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Chapter

Prospects for Using the Natural Antioxidant Compounds in the Obesity Treatment

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Abstract

Obesity is strongly associated with the insulin resistance development and is an integral pathogenic part of the metabolic syndrome, type 2 diabetes, atherosclerosis, and other comorbid pathologies. It is well known that the obesity causes the disorders in adipose tissue endocrine and metabolic activity, which results in the activation of free radical processes. The administration of natural origin substances with antioxidant properties could be a promising direction for obesity and overweight correction. The objective of the current study was to evaluate the usefulness of natural origin active substances with antioxidant properties' administration under the obesity and comorbid disorder treatment. This chapter presents the results of experimental studies that proved the usefulness of phenolic compounds from apple food concentrate (*Malus domestica* L.) and dry bilberry extract (*Vaccinium myrtillus* L.) application under experimental metabolic syndrome, insulin resistance syndrome, and type 2 diabetes mellitus, which are extremely associated with obesity. It has been found that due to expressive antioxidant activity, these compounds exhibit the high efficiency in carbohydrate metabolism disorders' correction (in cases of metabolic syndrome and type 2 diabetes), lipids metabolism disorders' correction (in case of metabolic syndrome), preventing of endothelial dysfunction under experimental insulin resistance, and normalization of antioxidant status in the liver (under experimental type 2 diabetes mellitus).

Keywords: obesity, apple polyphenol extract, blueberry dry extract, metabolic syndrome, insulin resistance, antioxidant activity, diabetes mellitus

1. Introduction

According to clinical and epidemiological studies, obesity refers to diseases with high medical and social significance and has a pandemic rate of expansion. Obesity is a component of the multimorbid disease pathogenesis, in particular of metabolic syndrome (MS), type 2 diabetes mellitus (DT2), also it is a predictor of atherosclerosis and cardiovascular complications [1]. It is known that an imbalance of antioxidant-prooxidant factors (with a predominance of prooxidants) is associated with obesity development due to pathological changes in metabolic activity of the adipose tissue [2].

The reactive oxygen species (ROS) production is primarily caused by the lipid accumulation, so it results in NADPH-oxidase excessive expression with the simultaneous decrease in the activity of antioxidant enzymes and nonenzyme antioxidants. Carbohydrate metabolism disorders lead to the activation of glucose metabolism polyol pathway and to protein pathological glycosylation along with metabolic end products accumulation. Oxidative stress leads to adipokines production dysregulation, in particular, adiponectin, plasminogen activator inhibitor-1, interleukin-6, and monocyte chemotactic factor. It plays a leading role in the involvement of other metabolic pathways to the pathological process and main disease pathogenesis complications [3, 4].

Free radical upbuilding is a major event involved in vascular endothelium damaging as a trigger mechanism for the endothelial dysfunction (ED) and cardiovascular complications development related to obesity and MS. As is well known, just cardiovascular events determine high premature mortality rates of these patient categories. In addition, the pathological changes of the antioxidant-prooxidant balance leads to the low-density lipoproteins (LDL) oxidative modification, which are the leading link to the proatherogenic process essential for obesity and associated pathologies [5].

The metabolic disorders' pathogenetic aspects that appear under MS are mainly based on the free radical oxidation (FRO) processes activation. Concerning the above mentioned, the antioxidant use is promising treatment strategy for correction of pathological states that developed under the insulin resistance (IR) [6].

Common therapeutic pharmacological correction strategies for obesity-associated diseases include the synthetic drug use, which are powerful microsomal oxidation processes activators that, in turn, initiates the FRO intensification and increases the ROS production. Recently, there is an increasing interest to the development and research of natural origin substances that can be used at the complex obesity-associated pathologies treatment. Plant polyphenols with expressive antioxidant activity can potentially demonstrate a therapeutic effect under MS pathogenetic manifestations and related comorbidities, including ED [7]. The mentioned metabolic disorders are mediated by chronic subclinical inflammatory process, which is intermediated by the adipocytes production of various active molecules, which, as usual, are factors of high cardiometabolic risk. That is why the evaluation of usefulness of natural origin substances with antioxidant properties administration under the obesity treatment was the aim of our work.

2. The preclinical study of phenolic compounds from apple food concentrate under experimental metabolic syndrome and insulin resistance syndrome

2.1 The effect of phenolic compounds from apple food concentrate on indices of carbohydrate and lipid metabolism under experimental metabolic syndrome

2.1.1 Materials and methods

Taking into account the significant role of reactive oxygen species (ROS) in the pathogenesis of obesity and associated diseases, the nutritional apple polyphenol food concentrate (APFC) investigation is seemed to be reasonable. Examined concentrate is rich in phenolic substances that reveal expressive antioxidant properties under the experimental MS. The investigated food concentrate was developed at the Pharmacognosy Department of the National University of Pharmacy under the supervision of Pharm. D. Koshoviy O.M. The food concentrate biological activity is primary caused by gallic acid, caffeic acid, chlorogenic acid, ursolic acid, quercetin,

epicatechin, leuco-anthocyanins, and ascorbic acid. The purified compound epigallocatechin gallate (EGCG, Sigma-Aldrich) was selected as a reference preparation because of its proven strong antioxidant properties.

