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EFFECT OF VITA GLUCAN ON SOME ANTIOXIDANT PARAMETERS OF THE HUMAN BLOOD. *IN VITRO* STUDY

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Abstract: Recently, β-glucan has been postulated to modulate antioxidant enzyme activity (superoxide dismutase-SOD) as well as to inhibit lipid peroxidation in studies concerning rats or rabbits. There are very few reports on antioxidant effect of β-glucan in the human blood. The study was aimed to estimate influence of Vita Glucan (VG) on SOD and catalase (CAT) activities as well as on total antioxidant power measured as ferric reducing activity and ascorbate concentration (FRASC) in the human blood *in vitro*. SOD activities were measured according to Fridovich's method, CAT activity by Aebi's and FRASC value by Benzi's one. Results of this study have shown that Vita Glucan at concentrations 42.5, 85, 170, and 340 mg × 100 mL^{-1} increased markedly activities of antioxidant enzymes and FRASC values in human red blood cells hemolysates.

Keywords: Vita Glucan**,** antioxidant properties, SOD, CAT, FRASC

Reactive oxygen species (ROS) (i.e. hydroxyl radicals, hydrogen peroxide and superoxide anion) are formed during partial reduction of molecular oxygen in the mitochondrial electron transport chain. Presence of unpaired electron on the outer orbitals of oxygen makes ROS highly toxic. Directly or indirectly, damage of cellular constituents by ROS contribute to various diseases such as inflammatory disorders, sarcomas, reperfusion injury after revascularization of coronary artery as well as many other disorders (1, 2).

In the defense against free radicals ROS are neutralized by enzymatic and non-enzymatic antioxidant systems. Among antioxidative enzymes, superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) play a key role. SOD converts superoxide into highly toxic hydrogen peroxide, which in the presence of CAT is decomposed into harmless water. In the non-enzymatic system, macromolecules such as albumin or ceruloplasmin and an array of small molecules, including ascorbic acid, α-tocopherol, β-carotene, reduced glutathione (GSH), uric acid, and bilirubin are involved (1, 2). Antioxidant activity of biological fluid is a combined effect of individual scavenging activity of these antioxidants and may provide insight into the overall prooxidant–antioxidant balance (3).

Vita Glucan is a natural drug received by extraction of Florida Oyster Mushrooms *Pleurotus ostreatus*. The main component of Vita Glucan is 1,3-D-glucan known as pleuran (pleuran-β). 1,3-D-glucans are water-soluble polysaccharides, whose molecules have β-1,3 substituents in the main chain and β-1,6 bonds in the branch points. This compound is also synthesized by fungi strains of *Basidiomycetes*. Other types of β-glucan isolated from medicinal mushrooms are lentinan from Shiitake mushroom (*Lentinus edodes*), schizophyllan from Split gill fungus (*Schizophyllum commune*), scleroglucan from Filamentous Fungi (*Sclerotium glucanicum*), and grifolan from Maitke mushroom (*Grifola frondosa*) (4). Vita Glucan is a good source of essential amino acids, minerals (selenium, magnesium, calcium, iron and zinc), folic acid and vitamins of B group (B_1, B_2, B_6), as well as ascorbic acid and cholecalciferol. (5)

A number of studies report that β-glucan plays a beneficial role in prophylactic treatment of differ-

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ent types of bacterial or viral infections such as influenza virus infection (6). It was also reported that anti-antibodies of β-glucan can efficiently immunize and protect against two major fungal pathogens by mechanisms that may include direct antifungal properties (7).

Recently, β-glucan has been postulated to modulate antioxidant enzyme activity (SOD) as well as to inhibit lipid peroxidation in different animal studies: rats or rabbits (8-13). There are very few reports on antioxidant effect of β-glucan in the human blood (14).

The study was aimed to estimate influence of Vita Glucan on SOD and CAT activities, as well as on total antioxidant capacity (TAC) expressed as total ferric reducing (antioxidant) activity and ascorbate concentration measurement (FRASC) in the human blood, *in vitro*.

