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RESEARCH ARTICLE

Oxidative Stress Induced Cell Damage and Antioxidant Enzyme Response in Human Lymphocytes

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ABSTRACT

Human lymphocytes were subjected to oxidative stress by exposing to hydrogen peroxide at concentrations varying from 0-250µM for time duration of 0-24h to evaluate cell viability. Trypan blue dye exclusion test indicated a loss of <5% cell viability on incubation with hydrogen peroxide for 24h; however MTT assay signified a decline of 55% activity after 12h or long with 200µM concentration. Redox status and activities of antioxidant enzymes were examined after incubation with hydrogen peroxide up to 200µM for 4h. Reduced glutathione level decreased with concentration dependent increase in lipid peroxidation, measured as MDA produced. Increase in LDH leakage from the cells with increasing hydrogen peroxide concentration in medium indicated considerable cell membrane damage. SOD and catalase activities increased at lower concentration of 50µM but at higher stress, a decline in activities of SOD, catalase and GST was observed. GPx increased with increasing hydrogen peroxide in incubation medium. The study shows that redox status declines and cell membranes become leaky with increasing oxidative stress. Antioxidant enzymes except GPx decrease under higher stress conditions.

Key words: Lymphocytes, oxidative stress, cell viability, cell damage, antioxidant enzymes

INTRODUCTION

Reactive oxygen species (ROS), which are normally generated inside the cell as byproducts of various metabolic processes, contribute to different intracellular functions, including cell signaling and gene expression. Under normal physiological conditions, the cellular redox balance is maintained by several antioxidant systems, among which the glutathione (GSH), thioredoxin (Trx), and peroxiredoxin (Prx) cycles play central roles [1]. Antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) neutralize prooxidants and maintain a state of equilibrium [2]. SOD catalyzes the dismutation of O_2^- to H_2O_2 [3] and inhibits the simultaneous presence of O_2^- and H_2O_2 derived from the hydroxyl radicals. Catalase degrades H_2O_2 to H_2O and a selenium-containing enzyme glutathione peroxidase catalyzes the reduction of H_2O_2 and lipid peroxides [4]. Glutathione S-transferase plays an important role in the elimination of toxic compounds by catalyzing the conjugation of electrophilic compounds to reduced glutathione (GSH) [5]. The excessive production of radicals and reactive oxygen species in the biological systems results in imbalance between the oxidants and antioxidants generating oxidative stress. The cellular antioxidant machinery insufficiently able to counterbalance the elevated ROS levels, resulting from the aberrant metabolic pathways, may contribute to progression of disorders such as neuro-de generation, muscle degeneration, cancer, diabetes, atherosclerosis, arthritis, kidney failure, and aging [3,6].

Immune cells, including lymphocytes are well known as an important source of both oxidant and pro-inflammatory compounds needed to support their functions. During normal lymphocyte cell life, processes including activation, proliferation, signaling pathways, antibody production and apoptosis are markedly dependent on ROS generation [7]. Several functions of the lymphocytes, such as clonal expansion, orchestration of the immune response and antibody production are strongly influenced by the intracellular and micro-environmental oxidant/antioxidant status [8]. However, these cells can also suffer the effect of oxidant

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overproduction. Thus, immune cells need to deal appropriately with this delicate balance between beneficial and hazardous effects of ROS [7, 8]. Lymphocytes the key cells of the immune system are exposed to chronic oxidative stress during various diseases and their treatment with antibiotics, chemical drugs or radiotherapies affecting the functioning of the immune system[9]. Deregulation of this redox homeostasis causes oxidative damage to the cellular building blocks, namely nucleic acids, proteins, and lipids, ultimately leading to cell death [8]. In search of compounds synthetic or natural, effective as antioxidants and protectors against the damage caused by oxidative stress, the effect of antioxidants on the cultured human lymphocytes exposed to H_2O_2 at concentrations varying from 50-250 µM for few minutes to hours is used by researchers to mimic acute or chronic stress[10]. Usually, the controls without stress for the same duration are used as reference but detailed information of the influence of a wide range of oxidative stress on lymphocyte antioxidant system will help to understand the mechanism of detrimental influence of stress, possible protection and structure mechanism relationship by various natural and synthetic compounds. Addition of hydrogen peroxide into culture of lymphocytes creates oxidative stress condition, which is an increase in the number of prooxidants[10]. The present study was taken up with an aim to study the influence of prolonged oxidative stress on the redox status and antioxidant enzymatic defense system of cultured human lymphocytes.

