UDC 615.322:615.451.1:616.379
DOI: 10.15587/2519-4852.2024.318509

EFFECT OF A NEW PHYTOCOMPOSITION BASED ON POLYPHENOLIC EXTRACT FROM CRANBERRY LEAVES AND AMINO ACIDS ON THE STATE OF THE PANCREAS IN METABOLIC SYNDROME

Mariia Anisimova, Nadiia Kononenko, Valentyna Chikitkina

The aim of this work *was to study the effect of a phytocomposition based on a polyphenol extract from large-fruited cranberry leaves and amino acids (L-arginine, taurine, glycine) on the state of the pancreas and liver in experimental metabolic syndrome in rats.*

Material and methods. *Metabolic syndrome was reproduced using a high-sucrose diet, which was provided by replacing drinking water with a 30 % sucrose solution in drinkers in a free-access mode for 8 weeks. A phytocomposition based on a polyphenol extract from large-fruited cranberry leaves and amino acids at a dose of 100 mg/kg and comparison drugs phytocollection "Arfazetin" and tablets "Metformin" were administered daily during the reproduction of pathology for 8 weeks. The ability of the phytocomposition to improve glucose tolerance was evaluated in an oral glucose tolerance test. Histologically, the state of the pancreas was assessed with staining sections with hematoxylin and eosin, aldehyde fuchsin according to Gomora, and morphometric measurements of pancreatic islets were carried out. The liver sections were subjected to the McManus PAS reaction to detect glycogen; to verify neutral fats, the liver sections were stained with Sudan IV.*

Results. *Preventive administration of a phytocomposition based on a polyphenol extract from large-fruited cranberry leaves and amino acids at a dose of 100 mg/kg for 8 weeks had significantly improved glucose tolerance, a positive effect on the morphological state of the insular apparatus of rats: in the overwhelming majority of islets, cells with normal density were observed, which were uniformly distributed over the entire area of the islet. Also, no signs of hypertrophy or dystrophy of β-cells were detected. Under the influence of the studied phytocomposition, liver hepatocytes had normal glycogen saturation and a minimum content of neutral fats in the cytoplasm. In terms of the severity of the protective effect on the insular apparatus of the pancreas of rats, the phytocomposition exceeded the "Arfazetin" collection and was not inferior to "Metformin" tablets, and in terms of the degree of restoration of impaired metabolic changes in the liver, it surpassed both comparison drugs.*

Conclusion. *Two-month maintenance of rats on a diet high in sucrose was characterised by impaired glucose tolerance, pathomorphological changes in the insular apparatus of the pancreas, impaired glycogen-forming function of the liver and development of steatohepatosis. Phytocomposition based on polyphenolic extract of cranberry leaves and amino acids at therapeutic and prophylactic administration on the background of a high-sugar diet significantly improved glucose tolerance, prevented dystrophic and necrobiotic changes of β-cells, depletion of glycogen stores and fat accumulation in the liver of rats. The phytocomposition based on the polyphenolic extract from large-fruited cranberry leaves and amino acids was superior to the reference preparation "Arfazetin" and practically not inferior to "Metformin" tablets in its ability to limit morphological changes in the pancreas tissue and exceeded both comparison preparations in the degree of restoration of disturbed metabolic changes in the liver. The results indicate the prospect of further experimental studies on the pharmacological properties of phytocomposition based on the polyphenolic extract of cranberry leaves and amino acids in order to create an effective phytomedicine for the correction of metabolic syndrome and type 2 diabetes mellitus manifestations*

Keywords*: metabolic syndrome, high sugar diet, large-fruited cranberry, amino acids, pancreas, liver*

How to cite:
Anisimova, M., Kononenko, N., Chikitkina, V. (2024). Effect of a new phytocomposition based on polyphenolic extract from cranberry leaves and amino acids on the state of the pancreas in metabolic syndrome. ScienceRise: Pharmaceutical Science, 6 (52), 47–59. http://doi.org/10.15587/2519-4852.2024.318509

1. Introduction

According to modern concepts, metabolic syndrome (MS) is a complex of metabolic, hormonal and clinical disorders that are high-risk factors for the development of cardiovascular diseases. The main manifestations of MS are considered to be abdominal obesity, insulin resistance and compensatory hyperinsulinemia, dyslipidemia, arterial hypertension, impaired glucose tolerance/type 2 diabetes mellitus, early atherosclerosis/ischemic heart disease, hemostasis disorders, hyperuricemia and gout, microalbuminuria, hyperandrogenism [1, 2].

Today, MS attracts the attention of many specialists because it precedes the onset of type 2 diabetes mellitus (type 2 DM) and atherosclerosis – diseases that are nowadays the main causes of mortality [3, 4]. The increased interest is also due to the fact that MS is a revers-

ible condition – with appropriate, timely treatment, it is possible to achieve the disappearance or marked reduction of its main manifestations.

The main goal of MS patients' treatment is correction of the main MS components - insulin resistance and hyperinsulinemia, arterial hypertension, obesity and lipid metabolism disorders. Along with lifestyle changes and medication correction, an important role in the complex therapy of MS and type 2 DM is given to herbal remedies, which, due to a wide range of pharmacological action, can regulate the parameters of carbohydrate and lipid metabolism, blood pressure, blood rheological properties, normalise weight [5, 6].

The only official herbal preparation for the therapy of metabolic syndrome and type 2 DM – collection, "Arfazetin", is registered in the pharmaceutical market of Ukraine.

The undoubted advantages of natural origin are a favourable safety profile and the possibility of use in complex therapy in combination with synthetic drugs. However, the disadvantage of phytosborations is the inconvenience of use by patients, which is associated with the preparation of the dosage form for consumption (e.g., decoction) at home, the lack of standardisation of the final dosage form, etc. These factors can significantly impact the effectiveness of therapy, which may be accompanied by impaired glycemic control, resulting in complications of the underlying disease and its progression. Because of the peculiarities of the pathogenesis of MS and type 2 DM and the leading role of lipid metabolism disorders and activation of free radical oxidation, the development of effective standardised agents of natural origin with distinct lipotropic and antioxidant properties for use in the complex therapy of these insulin-resistant conditions remains an urgent issue.

The choice of large-fruited cranberry leaves as a raw material for obtaining polyphenolic extract is due to its significant content of phenolic compounds (simple phenols, hydroxycinnamic acids, in particular, gallic and chlorogenic acids, flavonoids, ascorbic acid) [7, 8]. Large-fruited cranberry has a sufficient raw material base, is widely distributed in forest phytocenoses of the Carpathians and territories of Northern Ukraine.

Establishment of the peculiarities of the influence of phytocomposition containing polyphenolic extract from the leaves of large-fruited cranberry and amino acids (L-arginine, taurine, glycine) on the pathogenetic links of MS and mechanisms of its hypoglycemic activity is a priority and relevant, and the results obtained will contribute to the development of new phytopreparations with metabolitotropic action.

The **aim** of the study was to investigate the effect of phytocomposition based on the polyphenolic extract of cranberry leaves and amino acids (L-arginine, taurine, glycine) on the state of the pancreas and liver in experimental metabolic syndrome in rats.

2. Planning (methodology) of research

The study protocol describing the different stages of the present research work is presented in the following flow chart (Fig. 1).

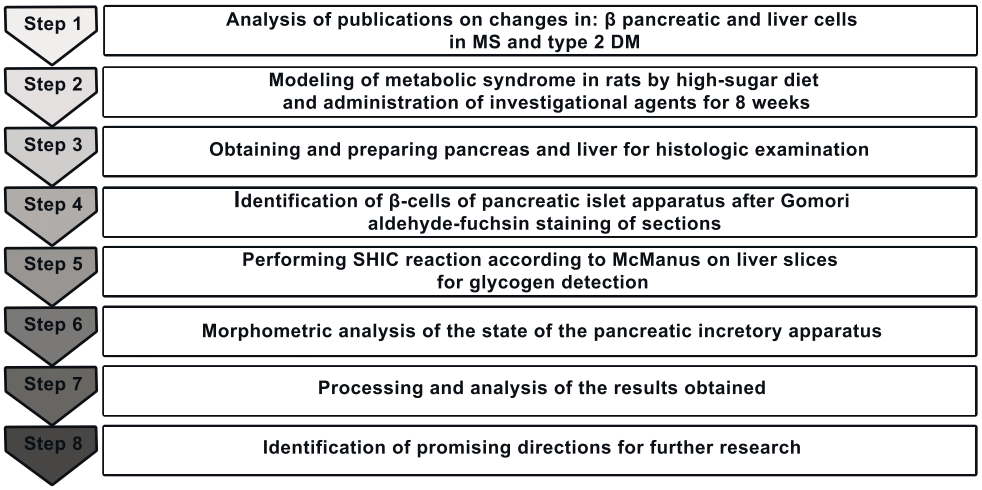


Fig. 1. Study protocol

3. Material and methods

The studies were conducted at the Department of Normal and Pathological Physiology and based on the Training and Research Institute of Applied Pharmacy of the National University of Pharmacy from February 1, 2022, to March 6, 2023.

Dried large cranberry leaves were ground to a 1–3 mm particle size and extracted three times with a 50 % ethyl alcohol solution at a total ratio of 1:10 at room temperature overnight.

