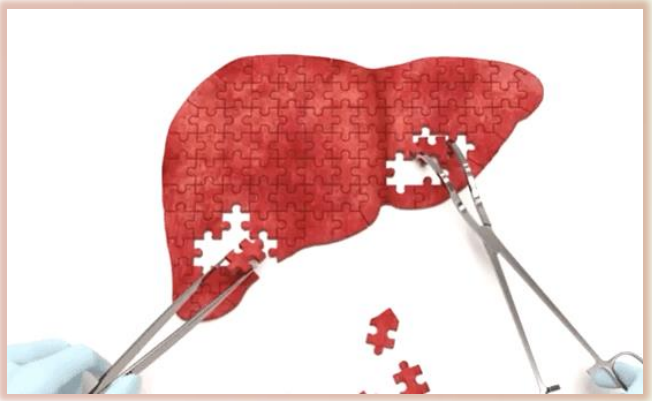
EVALUATION OF THE HEPATOPROTECTOR ACTIVITY OF NEW THIENO[2,3-B]QUINOLINE AND 1,4-DIHYDROPYRIDINE DERIVATIVES



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Abstract

Currently, liver diseases, their etiology, pathogenesis and morphological changes accompanying hepatobiliary pathology are becoming more relevant. It is important to search for drugs that have hepatoprotective properties and have several targets. In this work, we study the hepatoprotective activity of new derivatives of thieno[2,3-b]quinoline and 1,4-dihydropyridine with laboratory codes AZ-383, AZ-257, AZ-020 in a model of metabolic disorders in Wistar rats by evaluating morphological changes, biochemical and immunohistochemical parameters of the liver. These compounds were synthesized on the basis of the research laboratory “ChemEx" of the Lugansk State University named after Vladimir Dahl and according to the results of the experiment, they have hepatoprotective activity with alimentary and dexamethasone load, which is confirmed by a significant decrease in the activity of ALT, AST, the level of total bilirubin, morphologically and immunohistochemically.

Key words: high-fat diet, steroid load, metabolic disorders, cyanothioacetamide derivatives, hepatoprotective activity.

