PharmacologyOnLine
Archives • 2021 • vol.2 • 1192-1202

EFFECT OF COMPLEX PHARMACEUTICAL COMPOSITION AT THE HISTOSTRUCTURE OF THE PANCREAS UNDER THE CONDITIONS OF EXPERIMENTAL METABOLIC SYNDROME IN RATS

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Abstract

Metabolic syndrome (MetS) – it is a symptom complex characterized by insulin resistance, impaired prooxidant-antioxidant balance of the body with the development of subchronic inflammation, dyslipidemia and is a predictor of cardiovascular diseases and type 2 diabetes mellitus (T2DM). It was found that MetS causes a 5-fold increase in the risk of developing T2DM, a 2-fold risk of developing cardiovascular diseases over the next 5 to 10 years, 2-4 times increases the risk of stroke, 3-4 times myocardial infarction and twice the risk of death. Given the above, timely prevention and treatment of MetS is an important task.

Aim of this work is to study the effect of a complex pharmaceutical composition (CPC) (which contains antioxidants and metabolitotropic agents) as CPC is widely used in medical practice in Ukraine as a multivitamin complex, and to study its effect at the histostructure of the pancreas at the course of experimental metabolic syndrome in rats. Morphometric measurements were carried out on pancreatic sections: the total number of pancreatic islets (PI) in the micropreparation was determined.

An experimental model of metabolic syndrome (EMetS) in rats was caused by a high content of carbohydrates and fats in the diet by enriching the diet with fructose (adding fructose to the feed and replacing the drink with a 10.0% fructose solution - in total in the diet up to 20.0% of the daily caloric value) and animal fats (lard and fat in total in the diet up to 20.0% of daily calories) for 18 weeks

The studied vitamin E has a certain protective effect on the development of metabolic syndrome and manifestations of steatosis. Morphologically this manifests itself in a decrease in destructive-dystrophic changes and an increase in functionally more complete insulinocytes, and, as a consequence, restoration (to one degree or another) of the normal distribution of pancreatic islets in size and FI. CPC is inferior to comparison drugs of vitamin E and metformin in terms of expressiveness of antidiabetic and «antisteatous» activity. Regarding the comparison drug «Metformin», in terms of the expressiveness of the listed protective activities on this pathology model it is ahead of the studied CPC and vitamin E.

Keywords: *experimental metabolic syndrome in rats, complex pharmaceutical composition, histostructure of the pancreas.*

Introduction. Metabolic syndrome (MetS) – it is a symptom complex characterized by insulin resistance, impaired prooxidant-antioxidant balance of the body with the development of subchronic inflammation, dyslipidemia and is a predictor of cardiovascular diseases and type 2 diabetes mellitus (T2DM) [1, 2]. It was found that MetS causes a 5-fold increase in the risk of developing T2DM, a 2-fold risk of developing cardiovascular diseases over the next 5 to 10 years, 2-4 times increases the risk of stroke, 3-4 times myocardial infarction and twice the risk of death [1, 3]. Given the above, timely prevention and treatment of MetS is an important task.

Insulin sensitizers, in particular, biguanide derivatives - metformin, which is a drug with proven efficacy for the treatment of MetS, are used as drugs for the pharmacological correction of MetS [4]. Considering the importance of oxidative stress in the development of MetS, the use of agents with antioxidant activity is advisable for the prevention of MetS [5, 6]. These are plant phenolic substances (quercetin), as well as vitamins (A, E, C) and trace elements (zinc, selenium), correctors of mitochondrial function (coenzyme Q) [7, 8, 9, 10, 11, 12]. The described data highlight the positive effect of the individual compounds, but the effect of their combined use is unknown.

Aim of this work is to study the effect of a complex pharmaceutical composition (CPC) (which contains antioxidants and metabolitotropic agents) as CPC is widely used in medical practice in Ukraine as a multivitamin complex, and to study its effect at the histostructure of the pancreas at the course of experimental metabolic syndrome in rats.

Methods. An experimental model of metabolic syndrome (EMetS) in rats was caused by a high content of carbohydrates and fats in the diet by enriching the diet with fructose (adding fructose to the feed and replacing the drink with a 10.0% fructose solution - in total in the diet up to 20.0% of the daily caloric value) and animal fats (lard and fat in total in the diet up to 20.0% of daily calories) for 18 weeks [13].

