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IMPACT OF DRY EXTRACT OF GINGER ON MORPHOLOGICAL STATE OF PANCREAS OF SYRIAN GOLDEN HAMSTERS ON THE BACKGROUND OF HYPERCALORIUM DIET

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Мета – *дослідження впливу сухого екстракту імбиру на морфологічний стан підшлункової залози сирійських золотавих хом'яків на тлі ~~МС~~ метаболічного синдрому, індукованого гіперкалорійною дієтою.*
Матеріали і методи. *Метаболічний синдром моделювали шляхом утримання золотавих хом'яків-самців віком 20 тижнів на гіперкалорійній дієті, що збагачена джерелами енергії (містила 29 % жирів – переважно насичені ліпіди) та фруктозою (1 г на добу на 100 г маси тіла) протягом 6 тижнів. Сухий екстракт імбиру дозою 80 мг/кг, збір «Арфазетин» дозою 16 мл/кг та таблетки метформіну дозою 60 мг/кг уводили внутрішньошлунково один раз на день, починаючи з 4 тижня експерименту протягом 14 днів. На гістологічних зрізах підшлункової залози визначали загальну кількість панкреатичних острівців у мікропрепараті, вимірювали їх площу, проводили розподіл острівців на дрібні, середні та великі, визначали відсоткову частку кожної категорії панкреатичних острівців та стан β-клітин в них .*
Результати. *Тривале споживання хом'яками їжі, багатої на жири та вуглеводи, призводить до розвитку стану переддіабету, який характеризується морфологічним пригніченням інсулярного апарату: збільшення відносної частки дрібних та зменшення частки середніх панкреатичних острівців, зниженням наявності β-клітин та посиленням їх деструкції. За морфологічними характеристиками встановлено, що введення сухого екстракту імбиру дозою 80 мг/кг хом'якам з метаболічним синдромом відновлює стан панкреатичних острівців, їх площу, β- та α-клітини підшлункової залози до рівня інтактних тварин. Фармакологічний ефект сухого екстракту імбиру, ймовірніше за все, обумовлений фенольними сполуками, що входять до його складу - гінгеролом та шогаолом, які, можливо, модулюють вивільнення інсуліну за рахунок антиоксидантної дії.*
Висновки. *За виразністю протекторної дії сухий екстракт імбиру переважає препарати порівняння – метформін у дозі 60 мг/кг та збір «Арфазетин» у дозі 16 мл/кг. Отримані результати свідчать про перспективність подальшого експериментального та клінічного вивчення фармакологічних властивостей сухого екстракту імбиру з метою створення ефективного антидіабетичного фітопрепарату*
Ключові слова: *метаболічний синдром, висококалорійна дієта, сухий екстракт імбиру, підшлункова залоза, гістологічне дослідження*

1. Introduction

Expansion of type 2 diabetes mellitus (DM) has a pandemic nature. According to the International Federation of Diabetes (IDF), in 2015, there were 415 million patients with diabetes, in 2040, an increase of up to 642 million is projected [1]. In parallel, an increase in the number of states of glucose tolerance, which is almost always associated with a metabolic syndrome (MS), is recorded [2]. Metabolic syndrome is a complex of interconnected risk factors for cardiovascular diseases and type 2 diabetes, such as glucose metabolism disorders, high blood pressure, high levels of triglycerides, low level of cholesterol high density lipoprotein (HDL), and abdominal obesity. According to official medical data, in the western countries MS is observed in 20–25 % of the population, and at the age after 60 years – 45 %. On the background of MS mortality for various reasons increases 4 times, and cardiovascular pathology – more than 5 times, indicating a high social significance of this problem [3].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The morphological and functional status of the pancreas plays a significant role in the development of the main components of the MS (hyperinsulinemia, insulin resistance, glucose tolerance), and, conversely, existing metabolic changes (obesity, atherogenic dyslipidemia) contribute to the violation of the endocrine and exocrine function of the pancreas [4]. It is believed that the clinical and functional state of pancreas with MS represents dysmetabolic pancreatiopathy (steatosis, lipogenic pancreatitis, nonalcoholic fatty illness of pancreas), which consists in the diffuse development of adipose tissue in all organs of the body and is combined with inflammation on the background of obesity [5]. Therefore, under the condition of the MS, it is important to restore the status of pancreas, changes that are important in the development and complication of manifestations of the MS.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Modern therapy of MS is complex and involves the correction of violations of carbohydrate and lipid metabolism, the fight against excess body weight and the normalization of the morphological and functional state of the pancreas and liver. Along with standard drugs with different mechanisms of action (insulinosensitizers, hypolipidemic, antihypertensive, antithrombotic) it is expedient to use herbal substances that have a multifactorial effect on the body, which allows to level the main manifestations of the MS and eliminate or prevent the prevention of possible complications. Phytopreparations have a number of advantages over synthetic drugs: they are usually non-toxic, can be used for a long time without significant side effects and enhance the therapeutic effect of other drugs [6].

