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Differential Analysis of Chronic Toxic Hepatitis Caused by The Introduction of Heliotrin Solution in Various Ways

Aripov A.N., Akhunjanova L.L., Khamroev T.T.
Specialized Scientific and Practical Medical Center of Pediatrics of the Ministry of Health of the Republic of Uzbekistan, Tashkent e-mail: tolmas4th@mail.ru
Aripov Abdumalik Nigmatovich
Doctor of Medical Sciences, Professor.
Head of the Department of Clinical Laboratory diagnostics of Republican Specialized Scientific and Practical Medical Center of Pediatrics of the Ministry of Health of the Republic of Uzbekistan
Akhunjanova Lola Lazizovna
Candidate of Biological Sciences,
Senior Researcher of the Department of Clinical Laboratory Diagnostics of RSNPMTSP of the Ministry of Health of the Republic of Uzbekistan
Khamroev Tolmas Tolibovich
Junior Researcher of the Department of Pharmacology and Toxicology of the Institute of Plant Chemistry named after Academician S.Yu.Yunusov of the Academy of Sciences of the Republic of Uzbekistan

Annotation. In the present study, experimental models of chronic toxic hepatitis were induced by intragastric, subcutaneous and intraperitoneal administration of a solution of heliotrin. Hematological, biochemical and morphological research methods were used to assess the severity of liver damage. Based on the data obtained, toxic hepatitis was confirmed in three models. The most severe liver lesions occur with intraperitoneal administration of heliotrin. Oral or intragastric administration of heliotrin reduces the mortality rate (from 8% to 10%), avoids violation of the integrity of the skin and reduces the manifestation of pain syndrome in animals.

Key words: heliotrin, liver fibrosis, chronic hepatitis, liver cirrhosis, toxic hepatitis, subcutaneous, intraperitoneal, oral

Introduction.

In recent years, chronic hepatitis and cirrhosis of mixed etiology have become widespread [1, 2]. Cirrhosis of the liver (CL) is an urgent problem of modern medicine due to its widespread, continuously increasing morbidity and high mortality. About 10% of the world's population has chronic liver diseases, which is more than 500 million people, while more than 20 million people worldwide suffer from cirrhosis and (or) liver cancer. According to WHO forecasts, in the coming decades, the number of patients suffering from cirrhosis will increase by more than 60%, which is associated with the widespread spread of toxic and viral liver lesions. [8]. Widespread chronic hepatitis of viral etiology for the Central Asian region, including the Republic of Uzbekistan, there is a need for further fundamental research in this area. [5, 6, 9]. Fundamental research in this field has allowed scientists to decipher the mechanisms of development of microcirculatory disorders in the course, the features of the violation of rheological properties in the blood, metabolism, and others. Chemical and medicinal substances are often used to simulate liver lesions. Preclinical studies of promising hepatoprotectors are carried out, as a rule, on the classical model of toxic chronic hepatitis caused by intragastric, subcutaneous and intraperitoneal administration of a solution of heliotrin [3]. As a result, in order to obtain objective results, it is necessary that the chosen technique most fully reflects the processes of liver damage.

The object of the research:

To investigate the comparative characteristics of models of toxic chronic hepatitis caused by intragastric, subcutaneous and intraperitoneal administration of various doses and schemes of heliotrin solution based on hematological, biochemical and morphological parameters.

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Materials and methods.

The experiment was carried out on male mongrel rats weighing 100-150 g. All experiments were carried out in accordance with international standards adopted when working with experimental animals. Models of chronic heliotrine hepatitis (ChHH) are reproduced in different ways by administration of heliotrine in different doses according to the following schemes; with oral administration of a solution of heliotrin acidified with hydrochloric acid (pH 7.0) at the rate of 50 mg / kg (5 mg of heliotrin per 100 g) body weight was administered once a week for 42 days according to the method of N.H. Abdullaev et al. [3]. The animals were slaughtered for 60, 90 and 120 days from the beginning of the experiment;

with subcutaneous injection of heliotrin, the first week was administered 2 times at a dose of 10 mg / kg, then in a similar mode at a dose of 7.5 mg / kg and in the third week – 5 mg / kg [4]. After that , heliotrin was administered simultaneously with the substance under study for 25 days according to the scheme;

with intraperitoneal injection of heliotrin, it was administered in decreasing doses: in the first week 3 times at a dose of 25 mg / 100 g of mass, then 3 times a week at a dose of 15 mg / 100 g of mass for 2 weeks, in the fourth week 3 times - 10 mg / 100 g of mass, 3 mg / 100 g of mass. Chronic intoxication, confirmed by biochemical and morphological, was received on the 35th day.

