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EFFECT OF GENISTEIN AND OROBOL ISOFLAVONES ON FE^{2+}/ ASCORBATE INDUCED LIPID PEROXIDATION IN LIVER AND HEART MITOCHONDRIA OF RATS AND CENTRAL ASIAN STEPPE TURTLES

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ABSTRACT
This article studied the effect of genistein and orobol isoflavones on Fe^{2+}/ascorbate-induced LPO (lipid peroxidation) in mitochondria of the liver and heart of the Central Asian steppe turtle rat. In particular, it was studied the inhibitory effect of genistein and orobol on the swelling of liver and heart mitochondria in warm-blooded and cold-blooded animals with the help of Ca^{2+} ions. In addition, their inhibitory effect on Fe^{2+}/ascorbate-induced LPO and a decrease in the content of MDA (malonic dialdehyde) in mitochondrial membranes of rat liver and Central Asian steppe turtle were studied.

Keywords: mitochondria, genistein, orobol, mPTP, LPO, MDA.

INTRODUCTION

LPO products change the physicochemical properties of biological membranes [4]. For example, the introduction of LOOH changes the structural composition of membranes and damages them [3]. The main properties of membranes are fluidity and permeability, and the above substances ultimately lead to disruption of many of their functions [10]. After the attack of free radicals has stopped, LPO products must be removed from the damaged membrane in order to restore it.

The participation of iron in the LPO process is not limited to the beginning of the process. Further deepening of the LPO process also requires the participation of iron. Iron ions activate the breakdown of formed lipid hydroperoxides (LOON), they are converted into other lipid free radicals such as peroxyl (LOO•) and alkoxyl (LO•). As a result, the accumulated free low molecules and lipid radicals can damage almost all organic molecules (proteins, lipids, nucleic acids, etc.) and all biological structures. As a result, the accumulated free low molecular weight and lipid radicals can damage almost all organic molecules (proteins, lipids, nucleic acids, etc.) and all biological structures [11].

From many literature data, it is known that LPO-inhibiting compounds of the inner and outer mitochondrial membranes usually have an inhibitory effect on mPTP permeability [5]. Indeed, according to the results of our experiments, it was found that genistein and orobol isoflavones have an inhibitory effect on Ca^{2+} induced swelling of liver and heart mitochondria in rats and Central Asian desert turtles. It is not clear whether these compounds with an inhibitory effect [6] on mitochondrial RTR conductance have an inhibitory effect on Fe^{2+}/ascorbate-induced

LPO? To test this hypothesis, we studied the effect of genistein and orobol isoflavones on the content of Fe^{2+}/ascorbate-induced LPO and MDA in rat liver mitochondria.

The aim of the study was to study the effect of genistein and orobol isoflavones on Fe^{2+}/ascorbate-induced LPO in liver and heart mitochondria of rats and Central Asian desert turtles..

MATERIALS AND METHODS

Experiments were carried out in vitro on purebred white rats weighing 180–200 g and Central Asian desert turtles weighing 400–450 g. Rat and turtle liver mitochondria were isolated by differential centrifugation [9]. The Fe^{2+}/ascorbate system was also used to study the LPO process in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its barrier function, as a result of which the size of the organelle increased and the mitochondria swelled [7]. This change in volume was determined photometrically (V-5000Visible Spectrophotometer). IM: KCl - 125 mm, Tris-HCl - 10 mm, pH 7.4; Concentrations: FeSO4 - 10 μM, ascorbate - 600 μM; amount of mitochondria 0.5 mg/ml.

Mitochondrial protein was determined by the Peterson modification of the Lowry method [8].

Statistical processing of the obtained results and figures was carried out using the computer program OriginPro 7.5 (Microsoft, USA). In the experiments, the kinetics of mitochondrial swelling was calculated as a percentage of the maximum, as the arithmetic mean of 4-5 different experiments was calculated. The difference between the values obtained from the control and experimental+test material was calculated using the t-test. Here *P<0.05 and **P<0.01 represent statistical significance

The results obtained and their analysis. According to the results of the experiment, after the addition of Fe^{2+}/ascorbate to the incubation medium, the induced LPO process, that is, the rate of mitochondrial swelling, was taken as 100% (Fig.1). At the same time, Fe^{2+} in the incubation medium immediately forms chelates in mitochondria with a number of molecules of organic compounds, for example, citrate or adenosine diphosphate. Such chelates participate in Fenton reactions and promote the formation of PO [11]. The hydroxyl radical is capable of splitting off a hydrogen atom from polyunsaturated fatty acids and initiating lipid peroxidation [1]. Under the influence of Fe^{2+}/ascorbate, lipid peroxidation products disrupt the barrier function of the mitochondrial membrane, which leads to an increase in the rate of mitochondrial swelling compared to the control.

