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CHRONOPHARMACOLOGICAL STUDIES OF THE HEPATOPROTECTIVE ACTIVITY OF SILYMARIN UNDER EXPERIMENTAL TOXIC HEPATITIS IN RATS

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Abstract

Silymarin - a complex preparation of bioflavonoids of milk thistle, which contains silibinin A, silibinin B, silocristine, silidianin, isosilibinin A, isosilibinin B, isosilicristin, taxifolin. Chronopharmacological studies of carsil activity will establish a chronoportrait of this drug, which will help increase the effectiveness of pharmacotherapy of diseases of the hepatobiliary system. It was found that against the background of modeling hepatitis in the morning (9 a.m.) and evening (9 p.m.) silymarin was characterized by the most pronounced increase in the level of reduced glutathione and superoxide dismutase activity (group at 9 p.m.). It was investigated that the use of silymarin in the morning (9 a.m.) and in the evening (9 p.m.) was characterized by the most significant positive dynamics of reduction of transminase activity (1.2-1.5 times ($p < 0.05$), in the absence of significant changes at night and only the trend to their reduction on the background of daytime pathology (alanine aminotransferase by 16%). It is established that the effect of silymarin on energy and metabolic processes by increasing glycogen content by 1.2 times and decreasing uric acid content by 1.2 times is also observed in the morning (9 a.m.) and night (3 a.m.) period. Summarizing the results of the above chronopharmacological preclinical analysis of hepatoprotective properties of silymarin in the model of acute hepatitis modeled at different times of the day, the most pronounced pharmacological effect of the drug when taken in the morning (9 o'clock) and evening (9 o'clock).

Keywords: *silymarin, chronopharmacological studies, hepatoprotective activity*

Introduction

Silymarin - a complex preparation of bioflavonoids of milk thistle, which contains silibinin A, silibinin B, silocristine, silidianin, isosilibinin A, isosilibinin B, isosilicristin, taxifolin. The most active component of silymarin, which determines its therapeutic effect - silibinin. Systematic analytical reviews, which are devoted to the use of silymarin in various pathologies of the liver, are regularly published by specialists of the Cochrane Hepato-Biliary Group. The therapeutic efficacy of milk thistle preparations has been confirmed by repeated clinical studies and, according to the monograph «Milk thistle (Silybinum marianum)» (2011) bases of the body of integrative medicine «Natural Standard», It is noted that milk thistle has levels of evidence B (in chronic cirrhosis and other liver diseases) and C (in acute viral hepatitis, diabetes (in patients with liver cirrhosis), mushroom poisoning (pale toadstool) and liver damage drugs and toxins) [37].

The popularity of silymarin is confirmed by studies of the American Botanical Council, in particular, in 2010-2011 years, silymarin ranked fifth among herbal remedies in terms of sales, and among silymarin drugs - the drug "Carsil" (production Sopharma, Bulgaria) most often used as a drug of choice for self-treatment of hepatobiliary pathology. In particular, according to the results of marketing research in 72% of cases among the 60 proposed drugs for the treatment of diseases of the hepatobiliary tract, the drug of choice was "Carsil" [37, 38]. The above led to the choice of this drug among other preparations of milk thistle flavonoids as the object of chronopharmacological research.

Numerous clinical trials of this drug in various forms of liver disease have confirmed a high degree of safety of carsil, and among the manifestations of side effects were nausea, vomiting, loose stools, headache and skin rash. [36, 27]. In a double-blind, placebo-controlled study in 57 patients with acute viral hepatitis A and B, silymarin (a component of carsil) caused statistically significant positive changes in alanine aminotransferase and bilirubin at day 21 (20 of 27 patients), while reductions in and dosage form was biased. A double-blind study of the effectiveness of carsil in patients with acute hepatitis showed a reduction in alanine aminotransferase, bilirubin, reduced inpatient stay and reduced risk of complications of the underlying disease [14].