The obtained data were compared with similar indicators in the control group of animals and statistical processing of the results was also evaluated by the accuracy of the changes using the Arrow correction and the Litchfield-Wilcoxon method at P=0.05[10].

Research results and discussion:

The liver in the mammalian body performs complex and multifaceted functions aimed at preserving homeostasis, and is also actively involved in all types of metabolism. To characterize the degree of fibrosis of liver tissue, hematological, morphological and biochemical studies were carried out on the appropriate days in each of the above experiments. The obtained results of the conducted studies showed that in animals with chronic toxic hepatitis, the whole group has general weakness, ruffled and reduced shine of the coat, bloating, the development of ascites, and in this case the mortality of animals is noted.

With the introduction of heliotrin in the blood of experimental animals, a change in the content of erythrocytes was detected, not only qualitative, but also quantitative indicators of erythropoiesis change: the hematocrit index decreases. This fact is associated with an increase in tissue hypoxia, which leads to necrosis and apoptosis of cells. On the part of white blood cells, the most pronounced changes are observed in the blood of rats with ChHH, when the number of hepatocytes and medium cells increases in peripheral blood, which reflects the exudative phase of inflammation in response to damage to functional cells in the organ. This also reflects the proliferative phase aimed at phagocytosis of destructively altered hepatocytes of the liver. The decrease in platelets in the blood is apparently associated with a decrease in the functional activity of poorly differentiated hepatocytes (Table 1).

Table 1. Comparison of peripheral blood parameters of rats with ChHH

The name of the indicator	Intact animals	ChHH with oral administration	ChHH with subcutaneous administration	ChHH with intraperitoneal administration
Hematocrit index, %	44,46±1,01	42,7±2,2	40,3±1,88	38,11±1,48
Hemoglobin, g/l	145,2±0,16	140,75±3,7*	138,75±3,3*	127,23±0,45*
Red blood cells, T/l	8,85±0,24	8,2±0,31	7,98±0,29	5,85±0,35
Average volume of red blood cells, fl	50,24±0,44	54,56±0,66*	58,45±0,76*	61,24±0,48*
Average hemoglobin content in erythrocyte, pg.	16,42±0,34	17,3±0,35*	19,0±0,25*	21,0±0,44*

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The average concentration of Hemoglobin in the erythrocyte, g/ dl	32,7±0,53	34,86±0,25	36,25±0,15	39,96±0,42
Leukocytes, G/l	13,38±0,84	14,32±1,94	15,82±1,43	17,35±1,85
Lymphocytes, G/l	10,64±0,73	9,7±0,44	9,1±0,27	8,8±0,42
Lymphocytes, %	79,6±1,33	73,75±2,29	70,25±1,49	68,75±2,25
Average cells, G/l	1,66±0,11	2,94±1,49	3,54±1,15	4,14±1,22
Platelets, G/l	663,8±35,07	619,0±33,26	601,0±24,36	569,0±53,36

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

Modeling of toxic liver damage in rats caused by the introduction of heliotrin led to the development of disorders of the functional state of liver cells. In rats with ChHH, the activity of transaminases (AST and ALT) in the blood serum was compared with the level of these indicators in the control ($p<0.05$) (Table 2.).

Table 2. Comparison of biochemical blood parameters of rats with ChHH

The name of the indicator	Intact animals	ChHH with oral administration	ChHH with subcutaneous administration	ChHH with intraperitoneal administration
AsAT, E / l	146,72 ± 7,74	385,08 ± 44,39*	472,12 ± 38,7*	531,45 ± 27,12*
AlAT, E / l	86,35 ± 4,18	428,54 ± 27,08*	515,19 ± 12,24*	725,19 ± 12,22*
AlF, E / l	549,30 ± 21,84	1510,06 ± 33,4*	1602,50 ± 165,54*	678,50 ± 16,54*
Total bilirubin, mmol / l	15,58 ± 3,73	29,41 ± 1,38	38,37 ± 2,13*	55,21 ± 2,45*
Total protein, g / l	74,37 ± 6,02	66,14 ± 3,05	56,69 ± 7,25	42,14 ± 3,32

Note: * - reliability compared to control.

