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Antioxidant Complexes and Lipoprotein Metabolism – Experience of Grape Extracts Application Under Metabolic Syndrome and Neurogenic Stress

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1. Introduction

The oxidative hypothesis of atherosclerosis states that peroxide modification of LDL (or other lipoproteins) is important and probably required for the pathogenesis of arterial sclerotic disease; thus, there is an assumption that inhibition of LDL oxidation would increase or prevent atherosclerosis and its clinical consequences [1]. It is believed that the basis for the atherosclerotic plaque development is the foam cell formation from oxidized low-density lipoproteins (LDL) captured by monocytes and macrophages via scavenger-receptors.

Oxidation of LDL is also important for the healthy vessel functioning. High LDL concentrations can suppress the function of arteries in relation to release of nitric oxide from the endothelium, and many of such effects are mediated by the products of lipid oxidation [2]. Moreover, oxidized LDL inhibit the endothelium-dependent nitric oxide mediated relaxations in a rabbit isolated coronary arteries. Oxidized LDL induce apoptosis in the vascular cells, including macrophages, and this is prevented by nitric oxide [3].

One of the most important mechanisms of the inflammation proatherogenic effect is development of the systemic oxidative stress, and, as a consequence of proatherogenic abnormalities of the blood lipoprotein metabolism, there is appearance of antibodies to them, alterations of the main artery wall structure [4].

At the same time, on the one hand, a high atherogenicity of strongly oxidized LDL, especially tiny subfractions, has been confirmed; on the other hand, the oxidative stress is one of the causes of endothelial dysfunction.

Endothelium vascular wall cells are involved into the interaction with the pathogenic LDL [5]. While macrophages are being overloaded with esterified cholesterol, oxysterols and other biologically active substances, including powerful enzymes with a wide spectrum of action, a foam cell is formed from the macrophage. Yet so far to its apoptosis the foam cell secrets a wide complex of interleukins, enzymes, mediators. Many of them induce a local inflammatory process, destruction of the surrounding intercellular substance, damage of the fibrous structures and separate cells.

Many factors are considered as the most important factors for atherosclerosis development risk. Among such factors an important role belongs to the so-called proatherogenic states, including chronic stress and metabolic syndrome (MS) [6]. The proatherogenic character of stress is connected, first of all, with the activation of free radical oxidation and hyperlipidemia development. One of the principal statements of all contemporary conceptions of the atherosclerosis pathogenesis is thought to be the destruction of the cell membrane structure, which universal damage factor is peroxide oxidation of lipids (POL) [7].

It is well-known that free-radical processes play the leading role in atherosclerosis pathogenesis. So the antioxidants using in correction of proatherogenic states is fully explicable especially when we speak about natural antioxidants. Thus, the investigation of their biological effects under stress and metabolic syndrome is of grate interest and may be a perspective direction of research.

At the same time it is known that the enzymes associated with HDL, paraoxonase and PAF-acetyl hydrolase can hydrolyse biologically active lipids of mm-LDL, destroy monocyte aggregates and decrease the endothelial activation of mm-LDL [8]. HDL also contain a high concentration of tocopherol due to which they can be free radical scavengers as well.

Antioxidants protect LDL from peroxide oxidation and consequently from intensive uptake of LDL by macrophages decreasing the foam cell formation, the endothelium damage and possibility for lipids to infiltrate the intima. This condition supports the actuality of searching medicines for treating atherosclerosis, in which inhibition of the POL process plays an important part in the mechanism of their action [9]. Tocopherol, carotene, probucol, a number of plant medicines containing flavonoids are proposed as antioxidants.

The overwhelming majority of antioxidant substances used in pharmacotherapy are xenobiotics and so substrates of CYP system actvating ROS formation. Moreover some of them, such as probucol, leade to HDL-C decreasing.

Therefore, the substances of natural, in particular, plant origins that possess a complex activity draw attention of researchers.

Phenolic compounds are widely present in the world of plants; they are the most widespread product of the plant metabolism. Participation of polyphenols in redox processes to produce stable quinone structures by their phenolic forms reveals an antiradical direction of their action which provides their direct antioxidant activity. At present it has been proven that polyphenols as antiradical agents not only hinder the initiation of free radical oxidation, but also interrupt the chain of lipoperoxidation [10]. A great variety of

studies carried out both *in vitro* and *in vivo* supports the ability of polyphenols to inactivate ("to bind", "to scavenge") the radicals that initiate chains of oxidation. First of all, it relates to the primary ROS - $\cdot O_2^-$ and $\cdot OH$ [11].

There are some data that such natural polyphenols as catechins and procyanidins exposed to the human blood plasma produce certain complexes primarily with ApoA-1, i.e. with HDL.

One of the richest sources of polyphenols is *Vitis vinifera* and products of its processing, in particular wine.

Phenolic substances of grapes, including flavonoids and other polyphenols of grape, wine and grape seeds, are of a great interest due to their antioxidant properties and the ability to scavenge free radicals [12].

Studies *in vitro* have shown that grape, wine and grape seeds inhibit the oxidation of LDL. The activity of those substances as oxidation inhibitors in wine diluted 1,000 times markedly exceeded the analogous values for vitamins C and E [13]. It has been experimentally proven that red wine polyphenols slow down LDL oxidation processes and prevent platelet aggregation, thus preventing coronary heart diseases [14].

However, there is not a lot of research in this field yet. Arguments for anti-atherogenic properties of antioxidants are not enough. Results of convincing research are needed in order to decisively recommend antioxidants for treatment and prophylaxis of atherosclerosis.

2. Actuality

Taking into account the leading role of the free-radical processes in atherosclerosis pathogenesis one can make a conclusion about expediency of using natural antioxidants in prophylaxis and correction of this disease. [15]. Consequently, the study of the antioxidant influence on the development of stress-reactions and metabolic syndrome (MS) with the purpose of prevention of harmful complications for the cardiovascular system is of undoubted interest.

A number of studies also confirm the ability of a natural antioxidant α-tocopherol to reduce the risk of cardiovascular system diseases developed in patients with MS. It has been found that administration of α-tocopherol limits oxidation and cytotoxicity of LDL in the blood plasma significantly, supports the vascular endothelial function and reduces the intensity of systemic inflammation in the conditions of MS. The inhibiting effect of this antioxidant on aggregation and adhesion of platelets, adhesion of monocytes to endothelial cells and the smooth muscle cell proliferation has also been shown. However, numbers of experimental studies confirm that the single use of α-tocopherol is not enough for prevention of cardiovascular diseases in patients with MS [16]. It has been determined that the use of α-tocopherol in combination with ascorbic acid and aspirin (as a thrombolytic drug) is more effective [8]. In the ASAP study, the combination of vitamins E plus C was also tested, and this significantly decreased the intima-to-media progression rates in human. The ATBC clinical study used a combination of vitamin E and β-carotene in human as a secondary

prevention strategy; however, no benefit on major coronary events has been found. The large MRC/BHF Heart Protection Study (HPS) for secondary prevention also examined the benefit of the antioxidant combination (vitamins E and C and β-carotene).

A strong dose-dependent effect of α-tocopherol administration is one of the unwanted effects. It is known that even a slight increase of the α-tocopherol dose could affect lipoprotein oxidation, the endothelium function and the degree of systemic inflammation [12].

The results of Cambridge Heart Antioxidant Study (CHAOS) of using antioxidants in cardiology published in 1996 give the opportunity to say that in patients with true (confirmed by angiography) coronary atherosclerosis vitamin E administration (a daily dose of 544-1088 mg (400-800 MU) reduces the risk of non-fatal myocardial infarction. The overall mortality from cardiovascular diseases in this case does not decrease. A favourable effect is revealed only after one-year administration of tocopherol.

At the same time in the Heart Outcomes Prevention Evaluation (HOPE) study, which was devoted to the study of the action of both ramipril and vitamin E (400 MU/daily dose), it was found that use of this antioxidant during approximately 4.5 years did not cause any effect on either the primary (myocardial infarction, insult and death from cardiovascular diseases) or any other end points of research. In another large-scale study on the primary prophylaxis of atherosclerotic diseases in people at least with one risk factors (hypertension, hypercholesterolemia, obesity, preliminary MI of the closest relative or advanced age) vitamin E (300 ME/daily dose) was used during 3.6 years and did not reveal any effect on any of the end points (the incidence of cardiovascular events and death). The vitamin E effectiveness was also not confirmed for various other cases (hypercholesterolemia, the level of sportsmen training, sexual potency, retardation of aging processes, etc.).

Empirically vitamin E is used in various diseases; however, the majority of the reports about tocopherol effectiveness is based on the single clinical observation and experiment data. Nowadays there are no reliable results on the role of vitamin E in prevention of tumour diseases, though the ability to reduce formation of nitrosamines (potentially carcinogenic substances being formed in the stomach), to decrease the formation of free radicals and have antitoxic effects when using chemotherapeutic remedies is well-known. In addition, the long-term intake of vitamin E in the doses from 11 to 800 mg does not cause side effects.

In HDL Atherosclerosis Treatment Study (HATS) there was the treatment of atherosclerosis depending on the high density lipoprotein cholesterol (HDL-C) level; in 160 patients with coronary heart disease with the confirmed coronary artery stenosis and the low HDL-C level the higher (800 MU/day) dose of vitamin E than in HOPE was used. The treatment combination also included 1000 mg of vitamin C, 25 mg of β-carotene and 100 mg of selenium. The study lasted 3 years and revealed that antioxidants had no influence on the HDL-C level, but in combination with hypocholesterolemic drugs they reduced their effect on LDL-C and especially – on HDL-C.

The dominant carotenoid revealed in blood and various tissues (such as liver, kidneys, adrenal glands, ovaries and prostate) is lycopene. Due to its structure and mechanism of action lycopene belongs to the group of antioxidants; a lycopene molecule contains 13

double bonds, which can interact with free radicals. Like β-carotene lycopene can serve as a precursor of vitamin A. However, the lycopene antioxidant activity is two times stronger than that of vitamin A.

Lycopene is recommended as an adjuvant in the treatment of the following diseases: idiopatic male infertility, chronic prostatitis, preeclampsia and intrauterine growth retardation (IUGR), mastopathia, diabetes mellitus, cardiovascular diseases, leucoplakia, age-related degeneration of yellow spots and cataract [17]. As with other oxidants, lycopene is administered in immunodeficiency states against chronic infections and to reduce the harmful action of unfavourable environmental factors.

The most representative evidence of the antioxidants' positive role in cardiovascular diseases prophylaxis was obtained in the multicultural European community multicentre study on antioxidants, myocardial infarction, and breast cancer (EURAMIC), during which the relationship between the antioxidant status and acute myocardial infarction in patients from 10 European countries was determined. The protective action was proven only for lycopene. In the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) the high level of blood plasma lycopene is associated with decreased risk of acute coronary syndrome and insult. In Erasmus Rotterdam Health Study (ERGO, also called "Rotterdam Study") it has been proven that lycopene prevents development and progression of atherosclerosis.

The meta-analysis of 72 epidemiological studies conducted concerning the connection between the tomato intake and cancer has determined the associative feed-back between the blood plasma lycopene level and the risk of cancer in 57 studies and 35 from 57 obtained associations were statistically significant [18].

Probucol (phenbutol) is a hypolipidemic medicine and belongs to butyl phenol derivatives. Probucol is the medicine that is similar in structure to hydroxytoluene – the compound with the potent antioxidant properties.

The hypolipidemic effect of Probucol is caused by activation of non-receptor ways of LDL extraction from the blood. It is believed that the prominent antioxidant activity of probucol prevents LDL oxidation.

Probucol decreases the total cholesterol content in plasma due to intensification of the LDL catabolism at the final stage of cholesterol elimination from the organism. It also inhibits the cholesterol biosynthesis at early stages and to a small extent slows down the food cholesterol absorption. It does not influence the triacylglycerol and the VLDL content, but significantly decreases the antiatherogenic HDL level in the blood. It is believed that decrease of the HDL-C level reflects improvement of cholesterol esters transfer with HDL on acceptor lipoproteins due to increase of the cholesteryl ester transfer protein (CETP) activity.

In spite of undesired decrease in the HDL-C concentration probucol causes regression of xanthelasma; this effect is revealed best of all in patients with the most dramatic HDL-C decrease. This important observation demonstrates that the low HDL-C content is not undoubtedly a negative phenomenon. The data obtained in experiments with animals indicate that probucol due to its antioxidant properties prevents lipid peroxidation and thereby inhibits

the LDL uptake by macrophages, therefore, it inhibits atherogenesis. This allows suggesting that the therapeutic effect of the medicine may not be connected with its ability to decrease the LDL level. There is no clinical evidence of this hypothesis at the moment.

The medicine is absorbed slowly when taken internally, it is readily soluble in the adipose tissue releasing gradually into the bloodstream, and so its action is kept for a long time (up to 6 months after discontinuation of the treatment).

When using probucol in MultiVitamins and Probucol (MVP) research the renewal of the endothelium function in patients with IHD, decrease of restenosis cases after coronary angioplasty (when taking it at least 4 weeks before the procedure and further treatment during 6 months) was observed. Other antioxidants (α-tocopherol in high doses (700 mg per day), β-carotene and vitamin C) turned out to be ineffective.

Combined application of the endogenous antiradical antioxidants is of particular interest. In HPS (Heart Protection Study) along with the study of the simvastatin effectiveness the prophylactic action of antioxidants was investigated. The use of the vitamin complex (600 mg of vitamin E, 250 mg of vitamin C and 20 mg of β-carotene per day) lasted in average 5.5 years and did not reveal any differences in placebo groups and groups taking vitamins. Moreover, if the tendency exists, it reflects increasing of vascular events in the antioxidant intent-to-treat group. The action of antioxidants was compared with the effect of the combined use of simvastatin and nicotinic acid (niacin). Moreover, one of the groups received simvastatine+niacin and antioxidants. Angiographic and clinical data of this study were also disappointing with respect to the use of antioxidants.

Unfortunately, a great part of the compounds synthesized, which are used for pharmacocorrection of these states, are xenobiotics, so they can activate the free-radical formation process. Synthetic antioxidants, in particular probucol, can not be recommended for patient use because they decrease the HDL-C level.

