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RĪGAS STRADIŅA UNIVERSITĀTE

Alise Silova

CHANGES IN OXIDATIVE STRESS PARAMETERS AND ITS CORRECTION OPPORTUNITIES IN PARTICULAR PATHOLOGIES

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Biochemistry

Riga, 2015

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Doctoral Thesis has been worked at:
Rīga Stradiņš University Laboratory of Biochemistry, in collaboration with Biomedical Research Centre of Sheffield Hallam University and Randox Laboratories, Ltd, United Kingdom;
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The Doctoral Thesis is available at the library of RSU and on the home page: www.rsu.lv

ESF EIROPAS SOCIĀLAIS FONDS IEGULDĪJUMS TAVĀ NĀKOTNĒ EIROPAS SAVIENĪBA

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CONTENTS

ABBREVATION

ATP – adenosine 5'-triphosphate
AO – antioxidant
AOS – antioxidative system
NPP – Nuclear Power Plant
ROS – reactive oxygen species
ATP – adenosine triphospate
CEA – carcinoembryonic antigen
ChL – chemiluminiscence
COX – cyclooxygenase
Cu, Zn–SOD – superoxide dismutase
CNNP – Chernobyl Nuclear Power Plant
d – day
EGb – standardized Ginkgo biloba (*Ginkgo biloba* L.) leaves dry extract
ECs – standardized Green tea (*Camellia sinensis* L.) leaves extract
GB – *Ginkgo biloba*
GPx – Glutathione peroxidase
GT – green tea
GSH – reduced glutathione
GST − glutathione-S-transferase
GV – *Grinvitals Cereloba®Plus*
4–HNE – 4-Hydroxynonenal
IBF – Ibuprofen
IR – ionizing radiation
CF – clastogenic factors
LOX – lipoxygenase
LOOH – lipid hydroperoxides
LPO – lipid peroxidation
MDA – malondialdehyde
MT – mitochondria
N – count
NAD^+ – nicotinamide adenine dinucleotide
NADP+ – Nicotinamide adenine dinucleotide phosphate
NFkB – nuclear factor kappa–light–chain–enhancer of activated B cells
NSAID – non–steroidal anti–inflammatory drug
OS – oxidative stress
PC – protein carbonyl
PSA – prostate-specific antigen
Se – selenium
TAS – Total antioxidant status
TBARS_MDA – thiobarbituric Acid Reactive Substances

T2D − type 2 *diabetes mellitus*
VE – vitamin E

1. Topicality of the Problem

Nowadays, radioactive pollution is one of the global environmental problems, and the world was stricken by major technological Nuclear Power Plant (NPP) accidents (Chernobyl and Fukushima), as well as nuclear disasters can take place in other NPP; nuclear waste and threats created by nuclear weapons are just some of the most serious risks for increased radiation and subsequently adverse impact on living organisms, including human beings. On December 20, 2006, for the first time in human history, the United Nations recognized non-communicable diseases − diabetes as being one of the global health problems. UN pointed out that diabetes is a chronic disease with high care and treatment costs. It can lead to serious complications, so it creates major risks for families, countries and the entire world community.

The discovery of the role of free radicals in the pathogenesis of cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders, aging, and others has led to a medical revolution promising a new paradigm in healthcare. Epidemiological studies, such as “French Paradox” and “Spanish Mediterranean Diet”, as well as increased soil fertilization with selenium (Se) -containing mix of minerals in Finland, which allowed to reduce the onset of cardiovascular diseases and cancer in the Finnish population, have indicated a significant difference in the incidence of various diseases among ethnic groups with different lifestyles and exposure to different environmental factors. Free radical-induced oxidation reaction takes place in the processes which involve the oxygen / nitrogen and transportation of electrons. After incomplete oxygen / nitrogen reduction, there are formed free radical or non-radical molecules which are generally defined as reactive oxygen / nitrogen species (ROS / RNS). Reactive oxygen species participate in normal physiological processes; their enhanced formation is the result of many pathological effects and the external environmental changes. In the course of evolution, in order to neutralize ROS, cells have

developed antioxidant defence system (AOS), which takes part in maintenance of the free radicals and the induced oxidation reactions at an appropriate level. Oxidative stress (OS) develops at the molecular, cellular and organism level when there is an imbalance between ROS production and neutralization. Cells respond to OS with the adaptative response by activating reparations mechanisms, or in case of serious damage-inducing cell death. Thus, increased ROS formation and insufficient efficiency of AOS result in increased OS, which further contributes to the development of pathological processes.

Since Chernobyl Nuclear Power Plant (CNNP) clean-up workers returned home to Latvian to relatively clean ecological environment, changes in their body may be attributed to the remote effects of ionizing radiation (IR) and the long-term chronic OS is used. Ionizing radiation causes not only direct cell structure damage, but also has a long-term effects on the body since the radionuclides continue damaging cells and increasing the chances of developing cancer, including prostate cancer, diabetes, and other diseases associated with OS. Lung cancer occupies the first place among tumours with prostate and stomach tumours being the second most common form of tumors in CNNP clean-up workers from Latvia, while diabetes is one of the most widespread chronic non-oncological conditions among living CNNP clean-up workers. Despite a considerable number of studies, chronic effects of low-dose IR remain largely unclear. It has been proved that even 30 years after the atomic bomb explosion in Hiroshima and 10 years after the CNNP accident, clastogenic activity can be detected in human blood plasma, which is one of the risk factors of remote effects of IR. Clastogenic factors (CF) –substances – prooxidants that cause chromosomal damage have been known since the 70-ties of the last century when patients' plasma was studied after both therapeutic and accidental irradiation.

There has been found correlation between CF and OS. Formation of CF and subsequent chromosomal aberration are mediated by increased formation of superoxide anion radicals of different origins, which is neutralized by enzyme −

superoxide dismutase (SOD). On the other hand, CF themselves also trigger formation of the given radical from monocytes and neutrophils. Biochemical analysis of CF preparations identified three major classes of endogenous chemical clastogens: 1) lipid peroxidation products derived from arachidonic acid of membranes, and, in particular, the highly clastogenic aldehyde 4-hydroxynonenal; 2) cytokines, such as tumour necrosis factor alpha and 3) unusual nucleotides, such as inosine di- and triphosphate [*Emerit*, 2007].

The majority of studies have shown that development of type 2 *diabetes mellitus* (T2D) vascular complications is significantly influenced by chronic hyperglycaemia although its mechanism in this process is still not fully explored. One of the hypotheses assumes that hyperglycaemia which results in diabetic complications is affected by an increased OS, which is also enhanced by overproduction of superoxide anion radicals formed in mitochondria (MT) . Antioxidants may protect against auto oxidation of glucose, glycation and lipid oxidation since they play major role in protection against ROS to maintain the structural and functional integrity of endothelial.

In order to prevent or reduce both the radiation-induced damage and chronic complications of diabetes, it is essential to develop a pharmacological strategy to increase the therapeutic correction of ROS. In order to carry out an effective pharmacological interventions using AO, it is important to investigate the molecular mechanisms of damage caused by ROS. Despite the variety of methods available to measure the ROS-related parameters, currently there do not exist standardized OS assessment methods. The identification of the criterion for basal or physiological ROS levels in humans, may help to identify pathological ROS levels and timely initiate the required therapy. Thus there is still open the questions of where and how high the “red line” is. Antioxidant therapy is currently being considered as a prospective approach in treatment of the diseases which have OS as one of the pathogenic factors. Currently, there are no established guidelines for

drugs with AO potency, the time of their administration, dosage, beside thtat there have not been carried out pharmacokinetic studies in critically ill patients.

Controversial data obtained in clinical trials can be explained by the fact that there were used a variety of preparations, in different doses different time and route of administration, in different populations and the number of samples. Experimental models (on cells) showed that AO protect against oncogenic transformation caused by radiation, which did not show the same effect in human studies.

So far, AO have not been considered as an equivalent remedy in prevention and / or therapy of T2D and its complications. Despite the fact that the polyphenols as natural antioxidants are well represented, their significance in prophylaxis of diseases and improvement of health has been discovered relatively recently. Broader studies on the properties of flavonoids and other polyphenol AO and their role in prevention of diseases began only after1995 [*Scalbert et al.*, 2005]. Polyphenols according their structure show important antioxidant and advanced glycation end-product inhibitor properties *in vivo* and *in vitro* and as naturally occurring compounds with low toxicity could be promising for the treatment of diabetic complications, although their therapeutic potential in human remains to be investigated [*Peyroux et al.*, 2006].

Since prooxidant and AO balance is adjusted in several metabolic processes, it is virtually impossible to find a panacea that could radically normalize the situation, therefore it is recommended to use a complex of several AO both water and lipid soluble with different mechanisms of action.

As there are controversial data on efficiency of AO and OS induced changes in T2D patients and subjects who have received low-doses of IR, the doctoral thesis examines OS parameters (lipid and protein oxidative damage markers) changes and adjustment possibilities using AO in case of the above mentioned pathologies. In order to correct OS parameters in CNNP clean-up workers, hydrophilic and lipophilic AO (VE, Se, CoQ_{10}) were used together with one of

non-selective anti-inflammatory drugs (NSAID) – Ibuprofen (IBF), while T2D patients took preparations containing naturally occurring polyphenols (*Ginkgo biloba* L., *Camellia sinensis* L.).

Aim of the Thesis

1. To study the oxidative stress adjustment possibilities using:
   • non-steroidal anti-inflammatory drug – Ibuprofen in combination with hydrophilic and lipophilic antioxidants in patients who have received low-dose ionizing radiation in CNNP clean-up works;
   • polyphenol containing preparations in T2D patients.
2. To determine the differences in oxidative stress parameter changes in the CNNP clean-up workers with / without T2D and T2D patients who were not exposed to low dose ionizing radiation.

Objectives of the Thesis

1. To evaluate the effect of antioxidant vitamin E, selenium, CoQ_{10} and NSAID – Ibuprofen combination use on oxidative stress parameter changes in CNNP clean-up workers.
2. To investigate the changes in oxidative stress parameters in T2D patients and to evaluate the effect on oxidative stress parameter changes using different dosing of naturally occurring polyphenol-containing preparations – standardized *Ginkgo biloba* and green tea leaf extracts, and combination product *Grinvitals Cereloba®plus* in addition to the standard treatment.
3. To study the difference in oxidative stress parameter changes in CNNP clean-up workers with / without T2D and T2D patients who were not exposed to low dose ionizing radiation.

Hypothesis of the Thesis

1. Long-term use of vitamin E, selenium, and nonsteroidal anti-inflammatory drug Ibuprofen combination may reduce oxidative stress in Chernobyl clean-up workers.
2. Supplementing the use of Ibuprofen and selenium combination with CoQ_{10} may be significantly more effective in long-term oxidative stress regulation in CNNP clean-up workers.
3. T2D patients' long-term use of different doses of naturally occurring antioxidants (standardized green tea leaf extract, standardized *Ginkgo biloba* leaf extract, *Grinvitals Cereloba®plus* preparation) in combination with standard therapy may reduce oxidative stress more effectively than mono-standard therapy alone.
4. Oxidative stress parameter changes in CNNP clean-up workers with T2D differ from the ones experienced by T2D patients and CNNP clean-up workers without T2D.

Scientific Novelty of the Thesis

1. This is the first work which studies the effect of particular antioxidants and Ibuprofen combination on the regulation long-term oxidative stress induced by low-dose ionizing radiation.
2. For the first time, there has been identified the effect of polyphenol containing dietary supplements (*Ginkgo biloba*, green tea and preparation *GrinvitalsCereloba®plus*) on oxidative stress regulation in chronic T2D patients.
3. There have been found diferences in the intensity of oxidative stress between CNNP clean-up workers with and without T2D and it has been compared with T2D patients with no history of exposure to low-dose ionizing radiation.

Volume and Structure of the Thesis

The thesis is written in the Latvian language. It consists of nine parts: a literature review, work materials and methods, results, discussion, conclusions, practical recommendations, references, publications on the research topics, and attachments. The doctoral thesis is 153 pages long. The work contains 75 figures, 32 tables and 6 annexes. The list of references contains 221 scientific literature sources. The author has had 18 publications on the topic of the thesis.

2. MATERIALS AND METHODS

The doctoral thesis is based on:

- Study I − "Effects of Ibuprofen in combination with vitamin E and selenium on oxidative stress parameter changes in CNNP clean-up workers";
- Study II – "Effects of Ibuprofen in combination with selenium and coenzyme Q_{10} on oxidative stress parameter changes in CNNP clean-up workers";
- Study III − "Efficacy of long-term use of different dosing of particular polyphenol containing preparations on oxidative stress parameter changes in T2D patients";
- Study IV − "Oxidative stress differences in nuclear clean-up workers with and without T2D".

Studies was approved by RSU Ethics Committee (decisions 05.01.2005. and 11.03.2005). Each participant of the study was introduced to the study and gave a written consent. Study III was approved by Kaunas Regional Biomedical Research Ethics Committees (opinion 2009-04-15, Nr.BE-2-5) by the Lithuanian Bioethics Committee.

2.1. Design of the First Study

The study was conducted in the time period from 2005 till 2006, and it included people who took part in CNNP clean-up works in the time period from 1986 till 1989 and received no more than 200 mSv (135.72 ± 68.81, mean ± SD) of IR, the number of participants − 82, gender − male, age 38−65 years (49.9 ± 5.6, mean ± SD) .

