

# The effect of «Rheoambrasol» on morphological changes in the liver and kidneys in nitrite methemoglobinemia

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**Abstract.** We propose a new drug - "«Rheoambrasol»" which includes a substance of plant origin (complex compounds of polysaccharide and natural metabolite) non-toxic, environmentally friendly and has wide functional capabilities as a means capable of correcting the consequences of methemoglobinemia, restoring structural and functional properties of cells and preserving their viability. The aim of the work is to study the effect of the new blood substitute «Rheoambrasol» on morphological changes in the liver and kidneys in nitrite methemoglobinemia. The treatment of nitrite methemoglobinemia with rheoambrosol shows complete disappearance of general pathological phenomena both in the liver and kidney tissue. The drug Rheoambrasol containing a natural metabolite of the Krebs cycle in combination with polysaccharides increases the activity of the liver metabolizing function in toxic liver damage. These positive histostructural changes in the liver tissue and in the kidney tissue indicate an increase in the activity of metabolic function of the liver parenchyma and a significant decrease in the toxemia index.

## 1 Introduction

Toxic methemoglobinemia as a separate nosology and in combination with various pathological conditions, accompanied by acute and chronic oxygen deficiency, can significantly affect the formation and development of the underlying pathological process for a long time, being the cause of ineffective therapy and increased convalescence time [1]. It was found that methemoglobinemia is 2 times stronger, compared to a simple decrease in the level of functionally active oxygen, suppresses respiratory function of the blood [2-5].

As a remedy capable of correcting the effects of methemoglobinemia, restore structural and functional properties of cells and maintain their viability we propose a new drug - "Rheoambrasol". The drug "Rheoambrasol" includes a substance of plant origin (complex

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compounds of polysaccharide and natural metabolite). This compound is non-toxic, environmentally friendly and has a wide range of functions. "Rheoambrasol" is an infusion drug of a new class that has membranoprotective, biostimulating, antihypoxant, antioxidant and detoxifying actions.

## 2 Methods

The experiments were carried out on the basis of vivarium of the interuniversity scientific laboratory (ISL) of the Tashkent Medical Academy (TMA). 100 male rats weighing 190-220 grams were involved in the experiment. The laboratory studies were carried out on the basis of "Blood Substitutes Laboratory" of the Republican Specialized Scientific-Practical Medical Center of Hematology (RSSPMCH). Toxic methemoglobinemia in rats was created by daily infusion of sodium nitrite at a dose of 50 mg/kg for 30 days [6]. The severity of the animals' condition during the experiment was determined according to the criteria proposed by Ivanitskaya N.F. (1976). On the 30th day after the reproduction of methemoglobinemia, treatment was started.

Animals were treated for 5 days with blood substitutes: the new blood substitute «Rheoambrasol» in the experimental group and the preparation «Rheopolyglukin» in the comparison group. Blood substitutes were administered at a dose of 10 ml/kg.

Animals were divided into the following groups:

Group I - before methemoglobinemia (intact) (n=15);

Group II (control) - with methemoglobinemia without treatment (on the 30th day after sodium nitrite infusion);

Group III (comparison) - with infusion of «Rheopolyglukin» on the 30th day after sodium nitrite injection on the 5th day after the start of treatment, n=12;

Group IV (main, experimental) - with infusion of «Rheoambrasol» on the 30th day after the infusion of sodium nitrite – on the 5th day after the start of treatment, n=14.

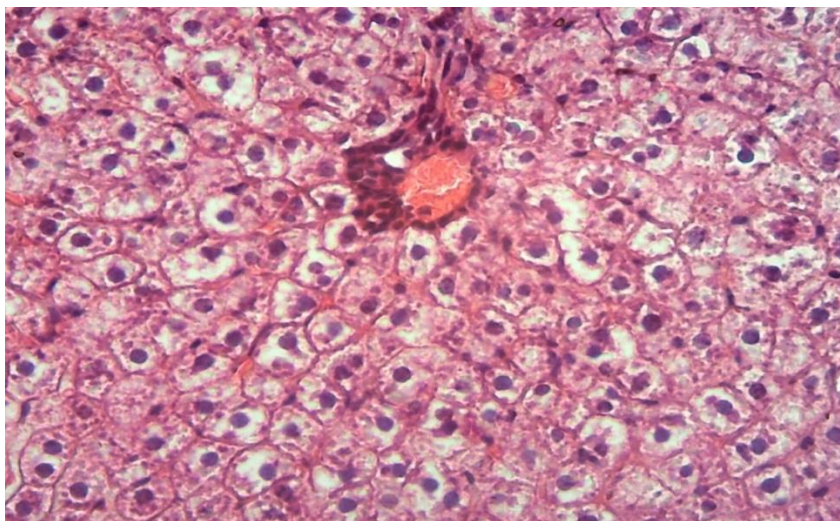
Histostructural features of the liver and kidneys during methemoglobinemia were investigated in the course of staging the methemoglobinemia model.

