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Correction of the Mitochondrial NADH Oxidase Activity, Peroxidation and Phospholipid Metabolism by Haplogenin-7-Glucoside in Hypoxia and Ischemia

Yusupova Umidakhon[1], Botirov Erkin[2], Gayibov Ulugbek[3], Niyazmetov Bahadir[1,*] and Almatov Karim[1]

[1]Department of Human and Animal Physiology, National University of Uzbekistan named after Mirzo Ulugbek, Tashkent, Uzbekistan
[2]Head of Chemistry Department, Surgut State University, Okrug, Russia
[3]Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

([*]Corresponding author's e-mail: physiologist0107@gmail.com)

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Abstract

In this study, it was used haplogenin-7-glucoside flavonol ($C_{16}H_{12}O_8$) isolated from the plant *Haplophyllum perforatum*. It was studied the effect of haplogenin-7-glycoside on NADH oxidase activity, peroxidation, and phospholipid metabolism in rat liver mitochondria under *in vitro* conditions. Incubation of mitochondria at 37 ℃ leads to disruption of the integrity of mitochondrial membranes, as a result, in cytochrome c desorption from the inner membrane into the intermembrane space, which leads to a decrease in the activity of rotenone-sensitive NADH oxidase and an increase in the activity of rotenone-insensitive NADH oxidase. Haplogenin-7-glucoside exhibits membrane stabilization by reducing these alterations. Under ischemia conditions, the effect of haplogenin-7-glucoside on the LPO process in mitochondria decreases LPO in mitochondria. Quantitative changes in phosphatidylserine in mitochondrial membranes under LPO conditions during incubation of mitochondria at 37 ℃ were observed to accelerate controlled (auto-oxidation) LPO over time (0 - 90 min). Peroxidation of phosphatidylserine in mitochondria when the addition of haplogenin-7-glucoside to the incubation medium at 30, 60 and 90 min, LPO was found to be reduced. The increasing amount of phosphatidylinositol has not happened depend on time. However, a slight increase was found under the influence of haplogenin-7-glucoside.

Keywords: Rotenone, NADH oxidase, Apoptosis, Hypoxia, Ischemia, Haplogenin-7-glucoside, Peroxidation of lipids (LPO), Phospholipids

Abbreviations:

LPO - lipid peroxidation
MDA - malondialdehyde
PLA2 - phospholipase A2
PLD - phospholipase D
ATP - adenosine triphosphate
OXPHOS - oxidative phosphorylation

Introduction

Hypoxia has a major pathogenic factor that plays a leading role in the development of many diseases. It may have an independent etiology, but is the result of poor function of the respiratory and cardiovascular systems, as well as associated with the transport function of the blood diseases and effects of other pathologies. Many scientists believe that environmental pollution, deterioration of extreme conditions can lead to various functional disorders that lead to hypoxic state. These pathological conditions include changes in various physiological activities, such as frequent and shallow breathing, decreased air exchange, the occurrence of cardiac tachycardia, followed by mental and emotional disturbances, weakness and loss

of sensation, heart failure, increased arousal in the brain, sleepless, headache, movement control disorders and even death [7].

This means that diseases such as stroke, heart attack and ischemia are observed due to the restriction of oxygen supply to living cells. Ischemic heart disease, chronic heart failure is primarily associated with circulatory disorders in the coronary arteries and the development of hypoxia. The nervous system is most sensitive to hypoxia: A few seconds after the complete cessation of blood flow, signs of damage to the cerebral cortex are observed. A 20 % reduction in oxygen consumption of brain tissue leads to loss of consciousness. In the medulla oblongata, deep structural changes of neurons are observed after 10 - 15 min, and after 56 min, brain anoxia occurs [2,21]. In a state of hypoxia, small necrosis centres appear in the heart tissue after 35 min, because of the development of ischemia and a large centralized myocardial infarction occur after 20 - 30 min [16]. However, studies conducted in the 1960s have shown that in the case of tissue oxygen deficiency and ischemia, primarily mitochondrial dysfunction are observed. Because of the slow oxidation of substrates, their ability to synthesize of ATP decreases depending on the level of oxygen consumption. In liver and brain tissue, significant mitochondrial damage is observed after 30 min hypoxia. After an hour, many mitochondria of liver cells terminate their vital activity to an irreversible extent [4,20]. Disorders of cells and tissue respiration result in multifaceted pathogenic factors, such as genetic, biochemical and structural-functional deficiencies of mitochondria, as a result it may cause to known as a typical pathological process of mitochondrial dysfunction. There are different types of mitochondrial dysfunction that result from congenital genetic deficiency or various factors such as hypoxia, ischemia, oxidative stress and pre-inflammatory cytokine expression [9].

