

**EFFECT OF HYD DRUGS ON HEMOLYTIC ANEMIA INDUCED
BY PHENYLHYDRAZINE HYDROCHLORIDE**Zayseva O.A.¹Makhsumov Sh.M.²Djanaev G.Yu.^{3,4}Mamadaliev Sh.M.⁵¹Associate Professor of Pharmacology Department of Tashkent Medical Academy²Associate Professor of Pharmacology Department of Tashkent Medical Academy³Assistant of the Department of Medical Biological Sciences of KIUT⁴PhD student of the Tashkent Medical Academy⁵Assistant of the Department of Pharmacology, Clinical Pharmacology and Medical Biotechnology of Andijan Medical Institute<https://doi.org/10.5281/zenodo.8368906>

Abstract. In conclusion, the conducted experiments showed that the histostructure of the vital internal organs of rats in the control and experimental groups was almost the same. At the same time, drug-related histomorphological changes in their histostructure were not noted. It should be noted separately that the study of the histostructure of the liver and kidney from the parenchymatous organs of the animals that received the studied tincture showed that some morphological signs indicating their functional activity appeared. Therefore, long-term administration of the antianemic compound - DOY tincture to the body does not cause chronic poisoning in animals and any negative reactions in the histostructure of vital internal organs and biochemical analyzes of peripheral blood.

Key words: *collection of medicinal plants, anemia, experimental animals, blood elements, hemolytic deficiency*

Introduction. Hemolytic anemia (Latin anaemia haemolytica from other Greek αίμα "blood" + λύσις "destruction, dissolution" + anemia) is a group name for rare diseases, a common feature of which is increased destruction of red blood cells, causing, on the one hand, anemia and increased the formation of erythrocyte decay products, on the other hand - reactively enhanced erythropoiesis. Anemia caused by iron and vitamin B12 deficiency is quite common. Anemia is also often observed in the case of long-term bleeding, piles, or stomach and duodenal ulcers. Anemia caused by iron deficiency is also common in women with long and heavy periods. Anemia associated with iron deficiency is caused by frequent pregnancy and long-term breastfeeding, because during pregnancy and breastfeeding, part of the iron reserve in the mother's body is transferred to the child[1,2,3,4,5,6]. Anemia observed in young children occurs due to improper feeding, as well as lack of food. A decrease in the amount of hemoglobin in the blood, with a slightly reduced or normal number of erythrocytes, is the main symptom of iron deficiency A. The patient is discolored, often complains of fatigue, headache, dizziness, blurred vision, hair loss, and brittle nails. Sometimes it becomes difficult to swallow, the patient craves things that are not usually eaten (chalk, lime, gilvata, etc.), likes spicy, salty foods. In the prevention and treatment of anemia related to iron deficiency, it is necessary to identify and eliminate possible sources of blood loss in time, achieve a certain level of planning of pregnancy and childbirth, and follow a balanced diet[7,8,9,10].

Anemia caused by vitamin B12 or folic acid deficiency is much rarer. This type of anemia has specific symptoms: sore tongue, signs of damage to the nervous system (funicular myelosis) are observed when the disease is missed. In order to prevent this kind of Anemia, it is very important to timely identify and treat chronic diseases of the gastrointestinal tract, especially those with diarrhea. It is necessary to take measures to prevent damage from them in the places where worms are spread, and when the disease appears, it is necessary to treat it in time[11,12,13,14,15]. There are many types of hemolytic anemia associated with extensive destruction of erythrocytes. They can be hereditary or acquired, and are usually characterized by yellowing of the skin and mucous membranes, and a decrease in the number of erythrocytes and hemoglobin. In all kinds of Anemia, it is necessary to consult a doctor and get proper treatment in time. Iron deficiency is one of the main causes of the symptoms and syndromes of anemia and anemic conditions. For this purpose, in the following years, a number of iron-sparing preparations were produced and applied to medical practice. But for the effective treatment of TTC, it is very necessary to have microelements of biological nature, which are involved in the formation of blood, along with the complex of BAMs. For this purpose, it is not appropriate to use any plants with antianemic effect separately, because the required polyvalent pharmacological effect can be achieved only on the basis of a combination of plants. Therefore, *Matricaria chamomilla* L. *Achillea millefolium* L., *Polygonum Hydropiper* L., *Polygonum aviculare* L., *Radix Glycyrrhizae* are medicinal plants with polyvalent pharmacological effects and their application in medical practice is of great importance[16,17,18,19,20,21,22].

Materials and research methods. The effect of DOY on the solution of anemia induced by phenylhydrazine hydrochloride was studied in a separate experiment in 35 laboratory rats weighing 165-200 g. For this purpose, hemolytic anemia was induced by injecting phenylhydrazine hydrochloride subcutaneously to experimental rats in doses of 25 ml/kg once a day for 5 days.

