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

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Hepatobiliary system diseases are medical and social problem at the present time. Important issue is the development of a new complex of herbal medicine and its pharmacoeconomic study.

The purpose of the work was an experimental substantiation of the hepatoprotective properties of the drug Lavaflam.

Methods of research: pharmacological, morphological, histological, methods of mathematical statistics.

Materials of research. Experimental pharmacological studies were performed on the white rats weighing 180–220 g. The study was carried out in the Central Research Laboratory (CRL) of the National University of Pharmacy, Kharkiv, Ukraine (Certificate No 023/13 of 05.03.2013) according to the methodological recommendations of the State Expert Center of the Ministry of Health of Ukraine. The rats were divided into four experimental groups: the first group – intact control; the second group – positive pathology – the animals were injected TChM; the third group – animals, received TChM and Lavaflam; the fourth group – animals, received TChM and reference drug Carsil.

Results. It has been found that the test drug Lavaflam showed hepatoprotective properties in experimental model of the subchronic hepatitis (diffuse inflammatory liver disease) in rats.

Conclusions. Lavaflam, in comparison with Carsil contributed to the elimination and reduction in a number of pathological changes, increased adaptive capacity of hepatocytes, and Lavaflam promoted the physiological regeneration of cells.

Relating to the effect of a positive influence on the hepatic parenchyma condition with induced subchronic hepatitis by TChM, the drug Lavaflam was not inferior to the comparison drug Carsil (by the effect on necrotic manifestations), or was superior to the comparison drug Carsil (by the zones of destruction spread, dystrophic changes in hepatocytes).

Key words: liver diseases, bile duct diseases, histology, Lavaflam.

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Hepatobiliary system diseases are medical and social problem at the present time as the diseases affect people of all countries and are common to all social strata and to all age groups. According to the current data by WHO hepatobiliary pathology has 30 % of the planet population [1]. In Ukraine the incidence of hepatitis has increased by 76.6 % over the past 10 years and prevalence – in 2.2 times. Mortality has doubled over the past 20 years, and 60% of patients are people of working age [1]. Hepatoprotective drugs are used for hepatobiliary pathology treatment regardless of the etiology. At the same time, the preparations of plant origin are played a significant role in this process. These preparations are with following herbs: Cynara scolymus, Silybum marianum, Helichrysum arenarium.

In the Ukrainian pharmaceutical market there is the A05 group of the drugs for treatment of hepatobiliary system diseases according to ATC-classification [2]:
1. A05A – drugs used for biliary pathology (Ursodesoxycholic acid, Allocholum, Arthol, Hepabene, Cholasasum, Chophytol, Species chologaogae, Helichrysum arenarium, Flamin);
2. A05B – drugs used for liver diseases, lipotropic substances (Arginine, Silymarin, Carsil);
3. A05C – drugs used for biliary pathology in combination with lipotropic substances (Hepadif, Eslidine).

A new combined herbal drug Lavaflam was developed under the direction of Doctor of pharmaceutical sciences, associate professor Bobrytskaya L. at the Department of Industrial Technology of Drugs in the National University of Pharmacy, Kharkiv (Ukraine). 1 tablet of Lavaflam contains 50 mg of Flamin and 20 mg of Lavender oil, medical form is tablets 0.6 grams № 50 (Table 1) [3]. Lavaflam will be produced by the pharmaceutical company “Zdorovye” (Kharkiv, Ukraine).

