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# HETEROSIDES, CONTAINING SULFUR AND NITROGEN HETEROCYCLES, HAVE ANALYPTIC PROPERTIES AND REGULATE THE FUNCTION OF LIVER DETOXICATION

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**A B S T R A C T**

The effect of Heteroside – 321 on the ethanol sleep model as an analeptic and its effect on liver detoxification function in mature rats of Wistar males was investigated. The duration of ethanol sleep, the frequency of respiration during sleep, the activity of alcohol dehydrogenase, alanine aminotransferase and aspartate aminotransferase, the content of aldehyde, serum creatinine and liver lipid peroxide were determined. Sulfocamphocain was used as a classic analeptic. It was shown that the synthesized in our laboratory Heteroside – 321 at a dose of 2 mg / 100 g of body weight had a pronounced analeptic activity, increased the function of liver detoxification and did not have negative side effects on liver function. The ethanol narcosis model can be used in the development and evaluation of new analeptics.



## INTRODUCTION

Liver is involved in the regulation of protein, carbohydrate and lipid metabolism, provides digestion and functions of almost all organism systems [1]. Moreover, xenobiotics and endotoxic compounds constantly forming in the organism are metabolized in the liver. Impaired detoxification function inevitably affect other systems of the organism [1,2]. It should be noted that with long-term chronic disorders of detoxification functions in the liver an inflammatory process is developed, which is fibrosis. This process can lead to liver cirrhosis. It should be noted,that it is an irreversible dangerous condition [3, 4].

According to medical statistics, about 6% of the world's population suffers from various liver pathologies [4]. Viruses, toxic compounds, and even some foods, including alcohol [5-7], can induce liver hepatitis and cirrhosis. Alcoholic beverages are the largest share among inducers of liver function pathology. The part of alcoholic liver damage accounts for up to 13.6% of all types of liver pathologies [8]. About 2 million people die from liver diseases due to alcohol in the advanced Western countries each year [9, 10].

In this regard, searchingof biologically active compounds and drugs regulating the detoxification function of the liver is a relevant and reasonable task.

It was previously shown that Heterosides synthesized in our laboratory have a pronounced hepatoprotective and hepatoreparative effect [11]. In addition, they can stimulate cell proliferation of bone marrow cells in the *in vitro* system [12]. In particular, they stimulate processes of liver repair against the background of hepatitis. Preliminary investigations have shown that Heterosides can also regulate detoxification functions [11-14].

It is known that the administration of 20% ethyl alcohol to rats at a dose of 4.5 mL/kg transfer them in the state of anesthetic sleep [13]. It was shown that the duration of alcoholsleep was 94 ± 8.8 min at this dose [11]. It is proved that 95% of the alcohol administered is metabolized in the liver, and there is a direct correlation between the duration of sleep and the rate of alcohol metabolism in the liver [14]. Consequently, the duration of ethanol anesthetic sleep can allow determining the rate of the liver detoxification function, and the administration of Heteroside – 321 on the background of anesthesia will allow evaluating the effect of the latter on the activity of the liver enzyme systems [11].

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The duration of alcoholsleep will also depend on the intensity of blood circulation and respiration. However, this will increase the rate of xenobiotics metabolism. As it is known, analeptics have a stimulating effect on the respiratory and vasomotor centers of the medulla oblongata [15].

It has been suggested that heterosides with a hepatotropic effect may also have analeptic effects and, as a result, affect liver function.

In this regard, we investigated the possible relationships between the function of liver detoxification and the analeptic properties of Heteroside – 321 in the present investigation.

One of the major liver enzymes is alcohol dehydrogenase (ADH) [EC 1.1.1.1] [16]. This enzyme provides alcohol oxidation and aldehyde reduction in the presence of NAD and NADH [17]. Moreover, it turned out that this enzyme performs a large number of other functions [18]. ADH is involved in the catabolism of the number of retinols, neurotransmitters, steroid hormones, etc. [18, 19]. The determination of ADH activity may indicate not only the rate of ethanol metabolism in liver cells, but also the overall functional activity of this organ.