MATERIALS AND METHODS

Chemicals; Ciprofloxacin, amphotericin-B, l-glutamine, Phytohaemagglutinin (PHA), phenylmethylsulfonyl fluoride (PMSF) and thiobarbituric acid (TBA) were purchased from Sigma(St.Louis,USA). Ethylenediaminetetracetate (EDTA), Trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2) and dimethylsulfoxide (DMSO) were from S.D. Fine Chemicals (Boisar, India). RPMI1640, Hisep, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) were procured from Himedia (India).

Collection of blood sample:

Blood samples of 3ml each were collected from median cubital vein of a healthy non-smoking individual of 18-25 years, in heparinised sterile glass vials.

Isolation of lymphocytes:

In a 15ml centrifuge tube blood and phosphate buffer saline (PBS) were mixed in 1:1 ratio (3 ml blood + 3ml PBS). Blood-PBS mixture was layered over 5ml Hisep solution in a centrifuge tube to centrifuge at 2000 rpm for 30min at 4°C. The buffy coat layer containing monocytes and lymphocytes was separated using a pipette in Biosafety cabinet in culture room. Cells are washed thrice using PBS and centrifuging at 1200 rpm for 5 min.

Cell Culture:

RPMI 1640 medium containing 1µg/ml of PHA (stock solution: 1mg/ml of PBS), ciprofloxacin (5µg/ml), amphotericin (2.5µg/ml) and l-glutamine (1.5 mg/ml) was used. Cell concentration was adjusted at 1×10^6 cells/ ml in the culture plate. Cells were cultured in media containing PHA. Primary cells were incubated in culture plate for 2-3h to separate lymphocytes from adherent monocytes at 37°C in presence of humidified 5% CO_2/95% air in CO_2 incubator. During this incubation period, monocytes in PBMCs get attached to the plate. Non-adherent lymphocytes in suspension were collected in a centrifuge tube and centrifuged at 1400 rpm for 5 min. The cell pellet was re-suspended in fresh culture media. The cells were counted in haemocytometer after trypan blue staining, plated with media (1×10^6 cells/ml) and kept in CO_2 incubator. The cells were multiplied for 72h. After every 24h, cell viability was checked and fresh media was added if needed. Secondary lymphocyte suspension was collected in a centrifuge tube and centrifuged at 1400 rpm for 5 minutes.

Secondary lymphocytes $5\text{-}6x10^5$ (300µl) in 48 well plate and $2\text{-}2.5x10^5$ (150µl) in 96 well plate were incubated for different durations (0-24h) with and without 50-200µM of H_2O_2. For antioxidant enzyme studies cells were incubated with specified concentrations of H_2O_2 for 4h.

Cell Viability Assay:

Trypan Blue Exclusion Test:

Trypan blue is a vital stain used to selectively colour dead cells blue. Since cells are very selective in the compounds that pass through the membrane; in a viable cell, trypan blue is not absorbed. The cell suspension is mixed with trypan blue solution (0.4% solution in PBS) in 1:1 ratio and incubated for 2 min. 200µl of this mixture was loaded onto the haemocytometer to visualize and count the cells under microscope [4].

Percentage of viable cells = No. of viable cells ÷ Total cells (Live + dead) x 100

MTT assay:

Cell viability assay was performed according to the MTT based method. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring yielding purple insoluble formazan crystals which were dissolved in DMSO. Briefly, 100µl lymphocytes culture was incubated with 100µl of 10% MTT for 2h. 200µl DMSO was added and read at 570nm.

Cell lysis:

Control and oxidative stress-induced cells were lysed by suspending in lysis buffer i.e. 50mM Tris, pH 8.0, 150mM NaCl, 10µg/ml leupeptin, 2µg/ml aprotinin, 0.3% NP-40 and 1mM PMSF. The homogenate was centrifuged at 10,000rpm for 15min at 4°C. Reduced glutathione content, lipid peroxides, protein content and antioxidant enzyme activities were assayed in the supernatant.