Almost 98 % absorption of oxygen taken in during respiration is associated with mitochondria. Because of these processes, 80 - 90 % of ATP accumulates in various tissue cells of mammals. Based on this function, which depends on the viability and vital activity of aerobic organisms, the delivery of oxygen to mitochondria and the maintenance of optimal oxygenation in the cell (respiration, oxygen transport through the lungs, cardiovascular circulation, erythrocytes, hemoglobin, blood mass transfer system) the most complex physiological systems are created. The organization of digestion, which involves the processing of food and their subsequent gradual enzymatic processing, is determined by the need to provide substrates in the respiration and OXPHOS reactions of mitochondria [13]. Excess production of NADH and NADPH under conditions of hypoxia and acidosis leads to an increase in reactive oxygen species [10]. In this case, the activation of free radicals leads to an increase in the concentration of Ca^{2+} ions in the cytosol. The result is a decrease in the ATP amount, an increase in the amount of the reactive oxygen species, and eventually cell death [19]. A 10 - 20% decrease for ATP in the cell leads to a 70 - 80 % decrease in the activity of energy-related processes. Lack of ATP leads to a decrease in anabolic processes, disruption of the activity of ion channels, resulting in disruption of cellular ionic homeostasis and, consequently, cell dysfunction [12]. Weakening of the mitochondrial antioxidant system under oxidative stress and the consequent decrease in phospholipid levels in the mitochondrial membrane, especially cardiolipin, disruption of mitochondrial DNA, resulting in accumulation of damaged mitochondrial DNA, leads to various pathological conditions in tissues [19]. One of the metabolic signs of hypoxia is a decrease in the energy supply of nerve tissue, as well as a decrease in the amount of creatine phosphate in the myocardium, which is a reserve source of macroergic phosphate binding and allows them to be transported from the cell to energy sites, [16], as well as a 70 % decrease in creatine phosphate in brain tissue after a few minutes, the loss of its complete reserve after 40 - 45 min [7].

Flavonoids are small molecular secondary metabolites synthesized by plants with various biological activities. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms (microorganisms, animals and other plants) and their reactions to environmental stresses. The majority of their functions result from their strong antioxidative properties. Although an increasing number of studies focus on the application of flavonoids in medicine or the food industry, their relevance for the plants themselves also deserves extensive investigations. [32]. More than 8,000 flavonoids with different chemical structures have been identified in plants [51]. Bioflavonoids were first discovered in 1930 by Nobel Laureate Albert Szent-Gyorgyi [46]. Flavonoids are not synthesized in human and animal cells; they enter the body through the consumption of plant products [41]. Flavonoids have anti-inflammatory, anti-allergic, antiviral, anti-cytotoxic and membrane stabilizing [35,49], anti-tumor, cytoprotective [27], neurocytoprotective [42], immunomodulatory [48] properties. However, the question of the effect of haplogenin-7-glucoside on mitochondrial structure and function remains open. Under hypoxia, it is important to identify the molecular mechanisms of various disorders in tissues, cells and mitochondria and to describe their corrective effect on biologically active compounds. For this reason, haplogenin-7-glucosoide was selected to study the corrective properties of mitochondrial membrane disorders in mitochondrial hypoxia and ischemia.

Materials and methods

Mitochondria of rat liver cells were isolated according to the method [50], with some modification [1,28]. Our experiments were carried out with frozen then melted mitochondria, 0.25 M sucrose, and 10 mM Tris-HCl (pH 7.4). The activity of the oxidases was determined after 180 min of incubation and flavonoid-containing mitochondria at 37℃. When mitochondria are stored at room temperature without oxygen and substrates, "non-stick" areas on the membrane begin to multiply, resulting in accelerated delivery of endogenous lipolytic and proteolytic enzymes activated by the Ca^{2+} cation to the active centre of the membrane-containing enzymes [1]. These alterations lead to the conversion of the activity of enzymes located in mitochondrial membranes. Inner and outer pathways of mitochondrial NADH oxidation were determined after freezing and thawing of mitochondria. Mitochondria were incubated in an incubator in the presence of quercetin at 37℃, at specified time intervals aliquots were taken for measurement of oxidase activity. NADH oxidation assessed by adding to the cell 3 μM of NADH. NADH oxidation was determined in the presence of 2 mg of rotenone. Incubation medium: 0.30 M of sucrose containing 10 mM of Tris-HCl, pH 7.4 [18]. Inner and outer pathways of oxidation NADH in mitochondria registered polarographically using the rotating platinum electrode under standard conditions of the cell of 1 mL polarography at 25 ℃.