Based on the data that the antioxidant properties of silibinin can lead to the restoration of free radical-damaged interferon synthesis [34], the effectiveness of silibinin injections in 36 patients with hepatitis C virus who did not respond to standard therapy with pegylated interferons and ribovir was studied. It was found that intravenous silibinin in monotherapy and in combination with standard treatment (according to the treatment protocol of this nosology) leads to eradication of hepatitis C virus (confirmed by immunological tests). Positive dynamics was registered when using small doses (5 mg / kg body weight) and higher (20 mg / kg body weight), which confirms the viability of this approach to improve the effectiveness of interferon therapy [30, 2]. The above is confirmed by data [11] in a clinical case in a patient with compensated cirrhosis of viral etiology who did not respond to interferon therapy: combined treatment with silymarin and ribavirin contributed to complete eradication of the virus on day 18 of therapy. The sources of the scientific literature present data on the successful use of silibinin for the prevention of HCV infection of transplanted liver in patients with HCV genotypes 1a / 4 and 3a [38]. The above results became the basis for the consideration of silibinin as an antiviral drug. An essential feature of silymarin is its ability to induce conjugation of bilirubin with glucuronic acid and inhibit γ-glucuronidase production by pathogenic intestinal bacteria [23]. Bilirubin and some glucuronic acid-related toxins are excreted more rapidly in the bile than silymarin is effective in jaundice. The possible choleretic effect of silymarin should be considered in the treatment of Oddi sphincter dysfunction, chronic biliary pancreatitis and in patients with intrahepatic cholestasis [29].

Thus, the above information on the established and confirmed by the principles of evidence-based pharmacological efficacy of plant hepatoprotector based on milk thistle flavonoids - "Carsil" (in some experiments of the basic substance silymarin) confirm that this drug is one of the most studied among registered hepatoprotectors. Chronopharmacological studies of carsil activity will establish a chronoportrait of this drug, which will

help increase the effectiveness of pharmacotherapy of diseases of the hepatobiliary system.

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 [25]. 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 [25].

It is well known that at therapeutic doses of paracetamol the drug is metabolized by binding to sulfuric (sulfation - 58-60%) and glucuronic (glucuronidation - 32-38%) acids, a small part is metabolized by the cytochrome system P450 (2-5 %). During biotransformation, the cytochrome system produces a toxic metabolite of paracetamol – N-acetylbenzoquinone, which is neutralized by the glutathione system. However, when taking toxic doses of this drug, the metabolism of paracetamol from sulfation and glucuronidation is redistributed towards metabolism by microsomal enzymes, and physiological reserves of carbohydrate glutathione are not able to neutralize the high content of toxic metabolite [1, 24, 15]. Thus, the destructive effect of paracetamol is realized by the interaction of the formed toxic metabolite (N- acetylbenzoquinone) with cytolemma and membranes of hepatocyte organelles [3, 13, 26]. The latter leads to the activation of lipid peroxidation of biological membranes, inactivation of membrane-bound enzymes, disorders of tissue respiration, plastic metabolism, oxidative phosphorylation. The above changes are reflected in a decrease in glycogen content, disruption of urea formation and breakdown of purine bases (observed changes in uric acid levels), increased activity of the dosage form, total bilirubin and cholesterol, and as a result of destruction of cytolemma and cell membranes. and aspartate aminotransferases) as a result of their release into the intercellular space and into the vascular bed [32, 5, 17]. Silymarin on the example of the drug "Carsil" (tab. 22.5 mg № 80 JSC "Sopharma") was administered in a therapeutic and prophylactic mode - 1 hour before the use of paracetamol and 2 hours after its introduction. A detailed scheme of the study of the daily dependence of the severity of the hepatoprotective activity of silymarin is shown in Table 2.

In animals of the control pathology groups and with the use of silymarin, blood sampling and isolation of the liver to continue the study was performed 24 hours after administration of paracetamol [25]. Whey was obtained from whole blood according to conventional methods [16].

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 alanine aminotransferase and aspartate aminotransferase by the Reitman-Frenkel reaction; albumin content - by reaction with bromocresol green, total protein - by biuret reaction, urea - by reaction with diacetylmonooxime, uric acid - enzymatically by the concentration of quinonymine formed, which is directly proportional to the activity of the enzyme; glucose level - glucose oxidase method; corticosterone - enzyme-linked immunosorbent assay; the content of total bilirubin with 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; activity of the dosage form - kinetically by the rate of formation of p-nitrophenol, which is directly proportional to the activity of the enzyme [16, 39]. The above indicators of our study are classical biochemical markers, the analysis of which allows to assess carbohydrate, protein, lipid metabolism, the state of excretory and detoxification processes in rats, in which the liver is directly involved [18]. Determination of the studied indicators was carried out according to the standard sets of production "Philisit-Diagnostics" (Ukraine), "SpineLab" (Ukraine) and Corticosterone EIA Kit – Enabling Discovery in Life Sciences (Japan).

