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Antifibrotic Efficacy of a New Phytocomposition of Essential Phospholipids with Glycyrrhizic Acid, Ecdysterone, Lycopene and Proanthacyanidin in Experimental Severe Chronic Hepatitis Compared with Phosphogliv

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Since the prevalence of acute and especially severe chronic liver diseases of various etiologies increases from year to year, this pathology is recognized as the main burden on health worldwide. Currently, it has been established that the use of drugs based on essential phospholipids and plant origin with antioxidant and hepatoprotective activity is very effective in the prevention and treatment of liver diseases. In this regard, we studied under experimental conditions the effect of phytocomposition of soy lecithin, glycyrrhizic acid, lycopene and ecdysterone (conditional name hepalipin) and proanthacyanidin (conditional name yantacin) isolated from the plant Alhagi pseudalhagi on cytolytic-cholestatic liver damage, as well as on the development of fibrosis. In this study, we evaluated the hepatoprotective and antifibrotic effects of a new combination called Hepatocin obtained in a 1:1 ratio (100 mg/kg of Hepalipin and 100 mg/kg of Yantacin). All the studies conducted were conducted on adult nonlinear rats, while the experimental animals were divided into a control group infected with heliotrin, a substance with hepatotoxic action, a group receiving hepatocin, and an intact group that was not infected with heliotrin at the same time. In the conducted studies, hepatocin significantly inhibited the development of cytolytic-cholestatic liver damage, helped to maintain the functions of the liver synthesizing protein and glycogen, and when administered to experimental animals against the background of a chronic disease developing as a result of damage by heliotrin, it has an antioxidant effect. In addition, the use of hepatocin for more than two months in chronic liver damage or early stage fibrosis led to the restoration of serum enzymes of experimental animals, as well as regulators of cytochrome P450 and b5 fibrogenesis in liver tissues, PCNA, PDGF-BB to levels almost close to the initial (intact) values. Thus, in experimental conditions of chronic severe hepatitis or early stage fibrosis, hepatocin showed statistically significant advantages over Phosphogliv in terms of the intensity of hepatoprotective or antifibrotic action.

Keywords: Experimental chronic heliotrine hepatitis; Hepatocin; Hepalipin; Liver fibrosis; liver cirrhosis; Proanthacyanidin.