The animals were divided into 4 groups of 6 rats: 1 - intact control (IC), animals that were kept on a standard vivarium diet and consumed a diet balanced in terms of the set of proteins, fats, carbohydrates, essential microelements and vitamins; 2 - animals with EMetS, in which the diet was enriched with fructose and fats (as indicated above) and which were not treated (control pathology group, CP); 3 - animals with EMetS, which were treated with CPC at a dose of 25.8 mg/kg intragastrically (w/w) [14]; 4 - animals with EMetS that were treated with vitamin E at a dose of 100 mg/kg intragastrically (w/w) [15]; 5 - EMetS animals that were treated with metformin at a dose of 60 mg/kg intragastrically (w/w) [16].

CPC, metformin and vitamin E were used in a therapeutic regimen, starting from the 15th week of modeling the control pathology for 4 weeks (28 days).

Euthanasia of female rats of all groups was carried out by use of decapitation under light chloroform anesthesia. The obtained pancreatic samples were fixed in 10% formalin solution, dehydrated in alcohols of increasing strength, embedded in paraffin. Paraffin blocks from the pancreas were cut on an MC-1 sled microtome, the sections were mounted on a glass slide, and stained with hematoxylin and eosin. [17].

Morphometric measurements were carried out on pancreatic sections: the total number of pancreatic islets (PI) in the micropreparation was determined.

For a more accurate analysis of the cytoarchitectonics of the islets, they were divided into classes depending on the number of beta cells [18]: I class - 5-16 cells (very small), II class - 16-30 cells (small), III class - 31-60 cells (medium), IV class - 61-100 cells (large), V class - more than 100 cells (giant); calculated the percentage of each category of PI [19]. Determined the functional index (PI) - the ratio of beta cells to alpha cells in the islet [20]. All obtained digital data were processed by the methods of variation statistics. [21].

Micropreparations were viewed with a Granum light microscope, microscopic images were photographed with a Granum digital video camera. DCM 310. The photographs were processed on a Pentium 2.4GHz computer using the Toup View software.

Results and Discussion. The glandular tissue is seen in micropreparations of the pancreas of intact animals, consisting of moderate-sized particles and a system of interlobular and intralobular excretory ducts, arteries and veins of various calibers. The connective tissue partitions between the lobules are

moderately expressed. A clear distribution of tissue into exo and endocrine components is visible in the lobules. The exocrine parenchyma of the gland consisted of the terminal secretory sections of the glands - acini with a high location density. Acini consisted of one layer of glandular cells, which are characterized by division into two zones with different colored stains: the basal basophilic zone, where there was a rounded, dense nucleus, and the eosinophilic central zone, containing small zymogen grains. The ratio of the zones ranged from 1:1.5 to 1:2.5. The acini lumen is small. The epithelium of most ducts is normal, rarely in a state of moderate proliferation. Depending on the caliber of the excretory duct (mainly interlobular), there was noted a different severity of the periductal stroma, sometimes single small lymphohistiocytic accumulations were found in it. The lumen of the ducts is often widespread, there are visible lumps of thickened eosinophilic secretion in some places. The condition of the arteries and veins is normal, some of them are full-blooded. The endocrine component of the gland is represented by PI of various sizes, the average total number of which reached 20.0 in a micropreparation. Most of the islets are clearly separated from the surrounding exocrine parenchyma, and had a rounded or oval shape. The bulk of the cells in the islets were beta cells, which are quite densely and evenly distributed in the central part. Sinusoidal capillaries are commonly seen between the cell strands in a number of islets. Alpha cells are arranged in a chain along the periphery of the islets (Figure 1). Such localization of beta - alpha cells in PI is typical for this animal species. [19, 22, 23]. The bulk of the islets corresponded to III class, these are medium islets, their percentage was 50%. Islets of the I - II class (very small - small) occupied a total of 27%, and in the IV - V class (large - giant) - 23%. The functional index (FI) was 4.5 (Figure 1, Table 1).