Experimental MS was modeled in Syrian golden hamsters (males, 20 weeks old) that were fed high-calorie diet enriched by energy sources (containing 29% fat, mostly saturated lipids) and fructose (1 g/100 g of body weight per day per) (as aqueous solution) for 5 weeks. Animals of intact control group were fed a standard balanced diet for hamsters during the experiment [8]. Basal blood glucose concentration in animal serum was determined using One touch ultra-easy glucose meter based on glucose oxidase method (manufactured by LifeScan, Johnson & Johnson, USA) [9]. The basal blood level of immunoreactive insulin (IRI) was determined using in vitro radioimmunoassay by the standard set of reagents (manufactured by Immundiagnostik, Germany). Insulin resistance index (HOMA-IR) was calculated from animal blood values of basal glucose and IRI indicators using Homeostasis Model Assessment algorithm (HOMA) [10]. The content of triacylglycerols (TAG) in serum and in the liver homogenate was determined using the standard set of reagents "KONE" (Finland) based on glycerol oxidation method. Determination of the free fatty acids (FFA) concentration in serum was carried out using a set of reagents produced by Kamiya biomedical company (USA). The concentration of total lipids was determined in liver homogenate using the standard kit produced by Eagle Diagnostics (USA) based on the reaction with a vanillin reagent. The content of high-density lipoproteins (HDL) and apo-B-containing lipoproteins (sum of low-density lipoproteins and very low-density lipoproteins—apoB-L) were determined in blood serum using turbidimetric method [11, 12].

Experimental animals were administered APFC for 2 weeks (since third week of pathology simulation) using a special iron catheter for intragastric administering. The dose of APFC was calculated as total content of polyphenols—9 mg/100 g of body weight (prophylactic treatment). The dose of reference preparation epigallocatechin gallate—EGCG (produced by Sigma-Aldrich, Germany) was 3 mg/100 g of body weight. Daily animal equivalent doses (AED) were calculated according to the latest recommendations for preclinical studies, taking into account the average daily dose for humans and interspecies difference in body weight and body surface area [13].

The results were statistically processed using the 4Pl statistical logistic method with free internet service of MyAssays® and nonparametric analysis methods (Mann-Whitney U-Test) with the standard software package STATISTICA 7.0 [14].

2.1.2 Results

The carried studies indicated expressive corrective effect of the test concentrate and EGCG on the markers of carbohydrate and lipid metabolism in the Syrian hamsters with experimental MS, which, however, had rather significant group differences (**Tables 1**–**3**).

Under the experimental conditions, APFC usage led to the normalization of glucose and IRI indices, in which content was significantly reduced by 19.5 and 21.02% respectively in comparison with animals of the model pathology group. The IR index significant reduction by 24.18% was a confirmation of the studied food concentrate therapeutic effect (**Table 1**).

EGCG also significantly decreased glucose and IRI levels by 10.9 and 10.1%, respectively, compared to animals with an experimental MS; index of IR also proportionally decreased (by 11.93%). It should be noted that the EGCG therapeutic effect was not so pronounced as after the apple concentrate of phenolic compounds administration. We suppose that it was mediated by complex multivector biologically active compounds effect in concentrate content.

Parameters	Intact control	Model pathology	APFC + model pathology	EGCG + model pathology
Glucose (mmol/l)	5.800 ± 0.440	11.200 ± 0.390*	9.021 ± 0.450**	9.983 ± 0.565**
IRI (pmol/l)	92.500 ± 2.305	138.280 ± 2.406*	109.220 ± 2.380	124.35 ± 2.250**
Index IR (HOMA-IR)	1.78	3.02*	2.29**	2.66**

n, number of animals in a group. Variation is statistically significant in comparison with intact control group indices ($p \leq 0.001$).*
** Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.001$).*

Table 1.
The effect of apple phenolic compounds food concentrate and epigallocatechin gallate on carbohydrate metabolism indices under experimental metabolic syndrome, n = 10.

Parameters	Intact control	Model pathology	APFC + model pathology	EGCG + model pathology
TAG (mmol/l)	1.950 ± 0.055	3.680 ± 0.146*	2.610 ± 0.017**	2.970 ± 0.012
FFA (mmol/l)	0.450 ± 0.020	1.438 ± 0.023*	1.015 ± 0.150**	1.109 ± 0.045
apoB-LP (mg/ml)	4.72 ± 0.23	6.68 ± 0.15*	5.09 ± 0.13**	5.44 ± 0.14
HDL (mg/ml)	1.11 ± 0.05	0.98 ± 0.07*	1.08 ± 0.02**	1.01 ± 0.03

n, number of animals in a group. Variation is statistically significant in comparison with intact control group indices ($p \leq 0.001$).*
** Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.001$).*

Table 2.
The effect of apple phenolic compounds food concentrate and epigallocatechin gallate on lipid metabolism indices in hamster's blood serum under experimental metabolic syndrome, n = 10.

Parameters	Intact control	Model pathology	APFC + model pathology	EGCG + model pathology
TL (mg/g)	108.25 ± 2.16	132.55 ± 2.35*	117.43 ± 1.86**	125.45 ± 1.67**
TAG (mg/g)	12.32 ± 0.75	14.24 ± 0.37*	13.18 ± 0.35**	13.47 ± 0.15**

n, number of animals in a group. Variation is statistically significant in comparison with intact control group indices ($p \leq 0.001$).*
** Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.001$).*

Table 3.
The effect of apple food concentrate of phenolic compounds and epigallocatechin gallate on lipid metabolism parameters in hamster's liver in experimental metabolic syndrome, n = 10.