Results. Peripheral blood analysis performed on the 6th day of the experiment showed a sharp decrease in the amount of hemoglobin and erythrocytes in rats. In particular, the amount of hemoglobin in the peripheral blood of experimental rats is up to 3.8 g%, and the amount of erythrocytes is 4.4 million. decreased to Neutrophil leukocytosis, paikilocytosis, hypochromia and anisatocytosis were noted in the blood.

The general condition of the experimental animals was significantly worse after the administration of phenylhydrazine hydrochloride, and their appearance was completely different from that of the rats in the control group. Their hair grew back, signs of inflammation around the nose and eyes were noted. The appetite of animals for food and water is greatly reduced.

Blood tests were carried out on the 15th and 30th days of the experiment, before the injection of phenylhydrazine hydrochloride and after the anemia was induced by the injection of phenylhydrazine hydrochloride, during the period of treatment with the study drugs. The main attention was paid to the general condition of the rats, the amount of hemoglobin and erythrocytes in the peripheral blood, the morphology of erythrocytes and the amount of leukocytes. The obtained results are presented in table No. 11 below.

In control rats, hemolytic anemia induced by phenylhydrazine hydrochloride was very severe. Changes in the morphology of red and white blood cells were seen as in anemia brought on by repeated bloodletting. A high degree of neutrophilic leukocytosis with left

absorption was noted in white blood cells. By the end of the experiment, 43% of the animals (3 out of 7 died) died, and the peripheral blood elements of the remaining animals could not reach their state.

Under the same conditions, the amount of hemoglobin in the peripheral blood of the animals that received the study tincture of DOY at a dose of 5 ml/kg increased by 0.9 g% on the 15th day of the experiment, the amount of erythrocytes increased by 0.3 million. rose to In animals that received the drug at a dose of 10 ml/kg, the amount of hemoglobin and erythrocytes accelerated and increased by 1.3 g% and 0.45 million, respectively.

Changes in the morphology of erythrocytes and leukocytes were significantly reduced. Leukoformula approached the physiological norm.

The amount of hemoglobin on the 30th day of the experiment was 2.3 g, compared to the results on the 15th day of the experiment. % and 3.4 g. %, the number of erythrocytes increased to 0.85 million and 1 million, the morphology of erythrocytes returned to normal, and leukocytes were around the physiological norm.

Comparative drug coamid in doses of 3 mg/kg of hemolytic anemia caused by phenylhydrazine increased the amount of hemoglobin in peripheral blood by 3.5% and the amount of erythrocytes to normal level on the 15th day of the experiment.

It should be noted that 2 and 1 rats died by the end of the experiment in the groups receiving DOY tincture at doses of 5 ml/kg and 10 ml/kg, respectively. No deaths were reported in the control group of rats receiving coamide.

In short, DOY tincture has a positive effect on hemolytic anemia, like coamide, which is widely used in medicine, but its effect on this anemia is lower than that of coamide. Therefore, the effect of the tincture on the course of hemolytic anemia is significantly weaker than its antianemic results in other models of anemia.

Taking into account the above, in order to apply DOY tincture to medical practice, its embryotoxic, teratogenic, and effects on peripheral blood elements and vital organs of the body were studied.

It has been noted in the above experiments that the investigated compound has a high level of anti-anemic effect in various models of anemia. Taking into account the above, we studied the effects of DOY tincture on the body when administered chronically.

The experiment was conducted on 42 laboratory rats with an initial weight of 140-156 g. ODY tincture was administered orally at 1 ml/100 g (10 ml/kg) and 2.5 ml/100 g mass (25 ml/kg) for 1 month. Animals in the control group were given distilled water.

In this, the main attention was paid to the general condition of the animals, the amount of peripheral blood cells, the mass of the animals, the amount of urine and the urine reaction. At the end of the experiment, the animals were euthanized under light anesthesia, and small pieces of internal vital organs were separated and given to histomorphologists in 12% pharmlin solution to study the histomorphological conditions of the organs.

Peripheral blood analysis was taken before the start of the experiment and on the 15th, 30th and 60th days of the experiment. Blood biochemical analyzes were performed on the 30th day of the experiment. The conducted analyzes showed that the amount of hemoglobin, erythrocytes and leukocytes in the peripheral blood of the animals did not change on the 15th day of the experiment, and they were the same as the amount of peripheral blood elements determined before the administration of the drug.

On the 30th day of the experiment, the erythrocytes and hemoglobin in the peripheral blood of the animals were compared to those of the control group.

