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MANIFESTATION OF TOXIC ACTION OF PARACETAMOL IN FEMALE AND MALE RATS DEPENDING ON THE CIRCADIAN RHYTHMS OF LIVER ACTIVITY

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Abstract

Today, the toxicity of paracetamol is very common, this is due to both the widespread availability of the drug and the misconception of its high safety. Paracetamol poisoning is the most common cause of severe acute liver damage.

Acute toxic hepatitis in rats of both sexes was simulated by administration of paracetamol at a dose of 1000 mg/kg of rat as a suspension in a 2% starch gel solution. The studied model of hepatitis was reproduced in chronodetermined mode, ie, the toxic dose of paracetamol was administered to rats at fixed hours and periods of the day: 09.00 (morning), 15.00 (day), 21.00 (evening), and 03.00 (night), so the model is interpreted as acute chronodetermined paracetamol-induced hepatitis (ACPH). In animals of the control pathology groups, blood sampling and liver isolation for further studies were performed 24 hours after administration of paracetamol.

Based on the analysis of features of liver biorhythms changes depending on the period when acute hepatitis was modeled the circadian dependence of hepatotoxic action of paracetamol has been established. It has been found that modeling of hepatitis at night (03.00) was characterized by a medium degree of hepatotoxicity of paracetamol during the day. It has been established that the morning period (similar to the night period) was also characterized by a medium degree of cytotoxicity of paracetamol. The most pronounced suppression of liver function by the value of markers of cytolysis was observed in the simulation of pathology in the evening (21.00) in animals of both sexes. It has been determined that the least pronounced cytodestructive effect of paracetamol in the simulation of toxic hepatitis was observed at daytime (15.00). There are no intersex differences in the hepatotoxic effects of paracetamol on the example of female and male rats under the conditions of administration of paracetamol to animals in doses that exceeded therapeutic levels and led to the development of acute paracetamol hepatitis modeled in chronodetermined mode.

Keywords: *toxic action of paracetamol, circadian rhythms, liver activity.*

Introduction

Today, the toxicity of paracetamol is very common, this is due to both the widespread availability of the drug and the misconception of its high safety [**Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**]. Although the National Institute of Health and Care Excellence (NICE) published a draft guide in August 2020 in which paracetamol is not recommended for the treatment of chronic pain due to its unfavorable benefit-risk profile [11], the latest statistics indicate a progressive annual increase in paracetamol use worldwide [10].

Paracetamol poisoning is the most common cause of severe acute liver damage [**Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**]. Some of such patients with acute liver poisoning develop metabolic acidosis, indicating mitochondrial toxicity of this xenobiotic [**Errore. L'origine riferimento non è stata trovata.**]. The formation of the toxic paracetamol metabolite - N-acetyl-p-benzo-quinone imine (NAPQI) occurs with the participation of the cytochrome P 450 system, namely its isoforms - CYP 2E1, CYP 1A2, and CYP 3A4 [**Errore. L'origine riferimento non è stata trovata.**]. At therapeutic doses of paracetamol, the formed metabolite is completely neutralized by binding to reduced glutathione (GSH), however, when toxic doses of paracetamol enter the body, the reserves of endogenous reduced glutathione are depleted and the formed NAPQI has a damaging effect on hepatocytes by activating the oxidative stress. People with reduced levels of reduced glutathione in liver cells (30% of normal content) are more prone to the possible development of hepatotoxic effects of paracetamol even at its therapeutic doses [**Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**]. Also in recent years, there have been data on the neurotoxic properties of paracetamol [**Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**, **Errore. L'origine riferimento non è stata trovata.**].

All the above data indicate the importance of finding promising ways to prevent the toxicity of paracetamol. One of the options to increase the safety of paracetamol is the use of the latter, taking into account the circadian features of the manifestation of its toxicity, ie the use of chronopharmacological approach [**Errore. L'origine riferimento non è stata trovata.**]. Therefore, this study aimed to investigate the diurnal (circadian) manifestation of the hepatotoxic effect of paracetamol in rats of both sexes when administering the latter to animals in doses exceeding therapeutic.

Methods

Acute toxic hepatitis in rats of both sexes was simulated by administration of paracetamol at a dose of 1000 mg/kg of rat as a suspension in a 2% starch gel solution [18]. The studied model of hepatitis was reproduced in chronodetermined mode, ie, the toxic dose of paracetamol was administered to rats at fixed hours and periods of the day: 09.00 (morning), 15.00 (day), 21.00 (evening), and 03.00 (night), so the model is interpreted as acute chronodetermined paracetamol-induced hepatitis (ACPH). In animals of the control pathology groups, blood sampling and liver isolation for further studies were performed 24 hours after administration of paracetamol [18].