In recent years, the incidence of complications of chronic liver diseases (ChLD), developing as a result of bad habits, infectious and non-infectious factors, has been increasing in different regions and demographic groups among different ages and genders. In turn, severe complications of ChLD, including cirrhosis of the liver (CL), hepatocellular carcinoma (HCC) and liver failure are among the causes of the global increase in mortality and are becoming an increasingly serious burden on the health system[1-6]. All over the world, a fairly large part of the population suffers from liver diseases of various genesis, unfortunately, almost half of patients with severe complications caused by chronic liver lesions and inflammation of various etiologies die every year, that is, about 2 million people. A significant part of the deaths observed due to complications of these liver diseases are due to CL, the final stage of liver fibrosis (LF), which develops as a result of a progressive violation of the architecture of the liver[2,4,7-10]. Deaths from HCC in recent years account for more than 30% of deaths from cancer worldwide, not only due to tumors arising in the gastrointestinal tract[3, 11, 12]. By country or region, the mortality rate from liver cirrhosis in China, the USA and Western European countries is significantly lower than in Central Asian countries. Differences in mortality rates from liver cirrhosis by country or region reflect differences in the prevalence of risk factors, which entails the need to strengthen preventive measures to control and reduce risk factors for liver cirrhosis in regions with high or rapidly increasing mortality rates. It is worth noting that approximately 30% of the world's population has LF or CL as a result of the death of hepatocyte cells due to prolonged exposure, there is also at least one risk factor that can lead to HCC[13, 14]. Chronic metabolic liver diseases, such as chronic viral and autoimmune liver diseases, which are growing among the population, chronic alcoholic hydrosystem, insulin resistance and non-alcoholic fatty liver dystrophy, which develop in connection with metabolic syndrome, can be included in the proposal of risk factors for LF, one of the main global public health problems[15-19, 24]. It is known that, although there are diseases such as compensated, decompensated and late decompensated CL, which combine various hemodynamic or clinical features, the risk of death from this disease does not always correspond to the described sequence. The specificity of its nature lies in the fact that it can always occur with high mortality in the short term with compensated cirrhosis, which is usually not diagnosed in time and patients receive a good quality of life, in which clinical signs do not appear compared to decompensated cirrhosis, in which the risk of death is considered high, and patients immediately seek medical help. The exacerbation of cases of this disease has led to the emergence of the concept of clinical cases of Cl. Due to the epidemiological features of cirrhosis, which is considered the leading cause of liver-related death worldwide, and its complications with changing trends, accurate clinical assessment and treatment of the current burden of ChLD, as well as prioritization of research and policy can be crucial. Thus, CL is the main, but largely preventable and underestimated cause of the deterioration of global health[7, 8, 13, 20-24]. Scientific discoveries of recent years have led to an improved understanding of cellular factors and molecular mechanisms of liver fibrogenesis, the successful introduction into clinical practice of progressive screening of fibrosis with the help of useful invasive, minimally invasive and non-invasive, as well as laboratory research methods that allow to detect fibrotic processes at early stages and prevent complications. In addition, although progress has been made in understanding the pathogenesis and clinical effects of LF, therapeutic strategies for this disease are limited. In particular, there is a great unmet medical need for antifibrosis therapy to prevent life-threatening complications, especially with progressive fibrosis[24, 15. 14, 4, 25]. This, in turn, means that patients with CLD need to be treated before complications develop, which can occur and are considered as the main cause of morbidity, disability and death worldwide. Therefore, the main way of progression of chronic liver diseases leading to its damage is the process of activation of fibrogenesis. Recently, the understanding of the mechanisms of fibrogenesis has expanded, which has led to an understanding of the reversibility of fibrosis and to fairly realistic expectations that effective therapy will provide a favorable prognosis even in severe stages of fibrosis. In this regard, a comprehensive study of the mechanisms of development of liver fibrosis remains an urgent task of modern medicine.

In this regard, in our country, in particular, in the Republican Scientific and Practical Pediatric Center, together with the Institute of Plant Chemistry, comprehensive research work is being carried out to develop and study the pharmaco-toxicological properties of soy lecithin, glycyrrhine acid, lycopene and the phytocomposition of ecdysterone (conditional name hepalipin). As a result of these studies, the intellectual property agency obtained a patent for an invention based on the higher hepatoprotective activity of Hepalipin compared to phosphogliv, widely used in medical practice, against acute and chronic toxic liver diseases in experimental animals under research conditions[26, 27, 29, 52]. Currently, in the prevention and treatment of fibrosis or cirrhosis of the liver, which develop as a direct complication of severe liver diseases, phosphoglyve-like phospholipid-containing drugs are mainly used.in particular, the use of agents with antioxidant activity leads to some positive results. Thus, he obtained a number of positive results regarding the study of the antitoxic hepatitis activity of natural proanthacyanidins with high antioxidant activity in liver pathologies, was isolated from *Alhagi pseudalhagi*[28,30-32]. In the manuscript presented by us, in order to develop a remedy that can prevent fibrosis or cirrhosis of the liver or stop fibrous processes in the liver, we made a combination of hepalipin with high hepatoprotective activity and plant proanthacyanidins with a natural base with high antioxidant activity. She also presented the results on antifibrotic activity with severe liver pathology of this combination, which was carried out on laboratory rats in the study conditions. In experiments in order to cause fibrous processes in the liver of the studied animals, due to the pronounced hepatotoxic activity, severe liver damage can cause cirrhosis of the liver, as well as liver failure, heliotrin, which is considered a pyrrosolidone alkaloid exhibiting genotoxic and carcinogenic activity, was also used[33-38]

MATERIALS AND METHODS

The combination called Hepatocin, in which antifibrotic activity was studied at the early stage of LF, consists of hepalipin [26] and a class of polyphenols – proanthacyanidin in a ratio of 1:1 (each substance in combination of a dose of 100 mg/kg). Proanthocyanidins obtained from the plant *Alhagi pseudalhagi*[27-29].

