International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 02, 2020
ISSN: 1475-7192

Activity of Monooxygenase and Nitrogenous Systems in Liver Microsomes Under the Influence of Inducers and Inhibitors of Medicinal Metabolism on the Organism in Conditions of Liver Pathology

Sayfullaeva Saida[1],Okhunov Alisher[2], Jumanova Nargiza[3]

Abstract— *In experiments on 48 white-breed rats of males with a mass of 180-250 g. it was found that benzonal in an effective dose of 75 mg/kg had a favorable effect on the monoxygenase and nitration system of hepatocyte microcircuits of rats with acute liver damage, while cimetidine in a dose of 10 mg/kg further suppressed the activity of MCO, increased the formation of toxic radicals NO, leading to an even greater aggravation of the severity of the animals.*

Keywords— *monooxygenase, NO-system, benzonal, cimethedrine, hepatitis, nitrogen oxide, endothelial nitric oxide synthase, inducible nitrogen oxide synthase, peroxynitrite, cytochrome P450.*

I. INTRODUCTION

In recent years, researchers have shown interest in intersystem and interorgan interrelations at the level of cellular activity, especially in the organs responsible for the functioning of the entire organism, maintaining its homeostasis in various pathological situations that arise both inside the organism and against the background of external causes [2,23]. Because of the special structurally functional position and liver value, hepatocytes occupy a special place, because their functioning is constantly influenced by endogenously formed and exogenously incoming xenobiotics [19, 21]. Liver parenchyma has the ability to level out all these threats. Functional activity of hepatocytes is determined by the solvency of enzymes of the monooxygenase system [16,17]. Physiological stability and flexible adaptability of this system can be fully assessed and traced against the background of the use of inducers or inhibitors of drug metabolism. It has been proved that the former increase the activity of monoxygenases, while the latter, on the contrary, reduce their activity [18,20]. However, there is still no answer to the question of how monoxygenase inducers and inhibitors influence the activity of the nitragic system. It is already known that the last markers of activity are nitrogen oxide (NO), endothelial (eNOS) and inducible NOS (iNOS) NO-synthase and peroxynitrite (ONO2-) [9,10].

[1]*Sayfullaeva Saida, Central Scientific Research Laboratory at the Tashkent Medical Republic of Uzbekistan Academy, e-mail: saida_sayfullaeva@mail.ru*
[2]*Okhunov Alisher, grant project manager, head of the Department of General and Pediatric Surgery of the Tashkent Medical Academy. Republic of Uzbekistan*
[3]*Jumanova Nargiza, Central Scientific Research Laboratory at the Tashkent Medical Academy, Republic of Uzbekistan e-mail: Nargizajumanova@icloud.com*

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020

The aim of the study is to study the activity of monooxygenases and NO-system parameters in microsomes of hepatocytes against the background of the action of benzonal inductor and cimethydine inhibitor in the conditions of liver pathology.

II. MATERIAL AND METHODS

The studies were carried out on 48 white, non-pedigree male rats weighing 180-250 g, which were divided into groups depending on the conditions of the experiment. The first group was the group of animals, which were intragastricly injected with 1% aqueous suspension of benzonal in doses of 75 mg/kg within 6 days; the second group was the group of animals, which were intragastric injected with 1% aqueous solution of cimetidine in dose of 10 mg/kg. Control for all groups - intact animals (8 individuals in each group). The animals were kept under standard vivarium and diet conditions. The experimental rats underneath the raush narcosis were slaughtered by instantaneous guillotine decapitation. The content of cytochromes P-450, P-420 and b5 by S method was determined in 105 000g of microsomal fractions of hepatocytes isolated with the help of preparative ultracentrifuge VAC 601 (Germany) on bovine spectrophotometer UV-2100 (Ltd, China). Omura and R. Sato (1964); activity of microsomal enzymes: HADP c-reductase was evaluated by C.H. Williams, H.Kamin (1961); benz(a)pyrengidroxylaze (B(a)PG) by C.H.Yang, L.P.Kicha (1978); N-demethylase amidopirine (N-AP) by A.Bast, J. Nordhosck (1981); anilingidroxylaze (AG) by A.I. Archakov et al. (1975); glucose-6-phosphatases (G-6-phases) - by N.S. Gnosh, N.S. Kar (1963). At the same time in the isolated microsomes the content of NO was determined by its main stable metabolites - ONO2- and ONO3- by P.P. Golikov et al. (2000); the activity of eNOS and iNOS was evaluated by V.V. Sumbayev, I.M. Yasinskaya (2000), concentration of ONO2- - according to the method of A.S. Komarin, R.K. Azimov (2005). Protein in microsomes was determined by O.N. Lowry and coauth method. (1951).

