EXPERIMENTAL STUDIES

THE FLAVONOIDS FISETIN, APIGENIN, KAEMPFEROL, NARINGENIN, NARINGIN REGULATE RESPIRATORY ACTIVITY AND MEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA AND INHIBIT OXIDATIVE PROCESSES IN ERYTHROCYTES

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Flavonoids, secondary plant metabolites, represent the most abundant heterogeneous group of phytochemicals. The aim of this study to compare antioxidant activity and regulatory properties of several representatives of different classes of flavonoids, fisetin, apigenin, kaempferol, naringenin, naringin, using liver mitochondria and erythrocytes as research objects. In the concentration range of 2.5–25 μM fisetin, apigenin, kaempferol, naringenin, and naringin dose-dependently prevented oxidative damage of erythrocytes induced by 700 μM *tert*-butyl hydroperoxide: accumulation of lipid peroxidation (LPO) products and oxidation of glutathione GSH. The IC_{50} values corresponding to the flavonoid concentration inhibiting the LPO process in erythrocyte membranes by 50%, were 3.9±0.8 μM in the case of fisetin, 6.5±1.6 μM in the case of kaempferol, 8.1±2.1 μM in the case of apigenin, 37.8±4.4 μM in the case of naringenin, and 64.7±8.6 μM in the case of naringin. The antioxidant effect of flavonoids was significantly higher in the membrane structures compared to the cytoplasm of cells. All flavonoids studied (10–50 μM) effectively inhibited the respiratory activity of isolated rat liver mitochondria and, with the exception of kaempferol, stimulated Ca^{2+}-induced dissipation of the mitochondrial membrane potential. Cyclosporine A and ruthenium red inhibited flavonoid-stimulated Ca^{2+}-dependent membrane depolarization, thus indicating that the mitochondrial calcium uniporter and the mitochondrial permeability transition pore opening were involved in the flavonoid effects. Flavonoids, as the redox-active compounds with antioxidant properties, are able to regulate mitochondrial potential and respiratory activity, and prevent mitochondrial oxidative stress. They can be considered as effective pharmacological agents or nutraceuticals.

Key words: flavonoids; mitochondria; red blood cells; glutathione; oxidative stress

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INTRODUCTION

Flavonoids, compounds belonging to the broad family of polyphenols, are secondary metabolites of plants. They represent the most abundant group of phytochemicals and are important components of the human diet [1, 2]. These compounds have high physiological and pharmacological activity both in plants and in animal and human tissues [3]. In plants, flavonoids provide protection from UV radiation, viruses and other phytopathogens, oxidative stress; they participate in the pigmentation of fruits and flowers, and act as signaling molecules (allelochemicals) [4].

Flavonoids share a common chemical structure constituted by a common skeleton of phenyl-benzo-γ-pyran (C6–C3–C6); the wide range of structural diversity of flavonoids is associated with varying degrees of hydroxylation, methoxylation, glycosylation or glucuronylation of these compounds [4]. This contributes to a wide variety of their biological properties. To date, more than 6000 compounds belonging to the flavonoid family have been identified [4]. The flavone molecule contains 15 carbon atoms and consists of a pair of aromatic rings (A and B) that are linked by an oxygenated heterocyclic C-ring. In turn, flavonoids are divided into subgroups depending on the type of heterocycles: flavonols, flavones, flavanones, isoflavones, flavanols, anthocyanins. The main biological activity of flavonoids is associated with their antiradical/antioxidant properties (redox potential), which are provided by a system of conjugated rings and hydroxyl groups [5]. Flavonoids are also able to activate free radical detoxification enzymes, such as NAD(P)H-quinone oxidoreductase, glutathione-S-transferase or UDP-glucuronosyltransferase [6], interact with proteins and membrane structures, regulate many signaling cascades, such as AMPK, MAPK, NF-kB-dependent pathways [7], and eliminate the toxicity of metal ions involved in redox transformations. It is known that flavonoids interact with the Nrf2-Keap 1 cascade, the main signaling cascade that regulates the cytoprotective response to oxidative stress [8]. The recommended daily intake of flavonoids (expressed as isoflavones) is 50 mg/day [9].

Results of numerous experimental and epidemiological studies demonstrate the beneficial effects of flavonoids in infectious (bacterial and viral), neurodegenerative, cardiovascular, age-related diseases, diabetes, cancer, and other diseases [10] and the absence of side effects.

At the same time, there are complex relationships between consumption of plant polyphenols and cell homeostasis, including metabolic, redox balance, proteostasis, and inflammatory reactions [11].

Despite numerous studies, the mechanisms of biochemical, physiological, and pharmacological effects of flavonoids require better understanding. This is necessary to elucidate the molecular basis for the successful use of flavonoids as pharmacological agents and nutraceuticals. Our studies of the interaction of a number of flavonoids (quercetin, naringenin, and catechin) with cell and artificial membranes have shown that flavonoids effectively inhibit lipid peroxidation (LPO), increase sensitivity to Ca^{2+}-induced opening of the mitochondrial permeability transition pore (mPTP) in isolated mitochondria, change membrane packaging and the hydration degree of liposomal membranes at different depths of the lipid bilayer [12].

