

# In the Dynamics of the Development of Hemolytic Deficiency Industried with Phenyhydrazine of the new Product “Reomannisol”

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## Abstract

The aim of the study was to evaluate the effect of local blood substitute rheomannisol on some metabolic, functional and structural parameters of the organism in the dynamics of development of experimental hemolytic anemia caused by phenyl hydrazine. **Objects & Methods of Research:** The object of research was white rats (n=120) in vivarian conditions of the tashkent medical academy. The study used experimental, biochemical and statistical methods. The practical results of the study are as follows: Data have been obtained that allow a deeper understanding of the formation of pathogenetic developmental mechanisms of hemolytic anemia. It is based on the use of a new blood-replacing drug Reomannisol to correct the degenerative mechanisms in hemolytic anemia, which has reduced the material costs and improved the quality of life in the treatment of patients and has been proven in practice.

**Keywords:** Hemolytic anemia; Experiment; Rats; Phenyhydrazine; Lipid Peroxide Oxidation (LPO)/Antioxidant system (AOT); Reomannisol

## Introduction

Currently, the main interest of scientists is to study the mechanism of development and treatment of hemolytic anemia, which accounts for 5.3% of all diseases of the circulatory system and 11.5% of cases of anemia in the world. <sup>[1,2]</sup> However, the study of the mechanisms of erythrocyte degradation, which provide natural tolerance in hemolytic anemia, as well as lead to hemolytic processes as a result of disruption of these mechanisms, is becoming more relevant. <sup>[3,4]</sup> In experimental hemolytic anemias, the identification of the mechanism of breakdown of erythrocytes associated with iron imbalance, antioxidant protection, lipid peroxidation and the development of endogenous intoxication syndrome allows to develop new ways to correct them.

The development of the medical sector of the country has a number of tasks aimed at adapting the medical system to world standards, diagnosis, prevention and treatment of various somatic diseases. Through the introduction, creation of a patronage service, support of a healthy lifestyle and prevention of diseases and effective diagnosis. <sup>[5]</sup> These tasks will reduce disability and mortality as a result of improving the use of modern technologies in the development of preventive measures to increase the effectiveness of local drugs in the treatment of hemolytic anemia in the population.

## Purpose of the research

The aim of the study was to improve the assessment of the effect of local blood substitute reamannisol on certain metabolic, functional and structural parameters of the body's vital activity in the development of the dynamics of experimental hemolytic

anemia caused by phenylhydrazine.

## Materials and Methods

The object of research was white rats (n=120) in vivarian conditions of the tashkent medical academy. Studies on the modulation of hemolytic anemia are devoted to design and methods. An experimental model of hemolytic anemia was created by administering a single dose of 50 mg/kg phenylhydrazine into the abdominal cavity. All experiments on animals were carried out in accordance with the international recommendations (code of ethics) for conducting medical and biological research using animals, developed by the council of international scientific organizations in 1985 (CIOMS, Geneva, 1985).

According to the purpose of the study, the studied animals (n=120) were divided into groups by random selection on day 1 of the experiment: Group I (n=10) healthy animals; Group II (n=35) with an experimental model of hemolytic anemia; Group III (n=25) after the introduction of saline (sodium chloride solution 0.9%) in the experimental model of hemolytic anemia; Group IV (n=25) after the introduction of the drug “Reosorbilakt” in animals with an experimental model of hemolytic anemia; Group V (n=25) after the introduction of a

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new locally produced blood substitute drug "Reomannisol" in animals with an experimental model of hemolytic anemia.

After administration of phenylhydrazine for 5 days, sodium chloride 0.9%, reosorbilakt, reomannisol were administered at a dose of 10 mg/kg body weight. In rats, studies performed decapitation of animals under mild ether anesthesia 1-5 days after phenylhydrazine administration. Liver and spleen of animals in all groups were morphologically examined and serum was used for biochemical studies.

Statistical processing of the results of the study was carried out using the application package of the personal computer "Statistica for Windows 7.0".