110 mongrel white male rats with a body weight of 100±10 g were used as the object of the study. All experimental animals were in the same conditions of maintenance and feeding. All laboratory animals used in the research, before and during the experiments, were kept in standard vivarium conditions, that is, they ate dairy products, fruits and vegetables, as well as special mixtures (compound feeds) prepared for rats. All experimental animals were provided with distilled water in special containers, which they could freely drink. A total of 110 rats were obtained in the studies. of this, 40 for the intact group and 70 for the control and experimental group: 40 of them were anesthetized under anesthesia during 5 weeks of the study, while the blood and liver parameters of the rats were monitored dynamically, and after 5 weeks the remaining animals were divided into 3 groups of 10 rats. Phosphogliv and another 1 group were given a hepatocyte for 2 months, at the end of the experiment, the rats were anesthetized under anesthesia and the necessary indicators were checked.

It is known that metabolic liver diseases in experimental animals in particular, there are various experimental models of ChLD or LF[39-48]. We mainly used heliothrin to cause chronic toxic liver damage[43, 49-51]. The hepatotoxicity of heliotrin (Uzbekistan) was shown by us in rats earlier, therefore, the model of heliotrin-induced LF is the most suitable model of toxic liver damage. To cause LF in experimental animals, first experimental animals were injected into the abdominal cavity through a less traumatic needle and did not cause stress, heliotrin was administered in highly toxic doses according to the following scheme[52]: 3 times a week at a dose of 250 mg/kg, then at a dose of 150 mg/kg 3 times a week for 2 weeks, the fourth week at a dose of 100 mg / kg 3 times a week, the last week at a dose of 30 mg/kg was administered 3 times. The hepatocyin was administered to experimental animals at a dose of 200 mg/kg or 20 mg/100 g (20 mg of hepatocin for every 100 g of rat weight) orally using a special probe for 2 months. At the same time, during screening studies, the most active of several dose combinations was selected, which demonstrated high activity in acute toxic hepatitis caused by heliotrin, and it was the

combination with high activity in these studies that was administered to rats using a special atraumatic gastric probe.

Chronic intoxication, confirmed morphologically, was obtained on the 35th day of the experiment. The mortality rate was up to 30%. It was also noticed that the motor activity of the experimental animals decreased, the consumption of food and water decreased by 35-40%, and body weight decreased by 25-30% compared to the initial indicators. To characterize the degree of fibrosis of liver tissue, we conducted a morphological study that the animals were taken out of the experiment under ether anesthesia (with chloroform), the liver was fixed and poured into paraffin (according to the standard method).

One of the morphological signs of HCG is intra-lobular lymphocytic infiltration, which is much more intense and uneven compared to the norm[50].

In rats with initial signs of fibrosis (F1), treated with heliotrin for 2-3 weeks (groups 2,3), there was an expansion of the portal tracts. But since the process of fibrosis in the liver of rats was unstable during these weeks, we chose the fibrosis stage after 5 weeks as an early stage[51, 46].

Analysis of the functional state of the liver was evaluated by determination of serum transaminases, determination of the level of total and direct bilirubin, total protein and its fractions (Human Diagnostics, Germany), alkaline phosphatase (Wuhan Fine Biotech Co., Ltd. (FineTest), China) and gamma-glutamyltransferase (Human Diagnostics, Germany), studies were conducted on a modular automated system for clinical chemistry and immunological analysis - Cobas 6000®. In addition, blood clotting factors were determined, since impaired liver function leads to a deficiency of clotting factors.

Determination of the total amount of cytochrome P450 and b5[53]. The activity of the monooxygenase system of the endoplasmic reticulum of hepatocytes In vitro was assessed by the content of cytochrome P_{450} and b_5 (Thermo Fisher Scientific Inc. USA).

Proliferating cell nucleus antigen (PCNA) (Sigma-Aldrich®, USA). The main purpose of using immunohistochemical studies in this work is to determine the biological markers of programmed death of liver parenchymal cells and the activity of proliferative-regenerative processes in the organ. Cell proliferation and organ regeneration The PCNA was used as biomarkers[54, 55].

Platelet Growth factor BB (PDGF-BB) (Sigma-Aldrich®, USA). Quantitative indicators of platelet growth factor BB (PDGF-BB) were determined by enzyme immunoassay in the blood serum of experimental animals[56, 57].

