Research Article

Protective effects of incretin mimetics in cardiomyopathy induced by doxorubicin

Alla P. Tarasova[1]*, Tatyana G. Pokrovskaya[1], Alexander V. Faitelson[2], Yury A. Khoshchenko[1], Alena S. Timokhina[1], Alla S. Kotelnikova[1], Anatoly V. Khavansky[1]

ABSTRACT

Introduction: The results of experimental and clinical trials make it clear that incretin mimetics possess pleiotropic effects and demonstrate the value in terms of assessment of their potential opportunities as cardioprotectors. **Research Tasks:** The aim is to study the cardioprotective effects of exenatide and vildagliptin on the model of doxorubicin-induced cardiomyopathy. **Materials and Methods:** The experiments on the Langendorff isolated rat heart were dedicated to the study of cardioprotective activity of exenatide (10 mcg/kg/day) ("Byetta®," Eli Lilly and Company, USA) and vildagliptin (0.2 mg/kg/day) ("Galvus®," Novartis, Switzerland), on the contractile function of the isolated heart which was previously perfused with doxorubicin (20 mg/kg, intraperitoneally before 48 h). The evaluation of cardioprotective activity was based on the findings of the functional trial with high-frequency stimulation (480 bmp) in hypercalcemia (5 mmol) perfusion. The complex evaluation of the myocardial damage in the flowing perfusate from isolated hearts included the assessment of creatine phosphokinase muscle brain isoenzyme (CPK-MB) and lactic dehydrogenase (LDH). The activity of lipid peroxidation (LPO) was evaluated by measuring the content of malondialdehyde (MDA) and diethenoid conjugant (DC). **Results:** Exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) demonstrate a cardioprotective effect on the model of doxorubicin-induced pathology, resulting in a decrease of diastolic dysfunction to 5.3 ± 0.1 units and 6.5 ± 0.2 units, respectively, compared to control 8.3 ± 0.1 units. The cardioprotective effect was confirmed by 27% and 19% decrease in the levels of CPK-MB marker damage, and by 11.8% and 9.6% decrease in LDH levels, respectively, in exenatide and vildagliptin series, compared to control. The cardioprotective effect was also confirmed by prevention of accumulation of LPO products of MDA and DC in the ventricular myocardium. **Conclusion:** Exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) decrease diastolic dysfunction, resulting in the recovery of the contractile function of the heart, reduction of the "diastole defect" (S_{tTTI}), and the decrease in irreversible damages of cardiomyocytes.

KEY WORDS: Doxorubicin-induced cardiomyopathy, Exenatide, Incretin mimetics, Isolated rat heart, Vildagliptin

INTRODUCTION

New substances with cardiotropic effects are being identified among various classes of chemical and pharmacological groups.[1-8] Undoubted interest is represented by incretin mimetics, as a fairly new group of hypoglycemic agents with pleiotropic effects, in particular cardioprotective.[9-12] The exact mechanisms underlying the effect of glucagon-like peptide-1 (GLP)-1 on the cardiac muscle have not yet been established.[13-15] It is suggested that GLP-1 can positively influence the apoptosis of cardiomyocytes, oxidative stress, and endogenous antioxidant defense mechanisms, having a beneficial effect on cardioprotection of the myocardium.[16-19]

The formation of free radicals leads to an increase in oxidative stress, the triggering of apoptosis-mediated through iron ions calcium, nitric oxide (NO)-oxidase, glutathione peroxidase, neuregulin-1, protein kinase B, growth factors, cytokines, and their receptors, which may be a direct cause of doxorubicin-induced cardiomyopathy. The theory of oxidative stress in the development of doxorubicin-induced cardiomyopathy is the most popular and often serves as an experimental model for studying the causes of apoptotic cell death and the selection of means for cardioprotection.[20,21]

Access this article online

Website: jprsolutions.info | ISSN: 0974-6943

[1]Department of Pharmacology, Medical Institute, Belgorod State National Research University, Belgorod, Russia,
[2]Department of Pharmacology, Kursk State Medical University, Kursk, Russia

***Corresponding author:** Alla P. Tarasova, Postgraduate Student, Department of Pharmacology, Medical Institute, Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia. E-mail: Tarasova_ap@mail.ru

Received on: 20-09-2017; Revised on: 22-10-2017; Accepted on: 27-11-2017

MATERIALS AND METHODS

The experiments were carried out on 40 mature Wistar rats of both sexes weighing 220 ± 20 g. All animal manipulations were carried out in compliance with the "European Convention for the Protection of Vertebrates used for Experiment or Other Scientific Purposes" (directive 2010/63/EU). All experiments were approved by the local Ethics Committee (Minutes No. 12-2016 of November 21, 2016).