In the liver homogenate were determined indicators that reflect the prooxidant-antioxidant balance of the body: the content of TBA-active products, carbohydrate glutathione, the activity of superoxide dismutase and catalase, as well as the classic indicator of carbohydrate metabolism - glycogen concentration [21, 20, 31]. TBA- reactants were determined by the method of [33], using the molar extinction coefficient of the trimethine complex 1.56 105 M–1 sm–1. The principle of this method is based on the formation of colored compounds of pink color by the interaction of malonic dialdehyde with thiobarbituric acid when heated.

To determine the carbohydrate glutathione used a modification of the method of G. L. Ellman [20], which consists in the precipitation of macromolecular compounds with a solution of sulfosalicylic acid. The reaction of sulfhydryl groups with Ellman's reagent destroys the disulfide bond in the reagent, forming a colored compound. The molar extinction coefficient of 2-nitro-5-thiobenzoate used in the calculations is 1,4 104 M–1 sm–1. Catalase activity was determined by the amount of hydrogen peroxide decomposed per unit time using the molar extinction coefficient of hydrogen peroxide – 2,22 104 mM–1 sm–1 [21]. Superoxide dismutase activity was determined kinetically by a modification of the method, which is to determine the degree of inhibition of the autooxidation of adrenaline by superoxide dismutase [31]. Glycogen content was diagnosed by the anthron method, the essence of which is the formation of a colored complex of blue color, the intensity of which is proportional to the glycogen content in the test sample [20]. Photometric measurements of the studied parameters were performed using a colorimeter-nephelometer KFK-2MP and spectrophotometers CF-46 та CF-26. Determination of serum corticosterone content was performed by enzyme-linked immunosorbent assay on the basis of Kharkiv National Medical University (enzyme-linked immunosorbent assay) FaxStart (USA)).

Analysis of circadian rhythms based on the values of the studied indicators in the morning, day, evening and night periods allows you to objectively assess the state of circadian rhythms, and the choice 9 a.m., 3 p.m., 9 p.m. and 3 a.m. for studies is justified by the average hours in the morning, day, evening and night, respectively [8]. The selection of both sexes of rats to study circadian rhythms of liver function in physiological conditions and in desynchrony on 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 different scientific sources of literature [28].

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 [8, 19, 12]. Mesor and amplitude were determined using a program Cosinor-Analisis 2.4 for Excel 2000/XP [10].

Chronopharmacological study was conducted in the spring season (March) 2015. 224 randomized rats (160 females and 64 males) weighing 190-210 g were used in the experiments. Therefore, not the same number of female and male rats were used in the experiment. The animals were in the vivarium of the Central Hospital of the National University of Pharmacy with controlled temperature and relative humidity, on a day / night cycle that corresponded to natural in the study season. In order 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 light region of the spectrum, and accordingly the process of melatonin synthesis is not disturbed [4, 6, 9, 7].

Results and Discussion

The use of silymarin in the treatment-and-prophylactic regime contributed to a clear tendency to increase the content of reduced glutathione onby 24% and 20% in the morning (9 a.m.) and evening (9 p.m.) accordingly, in the absence of such changes in

the drug groups at 3 a.m. and 3 p.m. (Table 3). Therefore, the mesor of this indicator changed insignificantly, with a simultaneous decrease in the amplitude (1.4 times) relative to the control pathology rats (Table 4). The acrophase of reduced glutathione when using silymarin is synchronous with intact animals and rats with hepatitis, while the bathyphase was shifted from 9 p.m. (in control and intact animals) at 3 a.m. in silymarin group (Table 3).

The activity of superoxide dismutase, with the use of silymarin did not change, except for the drug group at 9 p.m., in which there was a tendency to increase the activity of the enzyme by 14% relative to animals of control pathology (Table 3). The mesor of the rhythm of superoxide dismutase activity in the drug groups did not differ significantly from the mesor of the activity of this enzyme in animals with hepatitis (Table 4). There were also no changes in the amplitude of the rhythm of superoxide dismutase activity, which was almost at the same level in the group of silymarin and control animals (Table 4). The acrophase and bathyphase of the rhythm of superoxide dismutase activity in silymarin groups were synchronous in intact animals and rats with hepatitis and were observed in at 9 a.m. and 9 p.m., espectively (Table 3).

The introduction of silymarin did not contribute to changes in catalase activity in all study groups of the drug, which was reflected in almost the same values of the rhythm mesor of this enzyme in animals of control pathology and silymarin group (Table 3; Table 4), while the rhythm amplitude increased by 6.0 times in relation to animals with hepatitis (Table 4).

No synchrony of the acrophase activity of catalase was observed in the silymarin groups (9 p.m.) with intact or control animals, while the bathyphase was synchronous with intact rats (3 a.m.) (Table 3).