Normalization in lipid metabolism disturbances was observed under the influence of the APFC as well as EGCG administration, that was confirmed by the laboratory indices favorable dynamics in both serum and liver of animals (**Tables 2** and **3**). At the same time, a more pronounced effect was found in group of animals, which were administered with the apple concentrate.

Thereby, it was determined that in animals administered with the test concentrate, the content of FFA and TAG decreased, respectively, by 29.5 and 29.1%, compared with those in the model pathology group. Additionally, it was demonstrated

with more favorable lipoprotein fractions ratio in this group of animals treated by APFC. Thus, the content of the atherogenic fraction apoB-LP decreased (by 23.9%), while the level of antiatherogenic HDL was increased (content did not significantly differ from the same index in healthy animals), which indicates a reduction in the risk of proatherogenic changes and atherosclerosis development (**Table 2**).

The same tendency was observed under EGCG administration—the significant correction as for lipid metabolism pathological changes under experimental MS, which was demonstrated via decrease in TAG and FFA content by 19.3 and 22.9% respectively. In addition, under the EGCG action, the level of apoB-LP decreased (by 18.6%) and level of HDL slightly increased (by 3.06%), which is the evidence of MS-specific proatherogenic change reduction. However, the EGCG therapeutic effect was not so pronounced compared to the apple food concentrate of phenolic compounds.

Considering the fact that the studied food concentrate and EGCG showed pronounced corrective therapeutic effect as for pathological changes in blood lipid metabolism markers, it was appeared expedient to investigate the effect of these compounds on the lipid metabolism indices in the liver tissue.

There was a significant increase in the TL and TAG content in the liver in animals with a model pathology (in 1.22 and 1.16 times, respectively, in comparison with intact hamsters), which indicated the steatosis precondition formation, and primary was due to the lipid intensive flow from the bloodstream (**Table 3**).

The APFC administration caused substantial correction of the TL and TAG content in the liver, which did not significantly differ from the same indices in healthy animals (**Table 3**). This is a confirmation of the APFC beneficial effects as for preventing the fatty liver disease development, which is an MS pathogenesis integral component. This effect was probably mediated by lipid flow inhibition from the blood to the liver under the APFC action, which was evidenced by a decrease in the blood content of these compounds (**Table 2**).

The EGCG administration to animals with experimental MS also was accompanied with a significant decrease in the TL and TAG content in the liver tissue by 5.4 and 5.5%, respectively, indicating the ability of the test compounds to prevent the steatosis development under the MS. It should be noted that the TL level under EGCG administration was significantly higher than under APFC administration, in which prevention indicates about a less significant effect on liver fatty degeneration under experimental pathology. This dynamics is also a reason for EGCG's less effect on the pathological change correction in lipid metabolism in animals` blood specific to MS and for significantly higher lipid flow from the blood stream.

2.1.3 Discussion

The APFC therapeutic effectiveness is the result of the constituent substances complex synergistic effect that mediates various types of biological activity, in particular, a powerful antioxidant effect, the normalization of energy homeostasis and lipid metabolism.

It is well known that gallic acid, which amount is the main part of test concentrate quantitative content, has an ability to regulate body weight and glucose homeostasis via the way of AMP-activated protein kinase (AMPK) upregulation and modulation of mitochondrial functions through the stimulation of a gamma receptor coactivator-1α, which activates by peroxisomal proliferator (PGC1α). It is assumed that the main molecular mechanism of gallic acid action is the effect on the AMRK/Sirt1/PGC1α signaling pathway. In addition, gallic acid significantly improved homeostasis of glucose and insulin by the activation of AMPK (in the liver, muscle, and brown fat tissue) and the gene expression of uncoupling protein-1, which in general was manifested by an increase in energy expenditure and thermogenesis intensification without significant

effect on appetite. There is an evidence that gallic acid inhibits the gene expression of gluconeogenesis key enzymes and as a result makes a significant impact on the blood glucose level and downregulates the FFA content that was registered in our experiments. In general, these data show the gallic acid beneficial effect on energy homeostasis, making its usage sensible in treatment of IR and associated diseases [15–18].

Several studies show that another component of concentrate—quercetin—has therapeutic effect under MS. Quercetin manifests itself in improved insulin tissue sensitivity and in glucose level reduction that can be explained by α-glucosidase activity inhibition and stimulation of glucose absorption by skeletal muscle and liver cells [19]. Glucose is actively involved in glycolysis (due to increased activity of enzymes hexokinase and pyruvate kinase) and incorporated for glycogen synthesis in skeletal muscle and liver. In addition, quercetin corrects pathological changes in the cortisol and sex steroids content, counteracts the adipose tissue metabolic activity disorders, and normalizes cytokine profile under MS. The quercetin multivector effect leads to the normalization of carbohydrate and lipid metabolism, and reduced metabolic disorders caused by IR [20–23].

Ursolic acid in *in vivo* experiments in mice with simulated IR led to the inhibition of atherosclerotic plaque formation and systolic pressure reduction; however, mechanisms of these effects developing have not studied yet [24, 25]. Caffeic, chlorogenic, and ascorbic acids are powerful antioxidants that suppress the formation of free radicals, and ROS and strongly reduce the effects of oxidative stress, which is an MS pathogenetic component. In addition, phenolic acids inhibit the activity of α-glucosidase and sodium glucose transporter (SGLT1) [26, 27]. Leucoanthocyanins are able to induce autophosphorylation of the insulin receptor, resulting in improved insulin signaling and increased affinity of cells to its action [28].