Dry alcoholic extract of cranberry leaves with the addition of amino acids was obtained at the Department of Pharmacognosy of the National University of Pharmacy under the guidance of Professor Koshevoy O. M. The choice of extractant for obtaining the extract was carried out experimentally. Through experiments, it was found that the use of 50 % alcohol in the ratio of 1:10 (considering the absorption coefficient of the extractant) has the highest yield of dry extract from the leaves of large-fruited cranberry and the quantitative content of hydroxycinnamic acids 12.23 % and flavonoids 4.01 %, which are responsible for the hypoglycemic effect. The given ratio is sufficient for obtaining a remedy for correcting insulin-resistant states and optimal in technological terms from the point of view of obtaining the claimed remedy in industrial conditions and rational use of alcohol. The extracts were combined and purified by settling for 24 hours at room temperature, and the supernatant was separated by filtration, after which arginine, taurine and glycine were added in three times equimolar amount relative to the total amount of phenolic compounds in the extract, infused for 24 hours and evaporated to a dry extract.

The dry extract obtained from large cranberry leaves contains at least 10 % phenolic compounds in the form of gallic acid, at least 5 % hydroxycinnamic acids in the form of chlorogenic acid, and at least 2 % flavonoids in the form of rutin.

As reference preparations, we selected the phytoset "Arfazetin" (loose pack of 75 g, PJSC "Lectravy", Ukraine) and metformin (Metformin Sandoz, tablets, 500 mg, LEC S.A. Poland).

The choice of the collection "Arfazetin" is due to the fact that, at the moment, in the pharmaceutical market of Ukraine, it is the only preparation of plant origin with antidiabetic activity. The collection consists of blueberry shoots (20 %), husks of common bean fruits (20 %), rhizome and roots of Echinopanax high (lureberry) (15 %), rose hips (15 %), horsetail grass (10 %), St. John's wort herb (10 %), chamomile flowers (10 %).

Metformin, the antidiabetic agent of the biguanide group, is the first-line pharmacotherapy of type 2 DM and is used to correct insulin resistance in MS [9].

The doses of comparison drugs were calculated considering the species resistance coefficients of humans and rats. Metformin was administered at a dose of 60 mg/kg of rat weight, taking into account the average daily dose for humans of 2000 mg; Arfazetin collection was administered at a dose of 18 ml/kg.

The study was performed on male Wistar rats weighing 270–300 g, kept under standard conditions of vivarium NUPh at the temperature of 18–24 °C, 60–70 % humidity, natural light mode day/night in plastic cages no more than 4 animals in each. Animals were acquired in BioModelServis, Ukraine. The work with animals was carried out following the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), «Procedure for conducting experiments, experiments on animals by scientific institutions» [10]. Euthanasia was performed by an overdose of ether anesthesia or cervical dislocation. The Commission on Bioethics of the NUPh did not reveal any moral and ethical standards violations when planning and conducting research (No. 7 of 20.10.2022).

The metabolic syndrome was reproduced using a high sucrose diet (HSD), which was provided by replacing drinking water with 30 % sucrose solution in drinkers in a free-range mode for 8 weeks [11].

The investigated agents were administered intragastrically daily, once simultaneously with sucrose solution, for 8 weeks. The following groups of animals weighing 270–300 g were used: group 1 – intact control (IC); group 2 – animals of control pathology (CP), which received 30 % sucrose solution; group 3 – animals, which were administered (PC) at a dose of 100 mg/kg against sucrose [12], groups 4–5 – animals phytocomposition, which were administered comparison drugs against sucrose.

The glucose homeostasis of experimental animals was assessed by basal glycemia and carbohydrate tolerance levels, which were determined using the oral glucose tolerance test (OTTG) at the end of the experiment. Basal glucose concentration was determined on an empty stomach after an 18-hour fast in rat blood samples obtained from the tail vein. Then, glucose solution was administered intragastrically at a 3 g/kg dose for OTTG. Blood samples for glucose level determination were taken 30, 60, and 120 minutes after glucose loading [11].

The level of glucose in animals' blood was determined by the glucose oxidase method using chemical reagent kits "D-glucose" produced by Filisit-Diagnostika (Ukraine).

After all animals were removed from the experiment, liver and pancreas samples (body and tail parts extracted from the gastric-splenic ligament) were fixed in 10 % formalin solution and embedded in paraffin. Sections were stained with hematoxylin and eosin. Identification of β-cells of the pancreatic islet apparatus was performed after staining the sections with Gomori aldehyde-fuchsin, which stains basophilic granules of insulin in the cytoplasm of cells in blue-violet colour. This method allows us to determine the presence of cells in the islets of Langerhans where insulin synthesis occurs, thus assessing the functional state of β-cells [13–15].

Morphometric measurements were performed: on pancreatic sections (hematoxylin and eosin staining), we determined the optical density of pancreatic islets (PI – total number of islets in the microdissection), islet profile (number of β-cells in the islet). The islet profile was used to classify islets into small (up to 20 β-cells), medium (21–60 β-cells) and large (>61 β-cells) islets, and the percentage of each category of PI was determined [16]. According to the intensity of aldehyde-fuchsin staining and the character of distribution of basophilic insulin granules in the cytoplasm of insulinocytes, the morphofunctional state of β-cells in PI was assessed according to a 4-point system: 4 points – almost all β-cells are evenly filled with distinctly aldehyde-fuchsin stained granules; 3 points – a significant number of β-cells contain moderately aldehyde-fuchsin stained granules; 2 points – some β-cells are filled with moderately aldehyde–fuchsin stained granules, others are completely devoid of them; 1 point – cells with minimal amount of moderately or weakly aldehyde-fuchsin stained granules. All obtained numerical data were processed using variation statistics.

According to McManus, the SHIC reaction was performed on the liver slices to detect glycogen (control with salivary amylase). To verify neutral fats separately, liver samples were cut on a freezing microtome after fixation in formalin, and sections were stained with Sudan IV [14].

Microparaphrases were viewed under a Granum light microscope. Microscopic images were photographed using a digital video camera, Granum DSM 310. Photographs were processed on a Pentium 2.4GHz computer using the Toup View program.

Quantitative data were analysed using the standard statistical software package "Statistica 6,0". Quantitative data are presented as mean with standard error ($M \pm m$) and median with lower and upper quartiles (Me [Q25; Q75]). The parametric Student's t-test was used in the case of normal data distribution, the nonparametric Mann-Whitney U-test in the absence of normal distribution, and Fisher's angular transformation was used when considering the results in an alter-

native form. Differences were considered statistically significant if $p<0.05$.

4. Results

Maintenance of animals for 2 months in HSD conditions did not lead to the occurrence of fasting hyperglycemia. The level of basal glycemia after overnight fasting of rats did not go beyond the physiologic norm. It should be noted that in animals on the background of HSD received PC, Arfazetin collection and metformin, fasting blood glucose level was slightly lower but did not significantly differ from that in animals from IC and CP groups (Fig. 2).

During OTTG in rats of the CP group, a disturbance in glucose utilisation was found, which was manifested by an increase in blood glucose level by 120 % ($p<0.01$) compared to basal glycemia at the 30th minute and maintaining it throughout the test at a level that significantly exceeded IC and did not reach the physiological norm at the 120th minute of observation (Fig. 2). The rate and severity of impaired glucose tolerance may indicate the probable development of insulin resistance in animals of the CP group.

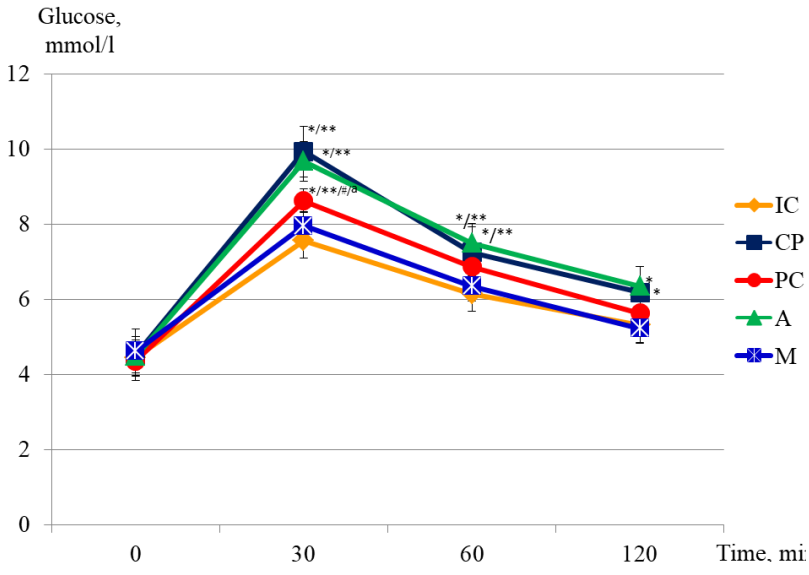


Fig. 2. Dynamics of glycemia during OTTG in rats after two months of maintenance on a high-sugar diet and exposure to phytocomposition, compared with the collection "Arfazetin" and tablets "Metformin" ($n=6$). IC – intact control; CP – control pathology; PC – phytocomposition at a dose of 100 mg/kg; A – Arfazetin collection; M – metformin tablets. Note: reliable differences: with baseline data – *$p<0.05$; with IC group – **$p<0.05$; with CP group – $^{\#}p<0.05$; with Arfazetin group – $^{a}p<0.05$

Under the influence of PC at a dose of 100 mg/kg against the background of two-month maintenance on HSD, a significant improvement of glucose tolerance was observed, which was manifested by a slower increase in blood glucose content at the 30th minute of testing by 93 % ($p<0.05$) compared to the basal level. At this test term, this index was significantly lower by 17.3 % ($p<0.05$) than the value of the CP group, statistically did not differ from the animals receiving Metformin and showed higher efficacy relative to Arfazetin. In the following terms of the study, blood glucose levels slowly decreased and, after 120 minutes, practically reached the values of the physiologic norm (Fig. 2).

