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BIOFLAVONOIDS COMPLEX USING IN THE PREVENTION AND TREATMENT OF DIABETES COMPLICATIONS

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Abstract

Aim: is the investigation the effect of different bioflavonoids combination on the indicators of blood coagulation in animals with diabetic nephropathy (DN).

Methods: the research was carried out on white nonlinear Wistar line rats, weight 240-280 g. A streptozotocin model was used to reproduce type 2 diabetes. For this purpose, rats were administered a single intravenous streptozotocin ("SigmaAldrich Chemie GmbH", Germany) in dose of 65 mg/kg. The streptozotocin administration was preceded by a high-fat diet.

Results: presents the results of a study of various bioflavonoids on the course of diabetic nephropathy against the background of type 2 diabetes. It was found that the most pronounced effect was shown by the use of the combination L-arginine with quercetin. The use of this combination led to an improvement in the indicators of coagulation hemostasis. Also using L-carnitine and quercetin led to the decreasing of von Willebrand factor on 68 % compared with the control pathology group which may indicate about endothelium protective action. The activity of this combination of flavonoids (quercetin) and L-arginine is related to their pharmacological properties. Quercetin has powerful antioxidant properties, while L-arginine, a substrate for NO synthase, is a precursor for NO synthesis in the vascular endothelium, and its use can increase the bioavailability of NO and dilate coronary arteries.

Conclusion: 1. Bioflavonoids effect on the coagulation link of hemostasis by regulating the balance of indicators that characterize the stages of blood coagulation. The most pronounced effect on the indicators of the coagulation system in DN was shown by the use of L-arginine in complex with quercetin. 2. The most pronounced endothelium protective effect was observed when using L-arginine in complex with quercetin, this indicator decreased on 68 % compared with the control pathology group. 3. The obtained results indicate that the complex of L-arginine with quercetin is promising in the complex treatment of diabetic nephropathy.

Key words: diabetes mellitus, diabetic nephropathy, coagulation, von Willebrand factor.

Introduction

Diabetes is a major public health problem that is approaching epidemic proportions globally. Worldwide, the prevalence of chronic, noncommunicable diseases is increasing at an alarming rate. About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors. Today, more than 1.7 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese. A diabetes epidemic is underway. According to an estimate of International Diabetes Federation comparative prevalence of Diabetes during 2007 is 8.0 % and likely to increase to 7.3 % by 2025 [9].

Almost 80 % of the total adult diabetics are in developing countries. The regions with the highest rates are the Eastern Mediterranean and Middle East, where 9.2 % of the adult population is affected, and North America (8.4%). The highest numbers, however, are found in the Western Pacific, where some 67 million people have Diabetes, followed by Europe with 53 million [3, 9].

Two major concerns are that much of this increase in Diabetes will occur in developing countries and that there is a growing incidence of Type 2 Diabetes at a younger age including some obese children even before puberty. In developed countries most people with diabetes are above the age of retirement. In developing countries those most frequently affected are in the middle, productive years of their lives, aged between 35 and 64.

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors, and life-style choices [3].

Diabetes is the fourth leading cause of death in most developed countries. Complications from Diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society. Diabetes is certain to be one of the most challenging health problems in the 21st century [7].

The number of deaths attributed annually to diabetes is around 3.2 million. Diabetic neuropathy is probably the most common complication. Diabetes is among the leading causes of kidney failure, but its frequency varies between populations and is also related to the severity and duration of the disease [9].

One of the obligatory components of the complex therapy of DM and its vascular complications is the use of antioxidant therapy. It is actual to study the effect of natural antioxidants by nature origin (plants flavonoids) on the processes of lipid peroxidation and the course of microangiopathy in patients with type 2 diabetes [5, 9].

Flavonoids are a large family of compounds that possess a common chemical structure and are synthesized by plants. Linked to their biological properties, polyphenols may be useful nutraceuticals and supplementary treatments for various aspects of diabetes mellitus. Based on several *in vitro* animal models and some human studies, polyphenols may play a role in many metabolic processes. They can modulate carbohydrate and lipid metabolism, attenuate hyperglycemia, dyslipidemia and insulin resistance, improve adipose tissue metabolism, and alleviate oxidative stress and stress-sensitive signaling pathways and inflammatory processes [6, 10].

Objective is the investigation the effect of different bioflavonoids combination on the indicators of blood coagulation in animals with diabetic nephropathy (DN).

