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ERYTHROCYTE BLOOD INDICES IN RATS UNDER CONDITIONS OF ACETAMINOPHEN-INDUCED TOXIC INJURY AFTER PARTIAL HEPATECTOMY

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The work focuses on investigating the erythrocyte indices of rat blood under conditions of acetaminophen-induced toxic injury following partial liver resection. Modeling of partial hepatectomy was performed using the method of Mitchell & Willenbring by surgically removing two-thirds of the liver. The determination of the erythrocyte count, their mean volume, hemoglobin concentration, its mean content, and mean concentration in erythrocytes was conducted at 24 (priming phase), 48 (active cell proliferation phase), 72 (termination phase), and 168 hours (distant period) after the performance of partial hepatectomy using an RT-7600 (Rayto) automatic hematology analyzer. It was established that after partial liver tissue resection in control rats, a decrease in hemoglobin levels and erythrocyte count occurs during the first three days, indicating the development of postoperative anemia, which is partially a physiological phenomenon. Instead, in animals with toxic injury, a decrease in hemoglobin concentration and a reduction in erythrocyte count take place throughout the entire regenerative period, with a deepening state of anemia and erythropenia during the termination phase (72 h) and the distant period (168 h). Liver tissue regeneration after partial hepatectomy in control rats is characterized by slight normochromia because of a decrease in the mean content and concentration of hemoglobin in erythrocytes at the early stages of regeneration, with a distant macrocytosis effect. Partial liver resection in animals with toxic injury is accompanied by the development of hypochromic microcytic anemia, with intensification during the terminal stages (72 h and 168 h) of the regenerative process.

Keywords: hemoglobin, erythrocytes, acetaminophen, partial hepatectomy, regeneration

Introduction. Currently, one of the most widely used medicinal xenobiotics is paracetamol (acetaminophen), which exhibits analgesic, antipyretic, and anti-inflammatory effects (Ishitsuka et al., 2020; Woodbury et al., 2024). At the same time, acetaminophen is used as a model hepatotoxin, allowing for the evaluation of mechanisms of toxic injury (Cai et al., 2022; Chidiac et al., 2023).

The process of reparative regeneration is part of liver remodeling, occurring in cases of cirrhosis as well as in the restoration of lost cells following hepatotoxic injuries. Liver resections are traditionally associated with significant mortality, particularly linked to intraoperative blood loss, where the development of anemia plays a key role (Huang et al., 2022; Verma et al., 2022).

Hematological parameters are among the indicators that allow for the assessment of the functional state of the body, differential diagnosis of diseases, and the severity of their course. Particular importance is attributed to the evaluation of the red blood cell count (RBC), their mean corpuscular volume (MCV), red cell distribution width (RDW), hemoglobin concentration (HGB), its mean content in erythrocytes (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Thus, the aim of the work was to investigate the erythrocyte indices in the blood of rats with acetaminophen-induced toxic injury following partial hepatectomy.

Materials and Methods. The research was conducted on white non-pedigree rats with a body weight of 150–170 g. The housing and all manipulations were carried out in accordance with international legal standards for carrying out animal experimentation, as outlined in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the norms of the "General Ethical Principles for Animal Experiments," approved by the First National Congress on Bioethics (Kyiv, 2001).

Modeling of acute toxic injury was performed by administering paracetamol to the experimental animals at a dose of 1250 mg/kg of the animal's body weight. The xenobiotic was administered per os in the form of a suspension based on a 2% starch gel solution during the two days preceding the scheduled liver resection of the animals (Kopylchuk

et al., 2021). Partial liver resection was performed by surgically removing two-thirds of the liver (Sahay et al., 2019).

The experimental animals were classified into two groups: group 1 consisted of control animals that had partial hepatectomy (C/PH); group 2 consisted of animals that had partial hepatectomy after acetaminophen-induced injury (TI/PH). The euthanasia of the animals was performed by cervical dislocation under light ether anesthesia at 24 h (priming phase), 48 h (active cell proliferation period), 72 h (termination phase), and 168 h (distant period). Intact animals (C) and rats with toxic injury (TI), which did not undergo hepatectomy, were used as experimental control groups at 0 hours.