## Results and Discussion

The theoretical significance of the study is that the experiment showed additional pathogenetic mechanisms of development of hemolytic anemia, as well as explained the relationship between hematological parameters of blood with changes in myelogram, iron and bilirubin metabolism, LPO/AOT and endogenous intoxication.

The practical significance of the study is based on the high therapeutic efficacy in the correction of complex disorders identified in hemolytic anemia based on experimental research, which in turn allows to prevent the development of complications in patients with this disease, as well as improve quality of life.

The general condition of rats with phenyl hydrazine-induced hemolytic anemia, hematological parameters of blood and bone marrow, morphological changes in the liver and spleen, indicators of iron and bilirubin metabolism, results of LPO/AOT and EU systems were covered. Studies have shown that hemolytic anemia, intoxication, and increased hemolysis of red blood cells caused by phenyl hydrazine lead to a worsening of the general condition of the animal (100.0%) to its death (20.0%).

Days 1, 2, and 5 of the experiment showed the development of circulatory, dystrophic, and destructive changes in the morphological study of structural components of the liver and spleen in rats with phenyl hydrazine hemolytic anemia. In addition, fatty dystrophy and sinusoids with pigmentation and extreme dullary hematopoiesis in the disse cavity have been reported to develop due to the accumulation of lipofucin and bilirubin in the cytoplasm due to proliferative processes in the liver by Kupfer cells. Destructive processes in the spleen were manifested by hemolysis of red blood cells in the sinus cavity and pulp, hyperpigmentation, and increased phagocytic activity of macrophages. Macrophage activity was observed in the white pulp of the spleen, especially in the germinative centers, expressed in the form of the formation of rosettes of macrophages with lymphoid cells [Figure 1].

The experiment revealed changes in hematologic parameters on the hemo gram and myelo gram, along with morphological

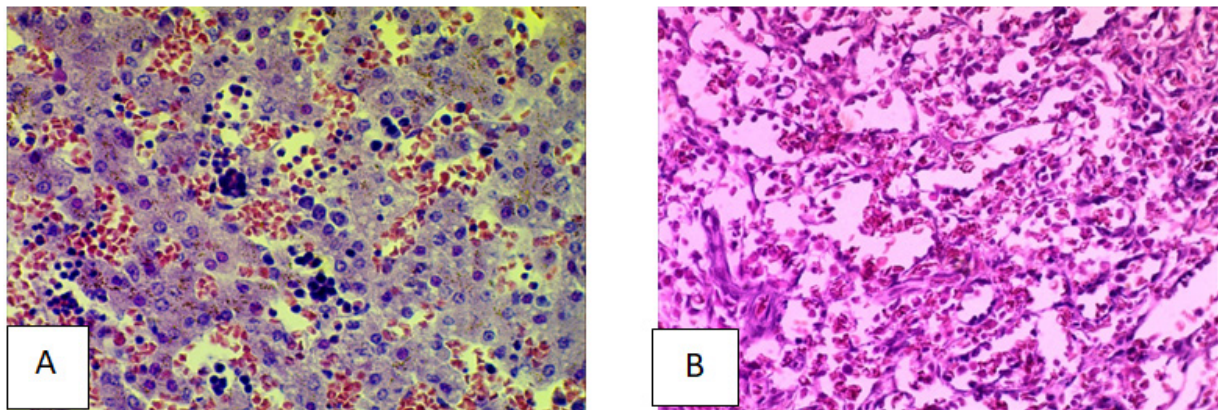


Figure 1: Morphological changes of the liver (A) and spleen (B) in phenyl hydrazine hemolytic anemia in rats.

