http://rjdnmd.org
Rom J Diabetes Nutr Metab Dis. **26**(4):393-399
doi: 10.2478/rjdnmd-2019-0042

THE QUERCETINE CONTAINING DRUGS IN PHARMACOLOGICAL CORRECTION OF EXPERIMENTAL DIABETES WITH MYOCARDIAL INJURY

Iryna Stechyshyn ✉***, Bohdana Pavliuk***

Department of Pharmacy Management, Economics and Technology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

received: November 09, 2019 *accepted*: December 14, 2019
available online: February 01, 2020

Abstract

Background and aims: *The prevalence of diabetes mellitus is becoming an epidemic. The diversity of etiological factors contributes to the fact that both types of diabetes mellitus occur among different age groups and among different segments of the population. Activation of various types of processes that lead to oxidative stress, endothelial dysfunction, the development of atherosclerotic changes, increases the risk of macro- and microvascular complications in diabetes mellitus.* ***Material and methods****: The experiments were performed on 172 white Wistar rats weighing 120–150 g. All animals were kept on a standard I. Horbachevsky Ternopil National Medical University (TNMU), vivarium diet. In our studies, for the first time was established a distinct cardioprotective effect of quercetin-containing drugs, Corvitin and Lipoflavon, for the purpose of normalizing disorders in type 1 and type 2 diabetes mellitus coupled with obesity, which opens wide opportunities for the introduction of these drugs into diabetic cardiomyopathy.* ***Results:*** *The results obtained are an experimental rationale for extending the indications of clinical use of Corvitin and Lipoflavon.* ***Conclusion:*** *Lipoflavon in the experimental type 1 and type 2 diabetes mellitus coupled with obesity significantly outperforms Corvitin in terms of TBA-active products and diene conjugates.*

key words: *antioxydants, obesity, hard disease, metabolic syndrome*

Background and aims

Diabetes mellitus, by definition of the World Health Organization, has acquired the character of a non-communicable epidemic, and its share in the structure of endocrine diseases reaches about 31.9 %. At the same time, almost 85-90 % are in patients with type 2 diabetes [1]. According to the World Diabetes Federation forecasts, the number of patients with diabetes will increase from 415 million people in 2015 to 642 million in 2040 [2]. The leading group of complications and the main cause of death in patients with diabetes is cardiovascular pathology, accounting for 80 % of all causes of death in such patients [3]. In particular, in type 1 diabetes chronic heart failure takes third place in patients with fatality, and fifth - acute myocardial infarction [3]. In patients with type 2 diabetes, death due to cardiovascular disease is in the first place (80 % of all cases): 65 % die from coronary heart disease, 15 % - from a

✉ Telephone: +38096-754-56-22. *corresponding author e-mail*: stechyshyn@tdmu.edu.ua

stroke [3]. In Ukraine, the main place in the mortality structure of patients with diabetes are occupying by myocardial infarction (55 %) and stroke (29 %), which is 70 times higher than the mortality rate from microvascular complications and diabetic coma (1-4 %) [4]. Diabetic cardiomyopathy is one of the leading forms of cardiac damage in diabetes, the main pathogenetic factor of which considering to be metabolic and structural disorders in the heart muscle, including through the discoordination of redox processes, the development of tissue hypoxia and to the activation of oxidative stress.

Given the high medical and social importance, one of the leading tasks of modern diabetes is to find new effective methods of correction diabetic heart disease. To prevent damage caused by oxidative stress, the use of drugs with antioxidant activity are warranting in diabetes treatment [5]. Quercetin is one of the modern remedies that have proven to be highly effective in a variety of pathological processes, including cardiovascular disease. The original domestic preparations of this flavonoid - water-soluble (Corvitin) and liposomal (Lipoflavon) - have been studied quite deeply in this research [6,7]. They have therapeutic effect in ischemic heart disease, including acute myocardial infarction, hypertension, and chronic heart failure [8]. Cardioprotective activity of quercetin-containing drugs are providing by their antioxidant, membrane-protective, angio-protective, neuro- and endocrine-modulating action, and ability to increase adaptation to hypoxia and stimulate synthesis of nitric oxide in the tissue of the heart [9].