Today, more than 150 types of medicinal plants that are hypoglycemic, hypolipidemic, anti-atherogenic [7] are known to medical science and can be used in the treatment of MS and DM-2 type. According to many researchers, phyto-preparations increase regeneration of β-cells of POs, promote the restoration of insulin products [7–9].

Due to the ability to reduce the activity of free radical oxidation, the enhancement of which is important in the induction of various components of the MS, in particular insulin resistance, endothelial dysfunction and prothrombotic state, medicinal plants have protective properties in relation to β-cells of pancreas, which are highly susceptible to oxidative stress due to low expression of antioxidant enzymes [10].

4. The field of research considering the general problem, which is described in the article

Among the many medicinal plants widely used in diabetes, blueberries and beans are considered to be the most effective, and the list of domestic officinal multi-component antidiabetic herbal preparations is limited to the species "Arphasetin", which indicates the relevance of the search and development of new phytopreparations for the treatment of DM type 2 and MS.

One of the promising medicinal plants with hypoglycemic action is ginger medicines (Zingiber officinale). In previous studies, we found the expressed antidiabetic properties of dry ginger extract on models of experimental DM 2 and MC [11–13]. Taking into account the above, it is expedient to estimate the protective properties of dry extract of ginger on β-cells of pancreas under the condition of experimental MS.

5. Formulation of goals (tasks) of article

The aim of this work was to investigate the effect of dry extract of ginger on the morphological state of the pancreas of Syrian golden hamster on the background of MS induced by hypercaloric diet.

6. Presentation of the main research material (methods and objects) with the justification of the results

The metabolic syndrome was modeled by keeping the hamsters on a high-calorie (hypercaloric) diet rich in energy (29 % fat - mostly saturated lipids) and fructose (1 g per day per 100 g body weight as aqueous solution) for 6 weeks [14].

The experiment used Syriac golden hamsters-males (20 weeks old at the beginning of the experiment) that were kept under the standard conditions of the vivarium of the Central Research Laboratory of the National Pharmaceutical University at a temperature of 22 ± 1°C, a moisture is 50-60 %, in a room with changing day-night regimes. Dry extract of ginger with a dose of 80 mg/kg, species of "Arphasetin" (production, series) at a dose of 16 ml / kg and metformin tablets (TN, production, series) at a dose of 60 mg/kg was administered intragastrically once daily from the 4th week of the experiment within 14 days. A group of animals of control pathology received similar purified water.

At the end of the experiment, the animals were decapitated under ethereal anesthesia and we received material for research. Since the pancreas of hamsters is very stretched (the head is located on the caudodorsal surface of the stomach, the body between the stomach and duodenum adjacent to it, the spleen, the ascending part of the colon, and the tail of the gland reaches the left kidney [15] for the study was taken part of the gland body, that was in the gastro-pleval connective tissue.