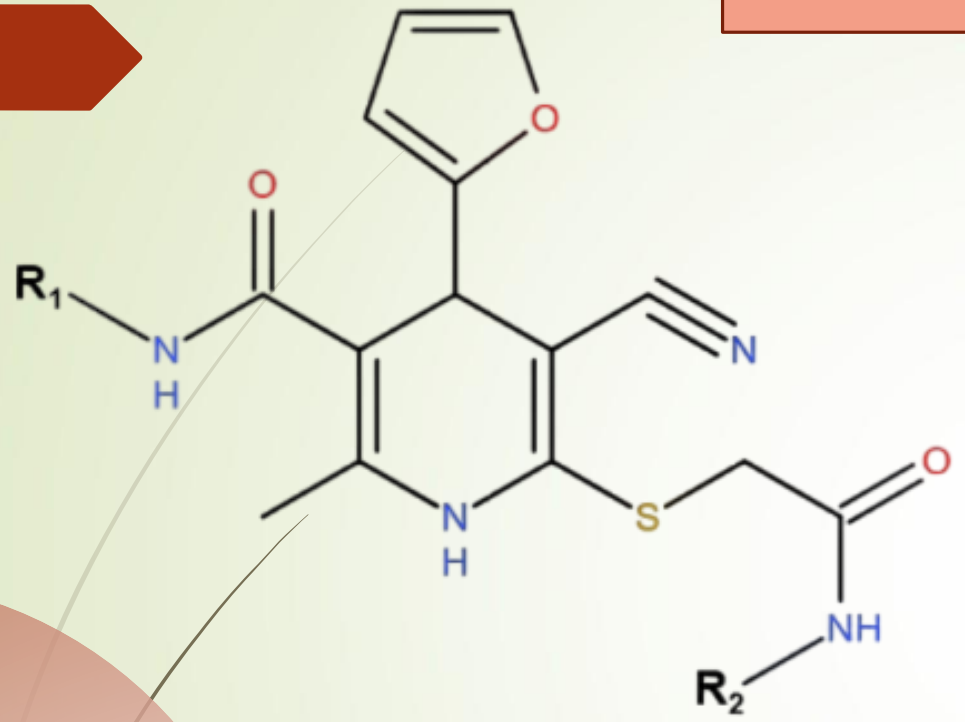
Relevance of the topic

- In the modern world, medical specialists are of particular interest to liver diseases, their etiology, pathogenesis and morphological changes that accompany hepatobiliary pathology.
- Due to the increase in frequency and significance

- polypharmacy in the pharmacotherapy of many diseases,
- malnutrition with a predominance of fats,
- the use of glucocorticoid therapy,

priority is given to **hepatoprotective activity** in the spectrum of pharmacological effects of drugs.

Relevance of the topic



- **Cyanothioacetamide derivatives** are the prospect of creating highly effective drugs that have a positive effect on metabolic processes while simultaneously having hepatoprotective activity.

- **Questions of the chemical synthesis** of cyanothioacetamide derivatives have been studied over the past three decades on the basis of the Research Laboratory «ChemEx» of the Dahl Lugansk State University.

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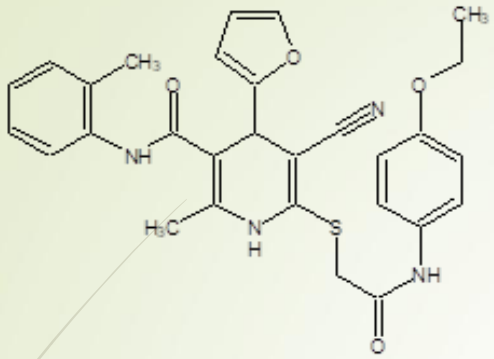
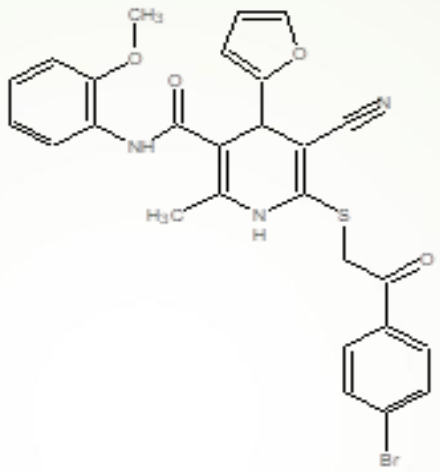


Compound **AZ-383**

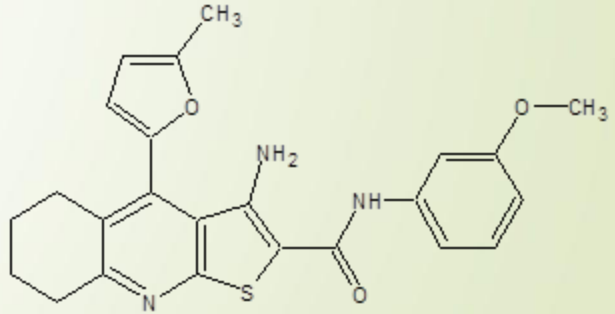
We have selected compounds with laboratory codes **AZ-383**, **AZ-257**, **AZ-020**, which have biotargets for influencing carbohydrate and lipid metabolism, as well as liver function.



Compound **AZ-257**

Expected pharmacological effects:

- hypoglycemic,
- lipid-lowering action,
- hepatoprotective,
- immunostimulating,
- protein-synthetic action,
- membrane protective activity,
- the ability to influence the level of appetite, body weight,
- restore β-cells of the pancreas.



Compound **AZ-020**

Chemical structure of the studied samples

Assessment of oral toxicity

- When assessing the acute oral toxicity of samples in laboratory animals, a **toxicity class was determined**.

- Substances with laboratory codes **AZ-383**, **AZ-257**, **AZ-020** are **low-toxic compounds** (LD50 ≥ 5,000 mg/kg).

