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FEATURES OF CIRCADIAN RHYTHMS, INDICATORS OF RAT LIVER FUNCTION UNDER PHYSIOLOGICAL CONDITIONS

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Abstract

The liver is an organ with an extremely broad functional and metabolic profile, which provides energy homeostasis of the body, it plays a leading role among peripheral oscillators Aim of this work was to study features of circadian rhythms of rat liver functions at physiological conditions in two sexes of animals.

Rats were decapitated with a biomaterial fence at the following periods and hours of the day: morning (9 a. m.), afternoon (3 p. m.), evening (9 p. m.) and night (3 a. m.). The evaluation of the daily activity of the liver was carried out by analyzing the dynamics of 16 indicators of carbohydrate, protein, purine metabolism, regulation of prooxidant-antioxidant balance, excretory and detoxification processes. In the blood serum activity of AlAT, AsAT, LPh, the content of total protein, albumin, uric acid, urea, cholesterol, total bilirubin, glucose, corticosterone were determined. In the liver homogenate the glycogen content, RG, TBA and the activity of SOD and catalase were examined.

Analysis of the results allowed to establish that the acrophase of rat liver activity was observed in the afternoon (3 p. m.) that is confirmed by the acrophases of the content of restored glutathione, cholesterol, total bilirubin and the activity of catalase and LPh indicating an increased activity of detoxification processes in this period.

Keywords: *circadian rhythms, liver function.*

Introduction

The ability to determine the dependence of the effectiveness and toxicity of drugs on the time of day or season is the most promising area of modern experimental and clinical pharmacology [18, 46]. The priority of chronobiological research was confirmed by the Nobel Assembly in 2017, which awarded a prize in medicine for research into the molecular mechanisms of control of circadian rhythms [38]. There is a lot of data on the influence of daily and seasonal rhythms on the pharmacological activity of certain drugs. However, the chronological profile of efficacy and safety for most drugs remains unclear [10, 34]. In particular, this applies to drugs that affect the liver, the functions of which are under control of circadian rhythms [45].

The liver is an organ with an extremely broad functional and metabolic profile, which provides energy homeostasis of the body, it plays a leading role among peripheral oscillators [8, 53]. Some scholars consider this organ, along with SCN, to be key in the organization of circadian rhythms. The liver creates a single metabolic and energy pool of the body for the metabolism of proteins, fats, carbohydrates [8, 57]. Therefore, the metabolism of proteins, fats, carbohydrates, enzymes, vitamins; regulation of water, mineral and pigment metabolism; activity of excretory and detoxification processes are characterized by circadian dependence [53, 34].

Aim of this work was to study features of circadian rhythms of rat liver functions at physiological conditions in two sexes of animals.

Methods. Serum was obtained from whole blood according to conventional methods [41, 26]. 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 [26]. 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 [4]. 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 [51], to determine GSH used a modification of G. L. Ellman's method [37], catalase activity was determined by the amount of hydrogen peroxide decomposed per unit time [36], and the activity of SOD was determined kinetically by determining the degree of inhibition of adrenaline SOD autooxidation [50]. Glycogen content was diagnosed by the anthrone method [35].

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 [10]. 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 [10, 13]. 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 [10]. 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 [55].

Chronopharmacological study was conducted in the spring season (March) on rats of both sexes weighing 220-250 g in compliance with all bioethical standards [16]. 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 [47].

Results. SOD activity in animals of both sexes was characterized by circadian stability, which is confirmed by the absence of statistically significant differences between the value of the enzyme activity at separately selected for the experiment time of day. Indistinct acrophase of SOD activity was observed in females and males at 09:00 with inverted bathiphase at 21:00 (differences in values are not significant (Table 1).

SOD daily activity was almost unchanged in rats of both sexes and was confirmed by an insignificant amplitude (Table 2), which is also clearly seen in the chronogram analysis of the enzyme activity in females and males.

The synchronicity of acrophase and bathiphase SOD activity (Table 1), similar architectonics of the rhythm and value of mesor (Table 2) of the enzyme activity in rats of both sexes indicate the absence of differences in daily fluctuations in SOD activity in liver cells of males and females.

It is important to study the circadian dynamics of catalase activity in liver cells, since hepatocytes are characterized by its high content and activity in relativeness to other cells of the body (red blood cells, bone marrow, kidneys and mucous membranes) [40, 48]. Analysis of the circadian rhythm of catalase activity indicates the presence of a significantly expressive acrophase at 15:00 in rats of both sexes with inverted bathyphase at 03:00 (Table 1). The mesor index of the enzyme activity was at the same level in rats of both sexes (Table 2). It should be noted that the activity of catalase in acrophase was by 1.6 times significantly higher than that in bathiphase in females and males, this was confirmed by the expressive amplitude of the rhythm, which is practically the same in animals of both sexes.

Analysis of the chronogram of the circadian activity of catalase in females and males indicates practically the same architectonics of the rhythm, values of this indicator at the same hours of the day do not differ significantly, which indicates the absence of sex differences in circadian fluctuations in catalase activity in rat hepatocytes.

