The ARRIVE Guidelines Checklist

	ITEM	RECOMMENDATION	Reported on page
Title	1	Influence of 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1) on free-radical homeostasis in the heart and blood serum of rats with streptozotocin-induced hyperglycemia	
Abstract	2	Diabetes mellitus type 2 is a pandemic metabolic disease, which is becoming a serious problem for health protection in consequence of global increase of its prevalence. It is stated that in case of DM2 the delivery of fatty acids to the myocardium is intensified and glycolysis is slowed down. At the same time, the concentration of reactive oxygen species which have a damaging effect on the lipids of cardiomyocytes membranes and contribute to the mitochondrion mechanism malfunction and, as a result, to the inhibition of ATP elaboration, increases. Taking into account the most important factors of free radical oxidation activation in the diabetes mellitus type 2 pathogenesis, as well as in the development of its complications, antioxidant therapy should be among the modern medical endocrinopathy treatment technologies. In this context, there is an urgent need for effective substances that would protect cellular structures from oxidative stress. 10-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1) is an aromatic cation (triphenylphosphonium) conjugated with 10-12 atom aliphatic compound, as well as plastoquinone, which is an active molecular component of this substance. When used in nanoconcentrations, this substance participates in the reactive oxygen species balance regulation as it has the ability to neutralize free radicals, including OH radicals in aqueous solutions. This may protect cells from apoptosis and necrosis induced by reactive oxygen species. The goal of this study was to evaluate the influence of SkQ1 on biochemiluminescence parameters, which reflect the free radical processes reactions rate and the total activity of the antioxidant system, the level of primary lipid peroxidation products such as diene conjugates, the activity of aconitase, which is the sensitive target of free radicals action and citrate content, the degree of protein oxidative modification, the activity of superoxide dismutase and catalase, and the level of their genes' transcripts in heart and blood serum of the rats with hyperglycemia induced by the administration of streptozotocin. To conduct the study laboratory rats have been selected of nursery rat males from Federal State-financed Organization of Health Service "Voronezh Hygiene and Epidemiology Center". The animals have been divided through stratified randomization by their weight and age. The rats used for the study weighed 200-250 g and aged 3-5 months. They had been kept at 12-h light day, room temperature and access to water and food at libitum for 2 weeks before the study. All treatments of the experiment were consistent with the requirements of the European legislation on the protection of animals (Directive 2010/63/EU). Hyperglycemia was induced by keeping rats on high-fat diet during one month with following two intra-abdominal injections of streptozotocin with a seven-day interval at a 30 mg/kg of animal weight dose with citrate buffer equal to pH 4.4. SkQ1 solution was administered intraperitoneally at a 1250 nmol/kg dose per day. Tissue samples were taken from control animals, animals with experimental hyperglycemia, rats with streptozotocin-induced glycaemia which were administered with SkQ1 solution, animals housed under standard vivarium conditions, which were administered with SkQ1, rats, which were administered intraperitoneally of citrate buffer equal to pH 4.4 once a	

		week during two weeks after one-month high-fat diet, and animals, which were administered intraperitoneally with appropriate amount of solution without SkQ1 (98% ethanol diluted eight times with normal saline solution). To determine the intensity of free radical oxidation and total antioxidant activity, we used the biochemiluminescence method. Aconitate hydratase, superoxide dismutase, catalase activities were estimated using the Hitachi U-1900 spectrophotometer supplied with software. The amount of citrate was determined by means of the Natelson method. Real-time PCR was being carried out using an amplifier ANK-32. The results of this research allow suggesting that the mitochondria targeted antioxidant — SkQ1, might be considered as a perspective substance for incorporation into the antioxidant therapy of diabetes mellitus type 2. The experimentally revealed ability of this compound to lower the intensity of free-radical processes, acting as the key component of the pathogenesis of the diabetes mellitus type 2, may serve as the reason for this conclusion. Thus, after the introduction of SkQ1 — to the animals with streptozotocin induced hyperglycemia, the values of the biochemiluminescence parametres reflecting the free radical oxidation intensity, the concentration of diene conjugates and carbonyl products of protein oxidation, and the AH activity and citrate content changed towards the control values. At the same time, the activity level of the antioxidant enzyme — superoxide dismutase and catalase, changed to the direction of norm.	
INTRODUCTION			
Background	**3**	a. It is known that the inclusion of high fat content in rat diet contributes to the occurrence of animal tolerance to insulin [Chukanov GN, Dvoracka M, Iskakova SS, Kurmambayev EZh (2004) Modeling of Type 2 Diabetes Mellitus for the Study of Medicines with Antidiabetic Activity. Nauka i zdravookhraneniye 4: 4 – 8.]. Small dose administration of streptozotocin leads to the moderate decline in insulin production, which is similar to a later stage of diabetes mellitus type [Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P (2005) Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. In Pharmacol Res 52(4): 313-320. http://dx.doi.org/10.1016/j.phrs.2005.05.004.]. Thus, the administration of streptozotocin causes a significant basal increase in the blood glucose level. It also causes glucose tolerance, increase in the glycated hemoglobin level and significant reduction of insulin concentration and resistance. It should be noted that under conditions of tissue tolerance to insulin, observed during type 2 diabetes mellitus (DM2), there is an increase in superoxide production by mitochondria [Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414: 813-820. http://dx.doi.org/10.1038/414813a.], cytochrome P450, xanthine oxidase and also by protein kinase C-dependent NADPH oxidase activation. Moreover, the free radicals which are generated during glucose autoxidation or glycosylation end products can initiate lipid peroxidation in lipoprotein particles [Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Nawata H (2000) High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 49(11): 1939—1945.]. The concentration of lipid peroxidation products through an increased hydrophilic	

