O. Ya. Mishchenko et al /J. Pharm. Sci. & Res. Vol. 11(5), 2019, 1687-1692

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Chronopharmacological dependence of the anticytolytic and antioxidant influence of silymarine and arginine glutamate under conditions of drug-induced hepatitis in rats

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Abstract:

One of the current trends in modern experimental and clinical pharmacology is the determination of the dependence of the effectiveness and toxicity of drugs on the time of day or season of the year.

The purpose of the research is to establish the characteristics of circadian rhythms of silymarine and arginine glutamate on the balance of antioxidant / prooxidant processes and the activity of cytolysis under conditions of drug-induced hepatitis in rats.

Materials and methods. Drug-induced hepatitis was caused by a single oral administration of paracetamol at a dose of 1000 g/kg of body weight of animals in the morning (09.00), day (15.00), evening (21.00) and night (03.00) periods. In order to describe the effect on lipid peroxidation/antioxidant system, the intensity of the lipid peroxidation was measured by the level of products tiobarbituric acid active products (TBA-AP). Determination of the level of recovered glutathione (RG), superoxide dismutase (SOD) and catalase activity in the liver homogenate allowed characterizing the functional state of the antioxidant system of the animal organism. The activity of cytolytic processes was determined by the level of alanine aminotransferase (ALT) cytolysis marker in the blood serum.

Results and discussions. We established the circadian mass peak of the activity of the antioxidant system of the liver under physiological conditions in rats in the morning-day time and the minimum – in the evening-night. Experimental paracetamol hepatitis was characterized by peaks of activity of cytolytic processes in the evening (21.00) and in the morning (09.00). The increase in ALT activity by 3.3 and 2.5 times ($p<0.05$) coincides with a peak reduction of endogenous antioxidant (RG) in the morning by 1,4 times ($p<0.05$) and minimal cytolytic changes during the day (15.00) and at night (03.00). We have established the effect of phytohepatoprotector silymarin on the balance of antioxidant / prooxidant and cytolytic processes in the morning (09.00) and evening (21.00) times. We observed an increase in the level of RG 1.2 times ($p<0.05$) and a decrease in the activity of ALT 1.2-1.5 times ($p<0.05$) in relation to animals with hepatitis when using silymarin. Arginine glutamate increased the activity of the antioxidant system and suppressed cytolytic processes during the day (15.00) and at night (03.00) against the background of paracetamol hepatitis: an increase in the level of RG by 1.4 times ($p<0.05$) and a decrease in the activity of ALT in 1.2-1.3 times ($p <0.05$), respectively.

Conclusion. The established circadian rhythms of the maximum severity of paracetamol hepatitis (at 21.00 and 09.00) should be taken into account in experimental pharmacology when reproducing this model of liver damage. The antioxidant and anti-cytolytic action of silymarin is maximally detected in the morning and evening, and arginine glutamate day and night. The established chronopharmacological peculiarities of the effect of the studied hepatoprotectors on antioxidant / prooxidant and cytolytic processes should be taken into account when using them as reference values in the preclinical study of promising hepatoprotectors and in further chronopharmacologic studies.

Keywords: *circadian rhythms, hepatoprotectors, paracetamol hepatitis, silymarin, karsil, arginine glutamate.*

INTRODUCTION

Knowing the circadian features of medicinal products action and their application considering these aspects contributes to improvement of their effectiveness and safety [1, 2]. In this view, one of the tasks of pharmacological research is the determination of circadian rhythms of the effect of new and existing medicines, in particular hepatoprotectors. The latter are widely used in complex therapy for both infectious and non-infectious liver diseases, in particular toxic drug hepatitis [3, 4, 5, 6]. Paracetamol hepatitis is one of the most common drug-induced hepatitis [7, 8, 9]. In the UK, annually 150-250 deaths from paracetamol are recorded, and paracetamol poisonings account for 50% of clinical cases of acute intoxication, in the United States – approximately 10% [10, 11, 12, 13]. In Ukraine, paracetamol poisoning statistics do not exist, and cases of paracetamol-induced hepatitis are largely undiagnosed [8].

The leading mechanism of hepatocytes damage to under the influence of various etiological factors, in particular toxic doses of paracetamol, is the depletion of antioxidant protection (AOP) reserves and the activation of lipid peroxidation (LPO) processes. The action of the majority of hepatoprotectors, respectively, is aimed at inhibition of these processes due to direct antiradical and/or indirect (increased antioxidant system (AOS) activity) effect [14, 15].

Taking into account that the features of circadian rhythms of modern hepatoprotectors silymarin and arginine glutamate are not sufficiently covered in the scientific literature and the dominative in the realization of their hepatoprotective effect of precisely the influence on the prooxidant-antioxidant imbalance and cytolysis activity, it is expedient to study these aspects.