The aim of this study was to compare the antioxidant activity and regulatory properties of a number of flavonoids belonging to different subgroups: the flavonols fisetin (7,3′,4′-flavon-3-ol) and kaempferol (3,4′,5,7-tetrahydroxyflavone), the flavone apigenin (4′,5,7-trihydroxyflavone), the flavanone naringenin (4′,5,7-trihydroxyflavan-4-one) and its glycoside naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) (Fig. 1). Rat liver mitochondria and erythrocytes were used as research objects.

MATERIALS AND METHODS

Reagents and Materials

The following reagents were used in this study: naringenin, fisetin, kaempferol, apigenin, naringin, sucrose, *tert*-butyl hydroperoxide (tBHP), disodium succinate, Tris(hydroxymethyl)aminomethane (Tris-HCl), ethylene glycol bis (2-aminoethyl ester)-N,N,N′,N′-tetraacetic acid (EGTA), adenosine diphosphate (ADP), safranin O, carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone (FCCP), thiobarbituric acid (TBA), trichloroacetic acid (TCA), cyclosporine A (CsA), ruthenium red (RuR) (Sigma-Aldrich Biochemie Gmbh, Germany or Sigma-Aldrich, USA); 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) (Chem-Impex International Inc., USA), potassium chloride, sodium chloride, calcium chloride, hydrochloric acid, sodium hydrogen phosphate, potassium dihydrogen phosphate, orthophosphoric acid, ethanol, dimethyl sulfoxide (DMSO) (REAKHIM, Russia). Solutions were prepared using water purified in the Milli-Q Direct system (Merck KGaA, Germany).

Preparation of the Biological Material for Analysis

Clinically healthy (Sanitary and Hygienic Conclusion No. 33-48/500 dated September 28, 2017, Center for Hygiene and Epidemiology of the Pervomaisky District, Minsk) outbred Wistar rats

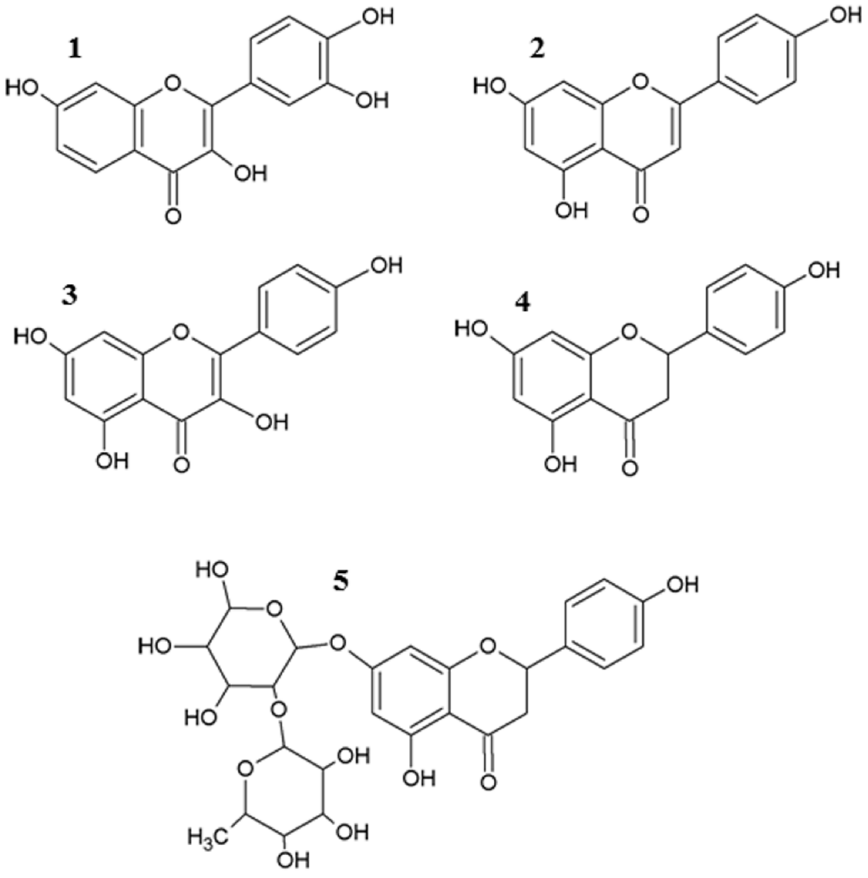

Figure 1. Chemical structures of flavonoids. *1* – fisetin, *2* – apigenin, *3* – kaempferol, *4* – naringenin, *5* – naringin.