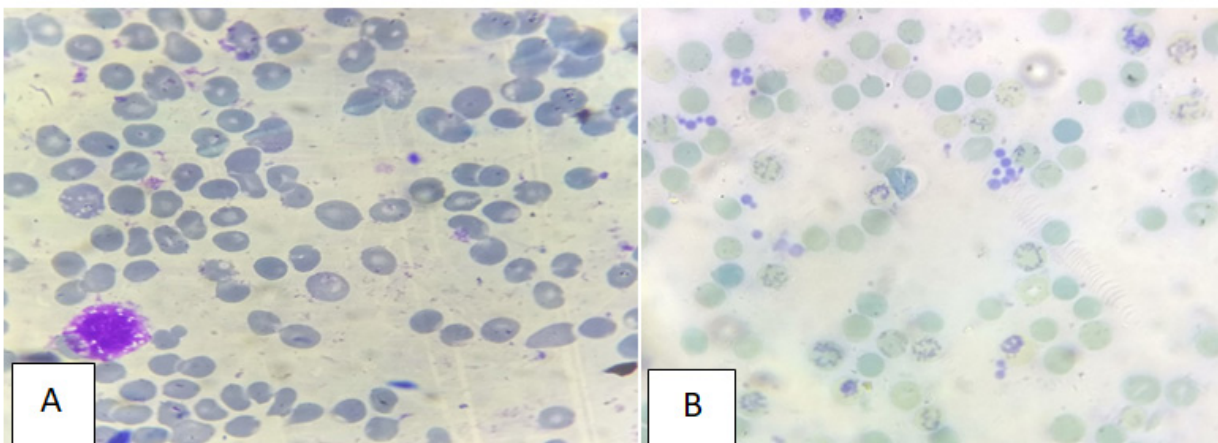


Figure 2: Morphological changes of the liver (A) and spleen (B) in phenyl hydrazine hemolytic anemia in rats.

signs of hemolytic anemia that occurred after administration of phenyl hydrazine to the animals. In particular, on day 5 of the experiment, hemolytic anemia caused by phenylhydrazine in rats with a healthy group ( $Hb 123.0 \pm 7.4$  g/l; erythrocytes  $3.6 \pm 0.16 \times 10^{12}/l$ ; erythrocyte sedimentation rate  $-5.0 \pm$  reached a peak of  $0.68$  mm/h), a significant 1.8 fold decrease in hemoglobin level ( $69.5 \pm 2.8$  g/l;  $r1 < 0.001$ ), a 2.18 fold decrease in the number of red blood cells in the blood ( $1, 65 \pm 0.09 \times 10^{12}/l$ ;  $r1 < 0.001$ ) decrease, ECG acceleration by 3.6 times ( $18.0 \pm 0.87$  mm/ch;  $r1 < 0.001$ ), anisocytosis and poikilocytosis [Figure 2].

On the fifth day, when analyzing the bone marrow erythroid cell series of rats with henoly hydrazine hemolytic anemia, a 2.7 fold increase in erythroblast levels ( $2.96\% \pm 0.04\%$ ) was observed. However, the composition of pronormocytes ( $5.97\% \pm 0.047\%$ ), basophils ( $7.97\% \pm 0.09\%$ ), polychromatophiles ( $18.83\% \pm 0.06\%$ ) and oxyphilic normocytes ( $14.66\% \pm 0.04\%$ ) was healthy. statistically 3.98 ( $1.5\% \pm 0.04\%$ ) compared to the group; 2.28 ( $3.5\% \pm 0.11\%$ ); 1.9 ( $9.6\% \pm 0.08\%$ ) and 3.96 ( $3.7\% \pm 0.07\%$ ) increases were observed. [6-11]