In this regard, investigation of ADH activity in animals after administration of ethyl alcohol and Heteroside – 321 will allow to evaluate the role of Heteroside – 321 in the function of liver detoxification in particular, and the general metabolism of this organ. The most important task in the development of new drugs is not only an assessment of their specific activity, but also an assessment of their biological safety.

From the above, we determined Heteroside – 321 influence on the time of onset of anesthesia and duration of ethanol, respiratory rate (analeptic effect), alcohol dehydrogenase activity, the acetaldehyde content, creatinine, and lipids hydroperoxides in the present study. Aspartate and alanine activity are indicators of the functional liver activity and possible side effects of Heteroside – 321

## MATERIALS AND METHODS

The evaluation of the detoxifying effect of Heteroside-321 was carried out on a model of alcohol anesthesia [13]. Experiments were performed using 2, 5 – 3 months old, male *Wistar* (weight 150 – 200 g). A comparative drug was a classic analeptic of the combined action of sulfocamphocain (SCC). This drug stimulates the respiratory and vasomotor centers of the medulla oblongata [14].

The animals were kept in accordance with sanitary and hygienic standards (temperature 20 - 24 ° C, humidity not more than 50%, natural day-night light mode), on a standard diet with free access to water [20].

The animals were divided into three groups of 6-8 rats each.All animals received intraperitoneally a 20% ethanol solution at a dose of 0.25 mL/per 100 g of body weight (Fig. 1). The dose and concentration of the ethanol solution was selected experimentally to achieve the optimum depth and duration of ethanol sleep. The first group was a control and received only an ethanol solution intraperitoneally. The second group received Heteroside – 321 at a dose of 2 mg / kg of body weight in the 30$^{th}$ minutes of ethanol sleep after taking a lateral position. The third group received 20 mg / kg of the SCC solution (Fig. 1) [13, 14].

Fig. 1. Experimental scheme of the investigation. The animals were divided into 3 groups, which received respectively: NaCl, Heteroside – 321 and SCC against sleep (30 minutes after the administration of ethanol). After awakening, the animals were decapitated, blood was collected and the liver was removed, followed by fractionation and determination of the investigated parameters.

The duration of sleep was defined as the difference between the time of awakening of the animal and the time tookingthe lateral position (the moment when the animals stopped responding to any stimuli and lost the inversion reflex). Thus, the period of rats in a lateral position reflects the duration of alcohol sleep [11, 12-14].

The frequency of respiratory movements (FRM) was determined immediately after the rats took the lateral position within 60 seconds. Then the FRM was measured every 10 minutes until the animal was fully awakened. After the awakening of each animal, decapitation was carried out and blood was collected, the liver was removed for further investigations (Fig. 1).

***Analytical Method.*** The determination of the activity of ALAT and ASAT in the serum was carried out according to the King method [21].

***Determination of the activity of alcohol dehydrogenase.***

The activity of alcohol dehydrogenase and acetaldehyde was determined in the liver homogenate. The liver was homogenized in 10 mL of Tris-HCl buffer, pH 7.4 containing 0.25 M sucrose in a ratio of 9 mL of buffer per 1 g of liver. The resulting homogenate was diluted 5 times with buffer and taken at 0.1 mL to determine the activity of the enzyme. 2.6 mL of 0.1 M glycine-NaOH buffer pH 9.0 and 0.1 mL + 0.04 M NAD, 0.1 mL of 0.75 ethyl alcohol was added to the reaction mixture. The amount of reduced pyridine nucleotides was determined at a wavelength of 340 nm using a Shimadzu UV-2700 spectrophotometer (Japan) during 1 min. The activity of alcohol dehydrogenase was expressed in nmol NADN / min per mg of protein of the sample according to the formula:

$$\frac{E \times V}{6.22 \times 10^{-3} \times T_1 \times A}$$

where$T_1$ is the time of the sample incubation, it is 1min, A is - the amount of protein in the sample (determined by the method of Lowry), V is thefinal sample volume in a cuvette 3.0 mL, $6.22*10^{-3}$is the nanomolecular extinction coefficient of the reduced form of pyridine nucleotides at a 340 nm wavelength, Eis the change in optical density of the sample for 1 minute[22].

