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INFLUENCE OF QUERCETIN AND DIHYDROQUERCETIN ON SOME FUNCTIONAL PARAMETERS OF RAT LIVER MITOCHONDRIA

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ABSTRACT

Flavonoids are proved to be prominent plant-derived compounds generally found in fruit- and vegetable- rich diets with free radicals quenching ability. Quercetin, a member of flavonoids subclass – flavonols, has gained major attention due to its overwhelming potential. Quercetin was demonstrated to preserve diverse degenerative diseases by inhibiting lipid peroxidation. In the present paper, we deal with two polyphenolic compounds' – quercetin and dihydroquercetin – influence on functional parameters of mitochondria. Thus, the fore mentioned flavonols showed the mitochondrial permeability transition pore (mPTP) inhibition and ATP-sensitive potassium channel activation capacities as well as strong antioxidant/antiradical activity. Improving functional parameters of mitochondria by both quercetin and dihydroquercetin formed a basic idea of the hypothesis that the studied flavonols may provide antihypoxic defense.

Keywords: flavonoids, antioxidant activity, antihypoxic agents, mPTP, mitoKATP

INTRODUCTION

Mitochondria play a major role in the apoptosis and necrosis regulation (**Dave, Byfield, *et al.*, 2008; Vladimirov, 2002; Shimizu, Tsujimoto, 2007**). The parameters like mPTP, oxidation phosphorylation, antioxidant defense systems, and others regulate the functionality of mitochondria (**Pozhilova, Levchenkova, *et al.*, 2014**). The swollen mitochondria initiates opening of mPTP that leads to the rupture of the outer mitochondria membrane as well as intermembrane components leakage into the cytosol provoking the process of cell apoptosis. The Ca^{2+} ions accumulation in the matrix of the mitochondria is also a direct regulatory mechanism for opening the mitochondrial pore, a key link in the launch of the cell apoptosis program (**Dave, Byfield et.al, 2008; Tsujimoto, Shimizu, 2007**). The formation and opening of mPTP is not the only mechanism for the leakage of mitochondria intermembrane components into the cytosol. However, the search for medicinal substances, the target of which will be the mitochondrial mega pore, can become a promising direction in the correction of many pathological conditions (**Novikov, Levchenkova, 2013**). Also, recently, interest in the study of the mitochondrial ATP-sensitive potassium channel (mitoKATP channel) has increased, since it has been established that its activation plays a key role in protecting organs and tissues from the effects of ischemia/reperfusion (**Akao, Ohler et .al, 2001**) and in addition to its important role, the mitoKATP channel plays a huge role in adapting the body to various stress factors (**Danilenko, Lokrovsky *et al.*, 2010; Hund, Mohler, 2011**).

It is known that the opening of the mitoKATP channel leads to depolarization of the membrane potential of mitochondria and causes the reversible oxidation of flavoproteins of the respiratory chain, thereby increasing the production of reactive oxygen species (ROS) in mitochondria (**Samavati, Monick et.al, 2002**). But in a review, **Pozhilova *et al.*** show that the natural metabolic activators of the mitoKATP channel, uridine and its nucleotides in micromolar concentrations, significantly reduce the area of myocardial infarction, normalize the level of ATP, creatine phosphate and antioxidant defense systems, reducing the formation of ROS, and normalize the heart rate by rat myocardial infarction models (**Pozhilova, Levchenkova *et al.*, 2014**). It is also mentioned that the opening of the mitoKATP channel leads to a decrease in ROS in tissue cells, thereby regulating it (**Ferranti, da Silva *et al.*, 2003; Facundo, de Paula *et al.*, 2007; Fornazari, de Paula et.al, 2008**) Thus, the activation of the potassium cycle upon adaptation to the stress factor and the subsequent decrease in ROS formation can explain the known protective role of the mitoKATP channel in ischemia/reperfusion (**Garlid and Halestrap, 2012; Meng, Ma et.al, 2016**).

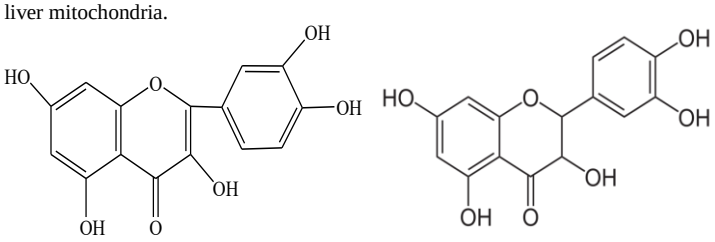
Activation of lipid peroxidation (LPO) is often one of the triggers of many diseases and is also an aggravating factor in many pathological conditions (**Mylonas, Kouretas, 1999; Lobo, Patil et.al, 2010; Chesnokova, Morrison *et al.*, 2009**). Moreover, the use of antioxidants in the treatment of various pathological conditions of the body slows down the course of pathological conditions caused by an excess of free radicals, since antioxidants can prevent the activation of induced free radical reactions (**Brewer, 2011**). Thus, it can be concluded from the above material that compounds with antioxidant activity can be considered as promising universal remedies promoting the prevention and treatment of a oxidative stress caused disorders.