The participants of the study were divided into four groups and received the following preparations: “SelenoPrecise” (Pharma Nord, Denmark) containing selenomethionine (Se-Met) -patented Se yeast (EP Patent No 1 478 732 B1); “Bio-E-Vitamin” (d-α-tocopherol) (Pharma Nord, Denmark); Ibuprofen (joint-stock Olainfarm, Latvia); *Placebo* − Se, vitamin E (VE), IBF (joint-stock Olainfarm, Latvia).

Table 2.1.

Use of Supplements and Distribution of Participants by Groups

Group	Selenium	Vitamin E	Ibuprofen
Se + VE (N = 21)	200 µg, one tablet in the morning	350 mg, one capsule in the morning	*Placebo*, one tablet in the morning
IBF (N =20)	*Placebo*, one tablet in the morning	*Placebo*, one capsule in the morning	200 mg, one tablet in the morning
Se+ VE + IBF (N = 21)	200 µg, one tablet in the morning	350mg, one capsule in the morning	200 mg, one tablet in the morning and one in the evening
Placebo (N=20)	*Placebo*, one tablet in the morning	*Placebo*, one capsule in the morning	*Placebo*, one tablet in the morning and one in the evening

Sample collection: venous blood samples were collected in the Centre of Occupational and Radiological Medicine of P. Stradins Clinical University Hospital (CUH) was at the beginning of the study, and after three, six and 12 months; the following parameters were identified − Se, VE, total antioxidant status (TAS), Thiobarbituric Acid Reactive Substances (TBARS_MDA), parameters of plasma chemiluminiscence (ChL), carcinoembryonic antigen (CEA), total prostate-specific antigen (PSA), free PSA. The study was conducted in collaboration with Sheffield Hallam University Biomedical Research Centre (UK) under the supervision of Professor Emeritus of Biomedical Science K. D. Rainsford.

During the study, the participants were observed by doctors, and there were not found serious deviations in the dynamics.

Exclusion factors: alcoholism, HIV / AIDS, tuberculosis, acute / chronic infectious diseases, severe cardiovascular and cerebrovascular diseases and mental illnesses.

Financial support:

• Pilot-investigation financed by Boots Healthcare Ltd. (UK) "The Effects of Ibuprofen Alone and with Anti-oxidant Mixture in Chernobyl Clean-up Workers Patients at Risk of Developing Prostate Symptoms", "Current Health Status of Chernobyl Liquidators, Opportunities for its Improvement Using Selenium Methionine, Vitamin E and Non-Steroidal Agent Ibuprofen" (2005−2006);

•The long-term study financed by MES project "Provision and Development of Scientific Infrastructure at Higher Education Establishments" "Antioxidant and Ibuprofen Effects on the Risk of Developing Prostatitis in Chernobyl Clean-up Workers" (2006−2007).

2.2. Design of the Second Study

The study was carried out from 2008–2009, and it included people who participated in CNNP clean-up works from 1986−1989 and received no more than 200mSv(135.72 ± 68.81, mean ± SD) of IR; the number of participants −75; gender − male; age 45–57 years (51.0 ± 6.0 avg. ± SD). The participants of the study were divided into three groups and received the following preparations: "SelenoPrecise" (Pharma Nord, Denmark) containing selenomethionine (Se-Met) – patented Se yeast (EP Patent No. 1478732B1); Coenzyme Q_{10} (CoQ_{10}) (Ubiquinol) (*Pharma Nord*, Denmark); IBF − and omeprazole (joint-stock company *Olainfarm*, Latvia).

Table 2.2.

Preparations and Distribution of Participants by Groups

Group	Selenium	CoQ_{10}	Ibuprofen	Omeprazole
Se+CoQ_{10}+IBF +Om (N = 25)	200 µg, one tablet in the morning	100 mg, one capsule daily	200 mg, one tablet in the morning and one in the evening	20 mg, one tablet in the morning
Se+CoQ_{10} (N =25)	200 µg, one tablet in the morning	100 mg, one capsule daily	NO	NO
CoQ_{10} + IBF + Om (N = 25)	NO	100 mg, one capsule daily	200 mg, one tablet in the morning and one in the evening	20 mg, one tablet in the morning

Sample collection: at the initial stage of the study and after three and six months, venous blood samples were collected at Centre of Occupational and Radiological Medicine of P.Stradins CUH. At the beginning of the study (month 0) and at the end of the study (month 6), the following parameters were identified − the activities of Cu, Zn-Superoxide dismutase (Cu, Zn-SOD) and glutathioneperoxidase (GPx), VE, malondialdehyde (MDA) and 4-hidroxynonenal (4-HNE). At the beginning of the study, after three and six months following indicators were identified − Se, reduced glutathione (GSH), triglyceride (TRG), cholesterol (CHOL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin (ALB). The study was conducted in collaboration with Sheffield Hallam University Biomedical Research Centre (UK) under the supervision of Professor Emeritus of Biomedical Science K. D. Rainsford. During the study, the participants were observed by doctors, and there were not found serious deviations in the dynamics.

Exclusion factors: analogous to the first study.

Financial support:

MES programme “Study of Exogenous and Endogenous Factors Endangering the Health of Population in Latvia”, grant “Effects of Antioxidants and Ibuprofen on the Risk of Prostatitis Development in Chernobyl Clean-up Workers” (2007−2009).

2.3. Design of The Third Study

The study was conducted from 2009−2011. All the subjects were outpatients of the Endocrinology Clinic, Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Lithuania. The subjects diagnosed with T2D (treated with Insulinum, Metforminum or combination of both), both sexes and followed up for diabetic retinopathy, nephropathy or neuropathy were enrolled into the study.

The exclusion criteria include: HbA1c > 13%, BMI > 45 kg / m^2, history of uncontrolled hypertension, other significant medical problems (major cardiovascular, hepatic, and other endocrine diseases), hypersensitivity to the test drug, and not being able to comply with the study protocol. The subjects were not deprived of taking their regular prescribed medications, but were advised to abstain from other dietary supplements rich in antioxidants.

All the patients were randomly allocated to receive standardized *Ginkgo biloba* L. dry extract − EGb, Green tea (*Camellia sinensis* L.) extract − ECs, combination of both − extract or placebo capsules. Placebo capsules were made from microcrystalline cellulose, a material indifferent to disease (Joint-stock company “Sanitas”, Lithuania). EGb capsule contains 80 mg of standardized dry extract of *Ginkgo biloba* leaves, adjusted to 19.2 mg *Ginkgo* flavone glycosides and 4.8 mg terpene lactones (ginkgolides, bilobalide) (Joint-stock company “Aconitum”, Lithuania). ECs capsule contains 200 mg standardized extract of

Camellia sinensis L. leaves, adjusted to 75% polyphenols (Joint-stock company "Sanitas", Lithuania). *Grinvitals Cereloba®plus* tablet containing 37.5 mg EGb, 37.5 mg ECs and 100 mg garlic (*Allium sativum* L.) extract (Joint-stock company "Grindeks", Latvia).

Table 2.3.

Use of Supplements and Distribution of Participants by Groups

Group	**Supplement**	**Avarage age, years**	**The average duration of illness, years**	**Medication in months**	
				0–9	**10–18**
GB (N = 26)	*Ginkgo Biloba* L. 80 mg	61.2 ± 10.6	10.5 ± 7.5	1 capsule / tablet twice daily	1 capsule / tablet three times daily
GT (N = 20)	*Camellia sinensis* L. 200 mg	63.7 ± 8.3	9.5 ± 5.2		
GV (N = 17)	*Grinvitals Cereloba®plus*	62.3 ± 6.5	10.5 ± 4.2		
Placebo (N = 26)	*Placebo*	61.8 ± 11.7	11.5 ± 8.0		

Sample collection: Venous blood samples were collected in Lithuania prior to the study, after nine, and 18 months, and RSU Biochemistry laboratory determined the following parameters TAS, Se (only at the initial stage of the study), the activities of Cu, Zn-SOD and GPx, VE, MDA, 4-HNE, a lipid hydroperoxide (LOOH).

Financial support:

This study is a part of international EUREKA project No.E! 3695 "Creation of the Methodology for Effects of Natural Antioxidants on the Development of the Diabetes Mellitus Complications".

2.4. Design of the Fourth Study

The study was carried out from 2010−2012 and it included males with / without T2D complications who had received low doses of IR as CNNP clean-up workers and they were compared with age-matched patients with T2D and practically healthy males.

Table 2.4.

Characteristics and Distribution of the Study Groups

Identification of groups	Description	Age, years	The average duration of illness, years
T2D (N = 36)	T2D patients	62.2 ± 9.3	10.5 ± 6.2
T2D + IR (N = 28)	CNPP clean-up workers with T2D	61.6 ± 7.9	10.7 ± 3.9
IR (N = 40)	CNPP clean-up workers without T2D	60.1 ± 6.6	No
C (N = 30)	practically healthy men	61.0 ± 7.9	No

Exclusion factors: for CNNP clean-up workers analogous to Study I; for T2D patients analogous to Study III. Sample collection: T2D patients' were selected from the Third Study, the blood samples of T2D +IR and IR groups were collected at P. Stradiņš CUH Occupational and Radiation Medicine Centre, C– different. The following markers were identified – the activities of Cu, Zn-SOD and GPx, TAS, Se, MDA, 4-HNE, protein carbonyls (PC).

Financial support: RSU Doctoral Study grants.

Biochemical tests were performed in a certified RSU Laboratory of Biochemistry (Register of Health Care Institutions, Enterprises and Practices No.0100-19100; Ministry of Health of Republic of Latvia certificate No. L-198-A).

The following tests were carried out at Randox Laboratories, Ltd. (UK): CEA, total PSA, and free PSA.

2.5. Statistical Data Processing

The biochemical tests yielded numerical (consisting of a numerical scale) values, which were not normally distributed and therefore non-parametric statistical methods were used.

The central tendency of the numerical scale was described using median values, while the results of the dispersion were represented as interquartile range (English abbreviation of this parameter IQR – interquartile range is also used in texts in the Latvian language). These descriptive statistical characteristics are also presented graphically (graphical mode -Boxplot), showing the minimum and maximum values of the given parameter − 25 and 75 percentiles, respectively (they represent IQR borders), as well as the 50^{th} percentile − the median value. In order to determine the differences in the numerical values of the study groups, independent samples were studied using non-parametric statistics − Mann-Whitney and Kruskal-Wallis test for. The above mentioned and other comparative tests were used to calculate the statistical significance (p-value). If the tests showed a p-value of less than or equal to 0.05, the differences were considered as being statistically significant. If the data of the numerical scale were recoded in the nominal scale then Hi-square and Fisher's exact test were used to compare the groups.

In assessing the differences between the results of repeated tests of the same patients, Wilcoxon and Friedman non-parametric statistical tests were used. Spearman correlation analysis was applied in order to determine the possible relationship between the characteristics of the samples. Statistical calculations and image creation was carried out using IBM SPSS 20.0 and MS Excel.

3. MAIN FINDINGS AND DICUSSION

3.1. Study I

As seen in Figure 3.1., in VE + Se group, the **amount of TAS** statistically significantly (p = 0.003) decreased in the third month compared with the initial period 1.57 (0.24) −1.44 (0.14) mM.

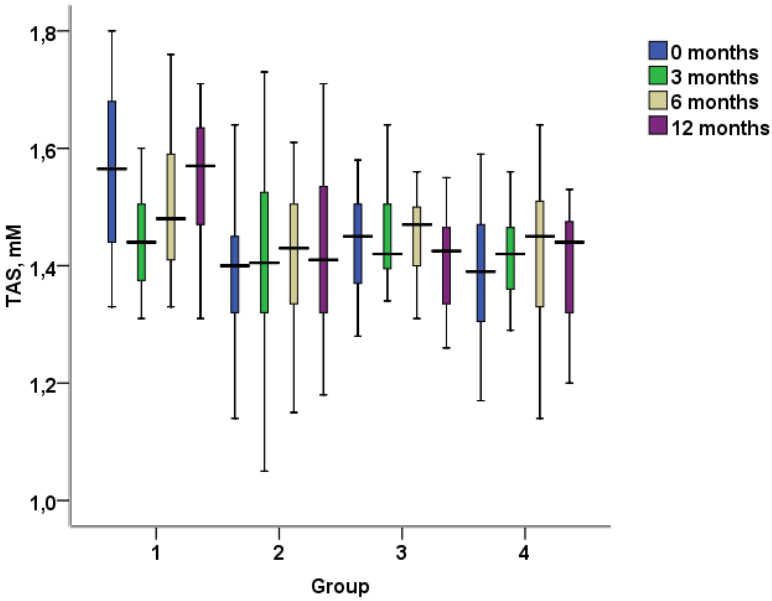

Figure 3.1. **Effect of Selenium, Vitamine E, Ibuprofen on the Amount of TAS**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – *Placebo* group.

It was followed by a statistically significant increase in the amount – to 1.48 (0.19) mM (p = 0.03) in the sixth month and in the 12^{th} month up to 1.57 (0.18) mM (p = 0.013) compared to the third month. In the *Placebo* group, there was a statistically significant increase in TAS amount in the third and sixth months, 1.39

(0.17) −1.42 (0.11) − 1.45 (0.19) mM (p = 0.024; p = 0.077), respectively, in comparison to the initial stage.

As shown in Figure 3.2., the amount of VE has statistically significantly increased in VE +Se group − 10.0 (7.2) −23.2 (8.9) − 23.2 (8.9) 24.1(9.3) μg/ml (p = 0.002; p < 0.001; p < 0.001) in the third, sixth and the 12th months, respectively, compared with the initial stage.