The results of the biochemical analyzes conducted on the 30th day of the experiment were also presented.

Based on the above results, the normal physiological functions of the liver are almost unchanged under the effect of antianemic compound DOH, but

under the influence of tincture, the process of protein synthesis of the liver increases, and the amount of proteins in the blood increases by 6% and 7.8%, respectively, compared to the control group.

So, the tincture under study significantly accelerates the process of protein synthesis of the liver.

Indicators of biochemical analyzes in the blood of animals chronically receiving tincture of ODY.

T/r	Indicators of biochemical analysis	Results of the analysis conducted on the 30th day of the experiment		
		In the control group	In the group receiving 5 ml/kg tincture	In the group receiving 10 ml/kg tincture
1.	AChAT mmol.ch.l	140,6±2,03	141,7±2,07	142,6±1,63
2.	Asat mmol.ch.l	143,0±1,87	144,5±2,56	145,1±1,57
3.	Proteins g./l.	59,6±1,66	63,2±1,64	64,0±1,36
4.	Cholesterol, mmol/l	1,72±0,01	1,76±0,041	1,75±0,067
5.	Sugar content, mmol/l	4,85±0,41	5,36±0,65	5,31±0,83

Analyzing the histostructure of the internal vital organs, the boundaries of the liver sections are not clearly visible in the livers of animals in the control group. The trabecular structure of hepatocytes is clearly visible and each section is surrounded by septa. The central veins of the liver are empty, the interlobular space is narrowed, the triad is well visible. Hepatocytes located in all parts of the liver have almost the same structure, their nuclei are clearly visible.

The capsule of the liver of the animals that received the study tincture is very thin, the liver section and the trabeculars of hepatocytes are the same as in the control group, the triads are well visible, the proliferation process in the hepatocytes around the triad and septa path, in some hepatocytes

2 cores were recorded.

Renal parenchyma is simple in structure and consists of central medulla and peripheral cortical matter. The capsule is very thin, the parenchyma stroma has a fine structure, the balls are the same size, round or oval in shape, the loop capillaries are thin and empty. Shumlyansky-Bauman capsules are free of pathological fluids. In the cavity of the nephron tubules, unsightly mesh-like, pinkish protein masses were noted.

The histostructure of the kidney of the animals that received the tincture of ODY was similar to that of the control group. Bauman-Shumlyansky capsules are empty, and some animals show significant swelling and metachromasia in the kidney. A state of proliferation is noted in fibroblasts in some areas of the interlobular stroma.

Mucous, submucosal, fleshy and serous layers of the organ-stomach wall that are in direct contact with the drug have a normal appearance. The structure of the intestinal walls is also simple, it is recorded in the form of a layer lying on the meat layer and a circular layer. The mucous layer of the intestine is full of blood, the villi are well developed in the absorbent form, the protoplasm of the epithelial layer is basophilic, and the nucleus is plasmatic. Fatty groups are gathered in the mucous layer, they are composed of goblet-shaped cells with cleft cytoplasm.

The mucus layer of the intestines of some animals that received tincture was well preserved, the villi consisted of plasmatic epithelia, their stroma was finely structured, the mucous layer of the intestinal glands was partially swollen, and the cell cytoplasm was vacuolated.

The intestinal lymphoid apparatus is well developed. Sometimes inconspicuous swellings are noted in the mucous layer.

The histostructure of the heart is normal, the fleshy layer of the heart - the myocardium is saturated with blood, cardiomyocytes are full of blood, and the nucleus is oval. Muscle capillaries are significantly hypertrophied, vacuoles are noted in their cytoplasm.

The histostructure of other vital internal organs was almost the same in animals of the control and experimental groups.

Conclusion. In conclusion, the conducted experiments showed that the histostructure of the vital internal organs of rats in the control and experimental groups was almost the same. At the same time, drug-related histomorphological changes in their histostructure were not noted.

It should be noted separately that the study of the histostructure of the liver and kidney from the parenchymatous organs of the animals that received the studied tincture showed that some morphological signs indicating their functional activity appeared. Therefore, long-term administration of the antianemic compound - DOY tincture to the body does not cause chronic poisoning in animals and any negative reactions in the histostructure of vital internal organs and biochemical analyzes of peripheral blood.