The liver tissue was sampled for histomorphological analysis. Pieces of liver tissue were fixed in 10% solution of neutral formalin, incubated for 2 days and then washed in running water, then dehydrated in alcohol with increasing concentration from 70% to 100% and in chloroform, followed by pouring of paraffin blocks of prepared tissues. The blocks were cut on a sledge microtome. The obtained histological sections were stained with Hematoxylin-eosin. Microscopy of finished preparations was performed with a «Leica» microscope under x10, x20 and x40 objectives [7-15].

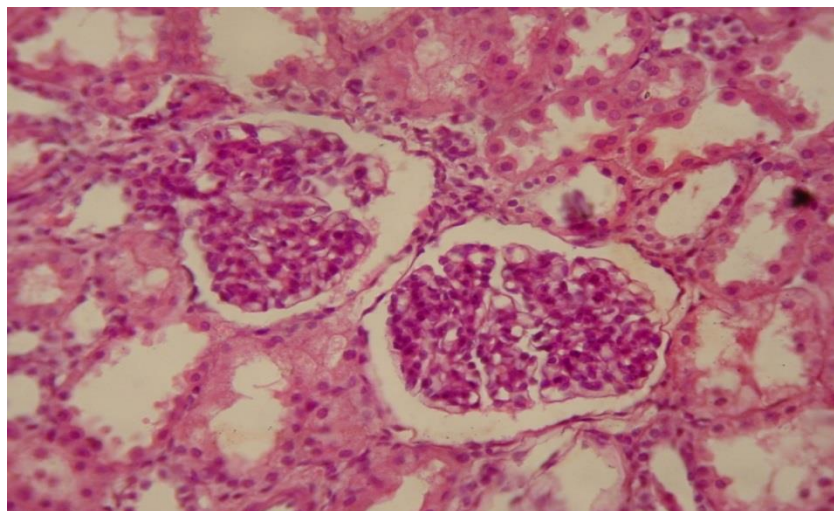
## 3 Results

The results of morphological study in toxic methemoglobinemia showed that the liver tissue showed the development of marked dyscirculatory, dystrophic and inflammatory changes. At the same time, the vessels of portal tracts and central veins are dilated and full of blood, in some places with diapedesis hemorrhages in liver parenchyma. Small lymphoid infiltration was detected in portal tracts. Severe dystrophic were noted in hepatocytes of all morphofunctional zones of liver lobules (Fig. 1). At the same time, hepatic cells were swollen due to large-volume vacuolar dystrophy, it was noted that some hepatocytes were transformed into balloon cells. Hepatocyte nuclei were located in the center of cells with signs of karyopycnosis and karyorexis. The renal tissue showed predominant edematous changes in the interstitium and tubule lumen. The tubules also increased in size due to loosening of the capillary network and edema of the lumen of Baumann-Schumlansky capsule. Due to edematous phenomena the external capsule of glomerules and interstitial

tissue of cortical layer are edematous with loosening of fibrous structures. Capillary network is multicellular with expansion of individual capillaries. Proximal and distal convoluted tubules are dilated, the lumen contains clumps of eosinophilic proteinaceous mass. The epithelium of the tubules is in a state of vacuolar and hyaline-drop protein dystrophy (Fig. 2).