### Table 1. Composition of the tablets Lavaflam

<table>
<thead>
<tr>
<th>Composition</th>
<th>g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavender oil</td>
<td>0.02</td>
<td>3.34</td>
</tr>
<tr>
<td>Beta-cyclodextrin</td>
<td>0.27</td>
<td>45.0</td>
</tr>
<tr>
<td>Flamin</td>
<td>0.05</td>
<td>8.33</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.20</td>
<td>3.33</td>
</tr>
<tr>
<td>Potato starch</td>
<td>0.022</td>
<td>3.67</td>
</tr>
<tr>
<td>Sodium croscarmellose</td>
<td>0.03</td>
<td>5.00</td>
</tr>
<tr>
<td>Polyethylene glycol 6000</td>
<td>0.002</td>
<td>0.33</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.006</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>0.6000</td>
<td>100</td>
</tr>
</tbody>
</table>

Analysis of the literature shows hepatoprotective properties of biologically active substances (flavones, flavonols, flavonones and chalcones, oxycinnamic acids, terpene compounds, glycosides, polysaccharides, alkaloid of coumarin etc.), which are the parts of Lavaflam tablets, and their wide application in hepatology [4]. Lavaflam is predicted to have choleretic, antisepic, antiapasmotic, antibacterial, anti-inflammatory effects, to improve the detoxification function of liver, normalize of gallbladder tone, increase biliary tract peristalsis, increase chologogue activity. So it made sense to study hepatoprotective properties of Lavaflam tablets and to determine a possibility to use it as a hepatoprotector.

Pharmacological study of the Lavaflam hepatoprotective properties was conducted as a preclinical study on the model of subchronic hepatitis in rats induced by tetrachloromethane (TChM).

The purpose of the study was histological evidence of the drug Lavaflam hepatoprotective properties in the experimental model of subchronic hepatitis.

**Research objectives:**
2. Comparison of hepatoprotective properties of Lavaflam and Carsil preparations on the basis of liver cells, portal vein, hepatic artery, hepatic venules, arterioles, sinusoidal hemocapillaries, bile duct epithelium histological examination.

**Object of study:** hepatoprotective properties of Lavaflam tablets.

**Methods of research:** pharmacological, morphological, histological, methods of mathematical statistics.

**Materials of research**

Experimental pharmacological studies were performed on the white rats weighing 180–220 g. The study was carried out in the Central Research Laboratory (CRL) of the National University of Pharmacy, Kharkiv, Ukraine (Certificate № 023/13 of 05.03.2013) according to the methodological recommendations of the State Expert Center of the Ministry of Health of Ukraine [5]. Rats that are included in the study were grown in the vivarium and before the experiment they were acclimated in the room for research during seven days. All studies were performed accordingly with the requirements of the European convention for the protection of...
vertebrate animals used for experimental or other scientific purposes [6].

Study of hepatoprotective activity of Lavaflam tablets was performed on the model of experimental subchronic hepatitis in rats caused by the intragastrically introduced TChM in the form of 50 % oil solution in dose of 0.4 ml/100 g of animal weight during 4 days by dint of metal probe [7].

The drug Lavaflam and the reference drug Carsil (Sopharma, Bulgaria, tablet 22.5 mg, series F45522) that were used for experimental subchronic hepatitis treatment, were studied on the next day after the pathology modeling. Lavaflam and Carsil tablets were administered prophylactically intragastrically once a day for a week prior to the onset of pathology development formation. In the last days of the experiment (7 days) the introduction was continued in the treatment regimen – intragastrically, once a day, for 4 days and 3 days after the formation of pathology. In general, the course of the studied drugs application was 14 days.

The rats were divided into four experimental groups, 10 animals in each: the first group – intact control; the second group – positive pathology – the animals were injected TChM; the third group – animals, received TChM and Lavaflam in a dose of 50 mg/kg body weight of the rats, the fourth group – animals, received TChM and reference drug Carsil in a dose of 50–5 mg/kg body weight of the rats. The doses of Lavaflam and the reference drug Carsil were calculated on the coefficients of species stability by Yu. R. Rybolovlev [8]. All the experiments were carried out according to the requirements of the EC Directions 86/609/EEC November 24, 1986 concerning care and use of laboratory animals.

Histological assessment of the liver morphological state was performed. Histological material was fixed in 10 % solution of neutral formalin and it was embedded in cellodin paraffin. Liver slices were stained with hematoxylin and eosin. Micropreparations were observed with the microscope Granum, microphotographs of images were taken using a digital video camera Granum DSM 310. Photos were processed on a computer Pentium 2.4GHz using the program Toup View.