Circadian rhythm of GSH content in liver cells was clearly expressed in rats of both sexes. The GSH content in acrophase (15.00) was 2 times significantly higher than that in the bathyphase in females (21.00) and 2.2 times in males (03.00), which was confirmed by the high amplitude of the rhythm (Table 2). The mezor index of this indicator was at the same level for both sexes (Table 2). Analysis of circadian chronograms of the GSH level of female and male rats indicates presence of such features of the circadian rhythm of this indicator: the evening-night time is characterized by a minimum GSH level in rats in both sexes, and with the begining of the morning period, the GSH level begins to clearly increase and reaches its maximum during the day. The architectonics of the GSH rhythm in females and males is similar, and the GSH values in the same study periods (hours) are very similar, which indicates the absence of differences in the circadian rhythm of the GSH level between animals of both sexes (Table 1).

Comparative analysis of the circadian rhythm of the AOS and LPO systems indicates the relationship of their circadian oscillations. In particular, in contrast to the circadian rhythm of GSH, the circadian rhythm of the TBA-AP was characterized by the maximum values in the evening-night time, with the minimum values of TBA-AP in the morning. The acrophase of the TBA-AP content in females and males was synchronous and was recorded at 21:00, the bathiphase at 09:00 (also synchronous in females and males) (Table 1).

The content of TBA-AP in the acrophase significantly exceeded its concentration in the bathiphase by 2.0 times in females and males, this is confirmed by the high amplitude of the rhythm, which is practically the same in rats of both sexes. The mesor of the TBA-AP content, as well as the amplitude value in females and males, differed insignificantly (Table 2). Almost identical architectonics of the GSH content rhythm was observed in females and males, which is clearly seen from the rhythm chronogram.

Summarizing the results of the analysis of the circadian dynamics of the constituent components of the LPO-AOS system, we can draw the following conclusion about the circadian rhythm of the prooxidant-antioxidant balance: it was established that the content of GSH, TBA-AP and catalase activity change their value during the day under physiological conditions, which allows us to assert the presence of a distinct circadian rhythm, with a clear acrophase and bathyphase. No pronounced circadian rhythm was found for SOD activity.

Circadian rhythms of indicators in animals of different sex were defined as very similar. They showed no significant difference. Circadian rhythms of LPO-AOS system in rats have no sex differences.

Analysis of results of the studies indicates that the blood serum glucose content in females was characterized by circadian stability: indistinct acrophase was recorded at 03:00 with bathyphase at 09:00 (Table 3).

Unlike females, males had a clearly expressive acrophase of glucose content at night (03.00) with bathyphase in the morning (09.00). The glucose content in the acrophase is reliably 68% higher than that in the bathiphase (Table 3). Slightly higher values of glucose in males relative to females are confirmed by a greater value of the mesorrhythm (1.3 times). The presence of a distinct circadian rhythm of glucose fluctuations in males compared to females is confirmed by the high value of the rhythm amplitude (1.7 times) of this indicator (Table 4).

Based on the analysis of the circadian rhythm chronogram of glucose in female and male rats, it can be established that the rhythm architectonics was identical in animals of both sexes, but the level of glucose in females was consistently higher than that in males (at 09.00 and 15.00 it was significantly higher by 62% and 29% respectively), which may be associated with the hormonal background of females, since estrogens exhibit a hyperglycemic effect (Table 3) [33, 21].

The glycogen content in the liver and the energy reserve of the body [17, 1] were characterized by the presence of a clearly expressive circadian rhythm. The maximum glycogen content in animals of both sexes was observed at night (03.00) with a subsequent tendency to its decrease in the morning. (09.00). There was a slight increase in the glycogen content during the daytime (1.2 times higher than the content obtained in the morning), in the evening it decreased again in rats of both sexes and in males it reached bathiphase (21.00). The mezor of glycogen content is almost the same in females and males, while the amplitude was quite high - the glycogen content in acrophase exceeded that in bathiphase by 1.6 times in females and by 1.7 times in males (Table 3, Table 4).

Based on the analysis of circadian chronograms of glycogen, it can be concluded that the architectonics of their daily rhythm are similar between females and males, which is indicated by the synchronicity of circadian fluctuations in animals and confirms the absence of significant sex differences in the dynamics of its change during the day.

Analysis of the obtained data indicates that the acrophase of the glycogen content in the liver is a synchronous acrophase of the serum corticosterone level in animals of both sexes (Table 3). In particular, at 03:00 the maximum content of corticosterone was observed in females and males, while the minimum at 15:00 - in females, and at 09:00 – in males. The mesor of corticosterone was at the same level in animals of both sexes (Table 4). There is confirmed a significant circadian changes of dynamic in content of corticosterone during the day by the high amplitude of the rhythm – the content of corticosterone in the acrophase exceeded that in the bathiphase by 2.7 times in females and by 2.5 times in males (Table 3).

The results of analysis of chronogram level of corticosterone in rats of both sexes indicate identical architectonics of the rhythm (with the exception of the bathyphase period, which was recorded in females at 15:00 and in males at 09:00),

which indicates the absence of significant sex differences in the circadian rhythm of corticosterone (Fig. 3). and also confirms the daily organization of the vital activity of animals with a nocturnal type of activity, which are characterized by the rhythm of synthesis and secretion of GCS [17, 1, 42].