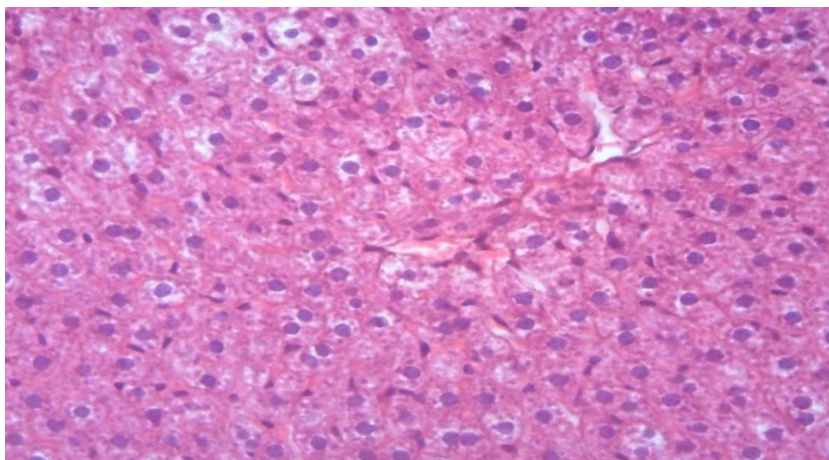
**Fig. 1.** Toxic methemoglobinemia. Severe vacuole dystrophy of hepatocytes. Staining: G-E. Uv: 10x40.



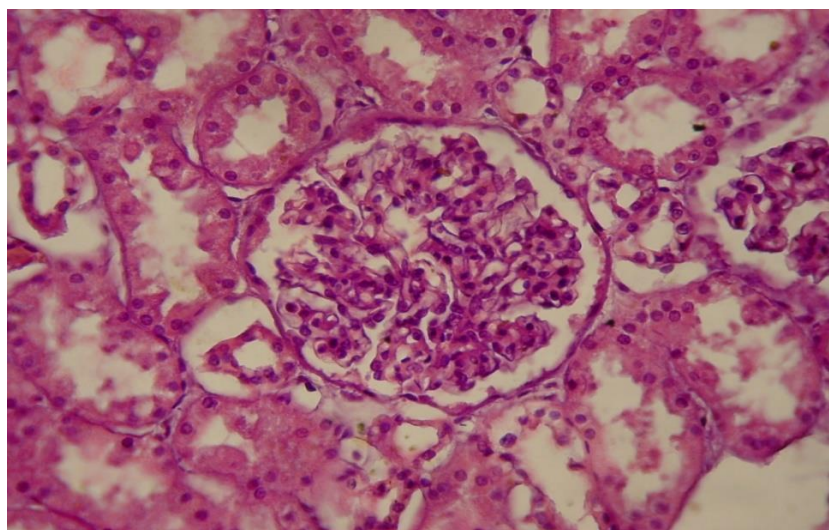
**Fig. 2.** Toxic methemoglobinemia. Interstitial edema, dilation of the lumen of the kidney tubules. Staining: G-E. Uv: 10x40.

The results of morphological study after «Rheopolyglukin» administration in toxic uterohemoglobinemia showed that in the liver tissue the phenomena of discirculatory, dystrophic and destructive processes are somewhat stabilized. No discirculatory changes were detected in the liver tissue vessels, only slight dilation of central veins was noted.

Liver cells remained swollen due to increased protein metabolism in the form of appearance of fine granularity in cytoplasm of hepatocytes (Fig. 3) with eosinophilic stained granules. Only in some hepatocytes vacuole dystrophy remained. Hepatocyte nucleus was rounded, located in the center of hepatocytes, in some of them perinuclear vacuolization of cytoplasm was detected. Moderate hypertrophy of Kupffer cells is noted.



**Fig. 3.** Infusion of «Rheopolyglukin». Stabilization of dyscirculatory and dystrophic changes in liver tissue. Staining: G-E. Uv: 10x40.



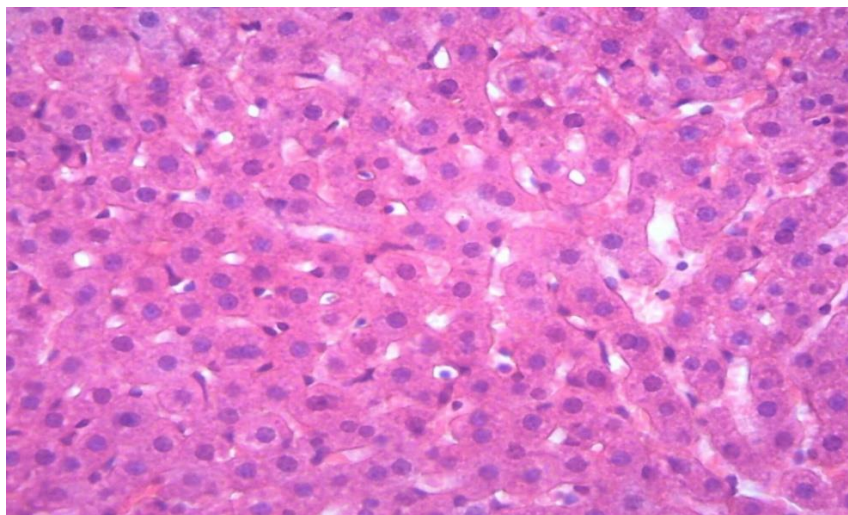
**Fig. 4.** Infusion of «Rheopolyglukin». Preservation of small interstitial edema. Staining: G-E. Uv: 10x40.