weighing 120–140 g, bred in the vivarium of the Institute of Physiology of the National Academy of Sciences of Belarus were used in experiments. After decapitation of rats, arterial blood samples were obtained by puncture of the abdominal aorta and stabilized with hirudin (50 μg/ml). Rat erythrocytes were washed three times in isotonic phosphate buffer (PBS) containing 145 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4 (ratio of 1:4). After decapitation of the rat, the liver was removed and placed in a buffer containing 0.02 M Tris-HCl, 0.25 M sucrose, pH 7.2, cooled to 4°C. All manipulations were performed on ice at 0–4°C. After washing from the blood, the liver was dried with a filter paper, weighed, and a liver sample weighing 6 g was cut with scissors and placed in a homogenizer (Teflon-glass) along with a five-fold volume of the buffer and homogenized at 600 rpm for 1 min. The fraction of cell nuclei was separated by centrifugation at 4°C at 600 *g* (centrifuge Hermle Z 32 HK, Hermle Labortechnik GmbH, Germany) for 10 min. Mitochondria from the resulting supernatant were sedimented by centrifugation at 4°C at 8500 *g* (centrifuge Hermle Z 32 HK, Hermle Labortechnik GmbH) for 10 min, then washed twice in the isolation medium at 4°C and resuspended in the isolation medium (to obtain the mitochondrial suspension with the protein content of 35–40 mg/ml) [13]. The protein content in the samples was determined using the Lowry method [14].

Freshly prepared 5 mM solutions of fisetin, apigenin, kaempferol, naringenin, and naringin in ethanol were used. Preincubation of erythrocytes and mitochondria with flavonoids was carried out for 5 min at 25°C. CsA, RuR were prepared on the day of the experiment in methanol and water, respectively. At the concentrations used, the solvents did not have any significant effects on the studied parameters. CsA (1 μM), RuR (1 μM) were added to the sample immediately before the start of the measurement.

Determination of Biochemical Parameters

Determination of the content of reduced glutathione (GSH) and lipid peroxidation (LPO) products in rat erythrocytes

Oxidative stress in rat erythrocytes was induced by adding a water-soluble analogue of lipid hydroperoxides, tBHP, to an erythrocyte suspension (5% hematocrit in PBS, pH 7.4). Erythrocytes were incubated in the presence of 700 μM tBHP for 1 h in the case of recording the LPO process and 100 μM tBHP for 5 min in the case of recording GSH oxidation (27°C). After exposure to the oxidizing agent, erythrocytes were treated with 20% TCA. The samples were centrifuged at 2500 *g* for 5 min (centrifuge Hermle Z 32 HK (rotor for microtubes), Hermle Labortechnik GmbH), 1.2 ml of 0.5 M phosphate buffer (pH 7.8) and 0.05 ml (5 mM) solution of Ellman's reagent were added to 1 ml of supernatant, GSH was determined by the optical density of the resulting product at 412 nm [15]. The concentration of stable products of membrane lipid peroxidation in rat erythrocytes (malondialdehyde and some minor low molecular weight dialdehydes) reacting with thiobarbituric acid (TBARS) was determined spectrophotometrically using the molar extinction coefficient $\varepsilon_{532}=1.56\times10^5$ $M^{-1}\cdot cm^{-1}$ of the resultant chromogen (TBA-malondialdehyde adduct) [16]. Erythrocytes were precipitated by adding 20% TCA. The precipitate was separated by centrifugation at 2500 *g* for 5 min (centrifuge Hermle Z 32 HK (rotor for microtubes), Hermle Labortechnik GmbH); 0.25 ml of 1% TBA in 0.05 M NaOH was added to 1 ml of the supernatant. The samples were placed in a boiling water bath for 20 min to form a colored adduct, and its optical density was determined using a Jasco V-650 spectrophotometer (Japan). IC_{50} was determined as the flavonoid concentration required for 50% LPO inhibition.

Determination of membrane potential and respiratory activity of rat liver mitochondria

The mitochondrial membrane potential was recorded spectrofluorimetrically using a positively charged lipophilic fluorescent probe safranin O in a medium containing 125 mM KCl, 10 mM Tris-HCl, 50 mM sucrose, 2.5 mM KH_2PO_4, 5 mM $MgSO_4$, pH 7.5 [17]. Voltage-dependent accumulation of the probe in mitochondria leads to fluorescence quenching. Safranin O fluorescence was excited at λ_{ex}=495 nm and recorded at λ_{em}=586 nm using a Solar CM 2203 spectrofluorimeter (Belarus). The medium contained the probe safranin O (8 μM) and the respiration substrate — succinate (5 mM). The reaction was started by adding rat liver mitochondria (0.3 mg/ml) to the medium; at the end of the measurement, the uncoupler FCCP (0.5 μM) was added. The values of the mitochondrial membrane potential (mV) were determined using a calibration graph representing the dependence of the fluorescence intensity of safranin O on the potential value (which varied depending on the extramitochondrial concentration of potassium ions) and calculated using the Nernst equation [17].