The resulting tissue material was fixed in 10 % formalin solution, dehydrated in spirits of increasing strength, poured into paraffin. Paraffin blocks with specimens of the gland were cut on the MS-1 snuff-microtubule, the sections were stained with hematoxylin and eosin [16]. The histological sections of the pancreas determined the total number of pancreatic islets (PI) in the slides. Using the Toupcam Granum program, the area of PI was measured, based on the area of PI, the islands were divided into: small (up to 4800 μm^2), medium (from 4801 to 6700 μm^2) and large (from 11000 μm^2), percentage of each category of pancreatic islets were determined [17]. All received digital data was processed by the methods of variation statistics [18]. The microscopic examination was carried out under the Granum light microscope, and photographs of the microscopic images were performed by a digital camcorder Granum DCM 310. The photos were processed on a Pentium 2.4 GHz computer using the Toup View program.

In intact hamsters, the exocrine component of the glandular tissue of the pancreas had a classic structure. The endocrine component of PI has predominantly an oval form, which is not always sufficiently clearly separated from the acinar tissue. The number of PI did not exceed 5-7 units per micropreparate (6.4 ± 0.2). Percentage of small software was 28.1 ± 3.0 %, the average – 65.7 ± 2.3 %, the largest – 6.2 ± 3.8 %. Their area was 4014.3 ± 243.1 μm^2, respectively; 6007.2 ± ± 208.7 μm^{2}and 16473.8 ± 3253.3 μm^2 (Tabl. 1). The islands are uniformly filled with α and β-cells, whose nuclei are quite distinct in size and intensity of color. The clear localization of cells with colored nuclei in the PI was not observed (Fig. 1).

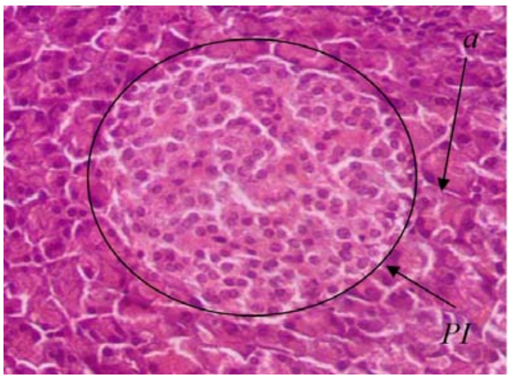


Fig. 1. The pancreas of intact Syrian hamster. You can see numerous acinus (a) and pancreatic islet (PI), evenly filled with cells whose nuclei differ in size and degree of color (×250)

According to the literature [16], smaller β-cells contain large dark nuclei, α-cells larger, with pale, smaller than β-cells, nuclei. The number of α-cells in PI is less than α-cells [16].

Prolonged use of the diet of hamster, saturated with fats and fructose, led to certain changes in both the endocrine and exocrine component of the pancreas. The number of PI on micropreparations did not change, however, the distribution percentage of them changed. So the share of small PI has increased by 1.55 times, the average, on the contrary, decreased by 1.4 times (a clear trend). The share of large PI has not changed, but among them observed the appearance of unusually large islands, resulting in an increase in the average area of these islands (although not likely) to 18642.5±5485.3 μm^2. The area of small PI has probably decreased to 2815.5±±196.8 μm^2, and the average (unlikely) – to 5771.77±±125.5 μm^2 (Table 1).

