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AGE-DEPENDENCE OF BIOCHEMICAL MANIFESTATIONS OF HEPATOTOXIC INJURY IN RAT EXPOSED TO XENOBIOTICS OF VARIOUS GENESIS

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Currently, the problem of liver diseases against the backdrop of the toxic effects of medicinal and industrial (herbicides) xenobiotics on the body is becoming increasingly relevant. The activation of compensatory mechanisms in response to the action of toxic agents – acetaminophen and/or diquat is closely related to age-specific features. Heightened focus on acetaminophen-induced injury is linked to the widespread use of this medication as an analgesic and antipyretic during pandemics of infectious and inflammatory diseases, especially under the conditions of warfare in Ukraine. In return, the widespread use of the herbicide diquat is accompanied by an increase in the registration of cases of diquat -induced acute poisoning.

The aim of study was to evaluation the biochemical parameters of the functional state of the liver of different-aged rats under conditions of acetaminophen- and diquat-induced toxic injury. Animals were divided into three age groups: adolescent (60 days), reproductive (150 days), and mature age rats (360 days). Acute toxic injury by acetaminophen was modeled by its oral administration through gastric intubation at a daily dose of 1250 mg/kg of the animal's body weight during the last 2 days of the experiment. Acute toxic damage by diquat was modeled by a single intragastric administration using a probe at a dose of 115.5 mg/kg of the animal's body weight. The functional state of the liver was assessed on an automatic biochemical analyzer NTI Biochem FC-120.

Acetaminophen intoxication led to an increase in the absolute liver mass indicator, organ index, changes in the macroscopic structure of the organ, increased serum activities of ALT and AST, total LDH, and a decrease in the De Ritis ratio amidst increased activities of ALP, GGT, and levels of total and indirect bilirubin in all age groups compared to the control. The most significant changes were observed in mature age animals (increase in ALT activity by 76%, AST by 56%, ALP by 51%, GGT by 51%, and a decrease in the De Ritis ratio by 47%). Under conditions of diquat-induced toxic injury, a slightly different trend and degree in manifestation of established changes are observed. A more pronounced hepatotoxic effect was registered in adolescent animals, manifested by the maximum increase in absolute liver mass by 26%, ALT activity by 71%, AST by 47%, ALP by 50%, Bili-T level by 69%. No statistically significant differences compared to the control and APAP-induced injury in the activity of total LDH and GGT upon administration of toxic doses of the herbicide diquat regardless of age category were observed.

Based on the experimental results, an age-related multidirectionality in the adverse effects of the medicinal xenobiotic acetaminophen and the industrial xenobiotic diquat on indicators of the morpho-functional state of the liver is observed. The most sensitive age group of animals to toxic injury by acetaminophen are rats of 360 days of age, while for diquat – 60 days of age.

Keywords: alanine aminotransferase, aspartate aminotransferase, de Ritis coefficient, alkaline phosphatase, gamma-glutamyltransferas, bilirubin, acetaminophen, diquat, toxic injury, liver

Introduction. Today, the problem of the frequency of functional changes development in the hepatobiliary system that occur due to the adverse effects of xenobiotics of various nature on the body is becoming increasingly relevant. Exogenous toxic agents entering the body by alimentary, percutaneous or inhalation routes contribute to homeostatic shifts, a decrease in the body's functional reserves, in particular, hepatic metabolism disorders, which is expressed by changes in biochemical parameters. The activation of compensatory mechanisms and adaptive reorganization aimed at maintaining homeostatic balance in response to toxic agents of medicinal and/or industrial origin are largely related to age-related features (Teshayev, 2023; Ketsa et al., 2022; Chidiac et al., 2023; Magalhães et al., 2018).

Among the medical xenobiotics, the scientific community pays important attention to acetaminophen (paracetamol, N-acetyl-p-aminophenol (APAP)), a popular over-the-counter drug that is widely used as an analgesic and

antipyretic agent and is relatively safe in therapeutic doses, which reduces vigilance regarding the risk of its xenobiotic effect realization (Chidiac et al., 2023). However, an analytical review of the literature shows ambiguity and contradictory opinions regarding the likelihood of developing acetaminophen-induced toxicity in different age groups (Caparrotta et al., 2017). On the one hand, the safety of systemic use of paracetamol in the treatment of acute or chronic pain syndrome of various etiologies, colds in different age groups, even in children under one month of age, is noted. In the context of the analysis of literature data in *PubMed* (Mast et al., 2018), acetaminophen is the first drug to treat chronic pain in the elderly.

On the other hand, repeated administration of doses exceeding the recommended doses over a certain period of time, along with its relatively low therapeutic index, is associated with the development of dose-dependent hepatotoxicity (Chidiac et al., 2023). Increased attention to this problem is due to the inclusion of APAP in the treatment protocols for coronavirus infection (COVID-19) and consumption in case of adverse reactions after immunization (Galluzzo et al., 2023). At the same time, modeling of acetaminophen-induced injury is of particular importance in connection with the use of acetaminophen for prehospital pain relief in the context of hostilities in Ukraine in accordance with the triple analgesia strategy, proposed by the Tactical Combat Casualty Care Committee (TCCC) and in accordance with the Order of the Ministry of Health of Ukraine No. 1122 (June 28, 2022) "On Approval of Methodological Recommendations for Pain Relief for Victims at the Evacuation Stage" (Chorna et al., 2023).