After 18 weeks of modeling the experimental insulin resistance induced by the high content of carbohydrates and fats in the diet in the structural organization of the endocrine pancreas of female rats, the following was observed: a significant decrease in the total amount of PI in the micropreparation (by 24% relative to the intact level). Among the remaining, there is a decrease in the number of III class islets and an increase in I and II class islets (especially very small). The percentage redistribution of their number according to the accepted classification in comparison with intact female rats was as the following: – the proportion of islets of I-II class (very small / small) increased among those found in the presence of PI by 11.82%; III class (medium) - decreased by 2.17 times. The PI proportion of IV - V class (large / extra large) did not change significantly. The FI of the endocrine apparatus decreased by 1.95 times compared with the intact control (Table 1), which is confirmed by light optics - in most islets therer is seen a noticeable focal sparseness in the arrangement of beta cells of the central zones, vacuolization of their cytoplasm. Part of the insulinocytes had hypertrophic nuclei, some of which were in a state of pycnosis or lysis (Figure 2). In a number of islets, proliferates of alpha cells of various expressiveness are seen in places of their typical localization. (Figure 3). In general, this PI position is a morphological reflection of the functional depletion of the endocrine apparatus. [24].

After the introduction of CPC at the background of modeling experimental insulin resistance in the studied female rats, the total number of PI in the micropreparation increased by 17%, although it was significantly less then at intact group. As for the proportion of islets of I-II class among them, their number was large (even in comparison with the control pathology). At the same time, the presence of III class islets was significantly increased by 33.33% (Table 1). Microscopically, a rather significant part of PI was characterized by the emptiness of the central zones from beta cells, signs of insulinocytes dystrophy. (Figure 4). This state of beta cells correlated with the FI index, which was 2.69, that is only 16.45% higher (statistically - a tendency to increase) than in the control pathology (Table 1).

Simultaneous 4-week administration of vitamin E to female rats at the background of experimental insulin resistance had a positive effect on the state of PI. In a significant number of islets, the state of the insulinocytes was visually normal, although some of them still had signs of moderate vacuolization of the cytoplasm. No expressive devastation of the islets was noticed. Focal moderate alpha cell proliferation persisted (Figure 5). Morphometric analysis showed that the total amount of PI in the preparation, compared with the

control pathology, was significantly increased by 18.42% and tended to recover relatively to the intact control. The relative share of I-II class islets did not decrease significantly (by 6.25%), while III class islets increased by 1.6 times. The number of islets of the IV-V class has not changed. FI on average in the group was 3.16, which is evidence of an improvement in the state of the endocrine apparatus by 36.8% (Table 1).

The use of the reference drug "Metformin" at the background of experimental insulin resistance clearly improved the state of the endocrine component of the glandular tissue in comparison with the control pathology. Both the average number of PI in the micropreparation (20.0) and their percentage distribution by class recovered to an intact level (a tendency to recovery). Compared to the control pathology, the I-II class islets are not likely to be reduced, and the islets of the III class are significantly increased by 2 times; IV-V class - not changes. FI increased to 4.22, which significantly corresponded to the intact level and was 1.83 times higher than the control pathology (Table 1). Microscopically, PIs were cell saturated, only a few of them had moderate signs of devastation and degeneration of insulinocytes (Figure 6).

Based on the obtained light-optical data, the following generalizations can be made: long-term maintenance of animals at the background of a diet high in carbohydrates and fats leads to a number of pathological changes in the endocrine component of the pancreas and in the liver of rats. Changes in the endocrine apparatus correspond to the signs of its activation described in the literature - the so-called concept of hyperfunction of beta cells. According to this concept, the introduction of significant amounts of carbohydrates leads to an overload of pancreatic beta cells: to inactivate high blood sugar, the body's need for insulin increases. The beta cells of the pancreatic islets begin to function in an enhanced mode. After a while, beta cells become depleted and subsequently die. In the end, as a result of the exhaustion of beta cells, the total mass of their population decreases and, as a result, the total capabilities of beta cells decrease. [24]. Morphologically this manifests itself in hypertrophy, cell dystrophy, varying degrees of islet devastation, and a decrease in their number in a micropreparation. An increase in the proportion of small cells, in our opinion, is also compensatory in nature, as that is an attempt by the organ to somehow increase / restore the total number of "working" beta cells. Such a microscopic picture reflects the development of the so-called «diabetogenic» state or prediabetes - metabolic syndrome [24].

The studied vitamin E has a certain protective effect on the development of metabolic syndrome and manifestations of steatosis. Morphologically this manifests itself in a decrease in destructive-dystrophic changes and an increase in functionally more complete insulinocytes, and, as a consequence, restoration (to one degree or another) of the normal distribution of pancreatic islets in size and FI. CPC is inferior to comparison drugs of vitamin E and metformin in terms of expressiveness of antidiabetic and «antisteatous» activity. Regarding the comparison drug «Metformin», in terms of the expressiveness of the listed protective activities on this pathology model it is ahead of the studied CPC and vitamin E.