		hydrocarbon tails contents, in turn, can lead to formation of membrane pores and membrane stiffening through down regulation of unsaturated fatty acids, and thus it can influence state of insulin receptors, repressing their hormone connection activity which is expressed as a reduction of the glucose consumption by cells [Mikaelyan NP, Potemkin VV, Frantseva EYu, Kulaeva IO (2012). Funkcional'noe sostoyanie membrano-receptornogo apparata kletok krovi pri vpervye vyyavlennom saharnom diabete tipa 2 [Functional state of the membrane-receptor apparatus of blood cells with newly diagnosed type 2 diabetes mellitus]. Problemy endokrinologii 4(2): 40-41.]. It is possible that reduction in glucose concentration during administration of SkQ1 to rats with DM2 may be related to the realization of antioxidant properties of this substance [Voronkova YG, Popova TN, Agarkov AA, Skulachev MV (2015) Effects of 10-(6'-plastoquinol)-decyltriphenylphosphonium (SkQ1) on oxidative status in rats with protamine sulfate-induced hyperglycemia. Biochemistry (Moscow) 80 (12): 1871-1879. http://dx.doi.org/10.1134/S0006297915120093.]. b. The use of animals for scientific purposes is both a longstanding practice in biological research. Anatomical and physiological similarities between humans and animals, particularly mammals, have prompted researchers to investigate a large range of mechanisms and assess novel therapies in animal models before applying their discoveries to humans. The human diseases often affect other animal species. It is particularly the case for Type 2 diabetes mellitus, hypertension, allergies, cancer, epilepsy, myopathies and so on.	
Objectives	4	The goal of this study was to evaluate the influence of SkQ1 on biochemiluminescence parameters, which reflect the free radical processes reactions rate and the total activity of the antioxidant system, the level of primary lipid peroxidation products such as diene conjugates, the activity of aconitase, which is the sensitive target of free radicals action and citrate content, the degree of protein oxidative modification, the activity of superoxide dismutase and catalase, and the level of their genes' transcripts in heart and blood serum of the rats with hyperglycemia induced by the administration of streptozotocin.	
METHODS			
Ethical statement	5	All treatments of the experiment were consistent with the requirements of the European legislation on the protection of animals (Directive 2010/63/EU)	
Study design	6	The laboratory rats were divided into four groups: group 1 (n=20) were animals housed under standard vivarium conditions (control group); group 2 (n=20) were animals with STZ injection-induced hyperglycemia; group 3 (n=12) were animals with streptozotocin-induced glycaemia which were administered with SkQ1 solution intraperitoneally at a 1250 nmol/kg dose per day, starting from the second week; group 4 (n=8) were animals housed under standard vivarium conditions, which were administered with SkQ1 at a 1250 nmol/kg dose per day, during the second week of conducting the experiment. Group 2 also included rats (n=8), which were administered	