Thus, the identified changes in the morphological structures of the liver and spleen, as well as in the hematological parameters of the blood and bone marrow, prove their involvement in the pathological process in hemolytic anemia. In addition, the above changes in GC in experimental animals were accompanied by disturbances in iron and bilirubin metabolism, and its severity increased until the fifth day of the study. For example, on days 1, 2 and 5, the amount of iron in the serum was 2.83 times ( $23.8 \pm 2.20$   $\mu\text{mol}/l$ ), 4.44 times ( $37.3 \pm 2.3$   $\mu\text{mol}/l$ ) and 6.43 times ( $54 \pm 2.6$   $\mu\text{mol}/l$ ), which increased the amount of ferritin by 6.5 times ( $16.0 \pm 1.4$  ng/ml) and 6.97 times ( $18.1 \pm 0.9$  ng/ml, respectively) and 11.7 times ( $30.3 \pm 2.3$  ng/ml), as well as common rail connecting system 2.4 times ( $190.70 \pm 26.8$   $\mu\text{mol}/l$ ), 2.5 times ( $198.1 \pm 23.0$   $\mu\text{mol}/l$ ) and 2.6 times ( $203.6 \pm 24.8$   $\mu\text{mol}/l$ ) on the first, second and fifth days of the experiment compared to those in healthy animals ( $8.4 \pm 0.50$  mmol/l,  $2.6 \pm 0.5$  ng/ml

and  $79.7 \pm 5.6$  mmol/l). However, changes in iron metabolism in animals with GK were accompanied by a significant increase in total bilirubin levels due to its unbound fraction. Thus, on day 1, the level of total bilirubin and its unbound fraction in group II animals increased to 3.6 ( $21.2 \pm 0.77$  mmol/l) and 3.61 times ( $15.9 \pm 0.70$  mmol/l), 6.05 ( $35.7 \pm 1.4$   $\mu\text{mol}/l$ ) and 5.27 times ( $23.2 \pm 1.04$   $\mu\text{mol}/l$ ) on day 2 and 16.76 ( $98.9 \pm 3.23$ ) on day 5.  $\mu\text{mol}/l$ ) and 14.61 ( $64.3 \pm 2.0$   $\mu\text{mol}/l$ ) compared to healthy animals (total bilirubin  $5.9 \pm 0.20$   $\mu\text{mol}/l$  and unbound bilirubin  $4.40 \pm 0.15$   $\mu\text{mol}/l$ ) increased [Figure 3].

Thus, intra-abdominal administration of a 2% solution of phenyl hydrazine at a dose of 50 mg/kg body weight is associated with the development of hemolytic anemia in rats, increased erythrocyte hemolysis and impaired iron and bilirubin metabolism, which on the fifth day ( $r=0.61$  and  $r=0.59$ , respectively), ferritin ( $r=0.46$  and  $r=0.65$ , respectively) and total bilirubin ( $r=0.48$  and  $r=-0.88$ , respectively). A study of the status of the LPO/AOT system of phenylhydrazine GK showed a disproportionate development in terms of its performance, which also led to maximal changes on day 5 of the study. In particular, this is due to a decrease in the functional activity of AOT, which is 2.25 times the amount of Superoxide Dismutase (SOD) in plasma ( $2.80 \pm 0.10$  units/mg protein unit;  $p1 < 0.001$ ), Glutathione Peroxidase (GPO) 4, 44 times ( $0.09 \pm 0.004$  condition. Unit/min  $\times$  mg Hb;  $p1 < 0.001$ ), Glutathione Reductase (GR) level 5.2 times ( $0.50 \pm 0.03$   $\mu\text{M}$  NADFN2/min  $\times$  g/Hb;  $r1 < 0.001$ ), catalase activity 2.48 times ( $14.3 \pm 1.2$  ng/mg Hb  $\times$  min;  $p1 < 0.001$ ), the concentration of Malonic Dialdehyde (MDA) against the background of activation of peroxide processes almost 2 times ( $5.10 \pm 0.21$  nmol/ml pl), the levels of diene conjugates and diene ketones were 2.64 times ( $3.70 \pm 0.24$  ratio) and 2.47 times ( $0.37 \pm 0.025$  ratio), LPO/AOT increased by a ratio of 5.57 times ( $0.518 \pm 0.025$ ).