The respiratory activity of isolated rat liver mitochondria was recorded polarographically at 26°C using a closed Clark electrode thermostated cell of 2 ml; measurements were carried out using a Hansatech Oxytherm+Chamber polarograph (United Kingdom), as we described previously [18]. The Clark electrode was calibrated using sodium sulfite. The required volume (≈50 μl) of mitochondrial suspension was added to a cell with a medium containing 0.05 M sucrose, 0.01 M Tris-HCl, 0.125 M KCl, 2.5 mM KH_2PO_4, 5 mM $MgSO_4$, pH 7.2 to obtaining a mitochondrial protein concentration of 1 mg/ml; after that the respiration substrate (succinate — 5 mM) and ADP (180 μM) were added.

Statistical Analysis

The normality of data distribution was evaluated using the Shapiro-Wilk test. Results were presented as mean ± standard error of the mean. The significance of differences was assessed using one-way analysis of variance (ANOVA) and the Tukey's test. Statistical analysis was performed using Microsoft Excel 2013 and STATISTICA 10.0 applications. Differences between parameter values in groups were considered as statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The Antioxidant Effect of Flavonoids In Vitro on Oxidative Damage to Rat Erythrocytes

In accordance with numerous observations [18, 23], tBHP incubation with rat erythrocytes was accompanied by intense peroxidation of membrane lipids and depletion of intraerythrocyte GSH (Fig. 2). The flavonoids, fisetin, apigenin, kaempferol, naringenin, naringin in the concentration range of 2.5-25 μM dose-dependently prevented oxidative damage to erythrocytes as evidenced by accumulation of TBARS and oxidation of GSH induced by exposure to the oxidant (tBHP) (Fig. 2). Our estimated IC_{50} values, corresponding to the flavonoid concentration inhibiting LPO in erythrocyte membranes by 50%, were 3.9±0.8 μM in the case of fisetin, 6.5±1.6 μM in the case of kaempferol, 8.1±2.1 μM in the case of apigenin, 37.8±4.4 μM in the case of naringenin and 64.7±8.6 μM in the case of naringin ($p<0.05$). The efficiency of inhibition of TBARS accumulation in rat erythrocytes decreased in the following order: fisetin > apigenin = kaempferol > naringenin > naringin (differences in the series were statistically significant, $p<0.05$). In the case of inhibition of intraerythrocyte glutathione oxidation, the effectiveness of fisetin, kaempferol, apigenin and naringenin differed insignificantly; the glycoside naringin did not have a protective effect. It should be noted that fisetin and kaempferol contain four OH groups, which act as hydrogen atom donors in reduction reactions, while the remaining flavonoids contain three OH groups. Previously, using the efficiency of reduction of the stable radical DPPH, the following order of increasing free radical scavenging activity was shown for a number of flavonoids: quercetin > catechin > luteolin > taxifolin > kaempferol > apigenin. This correlates with a change in the energy value of removing the hydrogen atom from the hydroxyl groups of polyphenols [19]. In a previous experiment, we also showed that the flavonoids quercetin and catechin, but not naringenin, effectively reduced the stable DPPH radical in a cell-free system; moreover, all tested flavonoids prevented the lysis of erythrocytes induced by hypochlorous acid [20].

The antioxidant activity of flavonoids in the erythrocyte membrane (inhibition of the formation of LPO products) was significantly higher than the effectiveness in the cell cytoplasm (prevention of glutathione oxidation) (Fig. 2). This reflects the high lipophilicity of the studied flavonoids [21].

Having a large number of hydroxyl groups and conjugated π-orbitals, flavonoids act as effective donors of electrons or hydrogen atoms [22]. In this case, it is possible for flavonoids to exist both in the form of a neutral molecule and in the form of a phenolate ion. Previously, we examined the electronic structure of molecules of a number of flavonoids, for example, quercetin and its semiquinone radical, assessed their antioxidant activity and showed that the active electronic orbitals (HOMO and LUMO) of quercetin and its semiquinone radical were delocalized over all phenolic rings, thus providing stabilization of the radical [23].