Another significant problem today is the excessive and uncontrolled use of xenobiotics of industrial origin, including pesticides (Magalhães et al., 2018; Sharma et al., 2019). According to Sharma A. et al (Sharma et al., 2019), about 2 million tons of chemical plant protection products are currently applied to the world's agricultural lands, of which 47.5% are herbicides. However, the increased irrational use of plant protection chemicals, including original and generic herbicides with diquat as an active ingredient, leads to an intensification of the potential herbicide load, bioaccumulation and progressive accumulation of pollutants and, accordingly, their accumulation in food raw materials and food products, which poses a direct threat to the proper functioning of the body (Magalhães et al., 2018; Sharma et al., 2019). It should be noted that as a result of the intensification of hostilities in Ukraine, potential risks of toxic effects of industrial xenobiotics on the body, in addition to the above, arise due to the destruction of storage facilities for plant protection chemicals (Baranov et al., 2023). Due to the widespread use of diquat, cases of diquat-induced acute poisoning are increasingly reported. Although diquat-induced toxicity can cause multiorgan failure, one of the main target organs of DQ, where its overwhelming amount is concentrated, along with the kidneys, is the liver (Magalhães N. et al., 2018; Wu et al., 2022). However, scientific sources contain only a small amount of information on biochemical changes in the body caused by the hepatotoxic effect of diquat-containing drugs. Taking into account the above effects of xenobiotics of medicinal and industrial origin, the aim of the study was to investigate the biochemical parameters of the functional state of the liver of different-aged rats under conditions of acetaminophen- and diquat-induced toxic injury.

Materials and methods. The experimental study was carried out on white non-breed rats housed in the vivarium of the Educational and Scientific Institute of Biology, Chemistry, and Bioresources at Yuriy Fedkovych Chernivtsi National University (Chernivtsi, Ukraine). Throughout the experiment, in accordance with group housing norms, the animals (2-3 individuals of the same sex) were kept in plastic cages for rodents with sandy bedding. Rats received pre-sterilized tap water *ad libitum*.

Care and manipulation of the rats were conducted in accordance with widely accepted recommendations, requirements, and provisions from Article 26 in Ukrainian Law No. 3447-IV of 21.02.2006, "On the Protection of Animals from Cruel Treatment" (as amended according to the law No. 5456-VI of 16.10.2012); "General Ethical Principles of Animal Experiments," adopted by the First National Congress on Bioethics (Kyiv, 2001); "The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and Directive 2010/63/EU on the protection of animals used for scientific purposes (22.09.2010). All procedures related to the animals were carried out in accordance with the ethical standards of the Committee on Bioethics of Scientific Research at Yuriy Fedkovich Chernivtsi National University (protocol No. 1 dated 04.04.2024).

The experimental animals were divided into three age groups by the method of random sampling: adolescent age rats (60-63 days) weighing 80–125 g, reproductive age rats (138–150 days) weighing 130–160 g, and mature age rats (348–360 days) with a weight of 210–250 g. The ages of the adolescent (60–63 days), young (138–150 days), and mature

(348–360 days) rats selected for our study are equivalent to the age stages of human development at 14–15 years, 24–25 years, and 44–45 years, respectively. The formation of animal age groups was based on recommendations, taking into account the table data on the correlation of rats' and humans' ages as presented in the literature (Ghasemi et al., 2021). During the entire experimental period, the rats consumed a dry semi-synthetic diet AIN-93, calculated according to the recommendations of the American Institute of Nutrition, taking into account the principle of pair-feeding (Kopylchuk et al., 2020).

Acute toxic injury from acetaminophen was modeled by its oral administration via gastric intubation at a daily dose of 1250 mg/kg of the animal's body weight during the last 2 days of the experiment as a 2% suspension of starch gel (Kopylchuk et al., 2020). Acute toxic injury from diquat in rats was modeled by a single intragastric administration using a probe at a dose of 115.5 mg/kg of the animal's body weight on day 29 of the experimental period (Sun et al., 2019).

Experimental animals of three age categories (adolescent, young, and mature age) were divided into 3 groups: Group 1 – animals that consumed a complete semi-synthetic diet (C); Group 2 – animals in which acute toxic injury was modeled by the action of acetaminophen after 4 weeks of consuming a complete semi-synthetic diet (APAP); Group 3 – animals in which acute diquat-induced injury was modeled after 4 weeks of consuming a complete semi-synthetic diet (DQ). Animals were removed from the experiment on the 29th (C), 30th (DQ) and 31st (APAP) days of the experiment by cervical dislocation in accordance with current recommendations and ethical standards.

After euthanizing the animals, the liver was separated, weighed, and used to determine the organ index. The liver index was calculated using the formula: liver index (%) = liver weight (g) / animal weight (g) × 100.

The functional state of the liver was assessed by the activity indicators of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and the levels of total, direct (conjugated), and indirect (unconjugated) bilirubin in the blood serum using the NTI Biochem FC-120 automatic biochemical analyzer with diagnostic reagent kits from High Technology (High Technology Inc., North Attleboro, MA, USA) according to the manufacturer's instructions. The content of indirect bilirubin was determined by the difference between total and direct bilirubin. To assess the degree of liver injury, the De Ritis ratio (AST/ALT ratio) was calculated.