It was studied an effect of 10-60 μM genistein concentrations on Fe^{2+}/ascorbate-induced LPO in rat liver mitochondria. In experiments, the effect of genistein at a concentration of 10 μM on Fe^{2+}/ascorbate-induced LPO in the mitochondrial membrane of the rat liver was insignificant compared to the control (Fig.1). As the concentration of genistein in the incubation medium increased, its inhibitory effect on lipid peroxidation became strongly manifested. Genistein concentrations of 20, 30, and 40 μM were found to inhibit Fe^{2+}/ascorbate-induced LPO in the mitochondrial membrane by 17.9%, 44.2%, and 59.8%, respectively, compared with the control. The concentration of 60 μM genistein isoflavone had the maximum inhibitory effect on the LPO process in rat liver mitochondria compared to the control, and the half-maximal inhibitory concentration (IC50) was 32.8 ± 2.5 μM (Fig.1). According to the results obtained,

genistein concentrations of 10–50 μM had an antioxidant effect on the mitochondrial membrane.

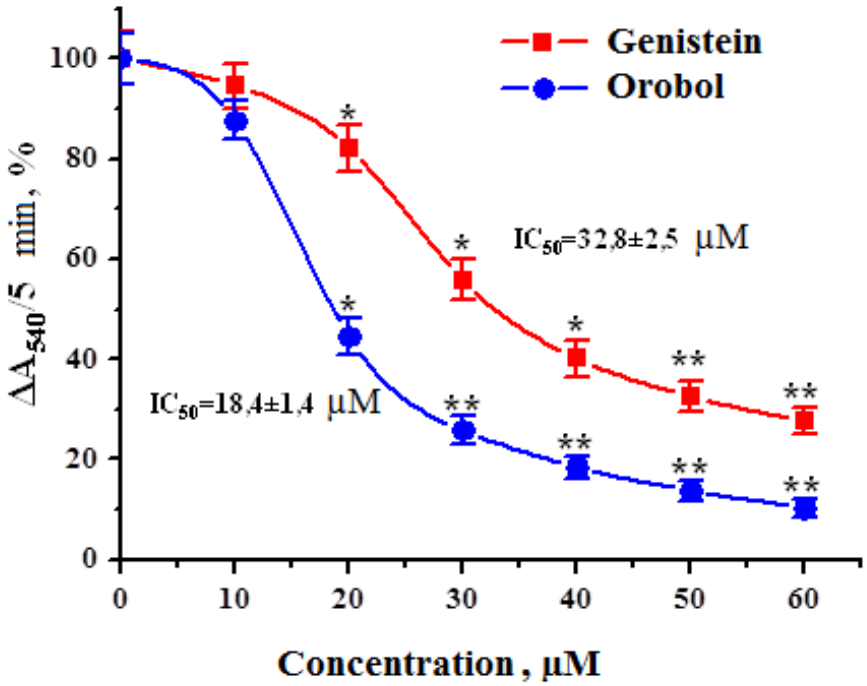

Figure 1. Effect of genistein and orobol isoflavones on Fe^{2+}+ascorbate-induced LPO in rat liver mitochondria (*P<0,05; **P<0,01; n=6).

While continuing the experiments, it was also studied the effect of orobol on Fe^{2+}/ascorbate-induced LPO in rat liver mitochondria. According to the obtained results, it was found that orobol in the presence of a concentration of 10-60 μM in the incubation medium has an inhibitory effect on LPO in liver mitochondria (Fig.1). Here it was found that orobol concentrations of 10, 20, and 30 μM inhibited Fe^{2+}/ascorbate-induced LPO in rat liver mitochondria by 12.4%, 55.5%, and 74.2%, respectively, compared with the control. As the concentration of orobol increased, its LPO inhibitory property became apparent. Here it was found that 60 μM orobol inhibited mitochondrial LPO by 89.7% compared with the control, and the half-maximal inhibitory concentration (IC50) was 18.4±1.4 μM (Fig.1). Thus, orobol isoflavone has an inhibitory effect on LPO in rat liver mitochondria, maintains the structure of the matrix, and contributes to the manifestation of its physiological function.