Purpose of the study

- To study the hepatoprotective activity of new heterocyclic alpha-cyanothioacetamide derivatives on a model of metabolic disorders, evaluating morphological changes, biochemical and immunohistochemical parameters.

Materials and methods

- The experiment was implemented at the Research Institute of Experimental Biology and Medicine, Voronezh State Medical University named after N.N. Burdenko on **72** mature Wistar rats.
- The evaluation of the hepatoprotective activity of new cyanothioacetamide derivatives was carried out on a model of metabolic disorders created by alimentary (high-fat diet for 8 weeks) and glucocorticoid (intraperitoneal administration of dexamethasone 0,125 mg/kg for 13 days) loads.

Materials and methods

INTACT GROUP	Rats on a **standard daily diet** - granulated feed and free water.
CONTROL GROUP	In addition to the standard daily diet + **high-fat diet** - palm oil at the rate of 30 g/kg for 8 weeks, then intraperitoneal administration of **dexamethasone** 0,125 mg/kg for 13 days.
COMPARISON GROUP №1	Pharmacological correction of modeled metabolic disorders with **Metformin** 300 mg/kg for 14 days.
COMPARISON GROUP №2	Pharmacological correction of modeled metabolic disorders with **Vildagliptin** 8 mg/kg for 14 days.
EXPERIENCE GROUP №1	Pharmacological correction of modeled metabolic disorders **AZ-383** at the rate of 1 mg/kg for 14 days.
EXPERIENCE GROUP №2	Pharmacological correction of modeled metabolic disorders **AZ-257** at the rate of 1 mg/kg for 14 days.
EXPERIENCE GROUP №3	Pharmacological correction of modeled metabolic disorders **AZ-020** at the rate of 1 mg/kg for 14 days.

Materials and methods

During the experiment, daily **physical examination and observation of behavioral reactions** were carried out.

The evaluation of the hepatoprotective activity of the compounds was carried out by determining the concentration of **ALT, AST and total bilirubin** in the blood.

Liver sections were stained with **Gill's hematoxylin and eosin** for further study of liver microarchitectonics.

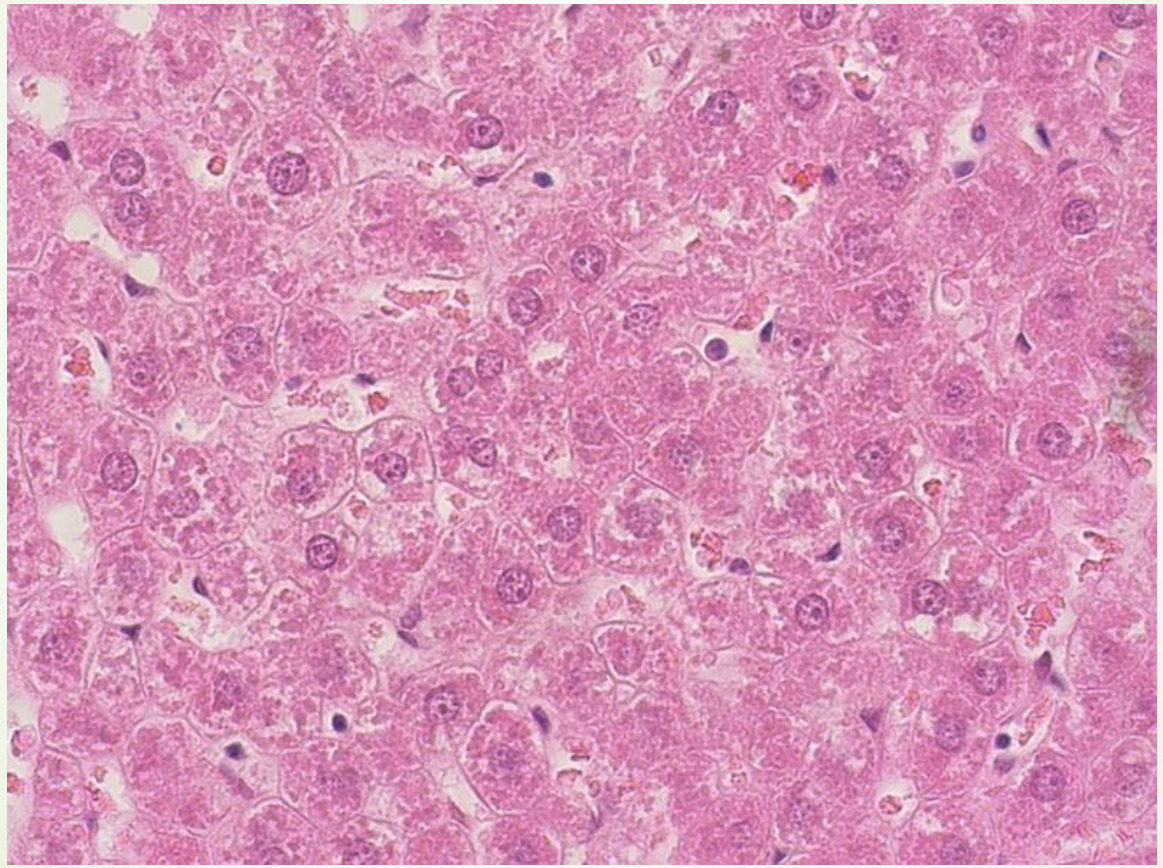
Spent immunohistochemical detection to **Ki-67** (SP6) rabbit monoclonal antibodies.