Serum was obtained from whole blood according to conventional methods [18, 24]. In all experimental groups of rats of intact control, control pathology and groups of animals injected with drugs in the serum were determined: the activity of ALAT and ASAT using the Reitman-Frenkel reaction; glucose level - glucose oxidase method; corticosterone - enzyme-linked immunosorbent assay; the content of total bilirubin by caffeine reagent by the Yendrashik method; cholesterol content - enzymatically, according to the concentration of quinonymine formed, which is proportional to the content of this lipid; ALP activity - kinetically by the rate of n-nitrophenol formation, which is directly proportional to the activity of the enzyme [24]. The above indicators of our study are the classic biochemical markers, the analysis of which allows assessing carbohydrate and lipid metabolism, the state of excretory and

detoxification processes in rats, in which the liver is directly involved [23]. Determination of the studied indicators was performed using standard kits by SPE "Philisit-Diagnostics" (Ukraine), LLC "SpineLab" (Ukraine) and Corticosterone EIA Kit - Enabling Discovery in Life Sciences (Japan).

Indicators that reflect the prooxidant-antioxidant balance of the body: the content of TBA-AP, GSH, SOD, and catalase activity and glycogen as an indicator of carbohydrate metabolism were determined in the liver homogenate. TBA reactants were determined by the method [30], to determine GSH used a modification of G. L. Ellman's method [26], catalase activity was determined by the amount of hydrogen peroxide decomposed per unit time [25], and the activity of SOD was determined kinetically by determining the degree of inhibition of adrenaline SOD autooxidation [29]. Glycogen content was diagnosed by the anthrone method [27].

Analysis of circadian rhythms based on the values of the studied indicators in the morning, day, evening, and night periods allows to objectively assess the state of circadian rhythms, and the choice of 09.00, 15.00, 21.00, and 03.00 hours for the study is based on average hours in the morning, day, evening and night, respectively [31]. The selection of both sexes of rats to study circadian rhythms of liver function in physiological conditions and in desynchrony against the background of paracetamol hepatitis is explained by the desire to study the state of circadian rhythms in the above conditions, taking into account the sex of animals, due to differences in literature [31, 32].

The analysis of the obtained experimental data was performed using the following chronobiological nomenclature: acrophase (AF) - time of day when the maximum value of the studied indicator is registered; bathyphase (B) - time of day when the value of the studied indicator is minimal; mesor (M) - the average value of the studied indicator during the day; amplitude (A) - the maximum deviation of the studied indicator in two directions from the mesor [31]. Mesor and amplitude were determined using Cosinor-Analisis 2.4 for Excel 2000/XP software [2].

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The nonparametric Mann-Whitney test was used. When comparing the statistics was to take the significance level $p < 0,05$ was taken [21].

Chronopharmacological study was conducted in the spring season (March) on rats of both sexes weighing 220-250 g in compliance with all bioethical standards [6]. The animals were in the vivarium of the NUPh CSRL with a controlled temperature regime and relative humidity, on a day/night cycle that corresponded to the natural one in the studied season of the year. To neutralize the influence of light factor on the synthesis of melatonin in the evening and night, the study was performed under an infrared lamp, the radiation of which does not fall in the wavelength range 450-485 nm, ie does not excite retinal ganglia containing melanopsin pigment sensitive to the light of this region of the spectrum, and accordingly, the process of melatonin synthesis is not disturbed [28].

Results and Discussion

The administration of paracetamol led to the development of acute toxic hepatitis in rats, which was characterized by desynchrony of the functional activity of the AOP system, which was reflected in changes in circadian rhythms of key components (catalase and SOD activity, GSH content). In particular, in females, regardless of the studied group of control pathology (03.00; 09.00; 15.00; 21.00) the content of GSH decreased almost equally - by 18-20%, while in males this decrease was different (table. 1). Thus, the modeling of pathology at 15.00 was characterized by a decrease in the content of GSH by 20% relative to intact animals, while at 09.00 and 21.00 - by 40% and by 48% at 03.00. In all the above cases, the decrease in GSH content in females and males of control pathology relative to intact animals had a clear trend but was not statistically significant (table. 1). The decrease in the content of GSH in circadian groups of control pathology was confirmed by changes in the mesor rhythm of this indicator - 1.3 times in females and 1.5 times in males relative to intact animals. The amplitude of the rhythm of GSH content in males remained unchanged, while in females it decreased by 1.3 times relative to intact rats (Table. 2). It should be noted that rats with hepatitis in animals of both sexes retained the architectonics of the rhythm of GSH content, which was characteristic of healthy rats, as evidenced by the synchrony of

acrophases (15.00) and bathyphases (03.00) of the content of this indicator between intact and control animals.