Comparative analysis of the chronogram of the content of glycogen and corticosterone in animals of both sexes indicates the synchronous fluctuations of these indicators during the day (with the exception of the evening period - 21.00, and daytime for females (15.00)), and confirms the well-known organ-specific effect of corticosterone [10] to stimulate the synthesis of glycogen in the liver. An evening decrease of glycogen in hepatocytes may be due to other mechanisms of regulation of its content in liver tissues [14, 43].

Analysis of the parameters of carbohydrate metabolism: the level of glucose, glycogen and corticosterone, as essential in the regulation of this metabolism, indicates the synchronization of the acrophases of these indicators in animals of both sexes at night (03.00), which may indicate the intensification of circadian processes of carbohydrate metabolism during this period. At 09.00, the content of glycogen and glucose in animals is minimal. Bathyphase of the corticosterone content was observed in males, which indicates antiphase of processes that take place during the period of acrophase (03.00).

The effect of corticosterone on the processes of membrane stabilization of cells is well-known [42]. Activity of serum aminotransferases depends on the GCS content, since under physiological conditions these enzymes are localized in large quantities in hepatocytes (ALT - in the cytosol; AST - in the cytosol and mitochondria), and their release into the intercellular space is the result of changes in the permeability of cell membranes [3].

Analysis of the data obtained indicates that the circadian rhythm of the activity of ALT and AST of blood serum in females and males is similar: the acrophases and bathiphases of the rhythm activity of cytolysis markers in animals of both sexes are synchronous. The acrophase of ALT and AST activity was observed during the day (15.00), and the bathyphase in the evening (21.00) (Table 5). Mesors of ALT and AST activity are almost the same in females and males, and the amplitudes of cytolysis markers are slightly higher in females relative to males in both indicators (Table 6).

It was experimentally established that the acrophase of ALT and AST activity in females and males was observed at 15.00 (Table 5) and is synchronous with the bathyphase of corticosterone content (Table 3), which can be explained by the effect of this corticosteroid on membrane stabilization, in particular by reducing permeability of cells [25].

Summarizing the results of the analysis of daily activity of carbohydrate metabolism and cytolysis markers, it should be noted that the content of glycogen, corticosterone and glucose in males changes during the day, it was characterized by a clear circadian rhythm, while the activity of cytolysis markers in animals glucose content in females was not observed. Circadian features of carbohydrate metabolism and activity of cytolysis markers are not characterized by significant differences between rats of both sexes (except for the circadian rhythm of glucose), circadian rhythms of the above indicators of carbohydrate metabolism and activity of markers of cytolysis in animals of both sexes are defined as very similar.

The content of total protein was defined as indicators of total protein metabolism, albumin and urea, as the main product of their metabolism. Analysis of the results shows that fluctuations in the total protein content were characterized by insignificant circadian dynamics (relative differences in the content of the indicator in the acrophase to the bathyphase (Table. 7). The acrophase of total protein in animals of both sexes is synchronous and was observed at night (03.00); bathyphase inverted in acrophase time and was recorded during the day (15.00) (Table 7). The mesor of the indicator is almost the same in animals of both sexes. Whereas the amplitude of the rhythm was slightly higher in males than in females (by 2.0 times), which indicates more pronounced fluctuations in the circadian rhythm of total protein in males relative to females (Table 8). It should be noted that in males the content of total protein in the acrophase is significantly higher than in the bathyphase by 25% (Table 7).

Albumin levels were also characterized by indistinct circadian dynamics during the day, there

were no significant differences between the content of the indicator in the acrophase and bathyphase. Acrophase in animals of both sexes was observed at 21.00, while bathyphase at 09.00 (Table 7). The amount of mesin albumin is almost identical in females and males at a slightly higher rhythm amplitude (1.2 times) in the latter relative to females (Table 8). It should be noted that no synchrony of acrophase and bathyphase of albumin and total protein was observed in rats of both sexes.

In contrast to total protein and albumin content, for which circadian rhythm was indistinct but observed, circadian rhythm was virtually absent for urea content. Acrophase in animals of both sexes was registered at 03.00, with almost the same urea content in females and males, while bathyphase at 15.00 in females and at 09.00 in males (Table 7). There were observed no reliable differences in urea content in the bathyphase between rats of both sexes (Table 4). Mesor and amplitude of urea rhythm in females were slightly higher than similar in males, in particular by 1.2 times larger mesor and by 1.8 times amplitude (Table 8).

Analysis of circadian rhythms of protein metabolism in serum of rats allows to make assumptions about the intensification of protein catabolism in the evening and night, as indicated by the acrophase of albumin in the evening and total protein at night, while the morning-day time is likely to be characterized by anabolic processes (bathyphase of albumin and total protein). Analysis of fluctuations in the daily content of urea indicates the absence of a pronounced circadian rhythm of this indicator in females and males.

Indicators of protein and purine metabolism include uric acid, which is a product of the breakdown of purine bases. Acrophase of uric acid content in females was observed at 09.00 and bathyphase at 15.00, and in males acrophase at 15.00, with bathyphase at 03.00 (Table 7)). Mesor of uric acid content is the same in rats of both sexes, and the amplitude is higher in males than in females (2.5 times) (Table 8). The absence of a significant difference between the values of uric acid content in the selected periods for study indicates the absence of a pronounced circadian rhythm of this indicator (Table 7).

According to the results of the analysis of circadian rhythms of protein and purine metabolism: total protein, albumin, urea and uric acid, we can say about their similarity in females and males.

