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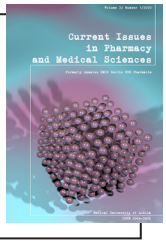

Biochemical research of hepatoprotective activity of Lavaflam tablets in rats with subchronic hepatitis

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ABSTRACT

Liver damage is a common problem all over the world. To optimize the drug provision in the limited financing of the Health Care System, it is necessary to develop new drugs with hepatoprotective properties that are highly effective and of low cost. That is why we developed the original drug Lavaflam. Lavaflam is a combination of the following substances: dry concentrate of Helichrysum arenarium and Lavender oil. The purpose of the research was to assess the biochemical evidence of the hepatoprotective properties of the drug Lavaflam on the experimental model of subchronic hepatitis in rats. Construction of an experimental model of subchronic hepatitis in rats, comparison of hepatoprotective properties of Lavaflam and Carsil preparations on the basis of biochemical research. The subject of the study was to determine the hepatoprotective properties of Lavaflam tablets. Experimental subchronic hepatitis induced in rats by way of intragastrical introduction of tetrachlormethane. The study drug Lavaflam in this experimental model of subchronic tetrachlormethane hepatitis in rats showed a positive effect on oxidant processes by increasing the compensatory mechanisms of antioxidant systems; inhibiting free radical pathology; having positive effect on the processes of biliary excretion, cholestasis, reduction of infiltrative, destructive and inflammatory process in the liver; decreasing the cytolytic process; restoring the structure of the membrane components of hepatocytes; stabilizing and enhancing functional activity of the liver; restoring its protein-synthetic function; and increasing the ability of the drug Lavaflam to restore metabolic and liver damage.

As a result of the performed biochemical study of the hepatoprotective action of Lavaflam, it has been found that in the developed drug Lavaflam, the level of hepatoprotective action corresponds with the reference drug Carsil.

INTRODUCTION

Liver damage is an important problem world-wide [1,2]. This is evidenced by the indicators of morbidity, disability and mortality of the population with this pathology [3,4]. Hence, there is a need to develop new drugs with hepatoprotective properties that are highly effective and of low cost. The drug Lavaflam was created in the department of Industrial Technology of Drugs, National University of Pharmacy, Kharkov (Ukraine) under the direction of Doctor of pharmaceutical sciences, Professor Bobrytska [5,6]. The drug resulted from the preliminary research of Prof. Georgievsky, Prof. Litvinenko, Prof. Spiridonov, Prof. Drogovoz, Prof. Hajja *et al*. in finding solutions to the problem of pharmacotherapy for hepatobiliary system diseases via integrated treatment of liver diseases. Such therapy had high efficacy, low toxicity and no side effects. Pre-clinical research *in vivo* was conducted under the direction of Prof. Drogovoz S.M. in the department of pharmacology of National University of Pharmacy, Kharkov, Ukraine – and confirmed the indicated effects.

AIM

The purpose of the research was to assess the biochemical evidence of the hepatoprotective properties of the drug

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Lavaflam on the experimental model of subchronic hepatitis in rats.

Research objectives

1. Construction of an experimental model of subchronic hepatitis in rats.
2. Comparison of hepatoprotective properties of Lavaflam and Carsil preparations on the basis of biochemical research.

Object of study

Ascertaining the hepatoprotective properties of Lavaflam tablets.

METHODS OF RESEARCH

Pharmacological, biochemical, methods of mathematical statistics. The study of the hepatoprotective action of Lavaflam was carried out via the model of subchronic tetrachlormethane (TChM) hepatitis in rats-males weighing 180-220 grams that were kept under standard vivarium conditions [7,8]. The rats were retained on a standard water and food ration with free access to water and food and natural change of day and night. Experiments were performed as per the requirements of the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC) [9], and according to the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001), as well as other international agreements and legislation of Ukraine in this area.

The condition of the liver was evaluated by the liver-mass index (LMI) [10] and other biochemical parameters. In the research, biochemical parameters were study in the blood serum, the liver homogenate and bile. The activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) were assessed in the blood serum by way of the Rietman-Frenkel method [11,12] and the ASAT/ALAT ratio (de Rhithis coefficient) [13] was established. Moreover, the level of cholesterol (CH) was ascertained by the Ilka method (using standard biochemical kits) and the activity of superoxide dismutase (SOD) was measured by applying the approach of Chevari *et al*. [14,15]. Furthermore, the level of total protein was determined by the biuret reaction, while the activity of alkaline phosphatase was found using the Bessel-Lowry-Brock method – with the help of biochemical sets of domestic production and urea [16,17]. In addition, the level of thiobarbiturate-active products (TB-AP) was investigated in liver homogenate by reaction with 2-tiobarbituric acid spectrophotometrically using the method of Stalna and Garishvili (1977), with the help of biochemical sets of domestic production [18,19]. Finally, the rate of bile excretion was determined by the method of Skakun and Oliynyk, and the content of bile acids and CH in bile were determined by the method of Miroshnichenko *et al*. (1978) [20]. We also calculated the cholato-cholesterol ratio (CCR) [21]. The results were calculated as the mean ± standard error, the statistical validity of intergroup differences was calculated according to t-Student criterion [22].