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Table 1. Influence of CPC, vitamin E and metformin on the state of the endocrine apparatus of the pancreas of female rats with the background of experimental insulin resistance in rats induced by a high dietary intake of carbohydrates and fats

Groups	Total number of pancreatic islets in a micropreparation	Distribution of pancreatic islets into classes depending on the number of beta cells			FI (ratio of beta / alpha cells)
		I (2-5 - very small) II (6-30 - small)	III (31-60 - medium)	IV (61-100 large) V (<100 -giant)	
Intact control (IC)	20,00±0,45	5,40±0,24	10,00±0,32	4,60±0,24	4,51±0,19
Control pathology (CP)	15,20±0,37*	6,40±0,24* (p=0,0555)	4,60±0,51*	4,20±0,37	2,31±0,23*
CP+Metformin	20,00±0,55**	6,00±0,32	9,20±0,20 T*(p=0,0952)/ **	4,80±0,66	4,22±0,12 **
CP+Vitamin E	18,00±0,63 T*(p=0,0555)/ **/ T***(p=0,0555)	6,00±0,32	7,40±0,51 */**/***	4,60±0,24	3,16±0,21 */**/***
CP+CPC	17,80±0,37 */**/***	8,00±0,32 */**/***/****	7,20±0,37 */**/***	2,60±0,87 T***(p=0,0952)	2,70±0,14 */ T**(p=0,0830)/ ***/****

Notes:
*- statistically significant value for IC,
**- statistically significant value for CP,
*** - statistically significant value for metformin,
**** - statistically significant value for vitamin E, p<0,050,
T – the values are sent to the statistical, 0,05<p<0,100.

Figure 1. Pancreas of an intact female rat. The pancreatic islet (PI) of III class is of a clear oval shape, evenly filled with acini cells of the exocrine parenchyma (A) with a clear two-zone stained cytoplasm. Hematoxylin-eosin. x250.