The lack of antioxidant medicines popularity and the absence of traditions of their common use in practical medicine are caused a number of reasons: unsatisfactory previous study of this issue, complexity of adequate estimation of oxidation state parameters in the organism and the absence of the effective medicines with the antioxidant activity that are able to quickly reduce the consequences of the oxidative stress.

Therefore, the main indications for using antioxidants are excessively activated free-radical oxidation processes accompanying different pathologies. The choice of specific medicines, correct indications and contraindications for their use has not been developed yet and require further research.

3. Experiment design

In our experiments we studied the indicators of lipid and lipoprotein metabolism in the blood plasma and the liver under the experimental metabolic syndrome (MS) in Syrian hamsters of different sex and age.

In the experiments purebred male rats with 180-220 g of the body weight were used. The animals were kept in vivarium on a balanced diet. During 21 days the animals were given low alcoholic beverages from grapes of red and white grades *per os* daily. These beverages were introduced in the maximum effective doses of 9 mg of polyphenols/100 g the body weight. Taking into account the fact that the polyphenol content in the beverages investigated was quite low, the effective dose was introduced 3 times a day by 2 ml of liquids per 100 g of the animal's body weight. Control animals were introduced the corresponding volume of the saline solution. Ethanol was given in the corresponding dose.

Stress was caused by immobilization on the abdomen for 3 hours [19]. Animals were decapitated 3 hours after the immobilization. The blood was collected to get the serum. The liver was perfused by the cold extraction medium (0.25 M sucrose in 0.025 M tris-HCl, pH 7.5), homogenized in the Potter homogenizer with 2 ml of the extraction medium per 1 g of the liver. All manipulations with animals were held under chloralose-urethane anaesthesia.

To distribute the plasma lipoproteins the samples were centrifuged at 65,000 rpm (342,000 g) for 4 h at 4°C in the Optima XL-100K ultracentrifuge (Beckman Coulter) set at slow acceleration and deceleration [20]. Samples were fractionated within 1 h of centrifugation.

Lipids were extracted with chloroform and methanol (1:2 v/v) twice, as described by Bligh et al [21], and the supernatant was collected for determination of TG and FFA. TG and FFA were determined by enzymatic colorimetric methods with commercial kits (Zhongsheng, Beijing, China). The total cholesterol content was detected with the help of standard enzymatic cholesteroloxidase kits of "Boehringer Mannheim GmbH diagnostica" firm (Germany). The total lipid concentration was determined with the help of a standard kit "Eagle Diagnostics" (USA) – the reaction with vanillin reagent.

Determination of the lipid peroxide product quantity was performed in heptane-isopropanol extracts [22]. The optical density was measured at the wavelength of 220 nm (for compounds with isolated double bonds), 232 nm (for diene conjugates) and 278 nm – for ketodienes and conjugate trienes.

The TBA content was determined on the spectrophotometer with the help of the reaction with thiobarbituric acid [23].

A modified version of the high performance liquid chromatography (HPLC) procedure developed by Stacewicz-Sapuntzakis et al. [24] was used to measure vitamins E in the plasma. The HPLC system included a 150 × 3.9 mm Nova-pak C18 (4 microns) column with a guard-pak pre-column (both from Waters, Milford, MA), Waters Millipore TCM column heater, Waters 490 multi-wavelength detector, Hitachi 655–61 processor, Hitachi 655A-11 liquid chromatography, and BioRad autosampler AS-100.

The serum ascorbic acid concentrations were measured as described by using HPLC [25] with salicylsalicylic acid as a deproteinizing agent, metaphosphoric acid as a stabilizer.

The serum PON1 activity was measured by the rate of generation of p-nitrophenol determined at 405 nm according to MacKness B et al. [26].

The plasma cholesterol ester transfer protein (CETP) activity was examined using the modifications of Khosla et al. [44]. The CETP activity in duplicate10-µL aliquots of the plasma was determined after incubations with ^{3}H-cholesterol ester (CE)-labeled HDL$_3$ and LDL. Radioactivity transferred from ^{3}H-HDL$_3$ to LDL (measured in the supernatant after precipitation with heparin/MnCl^{2+}) was used to calculate the CETP activity (expressed as the percentage of radioactivity transferred from ^{3}H-HDL$_3$ to LDL per 16 h of incubation).

To measure endothelium-bound LPL, the perfusion solution was changed to buffer containing 1% fatty acid–free BSA and heparin (5 units/ml). The coronary effluent was collected in timed fractions over 10 min and assayed for the LPL activity by measuring the hydrolysis of a sonicated [3H]triolein substrate emulsion [27].

The plasma LCAT activity was measured by determination of the amount of radioactivity in each spot calculating the free cholesterol/ total cholesterol ratio in each plasma sample before and after the LCAT reaction and thus estimating the esterification rate [28]. The fractional esterification rate $(\% \cdot h')$ expressed as the percentage of the free cholesterol esterified in the plasma sample per hour.

The HL activity was evaluated using the glycerol-stabilized emulsion of triolein and egg phosphatidylcholine containing glycerol-tri[9,10(n)-^{3}H] oleate by determination of the radioactivity amount during incubation [29].

Statistical analysis. All data were analyzed for statistical significance with SPSS 13.0 software. The data were presented as means ± standard deviation. Statistical analysis used one-way ANOVA. $P<0.05$ was considered to be statistically significant.

4. Discussion

The results of our studies suggest the existence of significant changes in the lipid metabolism, as well as sex and age differences in the lipid and lipoprotein metabolism both in healthy animals and in animals with MS.

In male hamsters fed with a high-calorie diet atherogenic dyslipidemia develops independently of age (Table 1). As it can be seen from the data obtained, increase of the total lipid content in the animal blood plasma is caused by increasing of the ApoB-containing lipoprotein (ApoB-LP) level since the HDL content is not changed. At the same time it has been found that the plasma TAG level in young (47%) and in adult animals (30%) increased in comparison with the intact group.

Increase of the TAG blood content in conditions of MS is considered to be a key factor for development of atherogenic dyslipidemia that is typical for this pathology [30]. A strong correlation between hypertriacylglycerolemia plus the HDL-C level decrease and accumulation of LDL*B* in the blood plasma has been demonstrated in many experiments and clinical studies [18].

It is assumed that atherogenic alterations occur as a result of lipoprotein disbalance in the blood plasma, i.e. because of predominance of the LDL and VLDL fractions over the

antiatherogenic HDL fraction (especially when the values of the LDL+VLDL/HDL index are higher than 3.5).

Age	Group	Parameters			
		TAG, g/L	Total cholesterol, mmol/L	ApoB-LP, g/L	HDL, g/L
4 weeks	Intact	1.06±0.07	2.93±0.19	4.72±0.23	1.11±0.05
	MS	1.56±0.09*	3.56±0.10*	6.68±0.15*	0.98±0.07
20 weeks	Intact	1.57±0.22	2.84±0.15	5.66±0.34	1.01±0.02
	MS	2.00±0.13*	3.71±0.18*	6.68±0.21*	0.85±0.08
1 year	Intact	1.50±0.10	2.73±0.02	5.21±0.06	1.74±0.13
	MS	2.27±0.13*	3.15±0.08*	7.00±0.22*	2.32±0.13*

The data presented as mean±SD

* –p≤0.05 versus intact animals

Table 1. Some plasma lipid values in male Syrian golden hamsters with MS (in each group n=10).

As it is known, there are 2 phenotypes of LDL: LDLA and LDLB that differ by size, density, the lipid content and the atherogencity coefficient. LDLB have less size (d 25.5-25.75) comparing to LDLA (d > 25.75) and are characterized by a lower content of polar lipids, as well as a higher content of cholesterol esters. Lipoproteins of this subfraction are slowly removed from the bloodstream that is caused by their low affinity to B/E-receptors for LDL, higher sensitivity to glycosylation and oxidative damage [31]; they also have a high affinity to scavenger-receptors of macrophages [32].

All this features explain a high atherogenicity of LDLB subfraction. Numerous clinical and epidemiological studies have confirmed that accumulation of LDLB in the blood is an independent risk factor for atherosclerosis occurrence [33].

Normally, there are predominantly LDLA in the blood plasma, and LDLB are present in a small percent of the total LDL, but in MS and insulin resistance the LDLB content increases significantly.

It is well-known that in MS the key factor for TAG and ApoB-LP accumulation in the blood is the VLDL hyperproduction by the liver [34]. According to our data, accumulation of ApoB-LP in the blood occurs parallelly with increase in the content of this lipoprotein fraction in the liver (Table 2).

These results allow us to make a suggestion that VLDL formation is activated in the liver of the animals fed with a high-calorie diet in our experiment.

The mechanisms of the VLDL hyperproduction by the liver in the conditions of FFA intensive supply to hepatocytes have remained still unclear. The stimulation of VLDL formation can occur both by using the elevated uptake of the blood FFA and via activation of fatty acid biosynthesis *de novo* because of hyperglycemia.

Age	Group	Parameters				
		Total lipids, mg/g liver	ApoB-LP, mg/g liver	HDL, mg/g liver	G6PDH, nmol/mg protein/min	Lysosomal lipase, nmol/mg protein/min
Week 4	Intact	104.24±2.52	11.46±0.37	1.25±0.14	3.74±0.33	0.67±0.03
	MS	124.16±2.05*	15.16±0.54*	1.11±0.07	2.80±0.17*	1.09±0.07*
Week 20	Intact	112.62±2.66	13.03±0.50	0.94±0.10	4.44±0.28	0.54±0.03
	MS	143.59±2.65*	15.69±0.36*	1.10±0.20	3.13±0.28*	1.27±0.09*

The data presented as mean±SD
* –$p \leq 0.05$ versus intact animals

Table 2. Some liver lipid metabolism values in male Syrian golden hamsters with MS used in the current study (in the crude tissue, in each group n=10).

It is known that in insulin resistance FFA that come to hepatocytes from the blood are primarily used for the TAG re-synthesis. It leads to increase in the intracellular TAG content and correlates with the increase of the VLDL secretion rate into the bloodstream. The VLDL morphology, which is specified predominantly at the second stage of their formation, depends significantly on the intracellular TAG content and hepatocyte sensitivity to insulin [35]. More active phospholipase D-dependent pre-VLDL lipidation takes place in the elevated intercellular TAG content and insulin resistance of hepatocytes [36]. Insulin blocks the VLDL1 formation in the liver. In the conditions of insulin resistance this effect and the elevated intercellular TAG content stimulate formation and secretion predominantly of VLDL1 by the liver [37].

The VLDL1 secretion increase leads to significant changes in the lipid and lipoprotein metabolism in the blood: the increased TAG content and accumulation of LDL*B* with high atherogenicity in the blood. These changes are typical for MS and considered to be separate risk factors for development of atherosclerosis.

Metabolism of ApoB-LP in the blood plasma is tightly connected with metabolism of HDL performing a reverse cholesterol transport from peripheral tissues to the liver. The leading factors in the process of transformation of VLDL into LDL in the bloodstream and determination of the LDL morphology are the rate of cholesterol esters transfer from HDL to ApoB-LP mediated by cholesteryl ester transfer protein (CETP), and the rate of TAG hydrolysis in the ApoB-LP composition mediated by lipoprotein lipase (LPL) and hepatic lipase (HL) [38].

According to data of many clinical studies, increase of the CETP activity of the HDL composition in most cases leads to decrease of the HDL-C level and accumulation of LDL*B* in the blood plasma. Moreover, a degree of these modifications correlates with the blood TAG level.

We observed significant changes in the cholesterol and HDL metabolism in the blood plasma in animals fed with a high-calorie diet. These changes have expressed the proatherogenic character and could be one of the causes for the LDL*B* accumulation in the blood.

Our results suggest that increase of the total blood cholesterol level in hamsters fed with a high-calorie diet is obviously connected with increase of the cholesterol content in the ApoB-LP composition as its level in the HDL composition decreases (Table 3).

Age (at the beginning of the experiment)	Group	Parameters			
		HDL-C, mkmol/L	HDL-CE, mkmol/L	LCAT, mkmol/l/h	CETP, mkmol/l/h
Week 4	Intact	174.17±18.99	1028.33±12.76	54.92±0.58	20.42±1.76
	MS	80.83±9.17*	810.00±22.78*	49.00±2.50	33.83±1.56*
Week 20	Intact	138.00±8.00	770.00±32.56	45.50±2.55	59.50±5.39
	MS	164.50±9.97	512.50±0.01*	20.25±2.28*	116.88±9.43*

The data presented as mean±SD

* –p≤0.05 versus intact animals, ᵃ –p≤0.05 versus intact animals 4 weeks.

Table 3. Plasma HDL-C and HDL-CE, cholesterol esterifying activity and CE transfer in Syrian golden hamsters with the experimental MS (in each group n=10).

Decrease in the HDL cholesterol level is apparently connected with increase of the transfer rate of cholesteryl esters from HDL to ApoB-LP. According to our data the rate of the cholesteryl esters transfer from HDL in the animals fed with a high-calorie diet grows to 166% and 199% compare to the values of young and adult intact animals, respectively (Table 3).

At the same time decrease in the free cholesterol and HDL esterified cholesterol levels was determined in young males, but in adult animals only the HDL esterified cholesterol content lowered. The cholesteryl ester transfer rate from HDL to ApoB-LP is activated when the TAG content increases in the blood, it is observed in the postprandial period, as well as in ApoB-LP metabolism abnormalities [39]. In both cases the cholesteryl ester transfer activation is a consequence of increasing the TAG-rich lipoproteins (TRL) in the bloodstream [40]. The latter is also confirmed by our data pertaining to the increase of the neutral lipids content in the ApoB-containing lipoproteins in hamsters with the experimental MS. These differences seem to be connected with the difference in the HDL free cholesterol esterification rate in males of various ages. This rate is primarily determined by the activity of LCAT – the enzyme associated with HDL [41].