Rate of the process of lipid peroxidation in the ascorbate-dependent system determined by thiobarbituric acid micro method developed by Vladimirov and Archakov [5]. The incubation medium contained 1 mL of 0.2 mM $Na_4P_2O_7 \cdot 10H_2O$; 1 mM of NADPH; 0.012 mM of Mohr salt ($FeSO_4(NH_4)_{26} \times H2O$); 50 μl of a suspension of brain tissue, thymus, adrenal gland, and liver; 50 mM of Tris-HCl buffer, pH 7.4 in the control sample was added all medium components except ascorbic acid. It was incubated at 37 ℃ for 20 min under constant shaking. The reaction was stopped by addition 1mL of 30 % trichloroacetic acid and the precipitate was removed by centrifugation for 10 min at 6000 g/min. Then to 1.5 mL of centrifugate was added 0.3 mL 0.6 M and 1.2 M of thiobarbituric acid. For color development tubes placed in a water bath at 100 ℃ and incubated for 10 min. The intensity of the color formation was measured with a spectrophotometer at 535 nm. The amount of MDA formed was determined using a molar extinction coefficient equal to 1.56/10 cm. The rate of reaction of lipid peroxidation expressed in nM MDA/mg of protein per hour.

Extraction of mitochondrial phospholipids and determination of their composition was carried out with some modifications based on the method of E.G. Bligh and W.J. Dyer [26]. The phospholipid composition of mitochondria was analyzed using 2-dimensional microlayer chromatography on silica gel-coated glass plates measuring 6×9 cm^2 [3]. Phospholipids were identified using special identifiers.

Induction of peroxidation of non-enzyme Fe^{2+}/ascorbate-bound lipids was performed by incubation by introducing mitochondrial suspension at the rate of 0.25 M sucrose, 10^{-5} M $FeSO_4$, and 6 - 8 mg of protein per 1 mL in a 2×10^{-4} M ascorbate medium. Incubation was carried out by continuous stirring in a water thermostat at 36.7 ℃. Phospholipids were extracted [26], the amount of MDA in the aqueous layer was determined [5], the aliquot of the chloroform layer was evaporated, and the dry residue was dissolved in ethanol. Diene conjugates of hydroperoxides and the amount of phosphorus in the alcoholic solution were determined [5]. Protein components in mitochondria were determined by [39]. The obtained results were processed by the Student-Fisher statistical method by calculating the arithmetic mean (M), mean error (m), reliability index (t and p). A value of R less than 0.05 was used as a reliable difference indicator.

Results and discussion

In liver mitochondria, NADH is oxidized both internally, that is, through the respiratory chain (the main chain that is inhibited by rotenone) and externally (an alternative that does not detect rotenone). In the internal pathway, NADH transfers electrons along the respiratory chain to molecular oxygen, while the external pathway of free oxidation begins with NADH-cytochrome-5-reductase located in the primary region of the respiratory chain [1]. The results obtained on the effect of haplogenin-7-glycoside on the activity of mitochondria sensitive to rotenone NADH oxidase and insensitive to rotenone NADH oxidases during hypoxia and ischemia are presented in **Figures 1** and **2**. When mitochondria were incubated with haplogenin-7-glucoside, the activity of rotenone-sensitive NADH oxidase was increased relative to the control value, while the activity of rotenone insensitive NADH oxidase was decreased. These changes were consistent with the amount of haplogenin-7-glucoside. If, at 10, 20, 30 and 40 min of control, the activity of rotenone-sensitive NADH oxidase was 33.5; 46.4; Decreased by 50.1 and 55.7 %, respectively, when incubated with 20 μg haplogenin-7-glucoside for each mg of mitochondrial protein 15.9; 25.2; 31.6 and 36.1 %, and with the addition of 60 μg - 6.0; 12.2; decreased by 18.0 and 24.8 % (**Figure 1**).