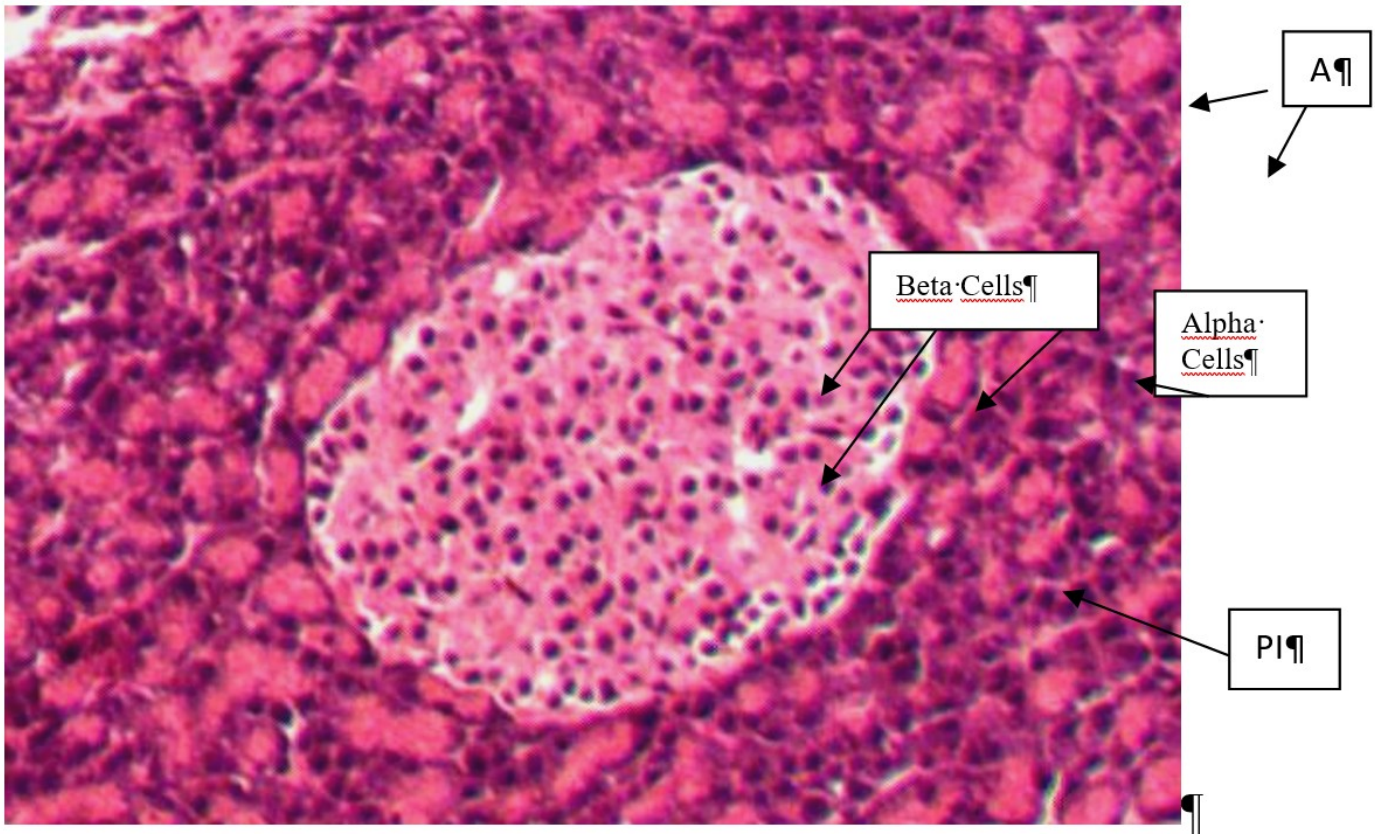

Figure 2. The pancreas of a female rat at the background of modeling of experimental insulin resistance induced by a high content of carbohydrates and fats in the diet. Devastation of the central zones (A-B) in the pancreatic islets (PI), nuclear hypertrophy, karyolysis of beta cells, vacuolization of the cytoplasm. Hematoxylin-eosin. x400.