However, information on the use and therapeutic activity of quercetin-containing drugs in cardiomyopathy, which develops in diabetes of various types, is limited. Therefore, it is promising to study the comparative effectiveness of water-soluble and liposomal drugs based on this flavonoid in heart damage, which develops in experimental type 1 and type 2 diabetes mellitus (DM) coupled with obesity, and their effects on metabolic disorders, including carbohydrate and lipid metabolism, in this pathology.

Materials and Methods

The experiments were performed on 172 white Wistar rats weighing 120–150 g. All animals were kept on a standard I. Horbachevsky Ternopil National Medical University (TNMU), vivarium diet. The studies were carried out in accordance with national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine № 3447-IV, 2006) and in accordance with the requirements of the Bioethics Commission of TNMU (Protocol, No. 29 of May 20, 2015).

All experimental animals were divided into groups: 1 and 5 - control; 2 and 6 are animals with diabetes; 3 and 7 - animals with diabetes receiving a water-soluble form of quercetin – “Corvitin” (BHFZ, Ukraine); 4 and 8 - animals with diabetes receiving liposomal preparation of quercetin - "Lipoflavon" (Biolik, PAT, Ukraine).

To simulate diabetes of both types, streptozotocin (STZ, “Sigma-Aldrich”, USA) was used, which was dissolved ex tempore and injected intraperitoneally on citrate buffer (pH 4.5). Type 1 DM in animals 2, 3 and 4 groups were caused by a single injection of STZ (50 mg/kg mass) [9]. To model type 2 DM coupled with obesity, which was caused by a 4-week high-energy diet [10] in 6, 7 and 8 groups of animals was conducted by a single STZ (30 mg/kg mass) injection [11]. An appropriate amount of citrate buffer (*p*H 4.5) was injected to the control animal groups. The administration of Corvitin and Lipoflavon to rats of 3 and 4 groups (with type 1 DM) was started 2 weeks after STZ

injection, animals of 7 and 8 groups (with type 2 DM) 10 weeks after STZ injection. Water-soluble and liposomal drugs were used at doses of 100 and 370 mg/kg, respectively (10 mg/kg in terms of quercetin), intraperitoneally, daily and for 14 days.

The development of diabetes confirmed by determining blood glucose and glycosylated hemoglobin (HbA1C) which presented in previous studies [12]. Also, serum determined concentration of TBA-active products (TBA) and catalase (Cat). In the heart was analyzed the activity of lipid peroxidation (LPO), lipid hydro peroxides (LHPO), TBA-active products and diene conjugates (DC), antioxidant system - by the activity of superoxide dismutase (SOD) catalase and the content of reduced glutathione (RG). The activity of succinate dehydrogenase (SDG) and cytochrome oxidase (CChO) were determined for the analysis of oxidative processes in the mitochondria of the heart. The total lipid composition of the blood was determined by the content of total cholesterol (CTC) (using the Liquck Cor-CHOL set, Cormay, Poland), triglycerides (TG) (using the Liquck Cor-CHOL set, Cormay, Poland), low-density lipoproteins (LDL) (using HC-LDL DIRECT kit, Cormay, Poland) and high-density lipoproteins (HDL) (using HC-LDL DIRECT kit, Cormay, Poland). The determination of leptin level (by standard DRG Leptin reagent kit, Sandwich ELISA, Germany) in the blood.

Statistical processing of the results was performed using IBM SPSS Statistica v.10.1 programs and Microsoft Excel XP. The obtained values by using the Student's t-test were compared and the non-parametric Mann-Whitney U-test. The difference between the studied parameters was considered statistically significant at $p \leq 0.05$.

Results

Our previous studies found significant changes in carbohydrate metabolism that indicated the development of diabetes [12].

Effect of Corvitin and Lipoflavon on blood biochemical parameters and morpho-functional status of the heart in type 1 diabetes mellitus.

In type 1 diabetes mellitus, the problems with lipid metabolism is also noted: TC, TG, LDL in serum increased by 44, 54 and 249 % respectively HDL, on the contrary, decreased by 64 % (the significance of the difference with the control group of animals was in all cases $p<0.001$) (Fig. 1).