Statistical analysis was conducted using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, California USA; http://www.graphpad.com). The data were analyzed using two-way ANOVA with Tukey's a posteriori criterion (Tukey's HSD post hoc test). The normality of distribution was tested using the D'Agostino-Pearson test, and the equality of variances was assessed using Fisher's test. Differences between groups were considered significant at $p \leq 0.05$. Data are expressed as mean ± SEM.

Results and Discussion. Experimental studies have shown that toxic exposure to medicinal (acetaminophen) and industrial (diquat) xenobiotics caused visible signs of intoxication and cases of animal death. During the experiment, the behavioral reactions of control animals of different age groups were adequate. Instead, in the experimental groups of rats of different ages after modeling acetaminophen- and diquat-induced lesions, depression of their general condition, dishevelment and loss of shine of the coat, and decreased skin elasticity were recorded. From the point of view of general observation, only in rats injected with toxic doses of diquat, regardless of age, digestive disorders in the form of flatulence, reflux and diarrhea were increased.

In the study of morphometric parameters in rats of the control group, we found the dependence of body weight, absolute liver weight and liver mass index of rats on the age of the organism (Table 1). If the average body weight of adolescent rats was (112.47 ± 6.59) g with an organ weight of (4.36 ± 0.37) g, reproductive rats – (144.87 ± 7.96) with a liver weight of (4.69 ± 0.30) g, then mature animals weighed (243.64 ± 12.84) g with a liver weight of (7.26 ± 0.35) g. Therefore, we noted that the age and body weight of the control group animals significantly correlated with liver weight. A significant percentage of increase in liver weight was recorded in mature animals compared to adolescent (40 %, $p \leq 0.05$) and reproductive animals (35%, $p \leq 0.05$), with no statistical differences between 60-day and 150-day old rats (Table 1).

After analyzing the absolute value of liver weight, it was found that in all age groups of rats with acetaminophen-induced lesions, an increase in the weight of this organ was observed compared to the control (adolescent – by 25 %, reproductive – by 38 %, mature – by 36 %, $p \leq 0.001$). A tendency to increase the level of this indicator is also observed in the group of animals injected with toxic doses of diquat (adolescent – by 26 %, reproductive – by 24 %, mature – by 19 %, $p \leq 0.001$).

Table 1.

Body weight, liver weight, and liver organ index in rats of different ages under conditions of acetaminophen- and diquat-induced toxic injury

Group	Body Weight (g)	Liver Weight (g)	Liver index
Adolescent age (60 days)			
Control	112.4 ± 6.6	4.4 ± 0.4	3.8 ± 0.2
APAP	86.4 ± 2.3 [a, c]	5.8 ± 0.3 [a]	6.7 ± 0.3 [a, c]
DQ	118.6 ± 2.6	5.9 ± 0.2 [a]	5.0 ± 0.2 [b]
Young age (150 days)			
Control	144.9 ± 8.0	4.7 ± 0.3	3.3 ± 0.2
APAP	135.0 ±4.8	7.6 ± 0.3 [a, c]	5.7 ± 0.1 [a, c]
DQ	149.3 ± 6.2	6.2 ± 0.2 [b]	4.3 ± 0.2 [b]
Mature age (360 days)			
Control	243.6 ± 12.8	7.3 ± 0.3	3.0 ± 0.1
APAP	235.3 ± 7.0	11.3 ± 0.3 [a, c]	4.9 ± 0.1 [a, c]
DQ	228.7 ± 10.1	8.9 ± 0.5 [b]	3.9 ± 0.2 [b]

Note (here and hereafter): a, b, c – values marked with these letter indices are statistically likely to different. a, b – statistically significant difference compared to control indicators (C); c – statistically significant difference compared to the indicators of the DQ group, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$.

If in the group of 60-day-old rats the difference in liver weight after hepatotoxicity caused by acetaminophen (5.76 ± 0.27) g and diquat (5.86 ± 0.19) g is not statistically significant, the administration of toxic doses of acetaminophen to reproductive (APAP, 150 days) and mature (APAP, 360 days) animals leads to an increase in organ weight (by 18.7 % – young, 150 days; by 21.2 % – mature, 360 days) compared to the corresponding values under the conditions of diquat-induced lesions in these age groups ($p \leq 0.05$) (Table 1).

Age-related sensitivity to xenobiotics of medicinal or industrial origin is partly explained by differences in their metabolic activity, which explains the maximum increase in the absolute liver weight in animals of reproductive (150 days) and mature (360 days) age with APAP lesions. Regarding toxic injury with diquat, we observed opposite changes in absolute liver weight, with the highest values in 60-day-old rats. Toxicity caused by diquat in adolescence can cause serious damage and be irreversible. There is no antidote for DQ poisoning, and the effectiveness of currently used therapeutic methods is still unsatisfactory. Its toxic potential is a consequence of diquat's ability to produce reactive oxygen and nitrogen species through redox cycling processes that can lead to oxidative stress and potential cell death (Magalhães et al., 2018).

The data presented in Table 1 show a significant increase in the liver organ index in all age groups of rats under both acetaminophen- and diquat-induced toxic injury compared to control values. However, the degree of manifestation of the identified changes is different. The relative coefficients of liver weight in the experimental groups of animals of 60 days of age were higher by 42.7 % in the APAP group and by 23 % in the DQ group; at 150 days of age – by 42 % in the APAP group and by 23 % in the DQ group; at 360 days of age – by 38.2 % in the APAP group and by 23.7 % in the DQ group compared to the control group ($p \leq 0.001$).