Analysis of cholesterol is of great practical importance, because this indicator is not only a characteristic of the state of lipid metabolism, but also reflects the state of excretory processes, because constant cholesterol levels are maintained by synthesis, catabolism and excretion of excess bile in the intestine [11, 28]. Acrophase of cholesterol in rats of both sexes is synchronous and was observed during the day (15.00) and the bathyphase at night (03.00) (Table 9).

Mesor of cholesterol rhythm is almost the same in rats of both sexes, while the amplitude is slightly lower in females than in males (by 1.7 times) (Table 10). Higher values of amplitude in males than in females are confirmed by the fact that in males the cholesterol content in the acrophase is significantly higher than in the bathyphase by 1.3 times, while in females this was not observed (Table 9; Table 10).

Circadian activity of the liver is characterized by daily peaks of anabolism and catabolism [56, 30], which directly allows to establish the acrophase and bathyphase of the most pronounced detoxification processes in the liver. The indicator that characterizes the excretory and detoxifying properties of the liver are direct and indirect bilirubin, which together make up the fraction of total bilirubin [39, 44]. We found a pronounced circadian rhythm of total bilirubin in the serum of female and male rats. The minimum content of total bilirubin in rats of both sexes was observed at night, followed by a rapid tendency to increase in the morning and during the day and reached the acrophase at 15.00. There was a gradual decrease in the content of this indicator in the evening, which continued during the night until 03.00. Mesor and rhythm amplitude of total bilirubin were almost at the same level in animals of both sexes (Table 10). High values of amplitude, which are confirmed by differences in the content of total bilirubin in the acrophase relative to the bathyphase (by 2.0 times in females and by 1.6 times in males), indicate the severity of circadian rhythm. Analysis of circadian chronograms of total bilirubin content of female and male rats indicates the identity of their circadian rhythms and confirms the absence of differences between the two sexes of animals.

AP is a sensitive indicator of excretory and detoxification processes [52, 7]. According to the results of the study, the acrophase activity of AP and bilirubin in the serum was observed during the day (15.00) in rats of both sexes, while the bathyphase inverted the acrophase and was recorded at night (03.00) (Table 9). Mesor rhythm of AP activity is almost the same in females and males, while the amplitude is 1.8 times greater in females relative to males. The amplitude of AP activity was characterized by high values, indicating a pronounced circadian rhythm, which is also evidenced by relative differences between the activity of the enzyme in acrophase and bathyphase (by 2.0 times in females and by 1.4 times in males) (Table 9, 10).

The analysis of circadian rhythms of indicators of excretory and detoxification processes of the liver indicates the synchronicity of the periods of maximum and minimum cholesterol, total bilirubin and AP activity, which can be explained by the systematic work of the liver in the implementation of detoxification function. Comparative analysis of circadian rhythms of cholesterol, total bilirubin and AP activity indicates the absence of significant differences in female and male rats values in the studied periods.

Discussion. As a result of the conducted studies there was established an existence of circadian dependency of activity of system LPO-AOS at intact rats of both sexes. GSH content was characterized by pronounced circadian changes throughout the day. Acrophase of this indicator was observed during the day (15.00) in rats of both sexes. The content of GSH in the acrophase was significantly higher (2.0 times in females and 2.2 times in males) than that in the bathyphase, which was observed in females in the evening (21.00) and at night (03.00) in males (Table 2).

The above circadian dynamics of GSH content may be due to the circadian rhythm of activity of enzymes of the pentose phosphate cycle (PPC), which is known to be the main generator of NADPH [24, 27]. The content of NADPH in cells plays a key role for the glutathione system, as it is required for the transition under the influence of the enzyme glutathione reductase oxidized form of glutathione to GSH. GSH acts as an «active stabilizer» of the LPO-AOS system and along with antioxidant enzymes (SOD, catalase) plays a leading role in ensuring the «stability of antioxidant homeostasis» of the body as a whole and, in particular, hepatocytes [5]. The study of the circadian rhythm of activity of key PPC enzymes: glucose-6-phosphate dehydrogenase (G-6-PHDH) and 6-phosphogluconate dehydrogenase (6-PHGDH), is actively conducted by scientists in animals with different activity levels in different seasons [14]. Systematization and analysis of the results on the acrophase activity of G-6-PHDH and 6-PHGDH show that in rats the acrophase activity of these enzymes is registered in the first half of the light period, which allows to substantiate the mechanism of the circadian peak of GSH content in hepatocytes during the day. (15.00).

The enzymatic part of the AOS system of cells is also provided by the activity of SOD and catalase – enzymes that usually work in tandem: the product of dismutation of superoxide anion (the reaction involves SOD) is hydrogen peroxide, which is the active substrate for catalase [6, 19, 20, 22]. It was established that SOD activity did not change during the day (indistinct acrophase in females and males was registered at 09.00, and bathyphase at 21.00 in animals of both sexes). Whereas for catalase a clear peak of activity in hepatocytes was registered during the day (15.00). In female and male rats, the activity of catalase in the acrophase was by 1.6 times significantly higher than that in the bathyphase, which was observed at night (03.00). It is reasonable to assume that the increased activity of catalase at 15.00 (acrophase) - is a reciprocal reaction to the increase in the content of hydrogen peroxide cells (active substrate of this enzyme) [20]. In our studies, it was found that at 15.00 in the serum of rats of both sexes there was a bathyphase level of total protein, which, in turn, suggests the intensification of anabolism and detoxification during this period [49]. Under the conditions of activation of detoxification processes from the systemic bloodstream, a large number of formed blood elements enters the liver buffer cells, and it is well known that the content of catalase in erythrocytes is quite high [14, 19]. This suggests that the high activity of catalase at 15.00 is not only due to hepatocyte catalase, but also to erythrocyte.

