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STUDY OF GLIKVERIN INFLUENCE ON CARBOHYDRATE METABOLISM AND HISTOSTRUCTURE OF RATS' PANCREATIC GLAND IN TERMS OF EXPERIMENTAL METABOLIC SYNDROME
ДОСЛІДЖЕННЯ ВПЛИВУ ГЛІКВЕРИНУ НА ВУГЛЕВОДНИЙ ОБМІН ТА ГІСТОСТРУКТУРУ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ

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Article

The article presents the results of the study of Glikverin impact on carbohydrate metabolism and morphological structure of the pancreatic gland in terms of metabolic syndrome in rats induced by high-sucrose diet. It was established that the use of combined agent Glikverin increased tolerance to carbohydrates and improved sensitivity of peripheral tissues to insulin. By expressivity of pharmacological action Glikverin prevailed comparator voglibose and metformin.

The results of histological examination of Glikverin showed that it improved morphological condition of β-cells of pancreas, prevented dystrophic and necrobiotic degenerative changes that helped to increase the number of islands, in contrast to the action of the reference drug metformin. This action Glikverin exhibited due to exposure to several pathogenetic chains of metabolic syndrome. The received results justify further pharmacological study Glikverin as a promising agent for treating the manifestations of metabolic syndrome.

Key words: metabolic syndrome, glikverin, quercetine, voglibose, metformin, pancreatic gland histostructure.

Реферат

У статті наведено результати вивчення впливу Гліквірину на вуглеводний обмін та морфоструктуру підшлункової залози за умов експериментального метаболічного синдрому у щурів, викликаного високоцукрозною дієтою. Встановлено, що застосування комбінованого засобу Гліквірину підвищувало толерантність до вуглеводів та поліпшувало чутливість периферичних тканин до інсуліну. За виразністю фармакологічної дії Гліквірин переважав препарати порівняння воглібоз та метформін.

За результатами гістологічного дослідження Гліквірин, завдяки впливу на декілька патогенетичних ланцюгів метаболічного синдрому МС, покращував морфологічний стан β-клітин підшлункової залози, запобігав дистрофічним та некробіотичним змінам, що сприяло збільшенню кількості острівців, на відміну від дії препарата порівняння метформіну. Отримані результати обґрунтовують подальше фармакологічне вивчення Гліквірину як перспективного засобу для лікування проявів метаболічного синдрому.

Ключові слова: метаболічний синдром, Гліквірин, кверцетин, воглібоз, метформін, гістоструктура підшлункової залози.

Реферат

ИССЛЕДОВАНИЕ ВЛИЯНИЯ ГЛИКВЕРИНА НА УГЛЕВОДНЫЙ ОБМЕН И ГИСТОСТРУКТУРУ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ КРЫС В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛИЧЕСКОГО СИНДРОМА.

В статье представлены результаты изучения влияния Гликверина на углеводный обмен и морфоструктуру поджелудочной железы в условиях экспериментального метаболического синдрома у крыс, вызванного высокосахарозной диетой. Установлено, что применение комбинированного средства Гликверина повышало толерантность к углеводам и улучшало чувствительность периферических тканей к инсулину. По выраженности фармакологического действия Гликверин превышал препараты сравнения воглибоз и метформин.

По результатам гистологического исследования, Гликверин, благодаря влиянию на несколько патогенетических звеньев метаболического синдрома, улучшал морфологическое состояние β-клеток поджелудочной железы, предотвращал дистрофические и некробиотические изменения, что способствовало увеличению количества островков, в отличие от действия препарата сравнения метформина. Полученные результаты обосновывают дальнейшее фармакологическое изучение Гликверина как перспективного средства для лечения проявлений метаболического синдрома.

Ключевые слова: метаболический синдром, Гликверин, кверцетин, воглибоза, метформин, гистоструктура поджелудочной железы.

Introduction. Metabolic syndrome (MS) is a complex of metabolic and hormonal disorders that increase cardiovascular risk factors twice, and development of diabetes mellitus type 2 (DM type 2) – five times in comparison with people without symptoms of this syndrome [1, 2]. MS has a pandemic incidence. It is observed in 20-30 % of adult population of majority world countries. Primarily decrease of physical activity and high-calorie diet are responsible for such statistics.

