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Research Article

STUDY THE EFFECT OF YANTACIN ON SOME INDICATORS OF CELLULAR RENEWAL AND ON THE LEVEL OF PROTEIN EXPRESSION ON RAT HEPATOCYTES IN CHRONIC HELIOTRINE LIVER DAMAGE

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ABSTRACT

The effect of plant proanthocyanidin – yantacin isolated from the camel thorn plant (Alhagi pseudalhagi) – Yantacin, when administered orally at a dose of 100 mg / kg, on possible changes in the early stages of liver fibrosis that occurred against the background of chronic heliotrine hepatitis in experimental conditions was studied. The studies were conducted on male rats with a body weight of 100 ±10 g. The effect of yantacin on the general and biochemical

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parameters of blood, as well as on p53, PCNA of liver tissue, mitotic and apoptotic index against the background of heliotrine liver damage was studied. As a result of the conducted studies, yantacin demonstrated a significant protective effect compared with the control group on the detected fibrosis, according to which certain indicators, against the background of chronic toxic liver damage. In this regard, a more extensive in-depth study of the protective and restorative effects of the studied plant proanthosianide substance on chronic changes in the liver, serious structural and functional disorders is being carried out.

KEYWORDS

Chronic hepatitis, liver fibrosis, liver cirrhosis, proanthocyanidin, p53, liver tissue PCNA, heliotrine lesion, yantacin, hepatoprotective, antifibrotic.

INTRODUCTION

It is known that chronic liver diseases associated with hepatitis B and C viruses are one of the urgent problems of modern medicine. The incidence of chronic hepatitis B and C in recent decades throughout the world, including in Uzbekistan, has a steady upward trend. Given the widespread prevalence of chronic HBV and HCV infections among children, the high frequency of adverse outcomes with disability and significant mortality in the final stages of the natural course, they represent one of the most pressing problems of modern pediatrics. Chronic HBV infection is observed in 5%-15% of patients with acute viral hepatitis; liver cirrhosis develops in 10% of them, and hepatocellular carcinoma forms in 8% of patients with liver cirrhosis [1, 2, 3]. Recently, significant progress has been made in the study of the pathogenesis of liver fibrosis in CHB. The widespread spread of chronic liver diseases, including hepatitis, fibrosis and insufficient effectiveness for predicting the course of the disease and timely prescribing their treatment require the development of new approaches to the therapy of this pathology. One of the promising directions in the search for treatment of chronic hepatitis is associated with the use of drugs, including those of plant origin, with the ability to reduce the degree of fibrous changes in the liver [4,5,6].

THE MAIN RESULTS AND FINDINGS

There are no effective methods of treating liver fibrosis. This makes it urgent to search for new methods aimed at the functional restoration of the liver and its regeneration. In connection with this, extensive pharmacological and toxicological and chemical-technological studies of Yantacin plant proanthocyanidin, which is isolated from the camel thorn plant (Alhagi pseudalhagi), widely distributed in Central Asia and Kazakhstan, were carried out in the laboratories of the Research Institute of Pediatrics and ICPS of the Academy of Sciences of the Republic of Uzbekistan in a new direction [7, 8].

The above was the basis for determining the purpose of this study and formulating tasks to achieve it.

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To evaluate the effectiveness of plant-derived yantacin of hepatoprotective and antifibrotic action in chronic toxic liver disease in rats.

The experimental part was performed on white mongrel male rats. An experimental model of chronic liver pathology was obtained in male rats weighing 100±10 g. The hepatotoxicity of heliotrin has been shown by us in rats before, so the model of heliotrin-induced hepatitis or liver fibrosis is the most suitable model of toxic liver damage. The chronic form of experimental hepatitis was caused by the administration of heliotrin in decreasing doses: according to the scheme: 10 mg/100 g of mass, 7 mg/ 100 g of mass, 5 mg/100 g of mass, 3 mg/ 100 g of mass [9]. Chronic intoxication, confirmed morphologically, was obtained on the 35th day of the experiment [10]. The mortality rate was 30%. On the 35th day of the experiment, the animals were taken out of the experiment under ether anesthesia, the liver was fixed and poured into paraffin according to the standard procedure. Also, to characterize the degree of fibrosis of liver tissue, a general and biochemical blood analysis, a morphological examination of the liver were performed simultaneously.

To determine the level of apoptosis, special research methods were used: determination of p53, PCNA of liver tissue of experimental animals was carried out by immunohistochemical method (ShiSh.R., 1991) [8].

With the introduction of heliotrin in the blood of experimental animals, a change in several indicators was detected; leads to a positive dynamics of morphological data, causes the reverse development of hyperfermentemia and hyperbilirubinemia; a significant decrease in cytokine levels; levels hyperlipidemia and hypoproteinemia; there is a tendency to normalize not only qualitative, but also quantitative indicators of erythropoiesis: the hematocrit index decreases, necrosis and fibrosis decreases.

