

Study Of the Effect of a New Blood Substituting Infusion Medical Drug "Reomannisol" On Biochemical Blood Parameters and Liver Morphology in Experimental Heliotrin Intoxication

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Abstract

The effect of the drug "Reomannisol" was investigated in experimental heliotrin intoxication on 90 rats. Infusion therapy was carried out by injecting a new blood substitute "Reomannisol" and a reference drug "Rheosorbilact" into the tail vein of rats at a dose of 5 ml / kg of body weight for 5 days. The biochemical parameters of the blood and morphological changes in the liver were studied.

Key words: blood-substituting infusion drug, "Reomannisol", heliotrinic intoxication, biochemical parameters, morphostructural changes

INTRODUCTION

Acute intoxication caused by various agents lead to serious metabolic disorders in the body and structural and functional changes in the liver [1], which require complex treatment, including infusion therapy, capable of correcting a whole range of disorders that occur in the body, not only on the functional, but also on the morphostructural level [2,3,4,5].

These drugs include the blood substitute "Reomannisol" developed at the Republican Specialized Scientific and Practical Medical Center for Hematology (RSNPMTSG) of the Ministry of Health of the Republic of Uzbekistan, which contains antioxidants [6]. Reomannisol, which has antioxidant, antihypoxant and detoxifying properties, is able to normalize metabolic disorders, positively affect energy production at the cellular level and normalize water-electrolyte metabolism [7].

Purpose of the study. To assess the effectiveness of the effect of the drug "Reomannisol" in experimental heliotrinic intoxication (HI) on the degree of restoration of blood biochemical parameters and morphostructural changes in the liver.

Materials and research methods.

Acute heliotrin intoxication was induced by a single subcutaneous injection of a sublethal dose of heliotrin to 90 rats, prepared at the rate of 40 mg per 100 g of body weight (Abdullaev N.Kh., Karimov Kh.Ya. 1989) [8, 9].

Infusion therapy was carried out by injecting a new blood substitute "Reomannisol" and a reference drug "Rheosorbilact" into the tail vein of rats at a dose of 5 ml / kg of body weight for 5 days.

The animals were divided into the following groups:

Group I - before reproduction of heliotrinic intoxication (intact) (n = 30);

Group II (control) - with heliotrin intoxication without treatment (n = 21);

Group III (control, comparison) – with heliotrin intoxication after administration of the drug "Rheosorbilact", within 5 days, 24 hours after the last injection (n = 26);

Group IV (main, experimental) - animals with heliotrin intoxication after the introduction of the blood substitute "Reomannisol", within 5 days, 24 hours after the last injection (n = 30).

24 hours after the final administration of drugs to determine the state of the monooxygenase system of the liver, the rats underwent "Hexenal" sleep according to the standard technique, the duration of which was determined after subcutaneous administration of 0.6% aqueous solution of "Hexenal" at a dose of 60 mg / kg, which reflects the detoxifying function of the liver [5] and 24 hours later, the animals were sacrificed under light ether anesthesia.

At the end of the experiment, the rats were slaughtered by decapitation under general ether anesthesia with blood sampling to study the parameters of biochemical analysis and liver for morphological study.

The conditions and use of all experimental animals involved in the experiment met the ethical requirements for biomedical research [10,11].

During the study, indicators of biochemical analysis were investigated: alanine aminotransferase (ALT) (U/l), aspartate aminotransferase (AST) (U/l), bilirubin (total, direct and indirect), glucose, urea, albumin, triglycerides (Tg), total cholesterol (TCh), albumin, globulin and calculated albumin-globulin coefficient (A/G) [12].

Biochemical studies of blood serum were carried out using commercial test systems HUMAN (Germany) according to the instructions supplied with the kits, with measurement of the results on a "BA88A" semi-automatic biochemical analyzer (Mindray, China).

After the slaughter of rats and blood sampling, the liver was removed and its macro- and microscopic examination was carried out. Pieces (lobes) of the liver, 1 cm² in size, were fixed in 10% neutral formalin. Finished blocks were cut using a microtome type (Russia). Histological preparations were stained with hematoxylin-eosin (H-E) and studied using a "Leica" microscope under objectives with a magnification of x10, x20, and x40 [13].