Methods

Experimental studies were performed on 36 white nonlinear rats weighing 240-280 g, which were divided into 6 experimental groups (6 animals each): group 1 - intact control - animals kept on a standard diet of vivarium; group 2 – control pathology – animals, which after the introduction of streptozotocin and nicotinamide reproduced the model of DN [1]. Next 4 groups of animals were treated with bioflavonoids and L-arginine: group 3 – animals with DN treated with flavicin (in dose 12 mg/kg); group 4 – animals with DN treated with quercetin (50 mg/kg); group 5 – animals with DN

treated with diosmin (in dose 100 mg/kg); group 6 – animals with DN treated with L-arginine (in dose 100 mg/kg) and quercetin (50 mg/kg).

A streptozotocin model was used to reproduce type 2 diabetes. For this purpose, rats were administered a single intravenous streptozotocin ("SigmaAldrich Chemie GmbH", Germany) in dose of 65 mg/kg. After 1 week a glucose tolerance test was performed to determine fasting blood glucose levels and 30, 60, 90 and 120 minutes after intragastric administration of 40 % glucose solution at a dose of 3 g/kg and blood glucose levels from 9.0 to 14 mmol/l [8].

To reproduce DN, the diet of experimental rats was based on a high-fat diet. At 35-40 weeks, the animals showed signs of DN – proteinuria, decreased glomerular filtration rate [2].

With the purpose of the coagulation system study were used platelet-poor citrate plasma and platelet-rich citrate plasma. Platelet-rich citrate plasma was obtained by centrifugation of citrate-stabilized blood (blood + 3.8 % sodium citrate in a ratio of 1:9) at 1000 - 1500 rpm for 5-7 min. Platelet-poor citrate plasma was obtained by double centrifugation: first, platelet-rich plasma was obtained, and then centrifuged again for 15 - 20 min at a temperature of + 4 ° C at 3000 - 4500 rpm [1].

In order to assess changes in the coagulation of hemostasis in complications of type 2 diabetes, the following indicators were used: activated partial thromboplastin time (APTT) by J. Caen; prothrombin time (PT) by A. J. Quick; thrombin time (TT) by the method of Biggs et al.; the level of fibrinogen by the gravimetric method according to RA Rutberg [1].

Determination of von Willebrand factor was performed on two-channel laser aggregation analyzer platelets.

During the work with animals we complied with the International Code of Medical Ethics (Venice, 1983), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the General Ethical Principles for Animal Experiments adopted by the First National Congress of Bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes.

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The probability of differences between the indicators of the control and experimental groups was determined by the criteria of Student and Fisher. The level of reliability was taken at $p < 0,05$.

Results

In animals with experimental diabetic nephropathy, disorders of coagulation (plasma) hemostasis were observed (table). The anticoagulant and antithrombogenic potential of the vascular endothelium shifted towards the shortening of the thrombin and prothrombin levels (by 21.5 and 8.2%, respectively), and activated partial thromboplastin time (by 37.8%), increased fibrinogen level by 4 times.

The obtained results indicate pronounced changes in the coagulation link of hemostatis in experimental DN in background of type 2 DM. It is indicating about development of endothelial dysfunction, which is consistent with literature data [4].

In animals who were treated with quercetin (group 4) and L-arginine+ quercetin (group 6) we observed a significant improvement of all coagulation hemostasis indicators. Under the effect of the studied flavonoids, a lengthening of APTT, TT, PT, a decrease in the level of fibrinogen was observed as compared with the group with DN (table). The obtained results indicate about partial restoration of the functioning of the plasma hemostasis system (internal and external cascade of plasma coagulation, the formation of a fibrin clot at the last stage of blood coagulation).

One of the specific markers of the development of endothelial dysfunction is an increase in the level of von Willebrand factor. In next stage of our investigation we study the changes of this indicator under the condition of DN development and bioflavonoids therapy (Fig.).

In the group of control pathology, the level of von Willebrand factor in the blood increased by 1.72 times, which indicated violations in the hemostasis system and the implementation of the antithrombotic function of the endothelium.

According to the results, the most pronounced endothelium protective effect was observed when using L-arginine in complex with quercetin, this indicator decreased on 68 % compared with the control pathology group. A less pronounced effect

was observed in the group of rats treated with quercetin.

Polyphenolic compounds interacting with free radicals, sharply slow down the process of lipid peroxidation in the body as a result of the formation of inactive phenolic radicals, as well as due to the acceleration of the utilization of preoxidized lipids. Drugs of the antioxidant group, due to their inhibitory effect on lipid peroxidation, stabilize the structure of cell membranes, normalizing their permeability, improving microcirculation, accelerating the utilization of toxic over-oxidized products; an integral result is the prevention of gross destruction of the organ and the stimulation of regenerative processes in it. These literature data are consistent with our experimental results [3, 10].

Discussion

1. Bioflavonoids effect on the coagulation link of hemostasis by regulating the balance of indicators that characterize the stages of blood coagulation. The most pronounced effect on the indicators of the coagulation system in DN was shown by the use of L-arginine in complex with quercetin.