Evaluation of erythrocyte indices (absolute erythrocyte count, hemoglobin concentration in whole blood, mean corpuscular hemoglobin concentration in erythrocytes, mean corpuscular hemoglobin in erythrocytes and mean corpuscular volume of a red blood cell) was performed using an RT-7600 automatic hematology analyzer manufactured by Rayto.

Results and Discussion. The research results showed that in animals of the C/PH group, a decrease in total hemoglobin level was observed during the first three days, with the lowest values at the initial stages of regeneration (24 h) compared to the control values (0 h). In contrast, in the group of animals with toxic damage after partial organ resection, this indicator decreased throughout the entire experimental period with the lowest values in the termination period (72 h) compared to the values of TI (0 h) (Table 1). A decrease in total hemoglobin content in the blood inevitably leads to the development of anemia, which may occur for various reasons. A key role is attributed to hepcidin, a hormone synthesized by the liver, which is a major regulator of systemic iron homeostasis. (Nemeth et al., 2021). The systemic inflammatory response following surgical intervention can cause excessive expression of hepcidin through the inflammatory mediator interleukin-6, as confirmed by previous studies of the scientific group under conditions of acetaminophen-induced toxic injury, thereby limiting the available circulating iron.

At the same time, we established that the number of erythrocytes in the blood of rats decreases after partial hepatectomy – in animals of the C/PH group within 72 h following the surgical intervention, while in rats with toxic injury, it persists throughout the entire experimental period, with the lowest values observed in the termination phase (72 h) and the distant period (168 h) compared to the values at 0 hours, indicating the development of erythropenia (Table 1). The main causes under these conditions may include blood loss, which was very characteristic of animals with toxic injury during surgical intervention, hemolysis of erythrocytes, or impaired of their production. We assume that under conditions of toxic injury, this fact is most likely due to the predominance of erythrocyte hemolysis, as confirmed by previous studies showing a decrease in osmotic resistance of erythrocytes in the group with toxic injury (Kopylchuk et al., 2022).

Table 1.

Erythrocyte indices in the blood of rats under conditions of acetaminophen-induced toxic injury following partial hepatectomy
(M ± m, n = 8)

Erythrocyte index	RBC	MCV	HGB	MCH	MCHC
C/PH					
0	8,52 ± 0,34	63,30 ± 1,52	152 ± 13	17,81 ± 0,83	281,24 ± 6,39
24	6,9 ± 0,30^{b}	65,85 ± 1,45	122 ± 11^{b}	14,9 ± 0,9^{b}	243,5 ± 6,42^{b}
48	6,8±0,40^{b}	65,5 ± 1,5	128 ± 4^{b}	14,5 ± 0,89^{b}	198,2 ± 5,42^{b}
72	7,01 ± 0,71^{b}	65,84 ± 0,705	132 ± 11^{b}	17,38 ± 0,30	227,9 ± 7,8^{b}
168	7,77 ± 0,09^{b}	77,39 ± 1,39^{b}	150 ± 7	17,62 ± 0,79	282,36 ± 6,36
TI/PH					
0	7,63 ± 0,96^{a}	55,3 ± 1,5^{a}	132 ± 8^{a}	15,5 ± 0,81^{a}	245,9 ± 5,9^{a}
24	6,1± 0,2^{c}	55,2 ± 1,6^{d}	110 ± 5^{c}	13,4 ± 0,81^{c}	208,3 ± 8,3^{c}
48	6,3± 0,4^{c}	49,8 ± 1,5^{d}	108 ± 8^{c}	11,2 ± 0,71$^{c, d}$	171,3 ± 4,3^{c}
72	5,14 ± 0,07$^{c, d}$	32,1 ± 1,2$^{c, d}$	92 ± 7$^{c, d}$	5,4 ± 0,79$^{c, d}$	122,9 ± 3,9$^{c, d}$
168	3,78 ± 0,26$^{c, d}$	29,8 ± 1,4$^{c, d}$	102 ± 3$^{c, d}$	5,3 ± 0,79$^{c, d}$	121,4 ± 4,4$^{c, d}$