Similarly, the activity of superoxide dismutase and catalase did not change the content of thiobarbituric acid-active products when using silymarin, except silymarin group at 3 a.m., in which the tendency to decrease in thiobarbituric acid-active products at reception of carsil by 14% concerning animals of control pathology was registered (tab. 3). This was reflected in the change in the magnitude of the thiobarbituric acid rhythm-active products when using carcil, which changed insignificantly (Table 4) and the amplitude, which was registered at the level of rats with hepatitis (Table 4). Acrophase and bathyphase of thiobarbituric acid content-active products are synchronous with intact and control animals and were observed about 9 p.m. and 9 a.m., respectively (Table 3).

Thus, according to the analysis of the effect of silymarin on the performance of the lipid peroxidation system of antioxidant protection in conditions of acute toxic hepatitis, simulated at different times of the day, there was a different circadian manifestation of antioxidant properties of this drug. Namely, taking it on the background of modeling the pathology in the morning and evening contributed to the growth of the content of reduced glutathione (only at 9 p.m.). However, the use of silymarin did not contribute to almost significant changes in catalase activity and thiobarbituric acid content of active products in all studied periods of the day. Beneficial effect of silymarin on prooxidant-antioxidant imbalance against the background of pathology modeled in the morning (9 a.m.) and evening (9 p.m.) The period was accompanied by a positive dynamics of changes in the activity of the markers of cytolysis in them. In particular, in a group of silimarin in the morning (9 a.m.) there was a significant decrease in the activity of transminases by 38% (alaninaminotransferase) and 37% (aspartatheminotransferase) and by 47% and 23% in the group of silimarin in the evening (9 p.m.), respectively. While, in the conditions of modeling of pathology in the afternoon (3 p.m.), the use of silimarin contributed to the tendency to reduce the activity of alaninaminotransferase - by 16%, and the activity of aspartatheminotransferase has not changed relative to animals of control pathology. Reception of the drug in the modeling of pathology at 3 a.m. did not affect the changes in the activity of cytolysis markers (Table 5).

The beneficial effect of silymarin on the dynamics of transminase activity was confirmed by a decrease in the mesor rhythm of the latter: alanine aminotransferase 1.4 times and aspartate aminotransferase 1.3 times relative to animals of control pathology. It was noted that under similar conditions the changes in the amplitude of transminases in the silymarin group were not

significantly significant (Table 6). In the silymarin groups, the acrophase activity of alanine aminotransferase was recorded at 3 a.m., and the bathyphase at 3 p.m. and was synchronous with rats with hepatitis. The acrophase of aspartate aminotransferase activity under the action of carsil was observed at 3 a.m., and the bathyphase at 3 p.m. (synchronous with intact animals), synchrony with rats of control pathology was not observed (Table 5).

Thus, according to the above analysis, the most significant decrease in transminase activity was observed when taking carsil on the background of modeling hepatitis in the morning and evening, which is correlated with the positive dynamics of changes in peroxidation of antioxidant lipids and probably due to maximal anticamidan.

The use of carsil on the background of acute paracetamol hepatitis modeled at different times of the day contributed to a decrease in glucose content by 7-17% in all study periods of the day (Table 7). The glucose rhythm mesor in the silymarin group was 1.2 times smaller than the glucose mesor in hepatitis rats and intact control animals (Table 6). The amplitude of the glucose rhythm in the silymarin group did not differ from the amplitude in intact control rats and control pathology animals (Table 6). The acrophase of glucose content in the silymarin group is synchronous with control animals and was observed about 9 p.m., whereas the bathyphase is synchronous with intact and control rats (9 a.m.) (Table 7).

Silymarin intake was characterized by a tendency to increase glycogen content by 16% and 18% in the silymarin group in the morning (9 a.m.) та ввечері (9 p.m.), accordingly, in the absence of changes in this indicator at night (3a.m.) and daytime (3 p.m.) periods (Table 7). Therefore, changes in the mesor and amplitude of the rhythm of glucose are insignificant (almost no different from those in animals of control pathology) (Table 8). Glycogen acrophase in the silymarin group is synchronous with control animals and was observed at 3 p.m., while the bathyphase is synchronous with intact and control rats (9 a.m.) (Table 7).

The content of corticosterone when taking carsil had no significant differences in animals of control pathology and intact, which is confirmed by almost identical mesors of this indicator in the above groups (Table 7; Table 8). There was a slight decrease in the amplitude of the rhythm of corticosterone content in the carsil groups (10% less than the corticosterone mesor of control animals, and 40% less than intact rats) (Table 8). Acrophase (3 a.m.) and bathyphase (3 p.m.) corticosterone content in the silymarin group synchronous acrophases and bathyphases of intact and control rats (Table 7).