It is also known that the detoxification function of the liver is very closely related to the excretory

(choleresis activity) [20, 49, 58]. The study of the circadian rhythm of total bilirubin, cholesterol and serum AP activity makes it possible to assess the daily severity of choleresis and, accordingly, to predict the intensity of detoxification processes in hepatocytes during this period. The circadian maximum AP activity found in our studies (15.00) was 2.0 times higher in females and 1.5 times higher in males than the activity of this enzyme in the bathyphase (03.00). The acrophase of AP activity is synchronous with acrophases of total bilirubin content (significantly 2.0 times higher total bilirubin content in females and 1.6 times higher in males than in the bathyphase) and cholesterol content (cholesterol batiphase in females and males) (Table 11) indicating the lowest rate of bile secretion during the day as a result of increased activity of detoxification processes that cause cholestasis [7].

The maximum content of total bilirubin recorded during the day (15.00) plays an active role in the course of detoxification processes, due to its inherent endogenous antioxidant activity [7, 31]. Regarding the activity of circadian rhythms of choleresis, it is reasonable to assume that the bathyphase content of total bilirubin, cholesterol and AP activity (Table 11) indicates a minimum circadian intensity of bile secretion, and possibly the lowest daily activity of detoxification processes at night. It should be noted that to ensure the proper course of detoxification processes, which include various reactions of biochemical transformations, it requires a high content of GSH, a key non-enzymatic component of AOS cells [9, 32], acrophase content, which is also observed during the day in rats of both sexes.

LPO processes are most active in the evening (21.00), which coincides with the daily period of vigor of rats. In the evening (21.00) there is an acrophase of TBA-AP content in animals of both sexes, and the value of this content significantly exceeds that in the bathyphase (09.00) by 1.9 times in females and by 2.1 times in males (Table 11). It is reasonable to assume that the presence of the evening maximum TBA-AP content is associated with catecholamine stimulation of LPO processes, because in rats the activation of the sympathetic-adrenal system is associated with the onset of the dark period of the day (according to S.S. Shapovalov, adrenaline acrophase was observed at 18.00, and the bathyphase - between 06.00 and 09.00) [58, 31]. According to sources in the scientific literature, lipid metabolism is more intense at night [23], which may also be due to the increased content of TBA-AP. The tendency to decrease the content of TBA-AP at 03.00 relative to 21.00 and reach its minimum at 09.00 may be due to increased nocturnal secretion and levels of melatonin – a strong endogenous free radical scavenger [12, 15].

The liver plays a leading role in the organization of carbohydrate metabolism [54]. It was established that the serum glucose level of females was characterized by circadian stability, while males had a pronounced acrophase at 03.00 in which the glucose content was by 68% higher than in the bathyphase, which was recorded at 09.00 (Table 11). Higher glucose levels in females in all study periods relative to the same in males (at 09.00 and 15.00 significantly higher by 62% and 29%, respectively) may be due to the hormonal background of rats, as estrogens can have a hyperglycemic effect [21]. Regarding the daily fluctuations in glycogen content, in our studies we found a clear circadian rhythm of the content of this indicator in both females and males. In particular, the level of glycogen in the acrophase (03.00) was significantly higher than that in the bathyphase by 2.7 times in females (09.00) and by 2.6 times in males (21.00) (Table 11). The established daily fluctuations of corticosterone content in rats of both sexes fully confirm the hypothesis [57] regarding the nocturnal period of activity of these animals, in particular, in female and male rats: acrophase of this indicator was registered at night (03.00), while bathyphase during the day (15.00) in females and in the morning (09.00) in males (Table 11).

Analysis of the circadian rhythm of activity of cytolysis markers revealed that the activity of AST in the acrophase (15.00) is significantly higher than in the bathyphase (21.00) by 1.9 times in females and by 1.5 times in males. A similar trend in circadian AST activity was observed with ALT activity in females: acrophase was recorded at 15.00, which is significantly higher than the bathyphase activity of this enzyme at 21.00 by 1.4 times. In males, ALT activity was not characterized by the presence of a pronounced circadian rhythm, however, similarly to females, the acrophase of enzyme activity was observed at 15.00, and the bathyphase – at 21.00

(Table 11). It should be noted that the acrophase activity of cytolysis markers is synchronous with the bathyphase corticosterone content, which can be explained by the manifestation of membrane-stabilizing properties of this GCS directly by regulating cell permeability (Table 11) [42, 43].