Experimental subchronic hepatitis in the rats was induced by the intragastrical introduction of TChM in the form of a 50% oil solution in a dose of 0.4 ml/100 g of animal weight during 4 days. Subsequently, the introduction of the experiment (7 days) was continued in the same treatment regimen intragastrically once a day for 4 days during, and 3 days after - the formation of the pathology (in general, the course of application of the investigational agents was 14 days). The study of the Lavaflam drug and the Carsil reference drug (Sopharma, Bulgaria, tablet 22.5 mg, series F45522) that were used for experimental treatment of subchronic hepatitis began the next day after the pathology modeling. The number of active compounds of the study drug was equivalent to the reference drug. The rats were divided into four groups: the first group – intact control; the second group – control pathology – the animals were injected with TChM; the third group – animals with experimental subchronic hepatitis and Lavaflam in a dose of 50 mg/kg body weight of the rats; the fourth group – animals with experimental subchronic hepatitis and reference drug Carsil in a dose of 50-55 mg/kg body weight of the rats [23]. The statistical processing of the obtained results was carried out utilizing Excel software. The condition of the liver was also analyzed by histopathological methods [24,25].

RESULTS AND DISCUSSION

Four groups of rats with experimental hepatitis were included in the investigation, and we assessed their survival, LMI and biochemical parameters in the serum of blood (Table 1), liver homogenate (Table 2) and bile (Table 3).

The study showed 100% survival of all animals. However, LMI was significantly increased ($p \leq 0.05$) in the 2nd group of rats (control pathology) with experimental hepatitis, in comparison with this indicator in the 1st group (intact control). Moreover, we saw an increase in infiltrative-destructive processes in the liver of rats with experimental hepatitis. In the 3rd and 4th groups of rats with experimental hepatitis (Lavaflam and Carsil, respectively), there was a decrease in LMI, indicating inhibition of infiltrative-destructive processes in the liver (Table 1).

We also noted that ALAT and ASAT activity was significantly increased and the de Rhithis coefficient was lowered in the 2nd group of experimental rats, as compared with the 1st group ($p \leq 0.05$), indicating the development of hepatocyte cytolysis. Furthermore, an increased level of CH was observed in the blood serum of the 2nd group, in comparison with this indicator in the 1st group ($p \leq 0.05$). This indicated a violation of lipid metabolism. In addition, development of cholestasis was made evident by the increased levels of alkaline phosphatase with decreasing rate of bile secretion and the lowering of the level of bile acids in the bile (Table 3) in the 2nd group of rats, as compared with the 1st group ($p \leq 0.05$). Liver metabolic disturbances were also reflected in the decreased level of total protein in the experimental rats of the 2nd group, as compared with those of the 1st group ($p \leq 0.05$).

In the case of use of Lavaflam (the 3rd group of experimental rats) and comparison of the biochemical changes in the rat blood serum, we saw positive changes in the activity of the indicators (p≤0.05): in ALAT, ASAT, levels of CH, total protein and alkaline phosphatase, which did not differ significantly from those in the 4th group of experimental rats (use of the reference drug Carsil) (Table 1).

Table 1. *Liver-mass* index and biochemical parameters of blood serum in rats with experimental subchronic hepatitis (M±m)

Parameters	n	Experimental conditions			
		group 1	group 2	group 3	group 4
Survival, %	6	100	100	100	100
Liver-mass index	6	3.303 ±0.09	4.961 ±0.23*	3.918 ±0.12*/**	3.925 ±0.1*/**
in serum of blood					
ALAT, mmol/hour ×ml	6	1.44 ±0.06	3.65 ±0.09*	2.60 ±0.28**	2.45 ±0.25**
ASAT, mmol/hour ×ml	6	1.29 ±0.04	3.48 ±0.21*	2.52 ±0.25**	2.35 ±0.25**
Rhithis coefficient	6	1.02 ±0.12	0.87 ±0.05*	1.00 ±0.14	1.00 ±0.11
CH, mmol/l	6	1.62 ±0.06	2.94 ±0.07*	1.60 ±0.21**	1.54 ±0.09**
Total protein, g/l	6	84.21 ±2.57	50.74 ±1.18*	66.26 ±1.38**	72.00 ±4.13**
Alkaline phosphatase, mkmol/s×L	6	4.19 ±0.61	8.03 ±0.37*	4.50 ±0.47**	5.88 ±0.28**
Urea, mmol/l	6	6.50 ±0.40	11.40 ±0.50*	9.60 ±1.2	8.50 ±0.8