Summarizing the above analysis of the effect of silymarin on carbohydrate metabolism on the background of acute paracetamol hepatitis modeled at different times of the day, we can conclude about the daily characteristics of the drug on the above metabolism. In particular, silymarin was found to increase glycogen levels in the morning and evening, moderately reduced glucose levels in all study periods, and did not affect corticosterone levels under similar conditions.

The absence of changes in the total protein content in the silymarin group was confirmed by the mesor rhythm of this indicator, which was almost at the same level in animals of control pathology, intact control and drug use (Table 9; Table 10). The rhythm amplitude of the total protein content in the silymarin groups increased 1.8-fold relative to animals with hepatitis and was at the level of intact control (Table 20). The acrophase of the total protein content in the silymarin group is synchronous with intact animals and was observed about 3 a.m., while the bathyphase at 9 p.m. (Table 9).

The content of albumin, as well as total protein, when using silymarin in comparison with animals of control pathology did not change, which is confirmed by almost the same mesor rhythm rhythm of albumin (Table 9). However, there was an increase in the rhythm amplitude of total protein in the silymarin group by 1.7 times relative to rats with hepatitis (Table 10). Acrophase of albumin content in the silymarin group synchronous with intact rats and was observed about 9 p.m., whereas the bathyphase at 3 p.m. (Table 9).

The use of silymarin contributed to a 20% increase in urea content only in the silymarin group during the day (3 p.m.), in the absence of significant changes in other periods of the day (Table 9). That is why the urea rhythm mesor in the silymarin group was practically at the level of control pathology rats.

There were also insignificant changes in the amplitude of the rhythm of this indicator when using silymarin in animals with hepatitis (Table 10). Acrophase and bathyphase of urea content in the silymarin group are synchronous with control pathology rats and were observed at 9 p.m., respectively (Table 9).

The introduction of silymarin helped to reduce uric acid content only in the morning (9 a.m.) and in the evening (9 p.m.) by 31% and 23%, respectively (Table 9). The mesor of uric acid in the silymarin group decreased insignificantly (1.1 times) with a decrease in amplitude by 26%, under similar conditions (Table 10). Acrophase and bathyphase index in the silymarin group are synchronous with intact rats and were recorded at 9 a.m. and 3 p.m., respectively (Table 9).

Thus, the use of silymarin on the background of acute paracetamol hepatitis simulated at different times of the day was not characterized by changes in total protein and albumin in all study periods, while urea levels increased only in the silymarin group during the day (3 p.m.) in the absence of significant changes in other periods. It should be noted circadian selectivity in the severity of normalization of uric acid content: in the silymarin group in the morning (9 a.m.) in the evening (9 p.m.) – the content of this indicator decreased, while in other periods it was not observed.

When using silymarin on the background of acute paracetamol hepatitis simulated at different times of the day, there was an insignificant decrease in cholesterol only in the morning (9 a.m.), in the absence of significant changes in other study periods (Table 11).

The magnitude of the rhythm rhythm of the cholesterol content under the action of silymarin was insignificant, but decreased (1.1 times), while the rhythm amplitude of this indicator was almost on a par with the control pathology rats (Table 6.10). Acrophase of cholesterol content in the silymarin group is synchronous with intact animals (3 p.m.), while the bathyphase - rats with hepatitis (9 p.m.) (Table 12).

The introduction of silymarin contributed to a moderate decrease in total bilirubin only in the silymarin group at 9 a.m. and 3 p.m. by 18% and 11%, respectively, as evidenced by a significant reduction in the mesor of this indicator in 1.1 times, relative to animals with hepatitis with a simultaneous decrease in amplitude (1.4 times) (Table 10). Acrophase and bathyphase of total bilirubin in the silymarin group are synchronous with intact and control rats and were observed at 3 p.m. and 3 a.m., respectively (Table 11). Under the action of Carsil, the activity of the dosage form decreased by 1.1-1.4 times depending on the study period, which was reflected in a decrease in the mesor of this indicator - 1.2 times and an increase in amplitude - 1.5 times (Table 12). The acrophase of activity of the dosage form in the silymarin group was observed at 3 p.m., and the bathyphase at 3 a.m. (Table 11).