Given the above, studies regarding the molecular and cellular mechanisms of plant compounds actions are currently considered relevant to detect potential pharmacological agents with effect on the organism. One of the potential pharmacological agents is polyphenol compounds of plants. Polyphenolic compounds have a very wide spectrum of action on biological objects, exhibiting antioxidant, hepatoprotective, antihypoxic, membrane-tropic and many other effects (**Asrarov, Komilov *et al.*, 2015; Rustamova, Irgasheva, *et al.*, 2005; Tsybulsky, Popov *et al.*, 2011**). The manifested physiological responds of cells exposed to polyphenols are observed due to polyphenols' ability to alter the mitochondrial membrane permeability with respect to particular ions that is crucial in preventing of different pathological conditions in an organism. It's been widely accepted that quercetin, typical component of onions, buckwheat and citrus fruit and utilized in traditional medicine as preventive agent against variety of diseases, such as cancer (**Zhang *et al.* 2011; Jovanovic, 1999; Mullen, 2008**), cardiovascular and nervous disorders (**Shankar, 2007; Labinskyy, 2006**), obesity (**Yang *et al.* 2008**), and chronic inflammation (**Teixeira S., 2002; Garcna-Mediavilla V., 2007**) possesses its therapeutic effect through immense antioxidant activity. Although the underlying mechanisms of biological action are largely under debate, the quercetin structure suggests multi-functional and mitochondria-mediated mechanism (**Karbarz M, 2008**).

Being a significant member of the flavonol subclass, quercetin, has attracted considerable attention. Moreover, quercetin and its glucosylated forms contribute to 60-75% of flavonoid intake (**Bouktaib *et al.* 2002**).

Oxidized low-density lipoproteins (LDL), that are hallmarks of certain diseases, such as cancer, atherosclerosis, and chronic inflammation (**Hollman and Katan 1997; Murota and Terao 2003**), were shown to be strongly inhibited by quercetin both by free radicals scavenging mechanism and chelating transition metal ions.

Thus, in this work, we studied the comparative mechanism of effect of two flavonoids (the quercetin and its derivative dihydroquercetin) on some functional parameters of mitochondria, such as mPTP, mitoKATP channel, and LPO of rat liver mitochondria.



A **B**

Figure 1 Chemical structures of quercetin (A) and dihydroquercetin (B)

MATERIALS AND METHODS

Mitochondria isolation

Mitochondria isolation protocol was followed as described by (**Schneider, Hageboom, 1951**) from rat liver tissue homogante (white outbred rats, both sexes, 180-200 g). In brief, rat liver tissue was homogenized on ice, medium A contained sucrose (250 mM), EDTA (0.5 mM), Tris-HCl buffer (10 mM) adjusted to pH 7.4, centrifuged at 1500 g for 7 min (–2 to –4 °C). After centrigugation supernatant was collected and mitochondria was precipitated at 6000 g for 15 min (–2 to –4 °C), resuspended in medium A lacking EDTA, and stored on ice until needed. The mitochondrial protein amount was measured by the Lowry protein assay with minor modifications **(Peterson, 1977)**. All experiments were performed at room temperature, on colorimeter Granat, Russia, 1991.

Mitochondria swelling assay

The opening of mPTP was observed through mitochondrial swelling by recording the spatial light scatter intensity at 540 nm in the presence of 10 mM Ca^{2+} as a control. 1 ml of mitochondrial solution contained 0.3-0.4 mg protein. The buffer contained sucrose (200 mM), EGTA (20 μM), succinate (5 mM), rotenone (2 μM), oligomycin (1 μg/ml), Tris (20 mM), HEPES (20 mM), KH_2PO_4 (1 mM), adjusted to pH 7.2 **(He, Lemasters, 2003)**. Polyphenols were added at the 1-100 μM concentration range.

mitoK_{ATP} current recording

mitoKATP channel current recording was monitored at 540 nm in external solution contained KCl (125 mM), Hepes (10 mM), succinate (5 mM), $MgCl_2$ (1 mM), K_2HPO_4 (2.5 mM), KH_2PO_4 (2.5 mM), rotenone (0.005 mM), oligomycin (0.001 mM) **(Vadzyuk, Kosterin, 2008)**. Polyphenols were added at the 1-50 μM concentration range. 10 mM of ATP was accepted as control.

Lipid peroxidation assay

Lipid peroxidation (LPO) was initiated by Fe^{2+}/ascorbate system in mitochondria suspension, external medium contained: KCl (125 mM), Tris-HCl (10 mM), pH adjusted to 7.4, according to **(Schneider *et al.*, 1948)**. 1 ml of mitochondrial solution contained 0.5 mg protein. 10 μM $FeSO_4$ together with 600 μM ascorbate were added to medium to generate process of mitochondrial swelling. The experiments were carried out 24-26°C.