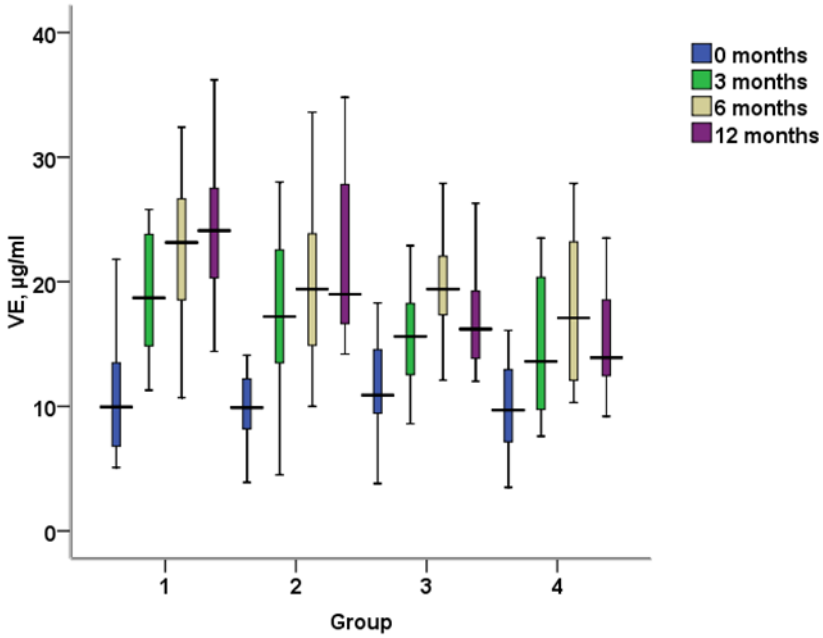

Figure 3.2: **Effect of Selenium, Vitamin E , Ibuprofen Use on the Amount of Vitamin E**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – Placebo group.

Comparison with the initial stage of the study shows that there was a statistically significant increase in VE − 9.90 (4.7) − 17.2 (9.5) − 19.4 (9.8) −19.0 (11.8) μg/ml (p <0.001) in VE +Se+IBF group over the whole period of the study.

In IBF group, VE statistically significantly increased in the third and the sixth months −10.9 (5.4) − 15.6 (6.7) − 19.4 (5.2) μg/ml ($p < 0.001$), respectively, compared to the initial stage of the study. At the end of the study, the amount of VE statistically significantly ($p = 0.011$) dropped to16.2 (6.3) μg/ml in comparison with the sixth month and remained statistically significantly elevated ($p = 0.001$) compared with month 0.

In *Placebo* group, VE statistically significantly increased in the third, and the sixth months − 9.70 (6.0) − 13.6 (10.9) − 17.1 (11.4) μg/ml ($p = 0.001$), respectively compared with the initial phase. At the end of the study, the amount of VE statistically significantly decreased ($p = 0.011$) to 13.9 (7.6) μg/ml, compared with the sixth month and remained statistically significantly elevated ($p = 0.002$) compared with month 0.

As shown in Figure 3.3., in VE +Se group, the **amount** of **Se** statistically significantly increased in the 12^{th} month – 80 (36) −123 (49) μg/L, ($p = 0.013$), if compared with the initial stage of the study.

Compared to the *Placebo* group, the amount of Se was statistically significantly higher in the third month ($p =0.01$) and 12^{th} month ($p = 0.002$).

In VE + Se + IBF group, the amount of Se, compared with month 0, statistically significantly increased in the sixth and 12^{th} months, respectively, 80 (19) – 99 (17) – 114 (40) μg/L ($p = 0.003$; $p = 0.0001$). In IBF group, the amount of Se was close to statistically significant decreased in the 12^{th} month – 76 (15) μg/L ($p = 0.093$) in comparison to month 0 − 91 (35) μg/L. The comparison with *Placebo* group did not yield statistically significant differences.

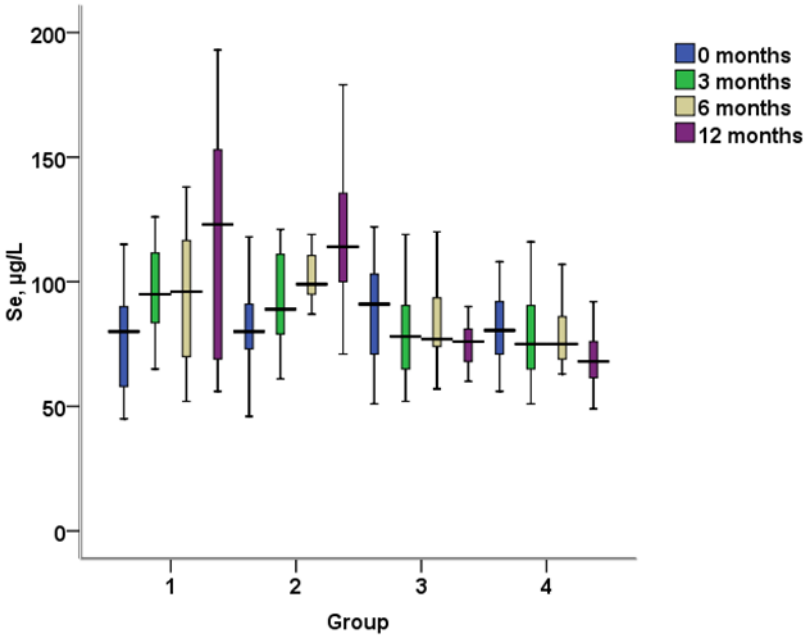

Figure 3.3. **Effect of Selenium, Vitamin E and Ibuprofen on the Amount of Se**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – *Placebo* group.

In the *Placebo* group, the amount of Se statistically significantly decreased in the 12th month 80.5 (24) – 68 (17) µg/L, compared with month 0 ($p = 0.007$).

As seen in Figure 3.4., in VE + Se group, the **total sum of lipid peroxides – $H_{max.}$** (conventional units – conv. unit) statistically significantly decreased in the 12th month 33.8 (31.4) in comparison with month 0 – 97.0 (63.1) ($p = 0.008$), as well as in comparison with the third month 99.7 (157.7) ($p < 0.001$), and in comparison with the sixth month 71.0 (98.0) ($p = 0.011$).

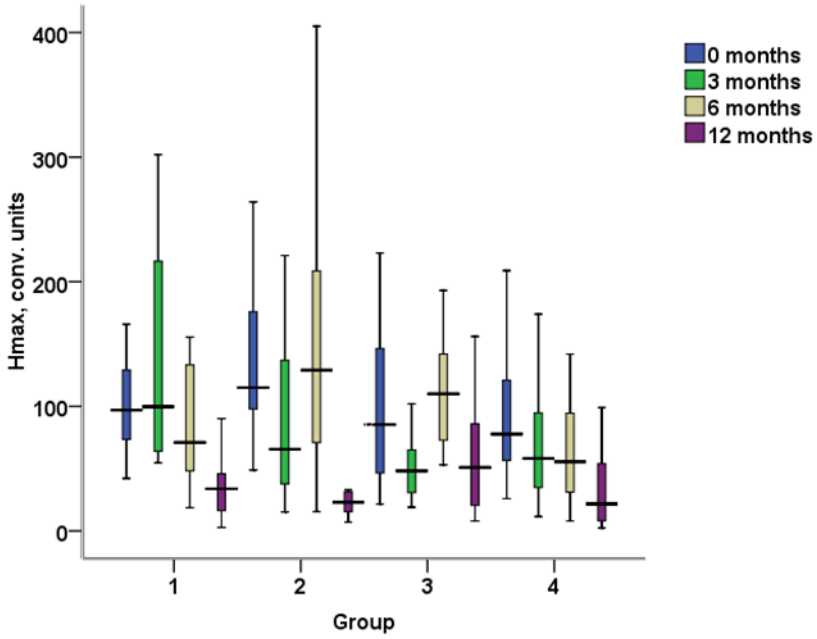

Figure 3.4. **Effect of Selenium, Vitamin E, Ibuprofen on the Chemiluminiscence parameter $H_{max.}$ or Total Sum of Lipid Peroxides**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – *Placebo* group.

$H_{max.}$ was statistically significantly higher in the third month in comparison with the *Placebo* ($p = 0.007$) and VE + Se + IBF groups ($p = 0.022$). At the end of the study, in VE + Se + IBF group, $H_{max.}$ 23.0 (17.7) conv. units, statistically significantly decreased ($p < 0.001$), in comparison with month 0, the third and the sixth months. If compared with *Placebo* group, $H_{max.}$ was statistically significantly increased in the sixth month ($p = 0.008$), in comparison with VE + Se group, $H_{max.}$ was statistically significantly lower in the third month ($p = 0.022$). At the end of the study, comparison of the groups VE + Se + IBF and IBF showed that $H_{max.}$ is lower in the group with antioxidant supplementation and the given difference was close to statistically significant ($p = 0.055$).

In the IBF group, $H_{max.}$ statistically significantly decreased in the third month − 48.3 (38.3) conv.units (p = 0.013) in comparison with month 0 − 85.5 (113.1). $H_{max..}$ statistically significantly increased (p = 0.015) in the sixth month 110.4 (91.6) in comparison with the third month, and in the 12th month – 51.0 (74.6) conv. units, statistically significantly decreased (p = 0.006), compared with the sixth month.

If compared with month 0, in *Placebo* group, − 77.8 (64.4) $H_{max.,}$ statistically significantly decrease (p = 0.020) in the sixth month to 55.7 (66.7), while in the 12th month up to 21.7 (46.4) conv. units (p = 0.001). At the end of the study, i.e. in the 12th month, the decrease of $H_{max.}$ was also statistically significantly lower compared with the third month − 58.2 (68.6) (p = 0.010) conv. units and the sixth month (p = 0.043).

As seen in Figure 3.5., in the 12th month, in VE + Se group, **blood plasma oxidizability – $S_{ox.}$** statistically significantly decreased 478 (307) – 140 (115) conv. units (p = 0.001), in comparison to the initial stage and the sixth month 367 (507) – 259 (235) conv. units (p = 0.026), if compared with the third month.

In VE + Se + IBF group $S_{ox.}$ statistically significantly decreased over the whole period of study 519 (260) – 307 (452) – 434 (344) – 104 (94) conv.units (p < 0.001), in comparison with the initial period.

In IBF group, $S_{ox.,}$ statistically significantly decreased in the third month 388 (375) – 209 (155) conv.units (p = 0.022) in comparison with month 0. In the sixth month, $S_{ox.}$ statistically significantly increased (p = 0.019) to 474 (242) conv. units, in comparison with the third month. In the 12th month, $S_{ox.}$ statistically significantly (p = 0.015) decreased to 223 (289) conv. units, in comparison with the sixth month, while there were no statistically significant differences in comparison with the initial stage.

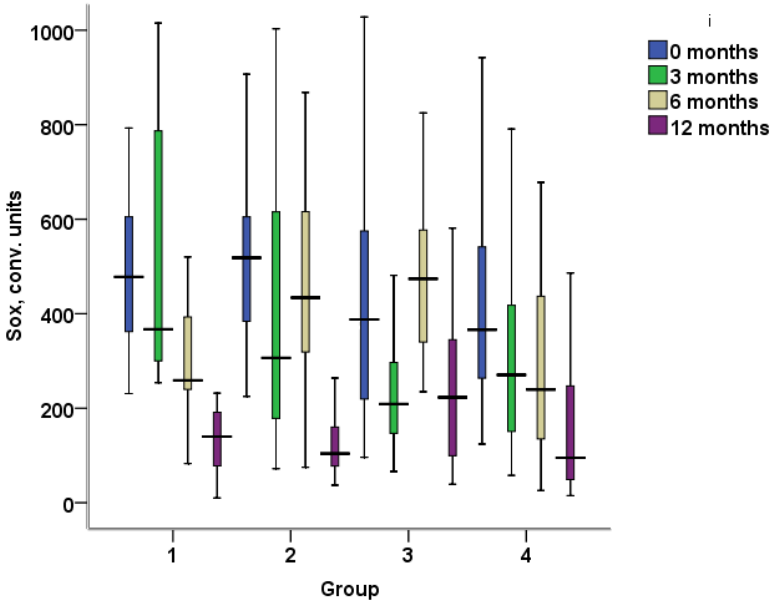

Figure 3.5. **Effect of Selenium, Vitamine E and Ibuprofen on Chemiluminiscence Parameter $S_{ox.}$ or Blood Plasma Oxidizability**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – *Placebo* group.

In *Placebo* group, $S_{ox.}$ statistically significantly (p = 0.020) decreased in the sixth and 12th month, 366 (284) –240 (304) – 95 (211) conv. units (p = 0.020; p = 0.001), respectively, in comparison with month 0.

As seen in Figure 3.6., in the VE + Se group, **lipid peroxidation processes ratio– tgα** statistically significantly decreased 6.97 (4.34) –3.02 (0. 60) conv. units, respectively, at the end of the study in comparison with the initial period (p = 0.001).

