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Study of antioxidant activity of liposomal forms of quercetin and curcumin in ischemic heart disease

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Abstract

Quercetin and curcumin are plant polyphenolic antioxidants with proven pharmacological efficacy. Their use is, however, limited due to low bioavailability and oral administration route. The encapsulation of lipophilic compounds in liposomes enables to increase their bioavailability and to create an injectable form. The present study aimed to comparatively investigate the antioxidant activity of a complex liposomal preparation containing two lipophilic antioxidants (quercetin and curcumin) and their monopreparations in liposomal forms. This study was conducted on Wistar line rats with experimental model of ischemic heart disease. Oxidative stress markers such as total antioxidant activity, malondialdehyde, and peroxidized proteins were analyzed in blood serum and cardiac tissue. Ischemic heart disease is accompanied with lipid peroxidation and changes in the activity of the antioxidant system. The obtained results demonstrated the antioxidant activity of monopreparations of curcumin and quercetin and their complex in liposomal forms. Quercetin and curcumin showed different antioxidant activities in terms of oxidative stress markers. The complex of the two antioxidants showed the synergistic effect of their lipophilic compounds in liposomal forms, which led to the normalization of test parameters according to the level of the control sample.

Key words: curcumin, antioxidant, quercetin, liposomes, oxidative stress markers

Introduction

Lipid peroxidation of biological membranes accompanies many diseases in cardiology, oncology, pulmonology, ophthalmology, etc. (Middleton et al., 2000; Pavlova, 2016). Antioxidants (AOs) of natural origin such as quercetin (Quer) (Ozgen et al., 2016; Xu et al., 2019), curcumin (Cur) (Liu et al., 2017; Rachmani et al., 2018), vitamin E (Di Vincenzo et al., 2019), and coenzyme Q_{10} (Tiano et al., 2007; Liu et al., 2016) may be effective for treating these pathologies. The high lipophilicity of these compounds, however, impedes their use as injectable preparations. It is believed that to increase the low bioavailability of lipophilic AOs, they should be used in liposomal (LS) forms. Several studies have been conducted for creating LS forms of lipophilic AOs (Alexopoulou et al., 2006; Ranjan et al., 2013; Shakhmaiev et al., 2015; Shvets et al., 2016; Pylypenko et al., 2019b). These studies followed two directions: the creation and study of LS forms of individual active pharmaceutical ingredients (APIs) (Shakhmaiev et al., 2015; Ng et al., 2018; Bavacad et al., 2019) and their complexes (Chavesa et al., 2018; Esteban et al., 2018; Pylypenko et al., 2019a). The pharmacological activity of natural bioflavonoids, in particular Cur and Quer, has been widely studied (Lakhanpal and Rai, 2008; Slesarchuk, 2014; Liu et al., 2015; Liu et al., 2017; Dong et al., 2018; Small et al., 2018); however, these compounds are plant lipophilic antioxidants and their use is limited due to extremely low bioavailability (Zhang et al., 2011; Riva et al., 2019).

Quer is a well-known exogenous AO that blocks the processes of chain reactions of free radical oxidation, thus preventing excessive oxidation of lipids, proteins, and nucleic acids and protecting cell membranes from damage by oxidants – Figure 1 (Middleton et al., 2000; Nakusov, 2010). Quer has angioprotective, AO, anti-inflammatory, wound healing, and antiviral effects (Lakhanpal and Rai, 2008; Ozgen et al., 2016; Dong et al., 2018; Xu et al., 2019). Currently, the world's first LS

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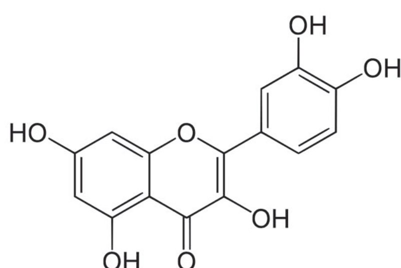

Fig. 1. Structure of quercetin

form of Quer ("Lipoflavon," Biolik, Ukraine) is used in clinical practice, for example, in cardiology, oncology, ophthalmology, type 1 and type 2 diabetes, psoriasis, pulmonology, renal failure, nephrotoxicity, and dentistry (Pasyechnikova et al., 2005; Ivanova and Yarosheva, 2008; Antipova et al., 2009; Belyaev, 2010; Asmolov et al., 2011; Ryabokon et al., 2011) .