The degree of liver damage in comparison with the groups of rats – intact control (the first group of animals) and positive pathology (the second group of rats) was taken into account to assess microscopic changes in the liver. Semi-quantitative assessments of the pathological process and the influence of Lavaflam on it were made on the liver slices. The following signs were evaluated: severity of fatty dystrophy, necrotic manifestations and existence of tissue pattern violation. Sokolovsky’s method was taken as the basis for semi-quantitative visual assessment of histochemical changes.

While applying the method of mathematical statistics, the significance level of p < 0.05 was accepted. In order to obtain conclusions when comparing statistical samples of relative variables, after the single-factor analysis of variance (or the Kruskal-Wallis criterion for data not subject to the normal distribution), differences were found between the experimental groups; the Newman–Keils criteria, the Student’s criteria for multiple comparisons or Mann–Whitney criterion, for nominal data – Fisher’s exact criteria were used. For the mathematical calculations the standard statistical software package Statistica 6.0 was used [9].

Results and discussion

Microscopic examination of experimental animals’ liver was performed after autopsy. In animals of intact control (the first group of rats) the liver had not express a typical structure segmental pattern of tissue. The segments borders were determined by triads, these zones were narrow. Endothelium conditions of the portal vein, hepatic artery, bile duct terminal branches, which are parts of triads, and other blood vessels, were normal. The radial direction of the hepatic plate system was not disturbed. Hepatocytes had a typical shape and size, distinct cell membranes. The nuclei of cells were oval and centrally located; they contain basically one, at least 2 nucleoli. Cell cytoplasm was uniformly colored; it did not contain any visible corpuscles on the optical level. The content of dual-core cells was normal. The cells that were in some distribution phases were not found in the micropreparate. Sinusoid capillaries were moderately dilated, sometimes with erythrocytes. The lymphoid cells number in the capillaries was moderate. The condition of stellate reticuloendotheliocytes (Kupffer cells) was normal (Fig. 1).

Explicit violation of the liver parenchyma was found in the rats of the second group (positive pathology group) 3 days after the last TChM administration. The changes were localized in centrolobular and perportal zones of the segments. The destructive zones size varied from 1/2 to 2/3 of hepatic lobules. Sometimes these zones merged with each other (Fig. 2).

We observed discomplexation, hepatic plate’s radial direction loss, scattered unicellular necrosis of hepatocytes, pronounced fat and protein degeneration of the cells. Manifestations of inflammatory response were minimal. Terminal hepatic veins were dilated; however sinusoidal capillaries were often compressed and poorly visible. Fatty degeneration in the destruction zones was mainly of medium and microvesicular character, in places small fat cysts were seen. Small groups of activated Kupffer cells were observed among necrotized hepatocytes (Fig. 3).

In the restricted parts of the lobules (the intermediate zone of the liver acini), the hepatic parenchyma was relatively retained. Hepatocytes of such zones were often swollen with blunted cell borders and fine cytoplasmic vacuolization. The radial direction of cells was not clear. There was a noticeable decrease in dual-core cells. At the same time, manifestations of regenerative processes in the form of moderate anisonucleosis were observed (Fig. 4).
In the group of animals that received TChM and Lavaflam (the third group of rats) we observed significant improvement of the hepatic parenchyma structural organization in most animals. Destruction zones are clearly reduced (Fig. 5).

Actually, it has been noted no protein degeneration of hepatocytes, fat dystrophy was reduced, there were fine vesicles, they do not violate cell integrity and fatty cysts were absent. Necrosis was in a small number of cells. Microcirculation was improved (Fig. 6).