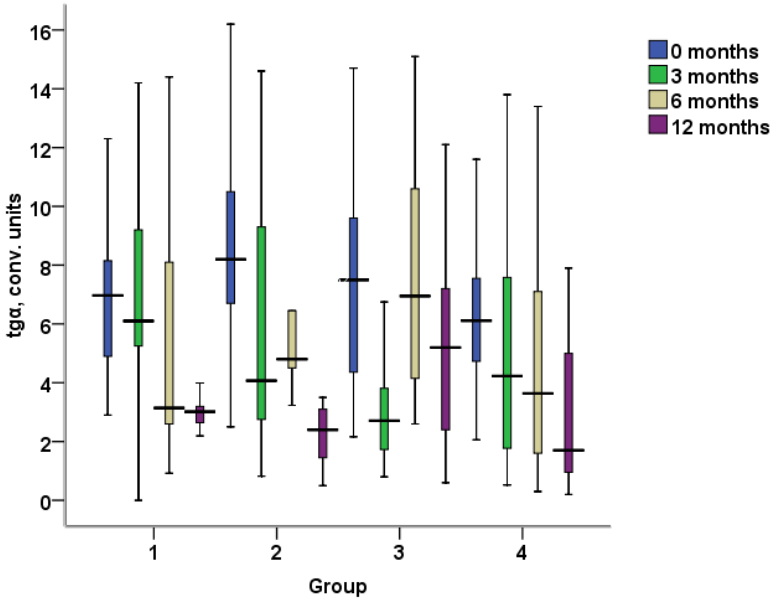

Figure 3.6. **Effect of Selenium, Vitamin E and Ibuprofen on Chemiluminiscence Parameter − tgα or Lipid Peroxidation processes ratio**
1 –VE + Se group.; 2 –VE + Se + IBF group.; 3 –IBF gr.; 4 – *Placebo* group.

In VE + Se + IBF group, tgα statistically significantly decreased at the end of the study – 8.20 (4.35) – 2.40 (2.10) conv. units respectively, in comparison with the initial period (p = 0.001).

In IBF group, tgα statistically significantly decreased in the third month, 7.50 (6.80) – 2.71 (2.52) conv.units respectively (p = 0.006) in comparison with the initial period.

In *Placebo* group tgα statistically significantly decreased in the sixth and 12th month, 6.11 (3.00) – 3.64 (5.70)–1.70 (4.30) conv. units, respectively, in comparison with the initial period.

Table 3.1.

Changes in the Amount of TBARS_MDA (µM)

Group	N	Month 0	3rd moth	6th month	12th month
Placebo	20	2.99 (0.68)	2.67 (0.64)	2.80 (0.61)	2.61 (0.43)* p = 0.014
IBF	20	3.20 (0.92)	3.04 (0.75) p = 0,074 *	2.82 (0.74)	3.12 (0.55)
VE+Se	16	3.23 (0.89)	2.83 (0.72)	2.93 (0.67)	2.99 (0.55)* p = 0.030
VE+Se+IBF	21	3.04 (0.64)	2.75 (0.41)	2.93 (0.80)	2.61 (0.79)+ p = 0.034

The results are presented as medians (interquartile range); *Statistically significantly differ from month 0; + significantly differ from the sixth month; N – number of participants.

Table 3.2.

Statistically Significant Changes in the Number of Onkomarkers After 12-Month Use of Selenium, Vitamin E, and Ibuprofen

Group	Parameter	Month 0	3rd month	6th month	12th month
Placebo N = 20	CEA ng/ml	1.94 (0.75)	1.91 (1.36)	2.26 (1.0) p = 0.07	2.58 (0.83)* p < 0.001
	PSA ng/ml	0.66 (0.69)	0.76 (0.90)	0.60 (0.68)	0.79 (0.79)* p = 0.001
	free PSA ng/ml	0.06 (0.08)	0.08 (0.07)	0.08 (0.07)	0.09 (0.10)* p = 0,008
IBF N = 20	CEA ng/ml	2.26 (1.26)	1.97 (1.12)	2.19 (2.32)	2.56 (1.43)* p = 0.002
	free PSA / PSA %	14.6 (13.0)	13.0 (8.1)* p = 0.020	11.7 (7.1) p = 0.025*	11.3 (9.6)* p = 0.020

The results are presented as medians (interquartile range).*Statistically significantly differ from month 0; N – number of participants.

In *Placebo* group, the amount of TAS (in the third month) and VE in the third and the sixth month) increased, which was followed by the decrease in all LPO parameters (the 12th month). The decrease in the amount of VE in the 12th month can be explained by the use of its endogenous reserves to inhibit LPO process, as well as its decreased regeneration. The depletion of Se and VE reserves could serve as the basis for further development of the OS, which was evident from

an average close to negative correlation between Se and Lipid Peroxidation processes ratio – tgα in the sixth month ($r_s = -0.487$, $p = 0.040$).

Evaluation of the oncomarkers in *Placebo* group revealed that despite the fact that the LPO markers decreased, the amount of CEA, PSA and the amount of free PSA statistically significant increased at the end of the study (see Table 3.2). Correlative analysis showed that, in month 0, there was a correlation between the parameters of oncomarker PSA and LPO − $H_{max.}$ and $S_{ox.}$ ($r_s = 0.588$, $p = 0.035$; $r_s = 0.483$, $p = 0.031$), and in the third month, there was a correlation with plasma total oxidizability − $S_{ox.}$ ($r_s = 0.487$, $p = 0.048$) thus proving the necessity to use AO in order to increase the antioxidative defence of the cells.

Evaluating the protective efficacy of VE and Se against OS, it was found that starting from the third month, LPO parameters − $S_{ox.}$ were first to decrease , $H_{max.}$ started to fall from the sixth month, followed by the decline in tgα and the end product of LPO − TBARS_MDA in the 12th month. At the end of the study, opposite *Placebo* group, with the reduction in LPO, AO defence (TAS, VE and Se) did not decrease, but on the contrary, increased. During the first three months of the study, there was observed correlation between TAS and TBARS_MDA ($r_s = 0.629$, $p = 0.012$), thus it is possible to assume that total antioxidants were maximally used to neutralize ROS before they attacked the cell membranes. Vitamin E and Se supplementation may have contributed to the increase in TAS at the latter stages of the research, achieving reduction in tgα as well. Vitamin E could also contribute to inclusion of Se into the active centre of GPx, thereby activating and increasing the enzymatic AOS protection and decreasing lipid hydroperoxide ($H_{max.}$) in the time period from the sixth till the 12th month. In the 12th month of the study, there was a correlation between VE and Se ($r_s = 0.506$, $p = 0.038$) with a statistically significant increases in plasma Se. It is known that CF formation is mediated and related to superoxide anions and their mechanisms of action; VE can inhibit NADPH oxidase and xanthine oxidase alongside with the formation of the superoxide anion radical formation. The active oxygen forms,

which are formed in presence of NADPH - oxidase, serve as the initiators of COX-2 expression, which promotes the development of cancer [*Harris et al*., 2005]. Oncomarkers PSA and CEA had not changed and remained within the normal range, which suggests that within 12 months of VE+Se use, there were no risks for triggering development of the disease. Thus, VE can also indirectly, by analogy to Se, regulate the activity of COX enzyme. According to the data given in the scientific literature, Se, by activating AMP-activated protein kinase, (provider of cell energy homeostasis) reduces COX-2 expression [*Hwang et al*., 2006]. Thus, AO defence, in this group, may not be explained by direct inhibition of COX-2, but decrease in superoxide anion radical formation and the effect of membrane stabilisation. The correlations between antioxidant parameters – TAS and prooxidant parameters tgα (r_s = 0.715; p = 0.003) and TBARS_MDA (r_s = 0.707, p = 0.002) at the end of the study, indicate the persistence of OS.

After the first three months of IBF use, all the ChL indicators statistically significantly decreased, which, to some extent, could be explained by specific AO properties of IBF which are expressed as the ability to intercept ROS and inhibit COX activity. In the latter stages of the study, these figures statistically significantly increased, and, in the 12^{th} month, they tended to decrease. It is possible to assume that in CNNP clean-up workers, as a specific group of patients, under certain circumstances, IBF may show prooxidative properties in the plasma, but due to the endogenous VE reserves, LPO level does not increase, but rather, there is a slight decrease in peroxidation process, maintaining the LPO markers at the level of the initial period of the study. It was found that, in the third month, there was a moderate positive correlation between VE and TBARS_MDA (r_s = 0.506, p = 0.027), as well as between VE and Se (r_s = 0.563, p = 0.009) which substantiates their synergy. Decrease in the level of Se at the end of the study, influenced GPx activity and thus enhanced increase in lipid hydroperoxide levels ($H_{max.}$) in IBF group. Significance of Se in LPO as well as *Placebo* group is evident from the correlation between Se and tgα (r_s = 0.525, p = 0.037) in the sixth month

of the study. Likewise *Placebo* group, IBF group also experienced decrease in VE after six-month long use of the preparation thus suggesting the depletion of endogenous reserves. Which means that IBF as mono preparation is effective for the first three months, in case of long-term use there is required supplementation with some of the AO, thus contributing to reduction of LPO product formation, and, at the same time it is essential to activate the enzymatic AOS, assuming that IBF is a weak AO, but in long-term use it may also turn into a prooxidant. If compared with the initial period, in, there was a significant increase in the amount of onkomarker CEA in IBF group the 12^{th} month, and it had a moderately positive correlation with the increasing amount of TBARS_MDA ($r_s = 0.643$, $p = 0.034$). Correlation analysis showed that, at the end of the study, in IBF group, there was a strong negative correlation between TAS and LPO parameters − $S_{ox.}$ ($r_s = -0.704$, $p = 0.003$) and tgα ($r_s = -0.755$, $p = 0.001$). Apparently, the existing level is insufficient to reduce LPO parameters.

Use of IBF as a mono preparation did not affect the amount of TBARS_MDA, whereas, in the groups where it was used in combination with VE + Se, decrease in the amount of LPO product began after 6 months, and at the end of the study, it was statistically lower in comparison with the initial stage of the study. In the complex group (VE + Se + IBF), Se concentration increased significantly at the sixth month, while ChL and TBARS_MDA parameters started to fall and were statistically significantly different at the end of the study if compared with the findings at the beginning of the research.

It is possible to assume that the increase in the amount of Se, promoted increase in GPx and its enzymatic activity, thus aiding faster and more complete neutralization of peroxides by converting them into less aggressive LPO end products. In contrast to VE + Se group, already at the sixth month of the study it was found that there is a positive moderate ($r_s = 0{,}534$, $p = 0{,}021$) correlation between Se and VE which is indicative of their synergy which resulted in enhanced Se inclusion in the active centre of GPx thus activating and increasing

antiperoxidative cell protection. If at the beginning of the study, in the VE + Se + IBF group, there was a negative correlation between TAS and LPO parameters − $H_{max.}$, $S_{ox.}$ and tgα, then in the sixth and 12^{th} months the correlation with the LPO parameter − TBARS_MDA was positive (r_s = 0,724, p < 0,01). It is possible that the increase in LPO parameters in the sixth month was initiated as a response reaction − AO expression in order to maintain red-oxide status in cells. Although it was found that the LPO metabolites decreased, the positive correlation between TAS and TBARS_MDA showed that the "tension" between antioxidants and prooxidants was still present.

According to literature data, NSAID, including IBF, take part in the pro-apoptotic activities in cells. Cyclooxygenase inhibition and hence decrease in prostaglandin synthesis result in accumulation of retained arachidonic acid which due to sphyngomyelinase is converted into ceramides that are able to quickly initiate the apoptosis, suggesting that ceramide may be a crucial mediator for the proapoptotic action of NSAIDs [*Adachi et al*., 2007].

The mechanism of VE and Se synergy may serve as a basis for prostate cancer prevention. Combination use of VE and Se promotes inhibition of cell growth, mainly in the increased apoptotic response reaction. Different caspases activation signalling mechanisms may be responsible for the induction of apoptosis mechanism [*Zu et al*., 2003]. As it was stated above, a positive correlation between VE and Se was found in VE + Se group in the 12^{th} month, while in VE + Se + IBF group it was seen in the sixth month already.

As seen in Table 3.2, the onkomarkers statistically significantly increased over the period of 12 months, in the study groups, which did not take AO; while in the *Placebo* group, there was an increase in CEA, PSA and free PSA, while CEA onkomarker increased in IBF group. No onkomarker changes were observed in VE + Se + IBF group. Since these changes were within the borders of reference values, it is not possible to claim that there were identified risks that could provoke and / or contribute to the incidence of oncologic diseases. If onkomarker changes were

compared among the groups, then combination use of IBF and VE + Se proved to be the most effective, as in this group there were not identified statistically significant changes in the amount of onkomarkers. The concept of IBF and antioxidant combination therapy has both preventive and therapeutic effect on the possibility of tumour prevention in patients with prolonged exposure to OS.

Taking into account the importance of IBF in anti-inflammatory activity and its impact on the processes associated with cell apoptosis mechanisms, it is likely that in case of IBF use in long-term, it is reasonable to combine it with membrane − stabilizing AO − both lipid soluble, for example, tocopherol, and water-soluble AO, such as Se compounds that also regulate red-ox status. Although, at the end of the study, LPO parameters decreased in VE + Se and VE + Se + IBF groups, the correlative analysis showed OS tension since there was found a positive correlation between antioxidants and pro-oxidants, i.e., in month 0, the third and the 12^{th} month − in VE + Se group; while in the sixth and 12^{th} month in VE + Se + IBF group. Apparently, AO is able only to delay, but not completely inhibit the excessive LPO.

3.2. Study II

As seen in Figure 3.7., in Se + CoQ_{10} + IBF + Om and Se + CoQ_{10} group, Cu, Zn-SOD activity statistically significantly increased after six month, 1378 (195) –1593 (171) U/gHb ($p < 0.0001$) and 1380(334) – 1506 (178) U/gHb ($p = 0.048$), respectively, in comparison with the initial period of the study.