In our next experiment, we studied the effect of genistein and orobol on Fe^{2+}/ascorbate-induced LPO in rat heart mitochondria. At the same time, the effect of genistein and orobol concentrations of 10-50 μM on lipid peroxidation of cardiomyocyte mitochondria was not clearly manifested due to the fact that the heart tissue is an excitable tissue due to the rapid implementation of calcium-ion-dependent signaling processes in them. Thus, it was shown that the effect of genistein and orobol on Fe^{2+}/ascorbate-induced lipid peroxidation of the mitochondrial membrane of the heart appears at slightly higher concentrations. Genistein concentrations of 20–100 μM had an inhibitory effect on lipid peroxidation of the rat heart mitochondrial membrane (Fig. 2). As the concentration of genistein increased, it was observed that its inhibitory property also increased. At the same time, the concentration of 20 μM

genistein did not affect LPO in rat heart mitochondria, while 40 μM genistein partially inhibited LPO. Genistein concentrations of 60 and 100 μM were found to inhibit cardiac mitochondrial lipid peroxidation by 42.2% and 66.6%, respectively, compared with the control. Therefore, genistein had an inhibitory effect on the process of lipid peroxidation in the mitochondria of the heart.

In continuation of the experiments, it was also studied the effect of orobol on the LPO process in rat heart mitochondria. According to the results obtained, it was found that concentrations of 20-100 μM orobol have an inhibitory effect on the LPO process with Fe2+/ascorbate of the mitochondrial membrane of the heart (Fig. 2).

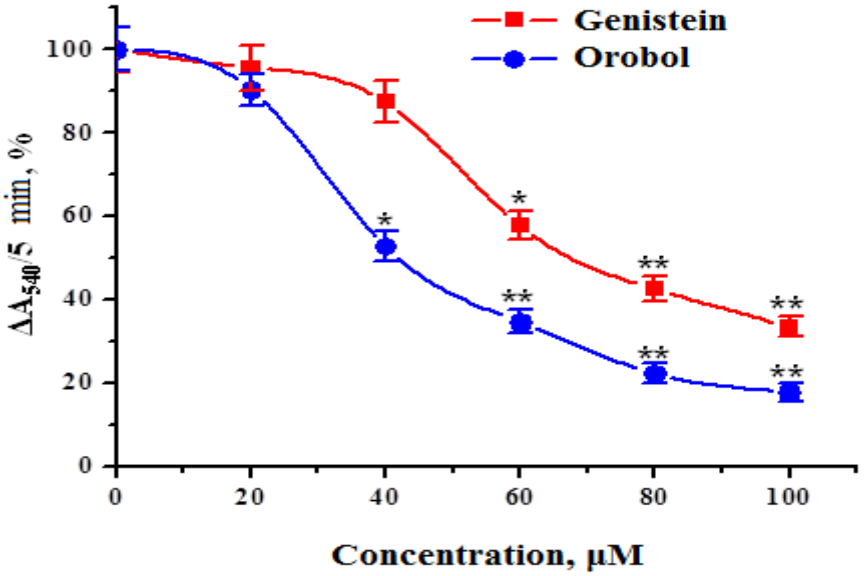

Figure 2. Effect of genistein and orobol isoflavones on Fe^{2+}+-ascorbate-induced LPO in rat heart mitochondria (*P<0,05; **P<0,01; n=6).

In this case, the concentration of orobol at a concentration of 20 μM did not clearly inhibit the mitochondrial lipid peroxidation of the heart. However, an increase in its concentration in the incubation medium exhibited an inhibitory effect on the LPO process with the Fe^{2+}/ascorbate in mitochondrial membrane. It was noted that concentrations of orobol at concentrations of 40 and 100 μM inhibited cardiac mitochondrial lipid peroxidation by 47.2 and 82.3%, respectively, compared with the control (Fig. 2). Therefore, it was observed that the inhibitory effect of orobol isoflavon on lipid peroxidation was more active than that of genistein. This may be due to the structure of the orobol. Because the number of hydroxyl groups and the position of the carbon ring in orobol isoflavone is slightly different from that in genistein. It was concluded that it is this chemical structure that may give orobol its active antioxidant properties.

The effect of genistein and orobol isoflavones on Fe^{2+}/ascorbate-induced LPO in liver and heart mitochondria of the desert turtle. The metabolic rate in rats is 7 times higher than in reptiles. The inner membrane of isolated rat liver mitochondria has a proton permeability 4-5 times higher than the proton permeability of the lizard liver mitochondrial membrane. The high permeability of rat mitochondria does not depend on the size of the inner mitochondrial membrane, but differences in permeability may be due to differences in the fatty acid composition of mitochondrial phospholipids. The high proton permeability of the inner

mitochondrial membrane can contribute to the high standard metabolic rate of mammals [2]. However, there are few published data on LPO processes in liver and heart mitochondria in the Central Asian desert turtle and the effect of biologically active compounds on them. A series of experiments was carried out to answer the question of whether there are differences in LPO from LPO from rat liver mitochondria in liver and heart mitochondria of the Central Asian desert turtle.