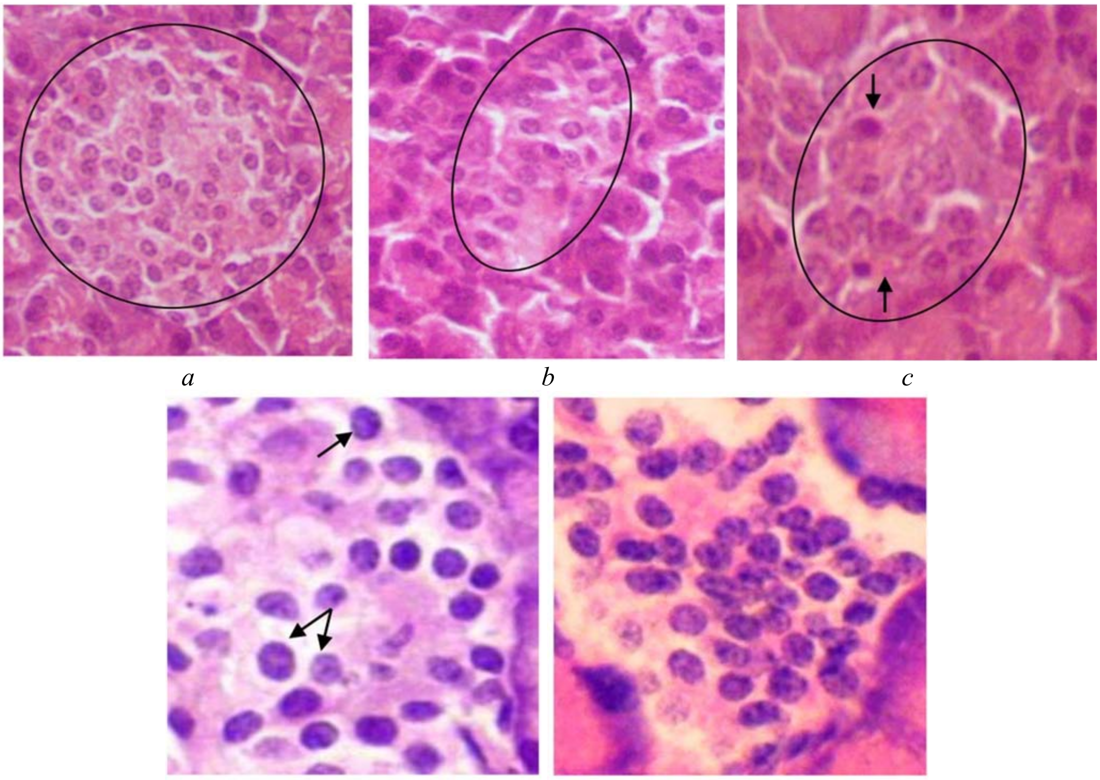
Table 1

Morphometric indices of the state of the enteric apparatus of the pancreas of hamsters in the conditions of long-term application of the diet saturated with fats and fructose, and the corrective action of the dry extract of ginger (n=5)

Group of animals	Total amount of PI in a slide	Differentiation of PI by area								
		small			average			large		
		square (μm^2)	quantity	%	square (μm^2)	quantity	%	square (μm^2)	quantity	%
Intact control	6.4±0.2	4014.3±243.1	1.8±0.2	28.1±3.0	6007.2±208.7	4.2±0.2	65.7±2.3	16473.8±3253.3	0.4±0.2	6.2±3.8
CP (diet)	6.2±0.2	2815.5±196.8*	2.8±0.2*	45.2±3.3*	5771.8±125.5	3.0±0.3*	48.6±5.5	18642.5±5485.3	0.4±0.2	6.7±4.1
CP + dry extract of ginger	6.2±0.2	3735.8±116.6**/#	1.8±0.2**	29.0±3.2**/#	5961.5±192.8	3.8±0.2	61.4±3.4	12572.7±839.3	0.6±0.2	9.5±3.9
CP + species «Arphasetin»	6.2±0.2	3245.0±113.7*/ψ	2.6±0.2 ψ	41.9±3.7*/ψ	6129.3±49.0	3.2±0.2*	43.1±10.1	13710.2±371.2	04±0.2	6.7±4.1
CP + methformin	6.2±0.4	3073.7±158.7*	2.4±0.2	38.5±2.2	6102.3±142.0	3.6±0.2	58.7±4.4	14362.2±0.0	0.2±0.2	2.9±2.9
p	0.9717	0.0003	0.0101	0.0024	0.5090	0.0142	0.0653	0.5927	0.8360	0.8103

*Note: p is the level of statistical significance obtained using ANOVA dispersion analysis when compared to all experimental groups; * – level of statistical significance when comparing experimental groups with intact control group; ** – the level of statistical significance when comparing experimental groups with the CP group; ψ – level of statistical significance when comparing experimental groups with the group of dry extract of ginger; # is the level of statistical significance when comparing experimental groups with the methformin group*