Statistical processing of the obtained results of biochemical analysis was carried out using the Excel programs and the "Biostatistics 4.03" application using the Student and Mann-Whitney test. P <0.05 was taken as a criterion of statistical significance.

Research results and their discussion.

With heliotrinic intoxication, a change in biochemical parameters was observed. In group II, the activity of ALT and AST increased 4.5 and 5.4 times (p <0.01), respectively. De Ritis coefficient decreased in group II relative to intact animals by 1.25 times (p <0.05). The concentration of total,

direct and indirect bilirubin in animals with heliotrin intoxication without treatment increased 2.9, 3.5 and 2.7 times ($p < 0.05$), respectively (Table 1).

In group II, there was also a statistically insignificant increase in glucose concentration by 1.1 times ($p > 0.05$). The concentration of urea increased by 1.2 times ($p < 0.05$), which may indicate an increased functional tension of the hepatic tissue.

It was also possible to observe an increase in triglycerides and total cholesterol by 1.3 and 1.7 times ($p < 0.05$), which also indicates lipid metabolism disorders caused by liver damage from heliotrin intoxication. It is necessary to note a decrease in the content of albumin in blood serum by 1.5 ($p < 0.05$), total protein by 1.3 times ($p < 0.05$), and a tendency towards a decrease in total globulin by 1.1 times ($p > 0.05$) which indicates a decrease in the protein-synthetic function of the liver caused by heliotrin poisoning. At the same time, A/G decreased by 1.3 times ($p < 0.05$) (Table 1).

In group II, expressed morphological changes were revealed in the liver of rats with heliotrin intoxication that did not receive treatment. The results of the morphological study showed that, in general, the morphological manifestations after intoxication with heliotrin are manifested by paralytic expansion of the venous vessels, violation of the beam structure of liver cells, especially in the periportal zone of the lobule, small- and large-droplet fatty degeneration of hepatocytes with the appearance of necrosis of single hepatocytes with a small accumulation in these areas the number of macrophages, lymphocytes, neutrophils, proliferation and hypertrophy of stellate reticuloendotheliocytes, edema and expansion of portal tracts with their infiltration with lymphohistiocytic elements and neutrophils, sometimes proliferation of periportal and intralobular bile ducts. On the part of hepatocytes, polymorphism is found (cells of various sizes, among them a large number of two- and multinucleated, nuclei of various sizes), their swelling, as a result of which the clarity of the beam structure is disturbed. Protein (hydropic, balloon) and fatty degeneration have a small focal character, and the severity of these changes, depending on the topographic zones, are manifested in different degrees of activity.

Morphological study showed that dystrophic and necrobiotic changes are more pronounced in the periportal zone of the liver lobule (Figure 2). Similar changes can be observed in the centrilobular zone of the liver tissue. In the second morphofunctional zone, more pronounced dystrophic and dysregenerative changes with the appearance of foci of necrobiosis and necrosis of hepatocytes are revealed. (Figure 1).

Table 1. Changes in the parameters of the biochemical blood test during heliotrin intoxication and after infusion of blood substitutes in rats ($M \pm m$)

Options	First group, intact, (n=30)	Second group, intoxication (n=21)	Third group, "Rheosorbilact" (n=26)	Fourth group "Reomannisol", (n=30)
Alanine amino transferase, U / l	20.1±0.7	91.5±2.4*	29.7±1.7*^	21.5±1.1^#
Aspartate amine transferase, U / L	14.8±0.4	80.6±2.0*	22.4±1.0*^	16.8±0.8^#
De Ritis, conv. units	1.38±0.03	1.14±0.02*	1.33±0.06	1.29±0.05^
Bilirubin, μmol / straight	3.7±0.1	14.7±0.5*	5.8±0.22*^	4.2±0.14^#

Table 1. Changes in the parameters of the biochemical blood test during heliotrin intoxication and after infusion of blood substitutes in rats (M±m)