First, the effect of genistein on lipid peroxidation with Fe^{2+}/ascorbate in turtle liver mitochondria was studied. After adding Fe^{2+}/ascorbate to the incubation medium, the induced LPO process, i.e. the swelling rate of mitochondria was taken as 100% and designated as control. It was noted that the intensity of the LPO process in turtles is slower than in rat liver mitochondria. According to the results obtained, it was found that genistein concentrations of 10-100 μM have an inhibitory effect on LPO induced by Fe^{2+}/ascorbate in turtle liver mitochondria. At the same time, it was found that the LPO process in liver mitochondria decreased by 12.6% and 32.3%, respectively, compared with the control in the presence of genistein concentrations of 20 μM and 40 μM in the incubation medium (Fig. 3). It was found that high concentrations of genistein, 80 and 100 μM, inhibit Fe^{2+}/ascorbate-induced LPO in turtle liver mitochondria by 56.7% and 62.3%, respectively, compared with the control. At the same time, it was noted that the half-maximal inhibitory concentration of genistein on lipid peroxidation of liver mitochondria was IC_{50}=35.7±2.7 μM (Fig. 3). Thus, genistein isoflavone has an inhibitory effect on the intensity of the LPO process in the mitochondrial membrane of the turtle liver.

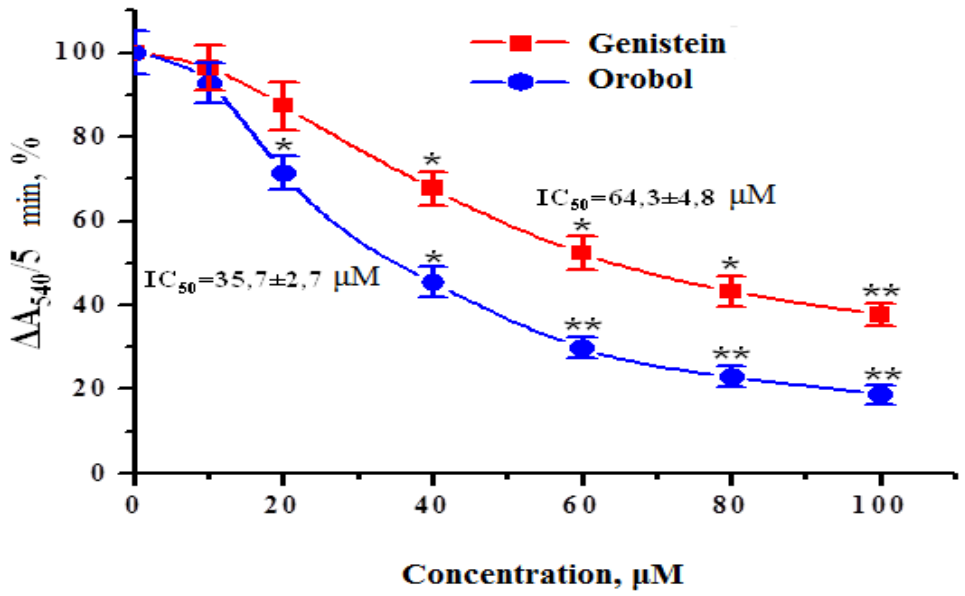

Figure 3. Effect of genistein and orobol isoflavones on Fe^{2+}+-ascorbate-induced LPO in turtle liver mitochondria (*P<0,05; **P<0,01; n=6)

In our next experiment, we studied the effect of orobol isoflavone on the LPO process in turtle liver mitochondria at concentrations of 10-100 μM. According to the results obtained, orobol isoflavone also has an inhibitory effect on the LPO process induced by Fe^{2+}/ascorbate in turtle liver mitochondria. It was found that the concentration of orobol at a concentration of 20 μM inhibited lipid peroxidation of turtle liver mitochondria by 28.6%, and at a concentration of

100 μM by 81.3% compared with the control. It was noted that the half-maximal inhibitory concentration of orobol at LPO in liver mitochondria is IC_{50}=64.3±4.8 μM (Fig. 3).