Main components for MS are insulin resistance (IR), abdominal obesity, atherogenic dyslipidemia [3]. There are IR and compensatory hyperinsulinemia which stand in the background of metabolic changes during MS. These two conditions lead to logical exhaustion of pancreatic β-cells and development of typical carbohydrate metabolism disorder – increase of fasting glycemia, failure in glucose tolerance and postprandial hyperglycemia. Correction of pointed disorders is performed by different groups of blood glucose lowering drugs.

In recent years it was proven that oxidative stress plays a significant role in MS components induction, particularly - IR [4]. This makes necessary to include medicines with antioxidant activity in complex therapy of MS.

Thus, treatment of MS has to be pathogenetically justified and has at the same time to correct all symptoms of MS which often leads to polypragmasy and development of side reactions.

Aiming to reduce medicines' loading at the standard therapy of MS it is reasonable to use combined medicine forms that combine different types of pharmacological activity.

In National University of Pharmacy we created new combined medicinal agent with conditional name Glikverin. This agent consist of antioxidant quercetine and well-known regulator of postprandial hyperglycemia - α-glucosidase inhibitor – voglibose.

The aim of this work was to investigate the influence of Glikverin on carbohydrate metabolism and on pancreatic gland morphological structure in terms of experimental metabolic syndrome. Ground for conducting of the study was significant pharmacological activity of Glikverin which were established in previous experiments [5].

Materials and methods. Pharmacological studies we conducted in Central Scientific Laboratory of National University of Pharmacy which was certified by State Expert Centre of Ministry of Health of Ukraine as a basis for experimental pharmacology (certificate № 008/11 dated 18.10.2011). The study was conducted on 42 male Wistar rats of 18months age, body mass 270-300 g. All procedures with animals were made according to rules of "European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) [6].

Metabolic syndrome was designed by high-sucrose diet (HSD), in which we substituted water with 30% solution of sucrose in water containers with free access to it during 8 weeks. Animals were held in a standard cell of vivarium. Investigational agents were administered simultaneously with sucrose solution intragastrically during 8 weeks. Next experimental groups were formed: 1 group – animals of intact control (IG), 2 group – animals of control group, that were administered 30% solution of sucrose, 3 group – animals that on the background of sucrose were administered Glikverin - mixture of quercetine (dose 50 mg/kg) and voglibose (dose 0,02 mg/kg), 4-6 groups – animals that on the basis of sucrose were administered reference agents (RA): quercetine substance in dose of 50 mg/kg (PJSC SIC «Borshchahivskiy CPP», Ukraine), voglibose substance in dose of 0,06 mg/kg (Ranbaxy, India) and standard agent – tablets "metformin" in dose of 200 mg/kg (Gedeon Richter, Germany).

In 8 weeks we evaluated condition of glucose homeostasis using the level of basal glicemia and with help of intraperitoneal glucose tolerance test (IPGTT, glucose in dose of 3 g/kg). Blood samples for glucose analysis were taken in 30, 60, 90 and 120 minutes after carbohydrates loading. Glucose level in blood was determined by glucose oxidase test using kit "D-glucose", Felicit-Diagnostics (Ukraine).

Areas under glicemic curves (AUCglu, mmol/l·min) were counted using statistical programs "MedCalk, v. 9.3.7.0".

Sensitivity of peripheral tissues to insulin action we determined with help of short insulin tolerance test. In this test the percentage of decrease of basal glycaemia was counted 30 minutes after intraperitoneally hormone administration in dose 1 IU/kg in relation to baseline level [7].

At the end of experiment animals were decapitated under ether narcosis. Histologically we investigated pancreatic glands (PG) of rats that received Glikverin and metformin [8]. On sections of PG we determined space density of pancreatic islets (general quantity of islets in sample) and percentage of islets with different content of β-cells: small (5-20 β-cells), middle (21-60 β-cells) and large (>60 β-cells). We conducted evaluation of microsamples under light microscope Granum, photographing of microimages was performed by digital video camera Granum ДСМ 310. Photo pictures were processed on a computer Pentium 2,4GHz using program Toup View.