All rats were divided into four experimental groups of 10 animals. The first group (n = 10), control, was intraperitoneally injected a saline solution. The second group (n = 10) was intraperitoneally injected with doxorubicin (Teva) at a cumulative dose of 20 mg/kg, once, the third (n = 10) - doxorubicin and intraperitoneal vildagliptin ("Galvus®," Novartis, Switzerland) at a dose of 0.2 mg/kg/day. The fourth (n = 10) - doxorubicin and exenatide ("Byetta®," Eli Lilly and Company, USA) subcutaneously once a day at a dosage of 10 mcg/kg/day. The doses of the drugs were calculated taking into account the coefficient of interspecific transfer of doses from the human body to the body of a rat.

The animals were withdrawn from the experiment after 48 h. The hearts were removed from the animals under zolestal anesthesia (30 mg/kg) and placed in an icy (2–4°C) Krebs-Henseleit solution of the following composition (mmol): NaCl-118.5; KCl-4.7; MgSO4/7H2O-1.2; KH2PO4-1.2; CaCl2-1.5; Glucose-11.1; NaHCO3-25.0. The pH level of the solution throughout the experiment was 7.4. After the termination of spontaneous contractions, the aorta was isolated, and the connective tissue was separated. The aorta was then cannulated, and retrograde perfusion of the heart was performed using the Langendorff method in a flow perfusion mode for 20 min with the Krebs-Henselite solution, saturated with carbogen (95% O2 + 5% CO2) at 37°C and 100 mmHg pressure and perfusate speed 10 ml/min.

The cardiac contractility was recorded with a latex balloon inserted into the left ventricle cavity and connected to a pressure sensor built into the device for physiological studies of the MP150 of Biopac Systems, Inc. (California, USA). The can was filled with distilled water, the volume of which was sufficient to create a diastolic pressure in the left ventricle at a level of 3–5 mm Hg. With the help of the original AcqKnowledge application of the company "Biopac Systems, Inc." (California, USA), the contractility indices in the rats were recorded: Left ventricular pressure (mmHg), heart rate (bpm), the maximum rate of contraction ($+dp/dt_{max}$, mmHg/s), and the maximum relaxation rate of the myocardium ($-dp/dt_{max}$, mmHg/s). To create a high frequency (480 bpm) a connector ground of an electric stimulator was attached to the metallized cannula and a connector plus was attached to the eye of the left atrium. After 20 min of perfusion with a high Ca^{2+} (5 mmol/L) solution, the heart was stimulated with electrical pulses using the stochastic threshold model 200-1 device from Biopac Systems, Inc. (California, USA) for 15 s.

To assess the functionality of the myocardium, the diastolic dysfunction ratio or "diastole defect" (S_{tTTI}) calculated from the intraventricular pressure curve was used. The area under the curve was calculated by folding the trapezium areas, which is equal to the product of its height on the middle line. The "diastole defect" (S_{tTTI}) was expressed in units. The cardioprotective effects of the drugs vildagliptin and exenatide were judged by their effect on the S_{tTTI} index.[22] The damage markers and the level of peroxidation were evaluated by conventional methods.[23,24]

The reliability of changes in absolute parameters was determined by the difference method of variational statistics with finding the mean values of the shifts, the mean of the arithmetic mean, and the probability of possible error (p) from Student's tables. Differences between the values of the indices were considered statistically significant at $P < 0.005$.

RESULTS AND DISCUSSION

Doxorubicin cardiomyopathy was characterized by a decrease in myocardial contractility [Table 1]. Conducting a functional test with high-frequency

Table 1: Effects of incretin mimetics exenatide and vildagliptin on indices of contractile function of the heart of rats with doxorubicin cardiomyopathy (M±m; *n*=10)

Groups of animals	LVP	$+dp/dt_{max}$	$-dp/dt_{max}$	HR
Intact animals	87.3±9.2*	1423±162.2*	−1265.2±173.2*	248±32.1
Doxorubicin control	64.5±11.2**	1025.7±154.3**	−1031.1±159.4**	247±29.4
Doxorubicin+exenatide (1 mcg/kg/day)	60.2±9.4**	1165.7±134.3**	−1119.9±119.4**	232±29.4
Doxorubicin+exenatide (10 mcg/kg/day)	76.8±7.4*	1302±169.2*	−1157.4±137.3*	231±26.9
Doxorubicin+vildagliptin 0.02 mg/kg/day	59.1±10.7**	1107.7±154.3**	−984.9±129.1**	227±29.4
Doxorubicin+vildagliptin 0.2 mg/kg/day	73.2±5.1*	1219±145.4*	−1108±169.3*	232±36.1