The circadian activity of SOD did not change significantly against the background of the introduction of paracetamol to females and males in all four study periods of the day (Table 1), which is also confirmed by the absence of changes in the mesor values of this indicator in rats of both sexes (Table 2). There was a decrease in the amplitude of the rhythm of SOD activity in males (3.4 times) with its invariability in females (Table 2). Also in males, there was a shift in the period of the bathyphase from 21.00 to 15.00 (Table 1).

In contrast to the circadian activity of SOD on the background of pathology, the rhythm of catalase activity underwent significant changes during the day. In particular, the circadian peak of activity (acrophase) of this enzyme, which was observed in intact animals of both sexes at 15.00, was leveled. Modeling of paracetamol hepatitis led to a significant decrease in catalase activity in this period by 1.6 times in females and 1.8 times in males, which was reflected in a significant decrease in the amplitude of the rhythm of this enzyme (Table 1; Table 2). It should be noted that the mesor of catalase activity rhythm did not decrease significantly: by 15% in females and by 18% in males (Table. 2). The acrophase of activity of this enzyme was shifted in males from 15.00 to 03.00, while in females it was as in intact animals. Also, under conditions of pathology, the bathyphase of catalase activity was shifted from 03.00 to 09.00 in females and from 09.00 to 21.00 in males (Table 1).

Against the background of paracetamol hepatitis, the level of TBA-AP in all studied groups of animals of control pathology did not change in comparison with intact control. Under conditions of pathology, periods of acrophase (21.00) and bathyphase (09.00) of TBA-AP content, which are characteristic of intact animals, were preserved (Table 1). This was also characteristic of the mesor and rhythm amplitude of this indicator - no significant differences were observed compared with intact rats (Table 2). It is known that the severity of the manifestation of hepatotoxicity of xenobiotics largely depends on the circadian rhythm of activity of the prooxidant-antioxidant balance of liver cells [4]. The circadian dependence of paracetamol cytotoxicity was assessed by changes in the activity of cytolysis markers under conditions of pathology. In particular, modeling of hepatitis at night (03.00) was characterized by an increase in the activity of ALAT and ASAT in 1.7 and 2.5 times in females and 2.7 and 3.1 times in males, respectively, compared with the intact control group (Table 3). The development of toxic hepatitis in the morning (09.00) was characterized by a significant increase in ALAT activity 2.5 times in females and 1.8 times in males, and ASAT activity 3.0 and 3.2 times, respectively. Simulation of hepatitis during the day (15.00) was associated with the least pronounced changes in ALAT activity, which did not change in females and increased 1.4 times in males, and ASAT activity increased 1.5 and 1.6 times, respectively, in these sexes. The toxic effect of paracetamol was most pronounced in rats of both sexes in the evening (21.00), which is confirmed by a significant increase in ALAT activity 3.4 times in females and 2.7 times in males and ASAT activity - 3.4 and 3.2 times, respectively (Table. 3).

The above analysis of the increase in the activity of transaminases in all studied groups of control pathology was confirmed by changes in the value of the mesor and the amplitude of the rhythm of these enzymes (table. 4).

The desynchrony of the circadian rhythm of transaminase activity was confirmed by the shift of the acrophase and bathyphase periods of the activity rhythm of these enzymes. In particular, the acrophase of the ALAT activity rhythm in females shifted from 15.00 to 21.00, in males from 15.00 to 03.00, while the bathyphase shifted from 21.00 to 15.00 in rats of both sexes. The acrophase of the ASAT activity rhythm was shifted from 15.00 to 09.00 in females and from 15.00 to 03.00 in males, and the bathyphase from 21.00 to 15.00 in animals of both sexes (Table. 4).

The shift of acrophase and bathyphase periods of cytolysis markers is due to different hepatotoxicity of paracetamol during the day. In particular, as noted above, in females and males the maximum toxic effect of paracetamol was observed at 21.00: ALAT activity is 232% higher than the activity of the enzyme in control pathology during the day at 15.00 (period of least damaging paracetamol) in females and 176% in males, and under similar conditions, ASAT activity was 122% and 128% higher, respectively.

Reproduction of control pathology in the morning (09.00) was characterized by a higher content of ALAT activity (relative to the daily group of control pathology) by 188% in females and 117% in males, and ASAT activity - by 156% and 150%, respectively, under similar conditions. In the simulation of hepatitis at night (03.00), the activity of ALAT was 33% higher in females and 89% higher in males than in pathology during the day (15.00), and ASAT - by 29% and 54%, respectively. Therefore, the least toxic effect of paracetamol was in the afternoon (15.00) in animals of both sexes, in which the bathyphase of ALAT and ASAT activity was registered (Table 3).

