Journal Healthcare Treatment Development
Vol: 03, No. 02, July-Dec 2023
ISSN: 2799-1148, DOI: 10.55529/jhtd.35.1.6

Research Paper

Assessment of the chronic process in the liver under study conditions using conventional and in-depth laboratory studies

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Article Info

Article History:
Received: 06 May 2023
Revised: 14 July 2023
Accepted: 22 July 2023
Published: 05 September 2023

Keywords:
Fibrogenesis
Hepatoprotectors
Heliotrin
Liver Fibrosis
Cirrhosis
Chronic Toxic Hepatitis

ABSTRACT

Background: Serious hepatic pathologies are, honestly, a huge clinical burden, and the creation of dependable experimental models that really mirror human liver disease, is basically crucial if we want to push hepatology research forward. Heliotrin, which is a pyrrolizidine alkaloid with well described hepatotoxic effects, might work as a practical tool for causing repeatable hepatic injury in animal models.
Objective: The aim was to look at the functional as well as the morpho logical changes in the liver that were produced by a subtoxic-dose regimen of heliotrin in white rats, and also to check how well lab blood indicators that hint at hepatic inflammation match up with histomorphological findings.
Methods: The experiments were carried out on white rats. Heliotrin was given using a subtoxic dose scheme, intended to induce severe liver pathology that is broadly similar to what is seen in human hepatic disease. For hepatic assessment, a mixed strategy was used, meaning conventional evaluation methods plus more extensive laboratory investigations. Blood parameters linked with inflammatory activity and hepatic functional status were measured, then correlated with morphological observations from liver tissue histology.
Results: After giving heliotrin at subtoxic doses, the rats showed pronounced functional and morphological alterations in liver tissue. Inflammatory process related markers, both the routine ones and the more detailed laboratory parameters, were significantly disturbed. Importantly, the changes seen in the lab indices matched the histomorphological findings in a consistent way, so this supports the idea that the biochemical markers do, indeed, reflect the deeper structural damage occurring in the liver.
Conclusions: Heliotrin, given at subtoxic levels in white rats, consistently triggers marked hepatic damage that looks a lot like human liver disease, with functional, biochemical, and morphological alterations all happening together. The fact that the blood test readouts in the lab really match the tissue

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findings, makes the experimental setup quite reliable for investigating hepatotoxic mechanisms, and it also backs up the value of these biochemical indices, as a sort of proxy for tracking how the condition is progressing during hepatological research.

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1. INTRODUCTION

The problem of treating hepatitis of various etiologies is an extremely urgent task of modern medicine. Currently, despite the large arsenal of hepatoprotectors, clinicians do not always manage to stabilize the course of hepatitis, increase regenerative activity and prevent the development of fibrosis and cirrhosis of the liver. Because the effect of many drugs positioned as hepatoprotectors in acute and chronic liver pathology is in many cases insufficient and is not confirmed by independent comparative studies [1], [2], [3]. In this regard, the search continues for new medicinal agents, including those of plant origin, with a wide range of pharmacological activity and economic availability. In addition, on the model of different etiological toxic hepatitis, it was shown that the introduction of different biological substances to animals contributes to the normalization of blood biochemical parameters [4], [5], [6]. Thus, studies of the hepatoprotective effect of drugs based on antioxidant properties remain an urgent direction in toxicology. Therefore, in order to further expand the pharmacological spectrum of action of this agent, studies of its effect on the processes of fibrogenesis and the formation of liver cirrhosis in a long-term experiment were conducted. To compare the hepatoprotective effect of drugs on structural changes in the liver, the carbon tetrachloride model, isoniazid and heliotrin are often chosen. In studies of hepatoprotective properties, functional changes are often considered together with structural changes, although some researchers have found discrepancies in the levels of enzymemia with morphological manifestations in chronic heliotrin intoxication of rats. Heliotrin, as carbon tetrachloride, damages the liver, initiating the formation of various radicals and reactive oxygen species, leading to inflammation and depletion of antioxidant protection for the second time [7], [8], [9], [10], [11], [12]. Thus, the increasing medical and social significance of chronic liver diseases requires new efforts in the development of issues of etiology, pathogenesis, immunology, diagnosis, treatment and prevention of these diseases. Currently, the radical surgical method of treating cirrhosis of the liver is orthotopic liver allotransplantation. However, its technical complexity, the shortage of donor organs, and the high cost of treatment limit the widespread introduction of this operation into clinical practice and stimulate the search for new available methods of treatment [13]. Therefore, reproduction in animal experiments of models of hepatitis and cirrhosis of the liver, close to clinical conditions, is still necessary [14]. Experimental models make it possible to give a comprehensive assessment and develop methods for adequate correction of liver failure, which is not always possible in clinical trials. For this reason, we used a method that is widely used in the context of the study of chronic toxic hepatitis.

1.1. The Purpose of the Work

Investigation of the effect of a toxic substance on the parameters of tumor necrosis Alpha factor-FNOAA, CD68, CD34 and CD95 in the blood of laboratory white rats under conditions of chronic heliotrine hepatitis.

2. METHODOLOGY

The experiment was performed on white female laboratory rats weighing 100 ± 10 g, obtained from a vivarium of used animals kept under standard conditions on a normal food and water diet. Manipulations with animals were carried out according to the manual [15]. Toxic liver damage was modeled by intragastric administration of heliotrin in low doses according to the scheme: 10 mg/100 g per kg of body weight, 7 mg/ 100 g per kg of body weight, 5 mg/ 100 g per kg of body weight, 3 mg/100 g per kg of body weight. All animals were divided into 6 groups of 10 individuals each. From the experiment, rats were removed by decapitation after 1, 3 and 5 weeks. The activity of ALT, AST, alkaline phosphatase, LDH, the level of total protein, direct bilirubin and glucose were determined in the blood serum. Chronic toxic liver damage or chronic intoxication was confirmed hematologically, biochemically and morphologically on the 35th day of the experiment [7], [16].