In the group of animals receiving the comparison drug Arfazetin at the 30th minute of OTTG, the degree of severity of hyperglycemia compared to basal was the highest among the studied means and did not differ from the CP group (116 %). After 60 and 120 minutes, the blood glucose level gradually decreased but did not normalise to the physiological norm and exceeded the baseline level by 38 % ($p<0.05$) (Fig. 2).

When the comparison drug Metformin was administered at the 30th minute after carbohydrate loading, the smallest increase in blood glucose level compared to basal level by 72 % ($p<0.05$) was detected, which acquired significant differences compared to CP by 19.9 % ($p<0.05$). In terms of severity of hypoglycemic effect, metformin, similarly PC, was superior to the efficacy of Arfazetine by 17.8 % ($p<0.05$). After 120 minutes of observation, blood glucose content under the influence of metformin acquired a physiologic norm.

Thus, the studied phytocomposition at 100 mg/kg dose significantly improves glucose tolerance, is more effective than the antidiabetic drug Arfazetin and is not inferior to the drug of first choice in treating type 2 diabetes mellitus – Metformin.

In a pathomorphologic study in rats of the IC group, the glandular tissue of the pancreas consists of moderately sized lobules and a system of inter- and intra-lobular ducts, arteries and veins of a different calibre. Connective tissue membranes between the lobules are moderately expressed. In the lobules, tissue is clearly distributed into exo- and endocrine components. PI represents the endocrine component of the gland, most of which are clearly delimited from the surrounding exocrine parenchyma and have a rounded or oval shape. The main mass of cells in the islets was β-cells, which were rather densely and evenly distributed in the central part. Widespread sinusoidal capillaries are visible between the cell masses in a number of islets. When staining with aldehyde-fuchsin, the cytoplasm of β-cells is uniformly stained in blue-violet colour, indicating a normal functional activity level. α-cells are located in a chain along the periphery of the islets (Fig. 3).

The optical density of the PI was 23,6 units. The bulk of the islets contained 21-60 β-cells, which are medium-sized islets. Their share was 55.1 %. The share of small islets containing up to 20 β-cells accounted for 29.67 %, and large islets (>60 β-cells) accounted for 15.2 %. The intensity of aldehyde-fuchsinophilic staining of β-cells was 3.3 points (Table 1).

The exocrine parenchyma of the gland consisted of the terminal secretory sections of the glands – acini with a high density of arrangement. The acini consisted of one layer of glandular cells, which were typically dis-

tributed into two differently stained zones: the basal basophilic zone, which contained a round, dense nucleus, and the eosinophilic central zone, which contained small zymogen grains. The ratio of zones varied within the range of 1:1.5–1:2.5. The lumen of the acini is small. The epithelium of most ducts is normal, in isolated ones – in a state of moderate proliferation. Depending on the calibre of the excretory duct (mainly interlobular), the periductal stroma is also expressed differently; sometimes, single small lymphohistiocytic clusters are found in it. The lumen of the ducts is often widespread; in some places, clumps of thickened eosinophilic secretion are visible. The condition of the arteries and veins is normal, some of them are full-blooded.

When rats were kept on HSD, the number of small PI increased visually, and the number of medium PI decreased. Against the background of unchanged islets, islets with a "rarefaction" in the arrangement of β-cells of varying degrees of severity were found. Among the visually normal β-cells, cells with hypertrophy of nuclei and with unclear pale nuclei (possibly at some stage of lysis) were visible; vacuolisation of β-cells was observed. The appearance of large "branched" islets, as if "spreading" in width or length, attracts attention. The "branched" segments of the islet were separated from each other by very scanty layers of connective tissue or one layer of acini (Fig. 4).

Aldehyde-fuchsin staining showed a distinct uneven staining of the insulinocyte cytoplasm even within a single islet, which is a morphological reflection of the cells' different functional statuses (Fig. 5).

Morphometric analysis showed that the optical density of the PI was at the level of the intact control (23.3 units). Still, there were changes in the percentage distribution of islets by the content of β-cells. The proportion of small islets increased by 1.5 times ($p<0.001$). At the same time, the appearance of very small islets containing only 2–4 β-cells was observed. The proportion of medium-sized islets decreased by 1.9 times ($p<0.001$), and large ones – by 1.6 times ($p<0.01$). The intensity of aldehyde-fuchsin staining of β-cells in the islets was estimated at 2.2 points (Table 1). No changes in the state of the exocrine parenchyma were noted. Simultaneous consumption of 30 % sucrose solution with the introduction of PC positively affected the morphological state of the insular apparatus of rats. Firstly, much fewer small PI were visualized. In the overwhelming majority of islets, β-cells with normal density are uniformly distributed over the entire plane of the islet. No signs of hypertrophy or dystrophy of β-cells were found. The shape of the islets was oval-round, only single ones had an "atypical" shape. Only in a small part of the islets were signs of depletion and changes in the morphological state of β-cells. Aldehyde-fuchsin staining showed that the cytoplasm of most β-cells in the PI was saturated with a specific blue-violet colour, which indicated a sufficient presence of insulin granules (Fig. 6). No changes in the state of the exocrine parenchyma of the glandular tissue were observed.

As morphometry showed, the volumetric density of the PI did not change and was at the level of 22.5 units. The number of small islets decreased in relation to the control pathology by 1.4 times ($p<0.001$), while the number of very small islets (up to 4 β-cells) became significantly less. The presence of medium islets became 1.7 times ($p<0.05$) greater. The proportion of large islets decreased by 1.8 times ($p<0.001$). The intensity of aldehyde-fuchsinophilic staining of β-cells was 3 points (Table 1). Against the background of the introduction of the collection "Arfazetin", a rather noticeable discrepancy in the state of the PI was observed: various types of emptiness, unevenness in location, vacuolization of the cytoplasm and degenerative changes in β-cells in some, morphological completeness of others. The content of specific insulin granules in β-cells also fluctuated according to the expression of aldehyde-fuchsinophilic coloration (Fig. 7).

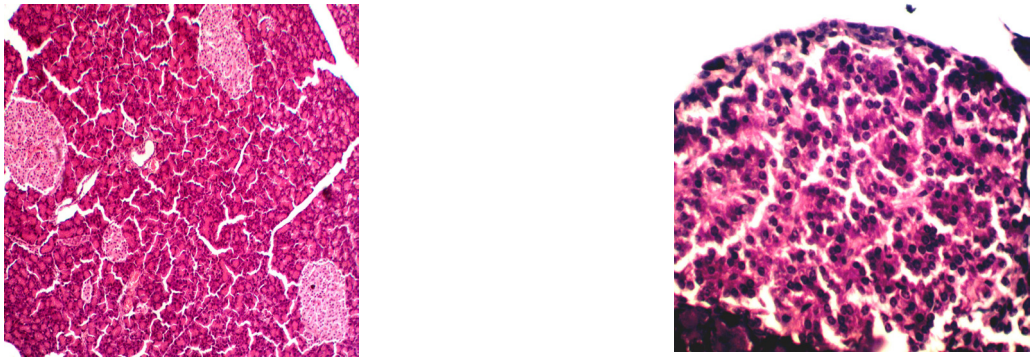
According to morphometry data, the proportion of small islets decreased by 1.3 times ($p<0.05$) and the proportion of medium islets increased by 1.3 times ($p<0.001$) in animals that were administered the "Arfazetin" mixture against the background of sucrose. The optical density of PI remained sufficient – 22.5 units in a micropreparation. The intensity of aldehyde-fuchsinophilic staining of insulinocytes was estimated at 2.5 points (Table 1).

Table 1

Morphometric analysis of the state of the endocrine apparatus of rats after two months of maintenance on a high-sugar diet and the influence of the phytocomposition in comparison with the collection "Arfazetin" and tablets "Metformin" (n=6)