LVP: Left ventricular pressure (mmHg), $+dp/dt_{max}$: Maximum contraction rate (mmHg/s), $-dp/dt_{max}$: Maximum relaxation rate (mmHg/s), HR: Heart rate (bpm). Doxorubicin was administered intraperitoneally 48 h before the experiment. The incretin mimetics - exenatide and vildagliptin - were administered twice at an interval of 24 h, respectively, intramuscularly and intrahepatically. **P<0.005 in comparison with the group of intact animals*P<0.005 in comparison with the control group

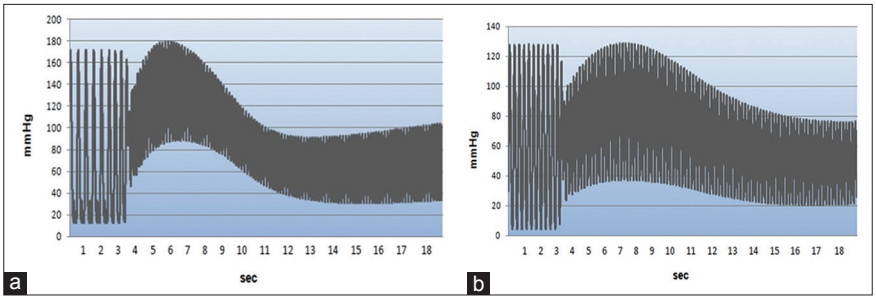

Figure 1: Exercise tolerance test under submaximal electrical stimulation of a rat heart isolated by Langendorff Ca^{2+} concentration in perfusate-5 mmol/L, intact group. Pressure profile in the left ventricle (mmHg) at imposing quickened heartbeat (480 bpm) within 15 s. Ca^{2+} concentration in perfusate-5 mmol/L. Doxorubicin (20 mg/kg) given at a single dose within 48 h (a), intact group (b)

stimulation revealed the development of the "diastole defect" [Figure 1a and b], and S_{tTTI} increased to 8.3 ± 0.3 units in comparison with intact animals-1.4 ± 0.1 units, in other words, it increased 8 times.

The incretin mimetics of exenatide in doses (1.0 mg/kg/day and 10 mcg/kg/day) and vildagliptin (0.02 and 0.2 mg/kg/day) did not affect the degree of decrease in contractility rates (as reflected in the table) and dose-dependently prevented a decrease in contractility when carrying out the test with high-frequency stimulation. Thereat, S_{tTTI} for exenatide 10 mcg/kg/day and vildagliptin 0.2 mg/kg/day were 5.3 ± 0.1 and 16.5 ± 0.2 units, respectively.

The ability of exenatide and vildagliptin to prevent damage to cell membranes was assessed by the change in the activity of creatine phosphokinase muscle brain (CPK-MB) and lactic dehydrogenase (LDH) in the perfusate during the reperfusion period [Figures 2 and 3].

Exenatide and vildagliptin contributed to a decrease in CPK-MB content by 27% and 19%, and in LDH-11.8% and 9.6% compared to the control group.

Similar changes were observed in the markers of products of lipid peroxidation [Figures 4 and 5].

The formation of a highly active hydroxyl radical in the Haber-Weiss reaction involving superoxide dismutase and ferrous ions is one of the presumed causes of doxorubicin cardiomyopathy.[25] By influencing the exchange of iron: Anthracyclines bind to Fe^{2+} ions, which leads to the formation of a hydroxyl radical, and promotes the release of Fe^{2+} ions from ferritin, further exacerbating oxidative stress.[26]

Therefore, if the conditions for chelation or oxidation of ferrous ions Fe^{2+} in the catalytically inactive state of Fe^{3+} ions arise in the cytoplasm of cells, this will create the conditions for achieving micromolar concentrations of reactive oxygen species in the cytoplasm of cells and reducing the damage to cardiomyocytes.[27] From the pharmacological point