Reduced glutathione:

Reduced glutathione (GSH) content was estimated as the total non-protein sulphydryl group by the standard procedure [11]. The proteins were precipitated by addition of TCA, centrifuged and the supernatant was collected. The supernatant was mixed with 0.2M phosphate buffer (pH 8.0) and 0.6mM DTNB dissolved in methanol, and allowed to stand for 8-10min at room temperature. The absorbance was recorded at 412nm. Standard GSH was used to calculate nmol of –SH content/mg protein.

Lipid peroxidation:

Peroxidative damage was estimated spectrophotometrically by the assay of thiobarbituric acid reactive substances (TBARS) and expressed in terms of nmoles of malondialdehyde (MDA) formed per mg protein [12]. In brief, to 0.2ml of sample, 1.5ml of 20% acetic acid, 0.2ml of 8% SDS and 1.5ml of 0.6% TBA were added. The mixture made 4.0ml with DW, heated in a boiling water bath for 1h, cooled to RT, centrifuged at 3000xg for 10min and supernatant was read at 532nm against DW.

Protein determination:

The protein contents were determined using bovine serum albumin (BSA) as the standard by Bradford [13] method.

Determination of Enzyme Activities:

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by slight modifications in the method used by Kakkar et al [14]. The reaction mixture containing 0.2M sodium phosphate buffer pH 8.0, 20mM PMS, 20mM NBT, 10mM NADH and cell lysate was incubated for 1min and acetic acid added to stop the reaction. Butanol was added to each test tube and centrifuged at 4500 rpm for 6min. The amount of pink formazan extracted in butanol was measured at 560nm and expressed as units/ mg protein.

Catalase (EC 1.11.1.6) activity was measured by the spectrophotometric method based on the decomposition of H_2O_2 [15]. Cell lysate (50µl) was mixed with 600µl of 15mM H_2O_2. The decrease in absorbance at 240nm (H_2O_2 decomposition) was determined for 3min. A cuvette containing only PBS pH 7.0 served as blank. A cuvette without a sample was used to ensure that H_2O_2 did not decompose spontaneously under the experimental conditions. Enzymatic activity was expressed as the rate constant of a first-order reaction (k) divided by the protein concentration. A_1 and A_2 refer to the absorbance before and after a given time interval of measurement (t), respectively. $k = (2.3/t)(\log A_1/A_2)$ (s^{-1}. mg protein^{-1}).

To estimate Glutathione peroxidase (Gpx, EC 1.11.1.9) activity cell lysate 0.2ml was added to 0.2ml of 0.4M phosphate buffer, pH 7.4, 50µl of 4mM GSH, 12.5µl of 2.5mM H_2O_2 and 0.1ml of 10mM sodium azide to incubate in a water bath at 37°C for 3min. 0.5ml of 10% TCA was added and centrifuged at 5000 rpm for 5min. 1.0ml of the supernatant was added to 2.0ml 0.4M Tris buffer, pH 8.0 and 50µl DTNB. Immediately read the OD at 412nm.The enzyme activity was expressed as nmol of glutathione oxidized/min/mg protein [15].

Glutathione-S-transferase (GST, EC 2.5.1.18) activity was assayed by measuring the increment of absorbance at 340nm due to the formation of 2,4-dinitrophenyl-S-glutathione from 1-chloro2,4-dinitrobenzene (CDNB) and GSH [15]. In brief, the reaction volume 1 ml containing 0.1M phosphate buffer (pH 6.5), 3mM CDNB in 95% ethanol and 30mM GSH were incubated at 37°C for 5min. The reaction was initiated by the addition of cell lysate and the activity was measured for 3min at 340nm. The specific activity of GST was calculated using the extinction coefficient 9.6 $mM^{-1}cm^{-1}$ at 340nm and expressed in terms of nmoles of CDNBGSH conjugates formed/min/mg protein.

The activity of Lactate dehydrogenase (LDH, EC 1.1.1.27) released into the medium as a result of membrane damage was assayed by measuring the rate of oxidation of NADH at 340nm [16]. The reaction mixture contained 100mM potassium phosphate buffer pH 7.6, 30mM sodium pyruvate, 6.6mM NADH and cell free media to make the final volume 1ml. The reaction was started at 25°C by addition of NADH and the rate of oxidation of NADH was measured at 340nm for 3-6 min. The enzyme activity was calculated using extinction coefficient 6.22 $mM^{-1}cm^{-1}$/mg protein.