		intraperitoneally with appropriate aliquot quantity of citrate buffer equal to pH 4.4 once a week during two weeks after one-month high-fat diet. Group 3 included animals (n=8), which were administered intraperitoneally with appropriate amount of solution without SkQ1 (98% ethanol diluted eight times with normal saline solution). The experimental unit - a single animal.	
Experimental	7	Rats had been kept in laboratory. The animals have been divided through stratified randomization by their weight and age. Hyperglycemia was induced by keeping rats on high-fat diet during one month. Streptozotocin was administered intra-abdominal at a 30 mg/kg of animal weight dose with citrate buffer equal to pH 4.4. The fact of rats being ill with hyperglycemia was verified by measuring the glucose level in the blood serum with usage of glucose oxidase test. SkQ1 solution was administered intraperitoneally at a 1250 nmol/kg dose per day. Appropriate aliquot quantity of citrate buffer equal to pH 4.4 was administered intraperitoneally.	
Experimental animals	8	To conduct the study laboratory rats have been selected of nursery rat males from Federal State-financed Organization of Health Service “Voronezh Hygiene and Epidemiology Center”. The rats used for the study weighed 200-250 g and aged 3-5 months.	
Housing and husbandry	9	Rats had been kept at 12-h light day, room temperature and access to water and food at libitum for 2 weeks before the study and further. Type of housing - plastic Bedding material - sawdust Number of cage companions - 2 rats per cage	
Sample size	10	The total number of animals used in experiment – 76 rats The number of animals in each experimental group: group 1 (n=20+8), group 2 (n=20+8), group 3 (n=12), group 4 (n=8). The number of animals was necessary for obtaining statistically significant results	
Allocating animals experimental groups	11	The animals have been divided through stratified randomization by their weight and age. The rats used for the study weighed 200-250 g and aged 3-5 months. The order in which the animals in the different experimental groups were treated and assessed: group 2 compared with group 1, group 3 compared with group 2, group 4 compared with group 1.	
Experimental outcomes	12	Weight gain, increased water intake, slowness in rats of the 2nd group. SkQ1 administration lowered glycemic level by 2.5 times, which was initially upregulated 2.7 times relative to the control. The glucose level of the animals of the 4th experimental group was	

		not significantly different from the stated value. Moreover, glucose concentration was within the stated value in rats of the second experimental group, having been administered citrate buffer and of the third group, having been administered aliquot of 12% ethanol.	
Statistical analysis	13	The unit of analysis - group of animals. Experiments were done at least in 8-20 biological and 2 analytical replicates. The results were compared with the control. The data were statistically analyzed a software package STATISTICA 6.0 with numerical variables – arithmetical mean (M), mean error (m) and statistical significance level (p). Normal distribution data were compared by applying Student's t-test for Bonferroni correction in independent samples. Significance level was set at $aP \leq 0.0167$ and $bP \leq 0.0167$.	
RESULTS			
Baseline data	14	The rats used for the study weighed 200-250 g. The rats used for the study weighed 200-250 g. group 1 - control rats with normal blood glucose levels. group 2 - glucose concentration upregulated 2.7 times relative to the control. group 3 - lowered glycemic level by 2.5 times relative to the control.	
Numbers analysed	15	The absolute number of animals in each experimental group: group 1 (28/28), group 2 (n=30/28), 2 rats died during the posing of the model group 3 (n=14/12), 2 rats died during the posing of the model group 4 (n=8/8).	
Outcomes and estimation	16	Significance level was set at $aP \leq 0.0167$ and $bP \leq 0.0167$.	
Adverse events	17	No modifications to the experimental protocols made to reduce adverse events.	
DISCUSSION			
Interpretation/ scientific implications	18	The results of this research allow suggesting that the mitochondria targeted antioxidants, for example SkQ1, might be considered as a perspective substances for incorporation into the antioxidant therapy of type 2 diabetes mellitus. The experimentally revealed ability of this compound to lower the intensity of free-radical processes, acting as the key component of the pathogenesis of the type 2 diabetes mellitus, may serve as the reason for this conclusion. Thus, after the introduction of SkQ1 — to the animals with streptozotocin induced hyperglycemia, the values of the biochemiluminescence parametres reflecting the free radical oxidation intensity, the concentration of diene conjugates and carbonyl products of protein oxidation, and the aconitate hydratase activity and citrate content changed towards the control values. At the same time, the activity level of the antioxidant enzyme — superoxide dismutase and catalase, changed to the direction of norm.	
Generalisability/ translation	19	The effects of SkQ1 on the stage of compensatory response occurrence in pathology may contribute to decrease of the degree of oxidative stress, normalization of antioxidant system functioning and blocking of the development of decompensation, characterized by the inhibition of protective	

		systems.	
Funding	20	This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.	