Of course, the glutathione system is not the only one in ensuring the homeostasis of cells AOP, but its role in paracetamol metabolism is crucial [8], which is confirmed by the obtained statistical results.

Thus, the development of acute paracetamol hepatitis was characterized by desynchrony of circadian rhythms of key components of the AOP system. The GSH content decreased most clearly (the results above), with a more pronounced decrease in males relative to females. Desynchrony of the circadian rhythm of SOD activity was characterized by a shift in the periods of acrophase and bathyphase with insignificant changes in the value of the mesor and the amplitude of the rhythm (males) of this enzyme. Under conditions of pathology, a pronounced acrophase of the rhythm of catalase activity in rats of both sexes, which was observed at 15.00, was leveled, so the rhythm of enzyme activity was "smoothed", which was confirmed by a decrease in rhythm amplitude. Regarding the circadian rhythm of TBA-AP content in animals of both sexes with pathology, it completely repeated the architectonics of the rhythm of this indicator in intact animals of the corresponding circadian groups (Table 3). Regarding the gender features of the implementation of the hepatotoxic effect of paracetamol on the state of prooxidant-antioxidant balance and the activity of cytolysis markers, they were practically absent.

Under conditions of acute paracetamol hepatitis development, the serum glucose content of animals of both sexes did not undergo significant changes in comparison with intact animals (Table 5). Desynchrony of glucose content rhythm was characterized by a shift in the acrophase period of glucose rhythm in animals of both sexes from 03.00 to 21.00, while bathyphase, as in intact animals, was observed at 09.00 in females and males (Table 5). Insignificant differences were in the values of glucose mesor: (4% in females and 10% in males) and the amplitude of glucose rhythm (12% in females and 31% in males) compared with intact animals (Table 6).

Under the conditions of experimental hepatitis, a decrease in glycogen content was observed in all pathologies of the studied periods. In particular, the simulation of pathology at 03.00 was characterized by a significant decrease in glycogen content by 39% in females and 53% in males relative to intact animals, while at 09.00 glycogen content in similar conditions significantly decreased by 44 and 48%, respectively (Table 5)

The minimum decrease in glycogen content was observed during the day (15.00) by 6% in females and by 15% in males and at night (21.00) - by 33% and 20%, respectively. 5). The decrease in glycogen in all circadian groups of pathology was accompanied by a significant decrease in the mesor rhythm of this indicator by 31% in females and 35% in males. The amplitude of the rhythm decreased 2 times in females and was almost unchanged in males. The acrophase of glycogen content in females and males was shifted from 03.00 to 15.00 with the preservation of the bathyphase (09.00), characteristic of intact animals in rats of both sexes (Table 6).

The content of corticosterone had no significant differences in animals of control pathology and intact control, which was confirmed by insignificant changes in mesor value (7%) in animals of both sexes and rhythm amplitude - more pronounced in females (21% relative to rats with hepatitis) and almost without changes in males (Table 4.7). Periods of acrophase (03.00 - females and males) and bathyphase (15.00 - females; 09.00 - males) of corticosterone content rhythm of control animals are synchronous with these indicators in animals of intact control groups (Table 6).

Thus, against the background of the development of acute paracetamol hepatitis, the content of glucose and corticosterone did not change significantly compared with the intact control groups, while the glycogen content decreased in females and males, but the circadian dynamics of

the indicators remained. The most pronounced decrease in the content of this indicator was observed in the morning (09.00) in females by 44% and at night (03.00) in males by 53% compared with intact animals. The daytime period (15.00) was characterized by a minimal decrease in glycogen content in animals of both sexes: 6% in females (unreliable) and 15% in males.

Regarding the sexual characteristics of carbohydrate metabolism disorders in experimental hepatitis, they are not significantly different between females and males.

According to the results, under the conditions of toxic hepatitis, there was no significant change in cholesterol. This indicator increased by only 6-15% in rats of both sexes, which was confirmed by the absence of changes in the size of the cholesterol rhythm mesor (Table 7; Table 8).

There was a tendency to reduce the amplitude of cholesterol rhythm in females and males by 21% and 29%, respectively, which indicates a "smoothing" of the rhythm on the background of pathology, ie desynchrony. The period of acrophase in animals of both sexes was shifted from 15.00 to 09.00 while maintaining the period of bathyphase characteristic of intact animals (Table 8).