A morphological study of the liver of experimental rats with chronic heliotrine intoxication for the presence of liver fibrosis was carried out. One of the morphological signs of ChHH is intra-lobular lymphocytic infiltration, which is much more intense and uneven compared to the norm [7]. The following changes were revealed: In rats with initial signs of fibrosis (F1), treated with heliotrin for 2-3 weeks, there was an expansion of the portal tracts (Fig.1). Fragmentation and degranulation of the rough reticulum are pronounced. Microvilli of bile capillaries are smoothed and their number is reduced, hepatocytes are sharply changed, partial necrosis phenomena are observed. Connective tissue strands are formed. The development of fibrous tissue was observed.

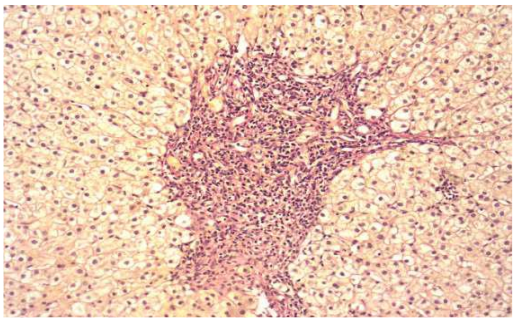

Fig. 1. Expansion of the portal tract stroma due to fibrosis, periportal segmental fibrosis (F1) in the liver in rats from X YY (group 3). Ocd.picrofuxin by Van Gieson. Uv.x200.

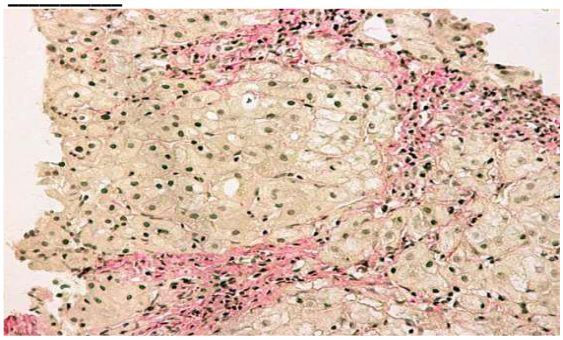

Fig. 2. Formation of fibrous septa in the liver of rats without signs of their vascularization by severe liver fibrosis (F3 according to Knodell). Ocd. picrofuxin according to Van Gieson. Uv.x 200.

A longer course of chronic hepatitis in rats naturally led to an aggravation of the severity of liver fibrosis (F3), characterized by the appearance of completed or incomplete non-vascularized port-portal or portocentral septa in the liver parenchyma, leading to a significant change in liver architectonics (Fig. 2). Additionally, the mortality rate of rats in the experimental groups was assessed. In the group of animals treated with heliotrin intragastrically, mortality was 10%, while with subcutaneous and intraperitoneal administration of heliotrin, mortality increased to 30-40%, respectively (Table 3.).

Table 3. **Mortality rate of animals with toxic heliotrin hepatitis**

The name of the indicator	Intact animals	ChHH with oral administration	ChHH with subcutaneous administration	ChHH with intraperitoneal administration
Number of experimental animals in the group	10	20	20	20
Number of dead animals	10	2	6	8
Mortality rate, %	0	10*	30*	40*

Note: * - reliability compared to control

According to the obtained laboratory data, toxic hepatitis is observed in the form of hyperfermentemia, hyperbilirubinemia, hypoproteinemia, both with intraperitoneal administration of heliotrin and with intragastric and subcutaneous. In three cases, the destruction of hepatocyte cell membranes, cholestasis, inflammatory, dystrophic and fibrotic changes in liver tissues are recorded.

Summarys.

1. Intragastric, subcutaneous and intraperitoneal administration of heliotrin causes laboratory-confirmed toxic hepatitis.
2. The most severe liver lesions occur with intraperitoneal administration of heliotrin.
3. Intragastric administration of heliotrin allows to reduce the mortality rate (from 8% to 10%), to avoid violation of the integrity of the skin and to reduce the manifestation of pain syndrome in animals.

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