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Study on Effects of Liposomal Quercetin on Biochemical Parameters of the Nigrostriatal System of Rats with Experimentally Induced Neurodegenerative Disease

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Abstract: The work was initiated to study effects of liposomal quercetin on lipid composition, lipid peroxidation and anti-oxidant system enzymes in the nigrostriatal system of rats with experimentally induced neurodegenerative disease. Despite considerable progress in studying roles of the amyloid-beta protein and the tau protein, microglia and inflammatory processes, as well as of sphingomyelin cycle and free radical processes in the onset and progression of neurodegenerative diseases, many issues relating to the significance of all these components remain open. The work is an attempt to study effects of changes in lipid composition of the nigrostriatal system and those of liposomal quercetin on the behavioral performance of animals with experimentally induced neurodegenerative disease. The neurodegenerative disease was induced by a 13-day intranasal administration of rotenone with addition of E.coli, a lipopolysaccharide (LPS), on the 14^{th} day. Findings from the study demonstrated changes in the lipid composition of the nigrostriatal system of animals with the experimentally induced neurodegenerative disease next to activation of lipid peroxidation and reduction in activity of the anti-oxidant system enzymes. The changes in the biochemical parameters of the system were considered as those causing deviations in behavioral performance of the animals. Liposomal quercetin administered in 30 minutes after administration of rotenone was found to reduce the neurotoxic effects of rotenone and LPS on the biochemical parameters of the nigrostriatal system and behavioral performance of

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the animals. Findings from the study demonstrated anti-oxidant and anti-inflammatory effects of liposomal quercetin blunting neurotoxic effects of rotenone and LPS; prospects to use it in generation of drugs for therapy of neurodegenerative diseases are under consideration.

Keywords: hyperphosphorylation, sphingomyelin, quercetin, nigrostriatal, dimethylsulfoxide, phosphatidic.

Introduction

Neurodegenerative diseases are figuring larger among underlying causes of work decrement and mortality increase worldwide. The findings from numerous studies aiming at studying causes underlying the onset of the degenerative nerve diseases helped to identify the stages in intricate processes of their onset and progression. Hyperaggregation of the amyloid-beta protein, hyperphosphorylation of the tau protein, free radical activation, changes in the sphingomyelin cycle, activation of microglia and inflammatory processes are thought to underlie the onset of neurodegenerative diseases [1,2,3,4]. Meanwhile, there is no one frame of mind on the root cause of the onset of a neurodegenerative disease ultimately resulting in death of the brain cells. With regard to the key role the free radical and inflammatory processes play in neurodegenerative diseases, studies on the effects produced by biologically active agents with anti-oxidant and anti-inflammatory properties are presently topical.

In the view of the aforesaid, the work was initiated to study effects of liposomal quercetin on lipid composition, activity of lipid peroxidation and anti-oxidant system enzymes of the nigrostriatal system in rats with the experimentally induced neurodegenerative disease, as well as those produced on behavioral performance of the animals. Today, liposomes are widely used as containers for targeted delivery of biologically active agents; liposomal quercetin is used in therapy of ophthalmic and skin diseases [5,6]. Anti-oxidant and anti-inflammatory properties of quercetin taken into account, we used liposomal quercetin trying to prevent changes in biochemical parameters of the nigrostriatal system in animals

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with the experimentally induced neurodegenerative disease.

Materials and methods

Animals

The outbred rats weighing 300-350g kept on a standard diet with addition of cholesterol making up 2% of the food volume were used in the experiments. The experiments with animals were conductedin compliance with the requirements of the Commission on Bioethics and Humane Treatment of Laboratory Animals at the Institute of Biophysics and Biochemistry under MizroUlugbek National University of Uzbekistan, in accordance with the Animal Testing Regulations. The animals were kept in the properly ventilated, illuminated and heated space with well-timed clean-up by 5 in one cage, and with free access to the food and water.