Against the background of pathology, there was an increase in the content of total bilirubin in some circadian groups with hepatitis. Thus, the simulation of pathology at 03.00 was characterized by a significant increase in the level of this indicator by 44% in females and 32% in males, at 09.00 the total bilirubin in similar conditions significantly increased by 45% in females and 42% in males. There was no significant increase in total bilirubin in pathology only at 15.00 in animals of both sexes. Administration of paracetamol to a group of animals of control pathology at 21.00 was characterized by an increase in total bilirubin by 24% in females and 37% in males (Table 8). A significant increase in total bilirubin on the background of pathology was confirmed by an increase in the mesor of the rhythm of this indicator by 27% in females and 28% in males.

The results of the data shown in table 8 show that the rhythm amplitude of total bilirubin did not have significant differences in control animals compared with intact rats of both sexes (Table 8). In females on the background of hepatitis, the periods of acrophase (15.00) and bathyphase (03.00) of the rhythm of the content of this indicator, which are characteristic of intact rats, were preserved, while in males the acrophase was shifted from 15.00 to 09.00 and the bathyphase (03.00) was preserved (Table 8).

Similar to the content of total bilirubin against the background of the acute paracetamol hepatitis development, a change in the activity of the cholestasis marker enzyme - alkaline phosphatase (ALP) was observed. In particular, in the control pathology at 03.00 the activity of this enzyme significantly increased by 44% in females and 68% in males, while in the morning (09.00) it significantly increased by 38% in females and trendy by 19% in males. However, against the background of the development of the pathological process in the afternoon (15.00), there was a trend to reduce the activity of ALP by 23% in females and 10% in males. Toxic hepatitis simulated in the evening (21.00), was characterized by an increase in ALP activity by 29% in females and the absence of significant changes in males (Table 7). The mesor of the rhythm of ALP activity increased by 15% in animals of both sexes, while the amplitude decreased 5.2 times in females and had no significant differences in males (Table. 8). The period of acrophase of the rhythm of ALP activity, which was registered in intact rats of both sexes at daytime (15.00) on the background of pathology was shifted to 09.00 in females and 03.00 in males, while the bathyphase at night (03.00) was preserved in females and shifted to the evening (21.00) in males (Table 8).

Regarding the sex differences of desynchrony changes in the indicators of excretory and detoxification processes against the background of ACPH according to chronoparameters, only differences in the amplitude of cholesterol rhythm (by 50%) and ALP (2.5 times) in males relative to females were recorded, at the same amplitude of total bilirubin and mesor rhythm of the above indicators in rats of both sexes. Thus, under conditions of acute toxic hepatitis caused by the administration of paracetamol at different times of the day, fluctuations in cholesterol levels were observed to a lesser extent in all studied periods of the day, while total bilirubin and ALP activity varied depending on the time of hepatitis simulation. In particular, in hepatitis, reproduced at 03.00 and

09.00, there was the most pronounced increase in total bilirubin and ALP activity compared with other study periods. Daytime hours (15.00) were characterized by the absence of changes in total bilirubin and ALP activity. While in the evening (21.00) these figures increased, but less clearly compared with animals with hepatitis, which was simulated at night and in the morning.

CONCLUSIONS:

Based on the analysis of features of liver biorhythms changes depending on the period when acute hepatitis was modeled the circadian dependence of hepatotoxic action of paracetamol has been established.

1. It has been found that modeling of hepatitis at night (03.00) was characterized by a medium degree of hepatotoxicity of paracetamol during the day: marked by an increase in transaminases activity (ALAT and ASAT 1.7 and 2.5 times in females and 2.7 and 3.1 times in males, respectively); a significant decrease in glycogen content (39% in females and 53% in males) was also observed a significant increase in total bilirubin and a clear trend to increase the activity of ALP in animals of both sexes.
2. It has been established that the morning period (similar to the night period) was also characterized by a medium degree of cytotoxicity of paracetamol: ALAT activity increased 2.5 times in females and 1.8 times - in males, and ASAT activity - 3 times and 3.2 times, respectively; GSH content decreased by 18% in females and 48% in males, glycogen levels by 44% and 48%, respectively. Under the conditions of modeling hepatitis during this period, the content of total bilirubin increased by 45% and 42% in females and males, respectively, while the activity of ALP increased by 1.2 and 1.4 times under similar conditions.
3. The most pronounced suppression of liver function by the value of markers of cytolysis was observed in the simulation of pathology in the evening (21.00) in animals of both sexes. The activity of ALAT and ASAT significantly increased 3.4 times in females and 2.7 and 3.2 times in males, respectively; GSH content decreased by 18% and 40% in females and males, and glycogen content decreased by 33% and 20% under similar conditions. The content of total bilirubin increased by 24% in females and by 37% in male rats, and the activity of ALP - 1.3 times in females and did not change in males.
4. It has been determined that the least pronounced cytodestructive effect of paracetamol in the simulation of toxic hepatitis was observed at daytime (15.00): ALAT activity of control females did not differ from that of intact animals, and ASAT increased 1.5 times, while in males - 1.4 times ALAT and 1.6 times ASAT activity. During this period, the GSH content decreased by 19% in females and by 20% in males. It should be noted about the leveling in this period in animals of both sexes of the circadian acrophase content of catalase and ALP activity characteristic of intact rats. In animals of both sexes, the content of glycogen, total bilirubin do not differ from similar indicators in intact animals.
5. There are no intersex differences in the hepatotoxic effects of paracetamol on the example of female and male rats under the conditions of administration of paracetamol to animals in doses that exceeded therapeutic levels and led to the development of acute paracetamol hepatitis modeled in chronodetermined mode.