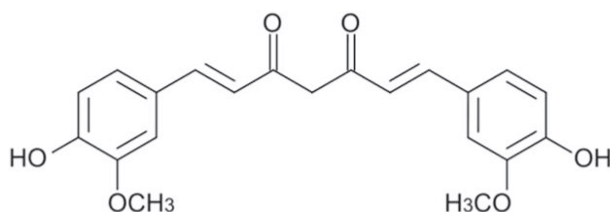

Fig. 2. Structure of curcumin

Cur is a pleiotropic compound that shows a wide spectrum of biological activities in an organism and exhibits AO, anti-inflammatory, and antitumor properties (Fig. 2) (Shimatsu et al., 2012; Alrawaiq and Abdullah, 2014). Extensive evidence on the pharmacological activity of Cur in oncology, ophthalmology, cardiology, and other pathologies has been accumulated (Cheng et al., 2001; Shimatsu et al., 2012; Mosovska et al., 2016; Liu et al., 2017; Pylypenko et al., 2018; Rachmani et al., 2018; Small et al., 2018). Long-term global data on the oral administration of Cur have confirmed its safety and efficacy (Cheng et al., 2001; Sanmukhani et al., 2014; Small et al., 2018). Although studies aimed at creating Cur nanoforms based on liposomal, polymer, or metal nanoparticles have been recently successfully completed (Feng et al., 2017; Ng et al., 2018), there is currently no commercial injectable preparation of Cur available in the market.

Considering the possibility of the effect of AOs on different components of the AO system, studies have focused on the complexes of these compounds with various APIs and their protective functions. Chaves et al. (2018) coencapsulated Cur and vitamin D3 in a multilamellar LS and showed that LS vesicles were stable throughout 42 days of storage and the efficiency of encapsulation of Cur and vitamin D3 was at least 89.6% and 88.3%, respectively. The effectiveness of using LS form of Quer obtained from plant extract for treating eye injury in rabbits was evaluated (Sotnikova et al., 2018). A comparative study of levodopa in a complex LS form containing Cur in the membrane bilayer and ascorbic acid and superoxide dismutase in the aqueous phase (Esteban et al., 2018) was carried out, and it was found that the co-loaded liposomes ensured the chemical stabilization of levodopa. The liposomes also exhibited a potential radical scavenging activity, mainly due to ascorbic acid. The effect of the complex LS preparation containing Quer and coenzyme Q10 was studied in rats with ischemic heart disease, and the synergistic effect of these two AOs has been proved (Pylypenko et al., 2019a). Another study of particular interest demonstrated a high AO effect of the LS complex of two polyphenols: Quer and a gallate-epigallocatechin isolated from green tea. The authors of this study prepared a stable LS form with mean size particle of 111.10 ± 0.52 nm and with at least 60% efficiency of encapsulation, and a synergistic effect of these two compounds in LS form was observed in 2,2-diphenyl-1-picrylhydrazyl assay (Chen et al., 2019).

A literature review showed that no studies have been conducted on the use of Quer and Cur complex in LS form for AO activity. The present study therefore aimed to investigate the AO activity of complex LS preparation containing two lipophilic AOs (Quer and Cur) tested on the rat model of ischemic heart disease (IHD).

