkach Jurkat

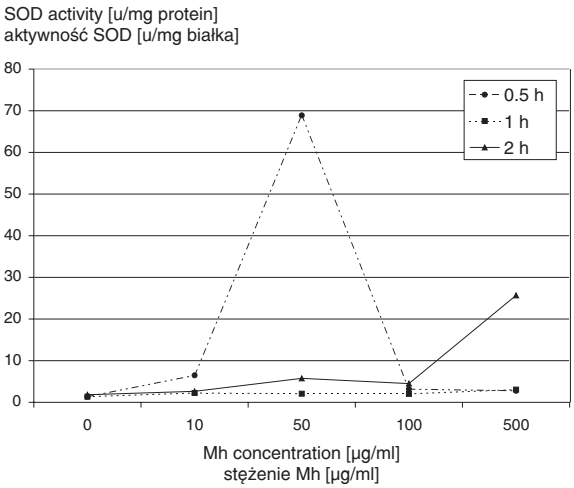


**Fig. 3.** Effect of *Majorana hortensis* on SOD activity in Jurkat cells

**Ryc. 3.** Wpływ *Majorana hortensis* na aktywność SOD w komórkach Jurkat

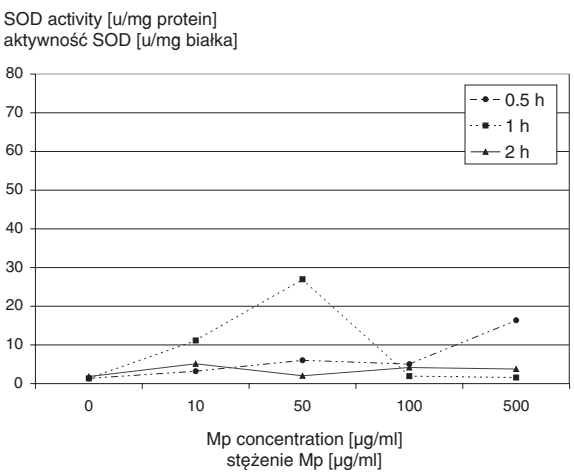


**Fig. 4.** Effect of *Mentha piperita* on SOD activity in Jurkat cells

**Ryc. 4.** Wpływ *Mentha piperita* na aktywność SOD w komórkach Jurkat

# Discussion

Among other species, *Thymus serpyllum*, *Thymus vulgaris*, *Majorana hortensis*, and *Mentha piperita* have demonstrated radical-scavenging activity [3, 6, own observations (paper in preparation)]. The possible impact of polyphenolic fractions derived from these herbs on SOD activity in a living cell system was evaluated here. Analysis of the obtained results suggests stimulatory effects of the extracts, most evident at shorter incubation times (0.5 and 1 hour) and at concentrations of 50 and 500 μg/ml. However, in the case of Ts and Mh a 2-hour incubation at the highest concentration (500 μg/ml) also resulted in a significant increase in enzymatic activity. On the other hand, the smallest concentration of the extracts (10 μg/ml) exhibited no significant effect on SOD.

Taking into consideration the quantitative and qualitative diversity in the polyphenol content of the studied plants, different patterns reflecting their effect on SOD activity in Jurkat cells were expected. Although all four plants belong to the same family (*Lamiaceae*), which indicates a high degree of similarity (e.g. they all contain luteolin-7-O-glucuronide and rosmarinic acid [8]), they also show disparate features. Certain phytochemicals occur exclusively in one species; *Mentha piperita*, for example, is an exceptional plant in this group because it contains luteolin-7-O-rutinoside and eriodictiol-7-O-rutinoside, which are not observed in the other three herbs [3]. *Majorana hortensis* is unique with respect to arbutin, not detected in the remaining herbs, but it is devoid of salvianolic acid, present in the others [8]. Obviously, besides the qualitative diversity, quantitative differences are also observed. Luteolin-7-O-glucuronide, for example, occurs in a much higher amount in *Thymus serpyllum* than in *Mentha piperita* [8]. Therefore, since in the present experiment mixtures of polyphenols (differing among the species) were tested, the obtained patterns reflecting their influence on SOD activity showed diversity among the herbs, and although the enzymatic activity was time and concentration dependent in the case of Ts and Tv, the relationships were not linear, but showed fluctuations. To clarify the picture of polyphenol/SOD interaction, in further studies the impact of selected polyphenols purified from the studied herbs will be investigated.

From the present observations indicating stimulatory effects of all four representatives of the *Lamiaceae* family it could be concluded that some antioxidative effects of polyphenols may result from direct interaction with SOD.

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