Received data we elaborated using analysis of variants. For determination of statistically significant differences between experimental groups we used Newman-Keuls and Mann-Whitney test. Differences were considered statistically significant within $p<0,05$.

Results and discussion. According to received data long-term HSD for animals didn't lead to change of basal glycemia (fig.1, tab.1). However the character of glycemic curves, received during conducting IPGTT, indicated the presence of impaired glucose tolerance in animals. Intraperitoneal administration of carbohydrates solution showed a sharp rise of glucose level in animals' blood. This concentration was statistically significant higher than value of intact group within all timelines (fig.1). Integrated index of area under glicemic curves (AUC_{glu}) was 1,6 times higher than corresponding index in intact group (tab.1) and this was also an evidence of glucose intolerance in control group animals as a result of violation of glucose utilization in liver caused by surplus consumption of sugars. Short insulin tolerance test allowed discover significant decrease of peripheral tissues sensibility to insulin action in animals of control group. Sensitivity index to insulin in control group was lower in 2,8 times in comparison with intact group (tab.1). Analyzing received data we can conclude that log-term keep of animals on HSD together with physical inactivity leaded to development of glucose intolerance, decrease in sensitivity of peripheric tissues to insulin. Beyond that taking into account the unchanged level of basal glycemia in animals of this group we can assume the presence of compensatory hyperinsulinemia. Due to this the level of basal glycemia in animals of control group remains on physiological level (tab.1).