DPPH assay

DPPH kinetics was carried out as described elsewhere **(Gayibova, 2019)**. Briefly, the kinetic results regarding DPPH (0.1 mM) decolorization in the presence of polyphenols (up to 10 μM) was recorded on SF-46 (LOMO, Russia, 1996). The spectroscopic data was fixed every 15 sec until the reaction reached steady state.

Conductometry

The conductivity of the solutions was measured using a Sartorius Professional meter PP-20 conductometer (USA) at a temperature of 25 ° C, at a constant salt concentration and a variable concentration of polyphenols. During the experiment, a weighed portion of polyphenols was dissolved in 20 ml of water, and then the conductivity was measured by successively diluting a solution of divalent metal salt (initial concentration of 10^{-3} M) with certain volumes of the initial solution of the substance (0.01 M) (**Andreev, 1971**).

Chemical reagent

EDTA, EGTA and cyclosporine A were purchased from “Sandoz”, Switzerland, rotenone and tris-HCl were from “Serva”, Germany, DPPH and $CaCl_2$ were from “Sigma”, USA. Other reagents were purchased from the local companies with grade marked as “chemically pure”.

Data analysis

A statistical analysis was based on three replicates computed on the statistical package Origin 6.1 (Origin Lab Corporation, USA). Statistical significance was performed as Student’s t-test. Mean values and standard deviations were calculated. Statistically significant results were expressed at * - $P<0.05$; ** - $P<0.01$; ***- $P<0.001$.

RESULTS AND DISCUSSION

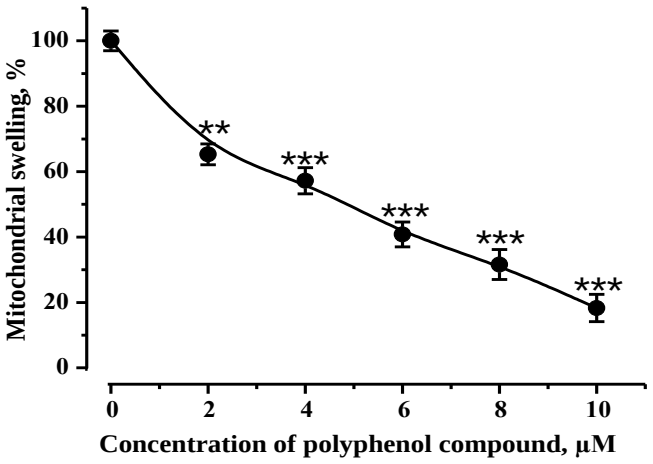
Antioxidant activity

Plant-derived compounds are an enduring source of phytochemicals possessing a list of therapeutic implementations.

In the molecular pathogenesis of plants’, animals’, and humans’ disorders, oxidative stress contributed with free radicals overproduction is suspected to play significant role. Therefore, the screening for the free radicals scavenging regulators of natural and synthetic nature and studying their antiradical processes remain relevant.

All plant compounds concerning animal organisms have biological activity of an extremely wide spectrum, due to the diversity of their chemical structure, and are currently in the center of scientific attention. In connection with the foregoing, the search for antioxidants and the study of their inhibitory effect on the processes of free radical oxidation. In this regard, the effect of various concentrations of quercetin and dihydroquercetin on preventing Fe^{2+}/ascorbate-induced mitochondria lipid peroxidation was studied *in vitro*.

The introduction into the external medium the system, including Fe^{2+}/ascorbate, activates lipid oxidation, that in turn destroys mitochondria membrane barrier function and leads to mitochondria swelling (Fig. 2). When quercetin and dihydroquercetin at a minimal dose of 2 μM are added into external solution, containing Fe^{2+}/ascorbate, the antioxidant properties of polyphenols are observed that is reflected in mitochondria swelling inhibition. The effect of quercetin and dihydroquercetin on the process of mitochondrial membranes peroxidation revealed dose-dependent manner. Also, at the concentration of 10 μM both compounds totally suppressed the mitochondria swelling proving the inhibition of lipid oxidation with IC_{50} values as follow 6.08 ± 0.06 μM (quercetin) and IC_{50}=8.1 ± 0.09 μM (dihydroquercetin).