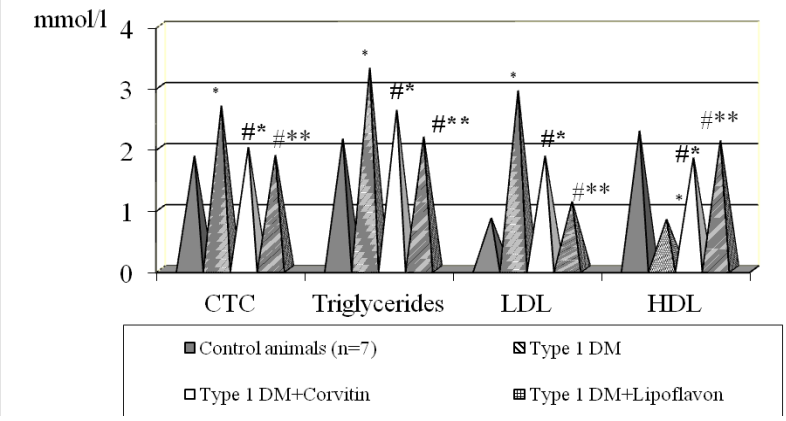

Figure 1. *Indicators of lipid metabolism in the serum of animals with type 1 diabetes mellitus and Corvitin and Lipoflavon administration.*
Notes: * - is significant in comparison with the markers of control animals; # - is significant between the type 1 DM; ** - is significant between the groups of type 1 DM + Corvitin.

In myocardium at type 1 DM significantly increased the content of products of peroxide degradation of lipid components of cardiomyocyte membranes LHPO - by 33%, TBA-active products - by 169%, DC - by 147 %. At the same time, there was an increase in the activity of Cat and SOD, but a decrease in the content of RG by 24 % (Table 1).

Table 1. The effects of Corvitin and Lipoflavon on the level of lipid peroxidation and antioxidant system in the animals heart with type 1 diabetes mellitus (M±m)

Index	Experimental group			
	Control (n=7)	type 1 DM (n=6)	type 1 DM + Corvitin (n=9)	type 1 DM + Lipoflavon (n=10)
LHPO, 10^3 standard unit/kg	5,70±0,10	7,60±0,17	6,03±0,17	5,88±0,05
P		<0,001*	>0,05	>0,05
p_1			<0,001*	<0,001*
p_2				>0,05
TBA-active products, mmol/kg	1,70±0,08	4,58±0,09	1,91±0,09	1,91±0,06
P		<0,01*	>0,05	>0,05
p_1			<0,001*	<0,001*
p_2				>0,05
DC, mmol/kg	4,04±0,10	9,97±0,15	5,04±0,19	4,40 ±0,08
P		<0,01*	<0,001*	<0,01*
p_1			<0,001*	<0,001*
p_2				<0,01*
SOD, standard unit /kg	1,94±0,15	2,56±0,08	2,45±0,23	2,00±0,25
P		<0,01*	<0,05*	>0,05
p_1			<0,05*	<0,01*
p_2				<0,05*
Catalase, cat/kg	7,71±0,17	11,28±0,51	9,16±0,21	8,91±0,12
P		<0,01*	<0,001*	<0,001*
p_1			<0,001*	<0,001*
p_2				>0,05
RG, mmol/kg	5,19±0,17	3,94±0,13	4,21±0,11	4,55±0,14
P		<0,01*	<0,001*	>0,05
p_1			<0,05*	<0,05*
p_2				<0,05*

Notes: 1. p - is significant in comparison with the markers of control animals; p_1 - is significant between the type 1 DM; p_2 - is significant between the groups of type 1 DM + Corvitin.

In type 1 DM, there was a decrease in the activity of the heart tissue of SDG and CChO (by 20 and 15 %, respectively).

In groups of animals with diabetes, where for correction was used quercetin-containing drugs, observed positive changes in lipid metabolism (see Fig. 1)

When using Corvitin, TC, TG, LDL content decreased by 25, 21 and 37 %, and Lipoflavon - by 30, 34 and 62 %, respectively. At the same time, the content of HDL increased by 123 and 157 %. At the same time under the influence of Lipoflavon was updated the lipid spectrum of the blood.

The investigational drugs at type 1 DM significantly reduced the manifestations of oxidative stress in the heart tissue (Table 1). Thus, the content of LHPO, TBA-active products, DC in myocardium under the influence of Corvitin decreased by 21, 58 and 49 %, Lipoflavon - by 23, 58 and 56 % respectively. With both drugs, LHPO and TBA-active products were back to normal. With the introduction of Corvitin and Lipoflavon, the activity of Cat decreased by 19 and 21 %, respectively. Lipoflavon reduced the level of SOD by 22 % and increased the RG by 16 % with normalization of both parameters (Table 1).