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Table 1. Changes in circadian rhythms of prooxidant-antioxidant balance in rats under conditions of ACPH (n = 6-8, *M ± SEM*)

Experimental Parameters		Hour of the day			
		03.00	09.00	15.00	21.00
		Females			
GSH, units	IC	87.52 ± 8.25	105.21 ± 11.65	154.25 ± 13.52$^{\#}$	78.33 ± 14.54
	CP	70.02 ± 19.78	75.49 ± 12.45	123.84 ± 8.57$^{*/\&}$	68.45 ± 16.93
SOD, units	IC	42.59 ± 3.18	48.83 ± 2.59	46.04 ± 3.63	39.36 ± 3.73
	CP	39.16 ± 3.94	43.48 ± 2.56	43.00 ± 2.84	35.34 ± 2.88
catalase, μkat/L	IC	43.60 ± 2.89	50.46 ± 4.24	78.22 ± 3.74$^{\#}$	50.08 ± 3.07
	CP	48.15 ± 6.76	46.27 ± 5.56	48.98 ± 8.19 *	46.58 ± 5.25
TBA-AP, μmol/g	IC	25.85 ± 2.85	16.24 ± 2.36	17.21 ± 3.37	30.51 ± 0.81$^{\#}$
	CP	29.06 ± 3.88$^{\&}$	16.23 ± 3.60	19.02 ± 2.03	33.12 ± 3.61$^{\&}$
		Males			
GSH, units	IC	70.74 ± 17.93	105.65 ± 11.0	157.75 ± 13.58$^{\#}$	89.71 ± 17.47
	CP	36.54 ± 12.99	62.98 ± 13.44	125.81 ± 14.82$^{\&}$	47.68 ± 13.27
SOD, units	IC	47.09 ± 3.65	48.35 ± 4.26	44.28 ± 3.92	40.16 ± 4.49
	CP	40.34 ± 4.98	43.16 ± 2.29	39.88 ± 2.50	40.67 ± 4.15
catalase, μkat/L	IC	45.77 ± 6.49	48.13 ± 5.86	81.37 ± 1.32$^{\#}$	50.27 ± 5.83
	CP	52.05 ± 5.96	44.80 ± 8.24	44.32 ± 9.64 *	40.81 ± 8.99
TBA-AP, μmol/g	IC	26.93 ± 2.24	16.12 ± 4.98	19.74 ± 7.08	34.40 ± 2.85$^{\#}$
	CP	28.20 ± 3.74$^{\&}$	16.02 ± 3.39	18.37 ± 4.00	31.84 ± 2.70$^{\&}$

Notes: ACPH - acute chronodetermined paracetamol-induced hepatitis; n is the number of animals in the group; * - deviation of the indicator is significant relative to the indicator of intact animals (p <0,05); # - deviation of the indicator is significant relative to the minimum indicator in the group of intact animals (p <0,05); & - deviation is significant relative to the minimum in the group of control pathology (p <0,05).

Table 2. Desynchrony of circadian rhythms of prooxidant-antioxidant balance indicators under conditions of ACPH according to Cosinor-Analisis 2.4 for Excel 2000/XP software

Experimental conditions (n = 6-8)	IC		CP	
	Mesor	Amplitude	Mesor	Amplitude
	Females			
GSH, units	106.33	35.97	84.45	27.14
SOD, units	44.20	5.04	40.24	4.50
catalase, μkat/L	55,59	17.31	47.49	0.44
TBA-AP, μmol/g	22.45	8.34	24.36	9.82
	Males			
GSH, units	105.96	44.23	68.25	45.29
SOD, units	44.97	4.33	41.01	1.27
catalase, μkat/L	55.38	17.83	45.49	4.35
TBA-AP, μmol/g	24.30	9.82	23.61	9,31

Notes: ACPH - acute chronodetermined paracetamol hepatitis; n is the number of animals in the group.