2. The most pronounced endothelium protective effect was observed when using L-arginine in complex with quercetin, this indicator decreased on 68 % compared with the control pathology group.

3. The obtained results indicate that the complex of L-arginine with quercetin is promising in the complex treatment of diabetic nephropathy.

Conflict of interest

The authors declsre that there are no conflicts of interest.

Relationship of the publication with the planned research works. The work presented is a fragment of the research project "Diabetic nephropathy pathogenesis and substantiation of chronic kidney disease diagnostics, № state registration 0120U102210.

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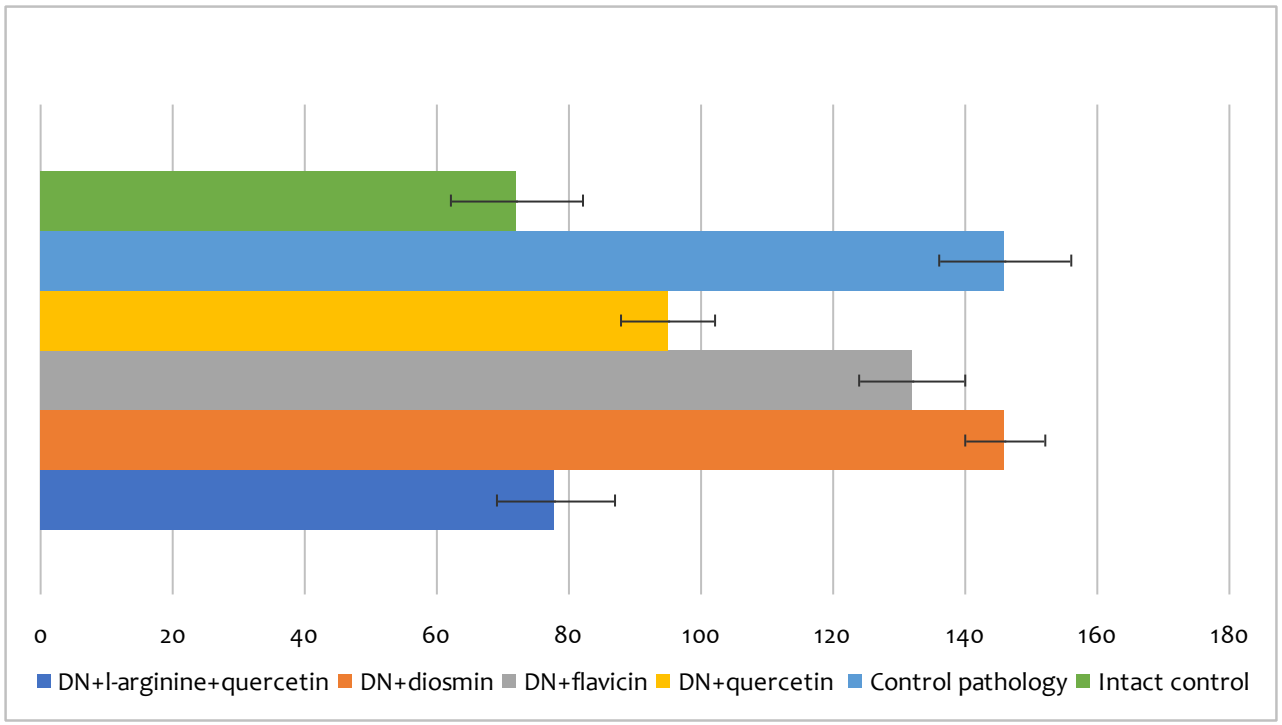
Table. Effects of flavonoids on the coagulation hemostatis in rats with diabetic nephropathy (X±Sx, n=6)

Group	Activated partial thromboplastin time, s	Prothrombin time, s	Thrombin time, s	Fibrinogen, g/l
Intact animals	23,6±0,8	12,2±0,5	32,6±1,2	4,6±0,4
Control pathology	32,4±2,2*	11,2±0,6	25,6±1,4*	18,6±0,9*
DN+ flavicin	30,9±2,1*	11,6±0,5	28,7±1,1	7,8±1,1*
DN+ quercetin	25,6±1,7	12,3±0,4	30,9±1,4	5,4±0,6
DN+diosmin	31,4±1,4*	11,4±0,4	27,6±1,3*	8,3±0,9*
DN+ L-arginine+ quercetin	24,2±1,2	12,7±0,6	31,8±1,5	4,9±0,5

Note. * - p <0.05 relative to the indicators of the intact group of animals

Figure. The effect of bioflavonoids on the level of von Willebrand factor in rats with diabetic nephropathy (X±Sx, n=6)



Notes:
1. * - p <0.05 relative to the indicators of the intact group of animals;
2. ** - p <0.05 relative to the indicators of the group of control pathology