The renal tissue showed some stabilization of dyscirculation and dystrophy processes. The tubules were rounded without capillary dilation, only slight edema of Baumann-Schumlansky capsule lumen was noted. Proximal and distal convoluted tubules were moderately dilated in the lumen, the epithelia were prismatic with uniform Eosin staining of

the cytoplasm (Fig. 4), the nuclei were rounded and located on the basal part of the epithelium.

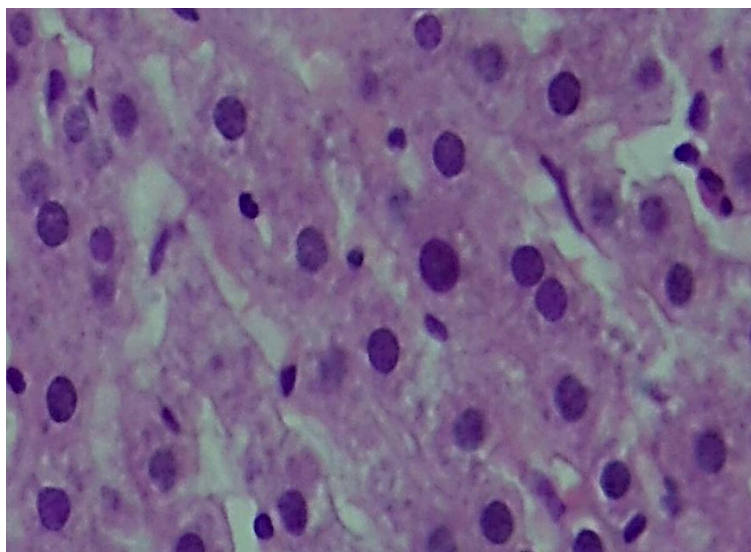
The drug «Rheoambrasol», containing a natural metabolite of the Krebs cycle in combination with polysaccharides, increases the low activity of pharmaco-metabolizing liver function in toxic liver damage. The new blood substitute «Rheoambrasol» significantly reduces the level of middle weight molecules and oligopeptides in plasma and erythrocytes. «Rheoambrasol» significantly reduces high indexes of toxemia in the liver.

The results of morphological study after reambrasol treatment of toxic methemoglobinemia showed that in the liver tissue almost completely stabilized manifestations of dyscirculatory, dystrophic and inflammatory changes. Only a slight enlargement of sinusoids and «Disse space» with appearance of single lymphocytes in their lumen was noted. Hepatocytes were arranged in beams, preserved their histotopography and relations with surrounding cells. Their cytoplasm is somewhat enlarged due to an increased number of eosinophilic fine-grained granules (Fig. 5).



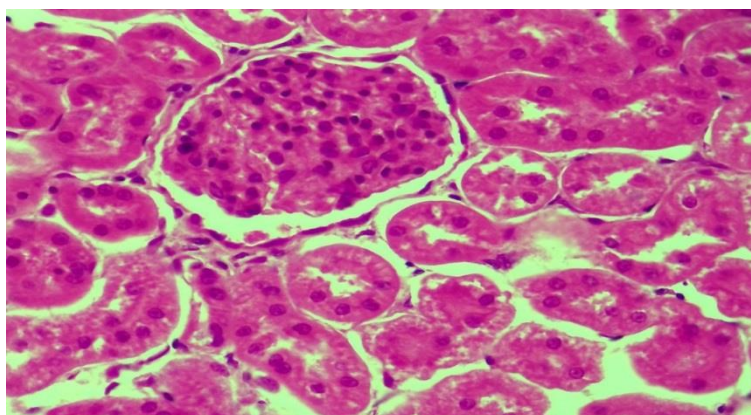
**Fig. 5.** Treatment with «Rheoambrasol» for toxic methemoglobinemia. Complete stabilization of all cellular and tissue structures of the liver. Staining: G-E. Uv: 10x40.