Design

Prior to induction of the neurodegenerative disease, all rats were subjected to the behavioral tests to be chosen for the experiment[7]. For the purposes of experiment, 26 animals were divided into groups, to name the active control group including 5 animals receiving normal saline with dimethylsulfoxide (DMSO) (8:2w/w) intranasally, and the exposure group consisting of 21 animals with the total score of 2.5 according to the McGraw Stroke Index Scale [8]. The animals in the exposure group were divided into 3 subgroups by 7 animals in a subgroup. In the 1^{st} subgroup the animals intranasally received rotenone in the dose of 1.5mg per kg of weight for 13 days; E.coli as a bacterial lipopolysaccharide (LPS) was added on the 14^{th} day in the dose of 200 mg/kg of weight [9]. In the 2^{nd} subgroup, liposomal quercetin in the dose of 3.0 mg per kg of weight was intranasally added in 30 minutes after administration of rotenone. In the 3^{rd} subgroup, intranasal administration of standard quercetin in the dose of 5.0 mg per kg of weight followed the administration of rotenone in 30 minutes. Effects of quercetinwere assessed on the 3^{rd}, 6^{th}, 9^{th} and 16^{th} day in the behavioral tests, such as the open field test, the elevated cross maze test, the passive avoidance test and the active avoidance test [10]. Muscle rigidity was assessed by the humpiness of a rat's body

comparing its length prior to induction of neurodegenerative disease and after that. Materials of the nigrostriatal system for biochemical analysis were taken on the 16th day after administration of rotenone. The animals were euthanized following anesthesia with observation of all animal testing regulations.

Substances and reagents administered to animals

Small single lamellar liposomes with sizes ranging from 150 to 200 nm were prepared from sphingomyelin (SPH) (0.053M), phosphatidylcholine (PC) (0.038M) and cholesterol (0.024M) with the molecular ratio of 7:3:3 and total concentration of lipids 50 mg/ml. After solvent drying, dissolved into the normal saline with dimethylsulfoxide (8:2) quercetin was added to the lipid film (SPH : PC : cholesterol) to be ultrasonicated at 22 kHz for 10 minutes [11]. Equilibrium dialysis was used to separate quercetin that was not included, while quercetin that was included (60% of the basal value) was determined by the thin-layer chromatography as describes elsewhere [12]. Sphingomyelin was isolated from the cattle brain. Phosphatidylcholine and cholesterol were obtained from Sigma Aldrich Chemical Co., USA. Quercetinwas kindly procured by the Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences.

Biochemical parameters and statistical data processing

The thin-layer chromatography was used for total lipid extraction and fractionation of lipids was performed as described elsewhere [13]. Lipid and total protein quantification was performed as described elsewhere [14]. Basal levels of the substrates reactive with the thiobarbituric acid were measured as described elsewhere [15]; activity of antioxidant enzymes, such as catalase, superoxide dismutase and glutathione reductase was estimated as described elsewhere, respectively [16,17]. Cary 60 spectrophotometer (Agilent Technologies, USA) was used to make optical measurements. The data were processed by means of the Student's t-test and the Origin 6.1 program. Significance level was at $p \leq 0.05$.

Results

Lipid composition of the nigrostriatal system in rats after experimentally

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induced neurodegenerative disease with symptoms of Parkinson's disease and after administration of liposomal and standard quercetincan be seen in Table 1.

Table 1. Lipid composition of the nigrostriatal system of rats after experimentally induced neurodegenerative disease, and administration of liposomal and standard quercetin

Lipids	Control group (n=5)	1st exposure subgroup: rotenone+LPS(n=7)	2nd exposure subgroup: rotenone + liposomal quercetin (n=7)	3rd exposure subgroup: rotenone+ standard quercetin (n=7)
LP,μg of P/g of tissue	10.3±0.8	14.1±1.2*	11.8±0.7	12.8±0.7
SPH, μg of P/g of tissue	225.2±7.5	208.1±6.1	215.2±6.3	210.2±5.3
PC, μg of P/g of tissue	559.8±10.6	532.0±9.5	550.8±11.5	540.8±11.5
PS, μg of P/g of tissue	86.5±5.5	70.4±5.1	77.5±4.5	72.5±5.3
PI, μg of P/g of tissue	88.7±6.8	95.5±7.5	90.5±6.8	91.7±7.3
PE, μg of P/g of tissue	491.4±21.4	488.3±19.1	493.4±21.4	493.4±21.4
Cardiolipin, μg of P/g of tissue	59.6±2.8	63.1±2.1	61.1±2.8	62.5±2.8
PA, μg of P/g of tissue	14.9±0.8	18.2±1.2*	15.2±0.8	16.4±1.1