The obtained data were processed by the method of variation statistics. The results satisfying $p<0.05$ were considered reliable.

III. RESULTS AND DISCUSSION

As liver pathology, we used the CCl_4induced OTG model. We observed a decrease in the content and suppression of the activity of the enzymes of the MCO (Table 1). Thus, in rats with OTH caused by the introduction of CCl_4, the content of cytochrome P-450 and b_5 decreased significantly in 1.83 ($P<0.01$) and 2.17 ($P<0.001$) times, amounting to 55 and 46.4% of intact rats. At the same time, the content of inactive form of cytochrome P-420 increased significantly in 1.55 ($P<0.05$) times, amounting to 156.4% of normal values. The activity of MCO enzymes was significantly inhibited 2-3 ($P<0.001$) times, amounting to 33.5; 27.1; 30.6; 39.1 and 25.3% of the values of intact rats, corresponding to the enzymes of NADP-cytochrome-s-reductase, B(a)P-hydroxylaze, amidopyrin-N-demethylaze, anilingidroxylaze and glucose-6-phosphatase. In our opinion, this was mainly due to the suppression of the synthetic function of hepatocytes, contributing to a decrease in the detoxifying function of xenobiotics.

Indicators of the nitrgic function of hepatocyte microcircuits were characterized by sharp inhibition of eNOS (decrease up to 48.7% relative to the initial parameters) and induction of the inducible form of nitrogen oxide synthase (increase up to 214.3% of the initial level) when using the model of liver damage with tetrachloromethane.

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020

Nitric oxide and peroxynitrite levels increased statistically by 1.8 (P<0.001) and 2.22 (P<0.001) times in tetrachloromethane hepatitis.

Table 1 Indicators of the nitriergic system in rat liver microsomes at its toxic lesions, M±m, n=6=7

Groups	**Nitrogen system**			
	NO, μmol/mg protein	eNOS, μmol/min/mg protein	iNOS, μmol/min/mg protein	ONO_2^-, μmol/mg protein
ОТГ, CCl_4	9,95±0,317*	8,48±0,306*	0,21±0,010*	0,18±0,007*
Control	5,52±0,164	17,42±0,627	0,10±0,002	0,080±0,0016

Note: * - differences between intact and experimental groups are reliable: p<0.05.

Therefore, in liver lesions, regardless of their genesis, the activity of hepatocytes MCO is significantly suppressed, and the inducible nitrgic enzyme system is sharply activated, significantly increasing the formation of toxic products of nitrogen oxide metabolism.

Of particular interest to us were the effects of the benzene in animals with OTG on the activity of the nitrogenous system. Benzonal was studied in an effective dose of 75 mg/kg, which was administered intragastricly for 6 days a day. The conducted studies showed that the benzonal inductor increases the activity of MCO enzymes in animals with CBF caused by tetrachloromethane (Fig. 1). Thus, the content of cytochromes P-450 and b5 increased statistically significantly in 2.07 (P<0.001) and 1.91 (P<0.001) times, cytochrome P-420 - decreased in 7.34 (P<0.001) times in relation to the untreated group. Activity of enzymes HADP-cytochrome-c-reductase, B(a)P-hydroxylaze, amidopirine-N-demethylaze, anilingidroxylaze and glucose-6-phosphatase increased significantly in 2.76 (P<0.001); 2.67 (P<0.001); 2.68 (P<0.001); 2.86 (P<0.001) and 2.83 (P<0.001) times, respectively, relative to untreated group values. Most of the studied parameters approached the values of intact rats.