Thus, the use of silymarin in the treatment-and-prophylactic regime in acute paracetamol hepatitis modeled at different times of the day was characterized by a tendency to lower cholesterol only in the morning, total bilirubin in the morning and afternoon and activity of the dosage form in all study periods.

Thus, the results of the analysis of the circadian dependence of silymarin activity on the imbalance of lipid peroxidation of antioxidant protection, cytodestructive changes and metabolic processes in acute toxic hepatitis established periods of the day when the drug most pronounced hepatoprotective effect.

Conclusions

1. It was found that against the background of modeling hepatitis in the morning (9 a.m.) and evening (9 p.m.) silymarin was characterized by the most pronounced increase in the level of reduced glutathione and superoxide dismutase activity (group at 9 p.m.).
2. It was investigated that the use of silymarin in the morning (9 a.m.) and in the evening (9 p.m.) was characterized by the most significant positive dynamics of reduction of transminase activity (1.2-1.5 times ($p < 0.05$), in the absence of significant changes at night and only the trend to their reduction on the background of daytime pathology (alanine aminotransferase by 16%).
3. It is established that the effect of silymarin on energy and metabolic processes by increasing glycogen content by 1.2 times and decreasing uric acid content by 1.2 times is also observed in the morning (9 a.m.) and night (3 a.m.) period.

Summarizing the results of the above chronopharmacological preclinical analysis of hepatoprotective properties of silymarin in the model of acute hepatitis modeled at different times of the day, the most pronounced pharmacological effect of the drug when taken in the morning (9 o'clock) and evening (9 o'clock).

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Table 1. Stages of research of circadian dependence of expressiveness of hepatoprotective activity of silymarin

Number stage	Features of each stage of research
I stage	Study of the circadian activity of the liver of female and male rats under physiological conditions to assess the circadian rhythms of carbohydrate, protein, purine metabolism, regulation of prooxidant-antioxidant balance, excretory and detoxification processes
II stage	Establishment of features of desynchronosis of liver function of female and male rats under conditions of toxic hepatitis simulated by administration paracetamol
III stage	Study of circadian dependence of hepatoprotective activity of silymarin on the background of acute chronodeterministic paracetamol hepatitis

Table 2. Scheme of research of circadian dependence of hepatoprotective activity of silymarin

Research period of the day	*1-day research*			
	night	morning	daily	night
Administration of drugs (hour of the day) in preventive mode	02.00	08.00	14.00	20.00
Introduction of paracetamol (year of age)	03.00	09.00	15.00	21.00
Administration of drugs (hour of the day) in the treatment regimen	05.00	11.00	17.00	23.00
	2-day research			
Administration of drugs (hour of the day) treatment regimen	02.00	08.00	14.00	20.00
Decapitation of animals (hour of the day)	03.00	09.00	15.00	21.00

Table 3. The effect of silymarin on circadian rhythms of prooxidant-antioxidant imbalance on the background of acute paracetamol hepatitis modeled at different times of the day (n=6-8, M±SEM)

Conditions experiment	Hour of the day			
	03.00	09.00	15.00	21.00
	Reduced glutathione, conventional units			
Intact control	87,52±8,25	105,21±11,65	154,25±13,52	78,33±14,54
Control pathology	70,02±19,78	75,49±12,45	123,84±8,57*	68,45±16,93
Silymarin	72,92±13,40	93,72±10,25	110,19±6,80	82,41±12,21
	Superoxide dismutase, conventional units			
Intact control	42,59±3,18	48,83±2,59	46,04±3,63	39,36±3,73
Control pathology	39,16±3,94	43,48±2,56	43,00±2,84	35,34±2,88
Silymarin	40,59±2,12	46,82±3,46	45,70±2,90	40,17±2,61
	Katalaza, mkkat / l			
Intact control	43,60±2,89	50,46±4,24	78,22±3,74	50,08±3,07
Control pathology	48,15±6,76	46,27±5,56	48,98±8,19*	46,58±5,25
Silymarin	42,75±6,90	46,98±6,02	46,64±4,19	50,54±4,73
	Thiobarbituric acid active products, µmol / g			
Intact control	25,85±2,85	16,24±2,36	17,21±3,37	30,51±0,81
Control pathology	29,06±3,88	16,23±3,60	19,02±2,03	33,12±3,61
Silymarin	25,00±2,70	16,02±2,62	17,31±2,33	30,77±2,12

Notes: n - the number of animals in the group, * - the deviation is significant relative to the rate of intact animals ($p<0,05$).