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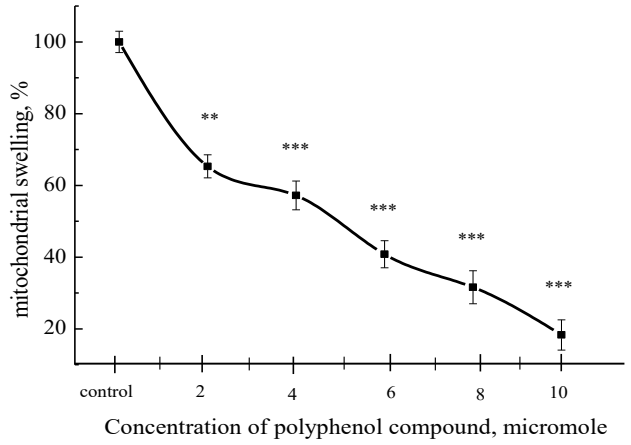


B

Figure 2 Influence of quercetin (A) and dihydroquercetin (B) on the Fe^{2+}/ascorbate generated mitochondria swelling. External medium was as follow: KCl (125 mM), Tris-HCl (10 mM), pH 7.4; $FeSO_4$ (10 μM), ascorbate (600 μM), protein content 0.5 mg/ml

Thus, obtained experiments show that quercetin has higher AOA compared to its derivative dihydroqercetine. The antioxidant properties of polyphenolic compounds are strongly up to the number of hydroxyl groups in the benzene ring, their position, degree of shielding, and also on the nature of other substituents that affect the electron density of the benzene ring (**Yashin, 2014**). The literature also provides data that the arrangement of hydroxyl groups in the benzene ring has a greater role in the manifestation of AOA than their number (**Amič, 2003**). The presence of several hydroxyl groups in the core, especially in the ortho- and para-positions to each other, causes special sensitivity to the action of oxidants. These phenols are extremely easy to oxidize and are good reducing agents. For example, ortho position substituted with hydroxyl groups undergo intramolecular repulsion, so their AOA increases compared to isomers in which the OH-groups occupy meta position. Probably, the higher AOA of quercetine depends on electron density of the benzene ring.

Conductivity

It is known that alkaline earth metal cations are capable, under certain conditions, of forming coordination bonds with oxygen-containing molecules. To obtain more direct experimental evidence on the complexing (chelating) properties of the studied polyphenols with cations of iron, the method of conductometry was used. As iron-binding property of polyphenols has been also proposed as their mechanism of cell protection against oxidative stress, the equilibrium constant between polyphenol and iron ions may provide insight into the mechanism of antioxidant effect.

The study of the electrical conductivity of divalent metal salts (Fe^{2+}) in an aqueous solution in the presence of studied polyphenols did not confirm the assumption that these compounds are capable of forming coordination type compounds. An analysis of experimental data shows that with an increase in the concentration of the studied polyphenol compound, as well as an increase in the concentration of divalent metal ions, the electrical conductivity of the solution containing Fe^{2+} cations does not change and does not reach a plateau (Fig. 3). However, presence EDTA in solution the electrical conductivity changes and reach plateau which indicates about chelating of metal ions with EDTA.

An analysis of experimental data shows that an increase in the concentration of polyphenols and divalent metal ions in a solution does not change the electrical conductivity.

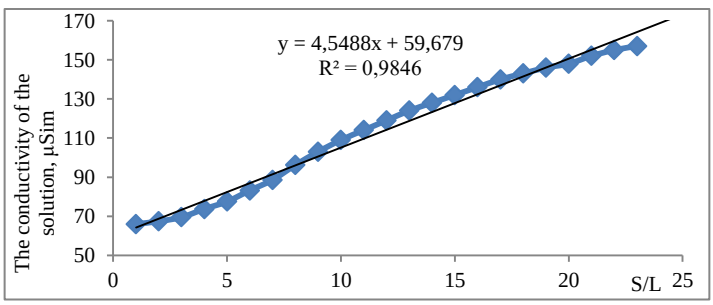


Figure 3 Typical conductometric titration curve of quercetin and dihydroquercetin with a Fe^{2+} solution (where S is the salt concentration, L is the polyphenol concentration, μM).

Thus, it can be concluded that AOA of polyphenols is not associated with the chelation of divalent cations by the studied polyphenols.

Antiradical activity

Determination of mitochondrial swelling induced by the Fe^{2+}/ascorbate system is one classic method for studying the antioxidant properties of biologically active compounds.

In the literature, AOA of polyphenol compounds is associated both with their ability to chelate various metal ions (**Tripathi, Rastogi, 1981**), and directly interact with reactive oxygen species: OH radicals (**Takahama, 1984**), O2• (**Takahama, 1985**), and singlet oxygen (**Youngman, Takahama et.al, 1984**). Besides, polyphenols can interact and/or bind the components of the reaction medium (**Riedl, Carando et.al, 2002**), which can lead to distortion of the results. The use of the Fe^{2+}/ascorbate-induced system does not directly assess the contribution of every single effect to the total antioxidant activity of the polyphenols.

Thus screening of compounds, that having free valence electrons are still stable organic radicals, seems reasonable (**Gayibov, Komilovi, *et al.*, 2017**). For example, ortho-substituted diphenols have four electrons that can reduce various radicals (**Pochinok, Tarakhovsky, *et al.*, 1985**). In this regard, the antiradical activity of polyphenols can be directly associated with their AOA.