The increase of the cholesteryl-ester transfer activity from HDL is mostly the result of the CETP activation. The increase of the CETP activity in MS was demonstrated in a great number of experiments [22]. It is known that the activation of CETP biosynthesis in the liver is primarily the cause for increasing the activity of this protein in the blood HDL composition, but mechanisms of CETP induction have been still unclear.

Thus, increase of the cholesteryl ester transfer rate from HDL on the background of hypertriacylglycerolemia, which is observed in our experiment in the animals fed with a high-calorie diet (Table 3), is atherogenic since the cholesteryl ester transfer predominantly to TAG-enriched lipoproteins leads to accumulation of CE-enriched VLDL1, which are major precursors of LDL*B*. Intensive TAG supply to HDL in exchange for cholesteryl esters results in accumulation of TAG-enriched HDL particles, which are the predominant

substrate for hepatic lipase (HL), in the blood. So, HDL particles are rapidly removed from the bloodstream and it leads to decrease of the HDL-C content.

That is why changes in the enzymes activity, which hydrolyze lipoprotein lipids in the bloodstream, in particular – in LPL and HL activity, affect significantly the lipoprotein metabolism in MS.

TAG in the TAG-enriched lipoproteins (chylomicrons and VLDL) are the substrate for LPL. FFA, released after hydrolysis under the action of LPL, come to adipocytes and muscle cells where they are deposited as the TAG component or used as a source of energy. TAG hydrolysis in the VLDL composition increases availability of cholesterol for its transfer to HDL, therefore, in this way LPL mediates the reverse cholesterol transfer. The LPL activity is regulated by the influence on transcription, translation and enzyme transport from the cells. Insulin is known to activate LPL that results in decrease of the total blood TAG level and stimulation of cholesterol reverse transfer [35].

According to our data, the plasma LPL activity decreased in young male hamsters fed with a high-calorie diet (Table 4).

Age (at the beginning of the experiment)	Group	Parameters	
		LPL (U/ml)	HL (U/ml)
Week 4	Intact	8±2	51±4
	MS	4±1*	91±3*
Week 20	Intact	83±2	3±1
	MS	129±3*	2±1

The data presented as mean±SD
* –p≤0.05 versus intact animals

Table 4. Postheparin plasma lipase activities in Syrian golden hamsters with the experimental MS (in each group n=10).

The results obtained are in agreement with the literature data about the reduction of the LPL activity in obesity and insulin resistance [42]. The mechanisms of the LPL activity inhibition in these conditions are still unclear though a definite contribution could be made by development of insulin resistance.

The increase of the cholesteryl ester transfer rate from HDL on the background of hypertriacylglycerolemia, which was stated in our experiment both in animals fed with a high-calorie diet and in chronic stress, is an atherogenic factor for two reasons. Firstly, the cholesteryl ester transfer predominantly to the TAG-enriched lipoprotein fractions leads to accumulation of VLDL1 enriched with cholesteryl esters, which are the main LDL*B* precursors. Secondly, the intensive exchange of cholesteryl esters in HDL for TAGs results in accumulation of TAG-enriched HDL in the blood, which are the predominant substrates for HL, and they are rapidly removed from the bloodstream, and it, in turn, causes decrease in the HDL-C concentration. The activation of the lipoprotein secretion by the liver is also observed in the conditions of the acute chemical and emotional painful stress. This fact may

be considered to be a sign of proatherogenesis since it is accompanied by hyperlipidemia development due to increase of atherogenic lipoprotein fractions.

As shown in our studies, decrease of the LPL activity in the blood plasma of young males fed with a high-calorie diet can be an additional factor for TAG accumulation in the blood and the HDL-C level reduction observed in our experiment.

HL mediates a selective transport of VLDL remnants to hepatocytes via LDL-receptors, takes part in reverse transport of cholesterol accelerating HDL coming into the liver via scavenger receptors (SRB1). Hydrolyzing TAG in the ApoB-LP composition HL plays a significant role in their re-modelling in the bloodstream. It is known that the HL activity specifies substantially the lipid composition, size and properties of LDL [43].

The HL activity is predominantly regulated at the transcriptional level under the influence of sex hormones, glucocorticoids and adipokines. The rate of the HL gene transcription is also dependent on the intercellular lipid content, primarily cholesterol in the hepatocytes [44].

In our experiment the blood plasma HL activity in male hamster fed with a high-calorie diet increased irrespective of age (Table 4), it corresponds to literature data. In a number of studies it has been shown that the HL activity increases in insulin resistance, obesity, and a high-calorie diet [45]. Moreover, it has been determined that increase of the HL mRNA content is observed when using a high-calorie diet; this is the evidence of the enzyme biosynthesis activation under these conditions. The authors associate this fact with the decrease in the blood plasma adiponectin level, which can inhibit the HL synthesis in hepatocytes.

Considering these data, as well as the data obtained in our studies about adiponectin decrease in the blood plasma in obesity (Fig. 1), we may suppose that one of the causes for the HL activity increasing when taking a high-calorie diet in our experiment is decrease of adiponectin secretion by the adipose tissue.

The HL activity increase is considered to be one of the key factors for the atherogenic dyslipidemia development in obesity and MS. In a number of works a clear correlation between the HL activity and the LDL*B* content in the blood plasma was demonstrated [19]. It is believed that namely HL activation results in the increased LDL*B* formation [33]. The latter occurs with increase of the TAG-enriched VLDL1 content in the blood and the CETP activation. Furthermore, the HL activity increase leads to decrease of the HDL cholesterol level [46]. This is associated with the fact that hydrolysis of TAG in the HDL3 composition results in their transformation into HDL2, which are rapidly removed from the bloodstream by the liver. Thus, the HDL-C level decrease that we determined in our experiment (Table 3) can be a consequence of the HL activity increase.

In the current study we have found that the blood FFA level increase is accompanied by the ApoB-LP synthesis activation in the liver of Syrian male hamsters fed with a high-calorie diet irrespective of age. This causes increase of the TAG and ApoB-LP level in the blood. Decrease of the HDL-C level is a consequence of the rate of cholesteryl ester exchange between HDL and LDL due to activation of CETP and HL. As a result of these changes the atherogenic dyslipidemia development, which is typical for MS, is observed.
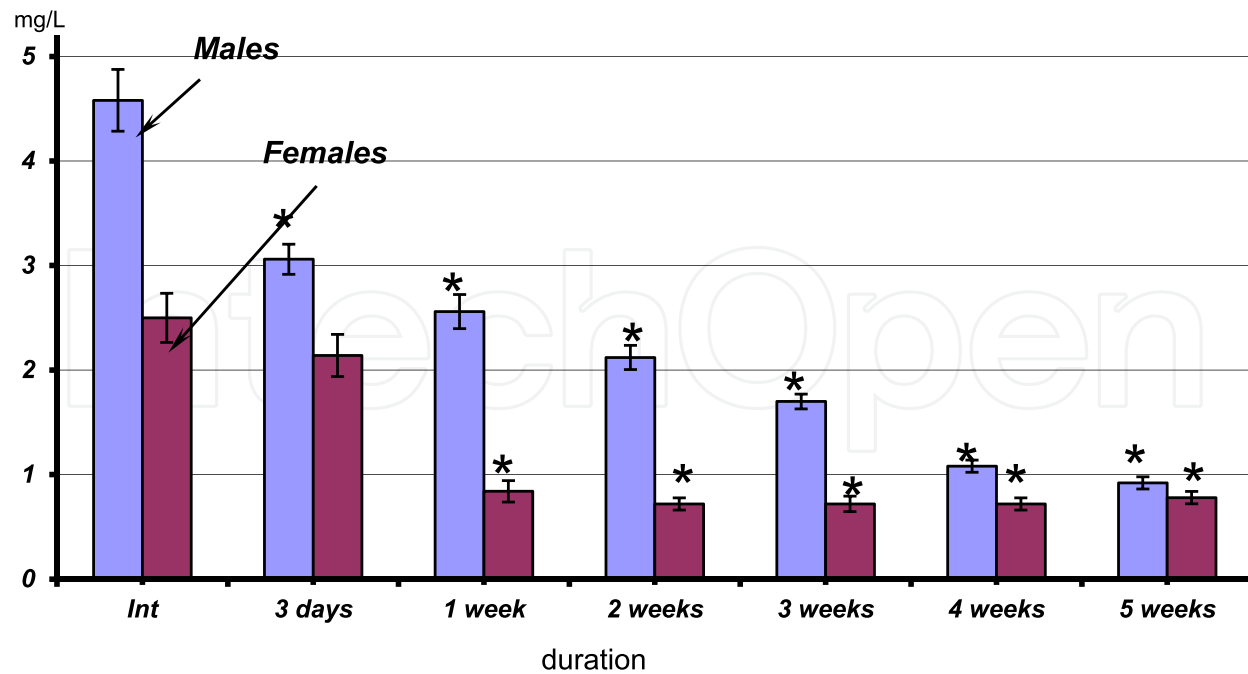
Figure 1. Plasma adiponectine level in Syrian golden hamsters with the experimental MS development (values are mean±SD; * –p≤0.05 versus intact animals, in each group n=10, * – p < 0.05 versus intact animals).

We have found the age differences of the lipid profile in the blood plasma of the normal male hamsters. So, in intact males (with age from 4 to 20 weeks) on the background of the constant content of total lipids and lipoproteins in the blood plasma there was increase of the FFA level (60% comparing to a 4-week intact), TAG (48%) and ApoB-LP (20%), and the HDL level showed a tendency to decrease. These results are the evidence of the lipidation increase with age. It has been also shown that in adult males the unesterified cholesterol and cholesteryl ester levels are lower than in young animals (20% and 25%, respectively), and the cholesteryl ester transfer rate from HDL in adult animals exceeds this index value in young animals (191%) (Table 3).

The data obtained correspond to the literature data about age-dependent changes in the lipid metabolism in males, which have the proatherogenic character [47]. It is known that with age the sex hormones level lowers in males and the glucocorticoid secretion level increases. The plasma lipid profile in males is determined, among other factors, by the secretion level of sex hormones possessing antiatherogenic properties. A lot of studies proved the presence of direct correlation between the blood testosterone plus the dehydrotestosterone level and the HDL-C content [48]. Moreover, the high level of sex hormones correlates with decrease of the TAG content and the total cholesterol in the blood. Thus, increase of the TAG level and decrease of the HDL cholesterol content in the blood plasma of males with age may be connected with reduction of the sex hormone secretion (Table 5). Changes of the lipid profile in the blood plasma of males with age may be also associated with increase of glucocorticoid secretion, which was observed in our experiment (Table 5).

Thus, with age the blood plasma lipid profile in males is subjected to unfavourable changes such as increase of the FFA and TAG content and decrease of HDL-C. The latter may be connected with decrease of the sex hormone level, and increase of the cortisol secretion.

However, despite the more favourable lipid profile in the blood plasma of young males comparing with normal adult animals, atherogenic dyslipidemia in obesity and insulin resistance develops irrespective of age.

In contrast with males, in females the atherogenic dyslipidemia development is significantly dependent on age (Table 6). In particular, while in males with age there are no significant changes in the liver ApoB-LP content, in females this index raises during maturation – in intact animals – to 20%, and in animals in the experimental MS – to 31%. That indicates intensification of lipolytic processes in the liver of females during ageing, and may serve as a manifestation of the lipid metabolism activation. The analogous changes in the total lipid content also proved this tendency (Table 6).

Sex	Parameter	Group	Age (at the beginning of the experiment)		
			4 weeks	20 weeks	1 year
Females	Estradiol, pmol/mL	Intact	0.55±0.05	0.64±0.06	0.54±0.05
		MS	0.63±0.06*	0.75±0.08	0.36±0.04*
	Cortisol, nmol/L	Intact	47.00±3.85	73.17±5.56	76.00±4.95
		MS	74.0±5.49*	87.5±4.45	117.00±2.63*
Males	Estradiol, pmol/mL	Intact	0.24±0.02	0.19±0.02	0.29±0.03
		MS	0.27±0.02	0.29±0.02*	0.20±0.03*
	Testosterone, pmol/mL	Intact	4.02±0.39	4.24±0.31	3.58±0.37
		MS	4.45±0.41*	3.51±0.40*	3.03±0.31*
	Cortisol, nmol/L	Intact	61.17±3.71	94.8±3.06	85.33±5.40
		MS	84.67±3.62*	132.00±7.88*	148.40±9.54*

The data presented as mean±SD
* –p≤0.05 versus intact animals

Table 5. Plasma sex hormones and cortisol levels in hamsters with the experimental MS (in each group n=16)

Age	Group	Parameters			
		Total lipids, mg/g	ApoB-LP, mg/g liver	HDL, mg/g	Lysosomal lipase, nmol/mg protein/min
4 weeks	Intact	117.67±4.72	8.87±0.24	1.27±0.08	0.34±0.03
	MS	144.34±5.00*	10.24±0.25*	0.65±0.05*	1.24±0.05*
10 weeks	Intact	137.54±3.91	10.65±0.46	0.89±0.07	0.83±0.04
	MS	179.22±3.44*	13.44±0.30*	0.46±0.06*	1.33±0.08*

The data presented as mean±SD
* –p≤0.05 versus intact animals

Table 6. Lipid metabolism parameters in the liver homogenate in Syrian golden female hamsters with MS (in the crude tissue, in each group n=16)

Oxidation of LDL and VLDL (i.e. ApoB-LP) is an alternative way of the lipoprotein catabolism, which leads to their uptake by macrophages via scavenger-receptors, and may

lead to the transformation of these cells into "foam" ones. That is why it is one of the factors of atherogenesis in MS.

In our experiment we also observed the composition changes in lipoproteins and in particular HDL particle enrichment with lipids (Table 7). However, the cholesterol content of these lipoproteins decreased in contrast to the ApoB-LP cholesterol content that was increased.