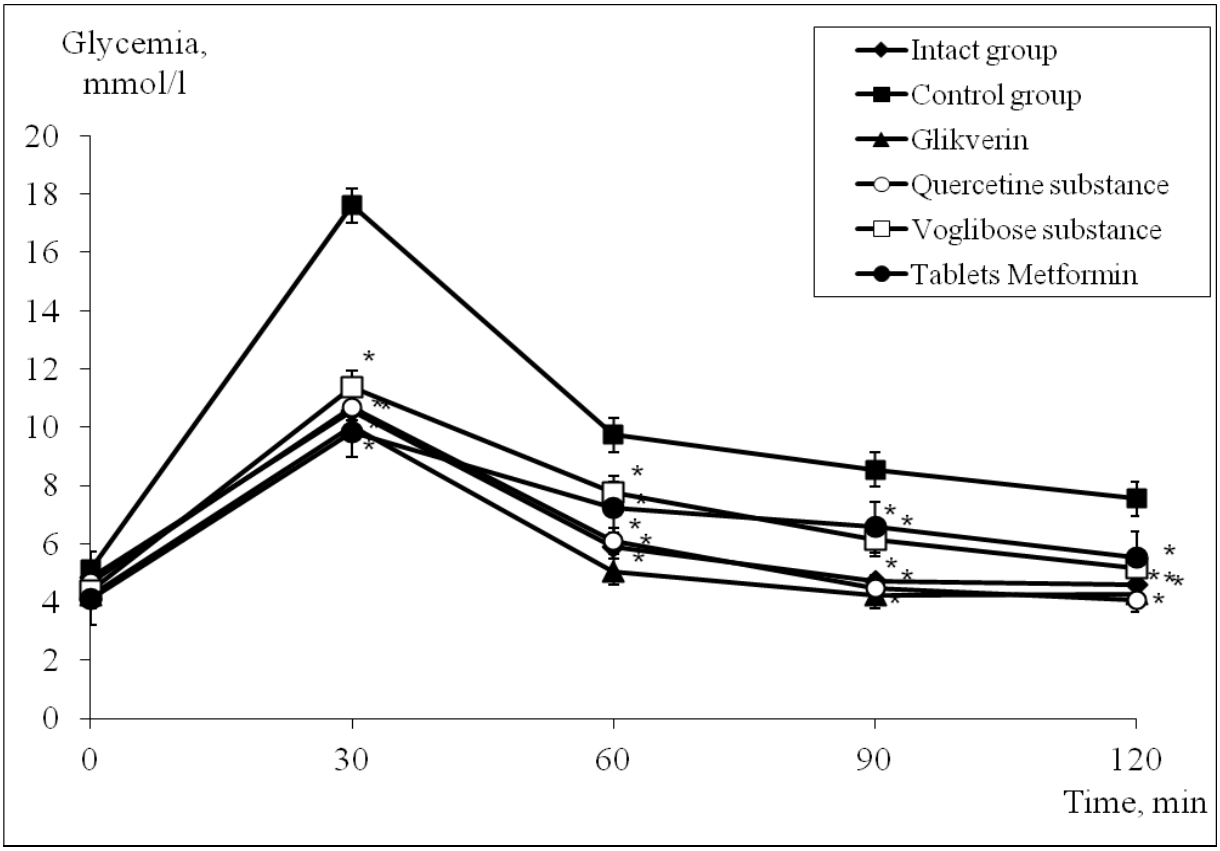

Fig. 1. Influence of Glikverin on dynamics of glycemia during IPGTT in rats in terms of metabolic syndrome, induced by 30 % sucrose solution, (X±Sx). Number of animals in each group = 7.

Table 1

Influence of Glikverin on indices of glucose homeostasis in rats in terms of metabolic syndrome induced 30 % sucrose solution (n=7), $X \pm S_x$

Study groups	Index		
	Basal glycemia, mmol/l	AUKglu, mmol/l×min (IPGTT)	CSI, %
Intact group	4,84±0,22	783,62±19,79	51,4±2,8
Control group	5,12±0,38	1290,24±55,02^{i}	18,2±3,3^{i}
Glikverin	4,24±0,73	733,65±15,32$^{p/v/m}$	49,6±7,7^{p}
Quercetine substance	4,68±0,25	768,21±29,44^{p}	46,6±3,8^{p}
Voglibose substance	4,37±0,24	900,21±40,43^{p}	42,3±5,4^{p}
Tablets Metformin	4,09±0,06	848,83±43,33^{p}	45,9±3,2^{p}

Notes:

i – differences are statistically significant in reference to values of animals from intact group, $p<0,05$;

p – differences are statistically significant in reference to values of animals from intact group, $p<0,05$;

v – differences are statistically significant in reference to values of animals treated with voglibose substance, $p<0,05$;

m – differences are statistically significant in reference to values of animals treated with metformin, $p<0,05$.

Chronic input of sugar in organism if animals caused changes in pancreatic gland in control group animals. Results of histological analysis correlate with received biochemical data and are an evidence for compensatory amplification of β-cells functional activity in order to maintain basal glucose level on physiological level. On the background of a normal space density of pancreatic islets (31,6 in microsample) we observed the rearrangement of islets within their sizes – the number of small islets increased, minor islets with only 2-4 β-cells appeared. This is a proof of enhanced regenerative processes in PG of rats for increase of functional capability of organ by means of excessive entrance of carbohydrates. There was also a grow in number of islet with nontypical shape – they appeared to spread in width, and this is also a sign of compensatory increase of insulinproductive islets' activity in terms of increase need for hormone for utilization of excessive carbohydrates. Along with this we observed decrease in number of big and middle islets (fig.2, b). We also observed islets with "exhaustion" and devastation of β-cells (fig.2, c, d). In some pancreatic islets (PI) nucleus of β-cells were hypertrophied, sometimes pyknotic, vacuolization of β-cells was observed (fig.2, e, f).

Thus, we revealed changes of carbohydrate metabolism in rats maintained on HSD. These changes suggest that there is a condition of "prediabetes" which is characterized by liver IR and strengthening of compensatory processes in pancreatic gland.

Using of Glikverin with solution of sucrose prevented development of metabolic disorders caused by long term keeping of animals on HSD. Evaluation of glucose homeostasis showed the improvement in glucose utilization in liver under the influence of a new combined agent. Increase of glucose tolerance spoke for that fact. While conducting IPGTT in rats that received Glikverin glycemia level was similar to glucose level in intact group and reference agent quercetin group and was statistically significant lower than in groups of voglibose and metformin (fig.1). Statistical analysis of values AUC_{glu} confirmed statistically significant advantage of Glikverin over reference agent voglibose and metformin (fig.1). Conducted short insulin tolerance test exhibited increase of sensibility of Glikverin and reference agents. Affected by investigational medicines КЧИ recovered to the level of intact animals (tab.1). Received data are associated with results of another studies of pharmacological properties of quercetin, voglibose and metformin in terms of metabolic syndrome [10, 11].