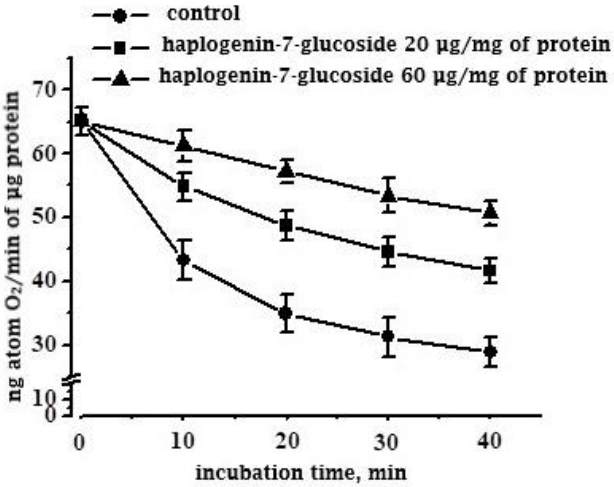

Figure 1 Effect of haplogenin-7-glucoside on rotenone-sensitive NADH oxidase activity during incubation of mitochondria for 40 min at 37 ℃ (in all cases $R < 0.05$; $n = 8$).

On the contrary, the activity of rotenone-insensitive NADH oxidase was 39.8 in the control; 52.8; Increased by 60.2 and 63.9 %, while the effect of 20 mcg on haplogenin-7-glucoside was partial, i.e., 18.8; 27.5; At 34.7 and 38.8 %, at 60 mcg - 9.6; 15.8; Increased by 21.2 and 26.1 % (**Figure 2**). Thus, in ischemia, disorders of mitochondrial membranes begin, resulting in desorption of cytochrome c from the inner membrane into the interstitial space, a decrease in the activity of rotenone-sensing NADH oxidase, and its restoration in the presence of haplogenin-7-glucoside. Under such conditions, the addition of flavin cytochrome c and cytochrome oxidase systems occurs. In addition, inhibition of rotenone-sensitive NADH oxidase is associated with a decrease in the transport of electrons from flavin mononucleotide (FMN) to CoQ [8]. In the oxidation of exogenous NADH in hepatic mitochondria, it was found that the transfer of electrons from the outer membrane to the inner membrane and from the inside to the outside is through cytochrome c [8,40]. Previously, it was hypothesized that the oxidation of NADH in damaged and injured mitochondria in the presence of externally added cytochrome c occurs only with cytochrome oxidase. Subsequently, the transport of electrons from the outer membrane to the inside has been shown to occur in intact, uninjured mitochondria.

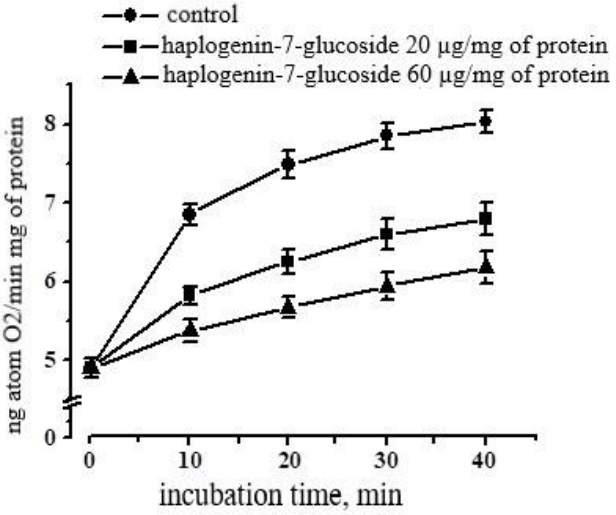

Figure 2 Effect of haplogenin-7-glucoside on the activity of rotenone-insensitive-NADH oxidase at the incubation by mitochondria at 37 ℃ for 40 min (in all cases $R < 0.05$; $n = 8$).