* – statistically significant differences in the 2nd group (control pathology) as compared with the 1st group (intact control) at the significance level p≤0.05 (Mann-Whitney Criterion)

** – statistically significant differences in the 3rd group (rats with experimental subchronic hepatitis and Lavaflam), in comparison with the 2nd group at the level of significance p≤0.05 (Mann-Whitney criterion)

*** – statistically significant differences in the 4th group (rats with experimental subchronic hepatitis and reference drug Carsil), in comparison with the 2nd group at the level of significance p≤0.05 (Mann-Whitney criterion)

As result of the study of the liver homogenate in the rats with experimental subchronic hepatitis (Table 2), we found that the level of SOD in 2nd group was significantly lower when compared with the 1st group (p≤0.05). This indicates the inhibition of lipid peroxidation processes (LPP). In the 3rd and 4th groups of rats with experimental subchronic hepatitis, there was an increase in the level of SOD. This show enhancement of the compensatory mechanisms of the antioxidant systems. Based on pathology (experimental subchronic hepatitis), there was a tendency to increased TB-AP in the rats of the 2nd group (p≤0.05). This confirmed the formation of a protective-compensatory reaction that is aimed at the containment of the LPP and stabilization of the membranes due to the inclusion of CH within them. In the 3rd and 4th groups of rats with experimental subchronic hepatitis, in contrast, there was a significant decrease in TB-AP (p≤0,05). In the case of administration of the drug Lavaflam (the 3rd group of experimental rats) and comparison of biochemical changes in the liver homogenate of the experimental rats, positive changes in study indicators were established (p≤0.05) in SOD and TB-AP that did not differ significantly from those in the experimental rats of the 4th group (use of the reference drug Carsil) (Table 2).

Table 2. Biochemical parameters in liver homogenate in rats with experimental subchronic hepatitis (M±m)

Parameters	n	Experimental conditions			
		group 1	group 2	group 2	group 4
in liver homogenate					
SOD, units/min/g of tissue	6	13.42 ±0.94	6.26 ±0.71*	16.42 ±1.04**	14.47 ±0.97
TB-AP, nmol/g	6	6.71 ±6.4	15.72 ±2.97*	7.47 ±0.17**	10.06 ±1.45

We also studied the indicators in the bile of experimental rats with subchronic hepatitis. These included the rate of secretion of bile, as well as the level of bile acids, CH and CCR. In the 2nd group of the rats, there were a significant decrease in all studied parameters (p≤0.05). This indicated a violation of the functional state of the liver. In the case of use of the drug Lavaflam, we observed restoration of the functional state of the liver wherein the rate of bile secretion was increased by 1.3 times. Furthermore, the level of CH and CCR in the bile increased significantly (p≤0.05). On comparing the biochemical changes in the bile of experimental rats, in using the drug Lavaflam (the 3rd group of experimental rats), positive changes in the research indicators were established (p≤0.05). These did not differ significantly from those in the experimental rats of the 4th group (use of the reference drug Carsil) (Table 3).

Table 3. Biochemical parameters in bile in rats with experimental subchronic hepatitis (M±m)

Parameters	n	Experimental conditions			
		group 1	group 2	group 3	group 4
in bile					
The rate of bile secretion, mg/min./100 g	6	5.5 ±0.45	3.05 ±0.57*	6.7 ±0.22*/**	4.02 ±0.35**
Bile acids, mg%	6	785.05 ±42.98	390.00 ±48.47*	820.50 ±33.24**	634.32 ±29.37**/***
CH, mg%	6	28.00 ±4.95	17.75 ±7.99*	27.00 ±8.66**	22.39 ±11.04**
Cholato-cholesterol ratio (CCR)	6	28.04 ±1.25	20.46 ±3.32*	30.39 ±4.68**	28.33 ±3.02**

CONCLUSIONS

1. Intragastric administration of TChM induced acute toxic lesions in the rat livers – experimental subchronic hepatitis.
2. The use of the study drug Lavaflam in the rats with experimental subchronic hepatitis showed positive effects on antioxidant processes by increasing the compensatory mechanisms of antioxidant systems on the processes of bile excretion and cholestasis indices, bringing about inhibition of the development of free radical pathology, reduction of infiltrative-destructive processes in the liver, reduction of inflammatory process in the liver, reduction of the cytolytic process, restoration of the structure of the membrane components of hepatocytes, stabilization and increased functional activity of the liver, as well as the restoration of its protein-synthetic function.
3. We found that the developed drug Lavaflam corresponds to the reference drug Carsil with regard to the level of hepatoprotective action.

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