Established efficacy of Glikverin was confirmed by histological study if pancreatic gland tissue. In Glikverin group majority of animals had the same morphological structure of PG (saturation with β-cells, uniformity of spreading, functional condition) as in intact group (fig.2, g). Only in one animal we observed vacuolization of β-cells in part of islets (fig.2, h). Space density of PI was also at the level of intact animals (31.3 PI vs. 31,6 PI in intact group), percentage distribution of PI in number of β-cells was restoring. Thus, the number of newly formed islets

decreased 3.4 times, number of small islets – 1.5 times comparing to control group. Number of middle PI raised 1.5 times and big – 4.1 times comparing to control group (tab.2).

Table 2

Morphometric analysis of condition of incretic apparatus in rats in terms of metabolic syndrome induced by 30 % sucrose solution

Study groups	Space density of pancreatic islets	Spreading of pancreatic islets according to content of β-cells			
		2-4 (very small)	5-20 (small)	21-60 middle)	> 60 (big)
Intact group	31,6 (27; 39)	0 (0; 0)	25,7 (19,4; 35,9)	46,8 (42,4; 51,6)	27,5 (20,5; 33,3)
Control group	29,9 (26; 33)	$17,2^{i}$ (12,5; 21,2)	$48,6^{i}$ (41,9; 51,7)	$29,6^{i}$ (23,1; 35,5)	$4,6^{i}$ (0,0; 7,7)
Glikverin	31,3 (29; 34)	$5,04^{i/p/m}$ (0,0; 6,7)	$32,9^{i/p/m}$ (25,0; 48,4)	$43,3^{p}$ (32,3; 53,1)	$18,7^{i/p/m}$ (12,9; 27,6)
Tablets Metformin	31,8 (29; 34)	$8,9^{i/p}$ (6,3; 12,9)	$40,9^{i/p}$ (35,5; 48,4)	$40,8^{p}$ (35,3; 44,1)	$9,3^{i/p}$ (3,2; 17,6)

Notes:

i – differences are statistically significant in reference to values of animals from intact group, $p<0,05$;

p – differences are statistically significant in reference to values of animals from control group, $p<0,05$;

m – differences are statistically significant in reference to values of animals treated with reference agent metformin, $p<0,05$.

Influenced by metformin number of newly formed islets decreased 1.9 times and number of small ones - 1.2 times. Number of middle islets increased 1.4 times and number of big – 2.0 times (tabl.2). Morphological condition of PI in different animals within group varied. Part of PI as for all parameters in all animals was close to physiological standard (fig.2, i). But there was a large part of islets which had “untypical” shape, exhaustion in different stage, chaotical distribution and vacuolization of β-cells cytoplasm (fig.2, , k-m).

Thus, Glikverin exhibited protective action towards β-cells. It enhanced β-cells morphological condition, prevented dystrophic and necrobiotic changes which contributed to increase in number of middle and decrease in number of small and very small islets. In

distinctiveness of protective action on insulinproductive cells PG of rats Glikverin was greater than reference agent metformin.

Conclusions

1. Keeping of animals on HSD during 8 weeks leaded to development of the complex of metabolic changes which had such characteristics as insulin resistance, intolerance to carbohydrates, compensatory raise in functional activity of insulin apparatus PG, with further exhaustion and destruction of β-cells.

2. Prophylactic administration of combined agent Glikverin increased tolerance to carbohydrates, enhanced sensibility of peripheric tissues to insulin, had protective action towards insulin apparatus PG.

3. Received data make evidence for further pharmacological study of Glikverin as promising agent for treatment of metabolic syndrome.