Materials and methods

Materials

Quer ($C_{15}H_{10}O_7$) was manufactured by PVP Sociedate Anonima (Brazil), and a highly purified curcuminoid complex was obtained according to the protocol of Pylypenko and Krasnopolsky (2019). In the product used, curcuminoids were represented by diferulomethane (Cur I – $C_{21}H_{20}O_8$) in the amount of at least 70–75%, demethoxycurcumin (Cur II – $C_{20}H_{18}O_5$), and bisdemethoxycurcumin (Cur III – $C_{19}H_{16}O_4$) – the last two fractions in the amount of 25–30%. On the basis of literature reports that the minor components of cur-

cuminoids might exhibit AO and anti-inflammatory properties, we used an API containing three curcuminoids to prepare the LS form. To prepare LS, the following phospholipids were purchased from Lipoid, Germany: phosphatidylcholine (PC) from egg yolk and dipalmitoylphosphatidylglycerol (DPPG); lactose from Sigma Aldrich, USA; and PBS. The standards of PC and lysophosphatidylcholine (lysoPC) manufactured by Sigma Aldrich were used for analytical studies.

Preparation of Liposomes

LS forms of Quer and Cur were prepared using a previously developed technological platform (Grygorieva et al., 2017; Pylypenko et al., 2019b).

Analytical studies

Chromatographic (HPLC and TLC) and spectrophotometric methods were used to qualitatively and quantitatively analyze the content of Quer and Cur. The analytical studies were performed according to the protocols of the State Pharmacopoeia of Ukraine (2015).

High-performance liquid chromatography (HPLC) analysis of Cur was performed using Shimadzu Prominence LC-20 chromatograph with an SPD-M20A diode array detector, a Shim-pack GISS C18 column (5 µm, 250 × 4.6 mm), a CTO-20AC column thermostat, column temperature of 30 °C; and a mobile phase of water : acetonitrile (54 : 46), adjusted with glacial acetic acid to pH 3.0 ± 0.05. The detection was performed at the wavelength of 427 nm. Sample volumes were 2–10 µl.

HPLC analysis of Quer was performed in the isocratic mode on a Shimadzu LC 20 chromatograph with a chromatographic column (250 × 4.6 mm) filled with L1 sorbent with particle size of 5 µm (Waters Xbridge) and a mobile phase of methanol : water : phosphoric acid (100 : 100 : 1). The flow rate of the mobile phase was 1ml/min, and the detection was performed at the wavelength of 255 nm, with column temperature of 30 °C. The sample volume was 20 µl. The average retention time of Quer was 38 ± 0.5 min, which corresponded to the retention time of the standard Quer sample. Impurities in Quer (kaempferol and isoramnetin) were not higher than 2.0%, which corresponded to the limits specified by the manufacturer.

Quer and Cur were quantified spectrophotometrically at 375 and 540 nm, respectively. Thin layer chromatography (TLC) analysis was performed using silica gel 60 aluminum-backed TLC plates (Sigma-Aldrich). The mobile phases were chloroform–methanol (98 : 2) for Cur and chloroform–methanol–water (65 : 25 : 4) for phospholipids.

The presence of residual solvents in LS was determined by gas chromatography on a Shimadzu GC-2014 ATF/SPL gas chromatograph with an AOS-6000 universal autosampler and an SH-Rtx-624 MS column (30 m, 0.32 mm, 5 µm). The following conditions were used: input method: Static Head Space; sample temperature: 100 °C (the sample was incubated with shaking for 15 min before the input); syringe temperature: 120 °C; injector temperature: 200 °C; detector temperature: 300 °C; carrier gas: helium; gas velocity: 35 cm/s; flow rate: 2.16 ml/min; solvent: DMF; volume of the injection: 0.5 ml.

The size of LS was measured on the Malvern Zetasizer Nano ZS nanosizer (UK) using a semiconductor laser at 375 nm wavelength and 30 °C. The oxidation index was determined by UV spectrophotometry at the wavelength of 210 and 233 nm. The suitability of the chromatographic systems was determined in accordance with the guidelines of State Pharmacopoeia of Ukraine (2015).