Thus, the APFC compounds administration under experimental MS significantly prevented an increase in glucose and IRI, corrected the manifestations of IR, decreased the content of FFA and TAG, probably due to the insulin counteraction on lipolysis and the modulation of the FFA metabolism under the effect of phenolic compounds, which reduced the flow of lipids to the liver (confirmed by a decrease in TL and TAG content in the animals' liver), normalized the lipoprotein blood spectrum (confirmed by a decrease in apo-LP and increased HDL contents), and improved the oxidative state in the liver. The pronounced therapeutic effect of the APFC could be mediated by the complex synergistic antioxidant effect of the constituent components—gallic, caffeic, chlorogenic and ursolic acids, quercetin, leucoanthocyanin, and ascorbic acid.

In general, observed changes indicate the ability of the studied concentrate to prevent the induction of proatherogenic disorders and the MS negative influence, such as the atherosclerosis development. These harmful factors are the main cause of the cardiovascular diseases development and their complications in patients with metabolic disorders [29].

According to the literature data, the mechanism of EGCG usage under the experimental MS is mediated by the effect on cellular mechanisms of glucose transporters translocation stimulation, in particular GLUT4 (mainly due to activation of AMPC and/or phosphoinositide-3-kinase), which is manifested by a hyperglycemia reduction due to increased glucose transport in cells of fat and muscle tissue. The activation of AMPK also correlates with a decrease in the accumulation of lipids in the liver. As for EGCG, there is also the ability to induce the activity of the glycogenesis enzymes (liver glucokinase) and suppress the expression of enzymes of gluconeogenesis. EGCG inhibits the activity of α-glucosidase to a lesser extent than chlorogenic, gallic, and caffeic acids, and it is due to a smaller length of the hydrocarbon chain of the polyphenolic molecule [7, 30].

Thus, EGCG in the experimental MS showed a therapeutic effect, which was mainly due to the normalization of glucose metabolism and inhibition of lipid accumulation in the liver, but anyway, the therapeutic effect of apple phenolic compounds food concentrate was more expressive.

2.2 The effect of apple polyphenolic food concentrate on the endothelial function under experimental insulin resistance

2.2.1 Materials and methods

The complications of cardiovascular system disorders' development are specific to the diseases associated with obesity and IR syndrome. The main pathogenetic component of cardiovascular pathologies is the progression of ED. This fact has been proved in our studies and was corresponded with the literature data. Concerning the glycemic profile positive changes under the influence of the studied APFC and EGCG, it was decided to investigate these compounds effect on the endothelium functional state under the experimental IR (**Tables 4** and **5**).

Experimental groups	Parameters		
	NO_2^- + NO_3^- (mmol/l)	Arginine (mmol/l)	Citrulline (mmol/l)
Intact control	122.20 ± 1.23	64.40 ± 0.65	41.30 ± 1.05
Model pathology	164.50 ± 1.15*	42.80 ± 0.43*	54.50 ± 1.21*
APFC + model pathology	125.82 ± 1.78**	69.50 ± 0.75**	43.39 ± 1.25**
EGCG + model pathology	136.85 ± 2.02**, #	58.25 ± 0.67**,#	46.13 ± 1.43**,#

n, number of animals in a group. *Variation is statistically significant in comparison with intact control group indices ($p \leq 0.001$).*
** Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.001$).*
# Variation is statistically significant in comparison with indices of animals that were on apple food concentrate of phenolic compounds ($p \leq 0.001$).*

Table 4.
The effect of test compounds on rat's NO-synthase system markers under experimental insulin resistance (the fifth week of experiment), n = 10.

Experimental groups	Parameters		
	ET-1 (pg/ml)	S-NO (mmol/l)	ET-1/S-NO
Intact control	2.29 ± 0.14	0.49 ± 0.03	4.67 ± 0.13
Model pathology	5.91 ± 0.23*	0.15 ± 0.04*	39.4 ± 1.05*
APFC + model pathology	3.60 ± 0.15**	0.32 ± 0.04**	11.25 ± 1.04**
EGCG + model pathology	3.88 ± 0.10**	0.26 ± 0.02**	14.92 ± 1.01**

n, number of animals in a group. *Variation is statistically significant in comparison with intact control group indices ($p \leq 0.001$).*
** Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.001$).*

Table 5.
The effect of examined compounds on rat's specific markers of endothelial function underexperimental insulin resistance (the tenth week of experiment), n = 10.

A balance between the content of vasoconstrictor and vasodilator substances play the key role in vascular tone regulation, progression and prognosis as for the course of endothelial dysfunction (ED) [5]. The IR syndrome was modeled on male Wistar rats weighing 160–200 g, 3 months of age by continuous intraperitoneally

dexamethasone administration in low doses (15 μg/kg), while feeding high calorie diet (containing 29% fat—mostly saturated lipids) enriched with fructose (daily 1/100 g of body weight as an aqueous solution) for 5 or 10 weeks [31].