The aim is to determine the peculiarities of circadian rhythms of paracetamol hepatitis and the influence of hepatoprotectors silymarin and arginine glutamate on the balance of antioxidant/prooxidant processes and the activity of cytolysis at acute paracetamol hepatitis in rats.

MATERIALS AND METHODS

Chronopharmacological study included three successive stages. In the first stage, the features of circadian rhythms of the LPO- antioxidant protection (AOP) system and the activity of cytolysis markers under physiological conditions

(in intact animals) have been investigated. In the second stage circadian rhythms of the prooxidant-antioxidant imbalance and cytolysis processes under conditions of acute toxic hepatitis caused by paracetamol have been studied. In the third stage, the influence of investigated hepatoprotectors on the prooxidant-antioxidant balance and the activity of cytolytic processes on the background of pathology and circadian features of this effect have been evaluated.

Drugs: hepatoprotectors wich are presented in the pharmaceutical market of Ukraine: silymarin containing flavonoids of thistle (trade name «Karsil», tab. 22.5 mg of thistle flavonoids Sopharma, Bulgaria) at a dose of 100 mg / kg and arginine glutamate (trade name Glutargin content L-glutamyl, L-arginine, tab. 250 mg in terms of arginine Pharmaceutical company Zdorovye, Ukraine) in a dose of 135 mg / kg. Drug doses were used according to the literature data [16].

Model pathology

Acute paracetamol hepatitis in rats was simulated by single intragastric administration of paracetamol (Sigma) (1000 mg / kg) in the morning (09.00), day (15.00), evening (21.00) and night (03.00) periods according to the formed groups of control pathology. Decapitation of animals for blood and liver tissue sampling was performed 24 hours after administration of paracetamol [17].

Determination of prooxidant-antioxidant parameters and cytolytic processes

The state of the LPO processes in hepatocytes was estimated by the content of thiobarbituric acid active products (TBA-AP), AOP system – by content of reduced glutathione (RG), superoxide dismutase (SOD) and catalase activity in hepatocytes. The level of RG was determined by the Ellman's method [18], the TBA-AP – by reaction with thiobarbituric acid [19], the activity of catalase – by reaction with hydrogen peroxide [20], and SOD – by inhibition of adrenaline autoxidation reaction [21]. The state of cytolytic processes was evaluated by the ALT activity by the Reitman & Frankel's reaction using the "Filisit-Diagnostica" kit (Ukraine) [22].

Chronopharmacological terminology

The circadian rhythms of the LPO processes and AOP system and the cytolysis activity were evaluated by the acrophase (AF) – the maximum value of the investigated parameters and the batiphase (BF) – the minimum value of the studied parameters for a certain hour of the day, the differences between which were statistically significant [1].

Statistical analysis

Statistical processing of the obtained results was carried out using the software "Statistic 8.0" according to the Mann-Whitney criterion. When comparing the statistical indicators, the level of significance was also taken as $p < 0.05$ [23].

Chronopharmacological study was conducted in the spring season of 2015. The animals were in the vivarium of the Central Scientific Laboratory of the National university of pharmacy (Certified by the State Expert Center of the Ministry of Health of Ukraine, the certificate No. 058/15 dated December 8, 2015, valid until December 07, 2019) with a controlled temperature regime and relative humidity, at day / night cycle, which corresponded to the natural time during the study season of the year. Work with animals was carried out in accordance with the requirements of GLP, the recommendations of the State Expert Center on the Ministry of Health of Ukraine, the National "General Ethical Principles of Animal Experiments (Ukraine, 2001), the Law of Ukraine No. 3447-IV of February 21, 2006, with amendments "On the protection of animals from hardship" and the adoption of the first national congress on bioethics (Kyiv, 2007) [24].

RESULTS AND DISCUSSION

Physiological conditions certain circadian rhythms of the LPO-AOP system and activity of cytolytic processes in the liver have been established in animals of normal (Fig. 1). AOP activity was maximal in the morning-day period and minimum in evening-night period, which is confirmed by AF and BF of two of the three studied system parameters: RG level (2.5±0.23 vs 1.41±0.13 μkmol/g, $p<0.05$) and catalase (78.22±4.74 vs 43.60±3.17 μkkat/l, $p<0.05$) activity respectively. The activity of the SOD did not have a pronounced circadian oscillation (39.36±3.74 and 48.83±2.84 conditional units). The expressiveness of the LPO processes was the highest in the evening-night period, as evidenced by the verified AF of the TBA-AP content at 21.00 (30.51±1.14 μkmol/g) with a diametrically opposed BF at 09.00 (16.24±2.58 μkmol/g) (Fig. 1). Thus, it has been established that the activity of the LPO processes depends on the state of AOP system and increases in the decrease periods of the latter.