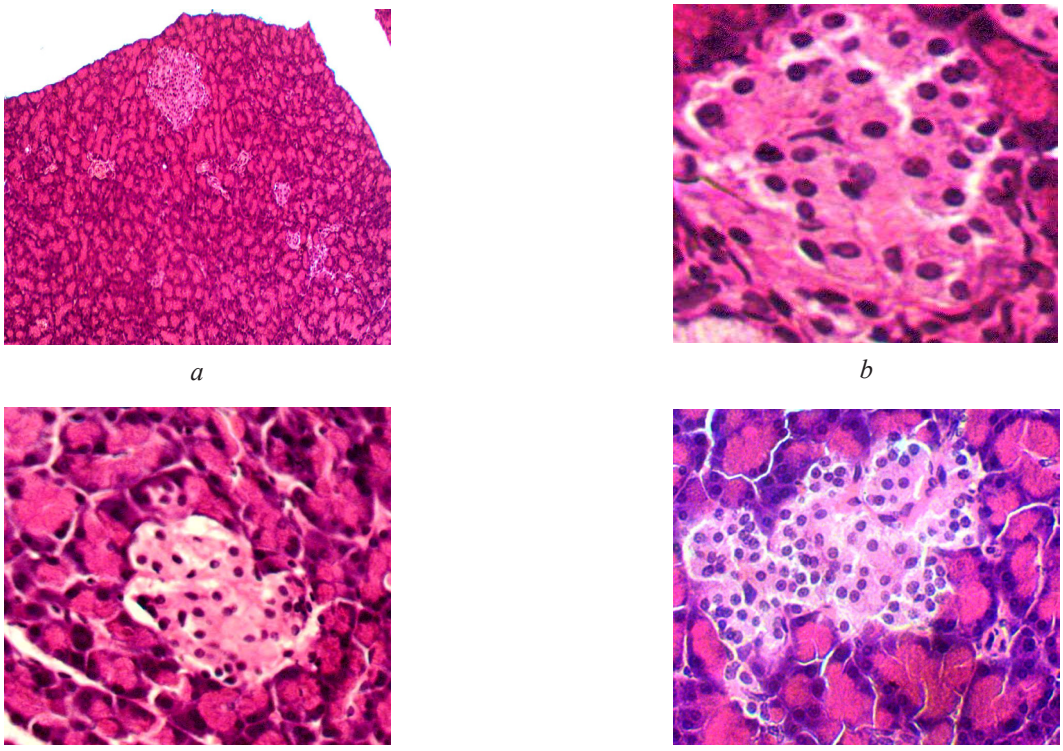
Group of animals	Optical density pancreatic islets	Distribution of pancreatic islets by β-cell content			Intensity of aldehyde-fuchsinophilic staining of β-cells (points)
		Small (to 20)	Average (21-60)	Big (>60)	
IC	23.7±0.42 [22; 25] 100 %	7.0±0.26 [6; 8] 29.67 %	13.0±0.26 [12; 14] 55.1 %	3.7±0.67 [2; 6] 15.2 %	3.3±0.21 [3; 4]
CP	23.3±0.49 [22; 25] 100 %	10.2±0.48* [9; 12] 44 %	7.0±0.26* [6; 8] 30.2 %	6.0±0.26** [6; 7] 25.8 %	2.3±0.21** [2; 3]
PC	22.5±0.43 [21; 24] 100 %	7.2 ± 0.17^{a} [7; 8] 32.14 %	$12.0\pm0.26^{\#/aa}$ [11; 13] 53.57 %	3.3 ± 0.21^{aa} [3; 4] 15.17 %	3.0±0.26 [2; 4]
Arfazetin	22.2±0.54 [20; 24] 100 %	$7.8\pm0.17^{\#}$ [7; 8] 35.1 %	$9.2\pm0.17^{\#\#}$ [9; 10] 41.44 %	5.2±0.31 [4; 6] 23.42 %	2.5±0.22 [2; 3]
Metformin	23.0±0.58 [21; 25] 100 %	7.2 ± 0.31^{a} [6; 8] 31.3 %	12.5 ± 0.43^{aa} [11; 14] 54.35 %	3.2 ± 0.54^{aaa} [1; 5] 13.91 %	2.8±0.31 [2; 4]

*Note: significant differences with intact control group – *p<0,001; **p<0.01; with control pathology group – $^{\#}$p<0,05; $^{\#\#}$p<0,001; with Arfazetin group – ^{a}p<0,05; ^{aa}p<0.001; ^{aaa}p<0.01.*



a *b*

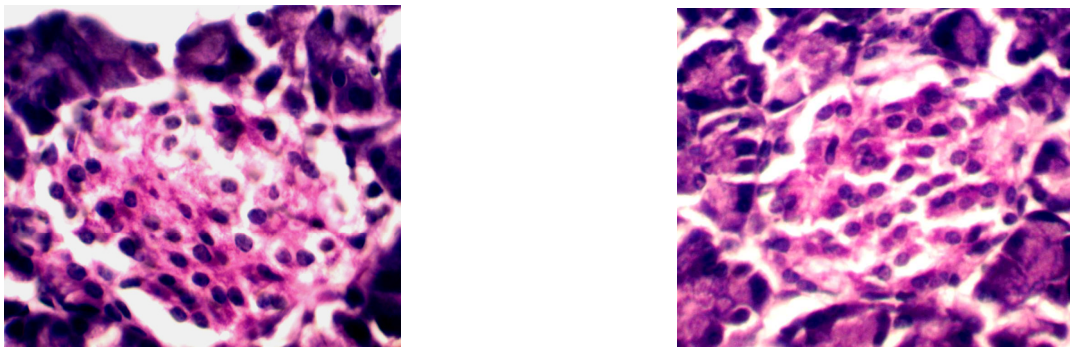
Fig. 3. Pancreas of an intact rat: *a* – pancreatic islets of different sizes among densely located acini are uniformly filled with β-cells (Hematoxylin-eosin. ×200); *b* – intense violet staining of β-cells (aldehyde-fuchsin according to Gomori, ×250)



a *b*

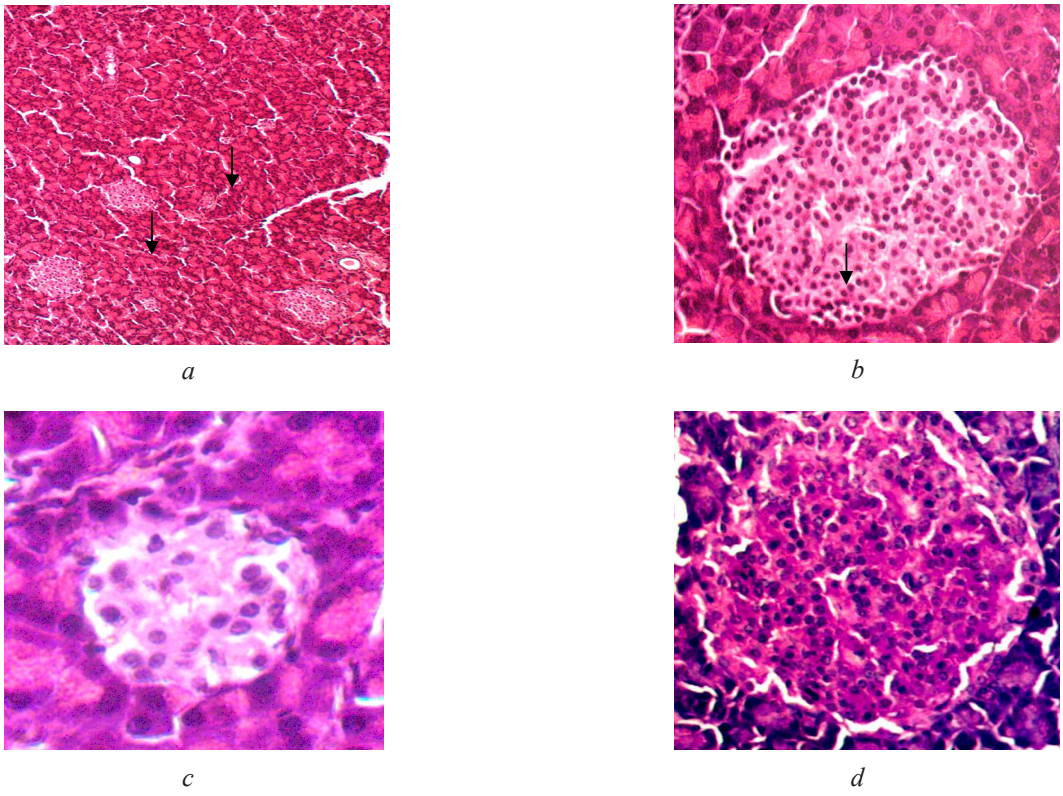
c *d*

Fig. 4. Rat pancreas after two months of exposure to HSD: *a* – distinct enlargement of small islets (×100); *b* – hypertrophy, pyknosis of some β-cell nuclei (×400); *c* – depletion, vacuolisation of β-cells (×250); *d* – uneven distribution of β-cells in the islet, the atypical shape of the islet (×250). Hematoxylin and eosin



a *b*

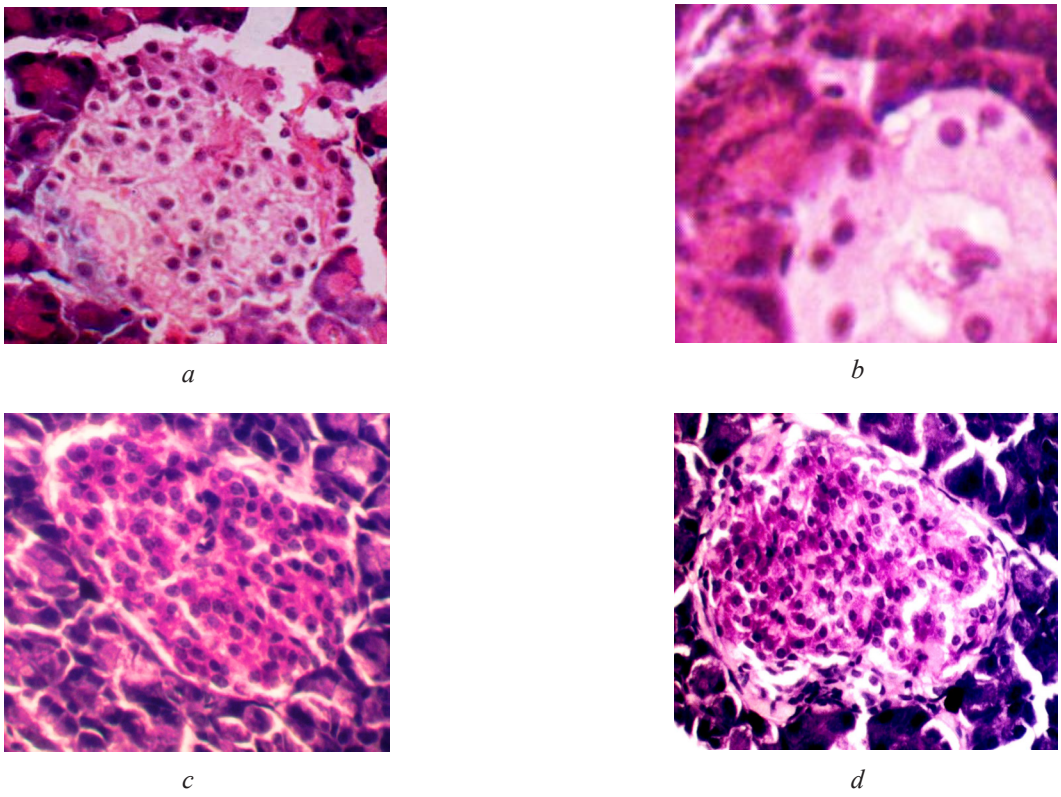
Fig. 5. Rat pancreas after two months of exposure to HSD. Uneven staining of the cytoplasm of β-cells of the islets (a, b). Aldehyde-fuchsin according to Gomor-hematoxylin, ×400