The results of the conducted studies, statistical processing done using the methods presented in R.B. Strelkova and statistical analysis of the results obtained were evaluated with an accuracy of $P>0.05$[58].

RESEARCH RESULTS

Hematological and biochemical analysis of rat blood during the development of chronic heliotrine hepatitis (ChHH)

Toxic damage to the liver in rats as a result of the introduction of heliotrin led to a violation of the functional state of liver cells.

Toxic damage to the liver in rats as a result of the introduction of heliotrin led to a violation of the functional state of liver cells. As a result of these functional disorders, the activity of serum transaminases (AST and ALT) in rats with ChHH was 238.6% and 281%, respectively, compared with the levels of these indicators in the control group. It was found that the total amount of protein decreased to 46% compared to the control group, while the total amount of bilirubin increased to 138.1%. There was also an increase in the amount of alkaline phosphatase and GGD to 10.3 and 145%, respectively, compared with the control group.

Along with this, it should be emphasized that there is also an increase in the level of alkaline phosphatase (APh), from 167.8 to 433.3, indicating a violation in the biliary system.

Thus, the administration of the Hepatocin to the studied animals reduced the activity of liver enzymes compared to the rats of the control group who did not receive this substance, while demonstrating indicators close to those of rats from the intact group (Table 1). However, the activity of Aph remained within normal limits.

The above data on the dynamics of the activity of incretorial and excretory enzymes in the blood serum of rats treated with these substances

under experimental conditions indicate that the administration of the amounts of Hepatocin had a normative effect on liver function.

Thus, the sum of Hepatocin was obvious in the model of chronic heliotrine hepatitis in terms of hepatoprotective properties compared with the control group, and compared with the comparative drug Phosphogliv, the drug showed similar or significantly higher hepatoprotective activity.

Investigation of the effect of the Hepatocin on the total amount of cytochrome P450 and b5 in ChHH conditions

The results of the experiment conducted to study the total number of cytochromes P450 and b5 showed that the total number of cytochromes P450 and b5 in the microsomal fraction of the liver increased by 41.7 and 24%, respectively, after administration of heliotrin in rats for 5 weeks.

The results of the experiments conducted to study the total amount of cytochrome P450 and b5 of the studied substances are presented in the table below.As a result of the administration of hepatocin for about a month, the total amount of cytochrome P450 in the microsomal fraction of the liver decreased to 22.4% and 26.5%, and the amount of b5 decreased to 6.5%. and 11.3%, under the influence of Phosphogliv, a decrease in the sum of these indicators was noted by 18.4% and 5%, respectively, compared with the control (Table 2).

Thus, the hepatocin showed significant activity in relation to the total amount of cytochrome P450 and b5 both compared to the control group and compared with hepalipin and the comparative drug phosphogliv.

Investigation of the effect of the Hepatocin on the PCNA protein, a marker of hepatocyte proliferation, in ChHH conditions

Studies have been conducted to study the PCNA protein, which is a marker of hepatocyte proliferation, against the background of treatment

Table 1. Comparison of hepatocyte and phosphogliv doses with high hepatoprotective activity by the activity of the functional state of liver enzymes in ChHH (n=10)

Experimental groups	Intact group	Control group	Hepatocin	Phosphogliv
Doses in mg/kg	Dist. water	Heliotrin	200	50
ALT u /l	95,8±2,4	268,9±9,6*	122,3±3,2*	144,5±2,4*
AST u /l	156,6±4,8	373,6±11,21*	174,7±9,6*	192,1±6,96*
Total protein (g/l)	85,6±4,8	58,7±2,9*	80,2±3,12*	71,5±2,4*
Albumin g/l	48,5±2,9	33,3±3,6*	45,8±2,9	40,5±2,48*
Total Bilirubin mkmol/l	2,15±0,96	5,12±0,96	3,1±,048	3,4±0,96
The de Ritz coefficient (AST/ALT)	1,63±0,22	1,35±0,11*	1,43±0,1*	1,33±0,11*
APh, u /l	394±4,48	434,4±6,52	401±4,48	401±5,22
LDG, u /l	625,5±11,6	1532,5±22,4	996,2±5,6	1105±44,8
GGD u /l	1,15±0,11	1,46±0,11	1,21±0,11	1,34±0,12
Amylase (u /l)	495,7±11,22	442,6±6,48	488,1±5,96	476,2±8,96

Note: * - the confidence level of the comparatively controlled (P>0,05).