Effect of Corvitin and Lipoflavon on biochemical parameters in the blood and morpho-functional status of the heart in type 2 diabetes mellitus coupled with obesity

Along with the increase of glucose levels and glycosylated hemoglobin [12] marked increase in serum adipocyte hormone - lipocytokine leptin by 139 %, compared with the control group of animals. (Table 2).

In animals with type 2 diabetes coupled with obesity, in the myocardium was detected an increase of products of peroxide degradation of the lipid components of the cardiomyocyte membranes: LHPO – by 26 %, TBA-active products – by 96 %, DC – by 116 % (Table 3).

At the same time, changes in the antioxidant system were observed in the heart: an increase SOD and catalase activity (by 46 and 32%), and a decrease the content of RG (by 21%) (Table 3). In the myocardium was reduced the activity of mitochondrial SDG and CChO.

There was a significant decrease in the concentration of leptin when administered Corvitin by 34%, Lipoflavon - by 42 %. Moreover, the activity of Lipoflavon significantly exceeded the activity of Corvitin, by influencing the content of leptin by 12 % (Table 2).

The use of Corvitin-containing drugs showed a significant decrease in myocardial content of GPL, TBA-active products and DC - by 19, 42 and 49 %, with the introduction of Lipoflavon - by 24, 52 and 54 %, respectively, both drugs contributed to the normalization of these indicators (Table 3).

With the introduction of Corvitin and Lipoflavon, the activity of Cat in the myocardium significantly decreased by 12 % and 14 % and did not differ from the level of control. Corvitin and Lipoflavon contributed to an increase in myocardial RG content of 14 and 20 %, respectively. In addition, Lipoflavon normalized the activity of SOD (Table 3).

Table 2. Effect of Corvitin and Lipoflavon on cytokine and C-reactive protein levels in animal blood in type 2 DM coupled with obesity (M±m)

Index	Experimental group			
	Control (n=7)	type 2 DM (n=8)	type 2 DM + Corvitin (n=10)	type 2 DM + Lipoflavon (n=11)
Leptin, ng/L	152,71±1,85	364,66±4,71	242,00±2,43	212,60±2,08
P		<0,001*	<0,001*	<0,001*
p_1			<0,001*	<0,001*
p_2				<0,001*

Notes: 1. p - is significant in comparison with the markers of control animals; p_1 - is significant between the type 2 DM; p_2 - is significant between the groups of type 2 DM + Corvitin.

Table 3. The influence of Corvitin and Lipoflavon on the performance of prooxidants / antioxidants in the myocardium in type 2 diabetes mellitus coupled with obesity (M±m)

Index	Experimental group			
	Control (n=7)	type 2 DM (n=8)	type 2 DM + Corvitin (n=10)	type 2 DM + Lipoflavon (n=11)
LHPO, $\times 10^3$ standard unit/kg	5,10±0,14	6,44±0,27	5,25±0,15	4,90±0,13
P		<0,001*	>0,05	>0,05
p_1			<0,01*	<0,001*
p_2				>0,05
TBA-active products, mmol/kg	1,80±0,10	3,52±0,09	2,05±0,17	1,69±0,20
P		<0,001*	>0,05	>0,05
p_1			<0,001*	<0,001*
p_2				>0,05
DC nmol/mg protein	3,74±0,07	8,09±1,00	4,15±0,35	3,74 ±0,14
P		<0,001*	>0,05	>0,05
p_1			<0,001*	<0,001*
p_2				>0,05
SOD standard unit/kg	2,34±0,05	2,87±0,17	2,62±0,17	2,35±0,20
P		<0,01*	<0,05*	>0,05
p_1			>0,05	<0,05*
p_2				>0,05
Catalase, cat/kg	8,41±0,30	10,00±0,21	8,85±0,16	8,61±0,18
P		<0,001*	>0,05	>0,05
p_1			<0,001*	<0,001*
p_2				>0,05
RG mmol/kg	4,22±0,09	3,33±0,08	3,81±0,10	3,99±0,05
P		<0,001*	<0,05*	<0,05*
p_1			<0,01*	<0,001*
p_2				>0,05

Notes: 1. p - is significant in comparison with the markers of control animals; p_1 - is significant between the type 2 DM; p_2 - is significant between the groups of type 2 DM + Corvitin

Discussion

Thus, Corvitin and Lipoflavon exhibit cardioprotective activity in type 1 and type 2 diabetes mellitus coupled with obesity. Both drugs, to a greater extent Lipoflavon, effectively suppress free-radical oxidation, normalize antioxidant status, reduces disorders of carbohydrate and lipid metabolism. In type 2 DM, it reduces leptin content.