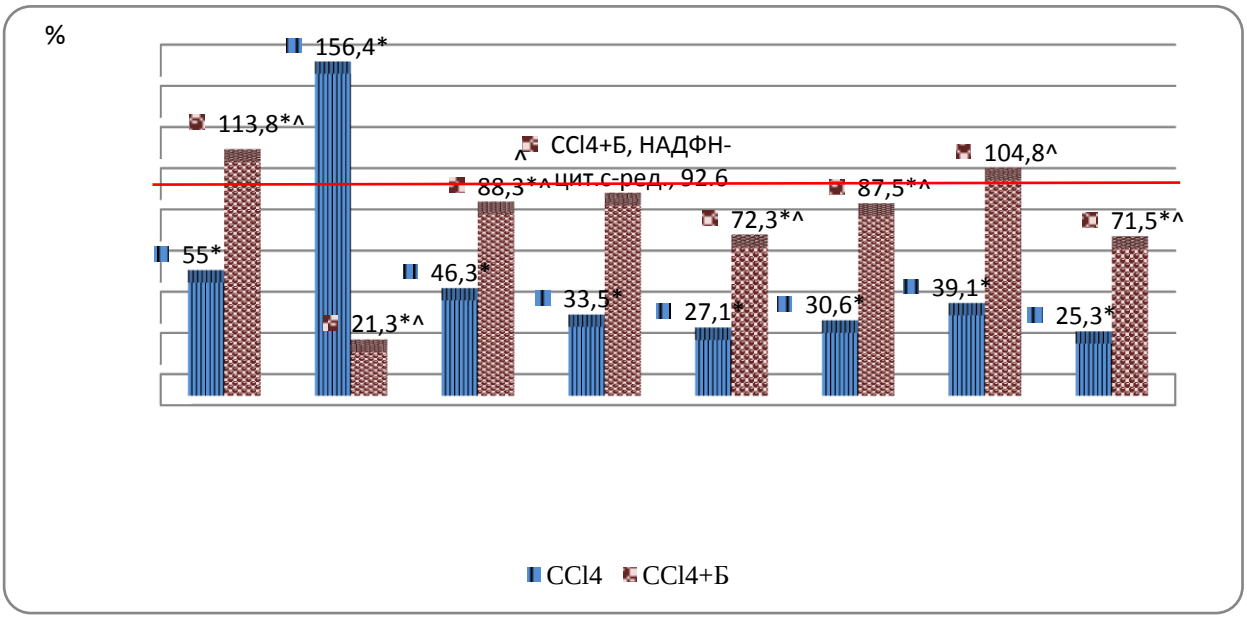

Fig. 2. Influence of benzonal on the liver microsystem in rats with OTG caused by tetrachloromethane administration.

Note: *-P<0.05 vs. control (100%), ^-P<0.05 vs. outcome (OTG).

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020

Consequently, benzonal activates the MCO of rat liver lesions, slightly reducing the imbalance in the nitrogen oxide production system. We have not revealed any recovery of the nitrigenous system.

The next stage of our studies was the study of the influence of cimetidine on the indices of monooxygenase and nitrgic systems of hepatocytes at acute toxic liver damage. Thus, cimetidine inhibitor in the dose of 10 mg/kg (less toxic dose) further suppressed the activity of MCO enzymes in animals with OTG caused by tetrachloromethane (Fig. 3). Thus, the content of cytochrome P-450 and b5 statistically significantly decreased by 1.73 (P<0.01) and 1.66 (P<0.01) times, while cytochrome P-420 increased by 1.32 (P<0.05) times relative to the untreated group. Activity of enzymes HADP-cytochrome-c-reductase, B(a)P-hydroxylaze, amidopirine-N-demethylaze, anilingidroxylaze and glucose-6-phosphate decreased significantly in 2.36 (P<0.001); 1.66 (P<0.01); 1.89 (P<0.01); 2.83 (P<0.001) and 2.61 (P<0.001) times, respectively, relative to untreated group values.