a *b*

c *d*

Fig. 6. Rat pancreas after simultaneous two-month maintenance on HSD and receipt of the phytocomposition: *a* – enlargement of medium-sized pancreatic islets (×100); *b* – islet with normal morphology (×200); *c* – destruction, dystrophy of β-cells (×400); *d* – restoration of specific aldehyde-fuchsinophilic staining of the cytoplasm of most insulinocytes (×250). *a, b* – hematoxylin-eosin; *c, d* – aldehyde-fuchsin according to Gomor-hematoxylin



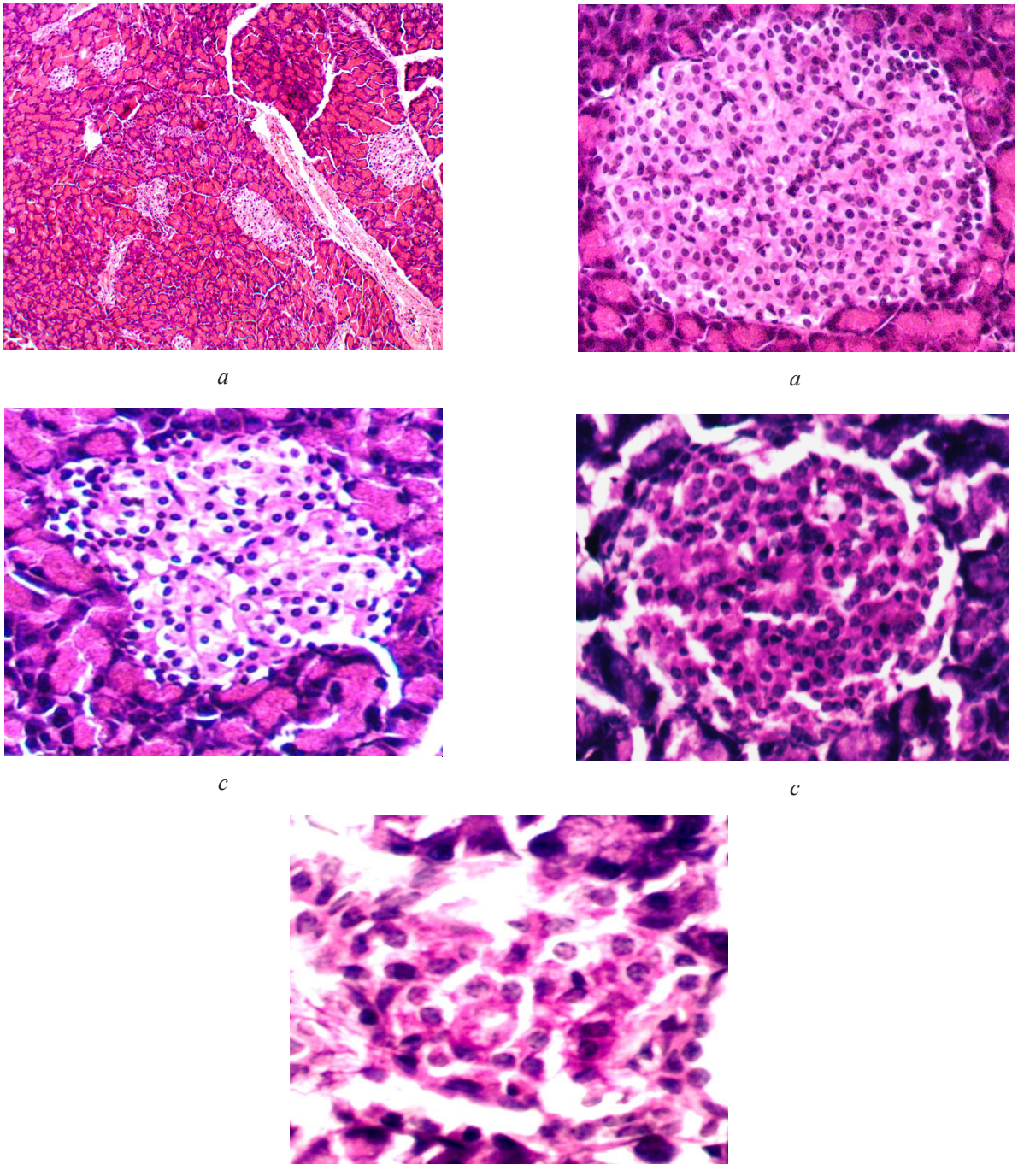
a *b*

c *d*

Fig. 7. Rat pancreas after simultaneous two-month maintenance of HSD and receipt of the "Arfazetin" collection: *a* – nested devastation, vacuolization of β-cells (×250); *b* – total devastation of the islet (×400); *c* – restoration of the intensity of specific staining of insulinocytes with aldehyde fuchsin (×250); *d* – uneven staining of β-cells with aldehyde fuchsin (×200). *a, b* – hematoxylin and eosin; *c, d* – aldehyde fuchsin according to Gomor-hematoxylin

After simultaneous two-month maintenance of HSD and receiving "Metformin" tablets, the morphological state of PI in different rats within the group varied slightly. Most of the islets in all animals corresponded to the physiological norm in all parameters. However, islets with varying degrees of emptiness, chaotic arrangement and vacuolization of the β-cell cytoplasm, "atypical" form were still observed. The intensity of aldehyde-fuchsin staining of the insulinocyte cytoplasm varied depending on the morphological state of the islet (Fig. 8). No changes were observed in the exocrine part of the glandular tissue.

The morphometric study showed that at an optical density of PI of 23 units, the proportion of small islets decreased by 1.4 times ($p<0.001$), medium islets increased by 1.8 times ($p<0.001$), and large islets decreased by 1.9 times ($p<0.001$). The intensity level of aldehyde-fuchsinophilic staining of β-cells was 2.8 points (Table 1).



a *a*

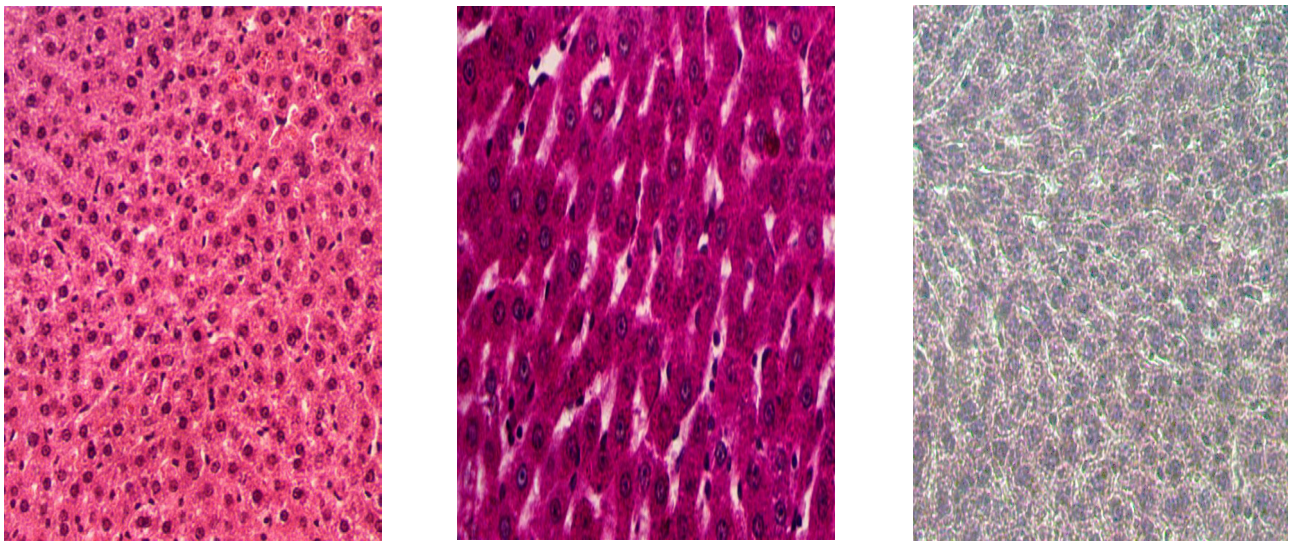
c *c*

e

Fig. 8. Rat pancreas after simultaneous two-month maintenance on HSD and administration of Metformin tablets:
a – enlargement of medium-sized islets (×100); *b* – unchanged islet (×200);
c – vacuolization, depletion of β-cells (×250);
d – rich specific aldehyde-fuchsinophilic staining of insulinocyte cytoplasm (×250);
e – depletion of specific aldehyde-fuchsinophilic staining of insulinocyte cytoplasm (×400);
a–c – hematoxylin-eosin; *d, e* – aldehyde-fuchsin according to Gomor-hematoxylin