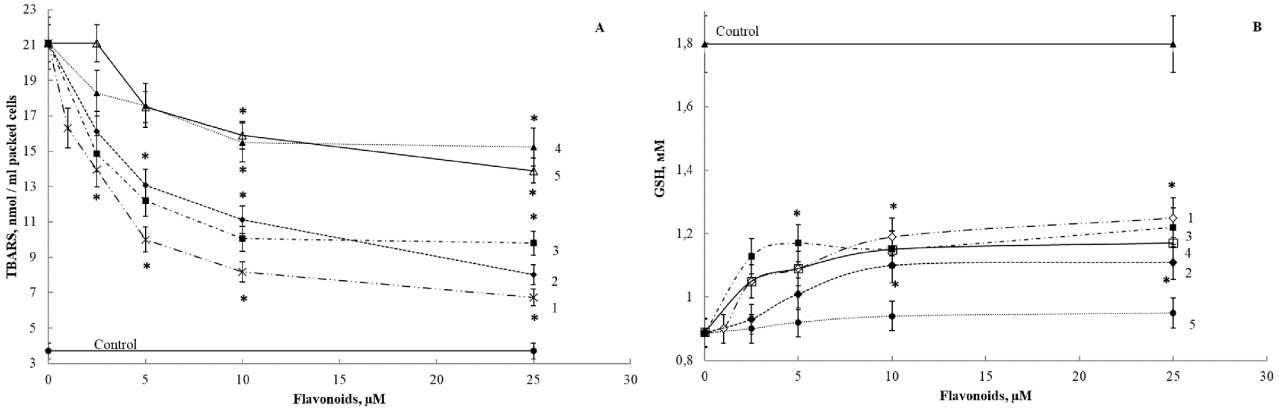

Figure 2. Inhibition by the flavonoids fisetin (*1*), apigenin (*2*), kaempferol (*3*), naringenin (*4*), naringin (*5*) of oxidative processes induced by tBHP in the rat erythrocytes: **A**) inhibition of the process of lipid peroxidation (LPO) and **B**) inhibition of GSH oxidation. Experimental conditions of erythrocyte incubation are given in the Materials and Methods section. A suspension of erythrocytes incubated with an oxidizing agent in the absence of flavonoids was used as a control. * – $p<0.05$ as compared with samples incubated in the absence of the antioxidants.

Regulation of Respiratory Activity and Membrane Potential of Isolated Rat Liver Mitochondria by Flavonoids

At concentrations 10–50 μM the studied flavonoids effectively inhibited the respiratory activity of rat liver mitochondria: in the presence of increasing concentrations of flavonoids, the rate of substrate-dependent oxygen consumption V_2 increased, while the rate of ADP-stimulated oxygen consumption V_3 markedly decreased, the respiratory control ratios V_3/V_2 and ADP/O also decreased (Fig. 3A-E), indicating impaired coupling of respiration and phosphorylation. Apigenin exhibited the most pronounced effect on the rate of mitochondrial oxygen consumption; the effects of naringenin, fisetin, and kaempferol were less pronounced. We did not find any significant change in the rate of substrate-dependent oxygen consumption and the ADP/O ratio in the presence of fisetin and naringenin and the ADP/O ratio in the presence of kaempferol; the glycoside naringin did not have any significant effect on respiratory parameters (Fig. 3A-E).