Cytolytic processes, which were determinated by ALT activity, were also characterized by day-to-day rhythms (Fig. 2). The highest severity of cytolytic processes activity was observed during the day at 15.00 (1.19±0.09 μkmol/h*ml), and the lowest in the evening at 21.00 (0.87±0.05 μkmol/h*ml). Thus, it has been established that under physiological conditions, circadian rhythms of activity of cytolytic and LPO processes do not coincide (Fig. 1; Fig. 2).

In the simulations of paracetamol hepatitis, violations of physiological circadian rhythms of the indicators of the LPO-AOP system (Fig. 1) and cytolytic processes were observed (Fig. 2). In the morning-day period (AF of RG under physiological conditions) a significant decrease in the content of RG in 1.2 (1.70±0.28 vs 1.23±0.12 μkmol/g, $p<0.05$) and 1.4 (2.50±0.23 vs 2.0±0.25 μkmol/g, $p<0.05$) times, respectively, and the activity of catalase in 1.6 times (78.22±3.74 vs 48.98±8.19 μkkat/l, $p<0.05$) at 15.00 in relation to similar indicators in physiological conditions were observed (Fig. 2). Chronogram of SOD activity in animals with control hepatitis was synchronous with that in animals of the normal control. The LPO processes activity and their circadian rhythm in animals of control hepatitis were similar to that of normal control, i.e., the activity of the LPO was the highest in the evening-night period (AF of TBA-AP level at 21.00 – 31.84±2.70 μkmol/g) and minimum in the morning (BF of TBA-AP at 09.00 – 16.24±2.36 μkmol/g) (Fig. 1).

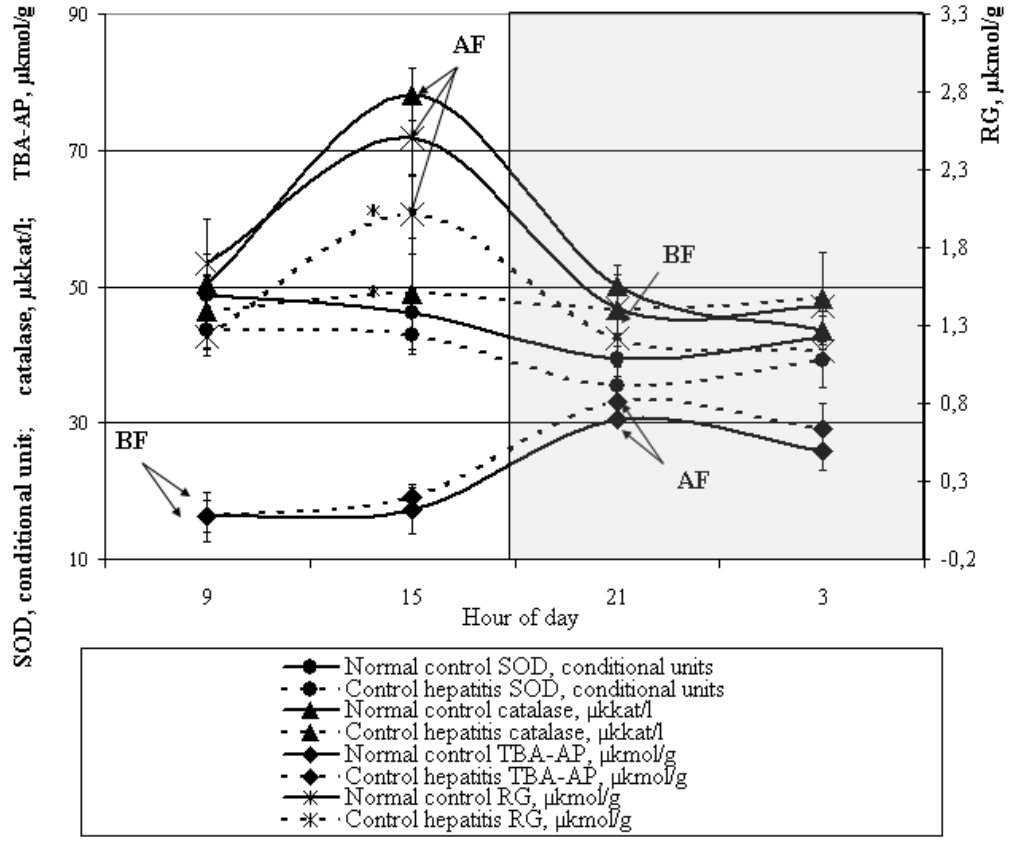

Figure 1. Circadian rhythms of the LPO-AOP system under the physiological conditions (intact rats – normal control) and against paracetamol hepatitis (control hepatitis).
Notes: * – the value of the indicator is reliable for normal control (p <0,05).