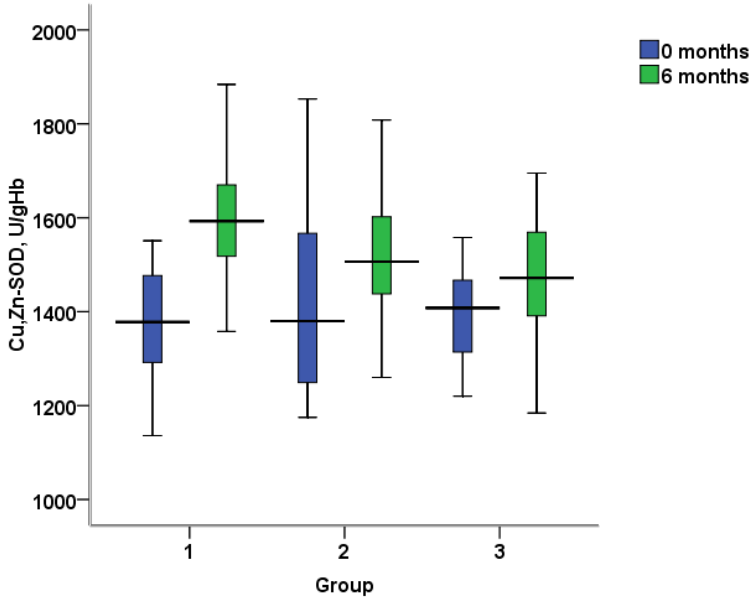

Figure 3.7. **Effect of Selenium, Coenzyme Q_{10} and Ibuprofen on the Activity of Cu, Zn-SOD**
1 – Se + CoQ_{10} + IBF + Om group; 2 – Se + CoQ_{10} group;
3 – CoQ_{10} + IBF+Om group

In CoQ_{10} + IBF + Om group, Cu, Zn-SOD activity did not change. In Se + CoQ_{10} + IBF + Om group, Cu, Zn-SOD activity was statistically significantly higher in comparison with Se + CoQ_{10} group ($p = 0.043$) and CoQ_{10} + IBF + Om group ($p = 0.013$).

As seen in Figure 3.8., in CoQ_{10} + IBF + Om group, which was not prescribed Se, **GPx** statistically significantly decreased, i. e. 6704 (2341) − 6216 (2213) U/L (p = 0.011). In the rest of the groups, GPx activity had a tendency to increase. In the sixth moth, in CoQ_{10} + IBF+ Om group, GPx activity was close to statistically significantly lower than in Se + CoQ_{10} group (p = 0.072).

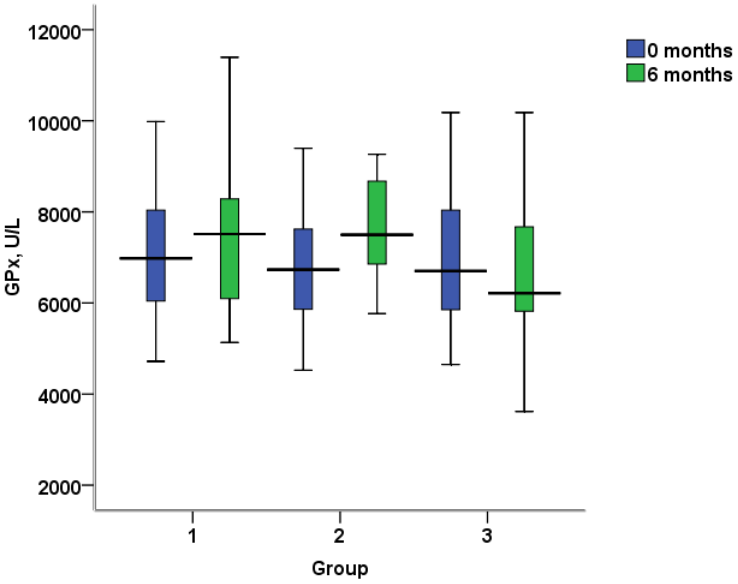

Figure 3.8. **Effect of Selenium, Coenzyme Q_{10} and Ibuprofen on the Activity of GPx**
1 – Se + CoQ_{10} + IBF + Om group; 2 – Se + CoQ_{10} group; 3 – CoQ_{10} + IBF+Om group.

As seen in Figure 3.9., after three months, in Se + CoQ_{10} + IBF + Om and CoQ_{10} + IBF + Om groups, the amount of **GSH** statistically significantly decreased 1.53 (0.23) – 1.40 (0.27) mM (p = 0.009) and 1.58 (0.28) – 1.48 (0.36) mM (p = 0.003) respectively. In the sixth month, the amount of GSH statistically significantly increased in all the groups in comparison with the initial stage: in Se + CoQ_{10} + IBF + Om group 1.53 (0,23) −1.61 (0.28) mM (p = 0.025); in Se + CoQ_{10}

group 1.56 (0,38) – 1.71 (0.20) mM (p = 0.001); in CoQ_{10} + IBF + Om group 1.58 (0.28) – 1.76 (0.44) mM (p = 0.003).

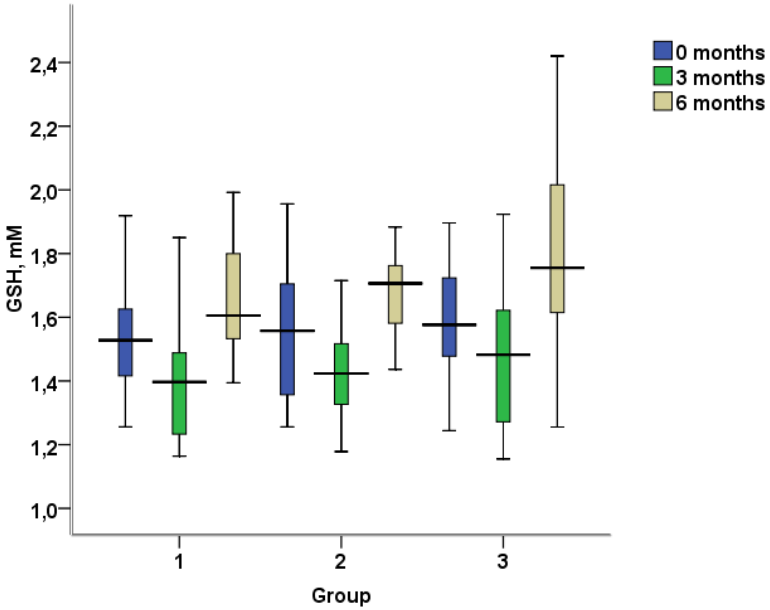

Figure 3.9. **Effect of Selenium, Coenzyme Q_{10} and Ibuprofen on the Amount of Reduced Glutathione (GSH)**
1 – Se + CoQ_{10} + IBF + Om group; 2 – Se + CoQ_{10} group;
3 – CoQ_{10} + IBF+Om group.

As seen in Figure 3.10., in Se + CoQ_{10} group, the amount of **Se** statistically significantly changed over the whole period of the study increasing in the third month, i.e. 96.0 (24.0) – 111.5 (28.5) µg/ml (p = 0.033) and in the sixth month, i. e. 133.0 (27.0) µg/ml (p = 0.008). In Se + CoQ_{10} + IBF + Om group, the amount of Se statistically significantly increased in the sixth month, i. e. 94.0 (29.5) – 124.5 (343.8) µg/ml (p = 0.011), respectively.

In CoQ_{10} + IBF + Om group, the amount of Se did not change statistically significantly.

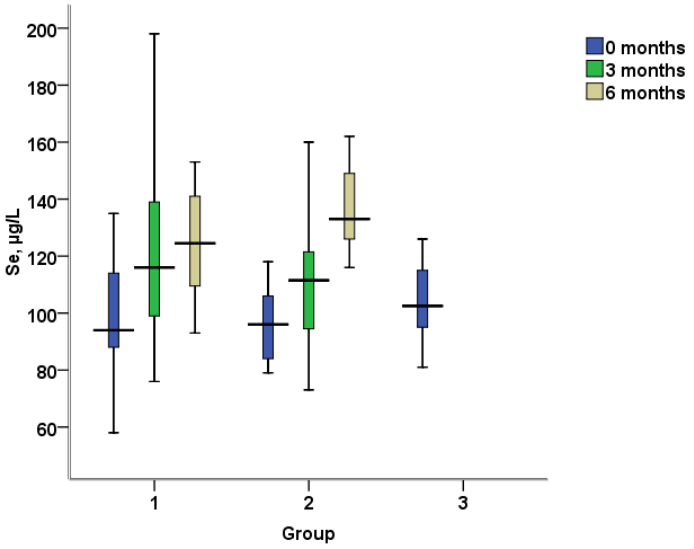

Figure 3.10. **Effect of Selenium, Coenzyme Q_{10} and Ibuprofen on the Amount of Se**
1 – Se + CoQ_{10} + IBF + Om group; 2 – Se + CoQ_{10} group;
3 – CoQ_{10} + IBF+Om group.

As seen in Figure 3.11., the amount of **albumin** (ALB) statistically significantly (p = 0,019) increased in the third month. In Se + CoQ_{10} group – 42.69 (2.47) – 43.84 (2.40) g/L, while at the end of the study, there were not found any statistically significant changes. In the third month, in the rest of the groups, changes in the amount of ALB were not statistically significant, while in the sixth month the changes were close to statistically significant. The comparison of the groups showed that the amount of ALB was statistically higher in CoQ_{10} + IBF + Om group than in Se + CoQ_{10} group (p = 0.037).

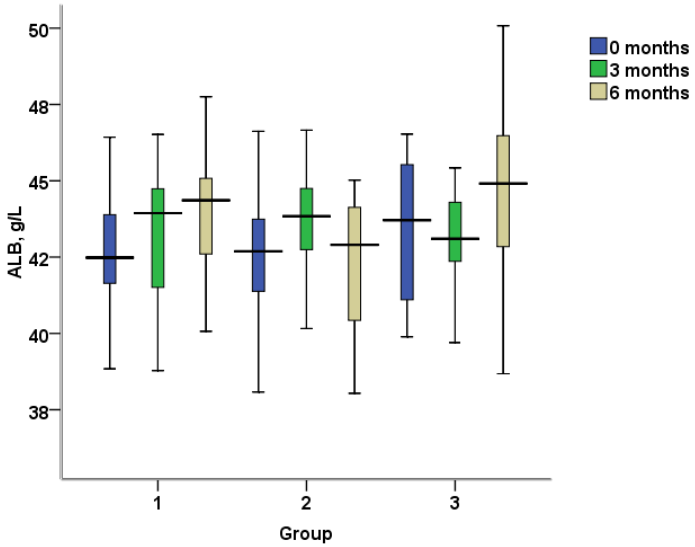

Figure 3.11. **Effect of Selenium, Coenzyme Q_{10} and Ibuprofen on the Amount of Albumine (ALB)**

1 – Se + CoQ_{10} + IBF + Om group; 2 – Se + CoQ_{10} group;
3 – CoQ_{10} + IBF+Om group.

Table 3.3.

Changes in the Amount of Malondialdehyde and 4-Hydroxynonenal

Group	MDA(μM)			4-HNE(μM)		
	N	Month 0	6th month	N	Month 0	6th month
Se+CoQ_{10}+IBF+Om	25	1.88(0.68)	1.65(0.64)* p = 0.050	13	4.62(5.07)	2.41(3.64)
Se+CoQ_{10}	21	1.80(1.00)	1.85(0.50)	15	4.62(7.54)	4.03(10.14)* p = 0.043
CoQ_{10}+IBF+Om	25	2.25(0.70)	1.59(0.59)* p = 0.005	18	4.08(4.96)	2.40(8.56)

The results are presented as medians (interquartile range). *Statistically significantly differs from month 0.

The **amount of VE** did not change during the study and remained within the reference borders.

In Se + CoQ_{10} group, CEA increased in the third month ($p = 0.037$). Statistically significant changes were not found in the amount of PSA and free PSA.

At the beginning of the study, it was found that there was a strong positive correlation between AOS parameters and LPO metabolites which indicates that, in response to the increase in OS, AO defence also increased: in Se+ CoQ_{10}+IBF+Om group, between VE and MDA and MDA + 4-HNE ($r_s= 0.557$, $p = 0.004$; $r_s= 0.630$, $p = 0.021$); in Se + CoQ_{10} group, between Cu, Zn-SOD and 4-HNE ($r_s= -0.633$, $p = 0.011$); in CoQ_{10}+IBF+Om group between ALB and 4-HNE ($r_s = 0,607$, $p = 0.008$).

The increase in the activity of Cu, Zn-SOD, in the study groups where CoQ_{10} administered in combination with Se, may have two explanations: either as activation of AOS (Cu, Zn-SOD gene expression is triggered due to the presence of Se) or as a response reaction to the increase of superoxide ion radical, which can appear in redox reactions, which catalyse flavin dehydrogenase, NAD-dehydrogenases, for example, glutathione reductase (GR). Glutathione reductase plays an important role in the maintenance of GSH potency supplying the reduced equivalents from pentose phosphate pathway and its activation may be associated with the necessity of substrate for the activity of GPx enzyme family. Selenium is an integral component of GPx which is indicated by the positive correlation ($r_s= 0.661$, $p = 0.004$), and it is not surprising that GPx activity had a tendency to increase in the groups where Se was used regardless of IBF administration in a particular study group. At the same time, it is possible to observe that, in CoQ_{10}+IBF+Om group, where Se was not used, GPx activity decreased significantly which may be a consequence of H_2O_2 tendency to increase and / or following inhibition of Cu, Zn-SOD activity with insufficient CAT activity. As the result of Se and CoQ_{10} use, there was found an increase in the level of plasma Se already after three months, while in combination with IBF− only after six months

of use. It was similar to the Study I, when IBF was administered in combination with AO – VE and Se; the amount of Se increased significantly in the sixth month.