The authors conclude that there is an electron transport chain called bi-transmembrane at the junction of the inner and outer mitochondrial membranes. This chain is composed of complex III, NADH - b_5 - reductase, exogenous cytochrome c, cytochrome oxidase that direct electrons from the outer surface of the outer membrane to the inner membrane matrix, and vice versa. The activity of this pathway depends on the activity of the respiratory chain, as well as mitochondrial intactness. Here, a sharp increase in control of rotenone-insensitive-NADH-oxidase activity was observed. In the presence of haplogenin-7-glucoside 20 and 60 µg, it was found to reduce the increase in rotenone-insensitive-NADH-oxidase activity. Thus, haplogenin-7-glucoside improves the stability of mitochondrial membranes, thereby reducing the hydrolytic activity of free radicals and endogenous phospholipases and proteases against phospholipids and proteins. We are familiar with the chain reactions of LPO that lead to the formation of a wide range of intermediate and final products that play an important role in the processes of modification of biological membranes and their properties. These are aldehydes, ketones, alkanes, various long-chain alkenes with branched-chain and cyclic ring protection, and single short (usually in the first case) and single long-chain (usually in the second case) phospholipids with polar branches at the end [11]. Lipids of biological membranes are probably one of the closest biological markers [15]. Oxidation of lipid molecules leads to irreversible changes and injury of membrane structures, disruption of their permeability to ions, formation of peroxides and increased hydrophilicity of molecules. Oxidation results in the formation of double-bonding molecules (diene conjugates), as well as toxic and mutagenic aldehydes. Accumulation of oxidized forms of lipids in the blood leads to destructive changes at the molecular (free radical modification of blood lipoproteins), cellular (structural and functional changes of biomembranes) and organ levels.

Normally in an LPO system, antioxidants are balanced and work on the feedback principle. Increased activity of antioxidants leads to a decrease in free radical reactions, which in turn alters the properties of lipids, resulting in more easily oxidized fractions, resulting in accelerated LPO. This accelerates the consumption of endogenous antioxidants and returns to the previous state. A constant level of activity of natural antioxidants is one of the main indicators of homeostasis [37]. In addition, the LPO process is extremely important in that it is one of the physiologically important modifications of the "bilayer" structure of phospholipids in biomembranes, participating in the breakdown of membrane structures and the renewal of phospholipids [5]. The LPO reaction in the membrane leads to the formation of various primary and secondary products. As a result, the structure of the biomembrane undergoes significant changes. Short acetyl chain phospholipids, like lysophospholipids, form micelles within the biomembrane [29]. Like lysophospholipids, they can be called natural detergents. LPO increases the polarity of the membrane and the viscosity of the lipid "bilayer" [5]. As a result of LPO, the flip-flop transition speed between the membrane "layers" increases, and the lateral mobility and rotation speed of the membrane proteins decrease [23,24]. it has been studied the effect of LPO on membrane asymmetry of MDA that is the final reaction product [24]. MDA has been shown to induce the migration of phosphatidylethanolamine and phosphatidylserine aminophospholipids from the inner layer to the outer layer of the dialdygide membrane "bilayer".

As shown in the work of Matsuzaki *et al*. [11], the intensification of free radical processes of peroxidation of semi-saturated fatty acids is observed in the development of stress, i.e. almost all acute diseases and conditions, exacerbation of chronic diseases, intoxications, burns, injuries, operations and others. The biological feasibility of this intensification is based on the synthesis of eicosanoids in extreme conditions, the renewal of membranes and the emergence of detoxification (detoxification) processes. Accumulation of significant amounts of reactive oxygen species (such a condition is observed under the influence of radiation, ultraviolet rays, hyperbaric oxygenation, intoxication, including alcohol) can lead to a number of negative changes:

1) disruption of the liquid crystal structure of membrane lipoproteins.

2) decrease in the strength of biological membranes: Membrane damage, mitochondrial swelling and disruption.

3) structural and functional disorders of the respiratory enzyme systems.

4) oxidation of sulfhydryl groups of glucose-6-phosphate dehydrogenase, glyceraldehyde phosphate dehydrogenase and succinate dehydrogenases.

5) disruption of transport mechanisms of ion transport (Na^+, K^+, Ca^{2+}, etc.).

6) imbalance of various metabolites between mitochondria and ribosomes.

7) inhibition of biosynthesis of proteins, nucleic acids and other compounds.

8) release of hydrolytic enzymes by injury (rupture) of lysosomes.

9) disruption of the membrane of erythrocytes, decreased respiratory processes and the development of hemolysis.