In some islands, viscous traces of different expressiveness were observed visually in the location of cells, there was a decrease in the presence of cells with dark cores (β-cells) or destruction or hypertrophy of parts of such nuclei (Fig. 2).



a *b* *c*

d *e*

Fig. 2. The pancreas of Syrian hamster who received a diet rich in fats and fructose: *a, b* – the dilution of cells in pancreatic islets, the practical absence of β-cells; *c* – destruction of β-cells in the islet; *d* – hypertrophy of the nucleus of β-cells; *e* – a certain "levelling" of the differential differences between the nuclei of the endocrinocytes; Hematoxylin-eosin; *a–c* – ×250; *d, e* – ×400

Similar changes indicate a certain exhaustion of the insulatory apparatus, which is a consequence of hyperfunction of β-cells against the background of hypercaloric diet. Under these conditions, the total potential of β-cells is reduced, however, in the blood of these animals hyperinsulinaemia is observed against the background of severe hyperglycaemia [13], which confirms the stresses of compensatory-adaptive processes, hyperfunction, and indicates the development of the pre-diabetes state.

In the exocrine part of the glandular tissue, the eosinophilic zone is often significantly enlarged in the pancreatic cytoplasm, the acinus lumen is narrowed and is surrounded by a secretion that disturbs the functional morphology of the cells. The cytoplasm is vaculated, the nuclei are hypertrophied (Fig. 3). These changes may be due to the development of peculiar changes in the external secretion department in conditions of excess fat and carbohydrates and relative protein deficiency [16].

Given the therapeutic and prophylactic administration of a dry extract of ginger with a dose of 80 mg/kg, visually virtually all PIs, β- and α-cells according to morphological characteristics corresponded to the state of the endocrine apparatus in intact hamsters (Fig. 4).

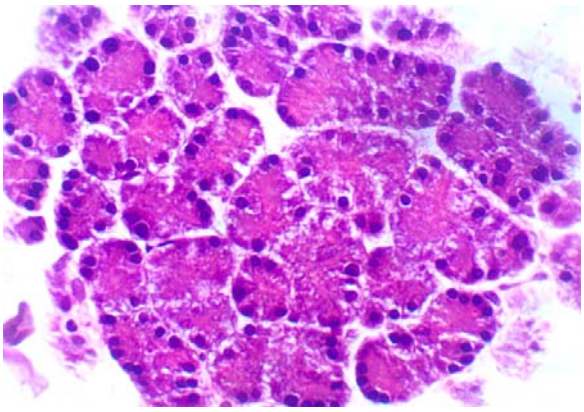
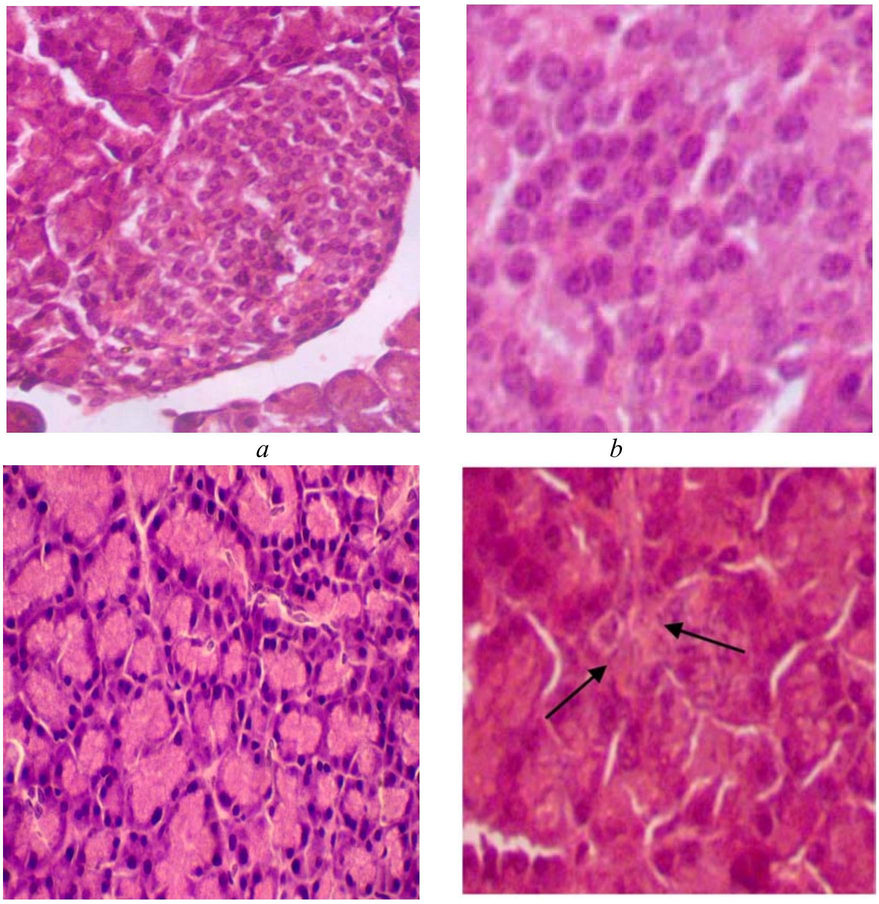