The liver organ index reflects the morphological and functional state of the organ, which is used to confirm pathological changes in the liver architecture and is considered a sensitive indicator of excessive toxic load on the liver (Zhou et al., 2023). A more pronounced increase in the hepatic coefficient is observed in animals administered high doses of APAP, regardless of age, compared with the values of controls and rats in which diquat-induced toxic injury was modeled. This is probably due to the fact that the main target organ of diquat due to its toxicokinetics and redox cycle is the kidneys, not the liver (Magalhães et al., 2018; Wu et al., 2022).

The changes in morphometric parameters established by us are supported by images of the macroscopic structure of the liver of rats of different age groups after toxic injury by acetaminophen and diquat (Figure 1). After the administration of toxic doses of acetaminophen to animals of different age groups, certain changes in the macrostructure of the liver were detected: color (in most animals with a yellowish tint), consistency, and size, which was reliably confirmed by weight indicators of the liver mass coefficient (Table 1). The most significant changes were recorded in animals of reproductive age (150 days) and mature age (360 days) with APAP lesions.

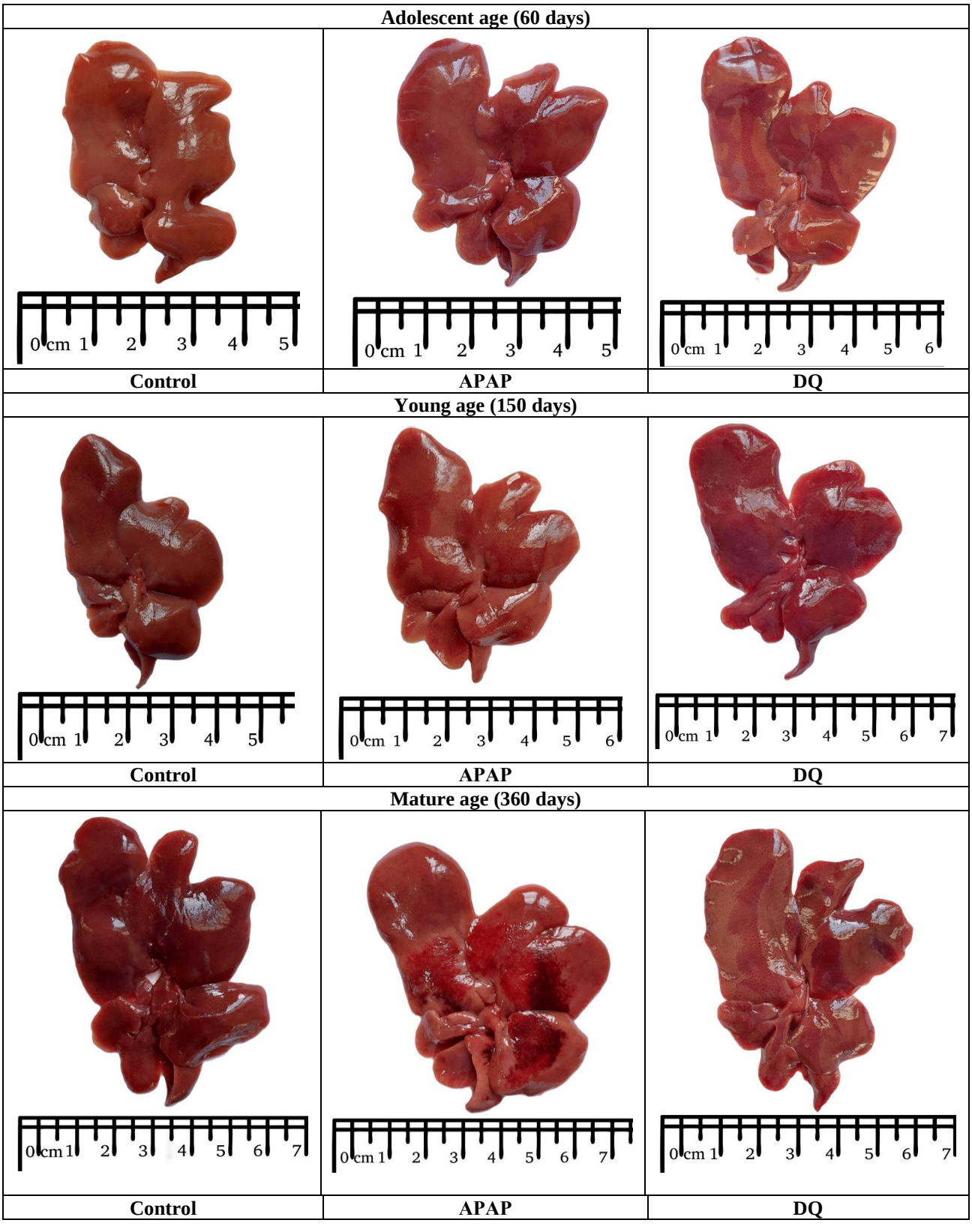

Fig. 1. Depiction of liver in rats of different ages under conditions of acetaminophen- and diquat-induced toxic injury

This may be due to the development of inflammation and swelling of the organ, as well as to the accumulation of glycogen. Under the influence of diquat, a pronounced difference was manifested mainly in determining the color of the parenchyma of the organ of experimental animals of different age groups and was most clearly observed in adolescence (60 days) and mature rats (360 days). The color of the liver was heterogeneous: foci of light brown color alternated with light red striations, the consistency was somewhat flabby. However, we noted that these changes in the macroscopic structure of the liver were combined with a maximum increase in the weight of the organ in

animals of 60 days of age, which was obviously reversed in response to a high load of industrial xenobiotics.