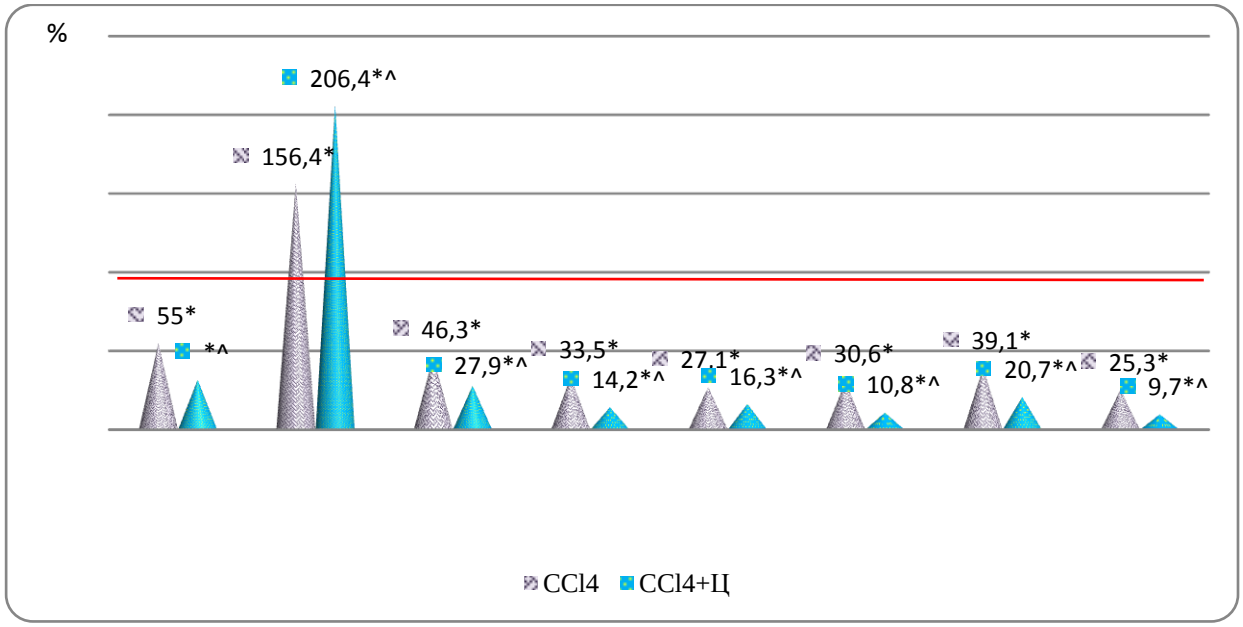

Fig. 3. Content of cytochromes and activity of monooxygenase enzymes in the liver of rats with OTH caused by tetrachloromethane administration against the background of cimetidine(C) application.

Note: *-P<0.05 vs. control (100%), ^-P<0.05 vs. outcome (CBF).

Therefore, the use of cimetidine in liver damage is not appropriate, as it further blocks the work of MCO hepatocytes.

Evaluation of the efficacy of cimetidine use in rats with liver damage in the correction of liver microsystem disorders has shown an even greater aggravation of the existing disorders. Thus, in rats with OTH caused by administration of tetrachloromethane, high activity of the inducible form of nitric oxide synthase after administration of cimethydine increased even more (1.14 times), low activity of eNOS decreased further (1.45 (P<0.05) times) in liver microsomes of experimental animals (Fig. 4). Nitrogen oxide and peroxynitrite content increased statistically by 1.19 and 1.26 times relative to untreated animal group values. The studied parameters differed from those of intact rats even more reliably.