Fig. 3. The Syrian hamster's pancreas that received a diet rich in fats and fructose. Hypertrophy and hyperchromia of nuclei, a distinct increase in the zymogen-retaining zone, vacuolation of the cytoplasm of pancreatitis. Hematoxylin-eosin. ×200

Morphometrically, the number and percentage of small islands, their area recovered to almost the level of intact control. A clear tendency towards the restoration of the share of middleware has been traced (Table 1). In the exocrine part, sometimes acino-islet cells are seen – mixed cells that produce both zymogen granules and hormones simultaneously (Fig. 4).



a *b*

c *d*

Fig. 4. The pancreas of Syrian hamsters receiving a dry extract of ginger on the background of a diet rich in fats and fructose: *a* – normal saturation, placement and differentiation of endocrinocytes in a pancreatic islet (×250); *b* – normal state of pancreatic β-cells (x400); c - the state of pancreatites corresponds to normal (×250); d – among acinus visible single actinost cells (×400). Hematoxylin-eosin

Under the influence of the drug comparison of methformin at a dose of 60 mg/kg, there was a visual improvement of the morphological characteristics of the inoculum apparatus of the hamsters. The islands were more evenly filled with cells, the most distinct were the differentiation of nuclei by staining, reduced the presence of hypertrophied and destructively altered β-cells (Fig. 5).