***Determination of acetaldehyde.*** Acetaldehyde (AA) was determined by the method, which is based on the derivatization of acetaldehyde, followed by HPLC analysis of 2,4dinitrophenylhydrazone acetaldehyde.

A microcolonial liquid chromatograph HR 1050/1100 was used for chromatography in the reverse phase version using a metal column of 8 cm x 2 mm with a nonpolar sorbent, Diasorb C16, 5-7 µm.

A mixture of organic solvents in isocratic mode carried out elution of the test substance. As a mobile phase, a mixture of purified and freshly distilled organic solvents was used - acetonitrile - distilled water (60:40) with the addition of 2.5% acetic acid and 1% diethylamine (volumetric %). The mobile phase was filtered through a Schott funnel (pore 16) and degassed with bubbling with helium for 5 minutes at a speed of 50-60 $cm^3$ / min.

The rate of the eluent in the column was 100 $mm^3$ / min. Introduction volume was 4.0 $mm^3$. Analysis of the substance after leaving the column was carried out using an UV detector at a wavelength of 358 ± 2 nm (accuracy of measurement of wavelength was 0.5 nm).

***Determination of creatinine***. Creatinine content was determined spectrophotometrically standard reagents as described ininvestigation [23]. 1 mL of distilled water and 0.1 mL of trichloroacetic acid at a concentration of 1.2 mol/L were added to 0.1 mL of blood serum. The samples were incubated for 10 min at 25 ° C and then they were centrifuged at 1000 g. Picric acid (35 mmol/L), sodium hydroxide (0.75 mol/L) were added to 1 mL of the supernatant and a creatinizer calibrator (177 µmol/L) was added to the reference samples. Experimental samples and reference samples were incubated for 20 min at 25 ° C and their optical density was determined on the spectrophotometer Shimadzu. Calculation of the concentration of creatinine was made according to the formula C = A / Akal * 177 (µmol/L), where A is the optical density of theinvestigated sample, Akal is the optical density of the calibration sample, 177 is the conversion factor.

***Determination of lipid peroxidation products***. The investigation of lipid peroxidation products is based on the determination of MDA (malonic dialdehyde), which isone of the intermediate peroxidation products, in the reaction with thiobarbituric acid (TBA).

3 mL of a 1% phosphoric acid solution, 1 mL of a 0.6% solution of TBA and 0.1 mL of an iron sulfate solution were added to 0.2 mL of blood serum to a final concentration of 1 µmol in the sample. Samples were kept in a boiling water bath for 1 hour. Then they were cooled and 4 mL of butyl alcohol were added. Intensively mixed and centrifuged for 10 min at 1000g. The optical density of the upper butanol phase was measuredon the spectrophotometer Shimadzu at a wavelength of 515 and 532 nm against butanol. The calculation of the products content reacting with TBA was carried out taking into account the MDA molar extinction coefficient of 1.56 • 105 mol $1cm^{-1}$:

$$A = \frac{\Delta E \cdot 10^6 \cdot 4}{1{,}56 \cdot 10^5 \cdot n},$$

whereAis MDA content (µmol / L); $10^6$isconversion factor (nmol); 4 is volume of butanol phase 6 mL;1,56 · $10^5$is MDA molar extinction coefficient;n is sample volume ($cm^3$); $E = E_{532} - E_{515}$.

Significant differences between groups were determined by a nonparametric Students test. All statistical analyses were performed using Statistica 10.0 program [24]. Differences between control and experimental groups were considered significant at $p \leq 0.05$.