Parameters	Group	
	Intact	MS
Total lipids, % of the total HDL composition	49.45±1.35	57.31±1.91*
Total cholesterol, % of the total HDL composition	14.97±0.23	11.21±0.76*
TAG, % % of the total HDL composition	1.75±0.07	3.08±0.15*
α-Tocopherol, mmol/L	8.02±0.39	5.70±0.35*
Isolated double bonds, U/ml	8.64±0.59	7.31±0.17*
Diene conjugates, mmol/L	18.88±2.10	31.68±1.65*
Ketodienes+conjugated trienes, U/ml	1.15±0.08	1.48±0.06*
Total hydroperoxides, mmol/L	69.04±3.46	78.31±1.33*

The data presented as mean±SD or percentage
* –p≤0.05 versus intact animals

Table 7. The plasma HDL composition in Syrian golden hamsters (1 year) with the experimental MS (in each group n=10).

There are several possible reasons for that phenomenon. One of them is a well-known fact that HDL contains high levels of both unsaturated fatty acids, which are rapidly utilized, and proteins, which hydrophilic properties compensate the lack of phospholipids, as well as α-tocopherol and enzymatic antioxidants, particularly paraoxonase, which protect these lipoproteins from peroxidation. There is no doubt that the changes in the cholesterol metabolism enzymes activity associated with HDL (CETP) are involved in this process (Table 3).

Nevertheless, the content decrease of compounds with isolated double bonds and accumulation of the lipoperoxidation products has been determined in the HDL fraction in MS (Table 7). Moreover, the data obtained have shown that the content of ketodienes and coupled trienes in the HDL fraction is 129% comparing to control; the content of diene conjugates – 168% and the content of the total hydroperoxides – 115%. It has been also found that there is decrease of the α-tocopherol content in HDL (41%) comparing to the control values (Table 7).

Thus, HDL can protect LDL from oxidation "providing" a cell with paraoxonase and PAF-acetyl hydrolase. However, this protective effect of HDL is reduced in response to induction of the stress acute phase in animal models [49].

As can be seen from our data (Table 8), the HDL-associated paraoxonase activity is generally decreased in experimental MS.

Sex	Age	Groups	Activity, nmol/mL/min
Males	4 week	Intact	80.78±3.69
		MS	67.06±3.70*
	20 week	Intact	62.19±2.63
		MS	37.29±3.33*
Females	4 week	Intact	104.41±2.95
		MS	75.45±2.21*
	20 week	Intact	127.27±2.95
		MS	121.93±3.05

The data presented as mean±SD
* –$p \leq 0.05$ versus intact animals

Table 8. The plasma HDL paraoxonase activity in Syrian golden hamsters with experimental MS (in each group n=10).

The data obtained show that application of antioxidant complexes for correction of unfavourable changes in proatherogenic states may be perspective since free radical oxidation activation is a common pathogenetic link of all those states; this link is not only involved in damage of cells and their components, but also as an alternative way of catabolism it accelerates the lipid recyclization.

At the same time, since a significant feature of proatherogenic states is the hormone status imbalance, polyphenolic antioxidants need special attention because these compounds along with the antioxidative activity also demonstrate phytoestrogen properties [50], and it may be an additional factor of the lipid metabolism regulation.

An important effect of flavonoids is scavenging of oxygen-derived free radicals. The experimental systems *in vitro* have also shown that flavonoids possess anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties. The so-called ''Mediterranean diet'' is thought to prevent cardiovascular diseases, as a consequence of its high content of antioxidants, which are crucial in ameliorating oxidative events implicated in many diseases. In addition to the antioxidant/antiradical activity, red wine polyphenols (RWPs) have been shown to possess many biological properties, including inhibition of platelet aggregation, the vasorelaxing activity, modulation of the lipid metabolism, and inhibition of the low-density lipoprotein oxidation.

In our research we have used wine, juice and polyphenolic extracts from grapes of different grades, and polyphenolic concentrates "Enoant" and "Polyphen" obtained from *Vitis Vinifera* grapes to correct the changes in the lipid metabolism in the conditions of the experimental metabolic syndrome, acute and chronical stress. All substances used in our research were developed in National Institute for Vine and Wine "Magarach" (Yalta, Ukraine). The studies carried out have specified that polyphenolic extracts and concentrates are quite active remedies that decrease negative effects in MS though the effectiveness of various substances administered are significantly different.

So, administration of any of the investigated substances has significantly decreased the total blood plasma lipoprotein content in hamsters with MS, but the use of "Cabernet" extract has the most pronounced effect (Fig. 2). The same tendency is observed in decreasing the ApoB-LP content, the total cholesterol and FFA level have also decreased, though practically no difference between the grape varieties investigated has found.
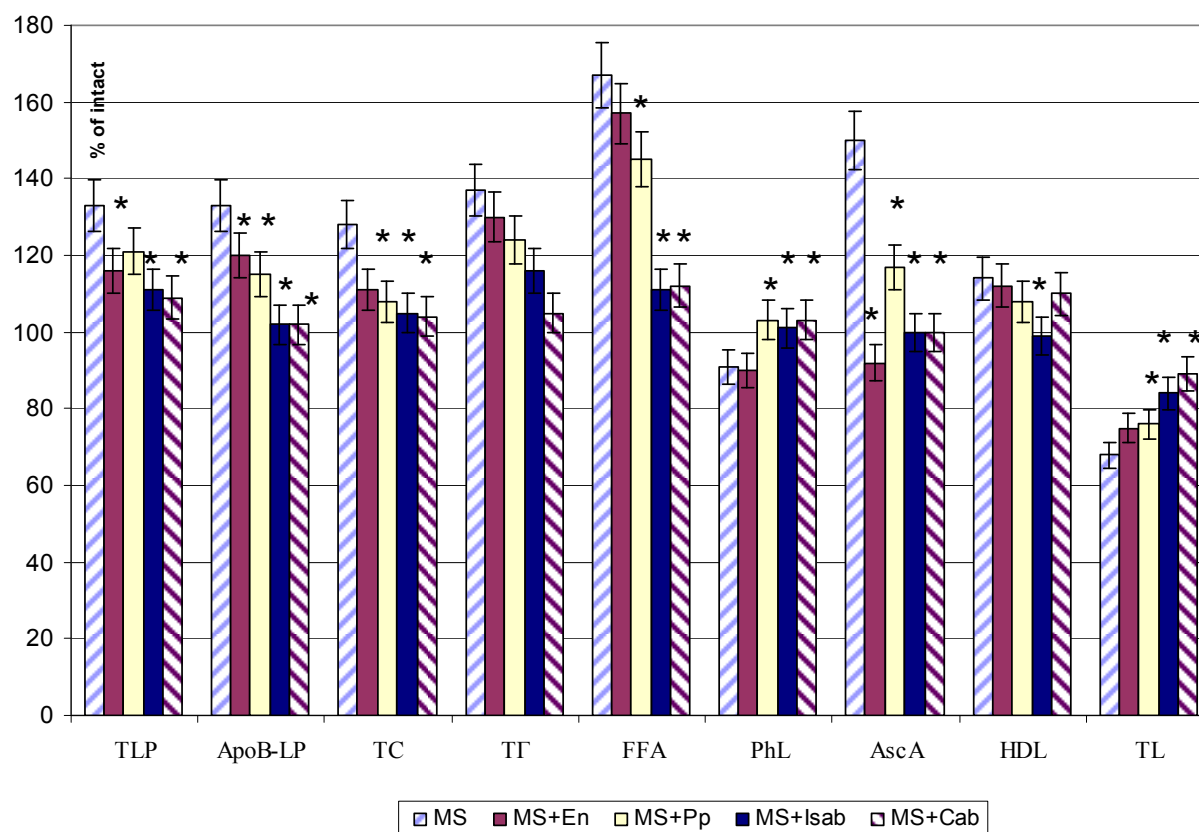
* – p≤0.05 versus intact animals.

Figure 2. The effect of *Vitis Vinifera* substances on some plasma lipid metabolism values in male Syrian golden hamsters (1 year old) with the experimental MS (in each group n= 7)

The non-enzyme antioxidant level (α-tocopherol, reduced glutathione and ascorbic acid) in the blood serum has also reached reference values under the influence of the polyphenolic extracts. This fact confirms the high antioxidant activity of the studied substances.

Normalization of the blood plasma phospholipid content under the influence of polyphenolic extracts arouses the interest. The phospholipid content returned to the intact level, which may be a result of their oxidation reduction, given that the unsaturated fatty acids in phospholipids are compounds that undergo oxidation by free radicals quickly and easily.

However, in spite of the quite favourable effect of "Isabella" extract, its administration also has negative consequences, particularly the HDL level decrease to the value observed in intact animals accompanied by the LDL content increase.

The investigated substances normalize also the blood lipoproteins composition. Thus, the total lipid and the total cholesterol content decrease in the ApoB-LP composition, moreover

"Enoant" lowers the cholesterol content in this atherogenic lipoprotein fraction even below the control level.

Generally, the TAG content is also normalized under the action of all the investigated substances, but taking into account the ratio – cholesterol/triacylglycerols, "Polyphen" has the most favourable effect.

The polyphenol extracts and concentrates have significantly improved the ApoB-LP oxidative status in animals with MS. The best results have been obtained when using "Cabernet", as well as for other indexes investigated (Table 9).

Parameter	Group				
	MS	MS +"Enoant"	MS +"Polyphen"	MS+ "Isabella"	MS+ "Cabernet"
Total lipids, % of the total ApoB-LP composition	88.87 ±0.71*	83.70 ±0.78 */**	83.12 ±0.37*/**	82.22 ±0.09*/**	81.00 ±0.19*
Total cholesterol, % of the total ApoB-LP composition	8.39 ±0.24	7.87 ±0.04*/**	8.13 ±0.04	8.06 ±0.12**	8.17 ±0.08*/**
TAG, % of the total ApoB-LP composition	37.55 ±1.89*/**	53.97 ±0.10*/**	53.02 ±0.14*/**	50.65 ±1.23*/**	49.57 ±0.40*/**
α - Tocopherol, mmol/L	2.68 ±0.08*	2.98 ±0.05*/**	3.06 ±0,04*/**	3.17 ±0,02*/**	3.19 ±0,05*/**
Isolated double bonds, U/ml	1.71 ±0.06*	1.84 ±0.03*/**	1.90 ±0.02*/**	1.97 ±0.03**	2.09 ±0.03**
Diene conjugates, mmol/L	37.25 ±1.50*	30.63 ±0.41*/**	29.54 ±0.34*/**	28.77 ±0.14*/**	26.68 ±1.94**
Ketodienes+conjugated trienes, U/ml	8.18 ±0.11*	7.49 ±0.04*/**	7.23 ±0.08*/**	7.17 ±0.08*/**	6.99 ±0.26**
Total hydroperoxides, mmol/L	108.25 ±1.39*	98.52 ±0.55*/**	94.97 ±0.15*/**	90.65 ±1.15*/**	89.30 ±1.06*/**

The data presented as mean±SD or percentage
* – p≤0.05 versus intact animals, ** – p≤0.05 versus model of MS

Table 9. The effect of *Vitis Vinifera* substances on the plasma ApoB-LP composition in male Syrian golden hamsters (1 year old) with the experimental MS (in each group n= 10)

The HDL composition in the blood is also affected by the substances studied. In these particles the total lipid content decreases and even reaches the level of intact animals when using "Cabernet" extract (Table 10).

The cholesterol level also changes: it decreases when using "Enoant" and increases under the action of "Isabella" and "Cabernet" extracts.

The HDL-C content decreases under the action of "Enoant" may occur due to peroxide processes inhibition since cholesterol accumulation in lipoprotein particles, as it was mentioned before, has a compensatory character in response to the phospholipid oxidation of the lipoprotein particle hydrophilic cover. The TAG content decreased under the action of

all substances, and "Isabella" was the most effective substance. The TAG content decrease is probably mediated by the phytoestrogenic action of polyphenols directed to lipolysis inhibition in the adipose tissue.

Parameter	Group				
	MS	MS +"Enoant"	MS +"Polyphen"	MS+ "Isabella"	MS+ "Cabernet "
Total lipids, % of the total ApoB-LP composition	57.31 ±1.91*	54.91 ±0.21*/**	53.09 ±0.08*/**	51.74 ±0.74*/**	49.20 ±0.42**
Total cholesterol, % of the total ApoB-LP composition	11.21 ±0.76*	10.54 ±0.30*/**	11.14 ±0.04*	12.05 ±0.21*	12.45 ±0.34*/**
TAG, % of the total ApoB-LP composition	3.08 ±0.15*	2.90 ±0.09*/**	2.11 +0.12*/**	1.92 ±0.04*/**	1.96 ±0.03*/**
α - Tocopherol, mmol/L	5.70 ±0.35*	7.47 ±0.20*/**	7.19 ±0.17*/**	7.36 ±0.11*/**	8.13 ±0.06**
Isolated double bonds, U/ml	7.31 ±0.17*	7.67 ±0.08*/**	7.69 ±0.07*/**	7.99 ±0.05*/**	8.15 ±0.01*/**
Diene conjugates, mmol/L	31.68 ±1.65*	24.85 ±0.35*/**	23.44 ±0.40*/**	22.55 ±0.34*/**	21.88 ±0.23**
Ketodienes+conjugated trienes, U/ml	1.48 ±0.06*	1.24 ±0.03**	1.32 ±0.03*/**	1.25 ±0.03*/**	1.54 ±0.47*/**
Total hydroperoxides, mmol/L	78.31 ±1.33*	75.26 ±0.31*/**	75.62 ±0.54*/**	74.48 ±0.55*/**	73.41 ±0.39*/**

The data presented as mean±SD or percentage
* – p≤0.05 versus intact animals, ** – p≤0.05 versus model of MS

Table 10. The effect of *Vitis Vinifera* substances on the plasma HDL composition in male Syrian golden hamsters (1 year old) with the experimental MS (in each group n= 10)

The exact bimolecular mechanisms for this cardioprotection are unclear, but it is likely that actions mediated both through the estrogen receptors, such as the beneficial alteration in lipid profiles and upregulation of the low-density lipoprotein (LDL) receptor, and independently of the estrogen receptors, such as antioxidant action, contribute to the cardioprotective effects of phytoestrogens observed.