Options	First group, intact, (n=30)	Second group, intoxication (n=21)	Third group, "Rheosorbilact" (n=26)	Fourth group "Reomannisol", (n=30)	
l	indirect	9.0±0.35	23.9±0.8*	12.8±0.5*^	9.6±0.4^#
	general	12.7±0.4	38.7±1.1*	18.6±0.7*^	13.8±0.5^#
Glucose mmol / l	4.9±0.19	5.9±0.13*	5.2±0.13^	5.1±0.15^	
Urea mmol / l	4.7±0.21	5.4±0.36	5.1±0.2	4.8±0.2	
Triglycerides, mmol/ l	0.89±0.01	1.2±0.04*	0.95±0.04^	0.90±0.03^	
Cholesterol mmol / l	1.9±0.02	3.2±0.09*	1.9±0.07^	1.8±0.07^	
Albumin g / l	29.6±0.2	19.0±0.3*	19.6±0.4*	20.9±0.4^#	
Globulin g / l	39.6±0.7	33.9±0.8*	34.4±0.5*	35.3±0.6	
Total protein g / l	69.2±0.6	52.9±0.7*	54.0±0.6*	56.2±0.6^#	
A / G, whisker ice.	0.75±0.02	0.57±0.02*	0.57±0.02*	0.60±0.02	

* - significance of the difference (p <0.05) when comparing the results with intact animals (the first group); ^ - the same (p <0.05) when comparing the results with the data obtained with heliotrin intoxication (second group); # - the same (p <0.05) when comparing the results with the data obtained after the infusion of "Rheosorbilact" (the third group).

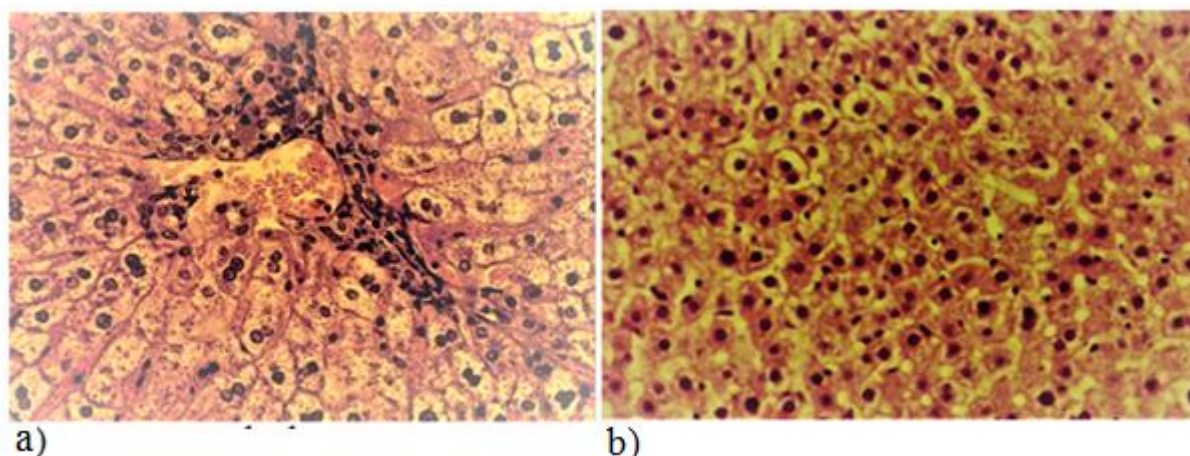


Figure 1. Heliotrin intoxication, no treatment

a) Lymphoid infiltration around the portal tracts, hydropic and fatty degeneration of the cytoplasm of hepatocytes; b) The second morphofunctional zone of the lobule, in hepatocytes, hydropic and fatty degeneration is more pronounced.