Determination of arginine content in blood serum was carried out using a photometric method based on the reaction with α-naphthol and hypobromite reagent. The citrulline content was determined by reaction with diacetyl monoxime in strongly acidic conditions [32]. The content of nitrites and nitrates in blood serum was determined by spectrophotometric method using Griess test. The method is based on the determination of the total level of nitrogen (II) oxide metabolites (blood serum is incubated with a Griess reagent without vanadium chloride (III), determined spectrophotometrically). To obtain the value of the content of nitrates, it has to subtract concentrations of nitrite ions from the obtained value (the blood serum was incubated with a Griess reagent and vanadium chloride (III), spectrophotometrically determined) [33]. The serum endothelin-1 (ET-1) content was measured by the immune enzyme method using a standard set of reagents (DRG, manufactured in Germany). The content of nitrogen (II) oxide stable active metabolites S-nitrosothiols was determined by fluorimetric method [34].

Experimental animals were administered APFC for 2 weeks (since third week of pathology simulation) using a special iron catheter for intragastric administration. The dose of APFC was calculated as total content of polyphenols—9 mg/100 g of body weight (prophylactic treatment). The dose of reference preparation EGCG (produced by Sigma-Aldrich, Germany) was 3 mg/100 g of body weight. Daily animal equivalent doses (AED) were calculated according to the latest recommendations for preclinical studies, taking into account the average daily dose for humans and interspecies difference in body weight and body surface area [13].

The results were statistically processed using the 4Pl statistical logistic method with free internet service of MyAssays® and nonparametric analysis methods (Mann-Whitney U-Test) with the standard software package STATISTICA 7.0 [14].

2.2.2 Results

Under our experimental conditions, it was observed a significant simultaneous increase in glucose and IRI level, which at the IR development initial stages (fifth week of experiment) mediated the nitrogen oxide production increase; it was confirmed by the nitrate and nitrite evaluation of the (NO-2 + NO-3) content. The last fixed fact was caused by the insulin inducing effect on the endothelial cell NO-synthase (iNOS) and the arginine flow activation (NOS substrate) into the cells (**Table 4**). Since citrulline is the another product of the NO-synthase reaction in addition to nitrogen (II) oxide, in our experiments, growth of this indicator was observed, which directly correlated with the increase in the content of NO-2 + NO-3 (**Table 4**).

According to the other studies in *in vivo* experiments, it has shown that insulin in physiological concentrations mediates NO-dependent vasodilation, which is accompanied by blood pressure normalization. The insulin vasoprotective action is based on the phosphatidylinositol-3-kinase activation in endothelial cells and microvessels. It leads to the endothelial NOS gene expression and insulin-mediated vasodilatation.

Hyperglycemia that developed under our experimental conditions led to the iNOS expression up-regulation and increased ROS production with free radical processes activation. Simultaneous increase in the nitrogen oxide and ROS content resulted in the peroxynitrite formation and significantly contributed in ED pathogenesis. The synchronous free radical formation under hyperglycemia caused damage to protein and lipid cell structures, which led to the highly toxic lipoperoxide

compound formation that enhanced the cell membrane destabilization. These pathological changes are accompanied by antioxidant defense dysfunction, which resulted in cell energy homeostasis disorders due to the tricarboxylic acid cycle suppressing and uncoupling of tissue respiration and oxidative phosphorylation. Furthermore, NO hyperproduction stimulated COX-2 expression, which in turn was accompanied by the vasoconstrictor factor formation intensification (thromboxane A2, an antagonist of NO). The combination of these factors creates preconditions for endothelium damage and the ED progression.

It is known that prolonged hyperglycemia stimulates the polyol pathway of glucose metabolism, leading to the depletion of glutathione reserves (potent antioxidant factor) and endothelial NOS-NADPH(H^+) cofactor in endothelial cells. The conditions of lasting hyperglycemia cause eNOS activity inhibition and, as a result, a decrease in the nitric oxide formation [35]. The above changes are corrected by increasing of diacylglycerol and protein kinase C activity. Literature data analysis indicates the prominent role of chronic hyperglycemia as a factor of the vascular complications pathogenesis under the treatment of IR and associated diseases, primarily angiopathy under diabetes mellitus. Lasting hyperglycemia is accompanied by the increase in the content of glycosylated hemoglobin and other end products of glycosylation. The last ones significantly reduce the availability of nitrogen (II) oxide, which is an important factor in endothelial function disorders. Hyperglycemia induces an increase in the lipid peroxidation products content that suppress the endothelium ability to vasodilatation [36].

At the tenth week of the experiment, under the lasting IR, we observed a significant increase in the endothelin (ET-1) content (in 2.58 times), while the concentration of stable NO metabolites (S-NO) was significantly lower (in 3.26 times) compared with indices in healthy animals. The ED development was confirmed by the calculation of the ET-1/S-NO ratio, which was 8.4 times higher in animals with IR than the similar index in healthy animals (**Table 5**).

ET-1 has a prognostic importance for cardiovascular pathologies. This vasoconstrictor factor is a marker of coronary atherosclerosis and coronary ED. The results of other authors' studies indicate a significant role of abdominal adiposity in increasing of the ET-1 production, which evidences about increased vasoconstrictor factors production under MS. The ET-1 content directly correlates with the average blood pressure. This marker is considered as an independent factor in the development and arterial hypertension progression in patients with obesity and MS [37].