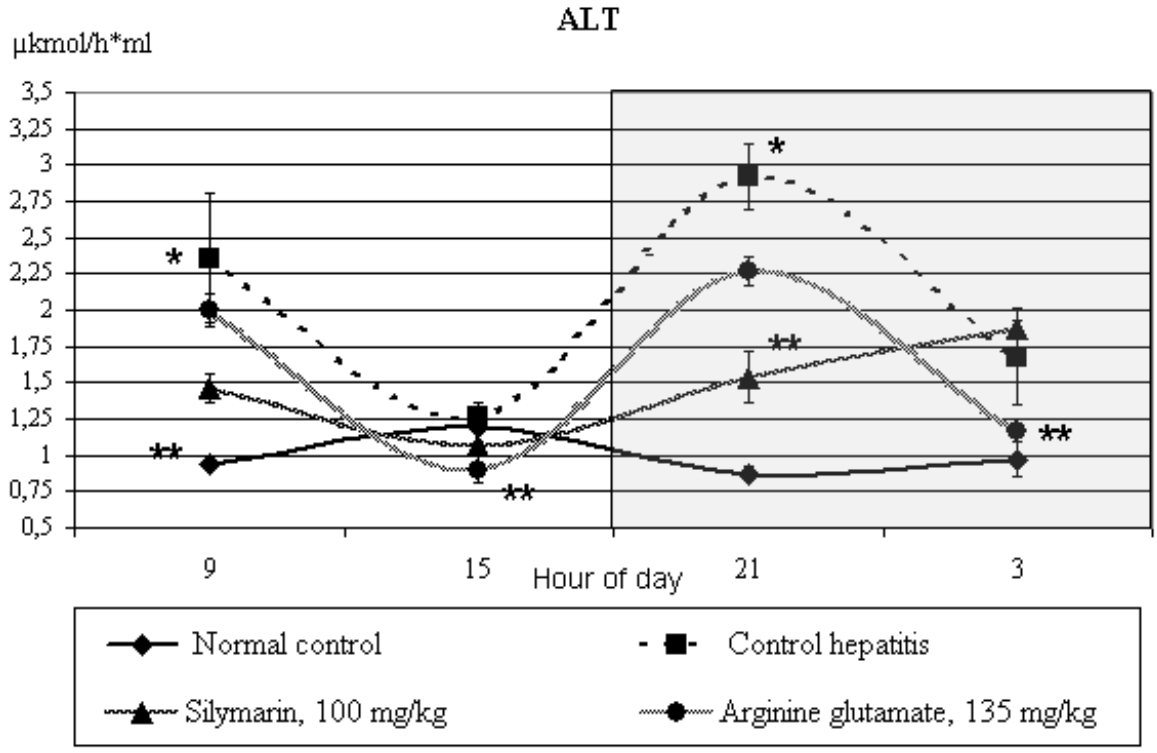

Figure 2. Circadian rhythm of ALT activity under the physiological conditions (intact rats – normal control) and against paracetamol hepatitis (control hepatitis).
Notes: * – the value of the indicator is reliable for rats of normal control (p <0,05).