Biochemical studies showed that after the use of the blood substitute "Reomannisol", compared with animals that did not receive treatment (Group II), there was a decrease in ALT by 4.3 times (p <0.01), AST by 4.8 times (p < 0.01), an increase in the "De Ritis" coefficient - by 1.1 times (p <0.05), a decrease in the concentration of urea by 1.1 times (p <0.05), triglycerides - by 1.3 times (p <0.05), total cholesterol - 1.8 times (p <0.05). There was also a tendency to an increase in albumin,

globulin and total protein, which may indirectly indicate the incipient restoration of protein-synthetic function. At the same time, relative to the indicators after the use of the comparison drug "Rheosorbilact", after the treatment of HI with "Reomannisol", the activity of ALT was lower by 27.6% ($p < 0.05$), AST - by 20.5% ($p < 0.05$), and the concentration direct, indirect and total bilirubin - lower by 27.6%, 25.0% and 25.8% ($p < 0.05$), respectively.

The results of a morphological study among animals that received treatment showed that "Reomannisol" with heliotrin intoxication in the liver tissue prevents the violation of lipid and carbohydrate metabolism, as indicated by the disappearance of signs of large-drop fatty and hydropic dystrophy in hepatocytes. The introduction of "Reomannisol" also normalized the discirculatory changes, as evidenced by the absence of any pathologies in the region of the central vein and sinusoids. When microscopic examination of liver tissue due to the expansion of the area of the cytoplasm and the nucleus, hypertrophy of hepatocytes is noted. The cytoplasm of liver cells is uniformly stained with eosin and only in some cells with preservation of loosening and peripheral edema. The nuclei of hepatocytes of different sizes, with the presence of hypertrophied among them, due to the increased amount of chromatin, nuclei. Also, among them you can find binuclear hepatocytes. The central vein and sinusoids are moderately dilated, without any pathology, Kupffer's cells are hypertrophied, and some of them have symbiosis with lymphoid cells (Figure 2).

Experimental rats receiving the drug throughout the experiment remained mobile and gained weight well. Hair and visible mucous membranes were unchanged.

The mass coefficients of the internal organs (liver) of the experimental rats remained stable at the control level.

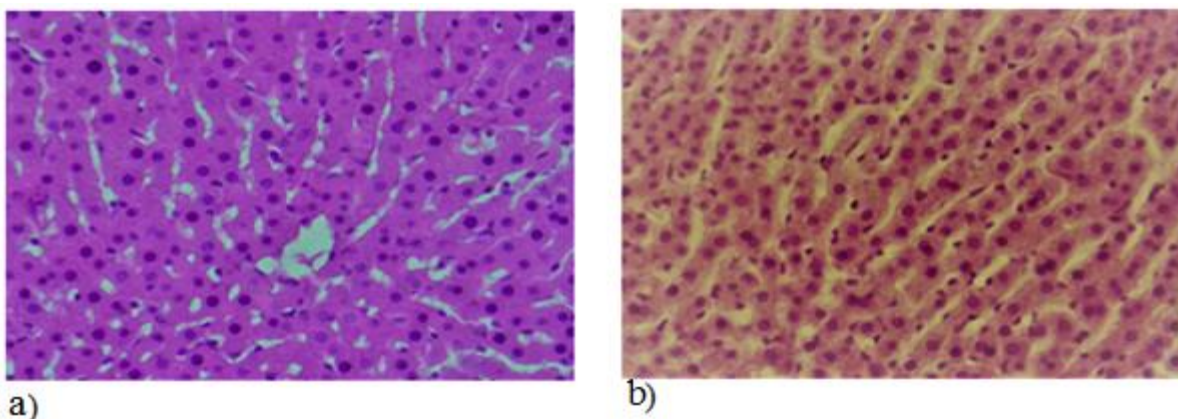


Figure 2. **Heliotrin intoxication, treatment with reomannisol**

a) Hepatocytes are hypertrophied, cytoplasm is eosinophilic, nuclei are hypertrophied; b) 2- and 3-morphofunctional zone, the cytoplasm is enriched with eosinophilic protein mass

Sleep caused by anesthesia under the action of "Hexenal" was carried out for all animals in order to study the detoxification effectiveness of the new blood substitute. If in the group of animals in group II with acute heliotrinic intoxication, who did not receive treatment, the duration of "Hexenal" sleep increased fourfold, relative to the group of intact animals, then after the infusion of the new blood substitute "Reomannisol" in group IV, "Hexenal" sleep decreased more than two fold, and after the use of the drug "Rheosorbilact", in group III - 1.5 times compared with intoxication (Group II).