We assume that the detected effect is an integral consequence of metabolic disorders in the liver, so the features of these changes were characterized by blood biochemical parameters (Table 2). It was found that in the blood serum of almost all age groups of rats under conditions of toxic injury by paracetamol and diquat, an increase in the levels of ALT and AST enzymes was observed compared to the control. Statistically significant differences in AST levels were not recorded only in young animals (150 days) under conditions of toxic diquat injury. As for animals with acetaminophen-induced lesions, ALT activity in the serum of adolescent rats increased by 52 %, reproductive rats – by 53 %, mature rats – by 76 %; $p \leq 0.001$) compared with the values of the corresponding control values. At the same time, the introduction of toxic doses of diquat was accompanied by a more pronounced increase in ALT in animals of 60 days of age by 71 % ($p \leq 0.0002$) and 150 days of age by 68 % ($p \leq 0.001$) compared to the control. In rats of 60 days of age, a more pronounced cytolytic effect was observed with the introduction of toxic doses of diquat (by 40 %, $p \leq 0.05$), while in mature rats (360 days) – under conditions of acetaminophen intoxication (by 22 %, $p \leq 0.05$). Instead, the activity of AST in the blood of rats of adolescent age (60 days) under 2-day acetaminophen administration increased by 35 % ($p \leq 0.05$), young age (150 days) – by 40 % ($p \leq 0.001$), mature age (360 days) – by 56 % ($p \leq 0.05$) compared to the control values. At the same time, in diquat poisoning, the level of AST in the groups of rats of adolescent age (60 days) and mature age (360 days) exceeds the control value by 47 % ($p \leq 0.05$) and 43 % ($p \leq 0.001$), respectively (Table 2).

Table 2.

Activity of marker enzymes of liver functional state in the blood serum rats of different ages under conditions of acetaminophen- and diquat-induced toxic injury

Group	ALT (U/L)	AST (U/L)	De Ritis ratio	LDH (U/L)	GGT (U/L)	ALP (U/L)
Adolescent age (60 days)						
Control	21.9 ± 4.9	77.3 ± 5.4	3.5 ± 0.1	116.0 ± 6.7	3.4 ± 0.3	102.4 ± 11.2
APAP	45.6 ± 5.5 [a,c]	119.0 ± 6.7 [a, c]	2.6 ± 0.1	162.6 ± 12.8 [a, c]	5.0 ± 0.5 [a, c]	185.4 ± 5.5 [a]
DQ	76.6 ± 10.0 [a]	146.1 ± 23.1 [b]	1.9 ± 0.2 [a]	107.6 ± 2.6	3.7 ± 0.3	204.9 ± 21.2 [a]
Young age (150 days)						
Control	25.7 ± 2.1	88.0 ± 5.3	3.4 ± 0.6	121.0 ± 5.8	4.5 ± 0.1	89.0 ± 4.9
APAP	55.2 ± 4.4 [a, c]	147.0 ± 16.2 [a, c]	2.7 ± 0.52	173.5 ± 14.1 [a, c]	6.5 ± 0.4 [a, c]	155.6 ± 8.3 [a]
DQ	79.6 ± 5.2 [b]	98.7 ± 8.8 [b]	1.2 ± 0.2 [a]	123.6 ± 3.3	3.8 ± 0.1	158.0 ± 7.5 [a]
Mature age (360 days)						
Control	29.2 ± 3.5	84.3 ± 5.6	2.9 ± 0.2	105.2 ± 5.5	4.7 ± 0.4	128.3 ± 9.4
APAP	123.2 ± 16.0 [a, c]	189.6 ± 15.5 [a, c]	1.5 ± 0.5 [a]	155.8 ± 17.2 [a, c]	9.6 ± 1.1 [a, c]	259.7 ± 40.4 [a]
DQ	96.5 ± 7.9 [b]	148.3 ± 8.5 [b]	1.5 ± 0.1 [a]	116.4 ± 11.9	3.9 ± 0.3	244.5 ± 26.9 [a]

The results of biochemical analysis of the blood of rats of different ages with acetaminophen-induced lesions indicate the development of cytolysis syndrome, which is maximally expressed in rats of 360 days of age (mature age), as evidenced by an increase in the activity of indicator transaminases – ALT and AST with a predominance of ALT percentage output. It is obvious that the maximum increase in ALT levels in 360-day-old animals with APAP lesions indicates the inflammatory nature of the disease, which develops rapidly and is a consequence of the destruction of parenchymal cells. It is worth noting that in response to diquat, the trend of changes in aminotransferase activity in animals is different. A more pronounced increase in ALT indicator compared to the output of AST was observed in adolescent rats.