The potential role of phytoestrogens, including isoflavonoids, as cardioprotective agents has been extensively reviewed. The data obtained in our experiments showed that in male hamsters with the experimental MS the treatment with grape extracts reduced VLDL cholesterol (VLDL-C) and TG by 30 and 40 % compared with the control animals. Furthermore, golden Syrian hamsters fed with red wine phenolics had a significant decrease in the plasma apo B concentrations. Similar to our previous study, grape polyphenols may have altered hepatic secretion of TG-rich VLDL. This reduction is evident when observing the decreases in both plasma apo B and apo E concentrations. The significant decrease in apo E concentrations may have further reduced plasma TG concentrations. In general, apo E displaces apo C-II from the VLDL particle, thereby inhibiting the lipoprotein lipase (LPL) activity and overall lipolysis. Furthermore, Huang et al. [51] showed that adding apo C-II to transgenic apo-E3–enriched

VLDL increased the LPL activity in a dose-dependent manner. The reductions in apo E and TG concentrations suggest less displacement by apo E, thereby promoting the grape polyphenols activity and further reducing the TG concentrations in the plasma.

Due to decreases in TG concentrations, administration of "Cabernet" extract was shown to affect the overall lipoprotein metabolism. Decreased concentrations of the plasma TG altered substrate availability in the delipidation cascade, leading to the decrease observed in LDL-C concentrations. After a 3-week treatment period the grape polyphenols treatment induced a significant decrease in the cholesteryl ester transfer protein (CETP) activity as well. Such decrease in the CETP activity may be partially a result of the substantial decrease in substrate availability, including both the plasma TG and LDL-C.

It is evident that grape polyphenols modify the packaging of VLDL through alteration in the hepatic enzyme activity and apo B secretion. These modifications seem to decrease the overall secretion of the VLDL particles and therefore, decrease plasma TG and related apo concentrations. Due to decrease of the TG substrate, further modifications in the lipoprotein metabolism may occur.

The alteration in the TG metabolism may not be the single mechanism driving the hypocholesterolemic effects of grape polyphenols. When golden Syrian hamsters were treated with dealcoholized red wine, red wine, or grape juice, similar significant reductions in both TC and LDL-C concentrations were apparent in all treatment groups compared with the control [51]. Although there was a trend for decrease in TG concentrations in all treatment groups compared with the control, the differences were not significant. That study, along with others, suggests the presence of an additional mechanism by which grape polyphenols exert the cardioprotective effect. In Hep G-2 cells, dealcoholized red wine was shown to upregulate significantly the LDL receptor activity. This significant increase in activity was similar to the increase seen when Hep G-2 cells were treated with atorvastatin. Furthermore, when Hep G-2 cells were treated with increasing doses of red wine, LDL receptor mRNA abundance was significantly increased in a dose-responsive manner. The increase of the LDL receptor activity and abundance may be a result of the homeostatic intracellular cholesterol feedback loop. In general, decrease in the intracellular cholesterol will upregulate the LDL receptor expression and activity, whereas increase in the intracellular cholesterol will downregulate the receptor [48]. Grape polyphenols were shown to decrease hepatic cholesterol concentrations; therefore, the liver compensates for this deficiency by upregulating the LDL receptor and the overall decrease in the plasma LDL concentrations occurs.

One possible explanation of the anti-atherogenic activity of grape polyphenols is the well-known HDL cholesterol-increasing effect of polyphenols in various species, including transgenic mice [52].

In our experiments it has been found that the grape extract treatment induced slight (15%) increase in HDL cholesterol concentrations is possibly related to the significant decrease in the hepatic lipase activity (Table 11). The reductions observed in both hepatic and LPL activities by grape polyphenols treatment may prevent formation of small atherogenic VLDLB particles and may also decrease their uptake by the LDL receptor -related protein.

In addition to increases in HDL cholesterol concentrations, grape extracts also change the size and quality of HDL particles [53]. Although the mechanisms by which polyphenols influence the metabolism of HDL particles are not clear, changes in LPL and cholesteryl ester transfer protein (CETP) may play an important role.

Polyphenols treatment in humans is associated with decrease in the CETP content correlated with the concomitant increase in HDL cholesterol concentrations [54]. Consistent with our findings, grape extracts caused a significant increase in the postheparin LPL activity and HDL cholesterol concentrations in patients with moderate hypercholesterolemia and in hamsters [39]. However, the HDL cholesterol-increasing action of polyphenols in animals (mouse, hamster and rat) without CETP in some cases [52] suggests that this effect is may be independent of the CETP activity.

milliunits	Control (MS) (n=50)	Grape extract "Cabernet" (n=50)
LPL	356.0±53.2	258.6±57.3*
Hepatic lipase	232.6±25.9	216.2±34.7

*P<0.05 versus control animals.

Table 11. The plasma postheparin lipases activity in male hamsters with MS (in each group n=10)

The "Cabernet" extract appeared to be the most effective substance in relation to the HDL defence from peroxidation, though the other substances revealed the same but not so high activity. They decreased the content of products (diene conjugates, ketodienes+coupled trienes, total hydroperoxides) effectively and increased – substrates (compounds with isolated double bonds) of lipoperoxidation, prevented decrease of the antioxidant level (α-tocopherol).

It should be pointed out that the level of lipid peroxidation secondary products (ketodienes+coupled trienes) decreased more effectively under the influence of "Enoant" (to intact values).

Under the action of the studied substances the lipoprotein supply to the liver also decreases, evidenced by the decrease of the ApoB-LP content in the organ. Moreover, in the composition of these lipoproteins the TAG content normalizes, and it indicates normalization of the activity of lipases catalyzing the lipoprotein metabolism in the blood (Table 12).

The liver oxidative status is also improved: the antioxidant levels almost restore, the peroxidation products content decreases, the content of compounds with isolated double bonds increases (Tables 3, 5, 9, 12).

Testing of the "Enoant" action – one of the substances studied – in female hamsters of different age with the experimental MS proved the effectiveness of the antioxidant therapy of this pathology.

So, the total lipids, TAG and FFA contents decrease in the blood plasma of those animals under the action of "Enoant" (Table 12). In addition, in adult females "Enoant" causes decrease in the ApoB-LP and total cholesterol content, and it, in turn, reduces atherogenic changes in MS.

Parameter	Group				
	MS	MS + "Enoant"	MS + "Polyphen "	MS+ "Isabella"	MS+ "Cabernet"
Total cholesterol, % of the total ApoB-LP composition.	7.18±0.06*	8.23±0.26*/**	8.46±0.05**	8.76±0.05**	9.10±0.13**
TAG, % of the total ApoB-LP composition	42.00±1.29*	44.64±0.52**	44.42±0.43**	45.95±0.50**	45.41±0.73**
Isolated double bonds, U/g	2.13±0.06*	2.39±0.04*/**	2.71±0.03*/**	2.99±0.09**	2.77±0.16**
Total hydroperoxides, mmol/g	101.03±2.00*	90.55±1.54*/**	88.69±1.02*/**	80.46±0.77*/**	78.83±2.71*/**

The data presented as mean±SD or percentage
* – p≤0.05 versus intact animals, ** – p≤0.05 versus the model of MS

Table 12. The effect of *Vitis Vinifera* substances on the liver cytosol ApoB-LP composition in male Syrian golden hamsters (1 year old) with the experimental MS (in the crude tissue, in each group n= 10)

The increase of α-tocopherol (the main lipid-phase antioxidant) in the blood plasma of animals that received "Enoant" proved its antioxidant activity in our experiment (Table 13).

Furthermore, the significant decrease of the body weight was observed in hamsters that received "Enoant" along with a high-calorie diet compared to the animals on a high-calorie diet alone.

Based on these findings, we may conclude that introduction of grape polyphenolic extracts and concentrates in MS can prevent the increase of the total lipid and ApoB-LP content in the blood plasma, prevent the activation of free radical processes in the plasma lipoprotein particles, and normalize the liver lipid metabolism. The ability of the investigated substances to reduce negative consequences of MS such as atherosclerosis development has been proven.

The last suggestion is confirmed by our results concerning the aorta wall lipid composition in the experimental MS. The introduction of "Enoant" for prophylaxis and treatment reduces significantly atherogenesis manifestations in the aorta, decreasing the aorta media lipidation and the neutral lipid content (Fig. 3, 4).

Thus, from our data, we can conclude that antioxidants, particularly grape polyphenolic concentrates and extracts, which have pronounced antioxidant, phytoestrogenic and stress-protector properties, should be included into a complex therapy of MS to reduce its negative effects.

The next experiment was designed to investigate the action of grape wines and polyphenolic concentrates on development of proatherogenic effects of the emotional-painful stress. In our experiments we used purebred female rats because, as it was shown in previous studies, the acute stress response in females was more expressive than in males.

Age	Group	Parameter						
		Total lipids, mg/ml	ApoB-LP, mg/ml	Total cholesterol, mmol/L	TAG, mg/ml	FFA, mmol/L	Diene conjugates in ApoB-LP, nmol/ml	α - Tocopherol, nmol/ml
4 weeks	MS	4.52 ±0.17	4.00 ±0.16	2.04 ±0.08	1.08 ±0.49	1.17 ±0.06	24.82 ±1.46	6.67 ±0.22
	MS+ "Enoant"	3.54 ±0.16**	3.47 ±0.13	1.88 ±0.06	0.91 ±0.02**	0.95 ±0.02**	22.27 ±0.99	9.79 ±0.77**
20 weeks	MS	7.75 ±0.20	3.84 ±0.11	2.58 ±0.07	1.40 ±0.04	1.42 ±0.04	23.58 ±1.35	10.49 ±0.82
	MS+ "Enoant"	6.88 ±0.14**	3.30 ±0.08**	2.22 ±0.05**	1.18 ±0.03**	1.15 ±0.03**	21.10 ±1.14	12.87 ±0.36**

The data presented as mean±SD or percentage
** – p≤0.05 versus the model of MS

Table 13. The effect of polyphenol concentrate "Enoant" on some plasma lipid metabolism values in female Syrian golden hamsters with the experimental MS (in each group n= 10)

Age	Group	Parameters			
		Total lipids, mg/g	α - Tocopherol, nmol/g	Ascorbic acid, mkmol/g	TBA active substances, nmol/g
4 weeks	MS	140.75 ±9,15	21.01 ±1.47	3.73 ±0.14	1.97 ±0.06
	MS+ "Enoant"	111.53 ±4.08**	25.31 ±0.34**	4.08 ±0.10	1.74 ±0.09
20 weeks	MS	154.18 ±2.70	19.59 ±0.39	6.10 ±0.35	1.95 ±0.09
	MS+ "Enoant"	121.04 ±4.18**	26.11 ±1.03**	5.96 ±0.24	1.73 ±0.09

The data presented as mean±SD or percentage
** – p≤0.05 versus the model of MS

Table 14. The effect of polyphenol concentrate "Enoant" on some liver lipid metabolism values in female Syrian golden hamsters with the experimental MS (in each group n= 10)

During 21 days animals were daily given *per os* grape wines of "Cabernet" and "Rkatsiteli" grades in the doses that corresponded to 300 ml of wine for a human of 70 kg. Other animals were given alcohol in the dose corresponding to 30 ml of alcohol for a human of 70 kg, as well as polyphenolic concentrates "Enoant" and "Polyphen" in the doses of 0.05 ml/kg of the body weight. The grape wines and polyphenolic concentrates were produced by the National Institute of Grape and Wine "Magarach". Control animals were given the corresponding volume of the physiological solution.
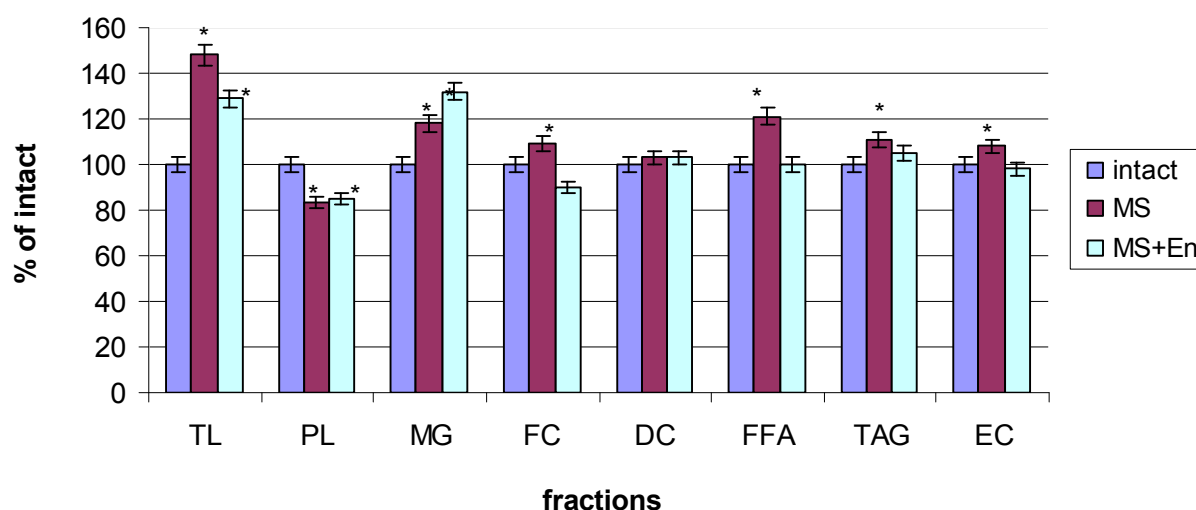
Figure 3. The lipid content in the aorta wall in male Syrian golden hamsters with the experimental MS and "Enoant" treatment (M±m, in each group n=10).
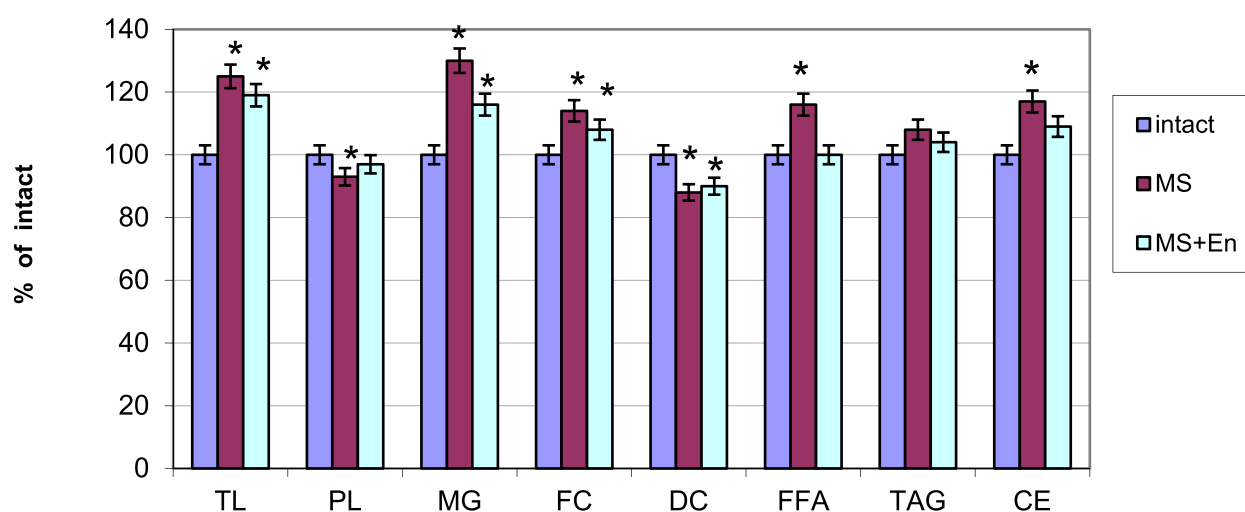

Figure 4. The lipid content in the aorta wall in female Syrian golden hamsters with the experimental MS and "Enoant" treatment (M±m, in each group n=10).