## RESULTS AND DISCUSSION

***The effect of Heteroside-321 on the respiratory center and detoxification function.***

The administration of 20% ethanol solution in a dose of 0.25 mL/kg to young intact animals accompanied by ethanol sleep , which occurred 2 minutes and 50 seconds after the administration of ethanol solution and lasted 132 minutes and 58 seconds (Table 1).

If Heteroside – 321 was administered at a dose of 2 mg/kg to animals in alcohol sleep, this reduced the sleep duration by 48.5% compared with the control animals, and the onset of sleep was observed after 3.5 minutes (Table 1). If animals were administrated with a known analeptic sulfocamphocain (SCC) at a dose of 20 mg / kg of body weight, the duration of sleep was reduced only by 23.2% compared with the control group of animals (Table 1). At the same time, the onset of alcohol sleep was 4, 1 min.
Table 1

The duration of anesthesia in the groups after the administration of 20% ethanol solution at a dose of 0.25 mL/kg, Heteroside – 321 at a dose of 2 mg/kg and SCC at a dose of 20 mg/kg

| Criteria | ExperimentalGroups | | |
|---|---|---|---|
| | 20% ethanol n=6 | ethanol+ Heteroside – 321 n=8 | ethanol+ SCC n=8 |
| Sleep time, min | **2, 5** | **3, 5** | **4,1** |
| Duration of anesthesia, min | **132, 6** | **68, 3*** | **102, 1*** |

* - compared with the control animals by the Student's test, p ≤0, 05

Therefore, Heteroside – 321 had a pronounced arousing effect. Analeptics are known to affect the respiratory and vasomotor centers of the brain [15]. In this regard,the effect of Heteroside – 321 on the respiration rate in the next series of experimentswas determined.Heteroside – 321 is involved in the regulation of the respiratory center and it has a pronounced analeptic effect (Fig. 2).

Administration of a 20% ethanol solution to the control group of animals was accompanied by a decrease in the respiration rate. It reached its minimum in 30–40 minutes after the onset of ethanolsleep (Fig. 2). Respiratory rate in the control group of animals remained lowered to 135-140 minutes. After 140 minutes of sleep, the FRM increased and exceeded the initial level by 160 minutes and, later, by 200 minutes it reached the initial level (Fig. 2).

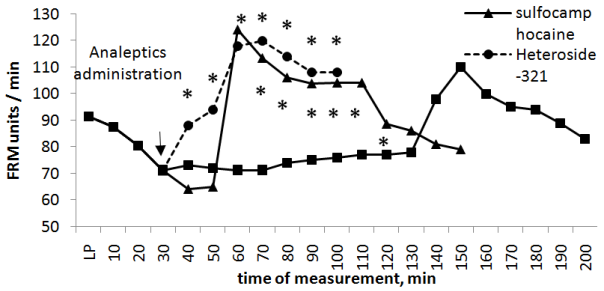


**Fig 2** Change in respiration rate after administration of a 20% ethanol solution at a dose of 0.25 mL/kg, Heteroside – 321 at a dose of 2 mg/kg and SCC at a dose of 20 mg/kg against ethanol anesthesia.

* – compared with the control animals by the Student's test, p ≤0, 05

In the case that animals who were in anesthesia the SCC wasintroduced on the 30th minute, the frequency of respiratory movements increased 30 minutes after the administration of the drug, i.e. on the 60th minute after sleep. Moreover, it significantly exceeded the initial level (Fig. 2). Later, after awakening of animals, it slowly decreased to control level (Fig. 2).

If the animals on the 30th minute of ethanolsleep were administrated with Heterozide-321, then already 10 minutes after the administration, their respiratory rate increased and at that time, it exceeded both the control and the group that received SCC (Fig. 2). Further, the respiratory rate in animals treated with Heteroside – 321 and SCC did not differ between themselves (Fig. 2).