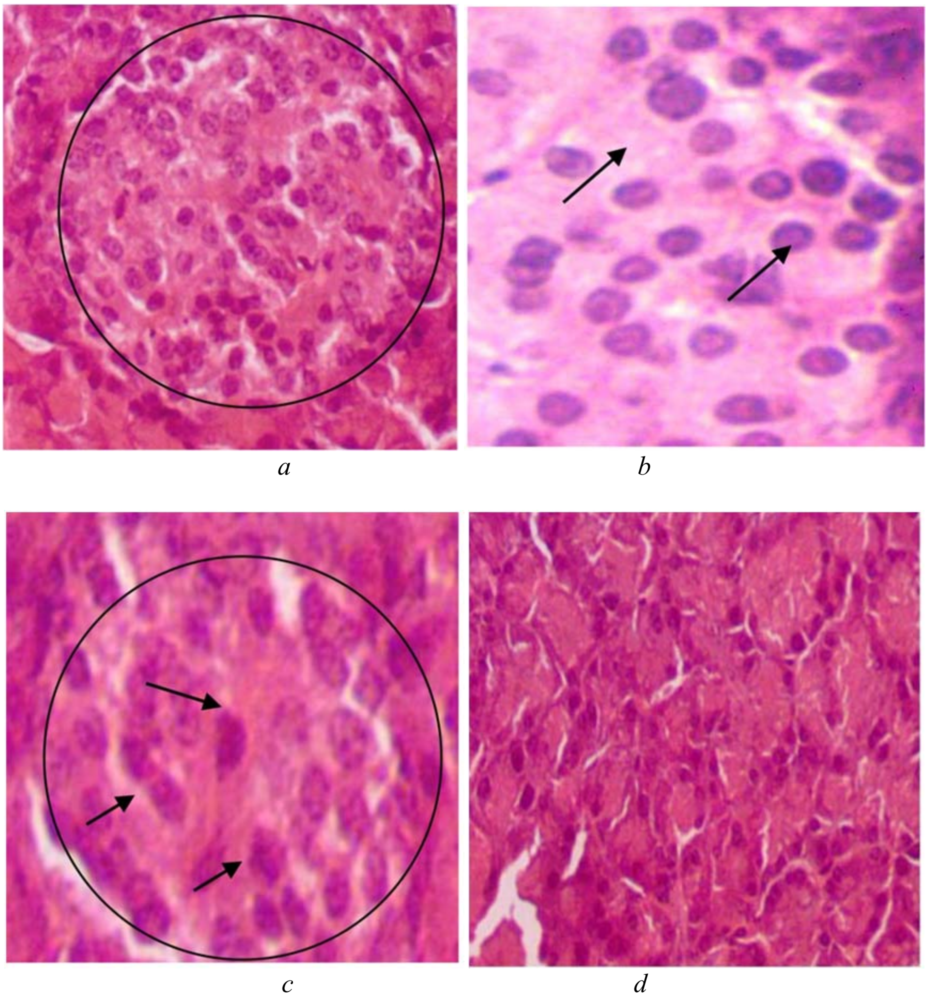


Fig. 5. The pancreas of Syrian hamsters, who received methformin on a background of fat-rich and fructose diets: *a* – less pronounced dilution of cells in a pancreatic islet, the absence of "levelling" the differential differences between the nuclei of endocrinocytes; *b* – a decrease in the presence of hypertrophic β-cells; *c* – in - the morphology of β-cells is close to normal; *d* – absence of cell vacuolization. Hematoxylin-eosin; *a* – ×250; *b, c* – ×400; *d* – ×200

However, morphometrically, the majority of the studied parameters did not differ from those of the control pathology, although some of them (the number and percentage of small and medium PI) had a clear tendency to recovery (Tab. 1).

After the introduction of a drug comparing the species "Arphasetin" to hamsters against a high-calorie diet, the amount of PI in the pancreas slides of animal remained stable. Morphologically, the changes of the in-vitro structures were less pronounced than in the hamsters of the control group, although some differences in the state of different PIs and β-cells were observed (Fig. 6).

The morphometric indices characterizing the endocrine component of the PI under the influence of the collection of "Arphasetin" coincided with those in the animals receiving methformin. The share and area of small PI was significantly lower than in the background of the action of ginger extract at a dose of 80 mg/kg (Tab. 1).

Exocrine parenchyma of PI was characterized by an unchanged acinus pattern. Pancreacites themselves were not overloaded with zymogen grains, the ratio of differently coloured zones in the cytoplasm of cells in comparison with intact control did not change significantly (Fig. 6).