Discussion

As you know, changes in the body that occur when the liver is damaged by heliotrin can directly affect the energy apparatus of the cell and the cellular respiration of hepatocytes [14,15], which leads to their functional and morphostructural changes.

Heliotrinic intoxication led to an increase in the main parameters of biochemical research: ALT, AST, which indicated cytolytic and necrotic changes in liver tissue; an increase in bilirubin fractions, which indicated severe liver damage and a violation of its functional activity; a decrease in the concentration of albumin and total protein reflect a decrease in the activity of the protein-synthetic function of the liver; an increase in triglyceride and total cholesterol levels - a violation of lipid metabolism due to liver damage.

In general, morphological manifestations after intoxication with heliotrin were manifested by paralytic dilatation of venous vessels, violation of the beam structure of the liver, especially in the periportal zone of the hepatic lobule - acinus, small- and large-droplet fatty degeneration of hepatocytes with the appearance of necrosis of single hepatocytes with an accumulation of macrophages in these areas, neutrophils, proliferation and hypertrophy of stellate reticuloendothelial cells.

Treatment of heliotrinic intoxication with the new blood substitute "Reomannisol" was accompanied by the normalization of biochemical parameters reflecting the functional activity of the liver. After the use of "Reomannisol", a decrease in the volume and degree of dystrophic and necrobiotic changes in the liver tissue was observed. At the same time, in hepatocytes, the histotopography and beam structure were preserved, and the content of eosinophilic protein mass increased in the cytoplasm, which indicates an increase in protein metabolism. The process of fatty and hydropic degeneration is stabilized.

When using the new blood substitute "Reomannisol" for heliotrinic intoxication, it was noted that its effective detoxification, hepatoprotective effect was accompanied by the disappearance of dystrophic, destructive and necrobiotic changes in hepatocytes. In the cytoplasm of hepatocytes, normalization of metabolism occurred, which was manifested by the restoration of lipid and carbohydrate metabolism, increased protein metabolism, as indicated by the filling of liver cells with an eosinophilic protein product, as a result of which the size of hepatocytes increased. On the part of the nuclei, an increase in metabolism was also noted, which manifested itself in the form of hypertrophy, hyperchromasia and the appearance of nucleoli.

Based on the above, we can conclude that the new domestic blood substitute "Reomannisol" can be considered the optimal means for correcting violations of biochemical and morphological parameters caused by heliotrin intoxication.

Conclusion:

1. The drug "Reomannisol" is more effective than the reference drug "Rheosorbilact", it restored biochemical parameters in experimental heliotrin intoxication;
2. "Reomannisol" has a positive effect on liver tissue at the morphostructural level, effectively correcting disorders caused by experimental heliotrin intoxication.

References:

1. [Alimova L.Kh., Gafarova S.M., Sayfullayev G.M. Disorders of energy metabolism in liver mitochondria in heliotrinous hepatitis. *Voprosy nauki i obrazovaniya*. 2018;10(22):14-15 (In Russ.)]