Consequently, Heteroside – 321 had a regulatory effect on the respiratory center of the brain. Moreover, its effect was more pronounced than in the case of the SCC. Such analeptic effect of Heteroside – 321 can be caused both by a direct effect on the brain and indirectly by accelerating the metabolism of ethyl alcohol in the liver.

One of the major liver enzymes is alcohol dehydrogenase (ADH), which provides for the metabolism of ethyl alcohol. [17-19].

It should be noted, the administration of Heteroside – 321 to experimental animals was accompanied by a38,2% increase in the activity of ADH compared with the control Administration of the SCC animals increased only by 28, 3% compared to the control (Table 2).

**Table 2** ADH activity in the liver homogenate and serum acetaldehyde content after administration of a 20% ethanol solution at a dose of 0.25 mL/kg, Heteroside – 321 at a dose of 2 mg/kg and SCC at a dose of 20 mg/kg against ethanol.

| Criteria | ExperimentalGroups | | |
|---|---|---|---|
| | 20% ethanol n=6 | ethanol+ Heteroside-321 n=8 | ethanol+ SCC n=8 |
| ADH activity, µg/mL | 1.49 ± 0.27 | 2.06 ± 0.73* | 1.92 ± 0.43* |
| Acetaldehyde (ACAD)content, µg/mL | 10,5 ± 0,9 | 7,5 ± 0,7* | 6,3 ±0,7* |

* – compared with the control animals by the Student's test, p ≤0, 05

The results showed a good correlation between the activity of ADH and the duration of sleep in experimental animals (Fig. 3).

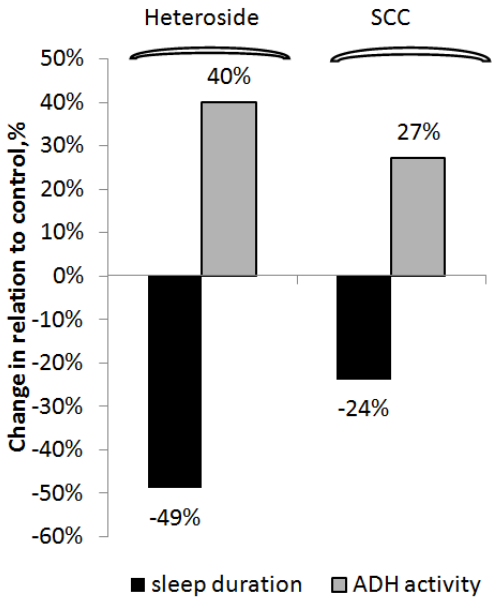


**Fig 3** The decrease in the duration of ethanol narcosis in animals treated with Heteroside-321 and SCC in percentage relative to the control, which is taken as 100%, against the increase activity of ADH in the liver in these animals.

The content of ethanol-acetaldehyde oxidation products decreased by 40% in the case of SCC and 28, 6% after administration of Heteroside – 321 (Table 2). The results suggest that Heteroside – 321 affects the functions of liver detoxification, activating the enzyme systems in this organ, and in particular alcohol dehydrogenase.

In this connection, the question of the possible side effects of the influence of heterosides on liver function is important.

### *About the Possible side Effects of Heteroside-321*

As it is known, liver dysfunction is most often argue by the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood serum [25].
It was found that Heteroside – 321 and SCC do not affect either the activity of ALT or AST; they did not differ from the indicators of the control group of animals (Table 3).