In the third month, the amount of GSH decreased significantly in the groups of patients where IBF was prescribed. This may have the following explanation: IBF and its metabolites have electron-deficient regions and thus it can partly react with GSH causing the decrease in its amount which would be followed by OS development [*Fazlul*, 2006]. Use of IBF in combination with antioxidants (Se, CoQ_{10}) can increase the amount of GSH in the time period from the third till the sixth month. In the sixth month, in CoQ_{10}+IBF+Om group, there was found a correlation between Cu, Zn-SOD and GSH ($r_s = -0.592$; $p = 0.006$) which is indicative of increase in GSH as compensatory response to Cu, Zn-SOD deficiency in cell protection. If in the third month, in Se + CoQ_{10} group, which did not use IBF, there was a strong positive correlation between GSH and PSA ($r_s = 0.556$, $p = 0.011$), then, in CoQ_{10} + IBF + Om group, this correlation was negative. It is related to a significant decrease in GSH ($r_s = -0.470$, $p = 0.049$). At the end of the study, the increase in the amount of reduced glutathione in all the groups may be ensured, as it was mentioned above, by GR, as well as ATP-dependant GSH−synthesis. According to the literature data, exogenous use of CoQ_{10} increases the production of energy in MT, since it is the main component of MT respiration chain during ATP synthesis [*Lenaz et al*., 1991*; Bhagavan*, 2006]; at the same time ATP is required for the biosynthesis of GSH as it is an energy-dependant process. The results of the study proved that the amount of VE did not change and was preserved at a sufficiently high level due to the presence of CoQ_{10} in all the study groups. As it is known, CoQ_{10} serves as a synergist of VE; it not only protects VE against oxidation caused by superoxide, but also reacts with VE in the antioxidative process. If in month 0, in Se + CoQ_{10} group, there was a negative correlation between Se and VE ($r_s = -0.571$, $p = 0.017$), then at the end of the study, after Se and CoQ_{10} use, this correlation was positive ($r_s = 0.603$, $p = 0.050$), which may be related to VE regeneration due to both $CoQ_{10,}$ and GSH. The

reduced glutathione take part in the regeneration of VE using ascorbic acid, which is evident from the positive correlation between GSH and VE in CoQ_{10} + IBF + Om group ($r_s = 0.451$, $p = 0.046$) in the sixth month of the study.

As seen in Table 3.3, after six months, decrease in MDA was present in the study groups which were prescribed IBF in combination with antioxidants. Although Se belongs to, so called, secondary group of AO, the analysis of quantitative MDA parameters did not reveal such changes in Se + CoQ_{10} group, which shows that LPO processes do not increase, but remain stable at the given concentration of Se and CoQ_{10} and the increased enzyme activity in AO protection system. Study I did not also show decrease in MDA in the given group which was prescribed antioxidants (VE+Se) after 6 months; it was seen only in the 12th month. Decrease in the amount of MDA in Se + CoQ_{10} + IBF + Om group ensures increase in the activity of Cu, Zn-SOD and GPx as well as increase in GSH, as well as IBF ability to intercept metals of changeable valence, as well as sufficiently high level of VE. In CoQ_{10} + IBF + Om group, which did not received Se and GPx the activity decreased significantly, it is possible to assume that the amount of hydroperoxides would increase with a subsequent increase in the level of MDA. It is apparent, that, in such situation, one of the key roles in LPO regulation is played by one of the enzymes from GPx enzyme family – Se-independent GPx, i. e. glutathione-S-transferase (GST), which breaks down lipid hydroperoxides and thus inhibits the formation of MDA –the end product of LPO. It must be noted that GST unlike GPx neither breaks H_2O_2 nor modifies it in any other way. In respect of another LPO metabolite – 4-HNE, there were detected changes opposite to MDA. There were significant changes in 4-HNE in Se + CoQ_{10} group, which may by partly explained by the increase in ALB in the given group in the third month. In the study groups which received IBF, increase in ALB was close to statistically significant reaching the maximum in the sixth month, there was also identified a close to statistically significant decrease in the amount of 4-HNE.

According to the literature data, IBF is not only able to inhibit COX1, COX2, and 5-lipoxygenase, but it is also able to increase the activity of 15-lipoxygenase [*Rainsford*, 2003], and as it is known, it is related to formation 4-HNE enzyme from arahidonic acid. It may be the reason why the decrease in 4-HNE was less expressed in the groups which were prescribed IBF. Ibuprofens property to reduce 4-HNE, as it is mentioned in the literature, may be related to its ability to intercept metals with a variable valence. The fact that 4-HNE did not increase in all study groups is connected with its elimination due to the increase in GSH. In general, the identified onkomarkers CEA, PSA and freePSA did not change, except for the Se + CoQ_{10} group where, in the third month, there was a statistically significant increase in the amount of CEA, which did not exceed the defined reference intervals (ne > 2,5 ng / ml). As these changes were within the limits of the reference values, it is impossible to claim that the identified risks may trigger and / or promote development of oncologic diseases.

3.3. Study III

Cu, Zn-SOD activity (Figure 3.12.) statistically significantly increased during the whole period of study in *Placebo* and GV groups, 1401 (166) – 1500(216) –1471(239) U/gHb ($p = 0.010$; $p = 0.042$) and 1347(186) − 1471(220) − 1591(263) U/gHb ($p = 0.011$; $p=0.003$), respectively.

In the first nine months, in GT and GB groups, there were found statistically significant changes – 1369 (148) – 1465 (258) U/gHb ($p = 0.031$) and 1404 (176) – 1487 (167) U/gHb ($p = 0.001$), respectively.

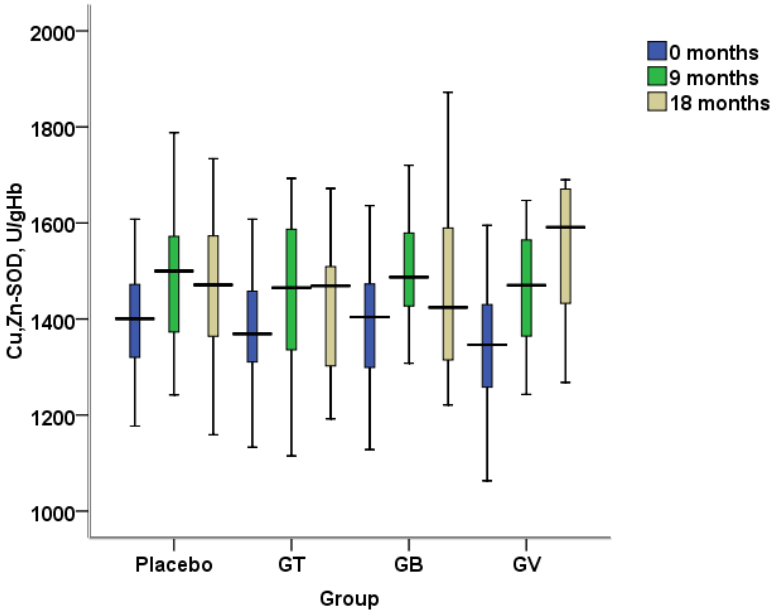

Figure 3.12. **Changes in Cu, Zn-SOD Activity After Use of Green Tea, *Ginkgo biloba* and *Grinvitals Cereloba® plus* Preparations**

GPx activity (Figure 3.13.) statistically significantly increased only in *Placebo* group in the first nine months, i.e. 5881 (3620) – 6609 (2522) U/L (p = 0.045), and in the 18th, it remained at the same level.

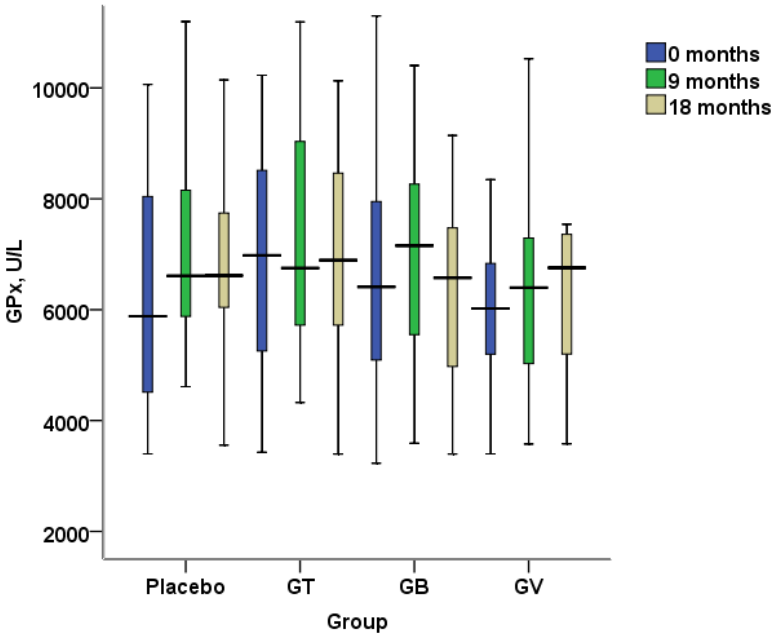

Figure 3.13. **Changes in the Activity of Glutathione Peroxidase (GPx) After Use of Green Tea, *Ginkgo biolobas* and *Grinvitals Cereloba® plus* Preparations**

Statistically significant differences in **CAT** (Figure 3.14.) activity were found in between the ninth and 18th month in *Placebo* and GB groups, 238 (106) – 184 (75) k/gHb (p = 0.025) and 188 (61) – 223 (102) k/gHb (p = 0.022) respectively.

The comparison of CAT activity changes in *Placebo* group with other groups showed that, in the ninth month, there were statistically significant changes in GB group (p = 0.023), while in the 18th month, there were close to statistically significant changes in GT group (p = 0.069), GB group (p = 0.082) and GV group (p = 0.056).

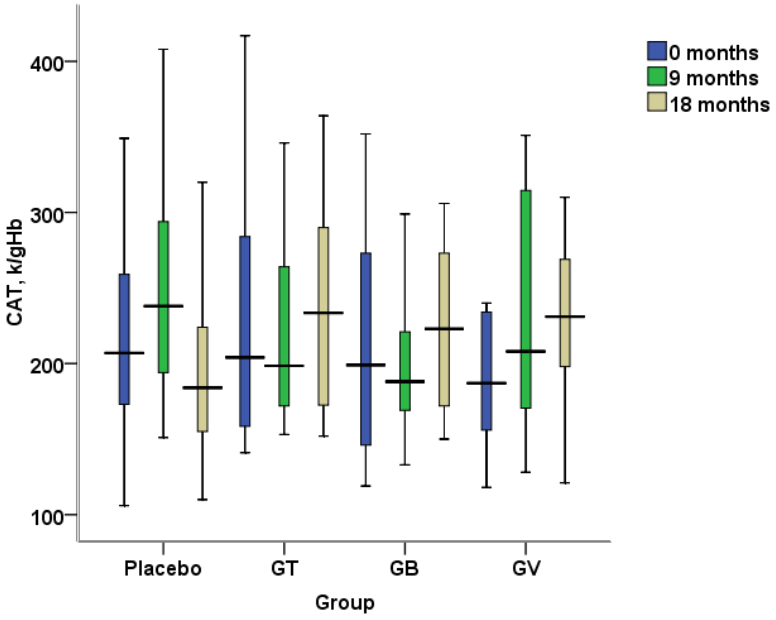

Figure 3.14. **Changes in Catalase (CAT) Activity After Use of Green Tea, *Ginkgo bioloba* and *Grinvitals Cereloba® plus* Preparations**

Total antioxidative statuss amount (Figure 3.15.) statistically significantly increased (p = 0.032) only in *Placebo* group till the ninth month – 1.56 (0.18) – 1.68 (0.21) mM, and in the 18th month it remained at the same level. There were no statistically significant changes in other groups.

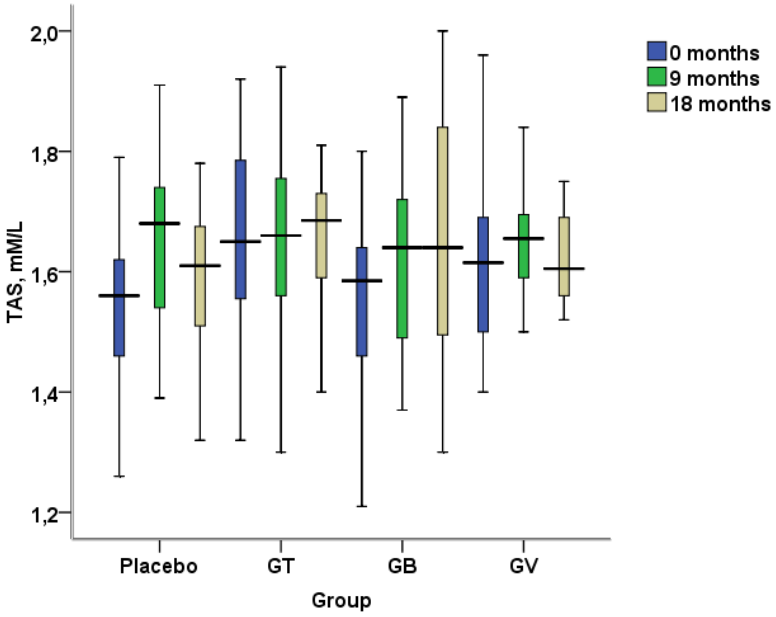

Figure 3.15. **Changes in Total Antioxidative Status After Use of Green Tea, *Ginkgo bioloba* and *Grinvitals Cereloba® plus* Preparations**

In *Placebo* group, the **amount of** VE (Figure 3.16.) statistically significantly decreased over the whole period of the study: 15.45 (4.60) – 12.80 (4.40) – 11.25 (3.23) µg/ml ($p = 0.004$; $p < 0.001$) respectively. In GB group the amount of VE statistically significantly decreased in the ninth month – 14.20 (4.10) –12.90 (5.70) µg/ml ($p = 0.037$) and remained at the same level also in the 18th month. In GV group, the amount of VE statistically significantly decreased from the ninth till the 18th month i.e. 12.35 (5.38) –11.60 (6.20) µg/ml ($p = 0.047$). In GT group, the amount of VE did not change.