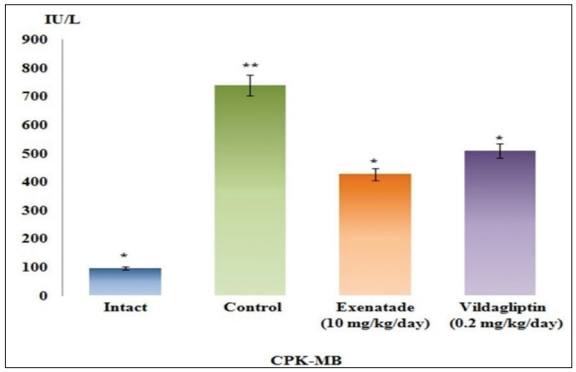

Figure 2: Content of creatine phosphokinase in the perfusate in groups under exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) on the background of doxorubicin cardiomyopathy. *$P < 0.05$ in comparison with the control. **$P < 0.05$ in comparison with the group of intact animals

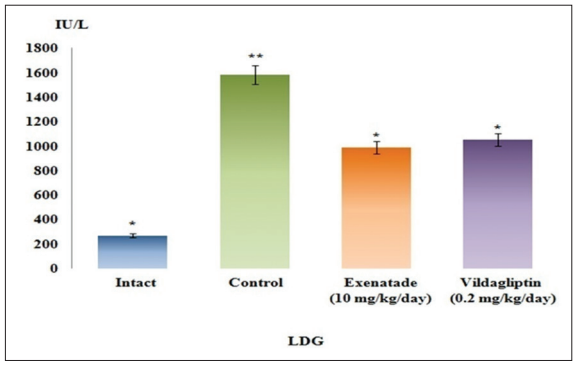

Figure 3: Content of lactate dehydrogenase in the groups under exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) on the background of doxorubicin cardiomyopathy. *$P < 0.05$ in comparison with the control. **$P < 0.05$ in comparison with the group of intact animals

of view, antioxidants are of interest as one of the promising groups of cardioprotective drugs that allow to preserve a viable myocardium, limit the size of damage and accelerate the restoration of contractile activity of the myocardium.[28,29]

In incretins, the presence of one of the ways of realization of cardioprotective effect is described the amplification of expression of heme-oxygenase-1

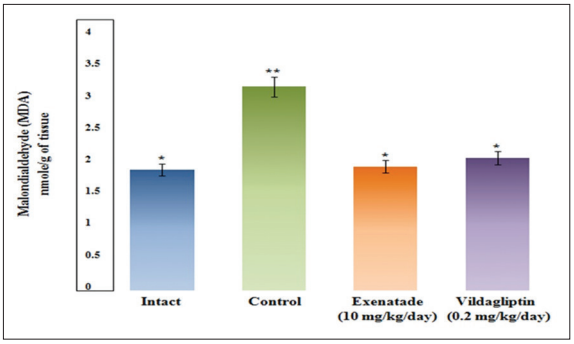

Figure 4: Content of malonic dialdehyde in the groups under exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) on the background of doxorubicin cardiomyopathy. **P* < 0.05 in comparison with the control. ***P* < 0.05 in comparison with the group of intact animals

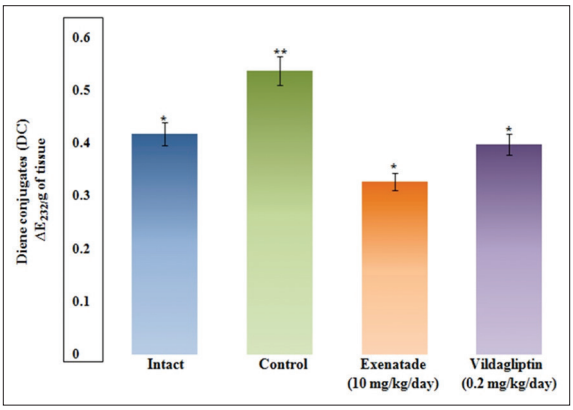

Figure 5: Content of diene conjugates in the groups under exenatide (10 mcg/kg/day) and vildagliptin (0.2 mg/kg/day) on the background of doxorubicin cardiomyopathy. **P* < 0.05 in comparison with the control. ***P* < 0.05 in comparison with the group of intact animals

(HO-1).[30] This enzyme prevents the heme-catalyzed formation of highly active hydroxyl radicals from hydrogen peroxide. Activation of HO-1 is associated with increased heme catabolism to bile pigments, which are potential endogenous antioxidants. In addition, induction of HO-1 is accompanied by an increase in ferritin activity, which has an antiapoptotic effect.[31] The enhancement of expression of HO-1 under conditions of oxidative stress can play an adaptive role in response to oxidative damage and diminish the death of cardiomyocytes.