It was noted that the intensity of membrane lipid peroxidation of turtle liver mitochondria was significantly lower than that of rat liver mitochondria. This can be explained by the adaptation of the turtle to hot climate conditions, inactivity and slow metabolism of substances and energy.

In our next experiment, we investigated the effect of genistein and orobol on Fe^{2+}/ascorbate-induced LPO in the heart mitochondria of the Central Asian desert turtle. Here we studied the effect of genistein and orobol concentrations of 10–100 μM on lipid peroxidation of cardiomyocytes' mitochondria (Fig. 4). Genistein concentrations of 10–100 μM had an inhibitory effect on lipid peroxidation of the mitochondrial membrane of the turtle heart. Genistein concentrations of 10 and 20 μM had no inhibitory effect on Fe^{2+}/ascorbate-induced LPO in turtle heart mitochondria. As the concentration of genistein increased, it was observed that its inhibitory property also increased. Here, genistein concentrations of 40 and 100 μM were found to inhibit cardiac mitochondrial lipid peroxidation by 27.4% and 58.5%, respectively, compared to control. It was noted that the half-maximal inhibitory concentration of genistein in relation to lipid peroxidation of heart mitochondria is IC_{50}=79.3±4.8 μM (Fig. 4). Therefore, genistein had an inhibitory effect on the process of lipid peroxidation in the mitochondria of the heart.

In continuation of the experiments, we also studied the effect of orobol on the LPO process in turtle heart mitochondria. According to the obtained results, it was found that concentrations of 10-100 μM orobol have an inhibitory effect on the LPO process of the heart mitochondrial membrane induced by Fe^{2+}/ascorbate (Fig. 4). It was noted that concentrations of orobol at concentrations of 40 and 100 μM inhibited cardiac mitochondrial lipid peroxidation by 47.2% and 78.9%, respectively, compared with the control. The half-maximal inhibitory LPO concentration in orobol heart mitochondria was IC50=43.1±3.4 μM (Fig. 4).

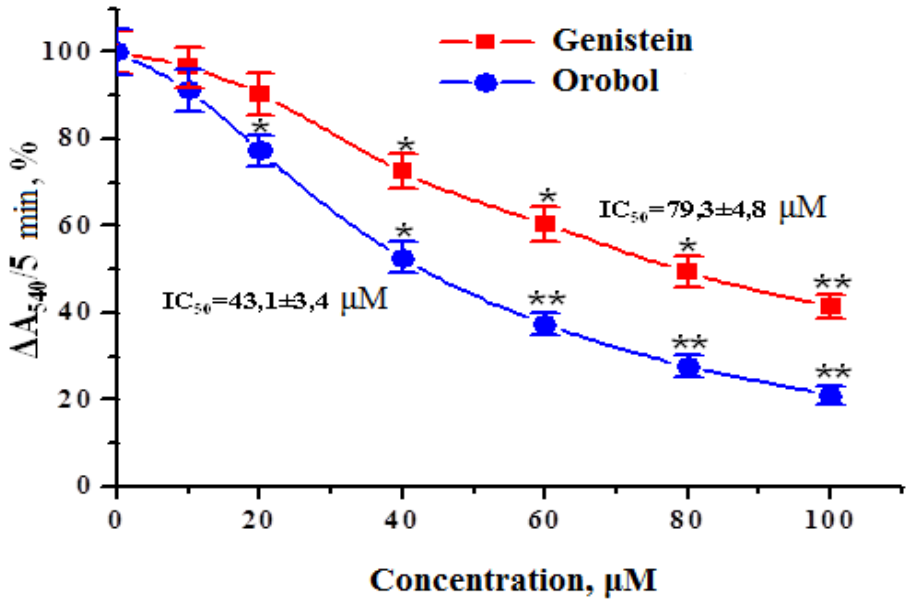

Figure 4. Effect of isoflavones genistein and orobol on Fe2++-ascorbate-induced LPO in turtle heart mitochondria (*P<0,05; **P<0,01; n=6).

Thus, the inhibitory effect of isoflavone orobol on lipid peroxidation was more active than that of genistein.

The inhibitory effect of flavonoids on the mPTP of liver mitochondria may be related to their antioxidant properties. Because flavonoid compounds have the ability to neutralize radicals and oxidants formed in mitochondria. The transition of a highly permeable mitochondrial pore to an open conformation can lead to an acceleration of electron transport along the respiratory chain and an increase in the formation of reactive oxygen species. We hypothesized that the inhibitory effect of the isoflavones genistein and orobol on liver mPTP may be due to their inhibition of lipid peroxidation in the membrane.

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