Table 2. Comparison of the hepatocin and phosphogliv by their effect on the activity of cytochrome P450 and b5 in chronic toxic liver damage (n=10)

Experimental groups	Doses in mg/kg	Cytochrome P450 protein, nmol/mg	Cytochrome b5 protein, nmol/mg
Intact group	Dist. water	0,69 ± 0,04	0,50 ± 0,04
Control group	Heliotrin	0,98 ± 0,12*	0,62 ± 0,06 *
Hepatocin	200	0,72 ± 0,08*	0,55 ± 0,05 *
Phosphogliv	50	0,80 ± 0,09*	0,59 ± 0,07 *

Note: * - the confidence level of the comparatively controlled (P>0,05).

of chronic hepatitis. The studied substances exhibit the ability to stimulate a number of positive reactions to the PCNA protein under the conditions of ChHH in rats with experimental liver pathology. At the same time, the increase in the number of experimental rats in all experimental groups was 30% compared to the control group. The results of studies in this direction are presented in Table 4. From the information provided, it follows that in chronic heliotrine hepatitis, significant changes in PCNA expression occur. However, in 70% of animals infected with ChHH, hepatocytes have a positive response to the PCNA protein, while in animals of the control group this was observed in all animals (100%). As can be seen from the presented data Table 3.

It follows that the studied Hepatocin stimulate the genetic mechanisms responsible for the proliferation of liver hepatocytes in experimental animals.

Along with the increase in the number of PCNA-positive animals against the background of Hepatocin, the quantitative level of PCNA protein also undergoes significant changes. So, especially Hepatocin and phosphogliv, increased the expression of this protein by more than 3.5 times compared to the group of untreated animals. The combination of Hepatocin increased up to 4.5 times, i.e. up to 1.2 times compared with phosphogliv, respectively.

The results obtained will undoubtedly help to understand the mechanisms of chronic liver damage in terms of cell death and regeneration, and will also become a theoretical prerequisite for the creation of new technologies for the treatment of liver pathology, especially chronic forms, as well as the initiation of fibrobiotic processes will serve as a reason for the development of measures to prevent the development of liver pathology in its early stages.

Analysis of the results of PCNA protein expression in the hepatocytes of experimental animals showed that the change in the amount of this protein in the hepatocytes of animals under the conditions of ChHH was manifested by a decrease of 4.5-4.6 times compared with the control group. Consequently, under conditions of toxic liver damage, there was a significant decrease in the expression of the marker of hepatocyte proliferation in hepatocytes. Indeed,

Table 3. Comparison of the hepatocin and the effect of Phosphogliv on the expression of PCNA protein in hepatocytes in ChHH (n=10)

Experimental groups	Doses in mg/kg	Number of animals	Positive expression of PCNA protein	
			Number of positive observations	The number of PCNA - positive cells, in %
Intact group	Dist. water	10	10	54,2±3,1
Control group	Heliotrin	10	7	9,7±1,28*
Hepatocin	200	10	10	44,1±1,12*
Phosphogliv	50	10	10	36,7±2,4*

Note: * - the confidence level of the comparatively controlled (P>0,05).

Table 4. Comparison of the effect of the hepatocin and Phosphogliv on platelet growth factor (PDGF) (n=10)

Experimental groups	Doses in mg/kg	platelet growth factor (PDGF) in pg/ml
Intact group	Dist. water	10,4 ± 0,8
Control group	Heliotrin	18,5±1,1*
Hepatocin	200	12,1± 0,8*
Phosphogliv	50	14,1± 1,1*

Note: * - the confidence level of the comparatively controlled (P>0,05).

the morphological picture of the liver studied in conditions of chronic heliotrine hepatitis also shows that structural and quantitative changes in conditions of liver pathology, such as the symptoms of mitosis characteristic of chronic hepatitis and, as a consequence, proliferation of hepatocytes, differ only in individual cases.