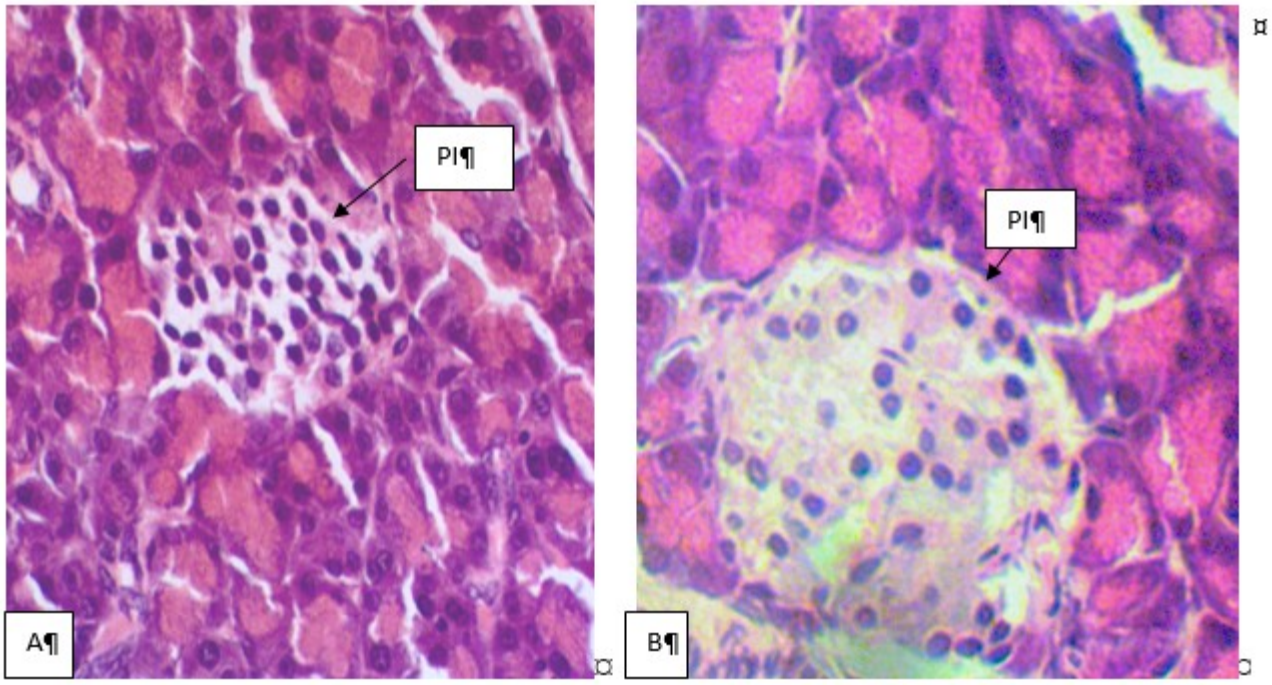

Figure 3. The pancreas of a female rat at the background of modeling of experimental insulin resistance induced by a high content of carbohydrates and fats in the diet. Focal proliferation of alpha cells (arrows), decrease in the number of beta cells (A, x400), vacuolization of insulinocytes (B, x200). Hematoxylin-eosin.

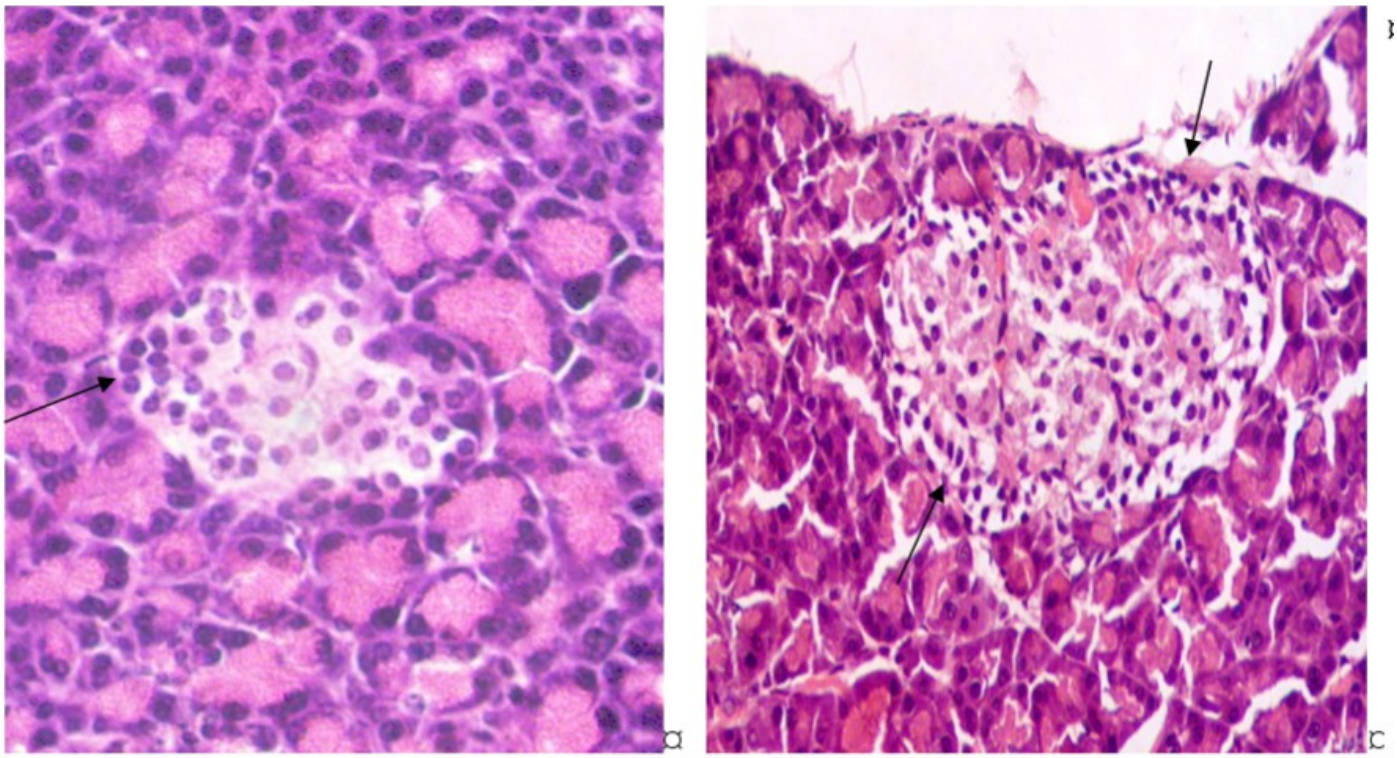

Figure 4. The pancreas of a female rat that received CPC at the background of experimental insulin resistance induced by a high dietary carbohydrate and fat content: a significant number of I-II class islands (A - arrows, x100); functionally visually normal pancreatic islet (B, x200), depletion of insulinocytes in the islets, hypertrophy of the nuclei, signs of dystrophy (C-D, x250, x400). Hematoxylin-eosin.