10) accumulation of intermediate metabolic products, including lactic acid, oxy-, ketoxic acids and the development of acidosis (as a result of disruption of redox processes).

11) glutathione, inactivation of lipolatic acids and others.

The results obtained under the influence of haplogenin-7-glucoside on the LPO process in mitochondria under ischemic conditions are given in **Figure 3**. Haplogenin-7-glucoside lowers mitochondrial LPO and increases the effect of flavonoid as its amount increases. If, when incubating the controlled mitochondria for 20, 40 and 60 min, MDA was 88.4; increased by 215.9 and 255.1 %, respectively, with the addition of 20 µg haplogenin-7-glucoside to each mg of mitochondrial protein 57.9, 126.0 and 178.2 %, respectively, in 60 µg/mg protein - only 23.2; increased by only 87.0 and 113.0 % (**Figure 3**).

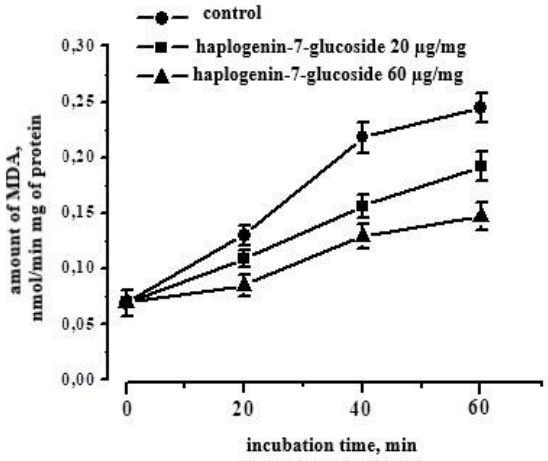

Figure 3 Effect of haplogenin-7-glucoside on LPO at incubation by mitochondria for 60 min at 37 °C. Mitochondria were stored at 37 °C for 60 min. The MDA amount was measured at an optical density of 532 nm. (pH 7.4) $R < 0.05$; $n = 8 - 10$.

Thus, the addition of haplogenin-7-glucoside to mitochondria leads to a decrease in MDA amount, a decrease in the LPO process, resulting in the transfer of electrons from substrates to the oxygen molecule in the respiratory chain and accelerated ATP synthesis. Lipids, and especially phospholipids, play a very important role in determining the functional state and structure of biological membranes. These properties of phospholipids are determined by the formation of a hydrophobic matrix in the membrane, which plays an important role in the functional state of the cell or their organelles [30,34].

Changes in the amount of phospholipids in mitochondrial membranes lead to structural and functional changes in the membrane. As a result, changes in the passage of ions in the membrane, the activity of receptors, OXPHOS, and the activity of various enzymes located there are observed. Therefore, we aimed to determine the structural changes in phospholipids when mitochondria were incubated in the presence of haplogenin-7-glucoside under conditions of peroxidation. In our next experiment, we corrected the quantitative change of phospholipids in mitochondria under LPO conditions with haplogenin-7-glucoside. At the same time, quantitative changes of phosphatidylserine over time (0 - 90 min) significantly accelerated LPO in the control (auto-oxidation). LPO induced by $FeSO_4$+ ascorbate was 25.2 compared to the control in the 0 - 90 min interval; 71.8 and 136.0 %, respectively. Peroxidation of phosphatidylserine in mitochondria with the addition of haplogenin-7-glucoside to IM 30; 14.3 compared to the group called LPO at 60 and 90 min; decreased by 91.4 and 108.0 %, respectively (**Figure 4A**). Phosphatidylserine contains glycerin, 2 fatty acids and serine. Serine-containing phosphatides are sour in nature due to the presence of amino acids. Phosphatidylserine retains many highly saturated fatty acids. Therefore, we suggest that an increase for phosphatidylserine increases the transferase activity of PLA2 and PLD [30,33] probably from acceleration. No time-dependent increase in phosphatidylinositol was observed in the control. However, it was found to be slightly increased under the influence of the compound (**Figure 4B**).