In this regard, the antiradical activity of quercetin and dihydroquercetin against DPPH free radical was studied. To do this, we used a technique based on antioxidants' ability to reduce the molecules of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (**Gayibov *et al.*, 2012**). We studied the kinetics of drug recombination with the stable radical DPPH. When the studied compounds are added to the alcohol solution of DPPH, the color of the solution changes, which corresponds to the transition of DPPH to a nonradical form. In fig. 4 (experimental points) presents the kinetics of changes in the DPPH optical density with the addition of the studied compounds.

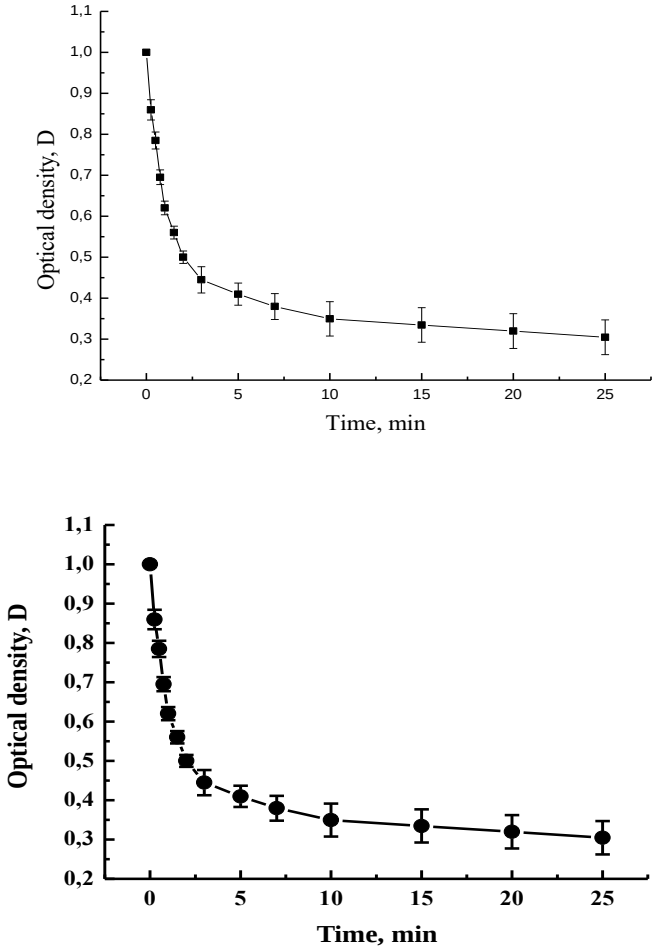


Figure 4 Kinetic curves of ARA of quercetin in ethanol solutions of DPPH. The concentration of polyphenol is 10 micromole.

To compare the ARA of the studied polyphenols, we selected a concentration of 50 μl for each compound from the prepared alcohol solution of the substance at a concentration of 10^{-3} M. Analyzing the results, we can conclude that the optical density of the ethanol solution of DPPH decreases which indicates their antiradical ability.

From experimental data, it follows that the studied compounds have a high ability to quench free radicals. To quantify the antiradical activity, we used the parameter t_{50} — the time required for the studied drugs to decrease the initial concentration of the radical to 50%, chemical reaction constant (k) and half inhibition concentration (IC_{50}).

$K\ 10^{-3}$, c^{-1}		IC_{50}, mcl		t_{50}, sec	
DQ	Q	DQ	Q	DQ	Q
1,2	5,3	14,3	7,2	105	9,6

Thus, the studied compounds are characterized by a strong ability of components to react with free radicals

The correlation coefficient between ARA and AOA of polyphenol compounds is **r=0.89**.

Our main interest in the investigation of AOA/ARA activity was to clarify the mechanism of AOA of two studied polyphenols depending on their chemical structure. It is well known that the antiradical/antioxidant of polyphenolic compounds is up to the number and location of hydroxyl groups in the benzene ring of polyphenols (**Yashin, 2014**). In the molecules of quercetin and dihydroquercetin there is the same number of hydroxyl groups. However, the antiradical activity of dihydroquercetin is significantly lower than the ARA of quercetin. Higher ARA of quercetin, might possibly due to the numerous existence of double bonds in comparison to the molecule of dihydroquercetin. Thus, we can assume that the high AOA/ARA of quercetin compared with its derivative is associated with the most energetically favorable state due to the distribution of electron density in the benzene ring.

When the quercetin donates an electron to free radical, initiating quercetin radical formation, due to resonance this newly formed radical lacks sufficient energy to approach reactive nature (**Mariani *et al*., 2008**). The structural groups that stabilize quercetin and specify its antioxidant features are considered to be the 3- and 5-hydroxyl groups together with 4-oxo group and B ring *o*-dihydroxyl groups (**Hollman and Katan, 1997**). This electron donating groups transfer electrons to the functional rings thus increasing the amount of resonance forms (**Mariani *et al*., 2008**).