In hepatocytes, a significant portion of AST (80% of activity) is found in the mitochondria (mitochondrial isoform), and only about 20% is localized in the cytosol. Minor and moderate inflammatory processes in hepatocytes are accompanied by the release of AST primarily from the cytosol, so the total amount of the enzyme that enters the bloodstream is small, unlike the level of ALT, which is completely localized in the cytoplasm (Botros et al., 2013). This example can be observed in both drug and industrial xenobiotic injury, when ALT levels are significantly higher than AST. It is known that the maximum increase in serum

aminotransferase activities is usually observed 48–96 hours after the administration of supratherapeutic acute doses of paracetamol. A few days after the consumption of excessive doses of APAP, liver failure may develop with an undetectable concentration of APAP in the serum (Fisher et al., 2019).

However, our results indicate age-related differences in the organ-specific response to paracetamol or diquat. The level of ALT is used to judge the biochemical activity in the course of liver disease. An increase of 1.5-4 times indicates low activity of the process, 5-10 times - moderate and more than 10 times - high biochemical activity [36]. Based on the results obtained, the degree of liver damage based on an increase in the level of transaminases in the blood serum can be considered as a phenomenon of moderate cytolysis (Contreras-Zentella et al., 2016). Elevated ALT and AST activity is considered an index marker of hepatotoxicity associated with oxidative stress caused by the toxins used in the study (Chidiac et al., 2023; Magalhães et al., 2018). However, a sharp increase in the activity of marker enzymes in the blood serum does not necessarily indicate liver cell death (Contreras-Zentella et al., 2016).

Regarding the de Ritis coefficient, when acetaminophen was administered in toxic doses, this indicator was reduced relative to the control only in rats of 360 days of age (mature age) – by 47 % ($p \leq 0.05$). Under conditions of toxic damage by an industrial xenobiotic, the de Ritis coefficient was significantly lower compared to the control in all age groups of rats (adolescent age – by 46 %, young age – 63 %, mature age – 47 %) (Table 2). Under the experimental conditions studied, even a decrease in AST/ALT reflects acute destruction of the cell membrane, but the deep structures of the hepatocyte remained intact, as evidenced by the predominance of ALT output. A characteristic change in the de Ritis coefficient in toxic damage by xenobiotics of medicinal or industrial origin is a reflection of the classic "hepatic" variant, when this index decreases compared to the norm, indicating a metabolic relationship of indicators through the glucose-alanine shunt, which ensures the integration of protein and carbohydrate metabolism. In this case, the source of alanine for glucose synthesis should be the pool of free amino acids and the entire protein pool, since both transaminase reactions are interconnected through glutamate, which allows for the efficient use of a minimum amount of aspartate. This process, necessary to maintain adequate blood glucose levels, leads to an increase in transaminase activity (Botros et al., 2013; Petersen et al., 2019).

At the same time, we recorded an increase in the level of total LDH activity only in rats treated with toxic doses of APAP compared to intact animals (adolescent rats – by 29 %, reproductive rats – by 30 %, mature rats – by 32 % ($p \leq 0.001$) (Table 2). Another important marker of tissue destruction accompanied by an increase in cell membrane permeability is LDH. Based on our data, the increase in LDH activity under conditions of acetaminophen intoxication in different age groups is a nonspecific sign, but may additionally indicate toxic liver damage due to the increase in isozymes LDH4 and LDH5 (Comandatore et al., 2022).

Among the biochemical parameters of cholestasis syndrome in rats of all age groups, regardless of the lesion with a drug or industrial xenobiotic, an increase in the level of ALP in the blood was observed compared to the control. Under the conditions of paracetamol intoxication, the highest values of this indicator compared to the control (by 51 %, $p \leq 0.05$) were recorded in rats of 360 days of age (mature age), and in the case of diquat administration – in rats of adolescent age (60 days) (by 50 %, $p \leq 0.05$). It should be noted that the activity of γ-glutamyl transpeptidase (GGT) is often used in clinical practice. We have found that statistically significant differences in the level of this enzyme compared with the control were detected only in rats with APAP lesions (adolescent – by 31 %, reproductive – by 32 %, mature – by 51 %; $p \leq 0.001$) (Table 2).

The analysis of the functional state of the liver in rats of different age groups under conditions of toxic injury with acetaminophen indicates the development of concomitant cholestasis syndrome. The markers of cholestasis are alkaline phosphatase and gamma-glutamyltransferase, the levels of which in our case significantly exceed the control values when administered toxic doses of acetaminophen. These enzymes are localized in the endothelium of the bile ducts and the epithelium of the liver sinusoids, so in cholestasis under the influence of bile components they are released into the blood (Lowe et al., 2024; Puukka et al., 2006).

The activity of the excretory enzyme of hepatocytes, alkaline phosphatase, was increased in all age groups of rats regardless of the nature of the toxin intake. Alkaline phosphatase is found in almost all organs, but its maximum activity is found in the hepatobiliary system. It is known that hepatic ALP is represented by two isozymes. The activity of the first isozyme increases in the blood serum during liver congestion and reduced elimination of the enzyme in bile. This is the main enzyme of biochemical importance for the diagnosis of pathologies of the hepatobiliary tract (Lowe et al., 2024).

We assume that the leading mechanism of GGT hyperenzymia occurrence is an increase in the

synthesis of the enzyme with subsequent translocation across the hepatocyte membrane. The main function of this ectoenzyme is to maintain intracellular concentrations of glutathione, the reserves of which are intensively used in acute APAP injury (Chidiac et al., 2023; Puukka K. et al., 2006). The increased activity of GGT may be a response to oxidative stress, promoting increased transport of glutathione precursors into the cell (Puukka et al., 2006). Based on the fact that the toxicity of DQ, in contrast to paracetamol, did not affect the level of GGT in the blood serum, it is likely that acetaminophen is a direct inducer of GGT synthesis in animals with APAP lesions, regardless of age. The most pronounced manifestations of intrahepatic cholestasis were observed in animals of 360 days of age with APAP lesions and in adolescent rats (60 days) after diquat administration.