Research results

Index 	ALT, U/l	AST, U/l	Total bilirubin, µmol/l	Glucose, mmol/l	Total cholesterol, mmol/l	Triglycerides, mmol/l
Intact group	59,53 ± 8,2	146,51 ± 16,84	10,88 ± 1,0	7,9 ± 0,7	1,3 ± 0,2	0,7 ± 0,3
Control group	105,29 ± 9,9*	192,65 ± 9,0*	23,72 ± 3,5*	11,44 ± 1,1*	2,0 ± 0,2*	1,9 ± 0,4*
Comparison group №1 (Metformin)	57,49 ± 6,6	181,67 ± 10,90*	10,70 ± 1,1	7,3 ± 0,5	1,6 ± 0,2	0,9 ± 0,2
Comparison group №2 (Vildagliptin)	53,23 ± 6,4	156,23 ± 8,5	11,93 ± 1,5	7,9 ± 0,4	1,6 ± 0,2	0,6 ± 0,2
Experience group №1 (AZ-383)	56,78 ± 6,6	155,87 ± 14,53	11,92 ± 0,7	7,9 ± 0,4	1,4 ± 0,1	0,7 ± 0,2
Experience group №2 (AZ-257)	55,85 ± 5,8	145,33 ± 12,67	12,54 ± 1,1	8,6 ± 0,6	1,2 ± 0,1	0,7 ± 0,2
Experience group №3 (AZ-020)	53,87 ± 5,1	138,21 ± 13,30	12,34 ± 0,8	8,3 ± 0,5	1,5 ± 0,2	0,8 ± 0,3