Changes in this system are associated with a decrease in the number of red blood cells, in particular, on the fifth day of the

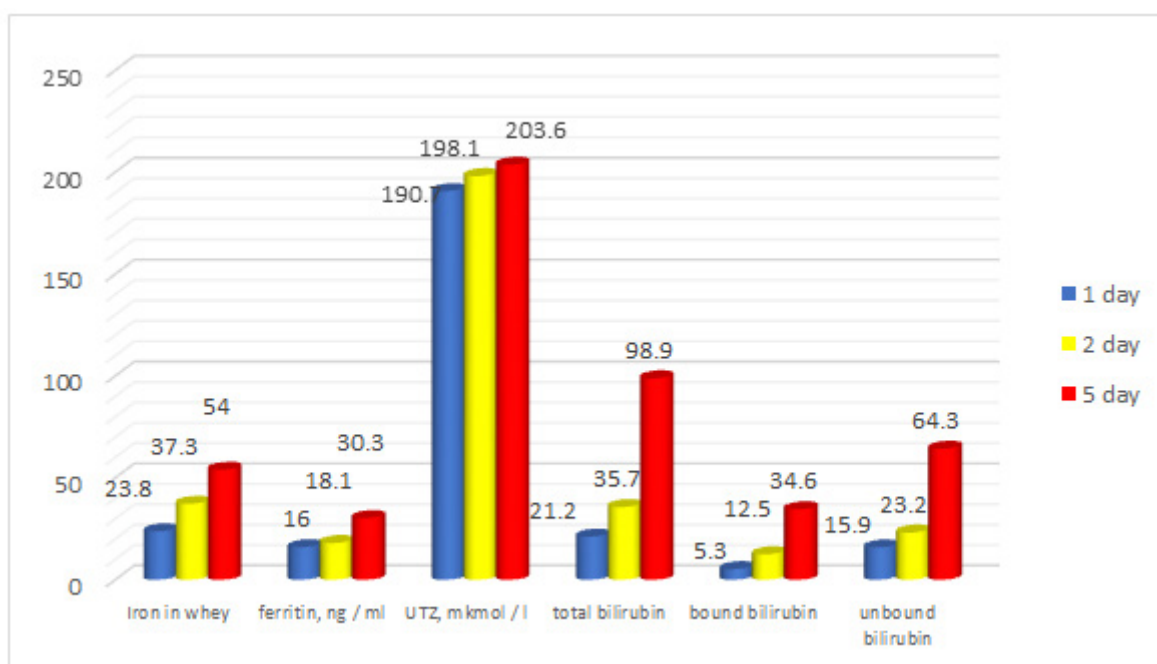


Figure 3: Dynamics of iron and bilirubin metabolism in rats with experimental hemolytic anemia.

**Table 1: Status of endogenous intoxication (M ± m) in hemolytic anemia (phenylhydrazine intoxication) in research groups in rats.**

| The indicators studied              | Group I (healthy) | Group II (GK)   |                  |                 |
|-------------------------------------|-------------------|-----------------|------------------|-----------------|
|                                     |                   | 1-day           | 2-day            | 5-day           |
| <b>In plasma</b>                    |                   |                 |                  |                 |
| Medium molecule                     | 10,6 ± 0,76       | 13,6 ± 0,81*    | 16,04 ± 0,7***   | 19,9 ± 0,8 ***  |
| Concentration of oligopeptides, g/l | 0,7 ± 0,04        | 0,91 ± 0,07 **  | 1,28 ± 0,13 ***  | 1,30 ± 0,08 *** |
| Toxemia index                       | 7,43 ± 0,6        | 12,3 ± 0,6 ***  | 20,53 ± 1,55 *** | 25,9 ± 1,3 ***  |
| Intoxication index                  | 20,5 ± 1,2        | 32,8 ± 2,02 *** | 57,5 ± 2,2 ***   | 54,9 ± 3,4 ***  |
| <b>In erythrocytes</b>              |                   |                 |                  |                 |
| Medium molecule                     | 13,9 ± 1,2        | 17,06 ± 0,9 *   | 18,6 ± 1,0 **    | 20,43 ± 0,9 *** |
| Concentration of oligopeptides, g/l | 0,94 ± 0,06       | 1,2 ± 0,08 *    | 1,9 ± 0,16 **    | 1,42 ± 0,07 *** |
| Toxemia index                       | 13,06 ± 0,76      | 20,5 ± 1,2***   | 36,9 ± 1,09 ***  | 29,01 ± 1,8 *** |
| Average volume of erythrocytes, %   | 20,6 ± 1,1        | 18,8 ± 0,9      | 23,76 ± 1,13 *   | 24,7 ± 1,28 *** |