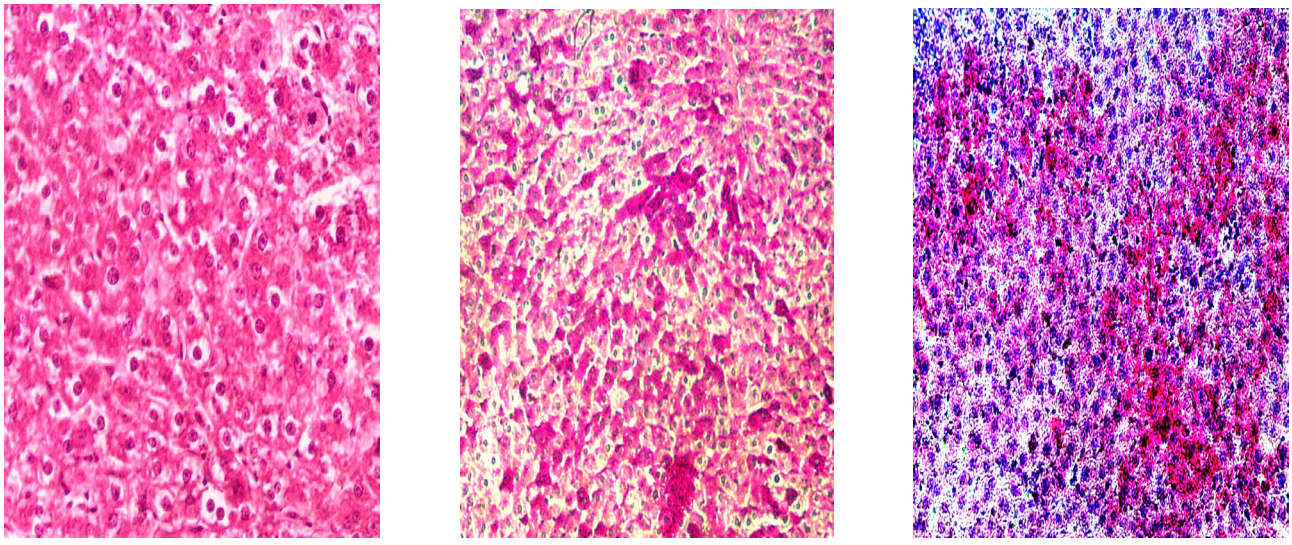
The histological structure of the liver of rats in the intact control group corresponded to the norm. The partial pattern of the tissue was not distinct. The liver particles consisted of strands of hepatocytes, which had a fairly clear radial direction. The boundaries of the lobules were determined by triads. The triad zones were narrow. The condition of the epithelium of blood vessels in the triads and other vessels was within normal limits. Intraparticle sinusoidal hemocapillaries were moderately dilated and contained a moderate number of lymphoid cells. Kupffer cells (stellate reticuloendotheliocytes) were common. Hepatocytes had a characteristic shape and size, the cytoplasm was uniformly stained, optically dense, did not contain inclusions that were visible under light microscopy. The nuclei of hepatocytes were normochromic, centrally located, contained 1, sometimes 2 nucleoli. The pool of binuclear cells was sufficient. The PAS reaction showed that the cytoplasm of hepatocytes was uniformly and densely filled with small glycogen granules, Sudan staining did not reveal fat accumulation in the cells (Fig. 9).

In the liver of rats kept on HSD, the cytoplasm of many hepatocytes became optically empty, did not stain or stained very weakly with eosin. The localization of such hepatocytes and the severity of the sign varied in different rats. In some changes, hepatocytes were affected mainly in the periportal zones, in others the changes were more diffuse or, conversely, focal. In all such zones, the radial direction of the cell strands was not observed, the cell boundaries were unclear. When setting the PAS reaction, a significant decrease in glycogen saturation of such cells or its absence is observed. Staining with Sudan IV revealed the presence of small drops of fat in the cytoplasm of many cells (Fig. 10).



a *b* *c*

Fig. 9. Liver of an intact rat: *a* – normal morphological structure of the tissue (hematoxylin and eosin, ×200); *b* – uniform replenishment of the cytoplasm of hepatocytes with glycogen granules (PAS reaction according to McManus, ×250); *c* – absence of fat accumulation in the cells (frozen section, Sudan IV, ×250)



a *b* *c*

Fig. 10. Rat liver after two months of exposure to HSD: *a* – hepatocytes with optically empty cytoplasm, unclear beam pattern (hematoxylin and eosin. ×250); *b* – decrease in glycogen content in hepatocytes (PAS reaction according to McManus. ×200); *c* – accumulation of fat in the cytoplasm of hepatocytes (frozen section, Sudan IV. ×200)

In the rat liver after the therapeutic and prophylactic administration of the phytocomposition, the general histoarchitecture of the parenchyma was not changed. In most animals, very small vacuolization of individual groups of hepatocytes was observed, which generally had almost no effect on the overall picture of glycogen accumulation. The content of neutral fats in the cytoplasm of most cells was minimal. In others, moderate-sized foci of hepatocytes with optically empty cytoplasm were observed, which was weakly stained with eosin (localization of such cells was mainly periportal). The PAS reaction showed a significant decrease in glycogen saturation of such cells, and staining with Sudan IV revealed the presence of fat in their cytoplasm (Fig. 11).

In the liver of animals receiving the "Arfazetin" collection, vacuolar dystrophy of hepatocytes had a focal character (from moderate to large size). In such hepatocyte cells, the glycogen content was reduced, fat accumulated (Fig. 12).

Metformin tablets' effect on the rat liver's condition was not as indicative as on the pancreas. In most rats, different-sized areas of liver parenchyma with optically empty cell cytoplasm were visible, depleted of glycogen and saturated with fat. The saturation of hepatocytes with glycogen outside the areas with optically empty cells varied – areas with normal accumulation alternated with areas with a depleted content of this carbohydrate (Fig. 13).