Pharmacological Studies

The experimental part of the studies was performed at the Biochemistry Department of Kharkiv National Medical University, Ukraine. The study was conducted on 30 Wistar line rats weighing 150–180 g, which were kept in standard vivarium conditions. The following groups of animals were used: 1) control group (n = 6); 2) rats with experimental IHD (n = 6); 3) rats with experimental IHD treated with LS form of Quer (n = 6); 4) rats with experimental IHD treated with LS form of Cur (n = 6); and 5) rats with experimental IHD treated with LS form of Quer and Cur complex (n = 6).

IHD was stimulated in IHD model rats following the procedure of Gaman et al. (2011): 0.1 ml of 0.1% adrenaline solution and 1 ml of 2.5% hydrocortisone acetate suspension were subcutaneously administered daily for 7 days into IHD model rats. After simulating the IHD, the test samples were intravenously administered daily for 5 days at the dose of 10 mg/kg.

Blood samples were obtained from the tail vein. Cardiac tissue samples were obtained by cardiac puncture in anesthetized rats using the following procedure: the

heart sample was washed in ice-cold 0.9% NaCl solution, weighed, and homogenized with three volumes of the solution. The homogenate was filtered and precipitated at 10 000 *g* for 10 min. The supernatant was used in the study.

The animals were kept under standard conditions in accordance with the regulations of the National Research Council (2010). All procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the experimental protocol was approved by the local animal ethics committee.

Determination of peroxidation and AO protection products

Malondialdehyde (MDA) concentration was determined by the thiobarbituric acid reaction method, and the absorbance of the colored complex was measured spectrophotometrically at 532 nm (Deryugina et al., 2010). Conjugated dienes (CDs) were extracted with the heptane-isopropanol mixture, and the amount of CDs was determined spectrophotometrically at 233 nm (Deryugina et al., 2010). The activity of superoxide dismutase (SOD) was determined spectrophotometrically at 450 nm. The method is based on the inhibition of nitroblue tetrazolium reduction in a nonenzymatic system of phenazine methosulfate and NADH (Matyushin et al., 1991). The catalase activity was determined spectrophotometrically; the method is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate with the maximum absorbance at 410 nm (Korolyuk et al., 1988). Determination of the SH-group concentration is based on the reaction of the sulfhydryl group with 5,5-dithiobis-(2-nitrobenzoic acid), which results in the formation of equimolar amounts of yellow-colored thionitrophenyl anion with the maximum absorbance at 412 nm (Deryugina et al., 2010). ATP concentration was determined based on the principle of glucose phosphorylation in the presence of hexoinase. The amount of ATP is equimolar to NADPH formed in the glucose-6-phosphate dehydrogenase reaction (Eschenko et al., 1982). The protein peroxidation (PP) level was determined based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine where protein-bound 2,4-dinitrophenylhydrazone is formed, which can be detected spectrophotometrically at 370 nm (Dubinina et al., 1995). The total antioxidant activity (TAA) in the blood serum was determined spectrophotometrically as a degree of inhibition of LOPs (thiobarbituric acid reactive substances) production in yolk lipoproteins. Lipid peroxidation in the yolk lipoprotein suspension was induced by the addition of $FeSO_4$ following the procedure of Kibanov et al. (1988).

Statistical analysis

The data are expressed as mean ± SEM. Statistical significance of the differences between the groups was analyzed using Student's t-test in accordance with the State Pharmacopoeia of Ukraine (2015).

Results

Table 1 shows the characteristics of the obtained monopreparations of Quer and Cur in LS forms and the LS preparation containing Quer and Cur complex. The particle size of the LS was 130–200 nm before lyophilization and 200–300 nm after lyophilization; this implies that it was suitable for intravenous administration. The efficiency of encapsulation of Quer and Cur into LS was at least 90 and 85%, respectively. All the preparations were yellow colored with different color intensities; pH values were between 6.6 and 7.2, and the time of emulsification of lyophilized LS preparations was maximum 5 min. Lyophilized preparations were stable for at least 12 months and contained a maximum of 5% water.