On the base of the conductometric titration, we considered that there are no complexes of polyphenols with Fe^{2+} ions which could decrease the LPO process in the membrane due to chelate of metal ions by polyphenols. It is reasonable to suppose that AOA of polyphenols related to the non-polar part of quercetin and dihydroquercetin molecules extend into the acyl chain region of mitochondrial membrane and quench free radicals.

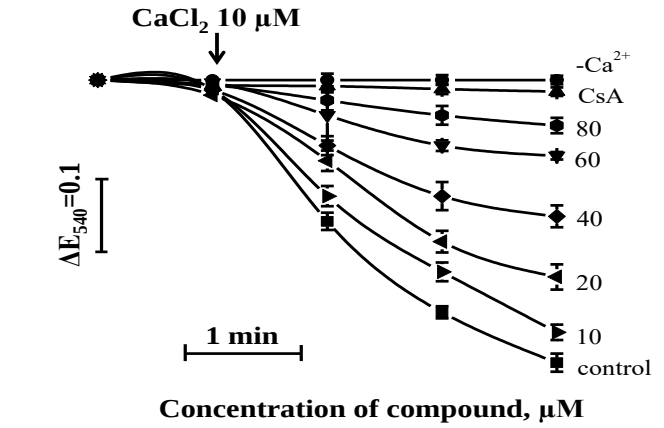
Inhibition of mPTP

Mitochondrial permeability transition pore (mPTP) approved to manifest the key role in the functional parameters of the cell. Thus, mPTP plays crucial part as trigger of apoptosis and necrosis under oxidative stress, ischemia/reperfusion as well as Ca^{2+} toxicity in liver cells **(Kim *et al*., 2013).** In this case the mechanism of cardioprotection of the most cardiovascular drugs is based on direct mPTP inhibition, as well as decreasing level of mPTP opening triggers, calcium overload and/or scavenging reactive oxygen species. According to the Akopova's data (**Akopova, Kolchinskaya *et al*., 2011**), mPTP goes into the open state as a result of the Ca^{2+} overload, induction of lipid peroxidation, the formation of ROS and closes in the presence of antioxidants or cation chelators (**Asrarov, Komilov, *et al*., 2015**).

Since Ca^{2+}-dependent mPTP is a key factor in ensuring the permeability of mitochondrial membranes (**Akopova, Nosar *et al*., 2013**), in the following experiments the effect of quercetin and its derivative dihydroquercetin on mPTP activity (Fig. 5) was investigated. The introduction of 10 μM Ca^{2+} ions into the external solution induces mitochondrial swelling (Fig. 5, cont.), which indicates that mPTP is in the open state. Cyclosporin A (CsA), a specific mPTP blocker, avoids mitochondria from being swollen under the above-mentioned conditions (Fig. 5, A), i.e. mPTP remains in closed state even when Ca^{2+} ions are in medium.

According to experimental data, in Ca^{2+} containing medium, quercetin at 50 μM inhibits opening of mPTP up to 18.6%, at 200 μM inhibits opening of mPTP up to 89.7% in comparison to control experiment. The data suggests that quercetin, depending on the concentration, inhibits the opening of rat liver mPTP. This is probably due to the antioxidant properties of quercetin (**Gayibov, Komilov *et al*., 2017**), that is universal mechanism of stabilizing membranes. Our further investigations were devoted to studying the influence of dihydroquercetin on mPTP activity on the mitochondrial membrane. We were interested in the comparison interaction of quercetin and its derivative dihydroquercetin with mPTP. The concentration of dihydroquercetin was the same (10-100 μM).

From the data in fig. 5 (B) inhibition of mPTP opening by dihydroquercetin is not much different from quercetin activity.



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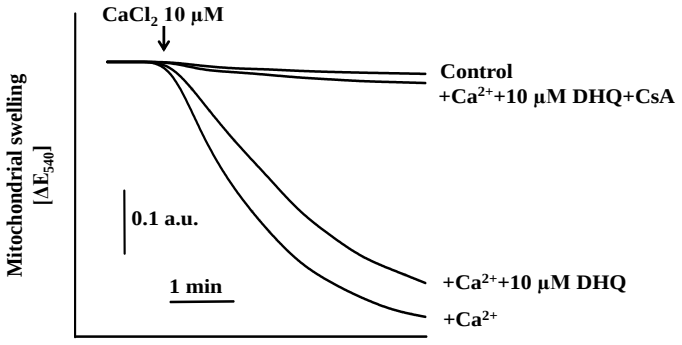