Proving the effectiveness of Corvitin and Lipoflavon conteining drugs at cardiomyopathy, which develops in experimental diabetes mellitus, substantiates the feasibility of further in-depth study of their properties with the

prospect of successful use as cardioprotective agents in this pathology.

Conclusions

Corvitin and, to a greater extent, Lipoflavon in type 1 diabetes mellitus contribute to a significant decrease in the blood content of total cholesterol, triglycerides, low-density lipoproteins with a simultaneous increase in the amount of high-density lipoproteins and decrease the level of TBA-active products. In the tissue of the heart - a significant decrease in the number of lipid hydroperoxides, diene conjugates, TBA-active products, restoration of the activity and content of components of the antioxidant system, improvement of the functional state of mitochondria, the level of synthesis of nitric oxide, against the background of endogenous intoxication.

Corvitin and, largely, Lipoflavon in type 2 DM coupled with obesity contribute to a significant decrease in blood leptin levels, a decrease the content of TBA-active products and catalase activity. In myocardial tissue – decrease in the content of lipid hydroperoxides, diene conjugates, TBA-active products, catalase activity, increase in the level of reduced glutathione, activity of mitochondrial enzymes, which occurs against the background of decreasing endogenous intoxication.

Lipoflavon in the experimental type 1 and type 2 diabetes mellitus coupled with obesity significantly outperforms Corvitin in terms of TBA-active products and diene conjugates.

Conflict of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Tkachenko VI. Draft Unified Clinical Protocol for Type 2 Diabetes Management at Primary Care. *Liky Ukrayiny* № 6 (162): 51–59, 2012.

2. Diabetes: facts and figures. International Diabetes Federation. *Available from*: http://www.idf.org/about-diabetes/facts-figures

3. Khutorska LA. Comparative analysis of mortality structure in patients with type 1 and type 2 diabetes mellitus. *Emergency medicine* № 7-8: 46–47, 2012.

4. Akhmetova ES. Pathophysiological and genetic changes in the body of a pregnant with gestational diabetes. *Journal of obstetrics and women's diseases* 66(I.5): 1694–1699, 2017.

5. Dewanjee S, Maiti A, Sahu R et al. Effective Control of Type 2 Diabetes through Antioxidant Defense by Edible Fruits of Diospyros peregrine. *Evidence–Based Complementary and Alternative Medicine* 2(11): 7, 2009.

6. Horoshko OM. Effect of lipin as a basis of liposomal lipoflavon on the functional state of kidneys in rats under a model of pathology. *Buk. Med. Herald* 3(71). 49-52, 2014.

7. Yaremenko O, Yordanova NKh, Kuchmerovskaya TM. Changes of amino acid blood plasma in patients with elevation myocardial infarction as a result of treatment by quercetin. *Bulletin of Scientific Research* 4: 45-49, 2015.

8. Jaiswal N, Rizvi SI. Protective effect of different layers of onion extracts (Allium cepa L.) on markers of oxidative stress in erythrocytes. *Cell Membranes and Free Radical Research* 3(2): 171-177, 2011.

9. Galenova TI, Konopelnyuk VV, Savchuk OM, Ostapchenko LI. Isolation of an experimental streptozotocin-induced model of type 2 diabetes in rats. *Physics of the living* T.18, № 3: 50-54, 2010.

10. Posokhova KA, Zozuliak NB, Cherniashova VV. Stechyshyn IP. *Method of experimental obesity modeling.* Patent of Ukraine 92289: 15, 2014.

11. Sun L, Li E, Wang F. Quercetin increases macrophage cholesterol efflux to inhibit foam cell formation through activating PPARγ–ABCA1 pathway. *Int J Clin Exp Pathol* № 8(9): 10854–10860, 2015.

12. Posokhova K, Stechyshyn I, Krynytska I, Marushchak M, Birchenko I, Klishch I. Comparative study of the effect of various forms of quercetin on experimental diabetes. *Romanian Journal of Diabetes Nutrition and Metabolic Diseases* 25(4): 383-388, 2018.