The nuclei are rounded, somewhat hyperchromic, located in the center of the cell, binuclear hepatocytes are found. Sinusoids are well defined, the wall is represented by a thin layer of endothelial cells with the presence of hypertrophied Kupffer cells. The mentioned positive histostructural changes in the liver tissue testify to the increased activity of metabolic function of the liver parenchyma and significantly decreased toxemia index. Under high magnification of microscope one can detect enrichment of hepatocyte cytoplasm with eosinophilic granules without vacuolization of matrix (Fig. 6). Hepatocyte nuclei were hypertrophied and hyperchromatous, binuclear cells were found.

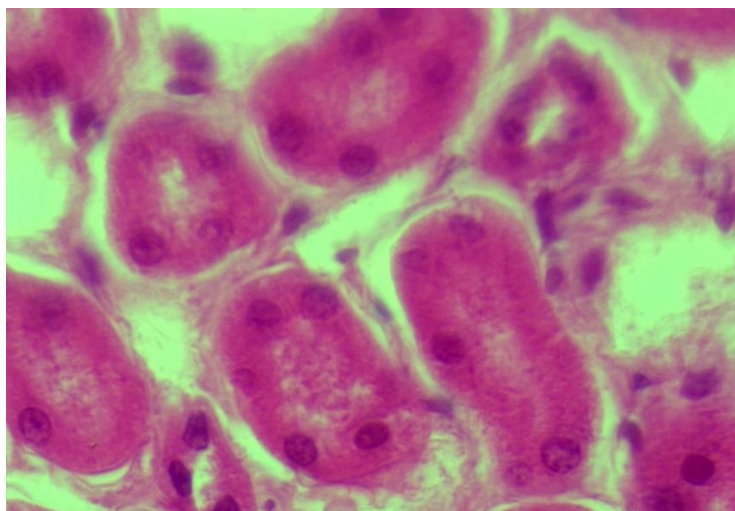


**Fig. 6.** «Rheoambrosol» treatment of toxic methemoglobinemia. Hepatocyte cytoplasm stained uniformly with eosin, nuclei hypertrophied. Staining: G-E. Uv: 10x100.

Stabilization of dyscirculatory, dystrophic phenomena with normalization of histotopography of tissue elements is also noted in renal tissue. The tubules were slightly reduced in size due to disappearance of edema. Capillary network was dense with proliferative activity of cellular elements. Crooked proximal and distal tubules without lumen edema, their epithelium swollen with increased eosinophilic staining of cytoplasm (Fig. 7). The epithelial nucleus is rounded and located in the center of the cell. When studying kidney tissue with a large microscope lens, intensive staining of cytoplasm of epithelium of convoluted tubules, preservation of histotopography of nuclear structures (Fig. 8). Interstitial tissue of the cortical layer of the kidney is moderately edematous, connective tissue structures retain their histostructures, fibrocyte nuclei are somewhat hypertrophied.



**Fig. 7.** Treatment with the drug «Rheoambrosol» toxic methemoglobinemia. Disappearance of dyscirurgical and dystrophic changes, increased staining of cellular elements of kidney tissue. Staining: G-E. Uv: 10x40.



**Fig. 8.** Treatment with «Rheoambrosol» for toxic methemoglobinemia. Increased eosinophilic cytoplasm of hepatocytes, moderate hypertrophy of stromal cells. Staining: G-E. Uv: 10x100.

At nitrite methemoglobinemia in the liver and kidneys develop discirculatory, dystrophic and destructive changes with lesions of both parenchymatous and stromal tissue structures. When «Rheopolyglukin» is administered, some stabilization of the above-mentioned general pathological changes is noted, but small interstitial edema and focal dystrophic changes of parenchymatous elements both in the liver and kidneys remain.

## 4 Conclusions

At nitrite methemoglobinemia in the liver and kidneys develop discirculatory, dystrophic and destructive changes with lesions of both parenchymatous and stromal tissue structures.

Application of the drug «Rheoambrosol» in nitrite methemoglobinemia promotes restoration of histostructural changes in the tissues of the liver and kidney, approaching the norm.

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