Total phospholipids, μg of P/g of tissue	1536.4±30.3	1489.7±25.3	1515.5±28.3	1500.3±28.3
Cerebrosides, mg/g of tissue	7.8±0.4	8.6±0.5	7.6±0.4	8.0±0.6
Sulfatides, mg/g of tissue	2.7±0.2	2.5±0.3	2.8±0.2	2.6±0.4
TC, mg/g of tissue	19.7±0.81	22.1±0.85	20.3±0.75	21.3±0.75

Abbreviations: LP – lysophosphatidylcholine, **SPH** – sphingomyelin, **PC** – phosphatidylcholine, **PS** – phosphatidylserine, **PI** – phosphatidylinositol, **PE** – phosphatidylethanolamine, **PA** – phosphatidic acid, **TC** – total cholesterol

Note: * significant differences between active control and exposure groups, significance level $p<0.05$

As it can be seen in Table 1, induction of the neurodegenerative disease model caused significant increase in concentrations of lysophospholipids (LP) and phosphatidic acid (PA), while there were insignificant changes in concentrations of other fractions and total available phospholipids. There was a tendency towards increase in concentrations of phosphatidylinositol (PI), while those of sphingomyelin (SPH) and phosphatidylserine (PS) tended to decrease; there was a 15%- increase of cholesterol in relation to the sum of phospholipids. Supposedly, after administration of neurotoxins, next to intensification of freeradical-induced lipid oxidation, the activity of phospholipases increased causing increase in concentrations of LP and PA; the microviscosity of nerve cell membranes seemed to change resulting in increase of cholesterol in the system under study.

Apparently, the changes in the lipid composition of the system have an impact on the receptor and synaptic parts of membrane, as well as on nerve cell signaling, probably, causing the changes in behavior of the animals with the experimentally induced neurodegenerative disease.

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Taking into account that quercetin and its metabolites demonstrate anti-oxidant properties, we made an attempt to study effects of liposomal quercetin on the activity of anti-oxidant system enzymes, such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GR) of nigrostriatal system in rats with the experimentally induced neurodegenerative disease.

Table 2. MDA and anti-oxidant system enzymes of the nigrostriatal system in rats with degenerative nerve disease model after intranasal administration of liposomal and standard quercetin

Groups of animals		**MDA µmol/mg of protein**	**Catalase (U/mg of protein)**	**SOD(U/mg of protein)**	**GR nmol/min /mg of protein**
1	Active control group (n=5)	2.42±0.15	54.12±3.15	55.42±4.16	35.2±2.42
2	1st exposure subgroup: rotenone+LPS (n=7)	4.35±0.52*	35.23±3.51*	41.73±3.64*	31.4±2.53
3	2nd exposure subgroup: rotenone + liposomal quercetin (n=7)	3.15±0.31*	46,.4±3.31*	49.23±2,80	33.2±2.31
4	3rd exposure subgroup: rotenone+ standard quercetin (n=7)	3.65±0.33	41.32±3.52	45.63±3.10	32.4±2.25

Note: * the differences are significant between the groups, significance level $p<0.05$

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As it can be seen, upon induction of the neurodegenerative disease, levels of LPO products reactive with thiobarbituric acid increased by 79.7%, while activity of catalase, SOD and GR reduced by 35%, 24.7% and 10.8%, respectively. Liposomal and standard quercetin caused reduction in concentrations of MDA in the nigrostriatal system of rats with degenerative nerve disease model, as compared to the group of animals in the exposure group not receiving quercetin, by 27.6% and 16.0%, respectively, to be 3.15 and 3.65 nmol/mg of protein, respectively. Liposomal quercetin administered after rotenone increased activity of catalase and SOD by 32% and 18%, respectively; while changes in the GR activity were insignificant both upon induction of the model and after administration of quercetin. As it can be seen, effect of the standard quercetin on the biochemical parameters of the nigrostriatal system was less significant than the one produced by the liposomal quercetin.