Table 3. Changes in the circadian rhythm of activity of cytolysis markers under conditions of ACPH (*n = 6-8, M ± SEM*)

Experimental Parameters		Hour of the day			
		03.00	09.00	15.00	21.00
		Females			
ALAT, µmol/h*mL	IC	0.97 ± 0.12	0.94 ± 0.05	1.19 ± 0.09$^{\#}$	0.87 ± 0.05
	CP	1.68 ± 0.33	2.36 ± 0.44$^{*/\&}$	1.26 ± 0.10	2.92 ± 0.22$^{*/\&}$
ASAT, µmol/h*mL	IC	0.74 ± 0.16	0.75 ± 0.09	0.95 ± 0.05$^{\#}$	0.51 ± 0.09
	CP	1.86 ± 0.22 *	2.25 ± 0.23$^{*/\&}$	1.44 ± 0.17 *	1.75 ± 0.23 *
Experimental conditions		Males			
ALAT, µmol/h*mL	IC	0.96 ± 0.06	0.90 ± 0.09	0.97 ± 0.13	0.88 ± 0.06
	CP	2.61 ± 0.09$^{*/\&}$	1.61 ± 0.34	1.38 ± 0.18	2.43 ± 0.20$^{*/\&}$
ASAT, µmol/h*mL	IC	0.72 ± 0.13	0.68 ± 0.07	0.90 ± 0.13$^{\#}$	0.58 ± 0.07
	CP	2.23 ± 0.21$^{*/\&}$	2.17 ± 0.13$^{*/\&}$	1.45 ± 0.19 *	1.86 ± 0.16 *

Notes: ACPH - acute chronodetermined paracetamol-induced hepatitis; n is the number of animals in the group; * - deviation of the indicator is significant relative to the indicator of intact animals ($p<0,05$); # - deviation of the indicator is significant relative to the minimum indicator in the group of intact animals ($p<0,05$); & - deviation is significant relative to the minimum in the group of control pathology ($p<0,05$).

Table 4. Desynchrony of circadian rhythms of cytolysis markers activity in the conditions of ACPH according to the software Cosinor-Analisis 2.4 for Excel 2000/XP *(n = 6-8)*

Experimental Parameters (n = 6-8)	IC		CP	
	Mesor	Amplitude	Mesor	Amplitude
	Females			
ALAT, µmol/h*mL	0.99	0.11	2.06	0.35
ASAT, µmol/h*mL	0.74	0.16	1.83	0.33
	Males			
ALAT, µmol/h*mL	0.93	0.01	2.13	0.90
ASAT, µmol/h*mL	0.72	0.10	1.90	0.44

Notes: ACPH- acute chronodetermined paracetamol hepatitis; n is the number of animals in the group.

Table 5. Changes in circadian rhythms of carbohydrate metabolism under conditions of ACPH (*n = 6-8, M ± SEM*)

Experimental Parameters		Hour of the day			
		03.00	09.00	15.00	21.00
		Females			
Glucose, mmol/L	IC	8.49 ± 0.50	7.04 ± 0.59	7.06 ± 0.49	8.16 ± 0.79
	CP	7.04 ± 0.49	6.79 ± 0.19	7.65 ± 0.37	8.27 ± 0.51
Glycogen, mg/g	IC	3.04 ± 0.20#	1.85 ± 0.18	2.25 ± 0.21	1.96 ± 0.19
	CP	1.85 ± 0.18*/&	1.03 ± 0.10 *	2.12 ± 0.16&	1.32 ± 0.19 *
Corticosterone, pkg/mL	IC	139.97 ± 6.35#	60.52 ± 1.03#	52.12 ± 0.81	106.80 ± 4.90#
	CP	121.48 ± 1.58&	67.12 ± 11.23&	50.35 ± 1.12&	98.17 ± 2.00&
		03.00	09.00	15.00	21.00
		Males			
Glucose, mmol/L	IC	7.07 ± 0.43#	4.34 ± 0.18	5.46 ± 0.35#	7.01 ± 0.70
	CP	6.42 ± 0.60	5.64 ± 0.34	7.46 ± 0.49&	7.56 ± 0.24&
Glycogen, mg/g	IC	3.02 ± 0.10 #	1.92 ± 0.15	2.40 ± 0.11 #	1.76 ± 0.06
	CP	1.43 ± 0.16 *	0.99 ± 0.10 *	2.04 ± 0.06 */&	1.41 ± 0.19
Corticosterone, pkg/mL	IC	146.12 ± 4.67#	57.17 ± 2.32	66.84 ± 6.80	121.65 ± 4.63#
	CP	131.94 ± 4.90&	65.33 ± 12.22	74.68 ± 9.40	124.85 ± 5.10&

Notes: ACPH - acute chronodetermined paracetamol-induced hepatitis; n is the number of animals in the group; * - deviation of the indicator is significant relative to the indicator of intact animals ($p<0.05$); # - deviation of the indicator is significant relative to the minimum indicator in the group of intact animals ($p<0.05$); & - deviation is significant relative to the minimum in the group of control pathology ($p<0.05$).