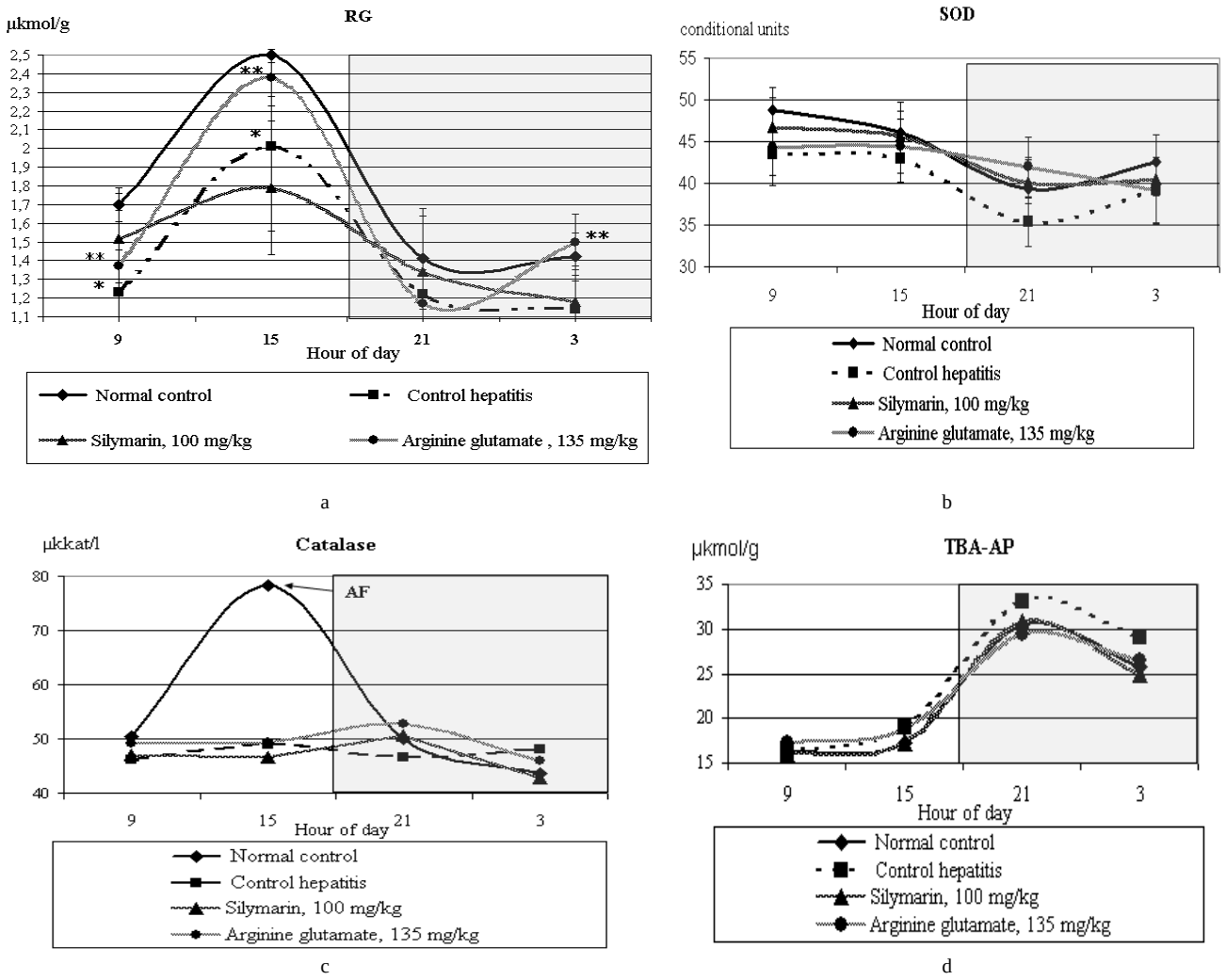

Figure 3. Influence of silymarine and arginine glutamate on circadian rhythms of LPO-AOP indices under the paracetamol hepatitis: a – content of RG; b – activity of SOD; c – activity of catalase, d – content of TBA-AP.

Notes: * – the value of the indicator is reliable for rats of normal control ($p<0,05$); ** – the value of the indicator is reliable for rats of control hepatitis ($p<0,05$).

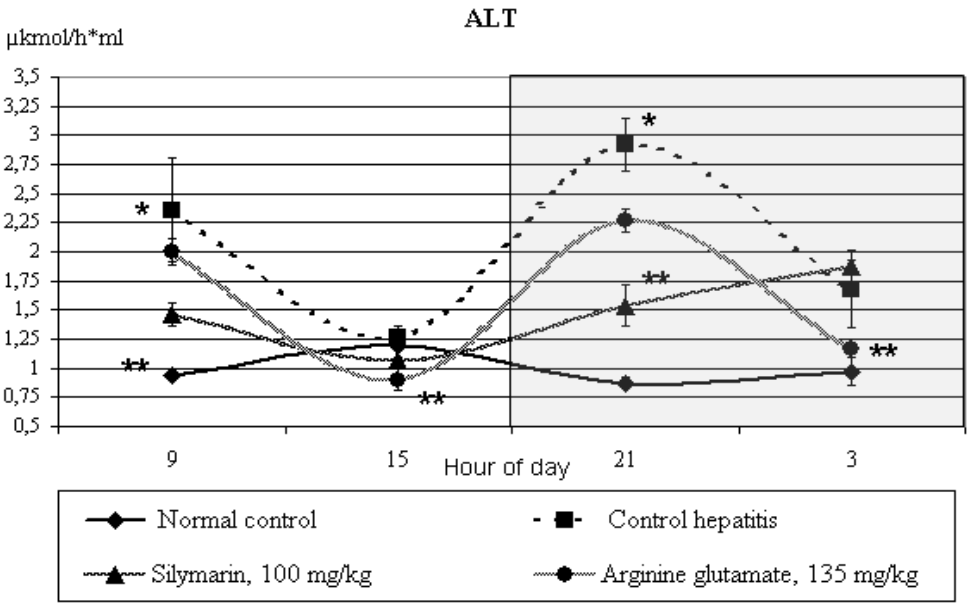

Figure 4. Influence of silymarine and arginine glutamate on circadian rhythms of ALT activity under the paracetamol hepatitis

Notes: * – the value of the indicator is reliable for rats of normal control ($p<0,05$); ** – the value of the indicator is reliable for rats of control hepatitis ($p<0,05$).