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020

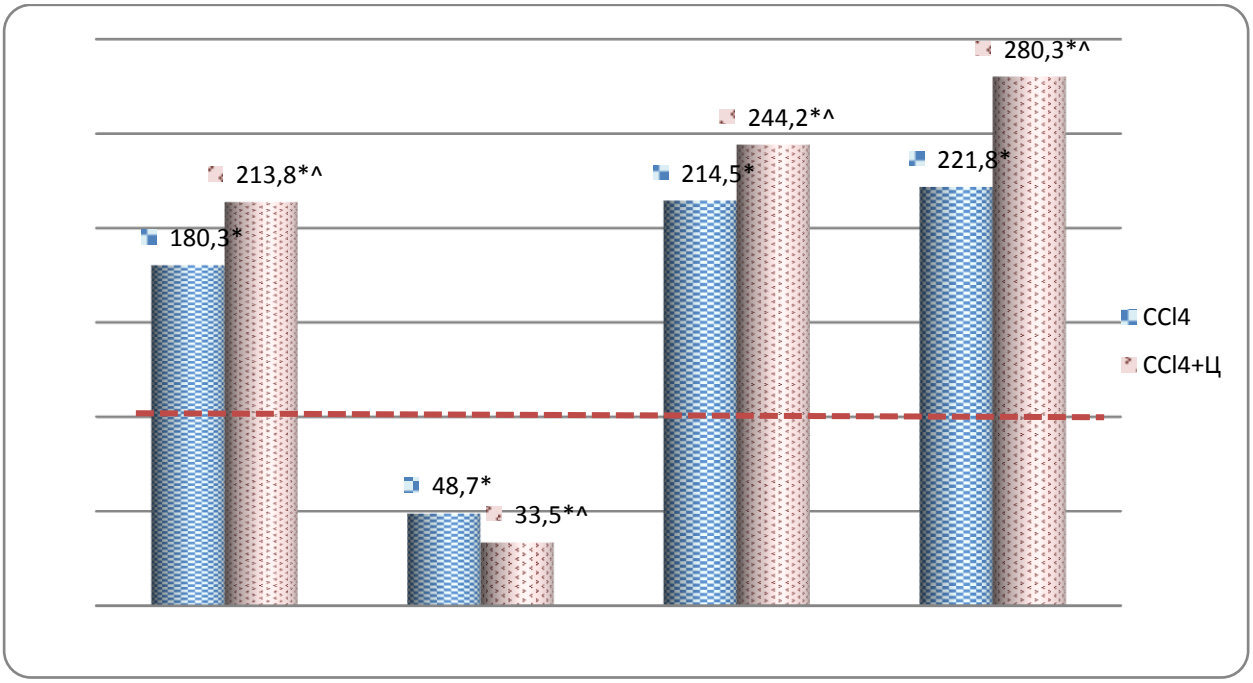

Fig. 4. Influence of cimetidine on liver microsystem parameters in rats with OTH caused by tetrachloromethane administration.

Note: *-P<0.05 vs. control (100%), ^-P<0.05 vs. outcome (OTG).

Therefore, the use of MCO inhibitor in liver diseases further aggravates the existing abnormalities in the nitragmatic systems of hepatocyte microcircuits.

Thus, the classic inhibitor of cimetidine drug metabolism suppresses the activity of liver MCO and has an inducing effect on the activity of iNOS, increases the formation of toxic nitrogen oxide radicals, which can lead to further aggravation of the condition of patients with liver diseases.

IV. CONCLUSION

Thus, the reaction of monooxygenase enzymes and NOS in microsomes under the action of a benzonal inductor is manifested in the synchronous mode of its intensification. Under the action of the inhibitor of the cimethydine monooxygenase system, the suppression of the rate of monooxygenase reactions is characterized by an increase in the activity of NOS both due to its constitutive (eNOS) and unconstitutional (iNOS) with simultaneous increase in the content of NO and ONO2- in microsomes. The revealed correlations between the indices of monooxygenase system and NOS testify to their clear mutual functional dependence, which, unfortunately, is still not taken into account in the experimental and clinical pharmacology, as well as in the treatment of patients who are prescribed inducers and inhibitors of drug metabolism.

Conclusions:1.In animals with OTG (CCl4), a benzonal dose of 75 mg/kg stimulating liver MCO, along with an increase in MCO enzymes, the activity of eNOS, simultaneously initiates the formation of NO, iNOS activity and the amount of ONO2.

The effective inhibitory dose of cimetidine 10 mg/kg potentiates the toxic effect of OTH, aimed at reducing both all the parameters of MCO and oppression of eNOS, expression NO, iNOS and ONO2-.