Alpha - factor of tumor necrosis in the blood-testing for FNOa and cd68+, CD34+ and CD95+. In diseases accompanied by inflammation, in particular, among the mechanisms leading to the pathogenesis of hepatitis, tissue damage is noted due to energy imbalance, excessive peroxidation and the development of secondary immunodeficiency. The following indicators were determined in the blood serum by the IFT method: the level of the main factors causing inflammation (Alpha - factor of tumor necrosis-fnoa), CD68+, CD34+ [17], [18], [19]. Based on the conducted studies, the obtained results were compared with the control group of mukhakamasi, and statistical processing of the results was carried out using the methods of R.B. Strelkov presented in [20].

3. RESULTS AND DISCUSSION

1. Hematological and biochemical analysis of rat blood during the development of chronic heliotrine hepatitis. 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 transaminases (AST and ALT) in the blood serum of rats with chronic gh 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 HHD to 10.3 and 145%, respectively, compared with the control group (Table *1*).

Table 1. Changes in the Functional State of Liver Enzymes in Chronic Heliotrine Hepatitis

Experimental Groups	**Intackt Group**	**Control Group**
Doses mg/kg	Dist. water	Heliotrin
ALT u /l	95,6±2,4	268,9±9,6
AST u /l	156,6±4,8	373,6±11,21
Total protein (g/l)	85,6±4,8	58,7±2,9
Albumin g/l	48,5±2,9	33,3±3,6
Total Bilirubin mkmol/l	2,15±0,96	5,12±0,96
Koeffitsient of de Rits (AST/ALT)	1,63±0,22	1,35±0,11
ALPh, u /l	394±4,48	434,4±6,52
LDH, u /l	625,5±11,6	1532,5±22,4
HHD u /l	1,15±0,11	1,46±0,11
Amilase (u /l)	495,7±11,22	442,6±6,48

Thus, as the degree of liver damage worsened, as can be seen from the table, significant changes in biochemical parameters were also observed. in subsequent studies, the amount of cytokines was mainly tracked dynamically, and it was analyzed whether these changes were proportional to biochemical changes in the blood.

2. Investigation of the effect of a toxic substance that damages the liver on CD68, CD34 indicators in conditions of chronic heliotrine hepatitis. In animals with chronic heliotrine intoxication, the relative number of CD68-positive lymphocytes increased inside liver fragments during 5 weeks of the experiment. The number of intrahepatic CD34-positive lymphocytes in experimental rats also increased significantly compared to the control group (Table *2*).

Table 2. Changes in CD68, CD34, in Conditions of Chronic Heliotrin Hepatitis

Experimental Groups	Doses in Mg/Kg	CD_{68}, % On the Square	CD_{34}, % On the Square
Intackt group	Dist. water	3,89±0,21	4,75±0,48
Control group	Dist. water	5,49±0,9*	27,68±1,15

Thus, in conditions of chronic heliotrine hepatitis, the hepatotoxic substance negatively affected the relative number of CD68 and CD34-positive lymphocytes in liver tissue.

3. Investigation of the effect of a toxic substance that damages the liver on CD68, CD95, as well as anti-inflammatory cytokine - fno - na in conditions of chronic heliotrine hepatitis. In chronic experimental hepatitis, there was a significant increase in proinflammatory cytokine - FHO - α in blood serum to 96.64 ±10.2 pg/ml compared with the norm ($P<0.001$). This is also relative to the control value ($p<0.025$), confirmed by a direct correlation between the value of fno-O and the severity of fibrosis (Table 3).

Table 3. Dynamics of Changes in Immunity Indicators in Blood Serum. Blood in the Process of Developing Chronic Heliotrine Hepatitis

Experimental Groups	CD_{68}, % On the Square	CD_{95}, % On the Square	ФНО-А (Ркg/Ml)
Control group	3,89±0,21	4,75±0,48	38,72± 2,54
Group 1	4,97±0,39	12,54±0,9	68,22±5,07
Group 2	5,08±0,45	16,44±1,25	75,9±6,22
Group 3	5,46±0,41	20,21±1,62	84,24±6,55
Group 4	5,81±0,61	22,65±1,49	86,61±5,62
Group 5	6,24±0,48	27,54±0,97	91,72±6,45
Group 6	5,49±5,9*	27,68±1,15	96,64 ±10,2

As shown in the Table *3*, chronic administration of a hepatotoxic substance led to an aggravation of the degree of liver damage and even to the appearance of fibrosis-specific assays.

4. CONCLUSION

Thus, in the conducted studies, a substance with hepatotoxic properties of heliotrin, when administered according to a subtoxic dose scheme, caused severe functional and morphological changes in the liver. It was found that the change in blood parameters characterizing inflammatory processes in the liver corresponds to morphological data.

Acknowledgments

The authors have no specific acknowledgments to make for this research.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
A. N. Aripov	✓	✓	✓	✓		✓		✓	✓	✓	✓			
L. L. Akhunzhanova	✓	✓	✓			✓	✓	✓						
O. A. Aripov		✓	✓	✓			✓	✓	✓		✓	✓	✓	
A. U. Nabiev	✓	✓	✓		✓	✓	✓			✓	✓	✓		

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to Cite: A. N. Aripov, L. L. Akhunzhanova, O. A. Aripov, A. U. Nabiev. (2023). Assessment of the chronic process in the liver under study conditions using conventional and in-depth laboratory studies. Journal Healthcare Treatment Development (JHTD), 3(2), 53–59. https://doi.org/10.55529/jhtd.35.1.6

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