In male intact rats, the content of total protein in the acrophase significantly exceeded that in the bathyphase 1.2 times, while in females it was registered indistinct circadian rhythm. Acrophase and bathyphase of total protein content in animals of both sexes are synchronous and were observed at 03.00 and 15.00, respectively (Table 11). Regarding the circadian rhythm of albumin content, in females the content of this indicator in the acrophase at 21.00 significantly exceeded (11%) the same in the bathyphase – at 09.00. Whereas in males the daily albumin content was characterized by indistinct circadian rhythms, but was synchronous with females (acrophase - at 21.00; bathyphase - at 09.00). The content of urea (protein metabolism product) and uric acid (the main metabolite of purine metabolism) were not characterized by pronounced circadian rhythms (Table 11). It should be noted that the minimum content of total protein in rats of both sexes was observed during the day (15.00), which coincides with a pronounced acrophase of catalase activity, AP activity, total bilirubin and cholesterol and probably due to intensification of excretory, detoxification and detoxification during this period.

Analysis of the data in table 11 allowed to establish the chronobiological features of indicators of prooxidant-antioxidant homeostasis, metabolic, excretory and detoxification processes, these 16 indicators partially reflect the state and activity of the studied processes and exchanges. In particular, in females and males acrophases are synchronous at 15 studied indicators, and bathyphases at 11. Intergender differences were recorded at the asynchrony of the acrophase of uric acid content in females and males, and the bathyphase of GSH, glycogen, corticosterone, urea and uric acid levels between animals of both sexes. Statistical analysis of these indicators in the bathyphase in females and males shows the absence of statistically significant differences between those, which suggests a virtually identical circadian rhythms in females and males. Comparative analysis of mesors of 16 studied indicators (Table 11) in females and males shows the identity of 15 of them. Differences were registered in the larger mesor of glucose rhythm by 1.3 times in females relative to males, which is due to slightly higher glucose content in females relative to males in all studied periods of the day.

Regarding the numerical ratio of the content of the studied indicators in the acrophase to the bathyphase (AF/B, Table. 11), according to 16 studied indicators, this ratio was the same or almost the same between females and males at 10 of them. The analysis also revealed a clear circadian rhythm (the value of the studied indicator in acrophase was significant relative to its bathyphase) in rats of both sexes, which is characteristic of GCS, TBA-AP, glycogen, corticosterone, total bilirubin and catalase and AST activities. No circadian rhythm was observed: SOD activity, urea and uric acid content in rats of both sexes, while ALT, AP activity and albumin content were characterized by pronounced circadian activity in females in the absence of similar in males. In addition, only males in contrast to females, pronounced circadian rhythm was characterized by glucose, total protein and cholesterol (Table 11).

Acrophase content of GSH, cholesterol, total bilirubin and activity of catalase and AP enzymes was observed, which was observed during the day (15.00), which indicates increased activity of detoxification processes and respectively circadian peak of liver activity in this period. Analysis of the above chronobiological analysis of circadian rhythms of liver activity of females and males indicates the absence of significant differences in their organization between animals of both sexes.

The relevance of the study to establish the chronobiological norm of functional activity of the liver is indisputable, because in order to obtain reliable results and their adequate interpretation of the chronopharmacological manifestation of hepatoprotective activity of drugs, it is necessary to take into account the initial chronobiological rhythm of liver function (state of biorhythms of a healthy organism) both for the analysis of desynchrony of biorhythms in the conditions of liver pathology and for the assessment of the influence of drugs on the prevention and treatment of pathology.

Conclusions. 1. The circadian harmony of homeostasis of the AOS system and the activity of

LPO processes is established. The highest «resilience» of AOS was observed in the morning, and the minimum in the evening and night. An acrophase of total protein and albumin content was established, which was recorded in the evening-night period, indicating a higher daily activity of catabolic processes in this period compared to anabolic (bathyphase of albumin and total protein) in the morning-day. Established relative acrophase of cholesterol, total bilirubin and AP activity, which was registered at 15.00 and synchronous bathyphase of total protein makes it possible to predict increased activity of anabolic and detoxifying processes during the day, compared with other studied periods of the day. It was found that the content of glycogen, corticosterone and glucose in males change their value during the day, as they are characterized by a clear circadian rhythm, while the activity of markers of cytolysis, urea and uric acid in animals of both sexes and glucose in females was not observed.

2. The circadian peak of liver activity under physiological conditions was established in rats, which is observed in the daytime period (15.00), and confirmed by acrophases of reduced glutathione, cholesterol, total bilirubin and catalase and AP activity, which indicates increased detoxification activity during this period.

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Table 1. Circadian rhythms of prooxidant-antioxidant indicators balance in hepatocytes (n=6-8, M±SEM)

Time of day		Females	Data analysis	Males	Data analysis
AOS		SOD, st.units			
	03.00	42,59±4,37		47,09±4,00	
	09.00	48,83±2,84	AF	48,35±3,73	AF
	15.00	46,04±3,98		44,28±3,67	
	21.00	39,36±3,74	B	40,16±4,09	B
		Catalase, μkat/l			
	03.00	43,60±3,17*	B	45,77±7,07*	B
	09.00	50,46±6,77*		48,13±6,33*	
	15.00	78,22±4,74	AF	81,37±1,43	AF
	21.00	50,08±2,94*		50,27±4,88*	
		GSH, st.units			
	03.00	87,52±9,04*		70,74±19,64*	B
	09.00	105,21±12,85*		105,65±11,98*	
	15.00	154,25±14,82	AF	157,75±14,87	AF
	21.00	78,33±15,93*	B	89,71±19,15*	
LPO		TBA-AP, μmol/g			
	03.00	25,85±3,13#		26,93±2,46	
	09.00	16,24±2,58	B	16,12±5,46	B
	15.00	17,21±3,58		19,74±10,04	
	21.00	30,51±1,14#	AF	34,40±3,13#	AF

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; * – deviation of the indicator in the group of one sex of animals is reliable relative to the indicator of acrophase ($p<0,05$); # – deviation of the indicator in the group of one sex of animals is reliable relative to the indicator of bathiphase ($p<0,05$).