Next, we studied effects of liposomal quercetin on the behavioral performance of animals with experimentally induced degenerative nerve disease in the open field test, the elevated cross maze test, the passive avoidance test and the active avoidance test.

Changes in behavioral performance of rats in the open field test before and after induction of degenerative nerve disease, as well as after administration of liposomal quercetincan be seen in Table 3.

Table 3. Behavioral performance of animals in the open field test

Behavioral activity	**Groups of animals (n=26)**			
	Active control (n=5)	**1st exposure subgroup: rotenone+LPS(n=7)**	**2nd exposure subgroup: rotenone+ liposomal quercetin (n=7)**	**3rd exposure subgroup: rotenone +standard quercetin (n=7)**

Latent period(s)	48.5±8.2	85.3±17.3*	58.2±9.2	69.2±6.3
Distance traveled(M)	11.2±2.5	5.4±1.7	8.1±2.3	7.2±2.5
Line crossings (n)	19.3±3.5	6.5±1.2*	14.2±1.7*	9.3±1.5
Squares transversed (n)	51.2±11.6	12.4±5.3*	33.1±8.4	20.3±7.6
Groomings (n)	2.3±0.3	1.3±0.2	1.9±0.3	1.5±0.2
Frequency of head-dipping (n)	23.1±3.1	9.2±2.5*	17.1±2.1*	11.4±23

Note:* statistically significant differences, significance level $p<0.05$.

The animals demonstrated the deferred reactions in the open field test; oligokinesia, an elongation of the latent period, a reduction in the number of line crossings, of the squares transversed and in the frequency of hear-dipping could be seen. On the 3rd day after induction of the neurodegenerative disease, 5% of animals in the 1st exposure subgroup demonstrated tremor of the extremities, in 10% muscle rigidity could be seen. In subgroups of animals receiving liposomal and standard quercetin, the behavioral performance parameters in the open field test changed insignificantly coming close to those in the controls (Table 3). The effects of liposomal quercetin were more significant than those of the standard one.

The findings from the study on behavioral performance (motivation and emotional tests) in the elevated cross maze test can be seen in Table 4; those from the passive avoidance test and the active avoidance test can be seen in Table 5.

Table 4. Behavioral performance of rats in the elevated cross maze test after administration of liposomal quercetin

Groups of animals (n=26)	**Latent period (s)**	**Time spent in a maze arm (s)**		
		Arm A	**Arm B (closed)**	**Arm C**

Active control (n=5)	8.7 ±2.4	151.3±21.3		148.3±21.2
1st exposure subgroup: rotenone+LPS(n=7)	13.2±2.5	131.2±23.5		135.3±24.3
2nd exposure subgroup: rotenone+ liposomal quercetin (n=7)	9.2 ±2.1	146.7±18.3		146.5±20.5
3rd exposure subgroup: rotenone +standard quercetin (n=7)	9.5 ±2.3	139.1±21.4		140.6±18.3
The test repeated in 24 hours				
Active control (n=5)	8.6 ±2.4	135.1±20.1	125.6±15.3	132.5±16.1
1st exposure subgroup: rotenone+LPS(n=7)	14.7±2.7	116.5±13.1	98.6±9.3	101.5±13.2
2nd exposure subgroup: rotenone+ liposomal quercetin (n=7)	8.2±2.4	125.6±16.5	120.1±10.1	121.5±12.1
3rd exposure subgroup: rotenone +standard quercetin (n=7)	11.8±2.3	123.7±12.5	115.3±11.5	108.5±13.1

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No changes could be seen in the behavior of control animals repeatedly placed in the elevated cross maze with the open arm; they examined and sniffed this arm with curiosity. Animals in the 1st exposure subgroup left the arm showing no interest to new conditions and smells, probably due to distortions of their emotion-motivation reactions. The rats receiving liposomal quercetin demonstrated longer period of examination of new arms than the animals not receiving quercetin; and this can be the evidence for the capacity of the agent to blunt the effects of neurotoxins on emotion-motivation reactions (Table 4).

The findings from the passive avoidance test and the active avoidance test after administration of liposomal quercetin to rats can be seen in Table 5.