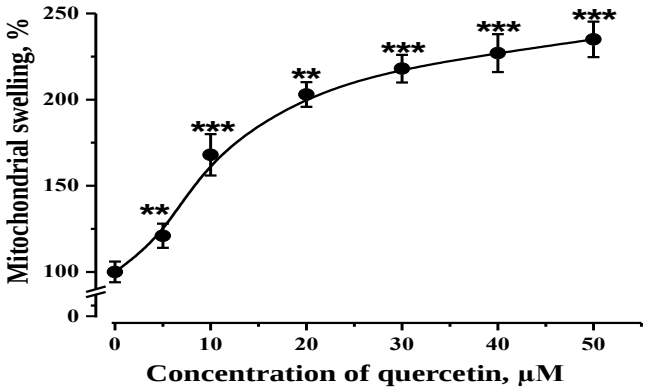
B

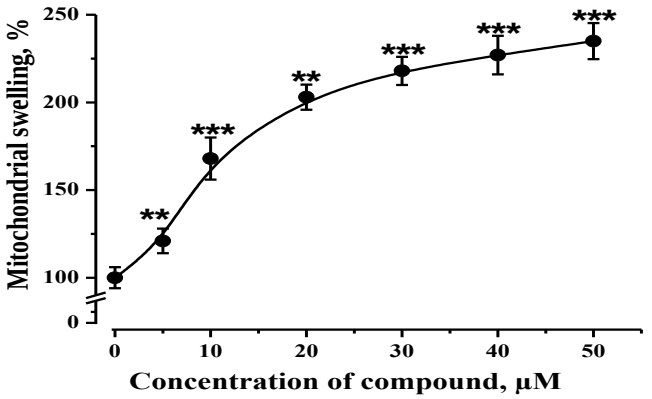
Figure 5 Influence of quercetin (A) and dihydroquercetin (B) on cyclosporine-induced mitochondria swelling. External medium was as follow: sucrose (200 mM), EGTA (20 μM), succinate (5 mM), rotenone (2 μM), oligomycin (1 μg/ml), Tris (20 mM), HEPES (20 mM), KH_2PO_4 (1 mM), pH adjusted to 7.2, protein content 0,3-0,4 mg/ml; (n=3-5).

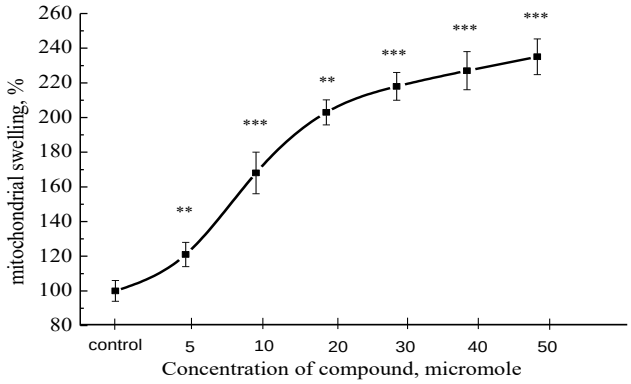
mPTP opening leads to mitochondrial dysfunction through ATP hydrolysis and oxidative phosphorylation uncoupling, ultimately resulting in cell death. The opening state of mPTP has been examined explored broadly in pathology of cardiovascular disorders such as ischemia/reperfusion and heart failure. Hence, mPTP is recognized as a therapeutic target for pharmacological and non-pharmacological strategies by blocking pore activation over direct blockage of mPTP components and/or indirect mechanism of blockage of mPTP inducers (**Javadov *et al*., 2009**). The list of indirect mechanisms of mPTP blockade includes ΔΨm modulation, redox state, mitochondrial mass regulation, calcium retention capacity and fusion/fission processes. Closer approach reveals alterations in protein expression, post-translational modifications and interactome disturbance, thus affecting signaling pathways.

mitoKATP activation

Potassium ions are the main ionic component of not only the cytoplasm but also the mitochondrial matrix. The direction of the electric field on the inner membrane of mitochondria favors the transport of potassium ions from the cytoplasm to the matrix. Thus, the presence of any K^+-selective channels, including the mitoKATP channel, in the inner mitochondrial membrane will be accompanied by changes in the mitochondrial volume and regulation of the state of the Ca^{2+} -dependent mPTP. Modulation of the conductivity of the mitoKATP channel underlies changes in mitochondrial functions under different pathologic conditions, however, the physiological role of this phenomenon is not entirely clear (**Akopova, Kolchinskaya et.al, 2011**). Fig. 6 shows experimental data using quercetin as mitoKATP channel activator. The data shows that quercetin, starting from a concentration of 10 and 20 μM, stimulates the mitoKATP channel opening (Fig. 6), and at concentrations of 80 μM or more, an exit to the board is observed. Regarding the action of quercetin on the activity of the mitoKATP channel, the experimental line observed is saturation curve line representing dose-dependent effect.