Table 2. Chronobiological characteristics of indicators of prooxidant-antioxidant balance (circadian rhythm) in rats (n=6-8) by the program Cosinor-Analisis 2.4 for Excel 2000/XP

Indicators, animal sex		Mesor	Amplitude
SOD, st.units	females	44,21	2,13
	males	45,88	4,69
Catalase, μkat/l	females	55,49	17,31
	males	56,39	17,83
GSH, st.units	females	106,33	35,97
	males	107,68	44,97
TBA-AP, μmol/g	females	21,94	8,91
	males	24,27	9,86

Notes: n – number of animals in the group.

Table 3. Circadian rhythms parameters of carbohydrate metabolism in rats (n=6-8, M±SEM)

Time of day	Females	Data analysis	Males	Data analysis
	Glucose, mmol/l,			
03.00	8,49±0,50	AF	7,07±0,43	AF
09.00	7,04±0,59	B	4,34±0,18*/^	B
15.00	7,06±0,49		5,46±0,35*/^	
21.00	8,16±0,79		7,01±0,70	
	Glycogen, mg/g			
03.00	3,04±0,20	AF	3,02±0,10	AF
09.00	1,85±0,18*	B	1,92±0,15*	
15.00	2,25±0,21*		2,40±0,11*	
21.00	1,96±0,19*		1,76±0,06*	B
	Corticosterone, pkg/ml			
03.00	139,97±6,35	AF	146,12±4,67	AF
09.00	60,52±1,03*		57,17±2,32*	B
15.00	52,12±0,81*	B	66,84±6,80*	
21.00	106,80±4,90*		121,65±4,63*	

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; * – deviation of the indicator in the group of one sex of animals is reliable relative to the indicator of acrophase ($p<0,05$); ^ – deviation of the indicator in the group within one study period is reliable between females and males ($p<0,05$).

Table 4. Circadian rhythms characteristics of carbohydrate metabolism parameters in rats (n=6-8) by the program Cosinor-Analisis 2.4 for Excel 2000/XP

Indicators, animal sex		Mesor	Amplitude
Glucose, mmol/l	females	7,69	0,91
	males	5,97	1,57
Glycogen, mg/g	females	2,2	0,40
	males	2,27	0,35
Corticosterone, pkg/ml	females	89,85	49,64
	males	97,94	51,09

Notes: n – number of animals in the group.

Table 5. Circadian activity of ALT and AST in the serum of rats (n=6-8, M±SEM)

Time of day	Females	Data analysis	Males	Data analysis
	ALT, µmol/h*ml			
03.00	0,97±0,12		0,96±0,06	
09.00	0,94±0,05*		0,90±0,09	
15.00	1,19±0,09	AF	0,97±0,13	AF
21.00	0,87±0,05*	B	0,88±0,06	B
	AST, µmol/h*ml			
03.00	0,74±0,16		0,72±0,13	
09.00	0,75±0,09		0,68±0,07	
15.00	0,95±0,05	AF	0,90±0,13	AF
21.00	0,51±0,09*	B	0,58±0,07*	B

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; * – the deviation of the indicator in the group of animals is relative ($p < 0,05$) to the acrophase index.

Table 6. Characteristics of circadian rhythms of ALT and AST activity in rats (n=6-8) by the program Cosinor-Analisis 2.4 for Excel 2000/XP

Indicators, animal sex		Mesor	Amplitude
ALT, µmol/h*ml	females	0,99	0,11
	males	0,93	0,01
AST, µmol/h*ml	females	0,74	0,16
	males	0,72	0,10

Notes: n – number of animals in the group.

Table 7. Circadian rhythm of total protein and purine metabolism in rats (n=6-8, M±SEM)

Time of day	Females	Data analysis	Males	Data analysis
	Total protein, g/l			
03.00	80,22±4,05	AF	84,79±5,83	AF
09.00	75,06±2,05		74,15±3,72	
15.00	71,06±4,16	B	68,37±1,73*	B
21.00	73,60±2,44		82,12±3,67	
	Albumin, g/l			
03.00	41,85±1,52		41,85±1,30	
09.00	37,31±1,18	B	38,03±1,59	B
15.00	42,26±0,91		38,91±3,46	
21.00	45,17±3,48	AF	47,56±4,02	AF
	Urea, mmol/l			
03.00	13,61±1,53	AF	11,02±1,46	AF
09.00	12,10±1,03		9,19±0,54	B
15.00	11,51±1,12	B	10,37±0,65	
21.00	12,43±1,12		10,20±0,97	
	Uric acid, µmol/l			
03.00	13,16±1,01		10,89±0,89	B
09.00	14,24±1,37	AF	11,74±1,05	
15.00	12,44±0,83	B	14,29±1,37	AF
21.00	13,07±0,73		11,44±1,21	

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; * – deviation of the indicator in the group of animals is relative (p <0,05) to the acrophase index; # – deviation of the indicator in the group of one sex of animals is relative (p <0,05) to the bathyphase index.