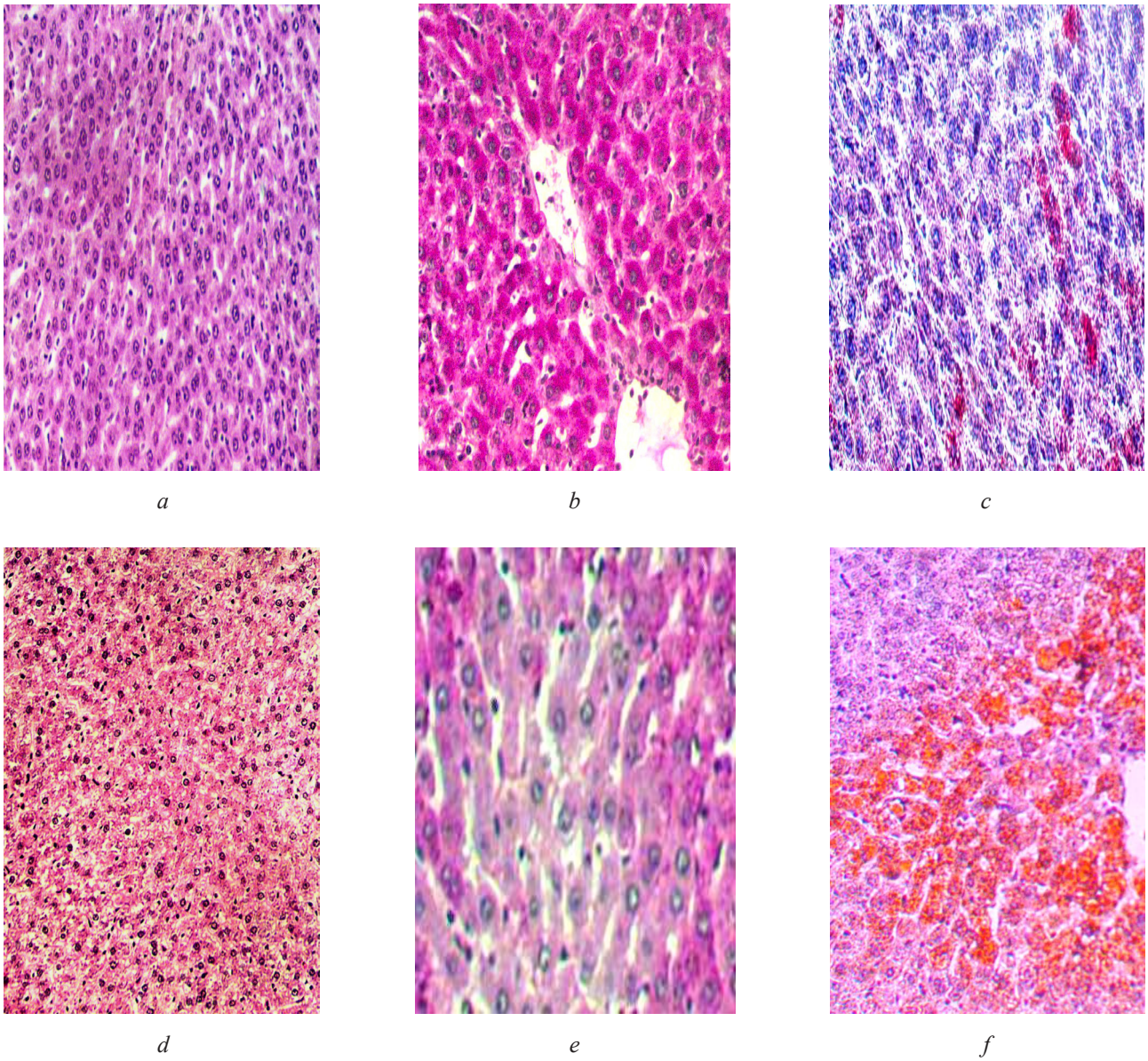
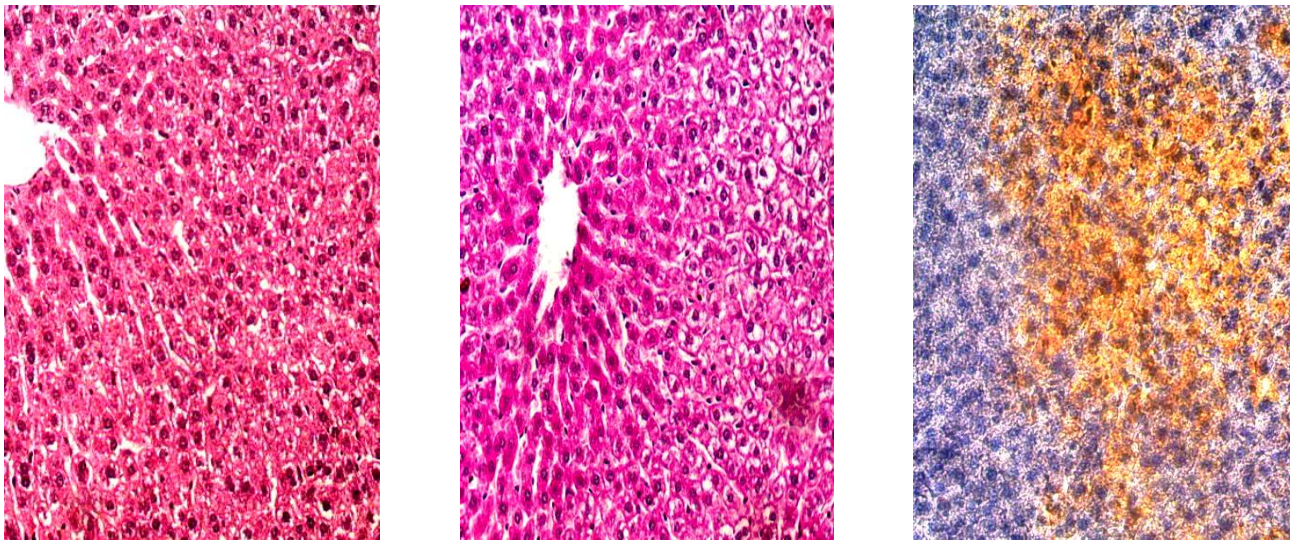
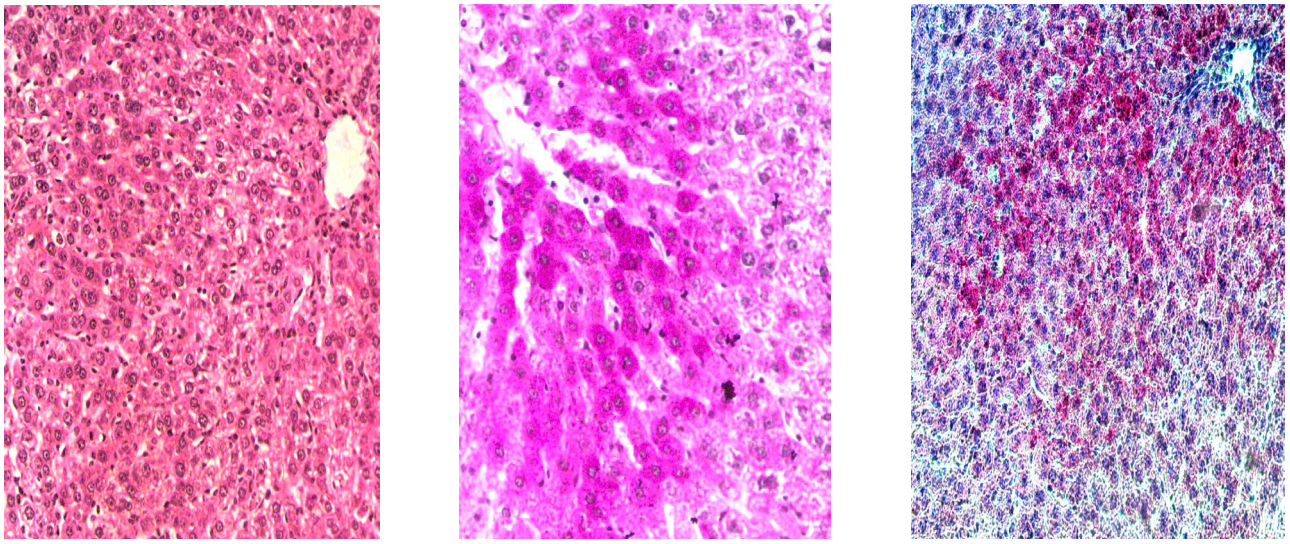


Fig. 11. Rat liver after simultaneous two-month maintenance on HSD and receipt of the phytocomposition:
a – fine vacuolization of individual groups of hepatocytes; *b* – restoration of glycogen accumulation;
c – absence of fat droplets in hepatocytes; *d* – hepatocytes with optically empty cytoplasm;
e – distinct decrease in glycogen content; *f* – fat in the cytoplasm of hepatocytes in periportal zones.
a, d – hematoxylin and eosin; *b, e* – McManus PAS reaction; *c, f* – frozen section, Sudan IV.
a, b, d, e – ×250; c, *f* – ×200



a *b* *c*

Fig. 12. Rat liver after simultaneous two-month maintenance of HSD and receipt of the "Arfazetin" collection: *a* – focal dystrophy of hepatocytes (hematoxylin-eosin); *b* – areas of decreased glycogen content (PAS reaction according to Man-Manus); *c* – accumulation of fat in hepatocytes (frozen section, Sudan IV, ×250)



a *b* *c*

Fig. 13. Rat liver after simultaneous two-month maintenance of HSD and administration of Metformin tablets: *a* – hepatocytes with optically empty cytoplasm (hematoxylin-eosin, ×200); *b* – absence of glycogen in some cells (PAS reaction according to McManus, ×250); *c* – fat in the cytoplasm of cells (frozen section, Sudan IV, ×200)

5. Discussion

The disorders occurring in rats during two-month maintenance on HSD are similar to the manifestations of metabolic syndrome in humans with prolonged excessive consumption of sugar-containing equal amounts of fructose and glucose, particularly insulin resistance with impaired glucose utilization [11].

In our study, PC based on polyphenolic extract from large-fruited cranberry leaves and amino acids significantly enhanced glucose utilization by peripheral tissues. This was confirmed by a significant decrease in glycemia during OTTG at the metformin level. According to this action, PC was significantly superior to the phytosanitary collection "Arfazetin".

Permanent significant manifestations of MS are insulin resistance and hyperinsulinemia, which cause initial pathologic changes in pancreatic islets and dysfunction of β-cells, key regulators of glucose homeostasis. Because β-cells are finely tuned to acute fluctuations in nutrient concentrations, chronic exposure to elevated levels of glucose and free fatty acids, as observed in MS, leads to the development of adaptive changes in β-cell mass and function [17].

In animals receiving only HSD, prolonged ingestion of excess carbohydrates led to the development of adaptive proliferative changes in pancreatic islets, which, probably, by activation are aimed at preventing the development of diabetes. Signs of compensatory-adaptive

changes in the insular apparatus were hypertrophy of the nuclei of a part of β-cells, increase in the proportion of small islets, and the appearance of "branched" large islets.

According to the literature [18], this microscopic picture reflects the development of the so-called "diabetogenic" condition - MS, eventually leading to the development of type 2 DM and its associated complications.

These pathologic changes coincide with the results of the study of pancreas samples from insulin-resistant patients without diabetes who underwent pancreaticoduodectomy and autopsy studies of obese and insulin-resistant people [19].

The studied phytocomposition based on the polyphenolic extract of cranberry leaves and amino acids, probably due to its complex composition, which determines a number of pharmacological effects, improved the morphological state of pancreatic β-cells. This was confirmed by an increase in the percentage of medium islets and a decrease in small islets (including very small islets, a decrease in large "branched" islets. According to the established effect phytocomposition was reliably superior to the reference drug collection "Arfazetin" and was not inferior to tablets "Metformin".

It is known that excessive intake of carbohydrates and, especially, fructose (it makes up 50 % of sugar) is one of the causes of oxidative stress and non-alcoholic fatty liver disease, which at the initial stages can often be corrected by diet and herbal medicines [20, 21].