2.2.3 Discussion

Under the conditions of experimental IR, the ED formation occurs gradually with the several pathological mechanisms involvement. On the one hand, there are disorders in the NO-synthase system functional activity. On the other hand, there is a marked imbalance of antioxidant-prooxidant factors in the direction of prooxidants content increasing.

According to some researchers, under the IR in the endothelium is stimulated the vasoconstrictor substances synthesis (ET-1, thromboxane A2, prostaglandin F2) and reduced the vasodilator production (prostacyclin and nitric oxide). Lasting IR syndrome is accompanied by a decrease in endothelial- and insulin-mediated vasodilation. The activation of the MAPK-passway by insulin via stimulation of the different growth factors is an important aspect of the pathogenesis of vessel damage caused by insulin under IR. Thus MAPK activation leads to stimulation of proliferative processes and the smooth muscle cell migration, strengthening of vascular remodeling, and proatherogenic changes. It is believed that one of the points of the arterial hypertension pathogenesis under MS is the ability of insulin to promote

hypertrophy of smooth muscle vessels. Under IR, the endothelium becomes a target for a number of damaging factors (oxidized LDL, free radicals, etc.), which is accompanied by disorders in the endothelium functional state and creates the basis for the cardiovascular complication development [38].

Our previous studies, which correspond with literature data, indicated that experimental IR is accompanied with proatherogenic changes in the blood profile, and the ET-1 index increase is a marker of hypoxic state that is specific to atherogenesis. Oxidatively modified very low-density lipoproteins (VLDL) are some from the ET-1 synthesis inductors [31]. Thus, under the conditions of our experiments, the ED formation was evident; it was confirmed by the corresponding changes in the studied parameters. These change dynamics proved proatherogenic disorders that took place under this modeled pathology. In general, the ED development mediated by atherogenesis is a main factor in the CVD complication pathogenesis, which caused high mortality rates.

The tested APFC administration to animals deeply corrected the pathological changes that are accompanied by the ED formation at different stages of the experimental IR development. This fact was proved by significant positive changes and normalization of the endothelium function marker content.

Under the action of food concentrate in animals, which were injected by dexamethasone for 5 weeks and fed with a high-calorie diet, the nitrate/nitrite and citrulline contents were reduced to almost healthy animals' levels. Naturally, there was a significant increase in arginine level that was not used as intensively as the substrate for NOS (as was in the case with animals of model pathology).

The above changes are the result of the complex APFC component therapeutic effects. The antioxidant-prooxidative balance was shifted, and free radical processes were activated under the IR. The formation of ED under lasting IR state was mediated by the induction of NADPH(H^+) oxidase endothelial expression, in which induction correlates with the high superoxide anion production. It, in turn, reduces the nitrogen oxide bioavailability and its stable metabolite content, which occurred in the conditions of our experiments (**Table 5**). The APFC administration to rats prevents these changes: the S-NO content significantly increased while the vasoconstrictor ET-1 level and their ratio were decreased. The possible mechanism can involve both direct antioxidant activity of phenolic compounds and suppressing of the NADPH(H^+)-oxidase expression, which plays a prominent role in the oxidative stress development in the endothelium. The ability of phenolic compounds in red wine and green tea to suppress the expression of NADPH(H^+)-oxidase subunits (in particular, p22phox and nox1) and, respectively, to inhibit the endothelial production of superoxide anion was proved [39]. Polyphenols are also characterized by an increase in catalase activity. Phenolic compounds, due to the phosphoinositol pathway initiation, stimulate the NO production and the endothelial hyperpolarizing factor. Taking into account the chemical composition of these substances and the APFC are similar, it is possible that their mechanisms for the realization of biological activity, in particular vasoprotective action, can be equivalent.

The concentrate contains the highest quantity of gallic acid, which mediates a variety of biological effects. According to literary data, gallic acid has powerful anti-inflammatory properties, which are mediated by several mechanisms. It has been demonstrated that gallic acid and its esters (gallates) have ability to inhibit NF-κB activation, mainly due to suppression of IL-1 and tumor necrosis factor (TNF) (including endothelial cells) production. Thus, gallic acid and its derivatives are capable of inhibiting the cytokine-induced nuclear translocation of NF-κB. It is assumed that galactic acid blocks the activation of the NF-κB and Akt signaling pathway through the suppression of COX and ribonucleotide reductase (It is

associated with the presence of anticarcinogenic properties). Some studies have shown the gallic acid antagonism against P-selectin, which, as known, mediate the blood-formed element adhesion, in particular, leukocytes and monocytes, to the endothelium, which is accompanied by the inflammation progression. Considering the fact that the inflammatory reaction in the endothelium is one of the main components in the ED formation, inhibition of this process prevents the disorders in its functional activity. Gallic acid reduces the secretion of monocyte chemotaxis protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), and vascular endothelial adhesion molecule 1 (VCAM-1) in endothelial cells [40].

The therapeutic effect on hyperglycemia and hyperinsulinemia mediated the peroxynitrite production suppression (specific to quercetin), which was resulted in the NO-synthase system improvement (at the initial stages of the model pathology development) [41].