The obtained results indicate that the administration of paracetamol resulted in a decrease in AOP activity without significant changes in the activation of the LPO process in comparison versus normal control. Such dynamics of indicators testifies to the peculiarities of the modelled pathology, when a certain reserve of AOP still does not allow significant increase in the processes of LPO. It is known that the key role in the toxic effects of paracetamol belongs to the enhancement of LPO, which are intensified against the background of the AOP system exhaustion, in particular, the RG, which is interacting with the toxic metabolite of paracetamol – *N-acetyl-para-benzoquinoneimine* [8, 26]. When taking therapeutic doses of paracetamol, the biotransformation of this xenobiotic occurs in three main paths: glucuration (58-60%), sulfation (32-38%), and metabolism by the cytochrome P450 system (2-5%). However, when using toxic doses of paracetamol, the pathway of its biotransformation is redistributed to the favour of metabolism by cytochrome P450 and as a result a significant amount of *N-acetyl-para-benzoquinoneimine* is formed that binds the endogenous reserves of RG. That is, when administering toxic doses of paracetamol, there is a depletion of RG reserves, which leads to the intensification of the LPO processes [25, 26]. According to the scientific sources [27, 28, 29, 30, 31, 32], the growth of cytolysis markers (ALT and AST) activity occurs as a result of cellular and subcellular membranes damage by free radicals and release of these enzymes in the intercellular space and in the vascular bed. The results of our study indicate that the peak activity of LPO and cytolysis in model pathology coincide in the evening period and are not common in other studied periods. A significant increase in ALT activity of in 3.3 times (2.92±0.22 vs 0.87±0.05 μkmol/h*ml, $p<0.05$) in the evening and in the morning was synphased to the BF of RG level in these periods (Fig. 2), that is, the activity of the cytolytic processes correlated with the decrease of RG level.

In the third stage, an assessment the effect of the hepatoprotectors on the prooxidant-antioxidant balance and the activity of cytolytic processes under the condition of pathology was made. With the use of hepatoprotectors, the daily dynamics of the parameters of AOS activity was similar to that of animals in normal control and animals with hepatitis, but the severity of antioxidant action of drugs was different (Fig. 3).

Phytohepatoprotector silymarin, which is able to increase the of antioxidant system activity due to direct and indirect antioxidant effect [32, 33, 34], has significantly increased the content of RG by 1.2 times (1.23±0.44 vs 1.52±0.24 μkmol/g, $p<0.05$) in relation to control pathology at 09.00 and in 1.2 times (1.22±0.42 vs 1.34±0.30 μkmol/g, $p<0.05$) at 21.00 when it was minimal, and did not change the AF of this indicator (Fig. 3a). Probably the indicated features of silymarin's action are the result of the predominant antiradical effect of flavonoids [32, 33, 34]. Silymarin most significantly inhibited the activity of cytolytic processes in the period of their maximum expression: in the morning (1,26±0,14 vs 2,36±0,44 μkmol/h*ml, $p<0.05$) and evening (1,82±0,14 vs 2,92±0,22 μkmol/h*ml, $p<0.05$), which is probably due to their membrane-stabilizing properties [16, 32, 34, 35] (Fig. 4).

Unlike silymarin arginine glutamate significantly increased the level of RG at 15.00 (1.2 times – 2.38±0.15 vs 2.01±0.45 μkmol/g, $p<0.05$) and 03.00 (by 1.3 times – 1.50±0.15 vs 1.14±0.18 μkmol/g, $p<0.05$), bringing the value of this parameter closer to the physiological norm (Fig. 3), that is, its effect was synchronous with the dynamics of RG level. Arginine glutamate also inhibited ALT activity statistically significantly in the day (15.00): 0.90±0.09 vs 1.26±0.10 μkmol/h*ml ($p<0.05$) and at night (03.00): 1.17±0.24 vs 1,68±0,33 μkmol/h*ml ($p<0.05$), that is, in periods of minimal manifestation of pathology. Such a physiological synchronous effect on the level of RG and synchronous to pathology inhibitory effect on the activity of cytolytic processes is due to the ability of arginine glutamate to normalize the impaired metabolic processes. Amino acids that are part of glutargin participate in metabolic processes, in particular in the ornithine cycle of ammonia neutralization [4]. Also, a constituent of the preparation, glutamic acid, in addition to antioxidant action, can stimulate the formation of nitric oxide [32]. L-arginine is a substrate for the formation of citrulline and nitric oxide, which, under certain conditions and concentrations, can exert antioxidant action [16].

Silymarin and arginine glutamate did not have a significant effect on the activity of catalase in condition of paracetamol hepatitis (Fig. 3c).