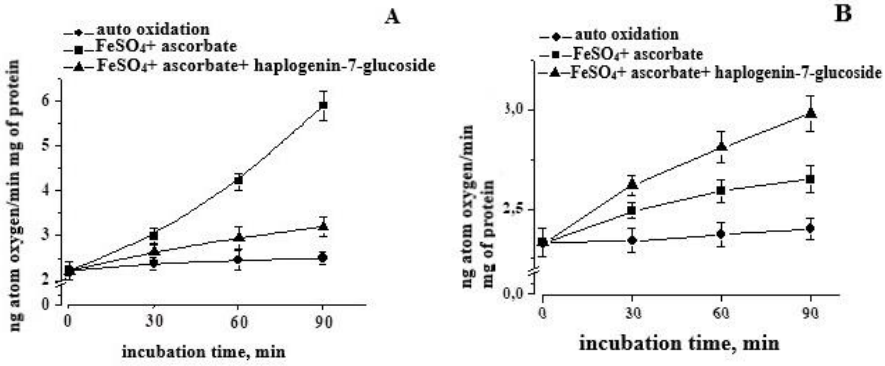

Figure 4 Quantitative correction of A) phosphatidylserine and B) phosphatidylinositol and under haplogenin-7-glucoside (60 μg/mg protein) under conditions of peroxidation of lipids in mitochondria at 37 ℃ ($R < 0.05$; $n = 6 - 8$).

Phospholipids that store inositol in biological membranes are in the form of triphosphoinositol, which contains salts of K^+, Na^+, Ca^{2+} and Mg^{2+}. Inositol-containing phospholipids occur in the form of K^+, Na^+ and Ca^{2+} mono-, di-, tri- and tetra-phosphatidylinositol. Phosphatidylinositol is involved in the transport of ions through biological membranes by special binding [47]. This is because, in our opinion, the transferase activity of PLA2 and PLD, as observed in small amounts of Ca^{2+} ions, first in phosphatidylserine [33] accelerates the synthesis of phosphatidylinositol because of enhancement, and most, conversely, slows it down or increases the activity of phospholipases relative to phosphatidylinositol. Hence, endogenous Ca^{2+} enhances phosphatidylinositol synthesis in the mitochondrial membrane, but an increase in Ca^{2+} ion reduces its amount. When mitochondria were incubated at 37 ℃, the amount of phosphatidic acid and lysophosphatidic acid in mitochondria under LPO conditions was also found to be less than control under the influence of haplogenin-7-glucoside. It was noted that the amount of phosphatidic acid accelerated the process of their peroxidation even without the addition of an LPO inducer to the incubation medium. Under the inductor effect ($FeSO_4$+ ascorbate), their LPO process was found to be 63.9 % higher than the control when the peroxidation process took 90 min. When mitochondria were incubated with haplogenin-7-glucoside, the amount of phosphatidic acid was 30 relatives to the group called LPO; 35.2 at 60 and 90 min, respectively; decreases were found to be 48.4 and 45.5 %, respectively (**Figure 5A**).

The amount of lysophosphatidic acid was also observed to increase relative to control under inducer action. At incubation an increase in lysophosphatic acid in mitochondria with haplogenin-7-glycoside, their peroxidation process was 30 relative to the group called LPO; the same effect was found at 60 and 90 min, i.e., an average decrease of 106 % (**Figure 5B**).

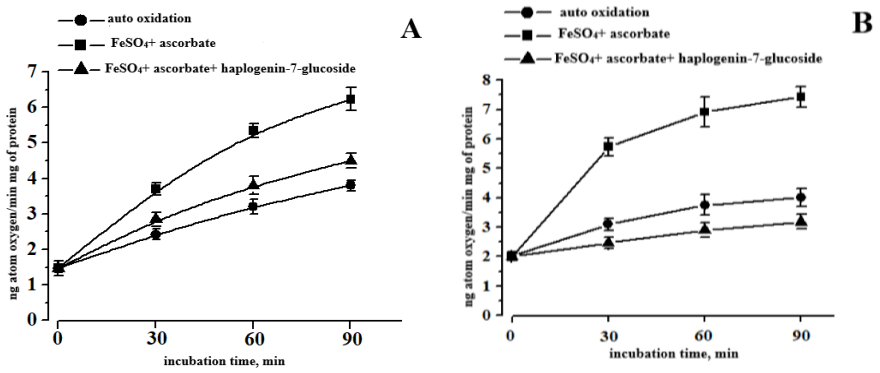

Figure 5 Correction of quantitative changes of A) phosphatidic acid and B) lysophosphatidic acid with haplogenin-7-glucoside (60 μg/mg protein) in the conditions of peroxidation of lipids in mitochondria during incubation of mitochondria for 90 min at 37 ℃.