Table 8. Characteristics of circadian rhythms of total protein and purine metabolism of rats (n=6-8) by the program Cosinor-Analisis 2.4 for Excel 2000/XP

Indicators, animal sex		Mesor	Amplitude
Total protein, g/l	females	74,98	4,64
	males	77,36	9,12
Albumin, g/l	females	41,65	3,94
	males	41,59	4,98
Urea, mmol/l	females	12,41	1,06
	males	10,20	0,6
Uric acid, µmol/l	females	13,23	0,69
	males	12,09	1,71

Notes: n – number of animals in the group.

Table 9. Circadian rhythm of excretory and detoxification processes in rats (n=6-8, *M±SEM*)

Time of day	Females	Data analysis	Males	Data analysis
	Cholesterol, mmol/l			
03.00	1,60±0,11	B	1,59±0,12*	B
09.00	1,70±0,06		1,75±0,07*	
15.00	1,89±0,06	AF	2,04±0,04	AF
21.00	1,66±0,13		1,60±0,11*	
	Total bilirubin, µmol/l			
03.00	9,01±0,39	B	9,85±0,61	B
09.00	12,46±0,97#		13,03±1,16	
15.00	17,82±1,16#	AF	16,28±1,88#	AF
21.00	12,12±0,98#		10,40±0,84	
	AP, U/l			
03.00	105,97±11,04	B	117,23±20,04	B
09.00	140,07±14,74		149,28±11,76	
15.00	219,27±38,30	AF	179,60±23,79	AF
21.00	140,07±21,27		143,63±17,10	

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; * – deviation of the indicator in the group of one sex of animals is relative ($p < 0.05$) to the acrophase index; # – deviation of the indicator in the group of one sex of animals is relative ($p < 0.05$) to the bathyphase index.

Table 10. Characteristics of circadian rhythms indicators of excretory and detoxification processes (n=6-8) by the program Cosinor-Analisis 2.4 for Excel 2000/XP

Indicators, animal sex		Mesor	Amplitude
Cholesterol, mmol/l	females	1,71	0,14
	males	1,75	0,24
Total bilirubin, µmol/l	females	12,85	4,41
	males	12,38	3,49
AP, U/l	females	151,34	56,65
	males	147,40	31,32

Notes: n – number of animals in the group.

Table 11. Circadian features of liver activity of healthy rats

Indicators	Animal sex (n=8)	Time of day				Mesor	AF/B (№ of times)
		03.00	09.00	15.00	21.00		
GSH, units	females	≠	≠	AF*	B	=	2,0
	males	B	≠	AF*	≠		2,2
SOD, units	females **	≠	AF	≠	B	=	1,2
	males **	≠	AF	≠	B		1,2
Catalase, µcat/l	females	B	≠	AF*	≠	=	1,8
	males	B	≠	AF*	≠		1,8
TBA-AP, µmol/g	females	≠	B	≠	AF*	=	1,9
	males	≠	B	≠	AF*		2,1
ALT µmol/year*ml	females	≠	≠	AF*	B	=	1,4
	males **	≠	≠	AF	B		1,1
AST µmol/year*ml	females	≠	≠	AF*	B	=	1,9
	males	≠	≠	AF*	B		1,5
Glucose, mmol/l	females **	AF	B	≠	≠	↑1,3	1,2
	males	AF*	B	≠	≠		1,6
Glycogen, mg/g	females	AF*	B	≠	≠	=	1,6
	males	AF*	≠	≠	B		1,7
Corticosterone, pkg/ml	females	AF*	≠	B	≠	=	2,7

	males	AF*	B	≠	≠		2,5
Total protein, g/l	females **	AF	≠	B	≠	=	1,3
	males	AF*	≠	B	≠		1,2
Albumin, g/l	females	≠	B	≠	AF*	=	1,2
	males **	≠	B	≠	AF		1,2
Urea, mmol/l	females **	AF	≠	≠	≠	=	1,2
	males **	AF	B	≠	≠		1,2
Uric acid, µmol/l	females **	≠	AF	B	≠	=	1,1
	males **	B	≠	AF	≠		1,3

Cholesterol, mmol/l	females **	B	≠	AF	≠	=	1,2
	males	B	≠	AF*	≠		1,3
Total bilirubin, kmol/l	females	B	≠	AF*	≠	=	2,0
	males	B	≠	AF*	≠		1,6
AP, U/l	females	B	≠	AF*	≠	=	2,0
	males **	B	≠	AF	≠		1,5

Notes: n – number of animals in the group; AF – acrophase of the studied indicator; B – bathyphase of the studied indicator; ≠ – the value of the studied indicator did not reach the acrophase or bathyphase; * – the value of the indicator in the acrophase is relative to the bathyphase of this indicator;** – the value of the indicator in the acrophase is not relative for its bathyphase; = almost identical mesor in females and males; ↑ – how many times the mesor of the indicator in females is higher than in males; AF/B – how many times the value of the studied indicator in the acrophase is higher than the bathyphase.