**Table 3** The activity of ALT, AST and creatinine content (conditional units/mL) in serum and the content of hydroperoxides lipids in the cytosol of the liver after the introduction of a 20% solution of ethanol at a dose of 0.25 mL/kg, Heteroside at a dose of 2 mg/kg and SCC in a dose of 20 mg/kg on the background of ethanol anesthesia.

| Criteria | ExperimentalGroups | | |
|---|---|---|---|
| | 20% ethanol n=6 | ethanol+ Heteroside-321 n=8 | ethanol+ SCC n=8 |
| ALT activity, µg/mL | 0.22 ± 0.015 | 0.24 ± 0.02 | 0.22± 0.03 |
| AST activity, µg/mL | 0.16 ± 0.06 | 0.14 ± 0.03 | 0.15± 0.04 |
| MDA, µmol/L | 0.268 ± 0.01 | 0.193* ± 0.03 | 0.232 ± 0.02 |
| Creatinine content | 0,58 ± 0,29 | 0,53 ± 0,22 | 0,52 ± 0,31 |

* – compared with the control animals by the Student's test, p ≤0, 05

One of the fastest and most common reactions to a variety of stress and functional changes in the liver is the formation of free-radical reaction products [26]. In determining the products of free-radical reactions, the content of lipid hydroperoxides is most often measured.

The determination of lipid hydroperoxides in serum showed that their content did not significantly change in animals treated with SCC (Table 3).

At the same time, the administration of the Heteroside – 321 to animals in ethanol sleepwas accompanied by a significant decrease in the content of lipid hydroperoxides by 28% compared with the control group of animals (Table 3). The main site of free radical formation is mitochondria and the endoplasmic reticulum of liver cells [27]. This effect of Heteroside – 321 on the content of lipid hydroperoxides may indicate the effect of Heteroside – 321 on the functional activity of the liver, which is consistent with previously obtained data [11].

Consequently, the test substances did not adversely affect liver function. However, these data do not exclude the possibility of Heteroside – 321 influence on other organism systems.One of them is the kidneys excretory system, the load on the excretory system increases after the administration of xenobiotics (ethyl alcohol, heterosides and SCC) to animals. Increase in the creatinine content in the blood can be an indicator of the action of toxic substances on kidney function, endocrine disorders and active degradation of muscle tissue caused by toxicants [28].

It was found that the administration of Heterosides, as the SCC, did not affect the creatinine content in the blood serum of experimental animals (Table 3).

Consequently, Heterosides do not affect the functional activity of the kidneys and do not show a toxic effect on the organism at a dose of 2 mg/100 g. However, they had pronounced detoxification and analeptic effects.

The results showed that the Heteroside – 321 synthesized in our laboratory at a dose of 2 mg/100 g had a pronounced analeptic activity, enhanced the function of liver detoxification against the background of ethanol anesthesia and did not show negative side effects on liver function and kidneys. Heteroside – 321 exerted a "regulatory" effect on liver function in animals under alcohol anesthesia, as may be shown by a decrease in the content of lipid hydroperoxides by 28% and an increase in ALD activity and aldehyde content.

The ethanol anesthesia model can be used not only in the development of new analeptic drugs, but also in the investigation of the fundamental mechanisms of the interrelation of various functional systems of the organism.In particular, it may be the intensity of respiration, blood circulation and detoxification function.

In recent years, searching for natural sources of Heterosides and the investigation of the mechanisms of their action have been conducted [29]. Such interest is due to a wide range of their biological effects. Thus, Heterosides have been shown to have anticarcinogenic [30], hepatoprotective [11], immunomodulatory [31], and other biological effects. The main source of natural Heterosides is plant objects. It should be noted, that the isolation, purification and standardization of natural compounds are laborious and difficult tasks. In this regard, the synthesis of Heterosides is of great interest.

It was shown that Heterosides synthesized in our laboratory have a broad spectrum of biological effects. For example, Heterosides in small concentrations ($10^{-4}$ –$10^{-5}$) after their administration into the culture of bone marrow cells were stimulated cells proliferation. However, they inhibited proliferative cell activity of the primary culture for 3-4 days [13]. The administration of Heterosides to experimental animals accelerated the rate of liver cells proliferation on the regenerating liver model and influenced the proliferation of spleen cells [11].