It was shown that all the substances investigated: polyphenolic concentrates "Enoant" and "Polyphen", 10% solution of ethanol, and grape wines "Cabernet" and "Rkatsiteli" possessed the stress-protective activity, which intensity was dependent on the substance used (Tables 15-17).

When introducing only "Enoant" and "Polyphen" to the animals these complexes did not cause any changes on the investigated indexes of the pro-oxidant and antioxidant status in the liver and it is an indication about safety of using these concentrates.

"Enoant" and "Polyphen" revealed the significant protective activity in the emotional-painful stress. It allows to use them as stress-protective, hepatoprotective and antiatherogenic remedies.

Parameter	Group									
	Stress + Enoant		Stress + Polyphen		Stress + ethanol		Stress + Cabernet		Stress + Rkatsiteli	
	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.
TL, mg/ml	4.14 ±0.45*	3.98 ±0.31 **	3.81 ±0.03 */**	3.82 ±0.48 **	5.50 ±0.74 */**	5.58 ±0.37*	3.41 ±0.66 **	3.46 ±0.43 **	3.43 ±0.15 **	3.57 ±0.54 **
TAG, mg/ml	0.76 ±0.08 */**	0.78 ±0.09*	0.51 ±0.08	0.71 ±0.09*	0.66 ±0.12 */**	0.91 ±0.05 */**	0.50 ±0.05	0.58 ±0.04 */**	0.54 ±0.10	0.54 ±0.05 **
Total cholesterol, mg/ml	70.76 ±9.34*	54.90 ±6.92 */**	74.14 ±8.21*	82.93 ±9.48 **	85.71 ±5.71	69.10 ±7.37 **	56.71 ±7.32*	62.25 ±6.50 **	97.51 ±9.66 **	80.83 ±8.74 **
HDL, mg/ml	0.98 ±0.14	0.89 ±0.08	0.96 ±0.05	0.88 ±0.08	1.10 ±0.19	1.00 ±0.12	1.26 ±0.15	1.04 ±0.08	1.08 ±0.12	0.96 ±0.05
ApoB-LP, mg/ml	1.56 ±0.12 **	1.89 ±0.26*	1.59 ±0.16 **	1.54 ±0.22 **	1.85 ±0.19 **	1.67 ±0.18 **	2.07 ±0.29*	1.67 ±0.18 **	1.86 ±0.20 **	1.63 ±0.18 **
Corticosterone, nmol/l	5.50 ±0.99 */**	5.87 ±1.08 */**	7.10 ±0.80 */**	29.33 ±8.58	15.00 ±1.72 */**	38.75 ±5.64 **	32.75 ±5.17 **	26.33 ±4.40 */**	16.93 ±2.43 */**	40.60 ±6.38 **

The data presented as mean±SD
* - p≤0.05 versus intact animals
** - p≤0.05 versus stressed animals

Table 15. The effect of grape polyphenol complexes and grape wines on the lipid metabolism and the plasma corticosterone level in rats with the neurogenic stress (in each group n=10).

Moreover, we have found out that the stress-protective activity of grape wines is equal to the polyphenolic concentrates activity given in the similar dose.

Wines of "Cabernet" and "Rkatsiteli" grades normalized the total lipid content both in a liver homogenate and in the blood plasma in stress; in addition, TAG levels also reached the control values.

Grape wine components prevented the FFA content increase noted when introducing the solution of alcohol. This fact may prove the protective action of the components mediated by inhibition of fatty infiltration of organs.

The latter is confirmed by the absence of influence of grape wine introduction on the NADPH-generating dehydrogenases activity in the liver.

The cholesterol content decrease in the blood plasma when introducing grape wines has attracted our attention, as well as a favourable redistribution of cholesterol in the LP fractions – decrease of ApoB-containing lipoprotein level with the unchanged HDL content.

Parameter		Group									
		Stress + Enoant		Stress + Polyphen		Stress + ethanol		Stress + Cabernet		Stress + Rkatsiteli	
		str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.
PON, nmol/ml/min		240.25 ±20.86 */**	216.00 ±16.24 */**	231.33 ±15.53 */**	231.25 ±15.52 **	197.50 ±14.37 */**	150.00 ±19.24 */**	239.25 ±24.18 */**	246.00 ±16.56 **	222.75 ±13.98**	215.25 ±13.78 **
Ascorbic acid, mkmol/ml		49.99 ±4.41*	43.20 ±4.16*	53.76 ±4.51 **	45.08 ±6.08 */**	38.04 ±4.12 */**	34.83 ±4.43*	48.07 ±2.13*	48.10 ±2.49 */**	55.87 ±6.65 **	53.41 ±5.42 */**
α-Tocopherol, nmol/ml		10.70 ±1.34 **	10.62 ±0.67 **	10.39 ±1.12 **	10.8 ±1.26 **	6.07 ±0.76 */**	5.35 ±0.78 */**	9.45 ±1.11	9.99 ±0.48 **	10.26 ±0.48 **	9.32 ±1.04
Oxidized ApoB-LP	Isolated double bonds, U/ml	2.76 ±0.36*	2.23 ±0.24*	3.14 ±0.46 **	3.51 ±0.42 */**	3.13 ±0.37 **	2.35 ±0.24*	3.25 ±0.38	2.64 ±0.28*	4.13 ±0.43 **	3.11 ±0.41*
	Diene conjugates, mmol/ml	22.47 ±3.20 */**	25.93 ±2.98 */**	21.54 ±1.22 */**	25.93 ±1.76 */**	30.44 ±2.20*	26.28 ±1.24*	23.05 ±4.48*	26.99 ±1.38*	19.54 ±0.93 **	21.56 ±0.99 */**
	Ketodienes+conjugated trienes, U/ml	2.31 ±0.14	2.58 ±0.29 */**	2.20 ±0.11 **	2.37 ±0.10*	3.01 ±0.50*	2.97 ±0.42*	2.29 ±0.16	2.60 ±0.31*	2.22 ±0.11	2.23 ±0.06 **

The data presented as mean±SD
* - p≤0.05 versus intact animals
** - p≤0.05 versus stressed animals

Table 16. The effect of grape polyphenol complexes and grape wines on the plasma oxidant/antioxidant status in rats with the neurogenic stress (in each group n=10).

Paraoxonase activity was normalized in the animals given wines and the antioxidant content both in the blood plasma and in the liver was significantly higher than in the control animals.

These effects together with much lower level of ApoB-LP oxidation in the animals given grape wines prove the high antiatherogenic potential of the wines investigated.

In addition, the grape wines have revealed a rather high level of the stress-protective activity and it is indicated by a significant decrease of the corticosterone content in the blood plasma in stressed animals given wines.

Since grape wines have shown a high level of the stress-protective activity we investigated how the ratio of wine components – polyphenols and ethanol – can influence the stress-protective activity of this complex.

As was shown in our experiments "Enoant" administration even in the combination with ethanol does not reduce the stress-protective action of it, but on the contrary – it intensifies this action preventing unfavourable effects of the alcohol. At the same time the TAG and FFA level in the liver tissue of rats given ethanol together with "Enoant" decreases even when using the lowest dose investigated (0.01 ml per 100 g) (Table 18). Since the content of TAG and FFA increases apparently due to the lipogenesis activation when using ethanol, which might lead to fatty infiltration of the liver, then reduction of this process activity could protect the liver.

Parameter	Group									
	Stress + Enoant		Stress + Polyphen		Stress + ethanol		Stress + Cabernet		Stress + Rkatsiteli	
	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.
Total lipids, mg/g	149.17 ±33.14	151.84 ±19.86 **	130.98 ±23.66	201.93 ±33.71 **	193.32 ±25.61 */**	220.54 ±23.22 */**	171.39 ±28.66 **	180.31 ±15.98 **	164.93 ±16.93 **	190.03 ±18.21**
TAG, mg/g	4.65 ±0.78	3.77 ±0.47	6.00 ±0.32	5.94 ±1.08 **	7.86 ±0.25 **	6.89 ±0.49 **	4.90 ±1.08	3.88 ±1.08	5.74 ±0.61	4.93 ±0.68
ApoB-LP, mg/g	4.58 ±0.06 **	4.43 ±0.07 */**	3.72 ±0.34*	3.15 ±0.44*	5.00 ±0.82	4.10 ±0.49 */**	3.06 ±0.16 */**	3.15 ±0.16 */**	2.87 ±0.30 */**	3.02 ±0.36*
FFA, mg/g	1.06 ±0.12	1.00 ±0.11	1.08 ±0.16	1.26 ±0.08*	1.35 ±0.15*	1.45 ±0.13*	1.05 ±0.22	1.22 ±0.20	1.15 ±0.13	1.40 ±0.31

The data presented as mean±SD
* - p≤0.05 versus intact animals
** - p≤0.05 versus stressed animals

Table 17. The effect of grape polyphenol complexes and grape wines on the liver lipid metabolism in rats with the neurogenic stress, in the crude tissue (in each group n=10).

Parameter	Group									
	Stress + Enoant		Stress + Polyphen		Stress + ethanol		Stress + Cabernet		Stress + Rkatsiteli	
	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.	str.	non-str.
GSH, mkmol/g	6.02 ±0.27 */**	3.70 ±0.42*	5.12 ±0.46	5.48 ±0.34	4.40 ±0.34	2.87 ±0.29 */**	4.64 ±0.70	4.38 ±0.35	4.47 ±0.51	3.59 ±0.50*
α-Tocopherol, nmol/g	27.82 ±2.86 */**	34.01 ±3.56 **	34.34 ±4.14 **	25.39 ±1.18 */**	6.55 ±0.61 */**	5.59 ±0.54 */**	26.95 ±1.32 */**	24.64 ±0.81 */**	29.24 ±1.82 */**	28.9 ±3.15 **
Ascorbic acid, mkmol/g	1.31 ±0.13 **	1.29 ±0.10 */**	1.17 ±0.23*	1.25 ±0.15 */**	0.59 ±0.07 */**	0.81 ±0.06*	1.04 ±0.21*	1.21 ±0.11 */**	1.24 ±0.20 */**	1.24 ±0.17 */**
Isolated double bonds, U/g	18.63 ±1.88 */**	15.99 ±1.50 */**	20.40 ±1.34 */**	18.66 ±1.52 */**	16.98 ±0.66*	13.89 ±1.73*	24.40 ±2.92 */**	16.15 ±1.76 */**	26.67 ±1.62 */**	18.82 ±1.90 */**
Diene conjugates, mmol/g	13.58 ±0.74 */**	12.87 ±0.54 */**	13.48 ±0.62 */**	12.85 ±0.26 */**	13.61 ±0.08 */**	13.20 ±0.58 */**	10.90 ±1.10 **	15.00 ±2.12*	10.66 ±1.88 **	14.32 ±1.62*
Ketodienes+conjugated trienes, U/g	13.57 ±0.54*	13.39 ±1.67	11.44 ±1.56 **	11.84 ±1.88 */**	12.68 ±1.91	13.04 ±1.31 **	14.20 ±2.02*	15.74 ±1.98	17.15 ±1.62*	16.04 ±1.78
TBA-active products, nmol/mg protein	0.21 ±0.02*	0.19 ±0.02 **	0.28 ±0.03*	0.22 ±0.03 **	0.55 ±0.08 */**	0.68 ±0.06 */**	0.15 ±0.02	0.28 ±0.04*	0.18 ±0.02	0.17 ±0.02 **

The data presented as mean±SD
* - p≤0.05 versus intact animals
** - p≤0.05 versus stressed animals

Table 18. The effect of grape polyphenol complexes and grape wines on the liver tissue oxidant/antioxidant status in rats with the neurogenic stress (in the crude tissue, in each group n=10).

It should be noted that the effect of high doses of "Enoant" (0.1 and 0.15 ml/100 g) was ambiguous. On the one hand, it caused α-tocopherol accumulation in the liver that might be an indicator of their protective action, but on the other hand, it probably revealed some

prooxidative effect initiating the increase in the content of the POL final products – thiobarbituric acid-active products, and also activating ApoB-containing lipoproteins oxidation. In this case the secondary oxidative stress developed. A tendency to decrease the lipid content in the liver and to increase it in the blood plasma testifies about it.

Such effect is typical for high doses of many antioxidants capable to reveal the prooxidant action, including α-tocopherol. These data indicate the necessity of reasonable attitude to antioxidants therapy, including "Enoant".