study, catalase, GR, GPO and SOD ( $r=0.45$ ;  $r=0.57$ ;  $r=0.54$  and  $r=0.34$ , respectively). and an increase in MDA levels, a correlation was observed between dien conjugates ( $r=0.62$ ;  $r=0.55$ , respectively) and a decrease in red blood cell levels. At the same time, an increase in lipid peroxidation processes and a decrease in the activity of AOT enzymes indicated a severe condition in animals with hemolytic anemia. By the 5th day of the experiment, the endogenous index in animals with hemolytic anemia was  $19.89 \pm 0.85$  TL, the average molecular weight of oligopeptides in plasma was  $1.30 \pm 0.08$  g/l, and the index of toxemia in plasma was 25, Reached  $86 \pm 1.35$  and increased its value in healthy animals. Also, after experimental hemolytic anemia in the second group, the content of medium-molecular erythrocytes in erythrocytes relative to the corresponding values of endogenous intoxication in group I was  $20.43 \pm 0.92$  units, oligopeptides in erythrocytes -1 to  $1.42 \pm 0.07$  g/l, index toxemia in erythrocytes increased to  $-29.01 \pm 1.77$ , the Average Volume of Erythrocytes (AVE)  $-14.69 \pm 0.84\%$ , Index Intoxication (II) increased to  $-54.87 \pm 3.41$  [Table 1].

Thus, the development of a pathological process in hemolytic anemia creates patho genetic conditions for the emergence of Endogenous Intoxication (EI) syndrome, which is an increase in the average molecule in plasma, the concentration of oligopeptides in plasma and oligopeptides in erythrocytes: erythrocytes ( $r=0.52$ ;  $r=0.92$ ) and  $r=0.92$ , respectively), a decrease in hemoglobin ( $r=0.58$ ;  $r=0.85$ ;  $r=0.83$  and  $r=0.92$ , respectively) and an increase in reticulocyte levels ( $r=0.64$ );  $r=0.73$ ; and  $r=0.75$ , respectively).

It is aimed at a comprehensive study of the main patho genetic mechanisms of the formation of pathological processes that play an important role in the development of hemolytic anemia. However, the mechanism of red blood cell breakdown in hemolytic anemia is extremely complex, involving many pathological processes such as LPO/AOT systems, iron, bilirubin metabolism, and EI that directly affect the morphological structure of the liver and spleen.

## Conclusion

Based on the research on the fifth day of hemolytic anemia in experimental conditions, Defects detected in the antioxidant enzyme system are probably associated with a sharp activation of free radical processes resulting from mass hemolysis

of erythrocytes. On the fifth day of the experiment, the development of circulatory and dystrophic changes in the liver and spleen parenchyma was clearly noted in rats with hemolytic anemia. Reomannisol has a more effective effect on the activity of antioxidant enzymes than rheosorbilact, which probably increases the level of antioxidant properties due to the synergism of the properties of mannitol and succinate. The combination of antioxidants is a multifunctional inhibitor of free radical lipid peroxidation and is an integral part of it, so that the stabilization of free radical processes leads to an increase in the resistance of erythrocyte membranes and a decrease in their hemolysis. Limiting the effectiveness of lipid peroxidation processes of biological cell membranes leads to an increase in their resistance to harmful actions. It is this factor that leads to a more effective normalization of circulatory and dystrophic changes in the studied organs under the influence of rheomannisol.

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