The influence of Quer and Cur monopreparations in the LS form and the LS preparation containing Quer and Cur complex on oxidative stress markers (concentrations of MDA, CDs, total antioxidant activity, SOD, catalase, SH-groups, and ATP) was analyzed in the IHD model rats, and the results were compared with those obtained for control animals. Table 2 shows the impact of LS preparations on oxidative stress markers in the blood serum of experimental animals.

An increase in the content of MDA and CDs by 180.9 and 73.1%, respectively, was observed in the blood serum of rats with IHD as compared to those in control animals ($P < 0.001$). The administration of monopreparations to the animals reduced the content of MDA and CDs by 56.7 and 33.0%, respectively, in the blood serum of the Quer group and by 30.5 and 12.6%, respectively, in the blood serum of the Cur group as compared to those in animals with IHD ($P < 0.001$). The administra-

Table 1. The characterization of complex LS preparation Quer and Cur and their monopreparations

Name of sample	API content [mg/ml]	PC content [mg/ml]	DPPG content [mg/ml]	Lactose content [mg/ml]	Encapsulation of API into LS [%]	LS size before lyophilisation [nm]	LS size after lyophilisation [nm]
LS-Cur	0.785	28.0	2.8	42.0	at least 85	130–150	200–280
LS-Quer	0.75	27.5	–	42.0	at least 90	160–180	180–220
LS-Cur + Quer	0.38 / 0.35	27.5	1.4	42.0	at least 85 and at least 90, respectively	160–200	240–300

Table 2. Results of the study of the AA using the IHD model: rat blood serum, 10 mg/kg daily, 5 injections

Indices	MDA [μmol/l]	CD [μmol/l]	SOD [RU/sec/l]	Catalase [μmol/l]	SH Groups [mmol/l]	TAA [%]	PP [U/mg protein]
Control	2.09 ± 0.07	51.33 ± 0.65	33.83 ± 1.01	20.62 ± 0.37	12.91 ± 0.12	52.32 ± 0.64	0.127 ± 0.004
IHD	5.87 ± 0.08	88.87 ± 0.38	40.80 ± 0.36	27.68 ± 0.29	7.16 ± 0.17	31.18 ± 0.47	0.336 ± 0.019
IHD + Quer	2.54 ± 0.12	59.53 ± 0.62	52.75 ± 0.78	38.77 ± 0.34	7.73 ± 0.23	57.04 ± 0.42	0.101 ± 0.006
IHD + Cur	4.08 ± 0.10	77.69 ± 0.71	44.55 ± 0.33	32.54 ± 0.39	10.05 ± 0.38	47.48 ± 0.50	0.206 ± 0.010
IHD + Quer + Cur	1.80 ± 0.03	47.83 ± 0.32	31.11 ± 0.44	19.22 ± 0.26	13.54 ± 0.29	49.54 ± 0.37	0.108 ± 0.005

tion of the LS form of Quer and Cur complex reduced the amount of MDA and CDs by 68.9 and 46.2%, respectively, as compared to those in animals with IHD ($P < 0.001$), which was almost at the level of the intact control (Table 2).

Regarding the total antioxidant activity, a decrease in activity by 40.4% was observed in the blood serum of rats with IHD as compared to that in control animals ($P < 0.001$). The administration of monopreparations increased the total antioxidant activity by 82.9 and 52.3% in the Quer and Cur groups, respectively, and the use of the LS form of Quer and Cur complex increased the total antioxidant activity by 59.0% as compared to that in animals with IHD ($P < 0.001$). Consequently, the use of Quer and Cur monopreparations as well as their complex in LS forms normalized the total antioxidant activity close to that of the intact control (Table 2).

The protein peroxidation level increased by 164.6% in animals with IHD as compared to that in the intact control ($P < 0.001$). The use of a complex LS preparation and the LS form of Quer monopreparation led to the normalization of the protein peroxidation level close to that of the intact control. The use of the LS form of Cur monopreparation showed an increase in the protein peroxidation level by 62.2% ($P < 0.01$) as compared to that in the intact control (Table 2).