Research results

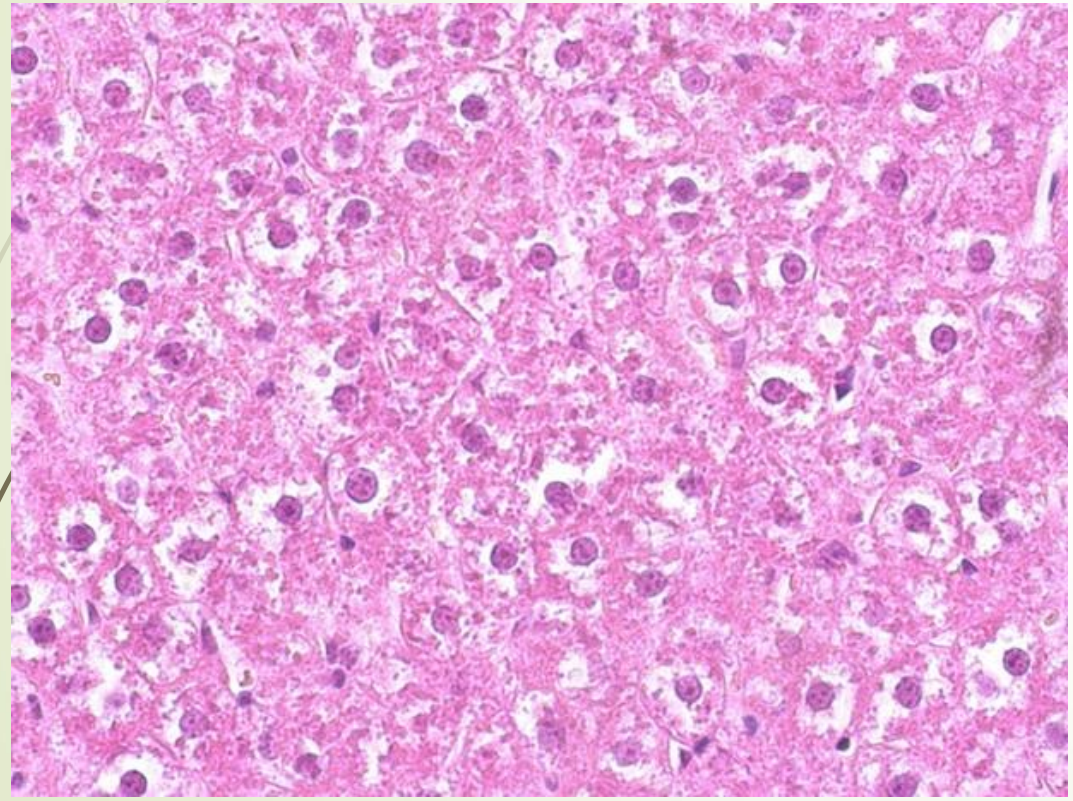
- Normal structural and functional organization of the liver of intact rats.