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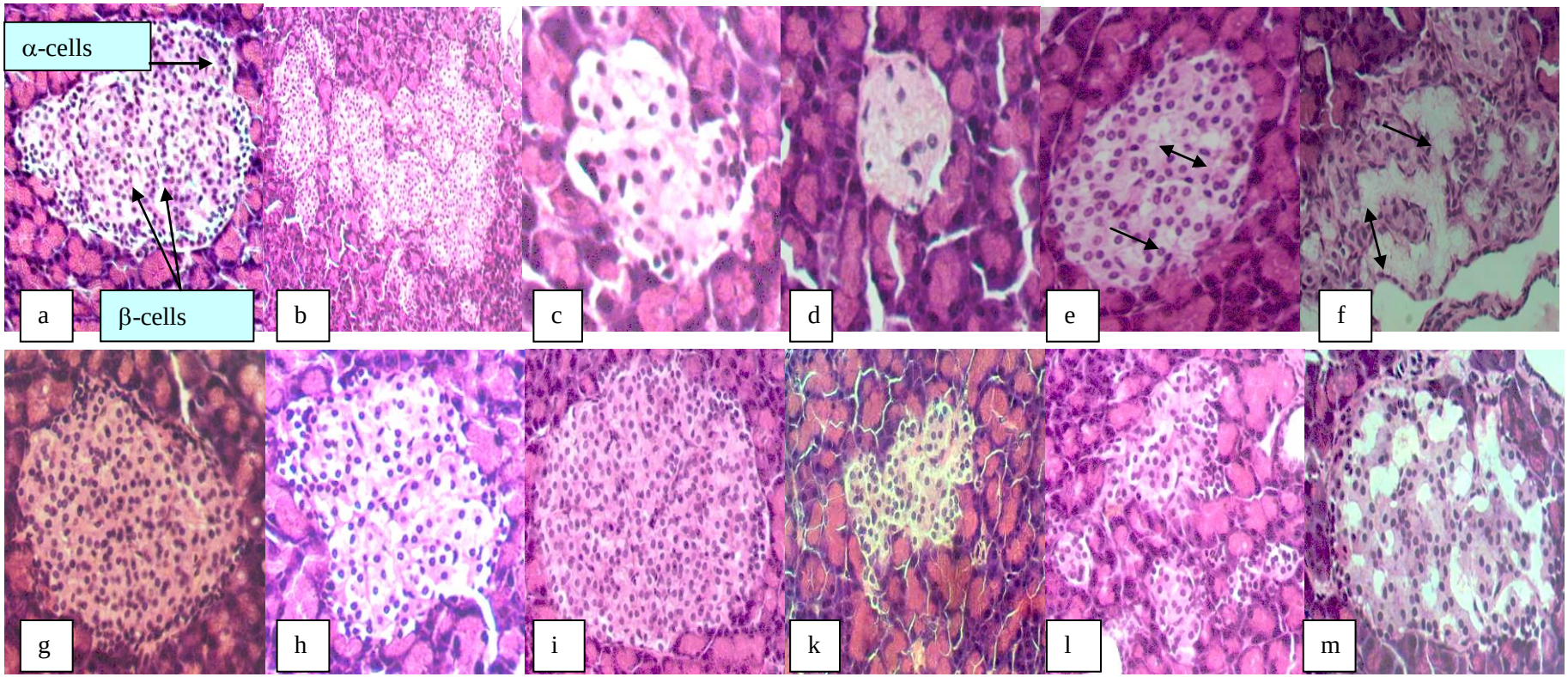

Fig. 2. Pancreas of rats from intact group, control group (sucrose) and rats that on the background of metabolic syndrome received experimental agents. Haematoxilin-eosine.

a – pancreatic islet from pancreas of intact animal: acinus with high density of spreading, pancreatic islet not of the precisely accurate form, evenly filled by β-cells. x200.

b-f – pancreas of rats' from control group (sucrose): «nontypical» shape of pancreatic islet (b); pronounced exhaustion of β-cells in pancreatic islet, hypertrophy of nucleus of remained cells (c); destruction of almost all pancreatic islet, pycnosis of nucleus of single β-cells (d); nesting, relatively moderate vacuolization of β-cells cytoplasm (e); very pronounced vacuolization (f). x250.

g-h – Glikverin, normal saturation and uniformity of spreading of β-cells in pancreatic islet of normal shape (g); vacuolization of β-cells of one animal (h). x250.

i-m – Metformin, physiologically normal pancreatic islet (i); «nontypical» shape of pancreatic islet (k); non-uniformal chaotic spreading of β-cells in different pancreatic islets (l); vacuolization of cytoplasm of β-cells (m). x250.