The content of SH-groups in the blood serum of animals with IHD decreased by 44.5% ($P < 0.001$). In rat groups administered with Quer and Cur monopreparations, the level of SH-groups remained below the intact control by 40.1% ($P < 0.001$) and 22.2% ($P < 0.01$), respectively. Furthermore, the administration of the LS form of Quer and Cur complex normalized the content of SH-groups in the blood serum to the level of control animals (Table 2).

Similar trends were also observed in the content of oxidative stress markers in the heart muscle (Table 3). An increase in the content of MDA and CDs by 136 and 88.5%, respectively, was observed in the cardiac tissue of rats with IHD as compared to those in the control animals ($P < 0.001$). The administration of monopreparations to animals decreased the content of MDA and CDs by 46.7 and 36.4%, respectively, in the cardiac tissue of the Quer group ($P < 0.001$) and decreased the content of MDA and CDs by 24.5% ($P < 0.001$) and 18.4% ($P < 0.01$), respectively, in the Cur group as compared to those in animals with IHD. The use of the LS forms of Quer and Cur complex reduced the amount of MDA and CDs by 52.1 and 49.0%, respectively, as compared to those in animals with IHD ($P < 0.001$), which was almost at the level of the intact control (Table 3).

Table 3. Results of the study of the AA using the IHD model: rat cardiac tissue, 10 mg/kg daily, 5 injections

Indices	MDA [mmol/g protein]	CD [mmol/g protein]	SOD [RU/g protein]	Catalase [μmol/g protein]	ATP [mmol/g protein]
Control	1.75 ± 0.02	15.27 ± 0.39	98.05 ± 0.41	24.68 ± 0.28	3.53 ± 0.19
IHD	4.13 ± 0.09	28.78 ± 0.64	116.82 ± 2.15	31.28 ± 0.43	1.47 ± 0.04
IHD + Quer	2.20 ± 0.08	18.31 ± 0.22	139.91 ± 1.33	44.26 ± 0.50	1.81 ± 0.04
IHD + Cur	3.10 ± 0.07	23.49 ± 0.72	128.67 ± 0.87	34.91 ± 0.49	1.65 ± 0.05
IHD + Quer + Cur	1.98 ± 0.04	14.69 ± 0.21	104.36 ± 1.62	27.57 ± 0.65	2.14 ± 0.05

To study the changes in the enzymatic AO defense system, the levels of SOD and catalase were determined. An increase in the content of SOD and catalase by 20.6 and 34.3%, respectively, was observed in the blood serum of rats with IHD as compared to those in the control animals ($P < 0.01$). The levels of SOD and catalase were similar to those of the intact control rat group administered with the LS form of Quer and Cur complex (Table 2). Similar trends were also observed in the cardiac tissue (Table 3). An increase in the content of SOD and catalase by 19.1 and 26.7%, respectively, was observed in the cardiac tissue of rats with IHD as compared to those in control animals ($P < 0.01$). Treatment with the LS form of Quer and Cur complex reduced the amount of SOD and catalase by 10.6 and 11.2%, respectively, as compared to those in animals with IHD ($P < 0.05$), which is almost at the level of the intact control (Table 3). In the rat groups administered with Quer and Cur, the monopreparations increased the levels of SOD and catalase even more than that in the rats with IHD in both blood serum and cardiac tissue.

The ATP level decreased by 58.4% in the cardiac tissue of rats with IHD as compared to those in control animals ($P < 0.001$). The administration of monopreparations increased the ATP level by 23.0 and 12.2% in the Quer and Cur groups, respectively, and the use of the LS form of Quer and Cur complex increased the ATP level by 45.5% as compared to that in animals with IHD ($P < 0.01$). The concentrations of ATP in groups of LS preparations were lower than those of control animals, but the use of a complex preparation was more effective than the use of monopreparations of Quer and Cur (Table 3).