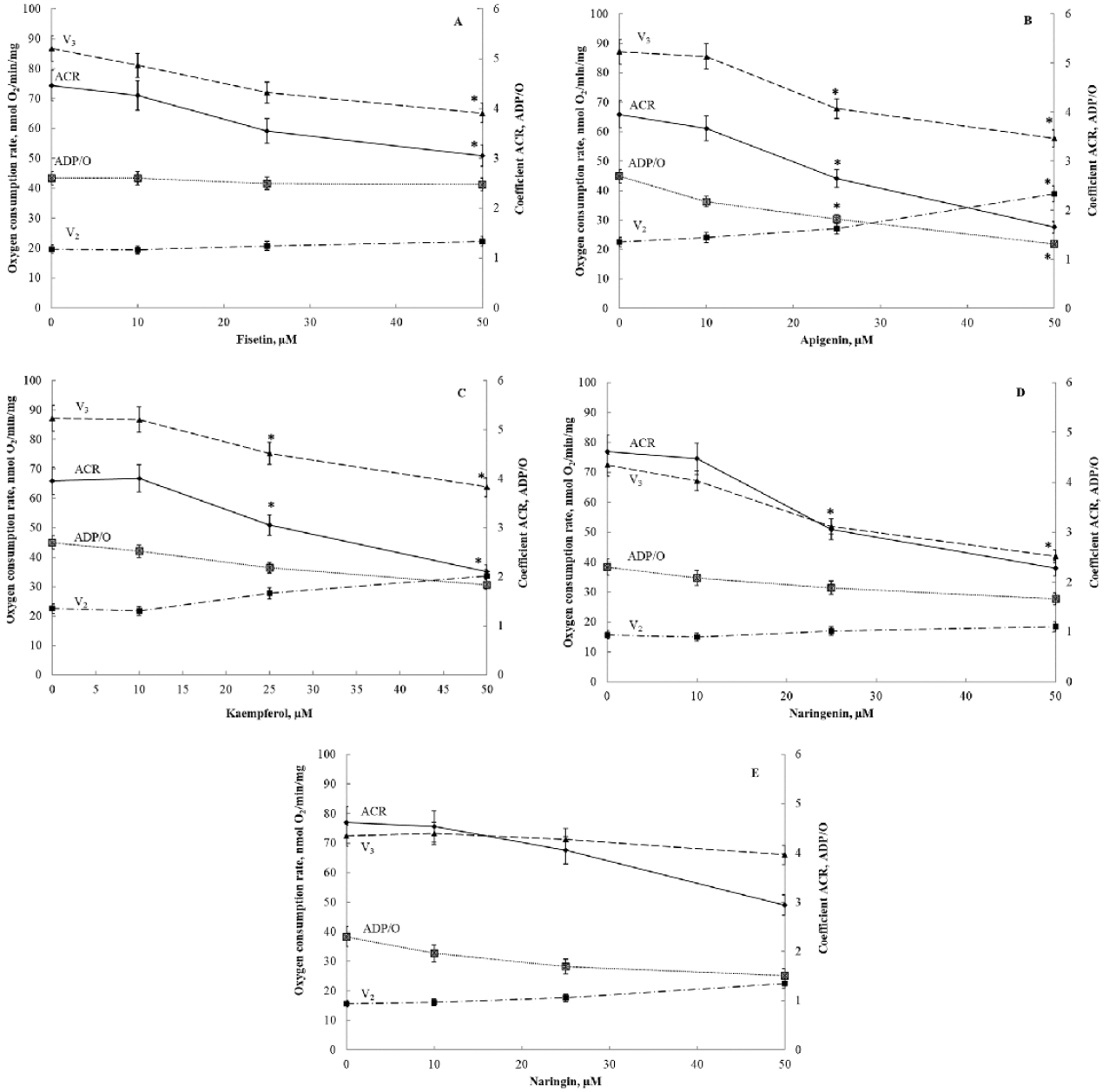

Figure 3. Respiratory activity of isolated rat liver mitochondria (1 mg protein/ml) in the absence and presence of the flavonoids fisetin (**A**), apigenin (**B**), kaempferol (**C**), naringenin (**D**), naringin (**E**) in the medium, containing 0.05 M sucrose, 0.01 M Tris-HCl, 0.125 M KCl, 2.5 mM KH_2PO_4, 5 mM $MgSO_4$, pH 7.2, 26°C, in the presence of 5 mM succinate as a substrate and 180 μM ADP. * – $p<0.05$ as compared with the values obtained in the absence of flavonoids.

One of the consequences of inhibition of the oxidative phosphorylation reaction in mitochondria is dissipation of the membrane potential. The mitochondrial membrane potential, generated during the mitochondrial electron transport chain operation, determines the functional state of these cellular organelles. In our experiment, apigenin, naringenin (10–50 μM), but not kaempferol, fisetin, and the glycoside naringin, in an EGTA-free medium caused dose-dependent dissipation of the membrane potential of mitochondria energized by succinate (Fig. 4A-E).

We suggest that flavonoids induce partial uncoupling of respiration and oxidative phosphorylation in rat liver mitochondria; this is accompanied by a decrease in mitochondrial membrane potential and impaired ATP synthesis. Such mild uncoupling, in turn, leads to a decrease in the production of reactive oxygen species by components of the mitochondrial electron transport chain thus preventing the development of oxidative stress and demonstrating the cytoprotective effect of flavonoids.