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020

References

1. A.I. Archakov, A.V. Lisitsa, N.A. Petushkova, I.I. Karuzina, R-450 cytochrome, medicinal disease and personalized medicine. Ч. 1 // Klin. med. - 2008. - №2. - p. 4-8.
2. Vanin, A.F. Nitrogen oxide - regulator of the cellular metabolism (in Russian) // Sorosovsky obrazovatelnogo zhurnal (in Russian) // 2001. - №11. - pp. 7-12.
3. Zinchuk, V.V. Endothelial dysfunction and oxygen-binding properties of hemoglobin // Cardiology. - 2009. - №7-8. - pp. 81-89.
4. Inzhutova, A.I.; Larionov, A.A.; Petrova, M.M.; Salmin, A.B. The theory of intercellular communication in the endothelial dysfunction development (in Russian) // Bulletin of Biol. and Med. - 2001. - №2. - pp. 165-170.
5. Kukes, V.G.; Sychev, D.A.; Hasanov, N.A. Problems of clinical pharmacokinetics at the present stage (in Russian) // Klin. - 2007. - №2. - pp. 58-63.
6. Kukes, V.G.; Sychev, D.A.; Shikh, E.V. Biotransformation study of the medicinal products is a way to increase the efficiency and safety of the pharmacotherapy (in Russian) // Doctor. - 2007. - №1. - pp. 2-5.
7. Lukyanova, L.D. (in Russian) // Proc. of 2007. The role of the bioenergetical disorders in the pathogenesis of hypoxia (in Russian) // Pat. - №2. - pp. 2-11.
8. Lukyanova, L.D.; Dudchenko, A.M.; Tsybina, T.A.; Germanova, E.L. Regulatory role of mitochondrial dysfunction in hypoxia and its interaction with transcription activity (in Russian) // MPEI Vestnik. RAMS. - 2007. - №2. - pp. 3-13.
9. Lyakhovich, V.V.; Vavilin, V.A.; Zenkov, N.K.; Menschikova, E.B. Activated oxygen metabolites in the monoxygenase reactions (in Russian) // Bul. SO RAMS. - 2005. - №4. - pp. 7-12.
10. Manukhina E.B. Downey H.F. Mallet R.T., Menshev I.Yu. Protective and damaging effects of the periodic hypoxia: the role of nitrogen oxide (in Russian) // MPEI Vestnik. RAMS. - 2007. - №2. -pp. 25-33.
11. Markov, H.M. Molecular mechanisms of vascular endothelial dysfunction (in Russian) // Cardiology. - 2005. - №12. - pp. 62-72.
12. Moiseyev, S.V. Medicinal hepatotoxicity (in Russian) // Klin. - 2005. - №14 (1). - pp. 10-14.
13. Osipov, A.N.; Borisenko, G.G.; Vladimirov, Yu.A. Biological role of nitrosyl complexes of hemoproteins (in Russian) // Successes of biol. - 2007. - T. 47. - pp. 259-292.
14. Pokrovskiy, V.I.; Vinogradov, N.A. Nitrogen oxide, its physiological and pathophysiological properties (in Russian) // Ter. - №1. - pp. 82-87.
15. Rice, R.H.; Gulyaeva, L.F. Biological effects of the toxic compounds. - Novosibirsk, 2003. –p.208.
16. Saratikov, A.S.; Novozheeva, T.P.; Vengerovskiy, A.I. Efficiency of the fermenting agents at the experimental liver damage with tetrachloromethanol (in Russian) // Expert and clin. - 2003. - №4. - pp. 48-49.
17. Sivkov, A.S.; Paukov, S.V.; Ruvinov, Yu.V.; Kukes, I.V. Individual safety of pharmacotherapy when evaluating the activity of cytochrome P-450 3A4 isoenzyme (CYP3A4) // Klin. med. - 2010. - №2. - pp. 61-067.
18. Simon, V.A.; Cytochrome, V.A., R-450 and interaction of the medicinal substances (in Russian) // Ros. zhurn. gastroenterol, hepatol., koloproktol. - 2002. - №6. – pp. 25-30.
19. Durante W., Johnson F.K., Johnson R.A. Arginase: a critical regulator of nitric oxide synthesis and vascular function // Clin. Exp. Pharmacol. Pgysiol. - 2007. - Vol. 34, №9. - pp. 906-911.
20. Getz G.S. Argenine/Arginase NO // Arteriosclerosis, Thrombosis and Vascular. Biol. - 2006. - Vol. 26. - pp. 237-240/
21. Kuczeriszka M., Olszynski K.H., Gasioroeska A. et al. Interaction of nitric oxide and the cutochrome P-450 system on blood pressure and renal function in the rat: dependence on sodium in take // Acta Pgysiologica. - 2011. - Vol. 201, №4. - pp. 493-502.
22. Lee W. Drug-induced hepatotoxicity // New Engl. J. Med. - 2003. - Vol. 349. - pp. 474-485.
23. Minamiyama Y., Jmaoka S., Takemura S. et al. Escape from tolerance of organic Nitrite by induction of cytochrome P450 // Free Radical Biology et Medicine. - 2001. - Vol. 31, №11. - pp. 1498-1508.

DOI: 10.37200/IJPR/V24I2/PR200351
Received: 16 Dec 2019 | Revised: 02 Jan 2020 | Accepted: 15 Jan 2020