Discussion

Many cardiological diseases are accompanied with changes in the levels of several oxidative stress markers (Middleton et al., 2000). Lipid peroxidation in the cardiac tissue leads to serious pathologies such as IHD, myocardial infarction, and arrhythmia (Rodrigo et al., 2013; Farías et al., 2017). Currently, various AOs with cardioprotective effect are being considered for the treatment of cardiac pathologies (Khalil and Sulaiman, 2010; Lopera et al., 2013; Rodrigo et al., 2013; Pavlova, 2017). Liposomes allow to create a water-soluble injectable form of lipophilic APIs and thus increase their bioavailability (Shvets et al., 2016). LS preparations with cardioprotective effect have been proposed for use in cardiology, for example, LS form of Coenzyme Q10 (Shakhmaiev et al., 2015), LS form of adenosine (Takahama et al., 2009), LS form of resveratrol and carvedilol (Alanazi et al., 2020), and LS form of Quer and Coenzyme Q10 (Pylypenko et al., 2019a).

A review of literature indicates that several studies have been conducted for obtaining monopreparations of LS lipophilic AOs (Cur or Quer) (Alexopoulou et al., 2006; Shaji and Iyer, 2012; Ranjan et al., 2013; Feng et al., 2017; Melnyk et al., 2018; Ng et al., 2018; Bavacad et al., 2019). However, the number of studies on complex LS forms is scarce (Ravichandiran et al., 2017; Sadeghi Ghadi et al., 2019). Nanoparticles containing Quer and Cur were prepared by the lipid film method with particle size of 261.8 ± 2.1 nm (Ravichandiran et al., 2017) and 260 ± 6.58 nm (Sadeghi Ghadi et al., 2019) and with the efficiency of encapsulation of Cur and Quer of 98.85 ± 0.55% and 93.13 ± 1.22%, respectively (Sadeghi Ghadi et al., 2019). Consequently, the physicochemical properties of our complex LS preparation was similar (particle size of LS preparations after lyophilization was 200–300 nm and the efficiency of encapsulation of Quer and Cur into LS was at least 90 and 85%, respectively – Table 1). Moreover, IHD is accompanied with lipid peroxidation and a change in the activity of the AO system

(Pavlova, 2017), as confirmed by the results of our experiments (Table 2 and Table 3).

The obtained results demonstrate antioxidant activities of monopreparations of LS forms of Cur, Quer, and their complex. Moreover, the monopreparations of Quer and Cur exhibited different antioxidant activities when compared with each other. A study on the influence of Quer and Cur in LS form on the activities of several oxidative stress markers showed that Quer exhibited a higher antioxidant activity, in particular for MDA, CDs, and protein peroxidation; in other cases, Cur was more effective, for example, for SH-groups. However, the most effective reduction in the levels of all studied oxidative stress markers in the blood serum and cardiac tissue of rats with IHD was achieved using an LS preparation containing the complex of both Quer and Cur. Apparently, AO activity depends on both the amount of AOs (Quer and Cur) used and their mutual synergy.

Several authors have indicated the synergistic effect of the AO activity of Quer and Cur on the basis of increased total AO activity and reduced MDA and lipid hydroperoxide levels (Liu et al., 2015; Balakina et al., 2017; Ravichandiran et al., 2017; Sadeghi Ghadi et al., 2019). In addition, the membrane-protective properties of Quer and Cur contribute to the increase in the protective and adaptive potential of the rat body (Balakina et al., 2017). Liu et al (2015) administered Quer and Cur separately and in combination *per os* to mice with lung cancer. They found that the use of both bioflavonoids separately and in combination improved the body weight of mice and reduced lung weight. Importantly, the co-administration of Quer and Cur reduced the levels of MDA and reactive oxygen species more effectively (Liu et al., 2015), which might be because Quer and Cur have different mechanisms of AO activity and have a higher therapeutic effect when administered in combination. It is noteworthy that the doses used *per os* (40 to 200 mg/kg) were significantly higher than those of Quer and Cur in LS form (10 mg/kg) that were administered in the present study. The encapsulation of lipophilic compounds into LS enables to increase their bioavailability and efficacy, and hence, it is possible to decrease the doses of APIs.