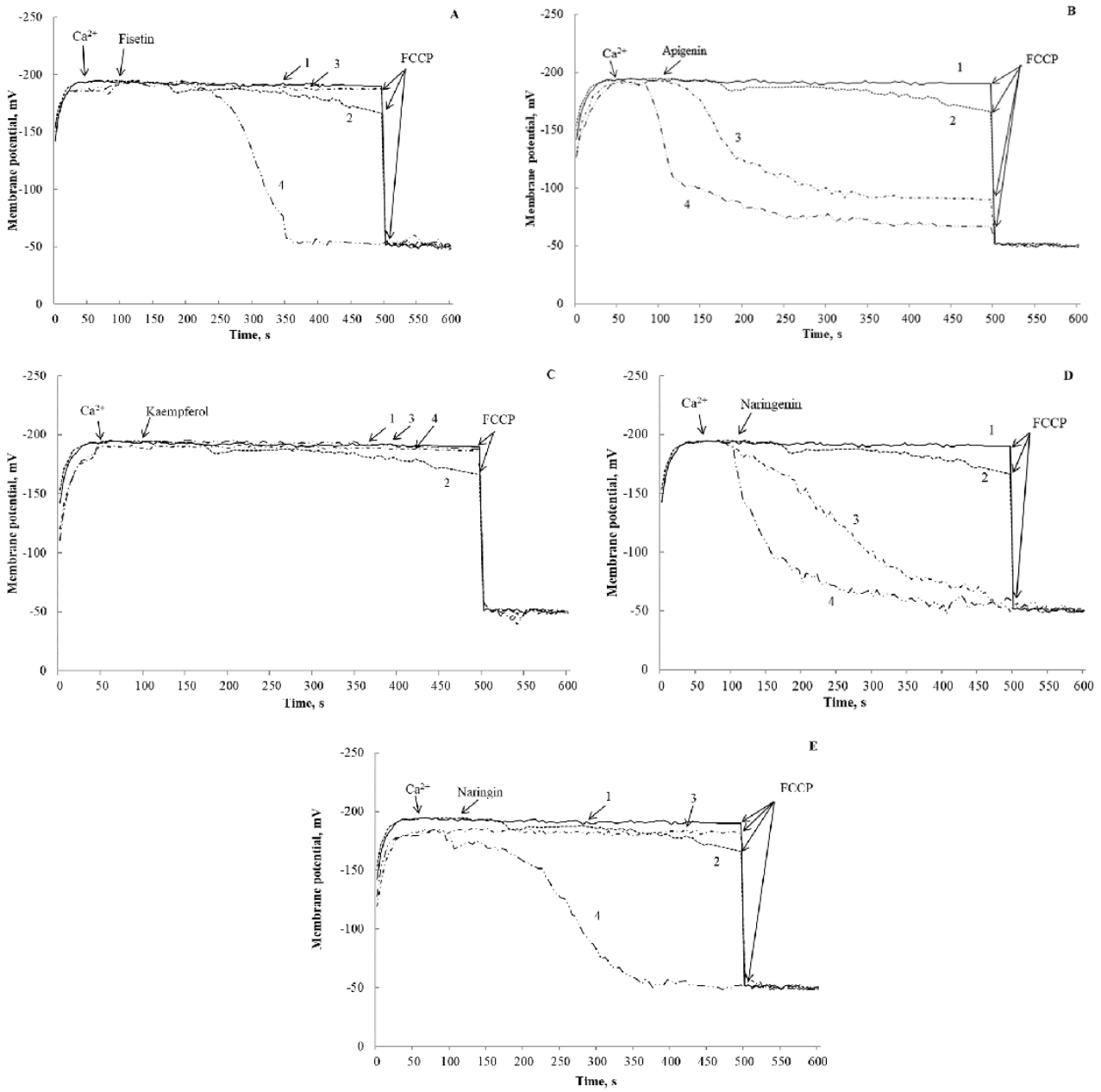

Figure 4. Typical kinetic curves of membrane potential dissipation in rat liver mitochondria, registered by changes in the fluorescence intensity of the safranin O probe, the effect of fisetin (**A**), apigenin (**B**), kaempferol (**C**), naringenin (**D**), naringin (**E**). Ca^{2+} ions (30 μM) and flavonoids (50 μM) were added to the mitochondrial suspension (0.5 mg/ml) in the medium containing 0.25 M sucrose, 0.02 M Tris-HCl 0.001 M KH_2PO_4 in the presence of 5 mM succinate. *1* – control, *2* – Ca^{2+} (30 μM), *3* – flavonoid (50 μM), *4* – Ca^{2+} (30 μM) + flavonoid (50 μM).

Addition of Ca^{2+} ions (30 μM) led to depolarization of mitochondrial membranes (Fig. 4A-E). Apigenin, fisetin, naringenin and naringin, but not kaempferol, stimulated the depolarizing effect of Ca^{2+} ions, possibly acting as calcium ionophores. In the case of assessing the joint effect of Ca^{2+} ions and flavonoids, Ca^{2+} ions were initially added to the mitochondrial suspension, and after 100 s flavonoids were then added. Previously, we showed that flavonoids, quercetin, catechin, naringenin (10–50 μM), increased the sensitivity of mitochondria to the Ca^{2+}-dependent process of mPTP formation [12].

RuR, a selective inhibitor of the mitochondrial calcium uniporter, prevented naringenin-stimulated Ca^{2+}-induced dissipation of the membrane potential; this suggests that flavonoids act on the calcium uniporter, responsible the transport of calcium ions into mitochondria (data not shown). Similarly, earlier we have shown that RuR effectively prevented Ca^{2+}-induced opening of mPTP in the presence of flavonoids, which we attribute to activation of the Ca^{2+} uniporter by flavonoids. Stimulation of the mitochondrial Ca^{2+} uniporter by a number of flavonoids was shown previously [24]. At the same time, one can assume a protonophore effect of lipophilic flavonoids, which are weak acids, in the inner mitochondrial membrane; this will lead to impaired coupling of respiration and phosphorylation and depolarization of membranes.

Cyclosporine A, an inhibitor of mPTP also completely prevented flavonoid-stimulated Ca^{2+}-dependent membrane depolarization. This suggests mPTP opening as a cause of the mitochondrial membrane depolarization.

The ability of flavonoids to modulate mitochondrial biogenesis, regulate mPTP formation, the generation of reactive oxygen species in mitochondria, membrane potential, electron transport chain activity, and mitochondrial calcium homeostasis is of significant interest [25, 26].