Note: C/PH – control animals that underwent partial hepatectomy; TI/PH – animals with toxic injury that underwent segmental partial liver resection; a – statistically significant difference between the indicators of rats in the TI group and control, P ≤ 0.05; b – statistically significant difference in the indicators of the C/PH group compared to the values of C at 0 h, P ≤ 0.05; c – statistically significant difference between the TI/PH groups compared to the indicators of rats in the TI group at 0 h, P ≤ 0.05; d – statistically significant difference between the TI/PH groups compared to the indicators of the C/PH group, P ≤ 0.05

In order to predict the course of the pathological process, erythrocyte indices have a significant role, among which is the mean corpuscular volume of a red blood cell. This indicator allows the classification of anemia into three main types: microcytic, normocytic, and macrocytic. We found that in the blood of C/PH animals, this indicator remains unchanged until 72 h after partial organ resection and significantly increases during the distant term of the regenerative process (168 h) compared to the control (0 h), indicating a probable development of macrocytosis (Table 1).

At the same time, in rats with toxic injury following partial hepatectomy, a reduction in the mean erythrocyte volume during the termination phase (72 h) and the distant terms (168 h) leads to the development of microcytic anemia, characterized by the formation of smaller erythrocytes (Table 1).

Conclusions. After partial liver tissue resection in rats with toxic injury, a decrease in hemoglobin concentration and a reduction in erythrocyte count occur throughout the entire regenerative period, with a deepening state of anemia and erythropenia during the termination phase (72 h) and the distant phase (168 h). Liver tissue regeneration after partial hepatectomy in control rats is characterized by slight normochromia due to a decrease in the mean content and concentration of hemoglobin in erythrocytes during the early stages of regeneration, with a distant macrocytosis effect. Partial liver resection in animals with toxic injury is accompanied by the development of hypochromic microcytic anemia, which intensifies during the terminal stages (72 and 168 h) of the regenerative process.

Conflict of Interest: *The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.*

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ЕРИТРОЦИТАРНІ ІНДЕКСИ КРОВІ ЩУРІВ ЗА УМОВ ТОКСИЧНОГО УРАЖЕННЯ АЦЕТАМІНОФЕНОМ ПІСЛЯ ЧАСТКОВОЇ ГЕПАТЕКТОМІЇ

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Робота присвячена дослідженню еритроцитарних індексів крові щурів із парацетамол-індукованим ураженням після часткової резекції печінки. Моделювання часткової гепатектомії проводили за методом Mitchell & Willenbring шляхом видалення 2/3 частин органу. Визначення кількості еритроцитів, середнього об'єму еритроцитів, рівня гемоглобіну, середнього вмісту гемоглобіну та середньої концентрації гемоглобіну в еритроциті здійснювали на 24 (період праймінгу), 48 (фаза активної проліферації клітин) та 72 (термінація) та 168 години (віддалений період) після проведення часткової гепатектомії на автоматичному гематологічному аналізаторі RT-7600 (Rayto). Встановлено, що в контрольних тварин після часткової гепатектомії впродовж перших трьох діб відбувається зниження рівня гемоглобіну та кількості еритроцитів, що засвідчує розвиток післяопераційної анемії, яка частково є фізіологічним явищем. Натомість у тварин із ацетамінофен-індукованим токсичним ураженням зниження рівня загального гемоглобіну та зменшення кількості еритроцитів відбувається впродовж усього періоду відновлення паренхіми печінки із поглибленням стану анемії та еритропенії на завершальних етапах регенерації – термінації (72 год) та віддалений період (168 год). Регенеративний потенціал після хірургічного видалення частини печінки у контрольних тварин характеризується незначною нормохромією, пов'язаною зі зниження середнього вмісту гемоглобіну та його середньої концентрації в еритроцитах на початкових стадіях ініціації регенерації з віддаленим ефектом макроцитозу. Проведення часткової резекції часток печінки тваринам, яким попередньо моделювали токсичне ураження медикаментозним ксенобіотиком призводить до розвитку мікроцитарної гіпохромної анемії, ознаки якої посилюються на завершальних етапах (72 год та 168 год) регенераційного процесу.

Ключові слова: гемоглобін, еритроцити, ацетамінофен, часткова гепатектомія, регенерація

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