Table 5. Results from the passive and active avoidance tests after administration of liposomal quercetin.

Groups of animals (n=27)	Number of the unlearned reflexes in the passive avoidance test (%)	Number of the acquired reflexes in the active avoidance test (%)	Number of refusals to perform the adequate response (%)	Number of animals reaching the adequate level of training) (%)
Active control (n=6)	21.2±2.7	78.1 ±13.5	8.1 ±3.4	79.0
1st exposure subgroup: rotenone + LPS(n=7)	77.5±13.4*	28.1±5.7*	65.1±7.5*	27.0

2nd exposure subgroup: rotenone+ liposomal quercetin (n=7)	35.3±6.2*	65.2±9.2	10.5±2.8*	68.0
3rd exposure subgroup: rotenone + standard quercetin (n=7)	45.2±6.5*	52.5±8.1	12.1±3.2*	55.0

Note: * the differences are significant between the groups, significance level at $p<0.05$

In the passive avoidance test, after 8 repeated placements of the control animals in the undimmed part of the chamber followed by their instinctive moving to the dimmed part of the chamber with pain stimulation, by the end of the experiment the animals entered the dimmed part of the chamber in 21.2% only; in 78.1% of cases the memory of the pain stimulation was preserved. In the 1st exposure subgroup, the animals moved to the dimmed part of the chamber in 77.5% of cases demonstrating that the acquired reflex to the pain stimulation was preserved in 28.1% of animals only. Intranasal administration of liposomal quercetin caused preservation of the unlearned reflex, that is, moving to the dimmed part of the chamber, in 35.3% of cases, in 65.2% of cases the animals avoided moving to the dimmed part of the chamber; memory of the pain stimulation was preserved. The rats administered with the standard quercetin avoided moving to the dimmed part of the chamber in 52.5% of cases; memory of the pain stimulation seemed not to preserve in other cases.

By the end of the active avoidance test, after 8 repeated placements 79% of control animals reached the adequate level of training, while the number of refusals

to perform the adequate response could be seen in 8.1%. Among animals of the 1st exposure subgroup, the adequate level of training was reached in 27%; while the number of refusals to perform the adequate response increased. After administration of liposomal and standard quercetin the adequate level of training could be seen in 68% and 55%/ respectively.

Thus, our results demonstrated protective effects of intranasally administered liposomal and standard quercetin reducing neurotoxic effects of rotenone and LPS; the former seemed to be more effective than the latter. Supposedly, this can be attributed to faster and proper crossing of liposomal quercetin through the blood-brain barrier, and additive effect of liposomes that may act as antioxidants adsorbing active radicals and build into the cell membranes.

Conclusion

Induction of neurodegenerative disease by rotenone and LPS in rats was found to cause activation of lipid peroxidation and reduction in activity of anti-oxidant system enzymes, resulting in changes of lipid profile in the nigrostriatal system of animals. Supposedly, these changes make an impact on the nerve cell signaling to reflect, and thus, on behavioral performance of animals with the experimentally induced neurodegenerative diseases. With oral administration of a biologically active agent, 80% of its active ingredient is known to decompose in the stomach and taken by the parenchymatous tissues, while only 20% of it arrives as intended.

According to literature, the intranasal administration is instrumental for biologically active agents to make their way to the central nervous system via olfactory region of the nasal cavity; lipophilic substances have advantages over the hydrophobic ones [18]. Presumably, this is why liposomal quercetin turned out more effective than the standard one against neurotoxic effects of rotenone and LPS. Our findings demonstrate that, possessing antioxidant properties and producing effects on the neurogenesis [19], liposomal quercetincan be used in generation of drugs for therapy of neurodegenerative diseases.

To sum up, intranasally administered quercetin facilitated the reduction in neurotoxic effects of rotenone and LPS on the behavioral and biochemical parameters of the animals with experimentally induced neurodegenerative disease. Liposomal quercetin turned out more effective than the standard one. Intranasal way of administration of antioxidants in the liposomal form may be considered useful in generation of drugs for therapy of neurodegenerative diseases.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript. The research did not receive specific funding but was performed as part of the employment of the authors (Institute of Biophysics and Biochemistry under MirzoUlugbek National University of Uzbekistan, Tashkent, Uzbekistan).