CONCLUSIONS

Flavonoids, secondary metabolites of plants, represent the most abundant group of phytochemicals and have high biochemical and pharmacological activity. In our experiment, a number of flavonoids in the concentration range of 2.5–25 μM dose-dependently prevented oxidative damage of rat erythrocytes and liver mitochondria as evidenced by accumulation of LPO products and glutathione oxidation induced by incubation of cells and mitochondria with the oxidant tBHP. The efficiency of inhibition of TBARS accumulation in erythrocytes decreased in the following order: fisetin > kaempferol = apigenin > naringenin > naringin. The antioxidant effectiveness of flavonoids in membrane structures was significantly higher as compared to the effectiveness in the cytoplasm of cells and the mitochondrial matrix. The studied flavonoids (10–50 μM) (except the glycoside naringin), effectively inhibited the respiratory activity of rat liver mitochondria, impaired coupling of respiration and phosphorylation. At the same time, apigenin, naringenin (50 μM), but not fisetin, kaempferol and the glycoside naringin, in an EGTA-free medium caused dissipation of the membrane potential of mitochondria energized by succinate. Apigenin, fisetin, naringenin and naringin also stimulated the depolarizing effect of Ca^{2+} ions in the mitochondrial membrane. It can be assumed that lipophilic flavonoids, which are weak acids, exhibit a protonophore effect in the inner mitochondrial membrane. This will impair coupling of respiration and phosphorylation. It is also possible that flavonoids stimulate the calcium uniporter. Flavonoids, as redox-active compounds with pronounced antioxidant properties, are able to regulate the process of mPTP opening, mitochondrial potential and respiratory activity, and prevent mitochondrial oxidative stress. They can be considered as pharmacological agents or nutraceuticals applicable for correction of mitochondrial dysfunction and related diseases.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

All manipulations with animals were carried out in compliance with generally accepted standards of humane treatment of laboratory animals, approved by the Ethical Committee of the Institute of Biochemistry of Biologically Active Compounds of the National Academy of Sciences of Belarus (protocol dated March 12, 2021 No. 29/21) and complied with GOST 33216-2014 “A Guide to the Maintenance and Care of laboratory animals. Rules for keeping and caring for laboratory rodents and rabbits” (Interstate standard, put into effect as a national standard of the Russian Federation on July 1, 2016).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ФЛАВОНОИДЫ ФИЗЕТИН, АПИГЕНИН, КЕМПФЕРОЛ, НАРИНГЕНИН, НАРИНГИН РЕГУЛИРУЮТ РЕСПИРАТОРНУЮ АКТИВНОСТЬ И МЕМБРАННЫЙ ПОТЕНЦИАЛ МИТОХОНДРИЙ ПЕЧЕНИ И ИНГИБИРУЮТ ОКИСЛИТЕЛЬНЫЕ ПРОЦЕССЫ В ЭРИТРОЦИТАХ КРЫС

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Флавоноиды — вторичные метаболиты растений — представляют наиболее распространённую гетерогенную группу фитохимических веществ. Целью настоящей работы было сравнительное исследование антиоксидантной активности и регуляторных свойств ряда флавоноидов, принадлежащих различным классам, — физетина, апигенина, кемпферола, нарингенина, нарингина, — используя в качестве объектов воздействия митохондрии печени и эритроциты крыс. Физетин, апигенин, кемпферол, нарингенин, нарингин в диапазоне концентраций 2,5–25 мкМ дозозависимо предотвращали окислительные повреждения эритроцитов: накопление продуктов перекисного окисления липидов (ПОЛ) и окисление глутатиона (GSH), индуцируемое 700 мкМ *трет*-бутилгидропероксидом. Значения IC_{50}, соответствующие концентрации флавоноида, ингибирующего на 50% процесс ПОЛ в мембранах эритроцитов, составили 3,9±0,8 мкМ в случае физетина, 6,5±1,6 мкМ в случае кемпферола, 8,1±2,1 мкМ в случае апигенина, 37,8±4,4 мкМ в случае нарингенина и 64,7±8,6 мкМ в случае нарингина. Антиоксидантный эффект флавоноидов был значительно выше в мембранных структурах по сравнению с цитоплазмой клеток. Все исследованные флавоноиды (10–50 мкМ) эффективно ингибировали дыхательную активность изолированных митохондрий печени крыс и, за исключением кемпферола, стимулировали Ca^{2+}-индуцируемую диссипацию мембранного потенциала митохондрий. Циклоспорин А и рутений красный ингибировали стимулируемую флавоноидами Ca^{2+}-зависимую деполяризацию мембран, что указывает на участие в эффекте флавоноидов митохондриального кальциевого унипортера и процесса открытия пор высокой проницаемости. Флавоноиды как редокс-активные соединения, обладающие антиоксидантными свойствами, способны регулировать митохондриальный потенциал и респираторную активность, предотвращать митохондриальный окислительный стресс. Их можно рассматривать в качестве эффективных фармакологических агентов или нутрицевтиков.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: флавоноиды; митохондрии; эритроциты; глутатион; окислительный стресс

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