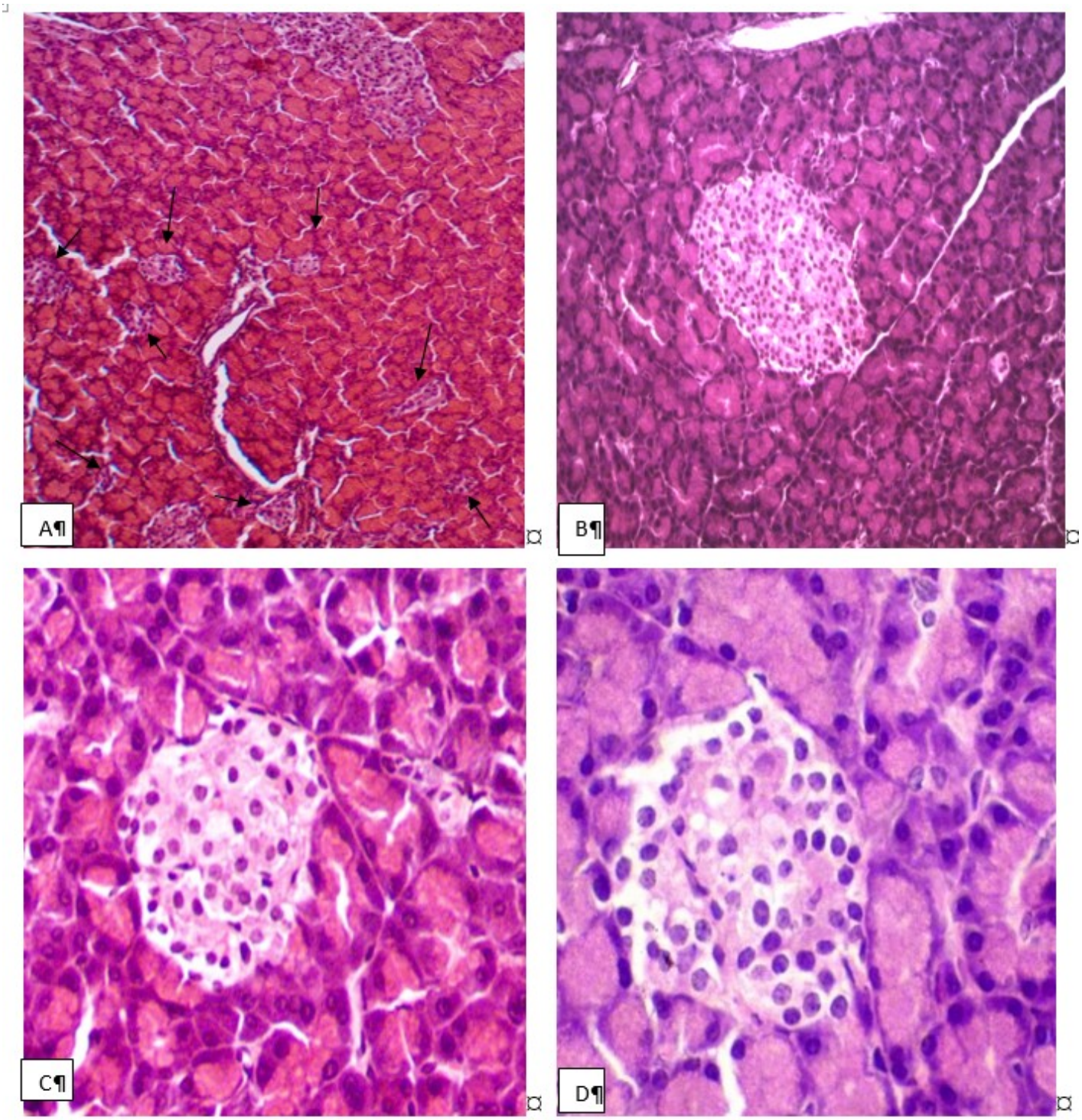

Figure 5. The pancreas of female rat, which received vitamin E at the background of experimental insulin resistance induced by a high content of carbohydrates and fats in the diet: normal IV-V class islet (A-B), moderate alpha cell proliferation (B, arrows). Devastation of the central zone of the islet (C), vacuolization of beta cells (D). Hematoxylin-eosin. A – x250, B, D – x200, C – x400.