Conflicts of Interest

The authors declare no conflicts of interest

References

[1] Jack C.R, Wiste H.J, Knopman D.S et al: Rates of b-amyloid accumulation are independent of hippocampal neurodegeneration. Neurology 2014; 82:1605-1612.

[2] Overk C.R, Masliah E. Pathogenesis of synaptic degeneration in Alzheimer's disease and Lewy body disease. Biochem. Pharmacol. 2014; 88:508-516.

[3] Pchelina S.N: Alpha-synuclein as a biomarker of Parkinson's disease. Annals of clinical and experimental neurology 2011; 4:46-51 (in Russian).

[4] Alessenko A.V. Potential role of sphingolipids in neuropathogenesis of Alzheimer's disease. Biomeditsinskayakhimiya (Biomedical Chemistry, Russia) 2013; 1:25-50 (in Russian).

[5] Jerdev V.P et al. Comparative pharmacokinetics of dihydroquercetin in rats after

administration of Flamen D in regular and liposomal forms. Eksperimentalnayaiklinicheskayafarmakologiya (Experimental and clinical pharmacology, Russia) 2010; 1:23-25. (in Russian).

[6] Naumov A.A, Potselueva M.M. Beneficial effect of liposomal dehydroquercetin on regeneration of skin after thermal burn. Tsitologiya (Cytology, Russia) 2010;52(4):311-316 (in Russian).

[7] Amikishieva A.V. Behavioral phenotyping: current methods and equipment. Vavilov Journal of Genetics and Breeding 2009; 3:529-542 (in Russian).

[8] McGraw C.P, Pashayan A.G, Wendel O.T. Cerebral infarction in the Mongolian gerbil exacerbated by phenoxybenzamine treatment. Stroke 1976;7 (5):485–488

[9] Moyseenok A.G, Omel'yanchik S.N, Shevalier A.A, Evkovich I.N. et al. Method for modeling of neurodegenerative disorder of central nervous system in rats. Patent BY 11160 C1 2008.10.30, Republic of Belarus (in Russian).

[10] Dubrovina N.I, Loskutova L.V. Dopaminergic mechanisms of memory and attention. Novosibirsk, Nauka, 2003 (in Russian).

[11] Yurasov G.N, Podgorny V.G, Kucheryanu V.S, Kudrin V.S et al. Effect of liposomal L-DOPA on dopamine, its metabolites and phospholipid exchange in the striatum of mice with experimental Parkinson syndrome. Byulleteneksperimentalnoybiologiiimedistiny (Bulletin of Experimental Biology and Medicine, Russia), 1996; 12: 614-617 (in Russian)

[12] Stefanov A.V et al. U.A Patent C2 76393, 2006; Method for the production of liposomal quercetin-containing medication (in Ukrainian).

[13] Mironov A.N et al. Guidelines on preclinical trials of drugs. Part I, Moscow, Grif I K Publisher, 2012, 223, 278-280 (in Russian).

[14]Morris Kates. Techniques of Lipidology. Isolation, analysis and identification of lipids. Elsevier Publishing Co., Inc. New York, 1972, (translation into Russian, Moscow, Mir, 1975).

[15] Handloser D, Widmer V, Reich E. Separation of phospholipids by HPTLC – an

investigation of important parameters. Journal of Liquid Chromatography&Related Technologies 2008; 31:1857–1870.

[16] Methodical regulations in study on processes of free-radical oxidation and antioxidant support system. Assay for malondialdehyde in blood. Voronezh, 2010, P. 37-39 (in Russian).

[17] Belikova M.V, Pastukhova C.A, Luk'antseva G.V, Krasnova S.P. Lutsenko AV: Effect of regular normobaric hypoxia on dopamine in the striatum upon ageing. Galitskyi Medical Bulletin 2015; 3 (1):28-30 (in Russian).

[18] Rice-Evance C.A, Diplock A.T, Symons M.C. Techniques in free radical research. Elsevier, Amsterdam 1991.

[19] Kravchenko I. Routes of drug administration. Ukraine, Odessa, Astroprint Publisher, 2009 (in Russian).