Parameter	Group						
	Stress +Ethanol	Stress+Ethanol+ Enoant, ml per 100 g of the body weight:					
		0.01	0.03	0.05	0.07	0.1	0.15
Total lipids, mg/ml	5.89 ±0.08*	5.69 ±0.06*	5.30 ±0.05*	4.82 ±0.15*	3.51 ±0.08	3.44 ±0.09	3.58 ±0.15
TAG, mg/ml	0.91 ±0.07*	0.99 ±0.04*	0.73 ±0.04*	0.52 ±0.03	0.43 ±0.03	0.39 ±0.02*	0.50 ±0.02
Total cholesterol, mg/ml	0.49 ±0.07	0.46 ±0.05	0.54 ±0.04	0.40 ±0.01*	0.55 ±0.09	0.55 ±0.03	0.56 ±0.02
HDL, mg/ml	0.93 ±0.02	1.00 ±0.06	1.02 ±0.07	0.91 ±0.05	1.81 ±0.03*	1.23 ±0.06*	1.32 ±0.04*
ApoB-LP, mg/ml	1.73 ±0.05*	1.84 ±0.05*	1.48 ±0.06	1.44 ±0.03	1.18 ±0.05	1.41 ±0.02	1.64 ±0.04*
α-Tocopherol, nmol/мml	4.11 ±0.34*	4.88 ±0.17*	5.84 ±0.14*	6.68 ±0.24*	8.20 ±0.34*	9.23 ±0.35	8.80 ±0.46
Ascorbic acid, mkmol/L	33.81 ±1.73*	34.04 ±2.73*	39.04 ±1.60*	49.25 ±1.10*	55.01 ±1.67	56.98 ±2.03	49.96 ±3.43
Diene conjugates in ApoB-LP, mkmol/L	28.11 ±0.34*	28.99 ±0.14*	29.35 ±0.80*	28.81 ±1.30*	23.91 ±0.51	20.60 ±1.43	28,27 ±1.35*
TBA-active products, mkmol/L	2.39 ±0.55*	2.01 ±0.30*	1.48 ±0.16*	1.11 ±0.04	1.18 ±0.34	0.77 ±0.21	1.31 ±0.20*
Corticosterone, nmol/L	35.25 ±4.27	24.00 ±3.03	28.25 ±4.54	16.00 ±0.44*	25.50 ±0.50	17.50 ±2.33*	21.60 ±6.00

The data presented as mean±SD
* - p≤0.05 versus intact animals

Table 19. The effect of different doses of polyphenol concentrate "Enoant" in combination with ethanol on the plasma parameters of the stress response development in rats with the neurogenic stress (in each group n=10).

At the same time small doses of "Enoant" have a relatively low biological activity; they do not reduce negative effects of ethanol intake and do not inhibit the stress response significantly.

Therefore, we can conclude that the most effective doses of "Enoant" are 0.05-0.07 ml/100 g of the body weight because with these doses "Enoant" has not only high stress-protective, antiatherogenic and hepatoprotective activities, but practically neutralizes negative effects of ethanol.

Thus, our results suggest that grape wines have a high stress-protective, antiatherogenic and hepatoprotective activity that is equal to grape polyphenolic non-alcoholic concentrates characteristics, and the wine components in the doses studied have prevented negative effects of ethanol. Introduction of ethanol to animals in the human equivalent dose – 0.43 ml/kg of the body weight increases their tolerance to stress, but is an unfavourable factor that could result in MS development, fatty infiltration of organs and other pathologies. The polyphenolic concentrates "Enoant" and "Polyphen" in the human equivalent dose – 0.3 ml/kg of the body weight reveal a significant stress-protective, hepatoprotective and anti-atherogenic activity under the action of the emotional-painful stress. Grape wines from "Cabernet" and "Rkatsiteli" grades in the human equivalent dose – 4.3 ml/kg of the body weight also reveal a high stress-protective, antiatherogenic and hepatoprotective activity equal to grape polyphenolic non-alcoholic concentrates, and the wine components in the doses used prevented the negative effect of ethanol.

The highest activity has been shown by the combination of "Enoant" and ethanol that corresponds to the ratio of components in dry red wines, as well as the absence of significant difference in the protective effects of red and white wines, in spite of the difference in the polyphenol content [55]. Based on these results, in the second series of our experiments we decided to investigate "Cabernet" and "Rkatsiteli" wine effects on the development of stress-reaction proatherogenic consequences under the action of the emotional-painful stress in different periods of introduction.

It has been shown that "Cabernet" had a higher level of the anti-atherogenic activity than "Rkatsiteli"; in relation to the stress-protective activity the wines of these grades did not differ markedly. Such effect is likely connected with accumulation of polyphenols in the organism.

To examine the last supposition it was necessary to determine how different periods of introduction of the investigated wines influenced the stress-reaction development. We have carried out the study of wine intake influence on the development of proatherogenic consequences of the emotional and painful stress in different terms after consumption.

The data obtained in the experiments showed significant improvement of the antioxidant status both in the blood plasma and the liver tissue one day after the introduction of "Cabernet" wine (tables 20, 21).

At the same time "Rkatsiteli" wine did not reveal such activity. A similar condition persisted for 2-5 days of administration.

Periods of time		Parameter					
		Total lipids, mg/g	TAG, mg/g	GSH, mkmol/g	α-Tocopherol, nmol/g	Diene conjugates, nmol/g	TBA-active products, nmol/g
Day 1	C+Str	94.94±5.65*	3.80±0.11*	2.12±0.17*	18.88±0.79*	14.93±0.37*	1.68±0.10
	R+Str	103.16±4.63*	5.15±0.61*	2.37±0.28*	15.60±0.39*	15.94±0.39*	2.24±0.32
Day 2	C+Str	105.17±5.12*	3.58±0.23*	2.51±0.34*	26.04±2.17*	14.13±0.09*	1.07±0.24*
	R+Str	106.93±9.42*	5.67±0.34*	2.77±0.87*	15.65±0.92*	15.10±0.07*	1.91±0.15
Day 3	C+Str	115.38±11.65*	3.92±0.13*	3.82±0.37*	23.69±2.18*	13.85±0.48*	1.70±0.11
	R+Str	103.28±5.81*	6.47±0.28*	2.47±0.26*	16.89±0.71*	14.44±0.25*	2.29±0.20*
Day 5	C+Str	139.14±8.06*	5.57±0.31*	2.43±0.37*	26.96±2.12	13.19±0.34	1.35±0.15
	R+Str	116.66±3.60*	6.88±0.37*	2.43±0.42*	18.64±1.18*	14.22±0.16*	2.06±0.13
Day 8	C+Str	161.18±6.05*	7.27±0.15	4.11±0.21	32.12±0.85	12.28±0.52	1.64±0.13
	R+Str	122.20±7.07*	6.35±0.45	2.33±0.28*	23.08±2.08*	12.93±0.44	1.73±0.04
Day 10	C+Str	181.82±9.24	8.04±0.63	5.15±0.42	30.62±2.53	11.86±0.12	1.30±0.19*
	R+Str	153.99±5.30*	8.41±0.56	3.22±0.48*	26.31±1.26*	11.84±0.48	1.29±0.14*
Day 12	C+Str	174.72±6.15	8.34±0.55	3.74±0.25	32.55±5.58	12.42±0.36	1.47±0.27
	R+Str	164.4±8.03*	8.63±0.47	3.25±0.17*	28.28±2.26	11.41±0.22	1.45±0.31
Day 15	C+Str	162.16±12.81	7.50±0.43	4.61±0.22	38.16±2.06*	10.50±0.52*	1.34±0.06*
	R+Str	172.13±10.42	7.68±0.69	4.42±0.34	41.99±2.42	10.27±0.63*	1.24±0.17

The data presented as mean±SD

* - p≤0.05 versus intact animals

Table 20. The effect of prophylactic administration of grape wines of "Cabernet" (C) and "Rkatsiteli" (R) grades on the stress response development in the liver tissue in rats with the neurogenic stress in different periods of time, in the crude tissue (in each group n=10).

Periods of time		Parameter						
		Total lipids, mg/ml	TAG, mg/ml	Total cholesterol, mg/ml	ApoB-LP, mg/ml	α-Tocopherol, nmol/ml	Diene conjugates, nmol/ml	Corticosterone nmol/l
Day 1	C+Str	5.15±0.50*	0.78±0.07*	0.94±0.04	1.53±0.02*	8.52±0.27*	31.49±2.58*	75.00±8.66*
	R+Str	6.15±0.44*	0.93±0.07*	1.08±0.05#	1.68±0.03*	6.29±0.47*	38.35±1.56*	111.70±10.00
Day 2	C+Str	3.87±0.23	0.71±0.04*	0.89±0.03	1.52±0.02*	8.90±0.72*	31.65±1.92*	96.67±21.86*
	R+Str	4.85±0.16*	0.84±0.06*	0.86±0.04	1.70±0.04*	7.77±0.27	32.17±1.71*	81.54±16.50*
Day 3	C+Str	3.42±0.32	0.56±0.03	0.73±0.05*	1.54±0.04*	10.20±0.52	24.72±2.89	35.67±9.49
	R+Str	4.45±0.41	0.70±0.04*	0.89±0.06	1.66±0.04*	8.49±0.38*	22.24±1.84	43.00±8.50
Day 5	C+Str	3.54±0.27	0.49±0.05	0.72±0.02*	1.45±0.06	10.28±0.65	28.34±1.77*	41.50±18.50
	R+Str	3.56±0.23	0.58±0.05	0.88±0.02	1.66±0.04*	10.01±0.27	20.46±2.03	57.10±3.00*
Day 8	C+Str	3.47±0.39	0.49±0.08	0.77±0.02*	1.36±0.03	12.02±0.35	20.47±2.03	42.50±10.61
	R+Str	4.08±0.31	0.4±0.06	0.84±0.03	1.55±0.04	10.04±0.68	20.15±2.61	40.50±9.19
Day 10	C+Str	3.73±0.24	0.54±0.05	0.69±0.02*	1.33±0.02	11.03±0.89	22.68±2.46	34.00±18.38
	R+Str	3.60±0.35	0.55±0.09	0.76±0.04*	1.43±0.04	11.39±0.47	22.90±1.93	38.00±2.82
Day 12	C+Str	3.71±0.35	0.49±0.07	0.65±0.03*	1.26±0.04	11.18±1.01	21.72±1.57	25.50±2.12
	R+Str	3.55±0.45	0.054±0.06	0.75±0.03*	1.35±0.03	11.40±0.93	20.08±1.45	27.00±1.31
Day 15	C+Str	3.78±0.36	0.59±0.04	0.61±0.02*	1.33±0.02	11.04±1.32	20.99±0.92	34.00±7.00
	R+Str	4.22±0.57	0.51±0.09	0.69±0.03*	1.29±0.06	11.72±0.93	20.59±1.92	31.00±10.82

The data presented as mean±SD

* - p≤0.05 versus intact animals

Table 21. The effect of prophylactic administration of grape wines of "Cabernet" (C) and "Rkatsiteli" (R) grades on the plasma parameters of the stress response development in rats with the neurogenic stress (in each group n=10).

However, on day 8 of administration the antioxidant and stress-protective effects of these wines were almost similar, and on the day 10 –they practically did not differ.

On days 12 and 15 there were also no differences as to the antioxidant and stress-protective action of the wines studied, which significantly reduced activation of the free radical oxidation under the action of stress normalizing the most of the indexes investigated.

Thus, the investigated wines are characterized by the high level of the antioxidant and stress-protective activity, and in the first days of introduction "Cabernet" wine improved more effectively the antioxidant status in the blood and the liver tissue than "Rkatsiteli" wine, but by day 10 the effects of the studied wines had no substantial difference.

Probably, these results are dependent on polyphenol cumulation in the organism because it is known that the polyphenol content of "Cabernet" is 10 times more than of "Rkatsiteli".

Thus, the results suggest that "Cabernet" and "Rkatsiteli" wines have already revealed the high stress-protective, hepatoprotective and anti-atherogenic activity in the conditions of the emotional-painful stress on the 2-3 days after introduction, and practically normalized the oxidative status and the lipid metabolism under the action of stress in prophylactic administration within 10 days. This indicates that grape polyphenols possess a high total antioxidant activity. At the same time the last suggestion required further research.

In order to examine the effects of wine stocks and polyphenolic concentrates obtained from other grape grades on development of proatherogenic consequences of the emotional-painful stress we investigated the action of substances obtained from the grapes of hybrid grades "Krasen", "Golubok" and "Podarok Magaracha" produced by the National Institute of Grape and Wine "Magarach".

In the series of experiments we used purebred male rats that during 21 day were given daily, *per os*, table wine stocks of the grades "Podarok Magaracha", "Krasen" and "Golubok" in the human equivalent dose corresponding to 300 ml of wine for a human with 70 kg of the body weight. Other groups of animals were given ethanol in the human equivalent dose corresponding to 30 ml of ethanol for a human with 70 kg of the body weight taking into account the species sensitivity coefficients, as well as the table wine stocks of the grades mentioned in doses equivalent to the polyphenol content of the given wines calculated by the polyphenol content in active doses (AD – 9 mg of polyphenols/100 g of the body weight).

The results have demonstrated that not only polyphenolic concentrates, but the table wine stocks also revealed a substantial stress-protective activity to a different extent (Tables 22-25).

In fact, "Krasen" table wine stock revealed the highest activity; the stress-protective activity was almost 2.4 times more the ethanol activity in the dose studied. This product effectively prevented the activation of free radical oxidation both in the blood (increased the level of compounds with isolated double bonds in the atherogenic ApoB-LP, decreased the content of peroxidation products – diene conjugates – almost 3 times comparing to the stressed animals, and 15% - comparing to the intact animals), and the liver tissue (prevented the antioxidant content decrease, particularly the content of α-tocopherol and ascorbic acid returned practically to the intact level, and there was 40% decrease of the diene conjugates level). At the same time this table wine stock prevented hyperlipidemia and the shift of

metabolism to the increased lipolysis, there was 60% decrease of the blood total lipid content comparing to the stressed animals, and 11% - comparing with the intact animals. At the same time the TAG content in the liver was equal to the intact level that also demonstrated the protective action of this table wine stock. Reduction of lipogenesis in the liver tissue under the action of this product is important, and it protects the organ from steatosis. It should be also mentioned that the given product normalized the cholesterol content in the blood plasma.