Statistical analysis was carried out using SPSS software for Windows, Chicago. Results are expressed as mean± SEM and a value of $P \leq 0.05$ was considered to be statistically significant. The statistical significance of the data was assessed by the one-way analysis of variance (ANOVA).

RESULTS

Starting with 3ml blood, 2-3 $x10^6$ lymphocytes could be isolated. Lymphocytes were cultured for three days in media containing 0.5µl/ml PHA. Cells multiplied two to three times in 24h. After three cycles of multiplication, lymphocytes cells could be sustained in media without PHA for 48h with 95% cell viability as checked by trypan blue exclusion cell counting and MTT assay. Therefore, experiments were planned in 24h after culture for three days with PHA. A linear increase in the absorbance at 570nm could be observed by MTT assay with increase in the number of cells/well up to $4x10^5$/150 µl in 96 well plate and up to $8x10^5$/300µl in 48 well plate (data not shown), therefore cell count $6.5x10^5$/300µl in 48 well plate were used in these studies.

Cell viability:

Lymphocytes were subjected to different concentrations of H_2O_2 with $6.5x10^5$ cells /300µl. Cells were counted using trypan blue exclusion assay at different time intervals. The results in Table 1 show that cell count was not affected adversely on inclusion of H_2O_2 in the culture medium up to 250µM concentration. More than 95% of lymphocyte cells were viable up to 24h with the tested concentrations of H_2O_2.

Table 1: Effect of H_2O_2 induced oxidative stress on lymphocyte count

Time of incubation(h)	Concentration of H_2O_2 in culture medium					
	Control	50µM	100 µM	150 µM	200 µM	250 µM
0	$66.0x10^4$					
3	$65.3x10^4$	$65.6x10^4$	$64.6x10^4$	$66.0x10^4$	$65.6x10^4$	$64.3x10^4$
6	$66.6x10^4$	$64.6x10^4$	$66.6x10^4$	$65.3x10^4$	$64.6x10^4$	$66.0x10^4$
9	$66.0x10^4$	$66.6x10^4$	$65.3x10^4$	$65.6x10^4$	$65.6x10^4$	$66.6x10^4$
12	$65.6x10^4$	$64.3x10^4$	$66.6x10^4$	$64.6x10^4$	$63.6x10^4$	$64.3x10^4$
18	$65.6x10^4$	$65.6x10^4$	$65.6x10^4$	$65.3x10^4$	$63.6x10^4$	$64.6x10^4$
24	$65.3x10^4$	$66.6x10^4$	$65.0x10^4$	$64.6x10^4$	$63.4x10^4$	$62.6x10^4$

The experiment is done in triplicates and the values are average cell counts.

Results in Table 2 show the effect of incubation of lymphocytes with different concentrations of H_2O_2 for increasing durations on cellular metabolism. Incubation of lymphocytes with 100µM H_2O_2 reduced the cell viability by 40% when incubated for 12h while incubation with 200µM H_2O_2 for the same period decreased cell viability by 60%. It is evident that increasing concentration of hydrogen peroxide in the culture medium affects the metabolism of lymphocytes. The inhibitory influence of oxidative stress increases with time of exposure to stress up to 12h. The increase in inhibition slows down after 15h. This may be due to a decrease in the effective concentration of hydrogen peroxide in the media after 12h.

Table 2: Effect of different concentrations of H_2O_2 on cell viability by MTT assay

Time of incubation (h)	Control	50µM	100µM	200µM	250µM
0	0.211±.05 (100)				
3	0.219±.06 (100)	0.191±.03 (87)	0.179±.04 (81)	0.174±.04 (79)	0.151±.02 (68)
6	0.207±.05 (100)	0.166±.04 (80)	0.142±.02 (68)	0.120±.03 (57)	0.107±.005 (51)
12	0.207±.07 (100)	0.144±.03 (69)	0.125±.03 (60)	0.089±.01 (43)	0.079±.006 (38)
15	0.202±.06 (100)	0.141±.04 (70)	0.110±.03 (54)	0.083±.009 (41)	.082±.008 (41)

Values in parenthesis represent percent of control i.e. without hydrogen peroxide. Values are mean± SD: n=3:p<0.5 compared to control

Redox status:

Lymphocytes were incubated with varying concentrations of H_2O_2 for 4h to examine the influence on redox status of the cells. Reduced glutathione and lipid peroxides produced were monitored as a measure of redox status. GSH

content decreased significantly even in presence of 50µM H_2O_2 (**Table 3**).