It is known that apoptosis is triggered either through receptors located on the surface of hepatocytes, or through structural and metabolic disorders in the mitochondria of cells. The receptor pathway of triggering apoptosis occurs due to the interaction of pro–apoptotic molecules (FasL ligand) with "death receptors" (FasR). Namely, the formation of a ligand receptor complex (FasR/FasL) contributes to the further launch of a cascade of intracellular reactions that ultimately ensure the realization of apoptotic death of hepatocytes. In the conditions of our experiments, the hepatocyte is influenced by a toxic factor – heliothrin, which can contribute to damage to the plasma membrane, including its receptor apparatus and, subsequently, disruption of ligand–receptor relationships. Although the main factors released by mitochondria into the cytoplasm are cytochrome C and apoptosis –indicating factor (AIF), as well as procaspases 2, 3 and 9, which trigger intracellular apoptosis and participate in many signaling pathways that ensure cell death, but this does not always end with the realization of cell apoptosis. Apoptosis and necrosis in the pathogenesis of experimental chronic liver damage. To assess the state of cellular renewal in conditions of chronic liver damage by heliotrin, we determined in the liver tissue of experimental animals the indicators of apoptotic index (AI) and hepatocyte necrosis as two main ways of cell death, as well as the mitotic index (MI) – an indicator of liver proliferative activity. In addition, the coefficients of the ratio of apoptosis to proliferation (AI/MI) and the ratio of apoptosis to necrosis were analyzed (Table 1).

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Table 1. Some indicators of cellular renewal in the liver in chronic heliothrin hepatitis against the background of the effect of Yantacin. (M±m).

Animal groups	Indicators				
	MI (mitotic index, per %)	AI (apoptotic index, per %)	Necrosis (per %)	Coefficients	
				AI/MI	Necrosis /AI
Control	4,2±0,30	6,10±0,25	--	1,2±0,12	--
Chronic heliotrical hepatitis	1,9±0,46*	10,2±0,32*	52,2±0,92	5,4±0,25*	5,1±0,36
Acute heliotrical hepatitis	2,4±0,40*	6,4±0,21*	58,2±1,51	2,7±0,21*	9,1±1,24
Chronic heliotrical hepatitis + Yantacin	3,1±0,32*	8,70,21*	33,1±1,24	2,81±0,45	3,81±0,92

Notes: - differences with intact ones are significant * ($p<0.05$).

Consequently, in conditions of chronic heliotrine hepatitis, the apoptotic death of hepatocytes increases, but, clearly, the death of hepatocytes due to necrosis increases almost 7 times. Such a pathway of liver cell death is practically not found in animals of the control group. Despite the high frequency of cell death (approximately more than 60 hepatocytes out of 100 in the field of vision are susceptible to death), the mitotic activity (MI) of hepatocytes is low -2.2 times lower than that in the control group (Table. 1), and the coefficient AI/MI, on the contrary, is 4.5 times higher.

Consequently, under conditions of prolonged exposure to relatively low doses of hepatotropic toxic factor, i.e., with chronic heliothrin hepatitis, there is a high level of hepatocyte death relative to the norm, but in intensity it gives way to necrosis, and the processes aimed at restoring hepatocyte deficiency not only do not have time to compensate for the missing cells, but even noticeably decrease, about as evidenced by the low mitotic activity of the liver and the high value of the AI coefficient/MI, there is a noticeable activation of hepatocyte apoptosis and necrosis. At the same time, AI in chronic heliotrine hepatitis is 1.7 times higher than the control value.

Under the conditions of HCG, necrosis exceeds apoptosis by 5 times, i.e. if 5 out of 6 cells subject to death undergo necrosis, then 1 undergoes apoptosis.

Consequently, in conditions of chronic heliotrine hepatitis, the apoptotic death of hepatocytes increases, but, clearly, the death of hepatocytes due to necrosis increases almost 7 times. Such a pathway of liver cell death is practically not found in animals of the control group. Despite the high frequency of cell death (more than 60 hepatocytes out of 100 in the field of vision are susceptible to death), the mitotic activity

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(MI) of hepatocytes is low. The positive effect of the studied substance on the apoptotic index, mitotic activity (MI) of hepatocytes, in this regard, the AI coefficient/MI and Necrosis/AI against the background of chronic heliotrine hepatitis in rats is presented in Table 1.

Based on the above, we studied the expression of protein p 53 on hepatocytes in conditions of chronic heliotrine hepatitis. The results of these studies are presented in table 2. The results of the conducted studies show that under conditions of toxic liver damage, certain shifts in gene regulation occur, in particular through the p53 protein that characterizes hepatocyte apoptosis.

Table 2. Expression of p53 protein on hepatocytes in conditions of acute and chronic heliotrine hepatitis and against the background of the effect of Yantacin. (M±m)

The studied groups of animals	Number of samples	Positive expression of p53 protein	
		Number of positive observations	Frequency of p 53 – positive cells, per %
Control	10	2	8,2±0,52
Chronic heliotrical hepatitis	10	5	14,11±1,09*
Chronic heliotrical hepatitis + Yantacin	10	4	11,24±1,21*

Notes: - differences with intact ones are significant * (p<0.05)

At the same time, it was found that if in healthy animals hepatocytes with a positive reaction to p 53 are found in 20% of animals, then with ChHH – in 50% of animals. Along with this, the level of p53 protein expression on the hepatocytes of experimental animals is also higher than that of the control (Table 2). The expression of p53 in ChHH exceeds the control value by 71.9%.