Table 4. The effect of silymarin on circadian rhythms of prooxidant-antioxidant imbalance in acute paracetamol hepatitis modeled at different times of the day by program Cosinor-Analisis 2.4 for Excel 2000/XP

Conditions experiment (n=6-8)		Reduced glutathione, conventional units	Superoxide dismutase, conventional units	Katalaza, mkkat / l	Thiobarbituric acid active products, µmol / g
Mezor	Intact control	106,33	44,20	55,59	22,45
	Control pathology	84,45	40,24	47,49	24,36
	Silymarin	89,81	43,32	46,73	22,27
Amplitude	Intact control	35,97	5,04	17,31	8,34
	Control pathology	27,14	4,50	0,44	9,82
	Silymarin	19,47	4,19	2,64	8,31

Note: n - the number of animals in the group.

Table 5. The effect of silymarin on the circadian rhythm of activity of markers of cytolysis under conditions of acute hepatitis modeled at different times of the day (n=6-8, M±SEM)

Conditions experiment	Hour of the day			
	03.00	09.00	15.00	21.00
	alanine aminotransferase, µmol / hour * ml			
Intact control	0,97±0,12	0,94±0,05	1,19±0,09	0,87±0,05
Control pathology	1,68±0,33	2,36±0,44*	1,26±0,10	2,92±0,22*
Silymarin	1,87±0,06	1,46±0,17	1,06±0,11	1,54±0,17**
	aspartate aminotransferase, µmol / hour * ml			
Intact control	0,74±0,16	0,75±0,09	0,95±0,05	0,51±0,09
Control pathology	1,86±0,22*	2,25±0,23*	1,44±0,17*	1,75±0,23*
Silymarin	1,66±0,10	1,42±0,17**	1,50±0,08	1,35±0,07

Note: n - the number of animals in the group; * - the deviation of the indicator is significant relative to the rate of intact animals (p <0,05). ** - deviation of the indicator is significant relative to the indicator in the group of animals with hepatitis (p <0,05).

Table 6. The effect of silymarin on circadian rhythms of activity of cytolysis markers according to the program Cosinor-Analisis 2.4 for Excel 2000/XP

Conditions experiment (n=6-8)		Alanine aminotransferase, µmol / hour * ml	Aspartate aminotransferase, µmol / hour * ml
Mezor	Intact control	0,99	0,74
	Control pathology	2,06	1,83
	Silymarin	1,48	1,40
Amplitude	Intact control	0,11	0,16
	Control pathology	0,35	0,33
	Silymarin	0,41	0,26

Note: n - the number of animals in the group.

Table 7. Influence of silymarin on circadian rhythms of carbohydrate metabolism on the background of acute paracetamol hepatitis simulated at different times of the day (n=6-8, M±SEM)

Conditions experiment	Hour of the day			
	03.00	09.00	15.00	21.00
	Glucose, mmol / l			
Intact control	8,49±0,50	7,04±0,59	7,06±0,49	8,16±0,79
Control pathology	7,04±0,49	6,79±0,19	7,65±0,37	8,27±0,51
Silymarin	6,60±0,64	5,66±0,15	6,14±0,59	7,00±0,42
	Glycogen, mg / g			
Intact control	3,04±0,20	1,85±0,18	2,25±0,21	1,96±0,19
Control pathology	1,85±0,18*	1,03±0,10*	2,12±0,16	1,32±0,19*
Silymarin	1,89±0,27	1,20±0,10	2,01±0,22	1,56±0,11
	Corticosterone, pkg / ml			
Intact control	139,97±6,35	60,52±1,03	52,12±0,81	106,80±4,90
Control pathology	121,48±1,58	67,12±11,23	50,35±1,12	98,17±2,00
Silymarin	114,98±4,64	65,04±12,78	54,13±2,12	99,42±1,87

Notes: n - number of animals in the group; * - the deviation of the indicator is significant relative to the rate of intact animals (p <0,05).

Table 8. Influence of silymarin on circadian rhythms of carbohydrate metabolism on the background of acute paracetamol hepatitis simulated at different times of the day according to the program Cosinor-Analisis 2.4 for Excel 2000/XP

Conditions experiment (n=6-8)		Glucose, mmol / l	Glycogen, mg / g	Corticosterone, pkg / ml
Mezor	Intact control	7,69	2,27	89,85
	Control pathology	7,44	1,58	83,83
	Silymarin	6,35	1,67	82,77
Amplitude	Intact control	0,91	0,40	49,64
	Control pathology	0,80	0,20	39,17
	Silymarin	0,71	0,19	35,57

Note: n - the number of animals in the group.