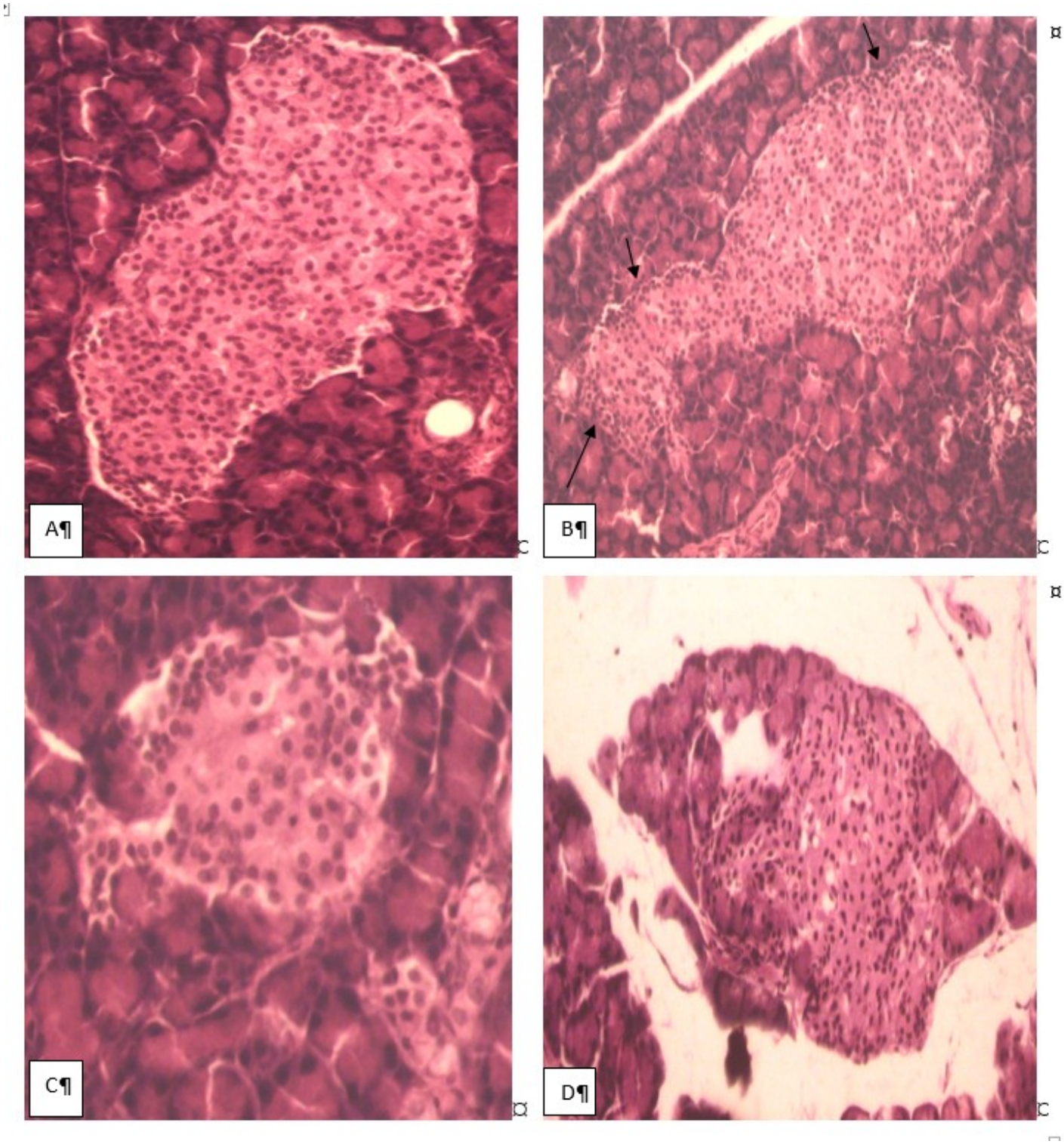

Figure 6. The pancreas of a female rat who received metformin at the background of experimental insulin resistance induced by a high content of carbohydrates and fats in the diet: functionally normal pancreatic islet (A, x200), devastation of insulinocytes in the islets (B, x250). Hematoxylin-eosin.