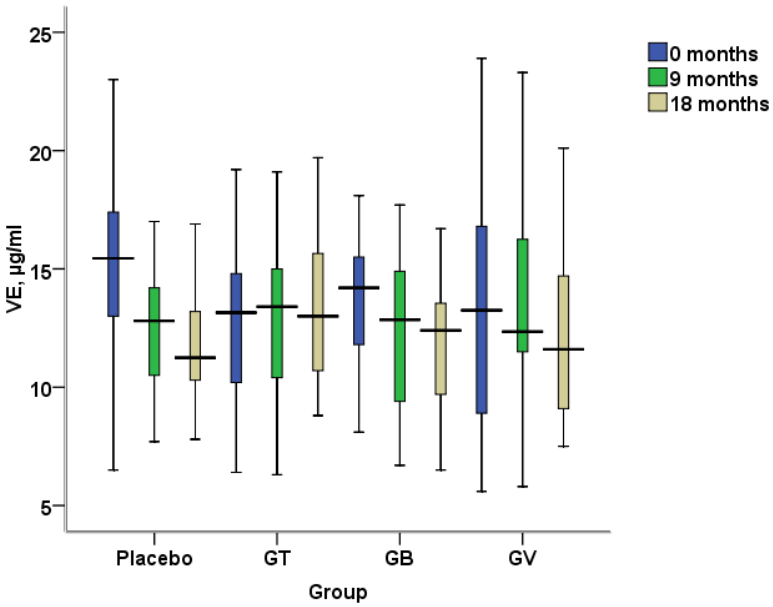

Figure 3.16. **Changes in the Amount of Vitamin E After Use of Green Tea, *Ginkgo bioloba* and *Grinvitals Cereloba® plus* Preparations**

Table 3.4.

Statistically Significant Changes in Dynamics of Lipid Hydroperoxide, Malondialdehyde and 4-Hydroxynonenal Amount in T2D patients after long-term use of different doses of GT, GB and GV

Para-meter	Gr.	Month 0	N	9th month	N	p	18th month	N	p
LOOH µM	GT	6,83(4.95)	20	6.61(3.51)*	17	0.019	6.34(8.81)	16	ns
	GB	7.32(3.20)	25	5.04(1.94)*	26	0.001	5.81(3.77)	20	ns
MDA µM	GT	1.77(1.10)	19	1.51(0.57)*	18	0.039	1.46(0.44)	16	0.084
	GB	1.70(0.65)	26	1.45(0.93)	26	ns	1.29(0.65)*	19	0.005
	GV	1.68(0.69)	18	1.48(0.63)*	16	0.012	1.48(0.75)*	14	0.041
4-HNE µM	GB	4.73(3.64)	21	4.32(3.94)	26	0.064	2.77(4.02)*	19	0.011

The results are presented as median (interquartile range); *Statistically significant in comparison to month 0; ns – data is not statistically significant, $p > 0,05$.

Use of 400 mg GT leaf standardized extract daily for the period up to the nine month proved to be the most efficient in addition to standard therapy. Content of the VE using GT did not change throughout the whole study compared with other study groups where its content decreased suggesting that the GT polyphenols can effectively protect biomembranes from VE depletion and engage in inhibition of LPO process. Such conditions develop due to inability of GT containing EGCG to form metal chelate complexes, to intercept superoxide anions, H_2O_2, ·OH and $O_2^{-\cdot}$, but also to regenerate tocopherols [*Ratnam et al.,* 2006*; Gawlik et al.*, 2007]. Decrease in the amount of lipid hydroperoxide in the ninth month allows to conclude that GT supplementation may interrupt LPO at it's "development or continuation stage" and hence reduce the formation of LPO end products. Findings of the study confirm that in the ninth month, the amount of MDA statistically significantly decreased, while the amount of 4-HNE had a tendency to decrease, and in the 18th month − to grow. Negative correlation between GPx and LOOH at the beginning of the study ($r_s = -0.469$, $p = 0.037$), and at the end ($r_s = -0.824$, $p < 0.001$) is indicative of the role of this enzyme in insufficient inhibition of LPO during both its stages. In the ninth month, there was found a negative correlation between TAS and 4-HNE, as well as MDA + 4-HNE ($r_s = -0.515$, $p = 0.029$; $r_s = -0.645$, $p = 0.005$) which confirms the fact that, in order to prevent 4-HNE formation, cells should contain sufficient amount of water − soluble antioxidant (including GSH) that are able to influence initiation of LPO process. The majority of experiments demonstrated that relatively low concentrations of flavonoids can stimulate the transcription of GSH synthesis gene [*Moskaug et al.,* 2005].

T2D patients who received standardized dry extract of GB leaves, containing 19.2 mg of Ginkgo flavone glycosides (quercetin, kempherol, isorhamnetin) and 4.8 mg terpene lactones (ginkolides, bilobalides), in addition to standard therapy, there was found a significant decrease in OS biomarkers − LOOH in the ninth month, while in MDA and 4-HNE − in the 18th month.

Decrease in lipid hydroperoxide in the ninth month, allows to draw a conclusion that supplementation with GB (in low doses), as well as GT may interrupt LPO at its "prolongated" stage and hence reduce the formation of LPO end products (MDA, 4-HNE). The correlation between Cu, Zn-SOD and LOOH, detected in the ninth month, indicates that with the increase in enzyme activity, dismutation of superoxide anion becomes more efficient which results in the decrease of LPO (r_s = −0.419, p = 0.033). According to literature data, quercetin possesses a greater potency than catechism to chelate copper and iron ions [*Paganga et al*., 1996], which may explain decrease in such significant LPO parameters as MDA and 4-HNE compared to GT activity. LOOH reduction may be also related to the activation of GPx although its increase was not statistically significant, in the given situation, it is possible to assume that there is a stable tendency to grow, as well as endogenous use of VE. The activity of GPx statistically significantly increased in the ninth month only in T2D patients who did not experience Se deficiency. Lipid hydroperoxide was broken by Se independent GPx, i. e. GST.

The given study showed that, in the first nine months, *Ginkgo biloba* activated Cu, Zn-SOD, and while in the 18th month this increase was close to statistically significant. Catalase activity increased significantly in the 18th month compared with the ninth month, which ensured breakdown of H_2O_2 into less toxic compounds and decreased dangerous to a cell ·OH production. At the end of the research, the negative correlation between the CAT and GPx showed prevalence of CAT in antiperoxidative activity. At the beginning and the end of the study, CAT activity had a moderately close positive correlation with the LPO parameters − the sum of aldehydes – MDA + 4-HNE and 4-HNE, respectively. It is possible that LPO metabolites induce the expression of CAT gene and thus illustrate cell adaptive response to OS. Although this study did not detected concentration of GSH, it is possible to assume that GB contributed to GSH synthesis *de novo* in T2D patients thus playing an important role in detoxification of 4-HNE creating the end products as non-enzymatic conjugates or through catalysis using GST. In

the ninth month, comparison of GB group with *Placebo* group patients who received standard therapy, showed a significant decrease in CAT ($p = 0.023$), the amount of LOOH ($p = 0.008$) and close to statistically significant 4-HNE ($p = 0.065$), and in the 18th month a statistically significant decrease in the amount of MDA ($p = 0.018$). If compared with GT group, the amount of LOOH was statistically significantly lower in the ninth month ($p = 0.042$). The comparison with GV group in the 18th month showed a statistically significant decrease in the amount of LOOH ($p = 0.050$) and the sum of aldehydes ($p = 0.044$).

Long-term combination use of preparation *Grinvitals Cereloba®plus* by T2D patients ensured a continuous Cu, Zn-SOD activation and decrease in the amount of MDA in the ninth month, which remained at the same level also in the 18th month. Differences in Cu, Zn-SOD activity were found after comparison with the level of plasma Se. In the ninth month, Cu, Zn-SOD activity in GV group was statistically significantly lower at the level of Se above 80 μg/L, compared with *Placebo* and GB groups ($p = 0.039$ and $p = 0.006$, respectively). However, at the end of the study, in GV group, Cu, Zn-SOD activity was statistically significantly higher in patients with the level of Se below 80 μg/L if compared with GT gr. ($p = 0.015$), GB gr. ($p = 0.027$) and *Placebo* group. ($p = 0.013$). Increase in Cu, Zn-SOD activity at the end of the study was possibly a compensatory response to the increasing Se deficiency since, at the beginning of the study, the level of plasma Se was below normal in 72.2% T2D patients in GV group. At the beginning of the study there was detected a moderately close negative correlation between Cu, Zn-SOD and MDA ($r_s = -0.484$, $p = 0.042$) suggesting the involvement of superoxide anion radicals in MDA formation. The correlation between MDA and VE ($r_s = 0.476$, $p = 0.046$) indicates the use of endogenous VE resources for LPO inhibition in response to the OS.

In the ninth month, in *Placebo* group T2D patients receiving standard therapy − metformin and insulin had significant increase in the activity of Cu, Zn-SOD, GPx and the amount of TAS, as well as decrease in VE, while in the 18th

month, there was a significant increase in Cu, Zn-SOD activity and decrease in CAT activity alongside with depletion of VE. Unlike in other groups, in *Placebo* group, significant changes in LPO metabolites were not found. In 65.4% of the T2D patients in *Placebo* group, Se content was lower than the normal. The detected negative Se correlation with LOOH and MDA + 4-HNE is indicative of the role of Se in the process of LPO (r_s= −0.485, p=0.012; r_s= −0.459, p = 0.036, as being an integral component of GPx it contributes to the increase in GPx activity. Under the conditions of Se deficiency and decreased GPx activity, partial cleavage of LOOH leads to both increase in LOOH concentration and the increase of other LPO products − MDA and 4-HNE. Comparison of T2D patients depending on plasma Se level, it was found that, in the ninth month, the patients with Se within the normal range had a lower content of LOOH (p = 0.022). The positive correlation (r_s = 0,458, p = 0,019) between CAT and LOOH at the beginning of the study evidences that with the increase in LPO process, there is also an increase in AOS anti-peroxidative activity. It is likely that LOOH induces expression of CAT gene and thus cell adaptive response to OS is manifested. Increase in CAT activity to balance red-oxide state may be also enhanced by the reduced amount of Se, which is evident from moderately negative correlation between the two indicators (r_s = −0.411; p = 0.037). The increase in the activity of GPx and the amount of TAS in the ninth month may be explained by the adaptive response to OS as evidenced by moderately close positive correlation between antioxidants and prooxidants: between GPx and 4-HNE (r_s = 0.438; p = 0.037) and between TAS and MDA (r_s= 0.434; p = 0.030). In the ninth and 18th months, there was a positive correlation between the lipophilic AO − VE and hydrophilic AO − TAS.

3.4. Study IV

As can be seen in Figure 3.17., **Cu, Zn-SOD** activity was lower in T2D group by 7.3%, i.e. 1365 (210) U/gHb (p = 0.002) compared with the control group where 1473 (181) U/gHb .

While in IR and in T2D + IR groups, i.e. in participants of the study who were exposed to IR the activity of the given enzyme remains at the level of the control group.

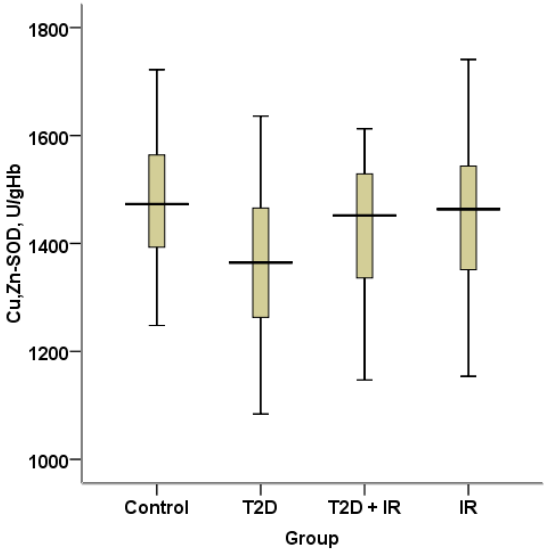

Figure 3.17. **Cu, Zn - SOD Activity**

Statistically significant differences in **GPx** activity were detected in diabetes-related groups: in T2D group GPx activity was decreased by 18%, i. e. 6151 (2349) U/L (p = 0.002) and T2D + IR group by 13%, i. e. 6558 (2124) U/L (p = 0.020) compared with the control group 7533 (2546) U/L (see Figure 3.18.).