Consequently, earlier studies and the results of this investigation suggest that the sulfur and nitrogen containingsynthesized in our laboratory Heterosides have a broad spectrum of biological action and a complex dose-dependent biological response. In this connection, a question may arise about the possible mechanism of polyfunctionality of Heterosides.

We suggest that the administration ofa single substance (Heteroside – 321) of different responses can be explained by the fact that for Heterosides there is a large number of receptors on different targets. It can also be explained by following, Heterosides act on the basic (evolutionarily ancient) mechanisms of cells regulation and organisms in general which trigger a cascade of metabolic changes [32 - 33]. The action of Heterosides by the third mechanism cannot be ruled out.This way can be named combined.

One of the evolutionary ancient regulatory mechanisms presented in all living organisms, regardless of their level of organization, is the redox system of the cell or organism [32, 33]. The redox system is the basic level of regulation, which dynamically changes under the influence of a huge number of endogenous and exogenous factors. The reorganization of the redox system leads to coordinated changes in all organism systems. In particular, it isthe body bioenergy system.

Previously it was shown that the Heterosides have a pronounced effect on the activity of antioxidant and energy-producing enzymes under conditions of cryogenic damage [34]. It was shown that, against the background of alcohol anesthesia, the content of lipid hydroperoxides decreased by 28%, which is consistent with previously obtained data
Heterosides, as low-molecular compounds containing sulfur and nitrogen, can have an antioxidant effect and even in lower concentrations, as in cell culture [13] or *in vitro* system, or 2 mg/100 g versus 20 mg/100 g of SCC. It can be assumed, that heterosides affect the redox system of the organism. These substances provide the functional activity of the respiratory center, the function of detoxification and other functional systems of the organism.

## References


1. Wong RJ, Gish RG. Clinical Epidemiology of Chronic Liver Diseases. Springer, Cham: 2018.
2. Scaglioni F., Ciccia S., Marino M., Bedogni G., &Bellentani S. Ash and nash. Digestive Diseases, 2011; 29(2): 202-210.
3. ChrostekL.,&Panasiuk A. Liver fibrosis markers in alcoholic liver disease. World Journal of Gastroenterology, 2014; 20(25): 8018.
4. Poynard T., Lebray P., Ingiliz P., Varaut A., Varsat B., Ngo Y., ... &Bismut, F. I. Prevalence of liver

fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). BMC gastroenterology, 2010; 10(1): 40.
5. Gao B.,&Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology, 2011; 141(5): 1572-1585.
6. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2008;134:1655–1669.
7. Altamirano J, Miquel R, Katoonizadeh A, Abraldes JG, DuarteRojo A, Louvet A, *et al*. A histologic scoring system for prognosis of patients with alcoholic hepatitis. Gastroenterology 2014;146:1231-1239.
8. O'Shea RS.,Dasarathy S., McCullough AJ. Alcoholic liver disease. Hepatology, 2010; 51: 307–328.
9. Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. Lancet. 2009;373:2223–2233.
10. Rehm J, Shield KD, Gmel G, Rehm MX, Frick U. Modeling the impact of alcohol dependence on mortality burden and the effect of available treatment interventions in the European Union. EurNeuropsychopharmacol. 2013;23:89–97.
11. Kabachnyy VI., Bozhkov AI., & Kabachna IV. Heteroside–321 hepatoregenerationactivityincases of hepatitisinducedbylocalhyperthermia. 2017; 12(3): 51-55.
12. Bozhkov AI., Kabachnyy VI., Bondar AY., Chumak IV., &Kolot NV. Hormesiseffectandtheinfluence of ultra-lowglycosidesdosesonthebonemarrowcellsproliferativeactivityinculture. 2011; 3(4): 154 – 166.
13. Kabachna IV., Drogovoz SM., Kabachnyy VI., & Serdiukova Y. Thestandardizedmodel of alcoholic anesthesia forthepurposefulscreening of analeptics. 2017; 21(4): 4 – 10.
14. Kabachna IV., Drogovoz SM., Kabachnyy VI., & Serdiukova Y. Thestudy of theanalepticactivity of derivatives of sulfur-andnitrogen-containingheterocyclesonthemodel of thiopentalnarcosis. 2018; 2: 54 – 60.
15. Haji A., Takeda R., &Okazaki M. Neuropharmacology of control of respiratoryrhythmandpatterninmaturemammals. Pharmacology&therapeutics, 2000; 86(3): 277-304.
16. AndronovaLM, UshakovaMM, KudriavtsevRV, Bark ovNK.Ethanol pharmacokinetics in narcotic action and endogenous ethanol in female ratsBiullEkspBiol Med, 1982: 94(12):60-2. (Article in Russian).
17. Crabb DW, Matsumoto M, Chang D, You M. Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. Proc NutrSoc.Feb, 2004;63(1):49-63.
18. Lee SL,Chau GY,Yao CT,Wu CW,Yin SJ. Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: significance of first-pass metabolism.Alcohol ClinExp Res. 2006;30 (7):1132-42.
19. Deacon RM.Housing, husbandry and handling of rodents for behavioral experiments. Nature Protocols, 2006: 1(2): 936–946.
20. Lang T. The Association for Clinical Biochemistry and Laboratory Medicine. National minimum re-testing interval project. Prepared for the Clinical Practice Group of the ACB and supported by the Royal College of Pathologists, 2013: 1-36.
21. PigolkinYUI., MorozovYUE, Mamedov VK. Forensicmedicaldiagnostics of acuteandchronicalcoholintoxication, 2012;55:1:30-33 (in Russian).
22. Coresh J, Astor BC, McQuillan G *et al*. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. Am J Kidney Dis, 2002; 39(5): 28-36
23. Mather K. Statisticalanalysisinbiology. Universitypaperbacks; London, 1965.
24. Mead R. Statisticalmethodsinagricultureandexperimentalbiology. ChapmanandHall/CRC, 2017.
25. Nyblom H., Berggren U., Balldin J., & Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol and alcoholism, 2004; 39(4): 336-339.
26. Bozhkov AI., Nikitchenko YV., Sheremet AA., & Bozhkov AA. The Prooxidant-Antioxidant System Is the Primary Stage of the Answer of Organism on Unbalanced Diet. Journal of Harmonized Research in Pharmacy, 2014; 3: 78-90.
27. ZhongH.& Yin H. Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. Redox biology, 2015; 4: 193-199.
28. Jhee J.H, Hwang SD, SongJH, Lee SW. Upper Normal Serum Creatinine Concentrations as a Predictor for Chronic Kidney Disease: Analysis of 14 Years' Korean Genome and Epidemiology Study (KoGES). J. Clin. Med. 2018; 7: 463.
29. Hagemann M., &Pade N. Heterosides–compatiblesolutesoccurringinprokaryoticandeukaryoticphototrophs. PlantBiology, 2015; 17(5): 927-934.
30. Oana C., Adriana T., Mircea C., Dragos S., & Monica H. Natural Macromolecules with Protective and Antitumor Activity. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2018; 18(5): 675-683.
31. Becker K., Hartmann A., Ganzera M., Fuchs D., &Gostner JM. Immunomodulatory effects of the mycosporine-like amino acids shinorine and porphyra-334. Marine drugs, 2016; 14(6): 119.
32. Marengo B., Nitti M., Furfaro AL., Colla R., Ciucis CD., Marinari UM., ... &Domenicotti C. Redox homeostasis and cellular antioxidant systems: crucial players in cancer growth and therapy. Oxidative medicine and cellular longevity, 2016.
33. Noctor G., & Foyer CH. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant physiology, 2016; 171(3): 1581-1592.
34. KabachnyVI.,GorbunovaNI.Heterozide derivatives as effective additives to cryoprotective media, 2004; 2: 28 – 35.

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