MATERIALS and METHODS

Vita Glucan was kindly provided by Pharmaceutical Enterprise Apipol-Farma (Myślenice, Poland). Epinephrine solutions, prepared for medical use, were obtained from Polish Pharmaceutical Corporation Polfa - Warsaw. Ascorbic acid, ascorbate oxidase and 2,4,6-tripyridyltriazine (TPTZ) were purchased from Sigma Aldrich. Other chemicals, such as acetate buffer (pH= 3.6), $FeSO_4$, and $FeCl_3$ were of analytical grade, purchased in Industrial and Commercial Company, Polish Chemical Reagents (Poland).

Study samples

Blood samples K_3EDTA remaining after diagnostic tests ordered in frames of routine medical checking were included in the present study. Only samples of clinically healthy subjects (16 women and 8 men) were used. Blood samples were divided into the control and the study samples. 0.9 mL blood of the control sample was incubated with 0.1 mL 150 mM/L NaCl, whereas 0.9 mL blood of the study sample was incubated with 0.1 mL of preparation Vita Glucan at concentrations 42.5; 85; 170; and 340 mg × 100 mL^{-1}. After incubation for 30 min at 37°C the samples were centrifuged at 2000 × g for 15 min. The plasma was removed and red blood cells were washed three times with sterile phosphate buffered saline (PBS). Aliquots of plasma were used for determination of FRASC values. Red blood cells were hemolyzed in total volume of 3 mL of ice-cold double-deionized water. Erythrocyte lysates were used for measurement of antioxidant enzymes activities.

Methods

CAT and SOD activities were determined according to the Fridovich's and the Aebi's method, respectively (15, 16). TAC, ferric reducing ability of plasma (FRAP), and ascorbic acid (AA) were measured using a modified version of the FRAP assay, known as FRASC. This method allows measurement of reduced form of AA simultaneously with the FRAP value (3).

CAT assay

The method is based on the decline of hydrogen peroxide. 0.1 mL of samples was diluted with 2.9 mL 50 mM phosphate buffer (pH=7.0) and mixed with 1 mL of hydrogen peroxide. Absorbance was measured at λ = 240 nm immediately after addition of hydrogen peroxide to study samples (A_0) and after 15 s (A_{15}). Assay reactions were performed at 25°C. CAT activity was calculated as follows:

$$U = 0.153 \cdot \log \frac{A_0}{A_{15}}$$

SOD assay

The method is based on the SOD ability to inhibit the epinephrine oxidation to adrenochrome. Assay reactions were performed at 37° in air. 0.1 mL of samples were diluted in 1.8 mL 50 mM carbohydrate buffer (pH = 10.2), mixed with 0.1 mL epinephrine and 1 mL of 10 mM EDTA (pH = 10.2). Absorbance was determined at λ = 480 nm immediately after addition of epinephrine and after 5 min. SOD in the sample competes for superoxide, inhibiting the epinephrine oxidation. The percentage of inhibition was calculated as follows:

$$\%inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

One unit of SOD was defined as the amount of enzyme that inhibits the oxidation by 50%. Blank sample was used in order to exclude different spontaneous degrees of oxidation. The absorbance of blank sample was subtracted from the absorbance of the sample to calculate the real absorbance for each sample.

TAC assay

The FRASC method is based on reduction of Fe^{3+}-TPTZ complex into Fe^{2+}-TPTZ, which is visible as blue, and the intensity of the color depends on the concentration of AA. AA is selectively decomposed by ascorbate oxidase. The absorbance is measured