Long-term quercetin and its metabolites supplementation, which are contained in the studied concentrate, caused AMPK and eNOS activation (probably via increasing phosphorylation) in endothelium cells. This activation leads to an increase in the S-NO concentration that was observed in the conditions of our experiments (**Table 5**). In this case, quercetin was able to reduce the content of ET-1, which was also determined in our experiments (**Table 5**), and it was corresponded with the literature data. It is supposed that quercetin reduces H_2O_2-induced expression of the mRNA of ET-1, and thus, it reduced ED severity mediated by ET-1 hyperproduction. In addition, quercetin reduces the p47phox subunit of NADPN(H^+)-oxidase expression by protein kinase C inhibition, which is resulted in an oxidative stress reduction. Quercetin supplementation leads to a decrease in platelet excessive aggregation, which leads to atherosclerosis complications and stenotic arteries embolism. According to the literature, quercetin exhibits distinct anti-inflammatory effects, reducing the activation of NF-κB, TNF-α, IL-1β, IL-6, and SRP. It is known that anti-inflammatory properties of quercetin are improved by ascorbic acid (the studied concentrate contains both components that suggested more pronounced anti-inflammatory effect) [21, 22].

Long-term supplementation of caffeic, chlorogenic, and ursolic acids exhibits a potent antioxidant effect and increases the nitrogen oxide bioavailability, which was proved by S-NO increase in our experiments. According to the other authors, chlorogenic acid is characterized by ability to decrease in the malondialdehyde content in LDL, which is evidenced about their stability against oxidative modification and, accordingly, prevention of proatherogenic changes [42].

The EGCG administration also improved the pathological changes in the endothelium functional state, mediated by the experimental IR, but this effect was less pronounced at all stages of the model pathology development than the effect of APFC. It should be noted that at the experimental IR initial stage (fifth week of the experiment), the effect on nitrates and nitrites level, arginine, and citrulline content under EGCG administration was significantly less compared with the results determined in the group of animals that administered the food concentrate.

Thus, the APFC administration prevented ED formation under experimental IR. We suppose that this effect was mediated by the complex synergistic effect of its constituent components including a potent antioxidant effect and abilities to modulate the activity of nitric oxide synthase and suppress the ET-1production, resulting in a vasoprotective effect. The EGCG administration was also accompanied by an improvement of the endothelium functional state under experimental pathology; however, the EGCG therapeutic effect was less pronounced compared with complex compound—the APFC, due to multivector effects on different parts of the ED formation.

3. The study of the blueberry (*Vaccinium myrtillus* L.) extract effectiveness under experimental diabetes mellitus induced by a high-fructose diet combined with dexamethasone injections

3.1 Materials and methods

DM2 is an endocrine disorder characterized by defects in mechanism of glucose insulin-mediated transport into the cells, resulting in persistent hyperglycemia. It is well known that glucose high levels lead to the free radical overformation, which activates lipoperoxidation—one of the main pathogenetic factors in atherosclerosis development [3, 4].

Bilberry leaf chemical composition usually consists of flavonoids, proanthocyanidins, triterpenes, and also phenolic compounds, in particular, of myricetin, which revealed an effectiveness at the DM2 initial stages of according to some authors [43, 44].

The experiment was conducted on male Wistar rats weighing 160–200 g. The experimental animals were divided into groups: (1) intact animals fed a standard vivarium chow; (2) animals fed a high-fructose diet (2 g of fructose/100 g of body weight daily) combined with daily subcutaneous dexamethasone injections in the dose of 2 mg/100 g body weight for 6 weeks; and (3) animals, which administered intragastric feeding of bilberry leaf dry extract in the dose of 9 mg/100 g of body weight for 2 weeks in addition to dexamethasone administration [45].

The glycosylated hemoglobin (HbA1C) concentration was determined in blood serum by immunoturbidimetric method. The fructosamine level was measured by the spectrophotometric method using nitro-blue tetrazolium chloride [46]. The area under the glycemic curves (AUC) during IGGTT was calculated using the computer software program MATLAB [47]. The glucose content was determined using standard kit of "Filisit-Diagnostika" (Ukraine). The basal level of immunoreactive insulin (IRI) was measured in the blood of animals by the in vitro radioimmunoassay using a standard set of reagents produced by "Immundiagnostik" (Germany) [10].

The lipoperoxidation markers were determined spectrophotometrically by the measuring of diene conjugates (DC) and TBA-reactive products (TBA-RP) content using reaction with thiobarbituric acid [48]. The antioxidant system status was evaluated via determination of reduced glutathione (GSH) concentration (spectrophotometrically by reaction with alloxan) and glutathione reductase (GR) activity [49]. Glucose homeostasis in test and control animals was evaluated at different times after the model inducing (7, 14, and 21 days) by basal glycemic indexes and intraperitoneal glucose tolerance test (IPGTT, 3 g/kg body weight) [50]. Statistical processing of the experimental data was performed using the STATISTICA software program (StatSoft Inc., USA, version 6.0). The significance of intergroup differences was estimated according to the Student's t-test [14].