Table 3: Effect of different concentrations of H_2O_2 on redox status of lymphocytes

Concentration of H_2O_2 (µM)	GSH (nmoles/mg protein)	MDA produced (nmoles/mg protein)	LDH (Units/ml)
control	0.403±.007(100)	3.77±.07(100)	0.06±.01[a](1.0)
50	0.383±.005(95)	4.42±.07(117)	0.16±.01[a](2.8)
100	0.352±.006 (87)	5.80±.09(154)	0.60±.01[a](10.5)
150	0.284±.006(70)	6.38±.07(170)	0.89±.01[a](15.6)
200	0.181±.004(45)	7.31±.05(194)	1.18±.02[a](20.6)

Values in parenthesis represent percent of control i.e. without hydrogen peroxide. Values are mean ±SD: n=3:p<0.5 compared to control:[a]p<.01 as compared to control. LDH in control cells is 5.72±.02

Incubation of lymphocytes with 200µM H_2O_2 decreased the GSH content by 55% as compared to control cells. Lipid peroxidation increased in a concentration dependent manner.70% increase in MDA content was observed in the lymphocytes incubated with 150µM H_2O_2 for 4h which further increased to 94% when H_2O_2 concentration was increased to 200µM. Similar results are reported by Ramteke et al [17], when increasing concentrations of H_2O_2 are added to the culture medium. A three-fold increase in thiobarbituric acid reactive substances on incubation of the cultured cells with $H_2O_2 \geq$ 250 µM for 90 min is reported by Martin et al in Caco-2 and Hep G3 human cell lines [18]. An increase in lipid peroxidation influences the lymphocyte membrane integrity and membranes become leaky as is evident from the increased LDH level in the medium (**Table3**). LDH release in the culture medium was negligible in lymphocytes under normal conditions, which is ≤ 2% of the total LDH in the cells i.e. 5.72units/ml. However, a significant increase in the level of LDH activity is observed in the incubation medium in presence of H_2O_2 more than 50µM. About 20% of the total LDH is observed in medium in presence of 200µM H_2O_2 although about 80% cell viability is maintained under the similar conditions as checked by MTT assay (**Table 2**). Similar release of LDH in the culture medium of Caco-2 cells [4] treated with 100µM or more H_2O_2 for 30min and lymphocytes [17] incubated with 0.1% or more H_2O_2 for 4h is reported. Results of this study indicate that the effect of H_2O_2 on lymphocytes is both time and concentration dependent. A decrease in cell viability, when exposed to oxidative stress (**Table 2**) also reflects influence of increased lipid peroxidation on membranes and related dehydrogenases.

Antioxidant Enzymes:

Activities of antioxidant enzymes viz. superoxide dismutase, catalase, GPx and GST in lymphocytes incubated with or without H_2O_2 for 4h are shown in (**Table 4**).

Table 4: Effect of different concentrations of H_2O_2 on antioxidant enzymes of lymphocytes

Concentration of H_2O_2 (µM)	SOD Units/mg protein	Catalase nmoles/min/mg protein	GST nmoles/min/mg protein	GPxnmoles/min/mg protein
control	982.9±7.3 (100)	65.2±2.1[a](100)	0.78±.03 (100)	223.5±2.2 (100)
50	1275.0±7.9(130)	98.5±2.3 (151)	0.68±.02 (87)	230.7±1.6 (103)
100	883.5±10.9 (90)	66.5±1.7[a](101)	0.54±.01 (69)	291.6±3.1 (130)
150	682.8±8.7 (70)	56.7±1.9 (87)	0.37±.02 (47)	349.6±3.0 (156)
200	512.3±8.2 (52)	48.3±2.1 (74)	0.26±.02 (33)	431.5±2.4 (193)

Values in parenthesis represent percent of control i.e. without hydrogen peroxide. Values are mean ±SD: n=3:p<0.5 compared to control: [a]p<.01as compared to control