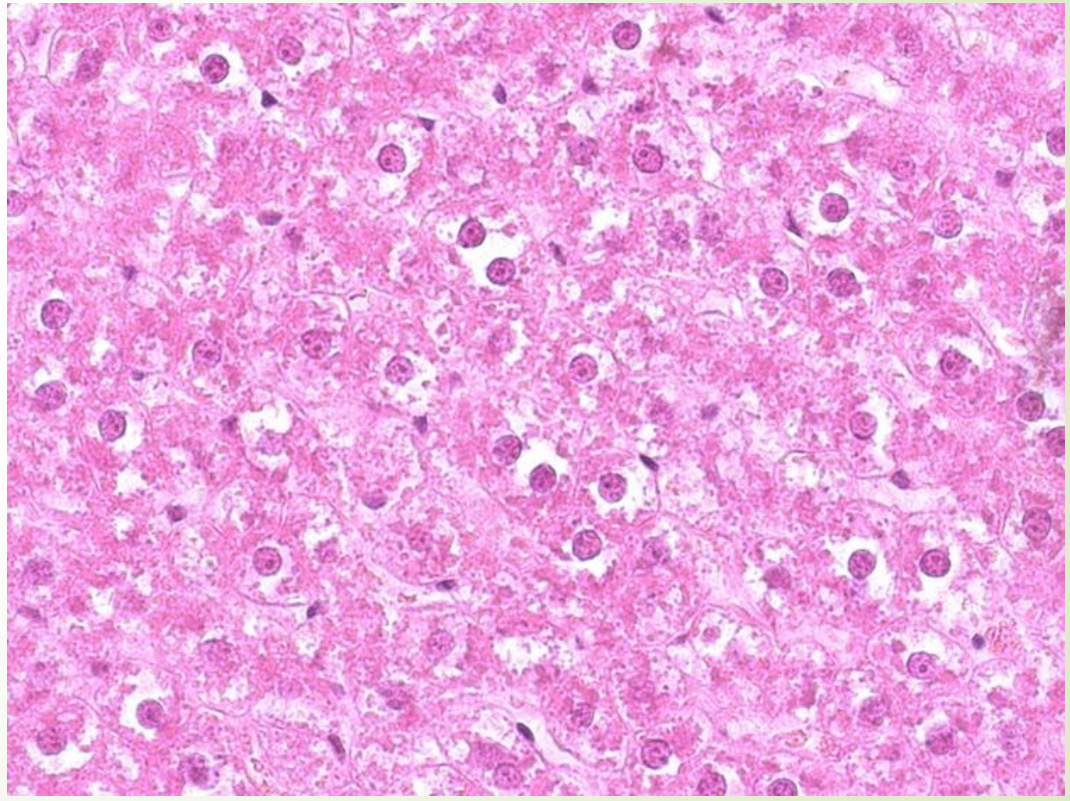


Rat liver intact group (x400 magnification, stained with Gill's hematoxylin and eosin).

Research results

- A high-fat diet and steroid load disrupted the structure of the liver, which was expressed in signs of protein, fatty degeneration, and hepatocyte necrosis.

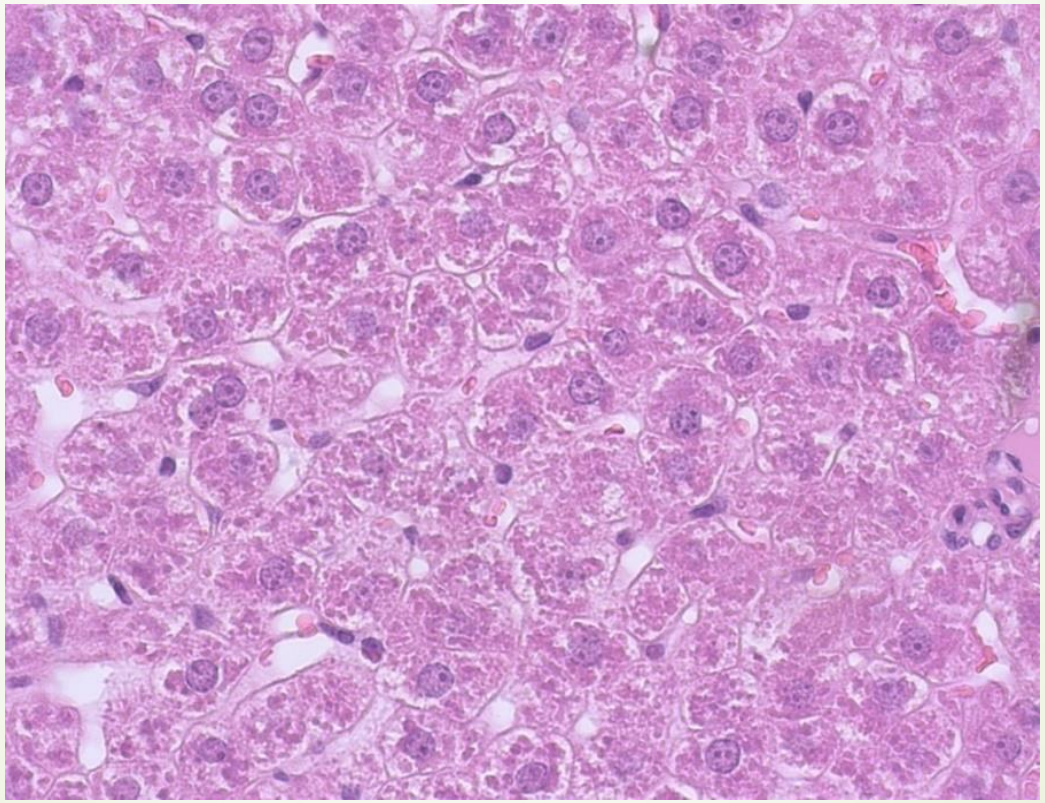




Control rat liver (x400 magnification, stained with Gill's hematoxylin and eosin).