References:

1. Toplicanin A. et al. Autoimmune Hemolytic Anemia in Inflammatory Bowel Disease—Report of a Case and Review of the Literature //Life. – 2022. – T. 12. – №. 11. – C. 1784.
2. FRANK M. M. et al. Pathophysiology of immune hemolytic anemia //Annals of Internal Medicine. – 1977. – T. 87. – №. 2. – C. 210-222.
3. Cappellini M. D. Coagulation in the pathophysiology of hemolytic anemias //ASH Education Program Book. – 2007. – T. 2007. – №. 1. – C. 74-78.
4. Ballas S. K. The pathophysiology of hemolytic anemias //Transfusion Medicine Reviews. – 1990. – T. 4. – №. 3. – C. 236-256.
5. Wapler U., Crubézy E., Schultz M. Is cribra orbitalia synonymous with anemia? Analysis and interpretation of cranial pathology in Sudan //American Journal of Physical Anthropology:

The Official Publication of the American Association of Physical Anthropologists. – 2004. – Т. 123. – №. 4. – С. 333-339.

6.Якубова Л. К. и др. Некоторые механизмы противовоспалительного действия препарата 3, 4-бис-(2-бромобензилоксиметил)-пиразола //Вестник Ташкентской медицинской академии. – 2013. – №. 1. – С. 31-35.

7.Haw A., Palevsky H. I. Pulmonary hypertension in chronic hemolytic anemias: Pathophysiology and treatment //Respiratory medicine. – 2018. – Т. 137. – С. 191-200.

8.Djanaev, G. Yu, O. A. Zaytseva, and O. O. Askarov. "Effect of Dry Extracts of Medicinal Plants on Urinary Excretion and Ion Exchange." European Journal of Medical Genetics and Clinical Biology 1.1 (2023): 90-97.

9.Khotimchenko S. A., Alekseeva I. A. Model of nutritional iron deficiency anemia in rats //Voprosy Pitaniia. – 1999. – Т. 68. – №. 5-6. – С. 13-15.

10.Махсумов Ш. М., Зайцева О. А. К механизму действия и некоторым токсикологическим характеристикам препарата 3, 4-бис-(2, 4-динитробензилоксиметил)-пиразола //Электронный инновационный вестник. – 2018. – №. 1. – С. 14-15.

11.Hashem W. M. et al. Microelimination of hepatitis C in patients with chronic hemolytic anemias: a single-center experience //Egyptian Liver Journal. – 2021. – Т. 11. – №. 1. – С. 48.

12.Махсумов Ш. М., Зайцева О. А. Противовоспалительная активность нового производного бензойной кислоты дифенил-/бис-(бензилокси)/-илона //ФЭн-наука. – 2015. – №. 6. – С. 41-44.

13.Aksan A. et al. Systematic review with network meta-analysis: comparative efficacy and tolerability of different intravenous iron formulations for the treatment of iron deficiency anaemia in patients with inflammatory bowel disease //Alimentary pharmacology & therapeutics. – 2017. – Т. 45. – №. 10. – С. 1303-1318.

14.Махсумов Ш. М., Зайцева О. А., Султанов С. А. О механизме противовоспалительного действия некоторых синтетических соединений : дис. – «Ліки-людині. Сучасні проблеми фармакотерапії та призначення лікарських засобів» Матеріали V Міжнародної науково-практичної конференції, 2021.

15.Balch A., Mackin A. Canine immune-mediated hemolytic anemia: pathophysiology, clinical signs, and diagnosis //Compend. – 2007. – Т. 29. – №. 4. – С. 217-225.

16.Djanaev, G. Y., Khakimov, Z. Z., Allaeva, M. J., Makhsumov Sh, M., Zaytseva, O. A., & Mamadjanova, M. A. Comparative Study of the Influence of Lesbochole. Misoprostol and Mucagen on the Gastric Mucous Barrier in Indometacin Gastropathy.

17.Weiss G., Ganz T., Goodnough L. T. Anemia of inflammation //Blood, The Journal of the American Society of Hematology. – 2019. – Т. 133. – №. 1. – С. 40-50.

18.Махсумов Ш. М., Зайцева О. А. Механизм противовоспалительного действия некоторых производных пиразола : дис. – Ташкентская медицинская академия, 2018.

19.Зокиров У. Б. и др. Изучение противовоспалительных свойств некоторых новых производных 3-(ароилоксиметил)-4-(ароилоксипропил)-1-пиразола //Фармацевтический вестник Узбекистана. – 2006. – №. 4. – С. 32-36.

20.Махсумов Ш. М., Зайцева О. А. Исследование противовоспалительного действия некоторых производных бензойной кислоты. – 2022.

21.Michel M. Warm autoimmune hemolytic anemia: advances in pathophysiology and treatment //La Presse Médicale. – 2014. – Т. 43. – №. 4. – С. e97-e104

22.Toplicanin A. et al. Autoimmune Hemolytic Anemia in Inflammatory Bowel Disease—
Report of a Case and Review of the Literature //Life. – 2022. – T. 12. – №. 11. – C. 1784.