The experimental studies demonstrate that when doxorubicin-induced cardiomyopathy is modeled in transgenic mice and animals with overexpression of HO-1, cardiac-specific hyperexpression of HO-1 prevents doxorubicin-mediated damage of sarcoplasmic reticulum and mitochondria in autophagic vacuoles.[32] Overexpression of HO-1 promotes mitochondrial biogenesis by enhancing the expression of the protein of the nuclear respiratory factor, the coactivator (PGC1α) and the mitochondrial transcription factor (TFAM) that are inhibited in transgenic animals with doxorubicin-induced cardiomyopathy. Simultaneously, overexpression of HO-1 inhibits the enhancement of the mitochondrial fusion mediator (Fis 1) and leads to an increase in the expression of the mediators of the synthesis of Mfn 1 and Mfn 2. This also prevents mutations in key mitochondria genes PINK 1 and PARKIN and ensures their normal functioning. This proves that NO-1 plays an important role in protecting the heart from oxidative damage by affecting mitochondria.[33] It can be surmised from the above that the mechanisms of antioxidant defense expression may take part in the mechanism of the protective action of the incretin mimetics in doxorubicin cardiomyopathy.

REFERENCES

1. Skachilova SY, Kesarev OG, Danilenko LM, Bystrova NA, Dolzhikov AA, Nikolaev SB. Pharmacological correction of L-NAME-induced oxide deficiency with derivatives of 3-(2,2,2-trimethylhydrazinium) propionate. Res Result Pharmacol Clin Pharmacol 2016;2:36-41.
2. Chernomortseva ES, Pokrovskiĭ MV, Pokrovskaia TG, Artiushkova EB, Gureev VV. Experimental study of cardioprotective and endothelioprotective action of macrolides and azalides. Eksp Klin Farmakol 2009;72:29-31.
3. Kochkarov VI, Molchanova OV, Pokrovskiy MV, Yakushev VI, Gudyrev OS. Endothelium-protective action of thioctic acid and rosuvastatin combination at concomitant hypoestrogen and L-Name-induced deficit of nitric oxide. Res J Pharm Biol Chem Sci 2014;5:1054-7.
4. Gumanova NG, Metel'skaya VA, Artyushkova EB, Kochkarov VI, Pokrovskaya TG, Danilenko LM, *et al.* Effect of antioxidants pQ510 and resveratrol on regulatory function of the endothelium in rats with modeled arterial hypertension. Bull Exp Biol Med 2007;143. Available from: http://www.elibrary.ru/contents.asp?issueid=436328&selid=9520932:619-22. [Last accessed on 2017 Mar 15].
5. Korokin MV, Pashin EN, Bobrakov KE, Pokrovskiy MV, Ragulina AV, Artjushkova EB, *et al.* Studying endothelioprotection and coronary action of derivatives 3-oksipiridin. Kuban Res Med Bull 2009;4:104-8.
6. Danilenko LM, Pokrovskiy MV. 3-(2,2,2-trimethylhydrazinium) propionate: New concept of realization of cardioprotective effect. Res J Pharm Biol Chem Sci 2014;5:1419-22.
7. Tsepeleva SA, Pokrovsky MV, Pokrovskaya TG, Korokin MV, Denisyuk TA, Kotelnikova LV, *et al.* Cardio- and endotelioprotective effects of arginase inhibitor L-norvalin at modelling L-NAME indused deficiency of nitric oxide. Kuban Res Med Bull 2011;4:185-8.
8. Danilenko LM, Pokrovskiy MV, Korokin MV, Gudyrev OS. Studyo fth emechanismsof cardioprotective effectof 3- (2,2,2-trimethylhydrazinium) propionate. Kuban Res Med Bull 2016;1:24-6.
9. Vlasov TD, Simanenkova AV, Dora SV, Shlyakhto EV. Mechanisms of neuroprotective action of incretinomimetics. Cardiol Diabetes 2016;19:16-23.
10. Trunina EN, Petunina NA, Chorbinskaya SA. Inhibitors of dipeptidylpeptidase-4 in the treatment of Type 2 diabetesmellitus. Possibilities of cardioprotection. Cardiol Diabetes 2011;2:59-64.
11. Tuchina TP, Zykov VA, Yu BA, Krylova IB, Lebedev DA. Evaluation of the cardioprotective effect of the preparation of glucagon-like peptide-1 in the experiment. Mod Med Top Issues 2014;37:11-19.
12. Tyurenkov IN, Bakulin DA, Kurkin DV, Volotova EV. Cardiovascular effects of incretinomimetics and their