Research results

- New derivatives of cyanothioacetamide with codes **AZ-383**, **AZ-257**, **AZ-020** showed hepatoprotective activity. The beam structure of the liver of rats of these groups was preserved. Hepatocytes had a polygonal shape, rounded nuclei, with a clear karyolemma and nucleoli. Glycogen granules were localized in the cytoplasm without an abundance of fat droplets and vacuoles.



Liver of rats of the experimental group treated with a new derivative of cyanothioacetamide with code AZ-383 (x400 magnification, stained with Gill's hematoxylin and eosin).

Research results

- Alimentary and dexamethasone loads led to an increase by 11,3% in the average size of hepatocytes, by 7,6% of the area of their cytoplasm and by 40% of the area of hepatocyte nuclei. Accordingly, there was a violation of the nuclear-cytoplasmic ratio, which increased by 30% compared with the intact group.

Group Index	Intact group	Control group	Comparison group №1 (Metformin)	Comparison group №2 (Vildagliptin)	Experience group №1 (AZ-383)	Experience group №2 (AZ-257)	Experience group №3 (AZ-020)
Hepatocyte cytoplasm area (μm²)	72,35 ± 0,39	77,82 ± 0,27	75,9 ± 0,34	76,19 ± 0,38	73,79 ± 0,29	75,68 ± 0,3	76,04 ± 0,35
Area of hepatocyte nuclei (μm²)	9,24 ± 0,2	12,97 ± 0,21	11,2 ± 0,25	10,98 ± 0,2	10,54 ± 0,22	10,86 ± 0,3	11,13 ± 0,24
Hepatocyte size (μm²)	81,59 ± 0,59	90,79 ± 0,48	87,2 ± 0,59	87,17 ± 0,58	84,33 ± 0,51	86,54 ± 0,6	87,17 ± 0,59
Nuclear-cytoplasmic ratio	0,13 ± 0,004	0,17 ± 0,004	0,15 ± 0,003	0,14 ± 0,003	0,14 ± 0,004	0,14 ± 0,003	0,15 ± 0,005

Research results

- When assessing the proliferation index KI-67 in the liver, a sharp (4,46 times) inhibition of the hepatocyte proliferation index was recorded in rats of the control group.
- The most pronounced proliferative activity of the liver was registered in rats that were injected with the compound with the code AZ-383.

Group	Mean number of hepatocytes per field of view	Mean number of KI-67-positive cells per field of view	Proliferation index
Intact group	40,92 ± 1,04	0,75 ± 0,04	1,83 ± 0,04%
Control group	43,68 ± 0,98*	0,18 ± 0,03*	0,41 ± 0,03%*
Comparison group №1 (Metformin)	45,7 ± 0,96*	0,54 ± 0,04*	1,18 ± 0,04%*
Comparison group №2 (Vildagliptin)	48,92 ± 1,08*	0,46 ± 0,02*	0,94 ± 0,02%*
Experience group №1 (AZ-383)	51,46 ± 1,09*	0,76 ± 0,03	1,48 ± 0,03%*
Experience group №2 (AZ-257)	43,9 ± 1,05*	0,42 ± 0,03*	0,96 ± 0,03%*
Experience group №3 (AZ-020)	45,72 ± 0,93*	0,48 ± 0,04*	1,05 ± 0,04%*

Conclusion

Analyzing the biochemical, morphometric and immunohistochemical parameters of the functioning of the liver under conditions of alimentary load and the intake of glucocorticoids, **the presence of hepatoprotective activity in new heterocyclic derivatives of cyanothioacetamide was established**.

The most pronounced hepatoprotective properties have a compound with the code **AZ-383** (5-cyano-6-({2-[(4-ethoxyphenyl)amino]-2-oxoethyl}thio)-4-(2-furyl)-2-methyl-N-(2-methylphenyl)-1,4-dihydropyridine-3-carboxamide).

These results illustrate the need for further preclinical studies of new alpha-cyanothioacetamide derivatives.

THANK YOU FOR YOUR ATTENTION