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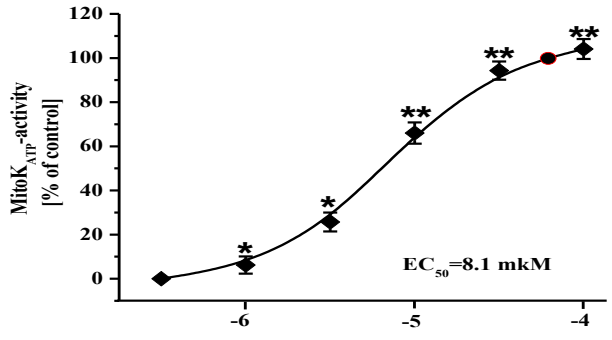

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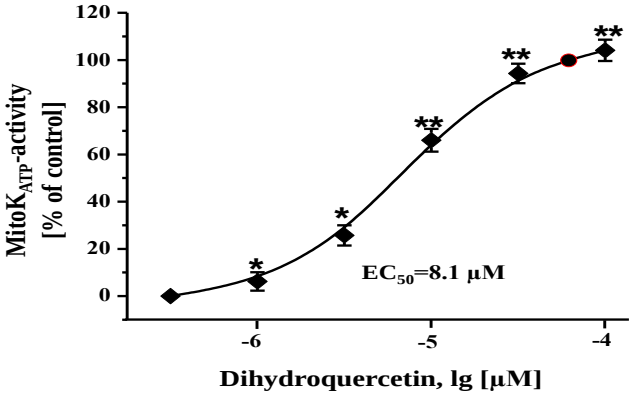

Figure 6 Influence of quercetin (A) and dihydroquercetin (B) on the mitoKATP channel activation. External medium was as follow (mM): KCl (125), Hepes (10), succinate (5), $MgCl_2$ (1), K_2HPO_4 (2.50, KH_2PO_4 (2.5), rotenone (0.005) and oligomycin (0.001), pH 7,4. (** - P<0,01, *** - P<0,001; n=4)

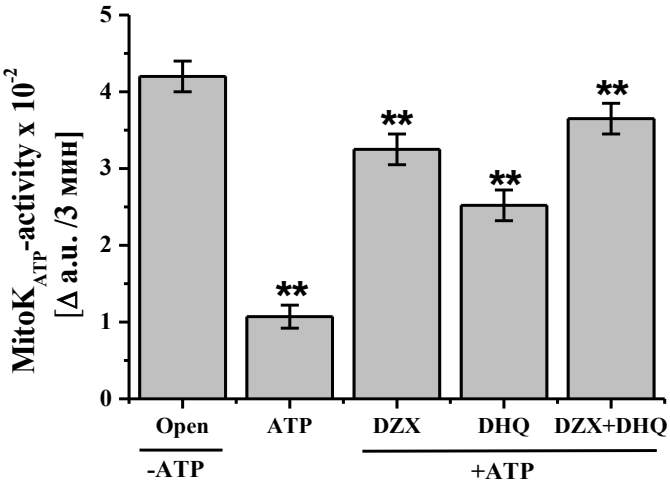

Figure 7 The effect of dihydroquercetin on the state of the mitoKATP channel of rat liver (** P <0.01; n = 3).
Open: open conformation, control without ATP (activity of the mito-CATF channel)
ATP: MitocATF channel state in the presence of 200 µM ATP
DZX: mitoKATP channel activity in the presence of 30 µM diazoxide + 200 µM ATP
DHQ: MitocATF channel activity with 80 µM DHQ + 200 µM ATP
DZX + DHQ: mitoCATP channel activity with 30 µM diazoxide + 80 µM DHQ +200 µM ATP.

The dual role of reactive oxygen species was widely accepted. On one hand in high concentrations ROS can damages cells; on the other hand they display messenger's activity in cellular signaling thus inducing cells survival in stress conditions. Interestingly ROS also can stimulate mitoKATP activation under oxidation stress in the heart and brain, conferring the cells protective effect (**Badziuk *et al.*, 1999**). The functioning of mitoKATP depends on the redox state of the active groups of the channel protein. It has been found that redox agents modulate the work of mitoKATP. For example, the electron donor n-dimethylaminoethyl benzoate activates mitoKATP, and the electron acceptors — pelargonidine inhibits the channel (**Grigoriev *et al*, 1999**), which is probably due to their effect on the SH groups of the channel. Perhaps the activation of this channel by quercetin and dihydroquercetin is related to the fact that they are electron donors (antioxidants).

CONCLUSION

It is known that polyphenolic compounds exhibit antioxidant, anti-inflammatory, immunomodulatory and many other actions, which creates the prerequisites for creating drugs based on them. In this case polyphenols are advantageous compounds due to their low toxicity and sufficient aqueous solubility.
Summarizing above mentioned data of polyphenol compounds activity on the mitochondrial level we can conclude that compounds are determined to some

extent by the influence on the mitochondrial membranes structure, free radical oxidation and oxidative phosphorylation processes.

As it was shown earlier by M.I. Asrarov *et al*. (**Asrarov, Komilov, *et al*., 2015**), a trend to improve some functional characteristics of mitochondria by the polyphenolic compounds provides the background to assume possible antihypoxic, cardio protective and antihyperglicemic effects of quercetin and dihydroquercetin. Further studies are required to validate the role of quercetin and dihydroquercetin as antihypoxic, cardio protective and antihyperglicemic agent on organismal level.

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