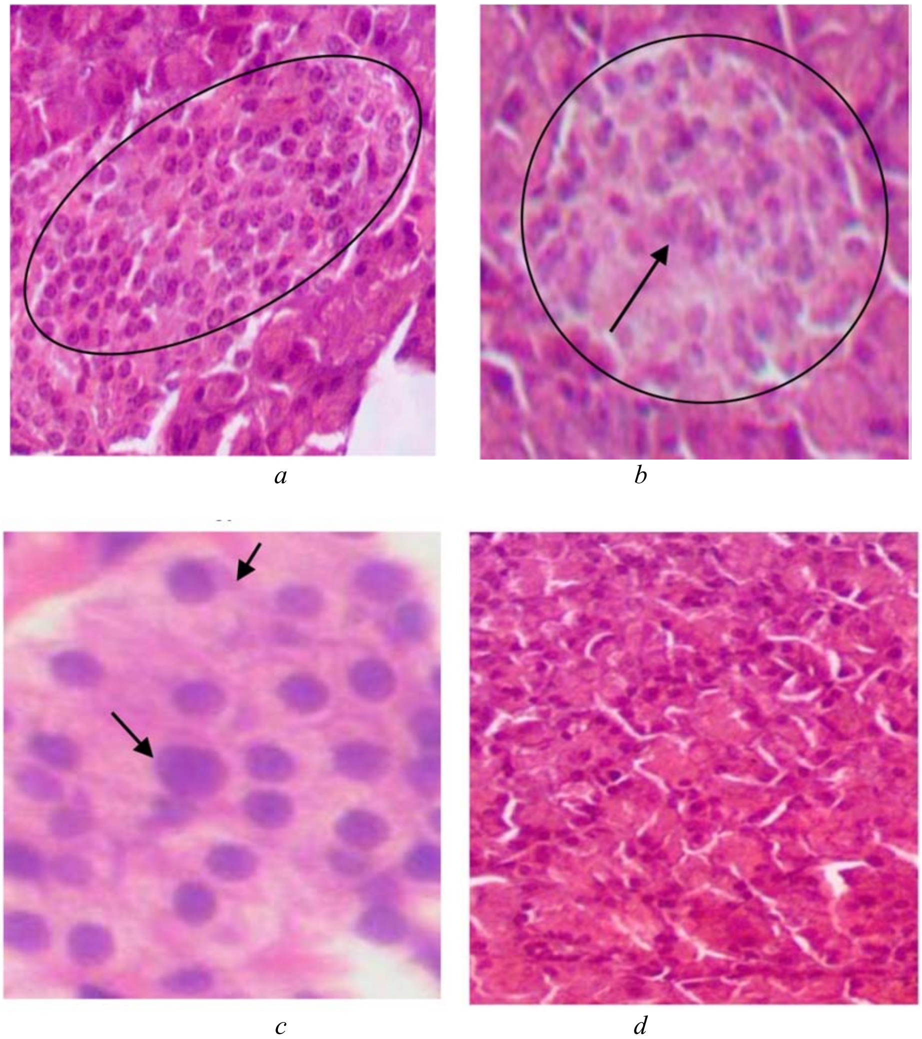


Fig. 6. The pancreas of Syrian hamsters, who received a species "Arphasetin" on the background of a diet rich in fats and fructose: *a* – the normal condition of the pancreatic islet and β-cells; *b* – focal devastation, destruction of β –cells *c* – hypertrophy of the β -cells; *d* – unchanged acinar parenchyma. Hematoxylin-eosin; *a, b* – ×250; *c* – ×400; *d* – ×200

7. Conclusions from the conducted research and prospects for further development of this field

Long-term consumption of food, rich in fats and carbohydrates by hamster leads to the development of pre-diabetes, which is morphologically characterized by inhibition of the insulatory apparatus: an increase in the relative proportion of small particles and a decrease in the proportion of middle pancreatic islets, a decrease in the presence and quality of β-cells in the islands.

The results of the morphological study showed the protective effect of a dry extract of ginger with a dose of 80 mg/kg on the pancreas and β-cells of pancreatic islets, manifested by the reduction of the number, percentage of small islets and their area to the level of intact control and a clear tendency to restore the proportion of middle pancreatic islets.

The established pharmacological effect of dry extract of ginger, most likely, is due to phenolic compounds, which are part of its composition gingerol and shgaalom, which may modulate the release of insulin due to antioxidant action [20].

According to the pronounced tread effect, the dry extract of ginger predominated comparisons - metformin at a dose of 60 mg/kg and the species of "Arphasetin" at dose of 16 ml/kg.

The obtained results testify to the prospect of further experimental and clinical study of the pharmacological properties of dry ginger extract in order to create an effective antidiabetic phytopreparatum.

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