Investigation of the effect of the hepatocin on platelet growth factor (PDGF) in chronic heliotrine hepatitis

Prolonged use of the hepatotoxic substance heliotrin in rats in descending order of high doses led to chronic liver damage and, as a consequence, to a number of changes in the body. Along with the aforementioned changes caused by the hepatotoxic substance, platelet growth factor (PDGF) levels also increased up to 1.8 times or up to 85% compared to the intact group, which was not injected with heliotrin. This can lead to an increase in PDGF levels and an increase in the process of fibrosis in the liver. That is, the processes of reparative regeneration in the liver slow down, which ultimately increases the likelihood of severe liver cirrhosis.

The use of the hepatocin in chronic heliotrine hepatitis led to a decrease in platelet growth factor (PDGF) up to 1.53 times compared with the control group (Table 4). The hepatocin showed up to 1.16 times higher activity compared with the comparable drug phosphogliv.

Thus, there was a decrease in PDGF to 53% when correcting chronic heliotrine hepatitis with the hepatocin. The studied hepatocinenhances the processes of reparative regeneration of hepatocytes in damaged liver tissue due to hyperplasia and their polyploidization. This can lead to a decrease in PDGF levels and blocking of fibrous processes in the liver.

The results obtained indicate the therapeutic effect of these drugs for the normalization of the morphofunctional state of the liver, metabolic shifts in chronic heliotrine hepatitis.

DISCUSSION

Exposure to the hepatotoxic substance heliotrin led to the development of cytolytic-cholestatic liver damage in rats and the emergence of the process of liver fibrosis as a result of chronic administration of a toxic substance. At the same time, a decrease in the motor activity of experimental animals, loss or barking of their fur also caused objective signs, such as a decrease in food and water intake and a significant loss of body weight, as well as the death of up to 30% of animals. Meanwhile, along with such changes in rat blood serum as hyperphenemia, hyperbilirubenemia, hyperprotenemia, sharp and serious changes in the levels of cytochrome P450 and b5 fibrogenesis regulators, PCNA, PDGF-BB were observed in rat blood and liver tissues. However, as a result of treatment with hepatocin for 2 months, not only the motor activity, the need for food and water of experimental animals improved, but also significant positive changes in body weight were observed. In accordance with the objective signs noted, the symptoms of cytolytic-cholestatic liver damage led to an almost complete restoration of all the studied parameters to their original (intact) values. This leads us to conclude that the studied combination of hepatocin helps restore impaired liver functions at the level of hepatocytes. However, in experimental conditions of chronic hepatitis or early stage of liver fibrosis, hepatocin showed statistically significant advantages over Phosphogliv in terms of the intensity of hepatoprotective or antifibrotic action.

CONCLUSIONS

1. Thus, prolonged administration of heliotrin, a substance with hepatotoxic effect, in descending order, starting with a subtoxic dose, led to the development of severe liver damage or liver fibrosis in rats. This was also confirmed by such indicators as significant changes in the objective signs of rats and obvious changes in laboratory diagnostic parameters.

2. It has been established that the severity of chronic toxic damage or fibrous disorders depends on the level of proliferating cell nuclear antigen (PCNA) in the liver under conditions of ChHH. Cytochrome P450 and b5, platelet growth factor in microsomal fractions, which are of diagnostic importance for the differentiation of stages of liver fibrosis, are confirmed by a direct relationship between the intensification of the fibrous process.

3. The hepatocyte in the studied dose, when administered to experimental animals

against the background of chronic disease, significantly suppressed the development of cytolytic-cholestatic liver damage in rats, had an antioxidant effect, helping to maintain liver function for protein synthesis. In addition, the total amount of cytochrome P450 and b5, considered regulators of fibrogenesis in liver tissues, normalized the levels of PCNA, PDGF-BB, which led to an almost complete restoration of all studied parameters to their original (intact) values in chronic toxic hepatitis, and in fibrosis showed significant changes close to the indicators of the Intact group. Experimentally, based on this activity, it was shown that in conditions of chronic hepatitis or liver fibrosis at an early stage, hepatocin has statistically significant advantages over the comparable drug Phosphogliv in terms of the intensity of hepatoprotective or antifibrotic action.

Conflict of Interest

There are no conflict of interest.

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