Table 9. Influence of silymarin on circadian rhythms of protein and purine metabolism on the background of acute paracetamol hepatitis simulated at different times of the day (n=6-8, M±SEM)

Conditions experiment	Hour of the day			
	03.00	09.00	15.00	21.00
	Total protein, g / l			
Intact control	80,22±4,05	75,06±2,05	71,06±4,16	73,60±2,44
Control pathology	69,97±1,41*	71,06±2,56	74,68±2,82	72,65±3,86
Silymarin	78,31±3,45	75,00±3,07	71,12±3,74	69,59±2,66
	Albumin, g / l			
Intact control	41,85±1,52	37,31±1,18	42,26±0,90	45,17±3,48
Control pathology	41,23±0,90	40,88±1,13	38,88±2,25	40,79±1,55
Silymarin	38,69±0,39	39,32±0,54	40,23±1,91	42,98±1,43
	Urea, mmol / l			
Intact control	13,61±1,53	12,10±1,03	11,51±1,12	12,43±1,11
Control pathology	9,75±0,21*	11,43±0,73	9,69±0,24	9,29±0,29*
Silymarin	9,14±0,41	12,30±0,65	11,63±1,22	10,14±0,77
	Uric acid, µmol / l			
Intact control	13,16±1,01	14,24±1,37	12,44±0,83	13,07±0,73
Control pathology	15,14±1,62	24,07±0,90*	11,00±1,90	21,73±2,16*
Silymarin	15,96±1,61	18,66±1,86**	12,62±0,93	17,58±1,92**

Notes:

1. n - the number of animals in the group;
2. * - the deviation of the indicator is significant relative to the rate of intact animals (p <0,05).
3. ** - deviation of the indicator is significant relative to the indicator in the group of animals with hepatitis (p <0,05).

Table 10. Influence of silymarin on circadian rhythms of protein and purine metabolism indicators against the background of acute paracetamol hepatitis modeled at different times of the day according to the program Cosinor-Analisis 2.4 for Excel 2000/XP

Conditions experiment (n=6-8)		General protein, g / l	Albumin, g / l	Urea, mmol / l	Uric acid, µmol / l
Mezor	Intact control	74,98	41,65	12,41	13,23
	Control pathology	72,09	40,44	10,04	17,98
	Silymarin	73,50	40,30	11,05	16,20
Amplitude	Intact control	4,64	3,94	1,06	0,69
	Control pathology	2,48	1,18	1,07	2,38
	Silymarin	4,50	1,99	1,31	1,75

Note: n - the number of animals in the group.

Table 11. Influence of silymarin on circadian rhythms of indicators of excretory and detoxification processes against the background of acute paracetamol hepatitis modeled at different periods of the day (n=6-8, M±SEM)

A group of animals	Hour of the day			
	03.00	09.00	15.00	21.00
	Cholesterol, mmol / l			
Intact control	1,60±0,11	1,70±0,06	1,89±0,06	1,66±0,13
Control pathology	1,82±0,09	1,95±0,08	1,94±0,06	1,76±0,12
Silymarin	1,73±0,09	1,74±0,07	1,89±0,10	1,68±0,19
	Total bilirubin, µmol / l			
Intact control	9,01±0,39	12,46±0,97	17,82±1,16	12,12±0,98
Control pathology	13,09±0,40*	18,12±0,93*	18,93±1,20	15,09±0,96
Silymarin	12,34±0,60	14,79±0,45	16,88±0,37	13,75±0,28
	Dosage form, U / l			
Intact control	105,97±11,04	140,07±14,74	219,00±38,30	140,07±21,27
Control pathology	152,53±12,55*	193,78±14,47*	169,00±47,90	180,40±16,39
Silymarin	118,98±8,50	144,10±14,55	151,43±22,82	142,45±9,50

Notes: n - number of animals in the group; * - the deviation of the indicator is significant relative to the rate of intact animals ($p < 0,05$).

Table 12. Influence of silymarin on circadian rhythms of excretory and of detoxification processes against the background of acute paracetamol hepatitis modeled at different times of the day according to the program Cosinor-Analisis 2.4 for Excel 2000/XP

Conditions experiment (n=6-8)		Cholesterol, mmol / l	Total bilirubin, µmol / l	Dosage form, U / l
Mezor	Intact control	1,71	12,85	151,34
	Control pathology	1,87	16,31	174,03
	Silymarin	1,76	14,44	139,24
Amplitude	Intact control	0,14	4,41	56,65
	Control pathology	0,11	3,22	10,77
	Silymarin	0,09	2,33	16,25

Note: n - the number of animals in the group