Group	Parameter				
	Total lipids, mg/g	TAG, mg/g	FFA, mmol/g	ApoB-LP, mg/g	Lysosomal lipase, nmol/mg protein/min
Str.+Con. Podarok Magaracha (AD)	149.03 ±2.59*,**	5.74 ±0.07**	4.46 ±0.04*	4.22 ±0.03*,**	0.45 ±0.03**
Str.+Con. Krasen (AD)	147.13 ±1.15*	4.27 ±0.02*,**	4.19 ±0.05*,**	4.61 ±0.01*,**	0.50 ±0.02
Srt.+Wine Podarok Magaracha	161.74 ±1.91*,**	6.33 ±0.05**	4.30 ±0.11*,**	4.43 ±0.03*,**	0.32 ±0.01*,**
Srt.+Wine Krasen	155.88 ±1.35*,**	6.04 ±0.16**	3.24 ±0.04**	4.50 ±0.07*,**	0.56 ±0.03**
Str.+Con. Podarok Magaracha (DW)	141.29 ±1.79*	4.95 ±0.15*,**	3.83 ±0.09**	3.18 ±0.03*	0.65 ±0.02**
Str.+Con. Krasen (DW)	145.87 ±3.19*	4.24 ±0.07*,**	4.23 ±0.07*,**	4.39 ±0.08*,**	0.55 ±0.03**
Wine Podarok Magaracha	182.4 ±3.08*	6.51 ±0.07**	2.70 ±0.08*,**	4.80 ±0.10*,**	0.37 ±0.01*
Wine Krasen	164.36 ±1.86	5.97 ±0.17	2.95 ±0.09*	4.93 ±0.18*	0.35 ±0.02*
Con. Podarok Magaracha (AD)	191.33 ±2.03*	6.47 ±0.04*	4.61 ±0.31*	4.33 ±0.08*	0.83 ±0.03*
Con. Krasen (AD)	170.4 ±2.09	6.15 ±0.14	3.22 ±0.05	4.97 ±0.11*	0.69 ±0.02#
Ethanol	229.76 ±3.39*	7.40 ±0.13*	4.57 ±0.13*	6.11 ±0.07*	0.38 ±0.02*

The data presented as mean±SD

* - p≤0.05 versus intact animals

** - p≤0.05 versus stressed animals

Table 22. The effect of grape polyphenol concentrates and grape wines on the liver lipid metabolism in rats with the neurogenic stress (in the crude tissue, in each group n=10).

It is also necessary to point out that the control intake of the investigated substances (Tables 22-25) did not reveal negative effects on the organisms of the experimental animals. Moreover, in addition to the antioxidant activity these substances revealed a significant hypocholesterolemic and anti-atherogenic action, which was more pronounced when using "Krasen" grade wine stock and the concentrate.

Group	Parameter				
	GSH, mkmol/g	α-Tocopherol, nmol/g	Ascorbic acid, mkmol/g	Diene conjugates, nmol/g	TBA-active products, nmol/ mg protein
Str.+Con. Podarok Magaracha (AD)	3.34 ±0.02*,**	24.01 ±0.47*,**	1.21 ±0.01*,**	14.96 ±0.22*,**	0.49 ±0,01
Str.+Con. Krasen (AD)	3.8 ±0.02*,**	26.71 ±0.46*,**	1.28 ±0.01*,**	15.03 ±0.11*,**	0,45 ±0.02
Srt.+Wine Podarok Magaracha	3.48 ±0.02*,**	26.71 ±0.34*,**	1.33 ±0.02*,**	13.81 ±0.18#,**	0.42 ±0.03**
Srt.+Wine Krasen	3.57 ±0.08*,**	28.46 ±0.75**	1.41 ±0.03*,**	13.36 ±0.40**	0.21 ±0.01*,**
Str.+Con. Podarok Magaracha (DW)	2.00 ±0.07*,**	21.72 ±0.51*,**	1.02 ±0.03*,**	16.05 ±0.11*,##	0.47 ±0.01
Str.+Con. Krasen (DW)	3.27 ±0.06*	21.12 ±0.39*,**	1.04 ±0.03*,**	16.72 ±0.16*,##	0.45 ±0.02
Wine Podarok Magaracha	4.68 ±0.10*,**	33.59 ±0.60	2.16 ±0.04*	10.42 ±0.53*	0.15 ±0.01*
Wine Krasen	4.56 ±0.24	35.48 ±0.78*	1.57 ±0.03	9.27 ±0.24*	0.13 ±0.01*
Con. Podarok Magaracha (AD)	4.46 ±0.13	35.29 ±0.45*	1.95 ±0.03*	10.87 ±0.41	0.20 ±0.01*
Con. Krasen (AD)	4.82 ±0.14*	27.84 ±0.39	2.00 ±0.04*	10.26 ±0.06*	0.15 ±0.01*
Ethanol	5.31 ±0.35*	24.16 ±1.40*	2.06 ±0.03*	12.62 ±0.60	0.46 ±0.02

The data presented as mean±SD

* - p≤0.05 versus intact animals

** - p≤0.05 versus stressed animals

Table 23. The effect of grape polyphenol concentrates and grape wines on the oxidant/antioxidant status in the liver tissue in rats with the neurogenic stress (in the crude tissue, in each group n=10).

Group	Parameter						
	Total lipides, mg/ml	TAG, g/ml	FFA, mmol/L	Total cholesterol, g/ml	HDL, mg/ml	ApoB-LP, mg/ml	Cortico-sterone, nmol/L
Str.+Con. Podarok Magaracha (AD)	3.89 ±0.08**	0.72 ±0.01*,**	1.40 ±0.02#,**	56.66 ±1.49*,**	0.79 ±0.02*,**	1.45 ±0.05*,**	47 ±2**
Str.+Con. Krasen (AD)	4.12 ±0.08#	0.67 ±0.01*,**	1.16 ±0.03*,**	58.70 ±1.77	0.82 ±0.03	0.,90 ±0.04*,**	47 ±3**
Srt.+Wine Podarok Magaracha	4.60 ±0.11*	0.65 ±0.02*,**	1.63 ±0.02*	63.32 ±1.01**	0.85 ±0.02**	1.21 ±0.02**	41 ±1*,**
Srt.+Wine Krasen	3.42 ±0.09*,**	0.52 ±0.03**	1.40 ±0.04**	64.69 ±1,70**	0.86 ±0.02	1.08 ±0.03#,**	42 ±1**
Str.+Con. Podarok Magaracha (DW)	5.25 ±0.07*	0.74 ±0.02*	0.74 ±0.02*,**	56.67 ±1.15*,**	0.87 ±0.03	1.75 ±0.07*,**	60 ±2
Str.+Con. Krasen (DW)	3.88 ±0.08**	0.67 ±0.01*,**	0.67 ±0.01*,**	55.58 ±1.21*,**	0.83 ±0.03**	1.06 ±0.03*,**	63 ±1*
Wine Podarok Magaracha	3.72 ±0.09	0.38 ±0.01*	1.14 ±0.02*	60.78 ±1.64	1.4 ±0.02*	1.11 ±0.02	54 ±2
Wine Krasen	3.76 ±0.03	0.36 ±0.01*	1.18 ±0.02*	57.21 ±0.81#	1.11 ±0.02*	1.14 ±0.01	44 ±4
Con. Podarok Magaracha (AD)	4.32 ±0.04*	0.56 ±0.02	1.23 ±0.01	68.70 ±1.22	1.04 ±0.02*	1.14 ±0.02	71 ±2*
Con. Krasen (AD)	3.78 ±0.04	0.39 ±0.02*	1.43 ±0.04*	60.89 ±1.67	1.16 ±0.02*	1.20 ±0.02	35 ±2*
Ethanol	4.05 ±0.09	0.74 ±0.03*	1.65 ±0.02*	55.80 ±1.46*	1.24 ±0.06*	1.32 ±0.02*	75 ±3*

The data presented as mean±SD

* - p≤0.05 versus intact animals

** - p≤0.05 versus stressed animals

Table 24. The effect of grape polyphenol concentrates and grape wines on the plasma lipid metabolism parameters and corticosterone level in rats with the neurogenic stress (in each group n=10).

Group	Parameter				
	PON, nmol/ ml×min	Ascorbic acid, mkmol/L	α-Tocopherol, nmol/ml	Isolated double bonds in ApoB-LP	Diene conjugates in ApoB-LP
Str.+Con. Podarok Magaracha (AD)	174 ±2*	37.26 ±1.60*,**	7.77 ±0.21*	2.07 ±0.09*	27.03 ±1.30*,#
Str.+Con. Krasen (AD)	194 ±3*,**	41.79 ±0.44*,**	8.25 ±0.66*,**	2.35 ±0.15*	26.86 ±0.43*
Srt.+Wine Podarok Magaracha	173 ±3*	40.08 ±1.72*,**	8.91 ±0.29**	1.92 ±0.25*	18.45 ±0.18*,**
Srt.+Wine Krasen	189 ±2*,**	54.30 ±0.97*,**	10.42 ±0.16*,**	2.47 ±0.03*,**	14.50 ±0.35*,**
Str.+Con. Podarok Magaracha (DW)	159 ±1*	35.46 ±1.10*	7.04 ±0.07*	1.52 ±0.06*	25.73 ±0.68*,**
Str.+Con. Krasen (DW)	179 ±2*,**	48.59 ±0.96*,**	8.03 ±0.06*,**	2.21 ±0.07*	27.68 ±0.60*,**
Wine Podarok Magaracha	223 ±6	75.95 ±0.74*	10.03 ±1.72	5.43 ±0.12*	15.67 ±0.15*
Wine Krasen	215 ±3*	74.79 ±0.34*	11.52 ±0.24*	5.55 ±0.15*	15.81 ±0.15*
Con. Podarok Magaracha (AD)	221 ±4	67.04 ±1.40	9.49 ±0.24	4.83 ±0.11	16.87 ±0.21
Con. Krasen (AD)	255 ±3	70.61 ±2.31	9.97 ±0.16	5.39 ±0.04*	15.47 ±0.34*
Ethanol	236 ±3	79.49 ±2.18*	10.22 ±0.36	5.75 ±0.17*	17.82 ±0.24

The data presented as mean±SD

* - p≤0.05 versus intact animals

** - p≤0.05 versus stressed animals

Table 25. The effect of grape polyphenol concentrates and grape wines on the plasma oxidant/antioxidant status in rats with the emotional-painful stress (in each group n=10).

5. Conclusion

Based on our findings, it is possible to state that antioxidant complexes, particularly polyphenol extracts and the concentrates obtained from *Vitis Vinifera*, which are safe and reveal the potent antioxidant and stress-protective activity, should be used for reduction of proaterogenic states consequences in the complex prophylactic and treatment of atherosclerosis as effective stress-protective remedies.

Thus, administration of *Vitis Vinifera* substances can prevent the increase of the total lipoprotein and ApoB-LP content in the blood, and prevent the free radical process activation in the plasma lipoprotein particles, and, in general, normalize the lipid and lipoprotein metabolism in the liver in metabolic syndrome. These results have proven the ability of the investigated complexes to reduce such negative consequence of metabolic syndrome as development of atherosclerosis.

In addition, according to obtained research data the polyphenolic concentrates possess a potent protective activity both in acute and chronical neurogenic stress.

Our studies suggest that multicomponent active substances with antioxidant properties are more effective in correction of the proatherogenic states caused by stress and metabolic syndrome negative effects in comparison with individual antioxidants (particularly, α-tocopherol). The research data suggest that the increased plasma antioxidant activity alone does not result in decreased foam cell formation, at least in the studied animal model. Moreover, *in vitro* studies have shown that α-tocopherol can be pro-oxidative rather than protective for lipids in isolated LDL. Similarly with vitamin E, vitamin C additives do not offer consistent benefit against atherosclerosis in animals.

The occurrence of tocopherol-mediated peroxidation and the mode of its prevention predicts that the balance of α-tocopherol and available coantioxidants, rather than α-tocopherol alone, determines whether LDL lipid peroxidation occurs in biological systems. Inhibition of the free radical process with the polyphenolic complexes administration can be associated with their ability to increase the level of antioxidants – α-tocopherol, ascorbic acid and reduced glutathione in the test animal liver tissue compared with the group of the stressed animals. The complexes obtained from *Vitis Vinifera*, in particular, polyphenolic concentrates "Enoant" and "Polyphen", as well as grape wines (particularly "Cabernet") with their moderate use revealed the potent antioxidant activity. The preliminary results also suggest that coantioxidants inhibit lipoprotein lipid peroxidation *in vivo*. Thus, if LDL oxidation causes atherosclerosis, the requirement for coantioxidants may explain why supplementation with individual antioxidants, particular vitamin E alone, overall has yielded inconclusive results in the controlled human and animal intervention studies.

In conclusion, our research results may be used for the development of the atherosclerosis prophylaxis strategy, and treatment of diabetes mellitus and metabolic syndrome because recent studies proved insufficient effectiveness of α-tocopherol and advantages of multicomponent antioxidant complexes administration. The high effectiveness of the polyphenolic complexes obtained from *Vitis Vinifera*, including polyphenolic concentrates

"Enoant" (from grape of "Cabernet" grade) and "Polyphen" (from grape of "Rkatsiteli" grade) produced by the National Institute of Grape and Wine "Magarach" has been proven. Our results also confirmed the high effectiveness of the antioxidant complexes from grapes in the correction of the endothelial dysfunction, thus, including these extracts in the treatment schemes is very reasonable. As it would be expected from our observations, increasing the antioxidant oxidant defense by antioxidant supplementation has the ability to restore the endothelial vasomotor function.

An important question to be asked is whether the polyphenol antioxidants exerted their inhibitory effect on lesion progression only because of their antioxidant properties or, possibly, because of additional biological properties, in particular – the phytoestrogen activity.

However, further studies, especially in humans, are required to validate the role of these antioxidants in inhibiting LDL oxidation.

Nevertheless, there are some limitations in the use of the concentrates produced from red grade grapes because of the uric acid content changes.

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