Detoxification of reactive oxygen species in cells is performed by both enzymatic and nonenzymatic systems, which constitute the AO defense system (Middleton et al., 2000). AOs decrease the level of free radicals by inhibiting the activities or expression of free radical generating enzymes or by promoting the activities and expression of antioxidant enzymes (Lü et al., 2010). The enzymatic system includes the following enzymes: SOD, catalase, glutathione peroxidases, and glutathione-regenerating enzyme (Middleton et al., 2000). SOD is one of the main enzymes of the AO system that are related to metal enzymes that catalyze the dismutation reaction of superoxide anion radicals to maintain their content in the cell at low levels and reduce the likelihood of formation of even more active singlet oxygen (Middleton et al., 2000; Nakusov, 2010). Catalase is a membrane-bound enzyme that is a major element involved in maintaining the intracellular concentration of reduced glutathione, which plays an important role in neutralizing free radicals (Middleton et al., 2000; Nakusov, 2010).

The data obtained in this study are consistent with the results available in the literature (Tiano et al., 2007; Gubareva et al., 2008; Nakusov, 2010; Orlova and Lazarchuk, 2010; Shakhmaiev et al., 2015). For example, Quer and dihydroquercetin drugs, which reduce the content of lipid oxidation products or inhibit their accumulation during the hypoxic action, increase the activity of AO enzymes by eliminating the peroxide metabolism products that inhibit them (Nakusov, 2010). As suggested by Nakusov (2010), it is also possible that flavonoids directly affect enzymes by interacting with amino acid radicals of the polypeptide chain, thereby changing the conformation of the protein molecule, which contributes to a change in the properties of the enzyme. This may be due to an increase in the SOD and catalase activity above the control level. Pavlova (2017) showed that 1–2 days after myocardial infarction in humans, the SOD level increased 2.1 times as compared to that in the control (healthy people); this may be associated with its compensatory allosteric activation in the conditions of hyperproduction of the superoxide anion radical that activates SOD and simultaneously inhibits catalase. In the subsequent 10–14 days, the SOD activity decreased; nevertheless, its content was 1.4 times higher than that of the control group (Pavlova, 2017). The authors showed an increase in the activity of SOD and catalase not only in patients with IHD but also in patients treated with AOs, which may be associated with the inhibition of the lipid peroxidation process. It has been established that strong AOs not only play a role in "trapping" free radicals, but they also increase the enzymatic activity of SOD (Middleton et al., 2000; Pavlova,

2017). Other authors showed an increase in the activity of SOD and catalase in cardiac pathology (Agarkov et al., 2017; Romuk et al., 2019). Some authors indicated an increase in the activity of SOD in the heart of rats, which was observed while studying markers of oxidative stress in streptomycin model of type 2 diabetes (Agarkov et al., 2017) and in cases of severe chronic heart failure (Pavlova, 2016).

Conclusions

A comparative study of the antioxidant activities of Quer and Cur monopreparations in LS form and the LS preparation containing Quer and Cur complex was conducted on the IHD model in rats. The administration of the complex showed a synergistic antioxidant effect of two lipophilic compounds in LS form. Additionally, Quer showed higher activity than Cur in the monopreparations. A statistically significant decrease was observed in the level of lipid oxidation products and an increase in the activity of the antioxidant activity system on the IHD model in rats using an LS preparation containing Quer and Cur complex as compared to the LS form of monopreparations. Currently, the use of LS antioxidant drugs is a promising area for pharmacotherapy in cardiology. On the basis of our results, the complex LS preparation containing Quer and Cur may be used to prevent free radical oxidation in cells during IHD. Future studies will assess the dose-dependent effect and the development of an optimal pharmaceutical form.

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