An additional confirmation of intrahepatic cholestasis manifestations is an increase in the level of total bilirubin in the blood serum of adolescent rats (60 days) by 52 %, reproductive rats (150 days) – by 65 %, mature rats (360 days) – by 60 % ($p \leq 0.001$) with APAP lesions compared with the values of the corresponding control values. At the same time, the introduction of toxic doses of diquat was accompanied by a more pronounced increase in total bilirubin in animals of 60 days of age (adolescent age) by 69 % and 150 days of age (young age) by 61 % compared to the control ($p \leq 0.001$) (Table 3).

Table 3.

Bilirubin levels in blood serum of rats of different ages under conditions of acetaminophen- and diquat-induced toxic injury

Group	Bili-T (μmol/l)	Bili-D (μmol/l)	Bili-ID (μmol/l)
Adolescent age (60 days)			
Control	1.5 ± 0.3	0,8 ± 0,1	0,7 ± 0,2
APAP	3.2 ± 0.7 [a]	1,9 ± 0,2 [a, c]	1,3 ± 0,6 [a, c]
DQ	4.9 ± 0.4 [a]	1,3 ± 0,1 [b]	3,6 ± 0,3 [b]
Young age (150 days)			
Control	0.8 ± 0.1	0,7 ± 0,1	0,2 ± 0,1
APAP	2.4 ± 0.3 [a]	1,4 ± 0,1 [a, c]	1,1 ± 0,2 [a]
DQ	2.2 ± 0.1 [a]	0,7 ± 0,1	1,5 ± 0,2 [a]
Mature age (360 days)			
Control	1,7 ± 0,2	1,1 ± 0,1	0,6 ± 0,2
APAP	4,3 ± 0,4 [a, c]	1,0 ± 0,1	3,3 ± 0,2 [a, c]
DQ	3,1 ± 0,3 [b]	0,8 ± 0,1	2.3 ± 0,3 [b]

In adolescent rats (60 days), an increase in total bilirubin levels under conditions of toxic damage to the APAP by 58 % ($p \leq 0.05$) occurs due to the content of direct bilirubin, while in case of poisoning with the industrial xenobiotic diquat, it is due to an increase in the level of its indirect fraction (by 80 %, $p \leq 0.001$) compared to control values. At the same time, in rats of 150-day (young age) and 360-day (mature age), regardless of the nature of the toxin intake, the increase in total bilirubin levels was mainly due to its indirect fraction (young age APAP and DQ – by 86% and 90%, mature age APAP and DQ – 81% and 72%, respectively, compared with the control) (Table 3).

The causes of the increase in total bilirubin levels in 150-day-old and 360-day-old rats, regardless of the nature of the toxin intake, mainly due to indirect bilirubin (indirect hyperbilirubinemia), may be excessive hemolysis and impaired capture and binding of free bilirubin by the liver. The increase in its concentration indicates the development of endogenous intoxication (Wolkoff, 2014). In the bloodstream, bilirubin binds to blood transport proteins, albumin. That is why indirect bilirubin is not filtered by the kidneys. Given that experimentally confirmed hypoalbuminemia occurs in APAP-induced lesions, indirect bilirubin does not have time to bind to blood proteins and quickly penetrates cell membranes to various tissues, which disrupts its uptake by hepatocytes. Bilirubin is displaced from plasma albumin, forms a temporary association with plasma membrane lipids, and passes through them via transport systems. Therefore, it is believed that high levels of bilirubin have a pronounced membrana toxic effect (Siqueira et al., 2019). It is worth noting that under the conditions of receiving supra-therapeutic doses of acetaminophen, when the pathway of its conjugation with glucuronate with the participation of the enzyme UDP-glucuronyltransferase is saturated, a significant

part is subjected to oxidative metabolism by the CYP450 system (Caparrotta et al., 2017). Given that UDP-glucuronyltransferase is simultaneously involved in the detoxification of indirect bilirubin, functional redistribution of this enzymatic activity may block the process of bilirubin conjugation. In turn, when diquat was administered in toxic doses, the most significant increase in the level of total bilirubin due to the content of its indirect fraction was observed in adolescent (60 days) and reproductive (150 days) rats. Given the above information, the fact we have established may be a sign of a functional disorder of the membrane-bound transport system involved in the uptake of indirect bilirubin. In addition to the changes we have shown, the literature reports that acetaminophen-induced injury leads to a decrease in iron levels in bile, whereas under conditions of injury with hepatotoxic doses of diquat, there is presence of a prolonged period of iron excretion in bile. It is noted that the observed changes likely indicate that a portion of the iron is excreted in bile as reversibly formed GS-Fe^{2+} chelates, with the inhibition of iron export by glutathione disulfide (GSSG) in the case of diquat-induced toxic injury or by 3-(glutathion-S-yl)-acetaminophen (GS-AAP) in the case of acetaminophen-induced toxic injury (Benzick et al., 1994).