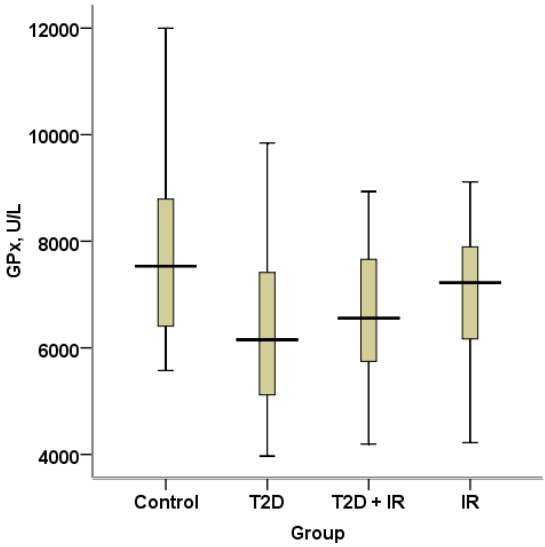

Figure 3.18. **Activity of Glutathione Peroxidase**

The amount of **Malondialdehyde** was statistically significant (p = 0.030) higher in IR gr. − by 18.8% i. e. 1.85 (0.88) μM and in T2D + IR group 16%, i. e. 1.81 (0.63) μM (p = 0.012) compared with the control group 1.56 (0.65 μM). In T2D group, the amount of MDA was close to statistically significantly higher (p = 0.076) than in the control group (Figure 3.19.), i. e. by 9%, respectively.

The amount of **4-HNE** was statistically significantly (p = 0.005) increased in T2D group − by 37%, i. e. 4.98 (3.74) μM compared with the control group 3.64 (1.26) μM. In T2D + IR group, the amount of 4-HNE was close to statistically significant (p = 0.094) higher by 24%, compared with the control group (Figure 3.20.).

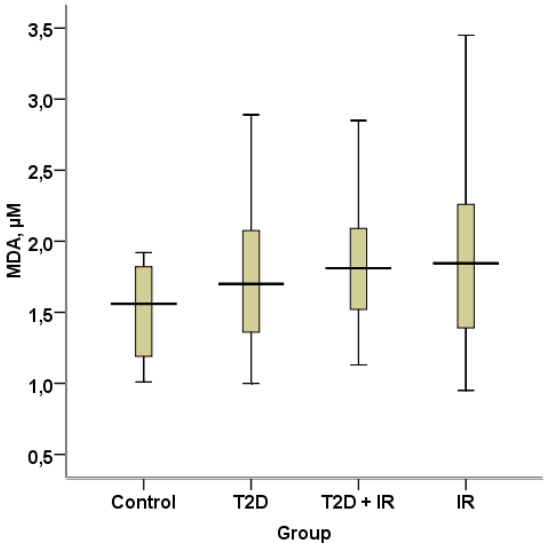

Figure 3.19. **Amount of Malondialdehyde**

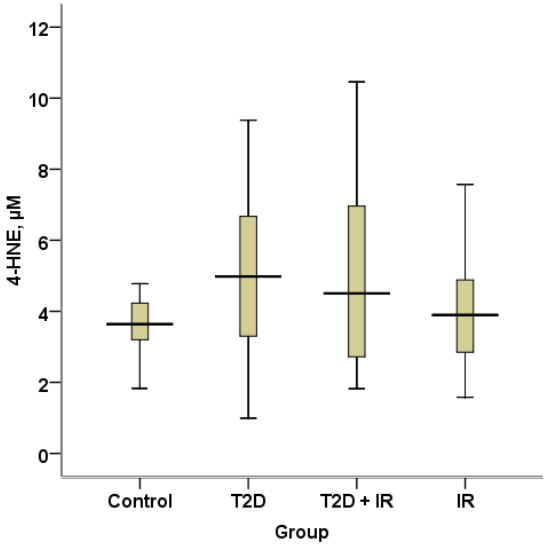

Figure 3.20. **Amount of 4-Hydroxynonenal**

In T2D group, the amount of **PC** was increased by 57%, i. e. 480 (160) pM/mg of protein ($p < 0.001$); in T2D + IR group by 37%, i. e. 420 (105) pM/mg of protein ($p = 0.001$); in IR group by 25%, i. e. 380 (95) pM/mg protein ($p < 0.001$) compared with the control group, 305(82.5) pM/mg protein. In T2D group and T2D + IR group the amount of PC was statistically significantly higher than in IR group, $p < 0.001$ and $p = 0.030$ (see Figure 3.21.), respectively.

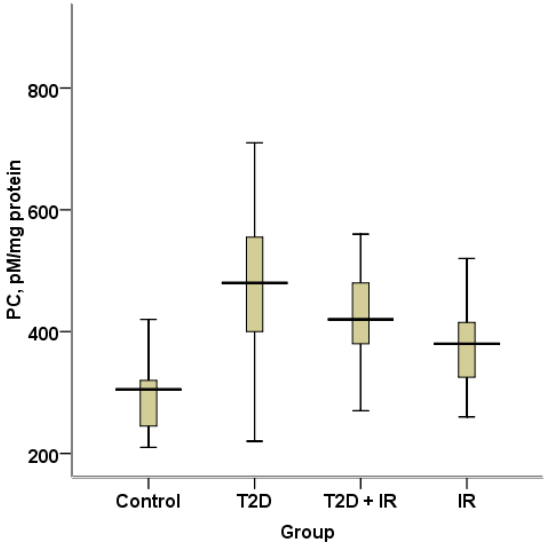

Figure 3.21. **Amount of Protein carbonyl**

In the groups T2D + IR and IR, there was a moderate positive correlation between TAS and 4-HNE (Figure 3.22. A and B).

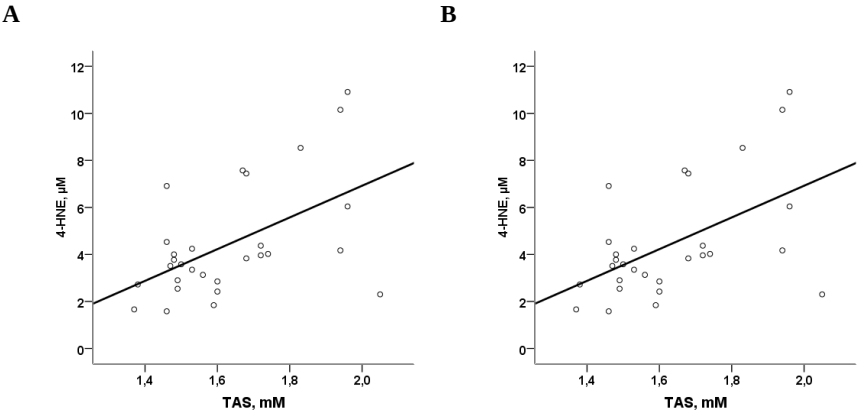

Figure 3.22. **Correlation between TAS and 4-HNE in T2D+IR (A) and IR groups**
A: Correlation: $r_s = 0.476$, $p = 0.011$
B: Correlation: $r_s = 0.447$, $p = 0.015$

It was found that the amount of Se in all the groups was below the accepted normal range (80−120 µg/L): in IR group 68.5 (18.75) µg/L; in T2D group 75.5 (17.00) µg/L; in T2D + IR group 66.0 (22.0) µg/L, and Se as the integral component of GPx should have influenced the enzymatic activity. The low Cu, Zn-SOD activity in T2D patients is associated with inhibition of the above mentioned enzyme, which can be caused by hyperglycaemia or excessive H_2O_2 accumulation in the body. The observed persistence of Cu, Zn-SOD activity in both groups which included CNNP clean-up workers might caused by increased enzyme gene expression, which could be considered as an adaptive response to OS. As it is known, Cu, Zn-SOD has radioprotective properties which play an important role in radioadaptive response [*Randah et al.*, 1997]. The reduced GPx activity in T2D and T2D + IR groups is associated with both Se deficiencies in the body, presence

of its substrate − reduced GSH, and with the inhibitory effect of hyperglycaemia on the expression of GPx, as well as overall destructive effect of ROS on the enzymatic systems responsible for utilisation of lipoperoxides. Thus, it is possible that the relatively low activity of GPx may lead to the increase of both H_2O_2, and LOOH which may be followed by increase in LPO end product − MDA and 4-HNE. In IR group, there was found a weak positive correlation between GPx and 4-HNE, that may be caused by the high GPx activity, increase in the amount of 4-HNE was not detected. Commonly, under the conditions of OS, increase in 4-HNE is indicative of increase in n-6 fatty acid oxidation, as well as the inability to inhibit decomposition of endoperoxides if GPx activity is reduced.

The amount of MDA significantly increased in CNNP clean-up workers with / without T2D, while in T2D gr. there was found a tendency for MDA to increase. Moderate correlation between TAS and 4-HNE levels (Figure 3.22.) in IR and T2D + IR groups shows activation of compensatory mechanisms, i.e. lipid oxidative damage is accompanied by the increase in AOS protection. Inhibition of 4-HNE elimination induced by GSH conjugate mediation may be associated with the increase in protein oxidation, as well as impaired GSH bioregeneration, especially, taking into account the fact that, in case of T2D, ATP formation is reduced, and GSH synthesis *de novo* is an energy-dependant process. Hyperglycaemia may indirectly affect GSH depletion, since the GSH depletion converts intracellular glucose into sorbitol using cofactor of the pentose phosphate metabolism cycle − NADP, which, in its turn, is required for GSH formation using GR. Increase in PC shows imbalance in AOS enzyme activity associated with insufficient activity of such enzymes as CAT and GPx that is followed by accumulation of H_2O_2. At low levels of H_2O_2, proteins have a high sensitivity to degradation in proteasomes, which in turn decreases at high H_2O_2 concentrations. 4-HNE − related proteins are resistant to degradation in the proteasomes and act as non-competitive enzyme inhibitors. Such an imbalance in the intensity of protein oxidative modification, its proteolysis and *de novo* synthesis serves as a tissue

damage marker. In IR group, in spite of normal activity of AO enzyme − Cu, Zn-SOD and GPx, the amount of MDA and PC was increased which may be the cause of OS and adaptation mechanism activation. It is known that MDA and 4-HNE are CF that persist for many years after exposure to IR and is a risk factor for late complications not only in individuals with post-radiation syndrome, but also in diabetes patients. CNNP clean-up workers with T2D had a reduced GPx activity, increased amount of MDA and PC, and 4-HNE that could be explained by the hyperglycaemic effect. Based on the findings of the given study and the literature data, it is possible to conclude that lipid and protein oxidation metabolites − MDA, 4-HNE and PC play an important role in the development of multifunctional diseases, including T2D, in CNNP clean-up workers. In such situations, it is advisable to use natural substances with antiradical activity and Se.

4. CONCLUSIONS

1. Adjustment of oxidative stress parameters using IBF (400 mg/d) in combination with a lipophilic antioxidant − CoQ_{10} (100 mg/d) and hydrophilic antioxidants − Se (200 µg/d) in CNNP clean-up workers was more effective than the combination with vitamin E (350 mg/d) and Se (200 µg/d).
2. Adjustment of oxidative stress parameters in patients with compensated T2D was more effective if the standard treatment (metformin, insulin) was supplemented with standardized *Ginkgo biloba* 80 mg dry leave extract, containing 19.2 mg of *Ginkgo* Flavon Glycosides and 4.8 mg of terpene lactones (ginkgolide, bilobalide) used one capsule three times a day for 18 months compared with the standard therapy supplemented with green tea and combination product *Grinvitals Cereloba®Plus*.
3. CNNP clean-up workers with T2D are adapted to oxidative stress conditions, and therefore there are less pronounced changes in the antioxidative system and oxidative stress parameters than in T2D patients who were not exposed to low doses of ionizing radiation, although they are higher than in CNNP clean-up workers without T2D and the control group.

Confirmation of Hypotheses of Doctoral Thesis

1. A long-term combination use of non-steroidal anti-inflammatory drug Ibuprofen, the lipophilic antioxidant − vitamin E, and hydrophilic antioxidant − Se normalized oxidative stress parameters. However, the correlation between antioxidants and pro-oxidants provided the evidence of oxidative stress preservation in CNNP clean-up workers.
2. Six month long combination use of non-steroidal anti-inflammatory drug Ibuprofen, lipophilic antioxidant − CoQ10, and hydrophilic antioxidant – Se

regulated the activity of antiradical anti-peroxidative enzymes in the anti-oxidative defence system, increased the amount of non-enzymatic antioxidant and hindered the increase in lipid peroxidation in CNNP clean-up workers.

3. Study of long-term efficacy of naturally occurring antioxidant preparations in T2D patients showed that use of *Ginkgo biloba* dry leave standardized extract 80 mg containing 19.2 mg Ginkgo Flavon Glucosides and 4.8 mg terpene lactones (ginkgolide, bilobalide) one capsule three times daily for 18 months was the most efficient in lowering oxidative parameters compared with both the applied standard therapy (metformin, insulin) and use of the green tea leaves in a standardized extract and *Grinvitals Cereloba® plus* product.
4. In general, the comparison of the groups showed that T2D patients had considerably more significant changes in anti-oxidative and oxidative stress parameters than CNNP clean-up workers with T2D, while in CNNP clean-up workers without T2D they are less expressed than in T2D patients and CNNP clean-up workers with T2D.

5. PRACTICAL RECOMMENDATIONS

1. Ibuprofen as a mono-preparation can be effectively used for the first three months, in case of a long-term treatment, there is a need for supplementation with one (or several) antioxidant, thus contributing to reduction of lipid peroxidation products formation and, at the same time, to activation of enzymatic antioxidant defense system.
2. Based on the findings of the given study and scientific literature data, it is possible to draw a conclusion that lipid and protein oxidation metabolites − malondialhyde, 4-hydroxynonenal and protein carbonyl play an important role in the development of multifunctional diseases, including T2D, in CNNP clean-up workers. In order to prevent such deseases, it is essential to use natural supplements with anti-radical properties and natural selenium preparations.
3. Firstly, it is essential to include natural antioxidants in the early stages of T2D treatment, before the onset of secondary complications. Secondly, if the blood tests show selenium deficiency, it must be compensated by using natural selenium supplements alongside with natural antioxidants as part of combination treatment.

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