therapeutic potential. Bull Russ Acad Med Sci 2011;72:66-75.
13. Spasov AA, Cheplyaeva NI. Potential for pharmacological modulation of the level and activity of incretinsin Type 2 diabetes mellitus. Biomed Chem 2015;61:488-96.
14. Liu Q, Anderson C, Broyde A, Polizzi C, Fernandez R, Baron A, Parkes DG. Glucagon-like peptide-1 and the exenatide analogue AC3174 improve cardiac function, cardiac remodeling, and survival in rats with chronic heart failure. Cardiovasc Diabetol 2010;9:324-33.
15. Luconi M, Cantini G, Ceriello A, Mannucci E. Perspectives on cardiovascular effects of incretin-based drugs: From bedside to bench, return trip. Int J Cardiol 2017;117:341-3.
16. Hull TD, Boddu R, Guo L, Tisher CC, Traylor AM, Patel B, *et al*. Heme oxygenase-1 regulates mitochondrial quality control in the heart. Cardiology 2016;1:378-83.
17. Lonborg J, Vejlstrup N, Kelbaek H, Botker WY, Mathiasen B, Jorgensen E, *et al*. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. Eur Heart J 2012;33:1491-9.
18. Nikolaidis LA, Hentosz T, Doverspike A, Huerbin R, Zourelias L, Stolarski C, *et al*. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. J Pharmacol Exp Ther 2005;39:303-8.
19. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, *et al*. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. Circulation 2004;285:955-61.
20. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad SV. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J Clin Invest 2014;124:617-30.
21. Kuznetsov AV, Margreiter R, Amberger A, Saks V, Grimm M. Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death. Biochim Biophys Acta 2011;1813:1144-52.
22. Kesarev OG, Danilenko LM, Pokrovskiy MV,Timokhina AS, Khovanskii AV. Study ofdose-dependent effect of 2-ethyl-6-methyl-3 hydroxypyridine succinate on the contractile function of isolated rat heat. Res Result Pharmacol Clin Pharmacol 2017;3:3-9.
23. Hrdina R, Gersl V, Klimtova I, Simunek T, Machacková J, Adamcova M. Anthracycline-induced cardiotoxicity. Acta Med (Hradec Kralove) 2000;43:75-82.
24. Wu ML, Ho YC, Yet SF. A central role of heme oxygenase-1 in cardiovascular protection. Antioxid Redox Signal 2011;15:1835-46.
25. Fogli S, Nieri S, Breschi MC. The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage. Faseb J 2004;18:664-75.
26. Corna G, Santambrogio P, Minotti G, Cairo G. Doxorubicin paradoxically protects cardiomyocytes against iron-mediated toxicity: Role of reactive oxygen species and ferritin. J Biol Chem 2004;279:13738-745.
27. Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (Adriamycin): A critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol Ther 2000;47:219-31.
28. Skachilova SY, Danilenko LM, Kesarev OG, Kochkarova IS. Pharmacological protection of the ischemic myocardium by derivatives of 3-(2,2,2-trimethylhydrazinium) propionate and evaluation of their antioxidant activity. Res Result Pharmacol Clin Pharmacol 2015;1:23-7.
29. Danilenko LM, Klochkova GN, Kizilova IV, Korokin MV. Metabolic cardioprotection: New concepts in implementation of cardioprotective effects of meldonium. Res Result Pharmacol Clin Pharmacol 2016;2:95-100.
30. Vavrova A, Popelová O, Sterba M, Jirkovsky E, Haskova P, Mertlíkova-Kaiserova H, *et al*. *In vivo* and *in vitro* assessment of the role of glutathione antioxidant system in anthracycline-induced cardiotoxicity. Arch Toxicol 2011;85:525-35.
31. Vivenza D, Feola M, Garrone O, Monteverde M, Merlano M, Nigro CL. Role of the renin-angiotensin-aldosterone system and the glutathione S-transferase Mu, Pi and Theta gene polymorphisms in cardiotoxicity after anthracycline chemotherapy for breast carcinoma. Int J Biol Markers 2013;28:336-47.
32. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, *et al*. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes 2009;58:975-83.
33. Vives-Bauza C. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A 2010;107:378-83.