Conclusions. The modeling of toxic damage by the medicinal xenobiotic acetaminophen and the industrial xenobiotic diquat causes the development of morphological and functional changes in the hepatobiliary system of rats of different age groups. Regardless of the nature of the xenobiotics used in the study as model toxins, the increase in the liver organ index illustrates an excessive toxic load on the organ in all age groups of animals - adolescent (60 days), reproductive (150 days) and mature (360 days). A more pronounced hepatotoxic effect under acetaminophen intoxication is most pronounced in 150-day and 360-day old animals, while under diquat-induced poisoning – in 60-day old rats, as evidenced by the corresponding percentage increase in the absolute liver weight.

Under the conditions of administration of toxic doses of acetaminophen, the most significant increase in the enzymes-markers of cytolytic syndrome – ALT and AST against the background of a maximum decrease in the de Ritis coefficient, indicates the predominance of hepatic ALT release into the bloodstream mainly in mature rats. In animals of this age group, the occurrence of the most pronounced cholestatic syndrome, manifested by an increase in ALP and GGT activities, and the level of total bilirubin due to its indirect fraction, was also recorded upon administration of supra-therapeutic doses of paracetamol. On the contrary, diquat-induced toxic injury is accompanied by a maximum increase in the indicator activities of cytolysis – ALT and AST, cholestasis – ALP and severe hyperbilirubinemia of the unconjugated type in adolescent rats.

Additional biomarkers of the pronounced hepatotoxic potential of the drug xenobiotic acetaminophen in contrast to the herbicide diquat are the increase in LDH and GGT activities in all age groups of rats. Thus, there are multidirectional age differences in the adverse effects of acetaminophen and diquat on indicators of the morphological and functional state of the liver. The most sensitive age group of animals to acetaminophen toxicity are rats of 360 days of age, and diquat – 60 days of age.

Interests disclosure. *The authors declare no conflict of interest*.

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БІОХІМІЧНІ ПРОЯВИ ГЕПАТОТОКСИЧНОГО УРАЖЕННЯ РІЗНОВІКОВИХ ГРУП ЩУРІВ КСЕНОБІОТИКАМИ РІЗНОГО ГЕНЕЗУ

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Нині проблема захворювань печінки на тлі токсичної дії на організм медикаментозних та промислових (гербіцидів) ксенобіотиків набуває все більшої актуальності. Активація компенсаторних механізмів у відповідь на дію токсичних агентів – ацетамінофену та/або диквату тісно пов'язані з віковими особливостями. Загострення уваги до ацетамінофен-індукованого ураження пов'язано з широким застосуванням даного медикаментозного препарату як анальгетика/антипіретика при пандеміях інфекційних і запальних захворювань, особливо за умов ведення бойових дій в Україні. Натомість, широке використання гербіциду диквату супроводжується зростанням реєстрації випадів дикватоіндукованих гострих отруєнь.

Метою роботи є оцінка біохімічних показників функціонального стану печінки різновікових щурів за умов ацетамінофен- та дикватоіндукованого токсичного ураження. Тварини були поділені на три вікові групи: щури підліткового (60 днів), репродуктивного (150 днів) та зрілого віку (360 днів). Моделювання гострого токсичного ураження ацетамінофеном здійснювали шляхом його перорального введення методом інтубації шлунку в добовій дозі 1250 мг/кг маси тіла тварини протягом 2 останніх діб експерименту. Гостре токсичне ураження дикватом моделювали внутрішньошлунковим одноразовим введенням за допомогою зонду в дозі 115,5 мг/кг маси тіла тварини. Функціональний стан печінки оцінювали на автоматичному біохімічному аналізаторі HTI Biochem FC-120.

Ацетамінофенова інтоксикація призводила до зростання абсолютного показника маси печінки, органного індексу, зміни макроскопічної структури органу, зростання у сироватці крові активностей ALT та AST, загальної LDH, зниження коефіцієнту де Рітіса на тлі зростання активностей ALP, GGT, рівня загального й непрямого білірубіну в усіх різновікових групах порівняно з контролем. Найсуттєвіші зміни простежуються у тварин зрілого віку (зростання активності ALT на 76 %; AST на 56 %; ALP на 51 %; GGT на 51 % та зниження коефіцієнту де Рітіса на 47 %). За умов дикват-індукованого токсичного ураження спостерігається дещо інша тенденція та ступінь прояву встановлених змін. Вираженіший гепатотоксичний ефект зареєстровано в тварин підліткового віку, що проявляється максимальним зростанням абсолютного показника маси печінки на 26 %, активності ALT на 71 %, AST на 47 %, ALP на 50 %, рівня Bili-T на 69 %. Статистично значущих відмінностей порівняно з контролем та APAP-індукованим ураженням у активності загальної LDH та GGT при введенні токсичних доз гербіциду диквату незалежно від вікової категорії не спостерігається.

Виходячи з результатів експериментів простежується вікова різноспрямованість у несприятливій дії медикаментозного ксенобіотика ацетамінофену та промислового ксенобіотика диквату на показники морфо-функціонального стану печінки. Чутливішою віковою групою тварин до токсичного ураження ацетамінофеном є щури 360-денного віку, тоді як дикватом – 60-денного віку.

Ключові слова: аланінамінотрансфераза, аспартатамінотрансфераза, коефіцієнт де Рітіса, лужна фосфатаза, гамма-глутамілтрансфераза, білірубін, ацетамінофен, дикват, токсичне ураження, печінка

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