3.2 Results and discussion

The dexamethasone low dose administration to laboratory animals under high-fat diet caused the multiple disorder formation specific to MS and DT2. In the rats' blood serum, there was a significant increase in the glycosylated hemoglobin level, fructosamine concentration, glucose content, basal glycemia, and increased area under glycemic curves. These changes were probably developed due to a decrease in the glucose utilization by peripheral tissues caused by inhibition of the glucose transporters expression (GLUT1 and GLUT4) under the influence of dexamethasone action. Change in HbA1C level is an objective indicator of carbohydrate metabolism in diabetic patients and the effectiveness of glycemic control. An increase in

Parameters	Intact control	Model pathology	BLE + model pathology
Glycosylated hemoglobin (%)	7.5 ± 0.5	9.6 ± 0.7*	8.4 ± 0.6*,#
Fructosamine (mmol/l)	1.91 ± 0.12	3.6 ± 0.29*	2.45 ± 0.24*,#
Basal blood glucose level (mmol/l)	4.04 ± 0.11	13.42 ± 0.38*	12.08 ± 0.24*
AUC (glycemic) (mmol/l/min)	625.44 ± 16.56	2092.25 ± 60.48*	1100 ± 61.56*,#
Glucose, mmol/l	4.6 ± 0.1	11.2 ± 0.2*	8.2 ± 0.1*,#
IRI (pmol/l)	221.74 ± 20.79	317.97 ± 39.72*	302 ± 36.70*

n, number of animals in a group. *Variation is statistically significant in comparison with intact control group indices ($p \leq 0.05$).*
#Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.05$).

Table 6.
The effect of bilberry leaf extract on carbohydrate metabolism indices under experimental diabetes mellitus induced by high-fructose high-calorie diet combined with dexamethasone injection, n = 10.

Parameters	Intact control	Model pathology	BLE + model pathology
TBA-AP (nmol/g)	0.96 ± 0.27	3.56 ± 0.41*	0.98 ± 0.39#
DC (nmol/l)	22.30 ± 1.42	28.5 ± 0.94*	26.9 ± 0.96
GSH (mmol/g)	0.25 ± 0.01	0.12 ± 0.02*	0.19 ± 0.03
GR (nmol/min mg) of protein	18.56 ± 0.64	14.20 ± 1.06*	17.9 ± 0.98

n, number of animals in a group. *Variation is statistically significant in comparison with intact control group indices ($p \leq 0.05$).*
#Variation is statistically significant in comparison with model pathology group indices ($p \leq 0.05$).

Table 7.
The effect of bilberry leaf extract on the specific markers of antioxidative-prooxidative status of rat's liver in experimental diabetes mellitus induced by high-fructose high-calorie diet combined with dexamethasone injection, n = 10.

the content of glycosylated hemoglobin is usually considered as an indirect marker for the retinopathy development, nephropathy, and other complications of diabetes. Due to the long-term hyperglycemia, albumin was subjected to nonenzymatic glycosylation, which was confirmed by the increase in fructosamine in our experiments (**Table 6**). The dry bilberry leaf extract (BLE) administration under model pathology was accompanied by a significant normalization of the studied indices (**Tables 6** and 7).

In particular, it was significant for the suppression of glycosylated hemoglobin (by 12.5%) and fructosamine (by 32%) concentration, hyperglycemia (by 26.8%), the area under the glycemic curves (by 47.4%), and the immunoreactive insulin level (by 5.1%) compared to untreated animals. In our opinion, it is due to the high content of different phenolic compounds in the bilberry extract, which have hypoglycemic and antioxidant effects. The mechanism of hypoglycemic effect of polyphenols is related to their impact on the process of glucose transport into the cell. The main role in hypoglycemic action of the extract of bilberry leaves belongs to myrtilline, which is a mixture of delphinidin and malvinidin esters that act as insulin synthesis activator [51].

It is proved that hyperglycemia is accompanied by free radical processes activation, which leads to complications in the main disease pathogenesis and tissue damage. Under the conditions of our experiments, the model pathology formation

was accompanied by the oxidative stress development, which was confirmed by significant increase in the content of TBA-AP and DC (basic lipoperoxidation products) in the liver—by 270.8 and 27.8%, respectively, compared with healthy rats. At the same time, antioxidant defense was reduced as evidenced by the decrease in the content of GSH and GR in the liver by 52 and 23.5%, respectively, compared to intact control animals. The above changes confirmed the oxidative stress development under pathology. The BLE administration under the model pathology led to the antioxidative-prooxidative balance normalization, which was evidenced by the significant suppression of lipoperoxidation and antioxidant status improvement, which was reflected in the relevant indicator dynamics. We suggested that these changes were the result of remarkable antioxidant and antiradical properties of the investigated extract components.

Thus, the effectiveness of the BLE under the experimental DT2 primary was due to the expressive antioxidant properties of the biologically active substances that are part of its composition.

4. Conclusions

Summarizing the above, the results of these and other experiments demonstrate that natural antioxidants of polyphenolic structure have a significant corrective effect on major factors in the pathogenesis of obesity. Firstly, polyphenolic antioxidants promote lipid and carbohydrate metabolism, which is very important for directional therapy of obesity. In the other hand, these compounds prevent comorbid conditions of obesity, such as endothelial dysfunction and atherosclerosis, which greatly increases the risk of mortality. Also, polyphenolic antioxidants reverse insulin resistance and its after-effects linked with oxidative tissue damage. The main explanation of such protective effects of natural polyphenolic compounds is based on their powerful antioxidative properties, because the pathogenesis of noted disorders is usually amplified by exponential releasing reactive oxygen species and their products. Consequently, the pool of current experimental data points out that concentrates of polyphenolic compounds could be used in adjuvant therapy of obesity, diabetes, and similar conditions, complicated by metabolic disorders and free radical oxidation.

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