2. [Kalinina Ye.Yu., Yagmurov O.D. Functional morphology of proximal and distal tubules of the kidneys and hepatocytes in the modeling of acute fatal poisoning with household gas. *Uchenyye zapiski SPbGMU im. IP Pavlova*. 2012; 19(1): 39-41 (In Russ.)]
3. Larisa Ivanovna Shevchenko, Umid Saidovich Ruziev, Khamid Yakubovich Karimov, Timur Raufovich Alimov Influence of New Blood Changer on the Activity of Lipid Peroxidation Process and Antioxidant Protection Process in the Liver and in the Heart in Hemorrhagic Shock // *Journal of Exercise Physiology online* April 2019, Volume 22 Number 2 P. 71-77. https://www.asep.org/asep/asep/JEPonlineAPRIL2019_RuzievUS_Alimov.pdf . (SCOPUS)
4. Larisa Ivanovna Shevchenko, Hamid Yakubovich Karimov, Ziyoda Bohodirovna Tolipova, Timur Raufovich Alimov, Olga Viktorovna Lubencova Study of efficiency of application of a new infusion drug containing amino acid in experimental protein deprivation // *Journal of Xi'an Shiyou University, Natural Science Edition*. - VOLUME 16 ISSUE 10 P. 340-348 URL: <https://www.xisdxjxsu.asia/viewarticle.php?aid=193> <http://xisdxjxsu.asia> <https://www.xisdxjxsu.asia/V16-10-43.pdf> . (SCOPUS)
5. Sharifa Mirkhamidovna Fazilova, Khamid Yakubovich Karimov, Timur Raufovich Alimov Infusion therapy of experimental hemolytic anemia with a new domestic drug // *International Journal of Pharmaceutical Research* July - Sep 2019 | Vol 11 | Issue 3 C. 1293-1299. . (SCOPUS)
6. [Karimov H.Ya., Shevchenko L.I., Staforova E.Yu., Kuzmicheva E.L. Composition of blood substitute. Patent No. IAP 05053 dated 17.09.2012. (registration date June 17, 2015). *Rasmiy akhborotnoma*, 31.07.2015;7(171):37-37 (In Russ.)]
7. Timur Raufovich Alimov, Larisa Ivanovna Shevchenko, Meri Givievna Lipartia, Hamid Yakubovich Karimov. Study of the effect of the drug "Reomannisol" on changes in the intensity of lipid peroxidation and the state of the antioxidant system in the myocardium and liver in acute blood loss. *Journal of critical reviews (JCR)*. 2020; 7(5): 1863-1868. [doi:10.31838/jcr.07.05.314](https://doi.org/10.31838/jcr.07.05.314). (SCOPUS)
8. [Shevchenko L. I., Khazbiyevich I. S., Ageyeva G. R. Investigation of the effectiveness of succivil in endogenous intoxication // *Eksperimental'naya i klinicheskaya farmakologiya*. 2003;66(4):53-55 (In Russ.)]
9. [Srov V.N., Yusupova S.M., Pulatova L.T., Polyarush S.V., Aripova T.U., Khushbaktova Z.A., Batirov E.Kh .. Study of the relationship between the structure of flavonoids and their hepatoprotective activity in conditions of experimental heliotrin hepatotoxemia. *Innovatsii v nauke*, 2019;6(94):30-34 (In Russ.)]
10. [Denisov A. V., Cheprakova V.A., Anisin A.V., Bezrukov S.I. Ethical aspects of the use of animals in modern experimental research. *Vestnik Rossiyskoy Voenno-meditsinskoy akademii*, 2018;3:238-242 (In Russ.)]
11. [Lipatov V.A., Severinov D.A., Kryukov A.A., Saakyan A.R. Ethical and legal aspects of experimental biomedical research in vivo. Chast' II. *Rossiyskiy mediko-biologicheskiy vestnik imeni akademika I.P. Pavlova* (In Russ.)] [DOI:10.23888/PAVLOVJ2019272245-257](https://doi.org/10.23888/PAVLOVJ2019272245-257)
12. [Kamyshnikov V.S. *Clinical laboratory research methods*. Moskva «MEDpress_inform». 2011: 1-750 (In Russ.)].
13. [Korzhevsky D. et al. (Ed.). *Morphological diagnostics. Preparation of material for histological examination and electron microscopy*. – S.-Peterburg, SpeLit, 2017 (In Russ.)]

14. [Alimova L.Kh., Gafarova S.M., Sayfullayev G.M. Voprosy nauki i obrazovaniya. 2018; 10 (22): 14-15 (In Russ.)]
15. [Kalinina Ye.Yu., Yagmurov O.D. Uchenyye zapiski SPbGMU im. IP Pavlova. 2012; 19(1): 39-41 (In Russ.)]