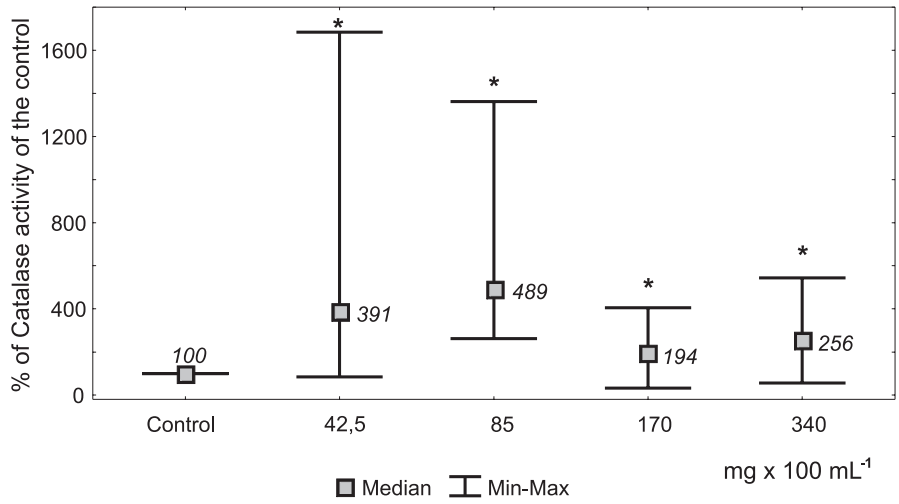

Figure 1. Influence of Vita Glucan concentration on CAT activity in the human blood.
* - Difference between study and control samples by the U test of Mann-Whitney significant at $p \leq 0.05$.

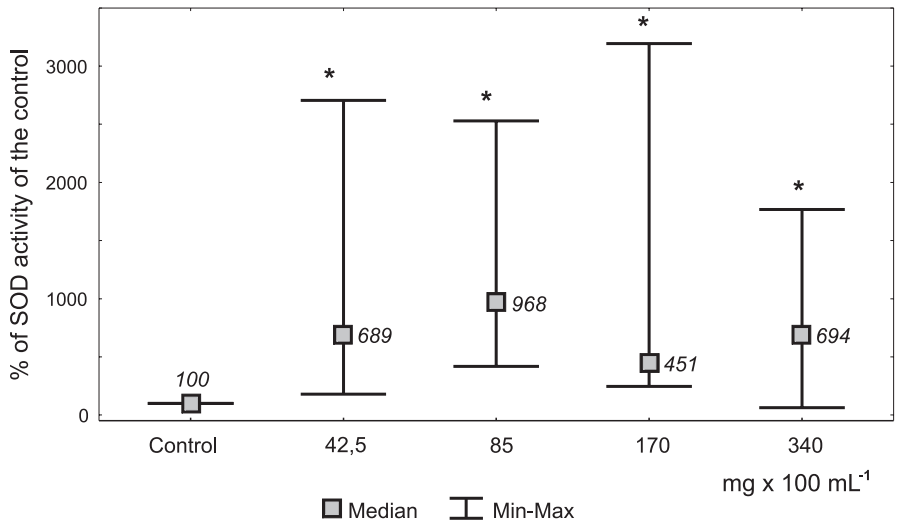

Figure 2. Influence of Vita Glucan concentration on SOD activity in the human blood.
* See Figure 1.

twice, directly after adding all components and 1 min. after (at λ=593 nm). The solution of AA should be prepared directly before the assay due to its liability at room temperature. FRASC values were obtained by comparing the absorbance of study samples at λ=593 nm with samples containing known concentration of ascorbic acid and ferrous ions (Fe^{2+}).

0.1 mL of sample was mixed with a 3 mL reagent mixture, which included 2.8 mL of acetate buffer (300 mM, pH = 3.6), a 0.17 mL TPTZ solution, and a 0.17 mL ferric (Fe^{3+}) solution. The reagents were warmed to 37°C. The calibration graph was prepared using 5, 10, 20, 30, 40, 50, and 100 µM/L aqueous solution of AA.

Statistical analysis

Differences between study and control samples were analyzed by the U test of Mann-Whitney, using STATISTICA v. 6.0 for Windows PL software (Poland).

RESULTS

The results are presented in Figures 1-3. It was found that Vita Glucan (VG) increased CAT and