The comparative analysis of the circadian dependence of the effect of the silymarin and arginine glutamate on the antioxidant/prooxidant balance and the activity of the cytolysis has allowed us to conclude about the chronoprofiles of sylimarin with the peak in ability of restoring the imbalance of the LPO-AOP system and reducing the cytolytic processes in the morning and evening, which is due to the presence of expressed direct and indirect antioxidant action in that drug. The chronoprofile of antioxidant and anticytolytic action of arginine glutamate is characterized by a peak in the day and night periods due to its metabolitropic mechanism of the prooxidant-antioxidant imbalance and cytolytic processes regulation.

CONCLUSION

1. The circadian activity peak of the antioxidant system of the liver in the morning-day period (AF of RG content and catalase activity), and the minimum in evening-night (the BF of RG content and catalase activity) have been established in the rat in physiological conditions. The activity of the LPO processes is antiphase to the activity of the AOP system with peak in the evening-night and minimum in the morning-day period (AF and BF of TBA-AP content).
2. Experimental paracetamol hepatitis was characterized by the activation peaks of cytolytic processes in the evening (21.00) and in the morning (09.00), coinciding with the reduction peak of the endogenous antioxidant

of RG in the morning, and with minimal cytolytic changes in the day (15.00) and at night (03.00). The established circadian rhythms of the paracetamol hepatitis severity should be taken into account in experimental pharmacology when reproducing this model of liver damage.

3. The most pronounced effect on the antioxidant and prooxidant activity of the phytohepatoprotector silymarin was observed in the morning (09.00) and evening (21.00), due to the presence of expressed direct and indirect antioxidant effects in that drug.
4. Arginine glutamate against the background of paracetamol hepatitis by its ability to regulate the prooxidant-antioxidant imbalance and cytolytic processes was characterized by peaks in the day and night periods, which is associated with its metabolitropic mechanism of action.
5. The established chronopharmacological peculiarities of the hepatoprotectors under study influence on antioxidant/prooxidant and cytolytic processes should be taken into account when conducting further chrono-pharmacological studies of these drugs, as well as using them as reference in preclinical study of promising hepatoprotectors.

REFERENCES:

1. Chronopharmacology for a doctor, pharmacist, student: a reference book / S. M. Drogovoz et al.; ed. prof.S. M Drogovoz. X .: "Titul", 2016. 376 p.
2. Dallmann R., Brown S. A., Gachon F. Chronopharmacology: new insights and therapeutic implications. *Annu Rev Pharmacol Toxicol*. 2014;. № 54. P. 339-361. doi: 10.1146/annurev-pharmtox-011613-135923.
3. Modern possibilities of metabolic therapy in the complex treatment of patients with chronic toxic hepatitis / N. V. Kharchenko et al. *Modern gastroenterology*. 2015. № 4. P. 43-50.
4. Kalko K. O. Chronopharmacological study of hepatoprotective agents activity : thesis. ... Candidate pharm. Sciences: 14.03.05 / NUPh. Kh., 2017. 187 p.
5. Bueverov A. O. Medicinal lesions of the liver. *RMZH*. 2012. № 3. P. 107-111.
6. Hepatoprotectors in the treatment of liver diseases: clinical and biochemical mechanisms of action / N. V. Kharchenko and others. *Health of Ukraine*. 2013. № 5. P. 28-29.
7. Drug-related hepatotoxicity / M. E. McDonnell et al. *N. Engl. J. Med*. 2014. Vol. 354. P. 2191-2193.
8. Side Effects of Drugs: Reference Book / S. M Drogovoz and others. X.: "SIM", 2015. 480 p.
9. Thomas M. Caparrotta1, Daniel J. Antoine, James W. Dear. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. *Eur J Clin Pharmacol*. 2018. № 74. P. 147-160.
10. Effect of the UK's revised paracetamol poisoning management guidelines on admissions, adverse reactions and costs of treatment / D. N. Bateman, R. Carroll, J. Pettie et al. *Br J Clin Pharmacol*. 2014. № 78. P. 610-618.
11. 2013 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 31st annual report / J. B. Mowry, D. A. Spyker, L. R. Cantilena, N. McMillan, M. Ford. *Clin Toxicol PhilaPa*. 2014. Vol. 52. P. 1032-1283.
12. Staggered overdose pattern and delay to hospital presentation are associated with adverse outcomes following paracetamol-induced hepatotoxicity / D. G. N. Craig, C. M. Bates, J. S. Davidson et al. *Br J Clin Pharmacol*. 2012. Vol. 73. P. 285-294.
13. Ferner R. E., Dear J. W., Bateman D. N. Management of paracetamol poisoning. *BMJ*. 2011. Vol. 342. P. 2218.
14. Ore A., Akinloye O. A. Oxidative Stress and Antioxidant Biomarkers in Clinical and Experimental Models of Non-Alcoholic Fatty Liver Disease. *Medicina (Kaunas)*. 2019. № 55 (2). doi: 10.3390/medicina55020026.
15. Hepatoprotective effects of blue honeysuckle on CCl_4-induced acute liver damaged mice / Y. S. Lee, I. J. Cho, J. W. Kim et al. *Food Sci Nutr*. 2018. № 7(1). P. 322-338.
16. Frolov V. M New domestic hepatoprotector glutargin: clinical efficacy and promising therapeutic uses. *News of medicine and pharmacy*. 2003. № 8. P. 5-6.
17. Manual for pre-clinical studies of drugs / AN Mironov et al.M .: Grif and K, 2012. 944 p.
18. Methodical instructions for performing experimental research on a large specialty practice book "Methods for assessing the state of oxidant and antioxidant systems of biological objects" / S. S. Chernedchuk et al.Odessa, 2010. 52 p.
19. Steel I. D., Garishvili T. G. Method of determination of malonic dialdehyde determination with the help of thiobarbituric acid. *Modern methods in biochemistry.M .: Medicine* , 1977. P. 44-46.
20. Method for determining the activity of catalase / M. A Korolyuk et al. *Laboratory work*. 1988. № 1. P. 16-19.
21. Sirota T. V. A new approach in the study of adrenalin auto-oxidation process and its use for measuring the activity of SOD. *Questions of medical chemistry* .1999 № 3. P. 263-272.
22. Clinical laboratory diagnostics (methods and treatment of laboratory investigations) / ed. prof. V. S. Kamishnykova. M .: MED-press-inform, 2015. 720 p.
23. Trukhacheva N. V. Mathematical statistics in medical-biological researches with application of the Statistica package. M.: GEOTAR-MEDIA, 2012. 379 p.
24. Pre-clinical research of drugs: method. recommendations / Stefanov O. V. et.al. ed. Corr. of AMS of Ukraine O. V. Stefanov. K. Avicenna, 2001. 528 p.
25. Bunchorntavakul C., Reddy K. R. Acetaminophen-related hepatotoxicity. *Clin Liver Dis*. 2013. Vol. 17, № 4. P. 587-607.
26. Evaluation of oxidative status in acetaminophen treated rat hepatocyes in culture / T. Rousar et al. *Physiol Res*. 2009. Vol. 58. P. 239-246.
27. Effect of toxic doses of paracetamol on circadian rhythm of prooxidant-antioxidant homeostasis / K. O. Kalko et al. *Pharmacology and drug toxicology*. 2016. № 1 (47). P. 81-86.
28. Chropharmacological characteristics oh the effect of hepatoprotectors in experiment? / N. D. Bunyatyan, E. A. Kal'ko, S. M. Drogovoz, and A. V. Kononenko. *Bulletin of experimental biology and medicine*. Vol. 165. № 6. P. 754-757. DOI. 10.1007/s10517-018-4258-8.
29. The circadian dependence of paracetamol toxicity in rats / E. A. Kalko, S. M. Drogovoz, A. Yu. Pozdnyakova, N. V. Zakharko. *Experimental and clinical pharmacology*. 2016. Vol. 79. № 7. P. 25-28.
30. K. O. Kalko, O. Ya. Mishchenko, L. V. Derymedvid, M. Yu. Zolotaikina, T. M. Gontova, V. V. Mashtaler, S. A. Kutsenko. A Screening Study of Hepatoprotective Activity of Liquid Extract from Common Tansy Tanacetum vulgare L. Herb in the setting of Subchronic Hepatitis in Rats. Research J. Pharm. and Tech 2018; 11(10): 4393-4396.
31. Kalko K. O., Drogovoz S. M. Cirkadian dependence of hepatoprotective activity of the antral on the model of acute paracetamol hepatitis in rats. *Pharm Drug Toxicol*. 2017. No. 2 (54). P. 62-68.
32. Kalko K. O., Mishchenko O. Y., Pozdniakova A. Y., Derymedvid L. V., Zolotaikina M. Y., Gontova T. M. et al. Screening studies of the common tansy grass (Tanacetum vulgare) liquid extract choleretic activity under subchronic hepatitis in rats. *Pharm Drug Toxicol*. 2018. № 1. P. 39-44.
33. Matveev AV Hepatoprotectors.Analysis of international research on medicines for liver.Simferopol: IT "Arial", 2013. 384 pp .
34. Effects and tolerance of silymarin (milk thistle) in chronic hepatitis C virus infection patients: a meta-analysis of randomized controlled trials / Z. Yang et al. *Biomed. Res. Int*. 2014. № 12. P. 41-85.
35. Hepatoprotective effect of silymarin / Nancy Vargas-Mendoza et al. *World J. Hepatol*. 2014. № 3 (6). P. 144–149.