Table 6. Desynchrony of circadian rhythms of carbohydrate metabolism in the conditions of ACPH according to the software Cosinor-Analisis 2.4 for Excel 2000/XP

Experimental conditions (n = 6-8)	IC		CP	
	Mesor	Amplitude	Mesor	Amplitude
	Females			
Glucose, mmol/L	7.69	0.91	7,44	0.80
Glycogen, mg/g	2.27	0.40	1,58	0.20
Corticosterone, pkg/mL	89.85	49.64	83.83	39.17
	Males			
Glucose, mmol/L	5.97	1.57	6.77	1.09
Glycogen, mg/g	2.27	0.32	1.47	0.37
Corticosterone, pkg/mL	97.94	51.09	104.68	48.49

Notes: ACPH- acute chronodetermined paracetamol hepatitis; n is the number of animals in the group.

Table 7. Changes in circadian rhythms of excretory and detoxification processes under conditions of ACPH (n = 6-8, M ± SEM)

Experimental Parameters		Hour of the day			
		03.00	09.00	15.00	21.00
		Females			
Cholesterol, mmol/L	IC	1.60 ± 0.11	1.70 ± 0.06	1.89 ± 0.06	1.66 ± 0.13
	CP	1.82 ± 0.09	1.95 ± 0.08	1.94 ± 0.06	1.76 ± 0.12
Total bilirubin, µmol/L	IC	9.01 ± 0.39	12.46 ± 0.97#	17.82 ± 1.16#	12.12 ± 0.98#
	CP	13.09 ± 0.40 *	18.12 ± 0.93*/&	18.93 ± 1.20&	15.09 ± 0.96
ALP, U/L	IC	105.97 ± 11.04	140.07 ± 14.74	219.00 ± 38.30#	140.07 ± 21.27
	CP	152.53 ± 12.55 *	193.78 ± 14.47 *	169.00 ± 47.90	180.40 ± 16.39
Experimental Parameters		03.00	09.00	15.00	21.00
		Males			
Cholesterol, mmol/L	IC	1.59 ± 0.12	1.75 ± 0.07	2.04 ± 0.04#	1.60 ± 0.11
	CP	1.69 ± 0.12	1.98 ± 0.08	1.96 ± 0.05&	1.77 ± 0.11
Total bilirubin, µmol/L	IC	9.85 ± 0.61	13.03 ± 1.16	16.28 ± 1.88#	10.34 ± 0.84
	CP	13.01 ± 0.67 *	18.53 ± 0.77*/&	17.74 ± 1.83	14.22 ± 0.80 *
ALP, U/L	IC	117.23 ± 20.04	149.28 ± 11.76	179.60 ± 23.79	143.63 ± 17.10
	CP	197.82 ± 23.54*/&	178.93 ± 18.77	162.80 ± 42.56	138.05 ± 22.01

Notes: ACPH - acute chronodetermined paracetamol-induced hepatitis; n is the number of animals in the group; * - deviation of the indicator is significant relative to the indicator of intact animals ($p<0.05$); # - deviation of the indicator is significant relative to the minimum indicator in the group of intact animals ($p<0.05$); & - deviation is significant relative to the minimum in the group of control pathology ($p<0.05$).

Table 8. Desynchrony of circadian rhythms of indicators of excretory and detoxification processes under conditions of ACPH according to the software Cosinor-Analisis 2.4 for Excel 2000/XP

Experimental conditions (n = 6-8)	IC		CP	
	Mesor	Amplitude	Mesor	Amplitude
	Females			
Cholesterol, mmol/L	1.71	0.14	1.87	0.11
Total bilirubin, μmol/L	12.85	4,41	16.31	3.22
ALP, U/L	151.34	56.65	174.03	10.77
	Females			
Cholesterol, mmol/L	1.75	0.24	1.85	0.17
Total bilirubin, μmol/L	12.38	3.49	15.88	3.20
ALP, U/L	147.44	31.32	169.40	26.91

Notes: ACPH- acute chronodetermined paracetamol hepatitis; n is the number of animals in the group.