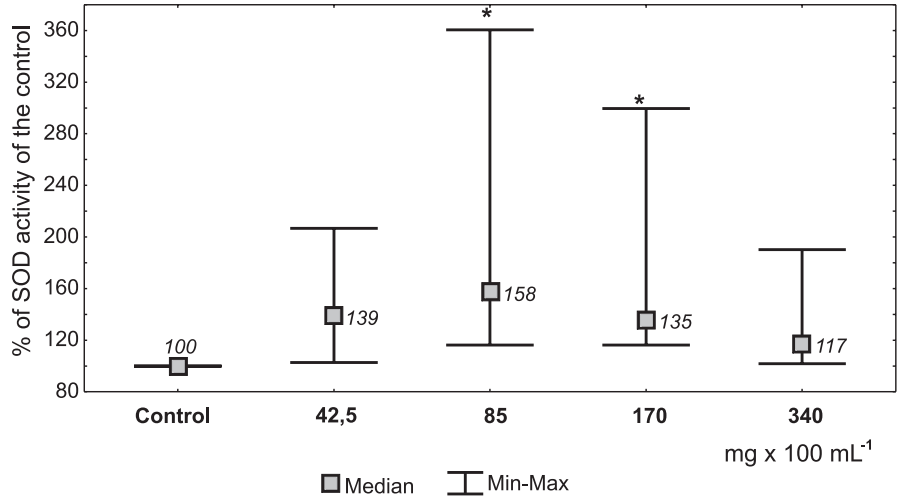

Figure 3. Influence of Vita Glucan concentration on FRASC value in the human blood.
* See Figure 1.

SOD activities in erythrocytes hemolysates and the FRASC value in plasma. CAT activity of red blood cells hemolysates before incubation with β-glucan was taken as 100% (Control).

Increases of CAT activity ranged from 194% for Vita Glucan at concentration of 170 mg × 100 mL^{-1} to 489% at concentration of 85 mg × 100 mL^{-1}. All changes of activity were statistically significant (at $p \leq 0.05$) (Figure 1).

Incubation of hemolysates with Vita Glucan resulted with a significantly higher activity of SOD than obtained from the control samples (Figure 2). The highest increase of SOD activity was observed after addition of Vita Glucan to the incubation medium at concentration of 85 mg × 100 mL^{-1}, whereas the lowest increase of SOD activity – though still statistically significant (at $p \leq 0.05$) – was found after incubation with concentrations of 170 mg × 100 mL^{-1}.

The changes of FRASC after incubation of blood with Vita Glucan at concentrations of 42.5 or 340 mg × 100 mL^{-1} were not statistically significant (at $p \leq 0.05$), however, the significance appeared at concentrations of 170 or 85 mg × 100 mL^{-1} of this medicine (Figure 3).

DISCUSSION

In this study human blood samples were used instead of rats' or rabbits' blood. Results of this study have shown that pretreatment of blood samples with Vita Glucan at given concentration increased markedly activities of antioxidant enzymes in red blood cells hemolysates.

Large variation of SOD activity and much smaller variation of CAT activity may be due to different induction of the expression of the antioxidant enzymes genes by the antioxidant (Vita Glucan). Scandlios (8) suggested that antioxidant-specific gene induction is mediated by the antioxidant responsive element (ARE) commonly found in the promoter region of genes. ARE was found in mammalian glutathione-S-transferase (GST), CAT and SOD genes. In mammalian systems ARE was reported to stimulate mitogen-activated protein kinase (MAPK), which in turn triggers two classes of transcription factors (nuclear factor kB and activator protein-1), leading to activation of antioxidant defense genes. The degree to which a given gene is activated depends on the cell type.

The effects of another β-glucan (lentinan) on SOD activity in human erythrocytes also have been studied. Lentinan at higher concentration increased the pathologically low SOD activity of erythrocytes of patients with cirrhosis of the liver (5).

Similarly to our study, pleuran effect on CAT and SOD has been reported (9). An increase in CAT activity in erythrocytes and an increase in SOD activities in liver were observed. In another study, in a model of acute colitis in rats, it was indicated that after pleuran administration, within a month, antioxidant defense (SOD activity) in erythrocytes was enhanced (10). However, the same author did not observe changes in antioxidant enzymes activities in blood of rabbits which received Oyster mushroom diet (11).

Campa-Cordova et al. investigated whether β-glucan induced immunostimulatory activity (12).

The authors found that immunostimulation with β-glucan was sufficient enough to generate an increase in the antioxidant activity of *L. vannamei* SOD for 48-120 h.

As a conclusion, the results of the present study support and confirm previous findings on antioxidant properties of β-glucan extract in different animal studies. Another aspect of this study which should be pointed out is that the most effective influence of Vita Glucan on antioxidant enzymes and TAC was observed at the concentration of 85 mg × 100 mL^{-1} of preparation. It would therefore appear reasonable to use Vita Glucan as an antioxidant relevant in limiting diseases, particularly based on impairment of oxidant/antioxidant balance.

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