Two-month experimental maintenance of rats on a high-sugar diet led to inhibition of glycogen-forming function and development of steatohepatosis, which is a confirmation of the development of hepatic insulin resistance in animals. These processes were significantly leveled against the background of administration of the studied phytocomposition based on polyphenolic extract from cranberry leaves and amino acids, which prevented the depletion of glycogen stores and accumulation of fats in the liver of rats. By the degree of recovery of disturbed metabolic changes in the liver the phytocomposition exceeded both comparison drugs collection "Arfazetin" and tablets "Metformin".

Thus, the ability of phytocomposition based on polyphenolic extract of cranberry leaves and amino acids to increase glucose utilization by peripheral tissues, improve the morphostructure of pancreas and liver tissue has been established in MS induced by high-sugar diet.

Practical relevance. The ability of phytocomposition based on polyphenolic extract of cranberry leaves and amino acids to improve the morphological state of the insular apparatus of the pancreas and liver tissue under a two-month high-sugar diet was shown on the model of metabolic syndrome, which indicates the prospect of creating a drug based on it for the treatment of metabolic syndrome and type 2 diabetes mellitus.

Study limitation. This study is limited by the absence of biochemical markers, which does not allow us to conclude about the hypoglycemic and hypolipidemic properties of the phytocomposition studied.

Further research prospects. Subsequent studies should aim to investigate changes in biochemical parameters of carbohydrate and lipid metabolism, the state of the antioxidant system and lipid peroxidation, and pathological changes in the tissue of the pancreas and liver in experimental models of metabolic syndrome and type 2 diabetes mellitus caused by various diabetogenic substances.

6. Conclusions

1. Two-month maintenance of rats on a high sucrose diet was characterized by pathomorphological changes in the insular apparatus of the pancreas, impaired glycogen-forming function of the liver and the development of steatohepatosis.

2. Phytocomposition based on a polyphenolic extract of cranberry leaves and amino acids prevented dystrophic and necrobiotic changes of β-cells, depletion of glycogen stores, and fat accumulation in rat livers during therapeutic and prophylactic administration on the background of a high-sugar diet.

3. The phytocomposition based on the polyphenolic extract of cranberry leaves and amino acids was superior to the reference preparation "Arfazetin" and practically not inferior to "Metformin" tablets in its ability to limit morphological changes in the pancreas tissue. It exceeded both comparison preparations in the degree of restoration of disturbed metabolic changes in the liver.

4. The obtained results indicate the prospects of further experimental research into the pharmacological properties of a phytocomposition based on a polyphenol extract from large-fruited cranberry leaves and amino acids to create an effective phyto-remedy for correcting the manifestations of metabolic syndrome and type 2 diabetes mellitus.

Conflict of interest

The authors declare that they have no conflict of interest related to this research, whether financial, personal, authorship, or otherwise, that could affect the research and its results presented in this article.

Funding

The study was performed without financial support.

Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

References

1. Ndumele, C. E., Neeland, I. J., Tuttle, K. R., Chow, S. L., Mathew, R. O., Khan, S. S. et al. (2023). A Synopsis of the Evidence for the Science and Clinical Management of Cardiovascular-Kidney-Metabolic (CKM) Syndrome: A Scientific Statement From the American Heart Association. Circulation, 148 (20), 1636–1664. https://doi.org/10.1161/cir.0000000000001186

2. Chasens, E. R., Imes, C. C., Kariuki, J. K., Luyster, F. S., Morris, J. L., DiNardo, M. M. et al. (2021). Sleep and Metabolic Syndrome. Nursing Clinics of North America, 56 (2), 203–217. https://doi.org/10.1016/j.cnur.2020.10.012

3. Frankenberg, A. D. von, Reis, A. F., Gerchman, F. (2017). Relationships between adiponectin levels, the metabolic syndrome, and type 2 diabetes: a literature review. Archives of Endocrinology and Metabolism, 61 (6), 614–622. https://doi.org/10.1590/2359-3997000000316

4. Di Pino, A., DeFronzo, R. A. (2019). Insulin Resistance and Atherosclerosis: Implications for Insulin-Sensitizing Agents. Endocrine Reviews, 40(6), 1447–1467. https://doi.org/10.1210/er.2018-00141

5. Rochlani, Y., Pothineni, N. V., Kovelamudi, S., Mehta, J. L. (2017). Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Therapeutic Advances in Cardiovascular Disease, 11 (8), 215–225. https://doi.org/10.1177/1753944717711379

6. Thomas, M. S., Calle, M., Fernandez, M. L. (2023). Healthy plant-based diets improve dyslipidemias, insulin resistance, and inflammation in metabolic syndrome. A narrative review. Advances in Nutrition, 14 (1), 44–54. https://doi.org/10.1016/j.advnut.2022.10.002

7. Koshovyi, O. M., Komisarenko, M. A., Kovaleva, A. M., Ilina, T. V., Vlasova, I. K. (2020). Mineral composition of aerial parts of cranberry. Fitoterapia, 1 (1), 46–49. https://doi.org/10.33617/2522-9680-2020-1-46

8. Ferlemi, A.-V., Lamari, F. (2016). Berry Leaves: An Alternative Source of Bioactive Natural Products of Nutritional and Medicinal Value. Antioxidants, 5 (2), 17. https://doi.org/10.3390/antiox5020017

9. LaMoia, T. E., Shulman, G. I. (2020). Cellular and Molecular Mechanisms of Metformin Action. Endocrine Reviews, 42 (1), 77–96. https://doi.org/10.1210/endrev/bnaa023

10. Poriadok provedennia naukovymy ustanovamy doslidiv, eksperymentiv na tvarynakh (2012). Nakaz Ministerstva osvity, nauky, molodi ta sportu Ukrainy. Nakaz No. 249. 01.03.2012. Available at: https://zakon.rada.gov.ua/laws/show/z0416-12#Text

11. Horbenko, N. I., Borikov, O. Yu., Ivanova, O. V. et al. (2019). Modeliuvannia metabolichnoho syndromu riznoho henezu u eksperymentalnykh tvaryn (metodychni rekomendatsii). Kharkiv, 38.

12. Chikitkina, V., Tanska, M. (2024). Screening studies of the hypoglycemic effect of a phytocomposition based on polyphenolic extract from cranberry leaves and amino acids. Modern Medicine, Pharmacy and Psychological Health, 1 (15), 119–125. https://doi.org/10.32689/2663-0672-2024-1-21

13. Maynard, R., Downes, N., Finney, B. (2014). Histological techniques: an introduction for beginners in toxicology. Cambridge: Royal Society of Chemistry, 334. https://doi.org/10.1039/9781839168895

14. Kiernan, J. A. (2015). Histological and histochemical methods: theory and practice. Banbury: Scion Publishing, 571.

15. Layton, C., Bancroft, J. D., Suvarna, S. K.; Suvarna, S. K., Layton, C., Bancroft, J. D. (Eds.) (2019). Fixation of tissues. Bancroft's theory and practice of histological techniques. St. Louis: Elsevier, 40–63. https://doi.org/10.1016/b978-0-7020-6864-5.00004-9

16. Horbenko, N. I. (2004). Patohenetychne obhruntuvannia efektyvnosti pokhidnoho yantarnoi kysloty – fensuktsynalu v terapii tsukrovoho diabetu ta yoho sudynnykh uskladnen (eksperymentalne doslidzhennia). [Extended abstract of doctors thesis].

17. Katsuda, Y., Ohta, T., Miyajima, K., Kemmochi, Y., Sasase, T., Tong, B., Shinohara, M., Yamada, T. (2014). Diabetic Complications in Obese Type 2 Diabetic Rat Models. Experimental Animals, 63 (2), 121–132. https://doi.org/10.1538/expanim.63.121

18. Weir, G. C., Bonner-Weir, S. (2004). Five Stages of Evolving Beta-Cell Dysfunction During Progression to Diabetes. Diabetes, 53(suppl_3), S16–S21. https://doi.org/10.2337/diabetes.53.suppl_3.s16

19. Hudish, L. I., Reusch, J. E. B., Sussel, L. (2019). β Cell dysfunction during progression of metabolic syndrome to type 2 diabetes. Journal of Clinical Investigation, 129 (10), 4001–4008. https://doi.org/10.1172/jci129188

20. Paternostro, R., Trauner, M. (2022). Current treatment of non-alcoholic fatty liver disease. Journal of Internal Medicine, 292 (2), 190–204. https://doi.org/10.1111/joim.13531

21. Alam, M. A., Subhan, N., Rahman, M. M., Uddin, S. J., Reza, H. M., Sarker, S. D. (2014). Effect of Citrus Flavonoids, Naringin and Naringenin, on Metabolic Syndrome and Their Mechanisms of Action. Advances in Nutrition, 5 (4), 404–417. https://doi.org/10.3945/an.113.005603

Received 27.08.2024
Received in revised form 06.12.2024
Accepted 19.12.2024
Published 30.12.2024

Mariia Anisimova*, PhD Student, Department of Physiology and Pathological Physiology, National University of Pharmacy, Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

Nadiia Kononenko, Doctor of Medical Sciences, Professor, Head of Department, Department of Physiology and Pathological Physiology**,** National University of Pharmacy**,** Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

Valentyna Chikitkina, PhD, Associate Professor, Department of Physiology and Pathological Physiology**,** National University of Pharmacy**,** Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

***Corresponding author:** Mariia Anisimova, e-mail: patology@nuph.edu.ua*