Consequently, in conditions of toxic liver damage, not only an increase in the number of animals with a positive reaction to p53 occurs, but the expression of this protein on hepatocytes is noticeably increased, and this is clearly seen in animals under prolonged exposure to a toxic agent, i.e. with chronic heliotrine hepatitis.

If this process proceeded in a balanced manner with the process of cellular renewal, i.e. with proliferation, then indeed, in such a change in the death of hepatocytes, as in ChHH, one could see elements of rationality. However, in the conditions of ChHH, the opposite picture is observed, characterized by further inhibition of mitotic activity, which increases to an even greater extent the imbalance between the death of hepatocytes and their renewal. Apparently, this is the essence of the complexity of the pathogenesis of chronic liver pathology, its progression, as well as the formation of the process of fibrogenesis with the transition to cirrhosis.

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Proliferation, as a compensatory process, allows under normal conditions to maintain homeostasis with natural cell renewal. In conditions of pathology, proliferation, which has complex mechanisms of regulation, is aimed at regenerating damaged tissue and maintaining its functional viability. We evaluated the proliferative activity of the liver parenchyma (hepatocytes) and one of the elements of its stroma (sinusoid cells) in conditions of ChHH. The positive effect of the studied substance on the level of expression of the p53 protein on hepatocytes against the background of chronic heliotrine hepatitis in rats is presented in Table 2.

It is known that apoptosis is triggered either through receptors located on the surface of hepatocytes, or through structural and metabolic disorders in the mitochondria of cells. The receptor pathway of triggering apoptosis occurs due to the interaction of pro–apoptotic molecules (FasL ligand) with "death receptors" (FasR). Namely, the formation of a ligand-receptor complex (FasR/FasL) contributes to the further launch of a cascade of intracellular reactions that ultimately ensure the realization of apoptotic death of hepatocytes. In the conditions of our experiments, the hepatocyte is influenced by a toxic factor – heliothrin, which can contribute to damage to the plasma membrane, including its receptor apparatus and, subsequently, disruption of ligand–receptor relationships.

How can we explain the low level of proliferative activity of hepatocytes detected by us in the conditions of chronic liver damage by heliotrin Indeed, in conditions of increased hepatocyte death, an increase in the rate of proliferation should also occur in order to maintain the balance of functioning hepatocytes. However, this does not happen. To clarify this issue, we studied the expression of another protein, PCNA, on hepatocytes in conditions of toxic liver damage, since this protein is widely used to assess the proliferative activity of cells. The results of the conducted research in this direction are presented in Table 3.

Table 3. Expression of PCNA protein on hepatocytes in conditions of acute and chronic heliotrine hepatitis and against the background of the effect of Yantacin. (M±m)

The studied groups of animals	Number of samples	Positive expression of PCNA protein	
		Number of positive observations	Frequency of PCNA – positive cells, per %
Control	10	10	54,2±3,1
Chronic heliotrical hepatitis	10	6	9,7±1,28*
Chronic heliotrical hepatitis + Yantacin	10	8	29,1±1,3

Notes: - differences with intact ones are significant * ($p<0.05$)

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As can be seen from the presented data, in conditions of chronic heliotrine hepatitis, noticeable changes in PCNA expression occur.

At the same time, 60% of animals with HCG have a positive reaction in hepatocytes to the PCNA protein, At the same time, in animals of the control group, this was the case in all animals (100%). Analysis of the results of PCNA protein expression on hepatocytes of experimental animals showed that it was also noticeably lower than in animals of the control group (Table 3). At the same time, if the expression of this protein on hepatocytes of animals with ChHH, it was 5.6 times lower than in the control group. Consequently, under conditions of toxic liver damage, there is a significant decrease in the expression of the hepatocyte proliferation marker on hepatocytes. Indeed, the morphological picture of the liver, studied by us in chronic heliotrine hepatitis, also indicates that in the conditions of the studied liver pathology, the structural changes characteristic of chronic hepatitis differ only in isolated cases - these are hepatocytes with signs of mitosis and, consequently, proliferation. The positive effect of the studied substance on the level of PCNA protein expression on the hepatocytes of experimental animals against the background of chronic heliotrine hepatitis in rats is presented in Table 3.

CONCLUSION

Thus, the obtained results showed that plant proanthocyanidin significantly reduced the death of hepatocytes and accelerated their regeneration in small quantities. In this regard, in order to recommend the use of yantacin for chronic liver diseases, administered for therapeutic purposes with toxic hepatitis, a more extensive in-depth study of the protective and restoring effect of the studied substance of plant proanthosianide on chronic changes in the liver, serious structural and functional disorders is carried out.

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