Hepatocytes on unchanged parts of the segments kept the radial direction in hepatic plates, they were morphologically more complete. The pool of dual-core cells was enlarged. We observed polymorphism of cells and nuclei and a lot of polypliod cells. They had an increased in size nucleus and cytoplasm, and hyperchromias were observed in them – an increase in the amount of chromatin in the nucleus (Fig. 7). This indicated a physiological regeneration of the liver. Moreover, the increase in the number of binuclear cells was regarded as an increase in their adaptive capabilities as they synthesize more protein than single-nucleated cells.

In the group of animals with induced subchronic hepatitis by TChM after administration of the reference drug Carsil (the fourth group of animals) there was a more noticeable fluctuation in the prevalence of the hepatic lobules parenchyma pattern zones of disturbance. Hepatocytes had middle-vesicular fatty degeneration in destructive zones. Protein dystrophy was not found in all animals. Necrotic cell changes were minimal. Outside the zones of hepatocytes destruction were morphologically more complete. Swelling of the cells was absent, cell membranes were distinct and cytoplasm vacuolation was not expressed. It was found that anisonucleosis was moderate, binuclear cells were noticeable in various zones of lobules in sufficient quantity. Vascular disorders, cellular response were not expressive in all animals (Fig. 8).

It has been revealed that Lavaflam significantly reduced the zones of destruction, the severity of hepatocytes fatty degeneration and necrotic manifestations. According to the expressive influence on necrotic manifestations, Lavaflam is not inferior to the drug of comparison Carsil and ahead of it in its effect on the destruction zones prevalence, and shows a tendency to reduce changes in lipid metabolism (Table 2).
Conclusions

1. Four-fold intragastric administration of TCHM caused the acute toxic lesion in rat liver – subchronic hepatitis.
2. The test drug Lavaflam showed hepatoprotective properties in the subchronic hepatitis experimental model.
3. Lavaflam, in comparison with Carsil, contributed to a number of pathological changes reduction and elimination, increased adaptive capacity of hepatocytes, and Lavaflam promoted the physiological regeneration of cells.
4. Relating to the effect of a positive influence on the hepatic parenchyma condition with induced subchronic hepatitis by TCHM, the drug Lavaflam was not inferior to the comparison drug Carsil (by the effect on necrotic manifestations), or was superior to the comparison drug Carsil (by the zones of destruction spread, dystrophic changes in hepatocytes).

Table 2. Correction by combined drug Lavaflam of pathological process in liver parenchyma of rats with TCHM lesion (balls, Me (LQ;UQ))

<table>
<thead>
<tr>
<th>Experiment group</th>
<th>Index</th>
<th>Severity of fatty dystrophy</th>
<th>Necrotic changes in hepatocytes</th>
<th>Prevalence of destructive zones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control (n = 6)</td>
<td></td>
<td>0 (0; 0)</td>
<td>0 (0; 0)</td>
<td>0 (0; 0)</td>
</tr>
<tr>
<td>Positive control (TCHM) (n = 6)</td>
<td></td>
<td>3 (3; 4)*</td>
<td>2 (1; 2)*</td>
<td>3 (2; 3)*</td>
</tr>
<tr>
<td>Lavaflam + TCHM (n = 6)</td>
<td></td>
<td>1 (1; 2)**</td>
<td>1 (0; 1)**</td>
<td>1 (1; 1)***</td>
</tr>
<tr>
<td>Carsil + TCHM (n = 5)</td>
<td></td>
<td>2 (2; 3)***</td>
<td>1 (0; 1)**</td>
<td>2 (2; 2)*</td>
</tr>
</tbody>
</table>

* differences are statistically significant with respect to the group of intact control (the first group of rats) at the level of significance P ≤ 0.05 (Mann–Whitney criterion);
** differences are statistically significant with respect to the group of positive pathology (the second group of rats) at the level of significance P ≤ 0.05 (Mann–Whitney criterion);
*** differences are statistically significant with respect to the group of rats with induced subchronic hepatitis by TCHM after administration of Carsil (the fourth group of rats) at the level of significance P ≤ 0.05 (Mann–Whitney criterion); n: number of animals in the experimental group.

References