SOD and catalase enzyme activities increased to 130% and 150% in presence of the low concentration of H_2O_2 i.e. 50µM. However, at higher concentrations the activities of these enzymes declined. SOD catalyzes the dismutation of superoxides to H_2O_2 which is in turn decomposed by catalase [3, 4, 6]. At higher concentrations of H_2O_2 these enzymes are adversely affected. SOD activity is reduced to 50% while catalase remains 75% in presence of 200µM H_2O_2 as compared to control cells. Subhashinee et al [4] have also reported an increase in SOD activity in Caco-2 cells when H_2O_2 up to 75µM is included in the culture medium, but a decrease is reported with higher concentrations. A lower level of SOD activity is reported in lymphocytes of cancer [15] and diabetic patients [19]. SOD and catalase are reported to decline under the chronic stress conditions [20]. GST activity decreases significantly in presence of H_2O_2 50µM or more. GST activity decreased to 33% as compared to control in presence of 200µM

H_2O_2. An increase in GST activity in cultured lymphocytes isolated from chicken with increase in H_2O_2 concentration is reported by Ramteke *et al* [17]. GPx activity is not significantly affected at lower concentration of H_2O_2 i.e. 50µM but increases to 130%-193% in presence of 100-200µM H_2O_2. An increase in GPx activity when Caco-2 cells were treated with H_2O_2 up to 250µM concentration for 1h is reported [4]. Increase in GPx activity with increased oxidative stress may be due to higher level of lipid peroxides, the substrate of the enzyme, as evident from the higher amount of MDA in lymphocytes incubated with H_2O_2.

DISCUSSION

Hydrogen peroxide is generated at low concentrations in aerobic cells non-enzymatically as well as enzymatically. In lymphocytes, ROS play vital role in signal transduction and immune response [7-9]. Hydroxyl radicals produced from H_2O_2 spontaneously attack various biomolecules to affect enzyme activities and membrane integrity [21]. The major reactive aldehyde resulting from the peroxidation of biological membranes is malondialdehyde (MDA) [3]. It is used as an indicator of tissue damage by a series of chain reactions [12]. Lipid oxidation products, such as 4HNE, is cytotoxic, hepatotoxic, mutagenic and genotoxic. MDA reacts with DNA and forms adducts with deoxyguanosine and deoxyadenosine [8, 9]. It can also modify RNA, proteins and other biomolecules. The change in permeability of membranes on exposure to H_2O_2 is attributed to the disruption of paracellular junctional complexes resulting from tyrosine phosphorylation of membrane proteins [22]. Since high and chronic stress is related to various pathological conditions, it is important to study the influence of long term oxidative stress on the antioxidant defense system of lymphocytes which are at the fore step exposed to stress and are crucial for immune system of an individual. Increased activities of antioxidant enzymes neutralizing superoxide and hydrogen peroxide at lower level of H_2O_2 may be an adaptive mechanism by the cellular system to balance the oxidative stress and maintain homeostasis. Decreased activities of SOD, catalase and GST at higher level of stress may be due to oxidative damage to the proteins or lack of availability of reducing power to balance the prolonged high stress. Glutathione S-transferases (GSTs) conjugate activated xenobiotics with glutathione; thus, GST reduction may impede detoxification and excretion of potentially harmful compounds.

GST and GPx enzymes use GSH as the coenzyme. High GPx activity may be resulting in lesser availability of GSH as reducing power and detoxifying agent in the cells. Reduced cell viability, high lipid peroxidation and increased leakage of LDH in the medium indicate damage to the cell membranes and cell integrity which may lead to increased inflow of toxic substances and finally cell death if the stress continues for a longer duration [4].

A synergistic interaction of the antioxidant enzymes protects them and the biological system from specific free radical attacks [3, 5, 10]. SOD protects catalase and GPx from superoxide radicals, whereas catalase and GPx protect SOD from hydroperoxides. These protective interactions may be playing an important role at the lower level of oxidative stress but at higher concentrations of H_2O_2, in the present study, even with high activity of GPx a detrimental effect is observed on SOD and catalase. It indicates that GPx alone is not sufficient to protect enzymatic proteins against oxidative damage in lymphocytes and some other factors may be responsible. The adverse influence of prolonged high oxidative stress on the antioxidant enzyme system of lymphocytes may further worsen the situation in chronic diseases. Strengthening of the antioxidant system of lymphocytes may be explored as a strategy to decrease cell damage and improve the immune system in various clinical situations or treatment where drug therapy involves high oxidative stress.

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