Thus, haplogenin-7-glucoside reduces the rate of peroxidation of phospholipids by liver mitochondria and has a corrective effect. The main content of the initial stages of apoptosis induction resulting from the occurrence of respiratory and oxidative stress in mitochondria has not changed [14]. In addition, a number of experiments have found important "details" proving the oxygen-peroxide mechanism of apoptosis [14]. For example, during the development of the nervous system in vertebrates, in the state of apoptosis, neurons lose a slightly oxidized phospholipid - cardiolipin, which is part of the inner membrane of mitochondria. These cells show a decrease in the amount of cardiolipin with the loss of mitochondria, with a slight decrease in the mass of mitochondria with the loss of cardiolipin. The fact that the loss of cardiolipin is associated with an increase in LPO by the ROS development by mitochondria in a competitive manner suggests the involvement of free oxygen radicals in these processes [36]. In our opinion, the increase in phosphatidic and lysophosphatidic acids in mitochondria under these conditions may be due to an increase in the synthesis of these phospholipids in the membrane, or an increase in the hydrolytic activity of PLD.

Conclusions

When mitochondria are stored under hypotonic sucrose (hypoxia and ischemia) (36.7 °C), disorders of mitochondrial membranes begin, as a result, cytochrome c desorption from the inner membrane into the interstitial space leads to a decrease in the activity of rotenone-sensitive NADH-oxidase and an increase in the activity of non-rotenone-sensitive NADH-oxidase. Haplogenin-7-glycoside, on the other hand, showed membrane-stabilizing properties by significantly reducing these changes; the addition of haplogenin-7-glucoside to mitochondria leads to a decrease for MDA, a decrease in the LPO process, resulting in the transfer of electrons from substrates to the oxygen molecule in the respiratory chain and accelerated ATP synthesis (**Figure 6**).

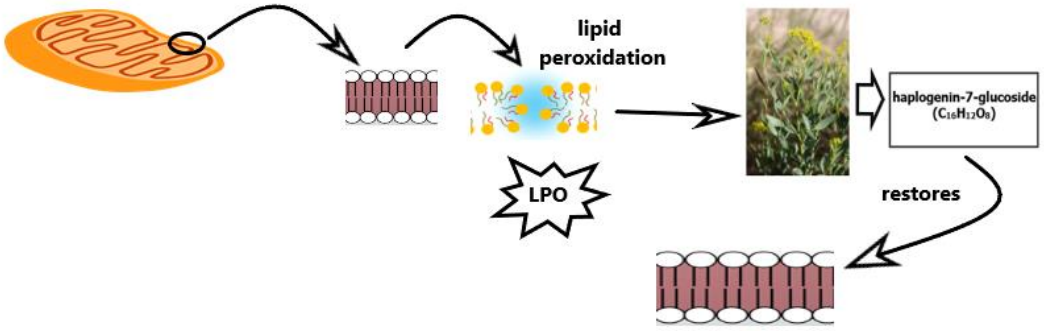

Figure 6 The transfer of electrons from substrates to the oxygen molecule in the respiratory chain and accelerated ATP synthesis.

Quantitative changes in phospholipids under LPO conditions in mitochondria were corrected with haplogenin-7-glucoside. Peroxidation of phosphatidylserine in mitochondria when haplogenin-7-glucoside is added to the incubation medium 30; 14.3 compared to the group called LPO at 60 and 90 min; 91.4 and 108.0 %, respectively. No time-dependent increase in phosphatidylinositol was observed in the control. However, a slight increase in the effect of haplogenin-7-glucoside and membrane-stabilizing effect was found.

When mitochondria were incubated at 37 °C, the amount of phosphatidic acid and lysophosphatidic acid in mitochondria under LPO conditions was also found to be less than control under the influence of haplogenin-7-glucoside. Thus, haplogenin-7-glucoside reduces the rate of peroxidation of phospholipids by liver mitochondria and has a corrective effect.

From the above data, it is clear that the results obtained based on experiments show a number of new aspects of the effect of haplogenin-7-glucoside. These help to understand the mechanisms of their action at the level of mitochondria in various physiological and pathological processes. The pharmacology of this compound can be further studied and used in the production of drugs that will be used in the treatment of liver diseases in the future.

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