BioNanoSci. (2017) 7:616–622
https://doi.org/10.1007/s12668-017-0461-8

Comparative Evaluation of Hepatoprotective Activity of Xymedon Preparation Derivatives with Ascorbic Acid and Methionine

Alexandra Vyshtakalyuk[1] · Andrey Parfenov[1,2] · Lilya Gumarova[1] · Nail Nazarov[1,3] · Vladimir Zobov[1,3] · Irina Galyametdinova[1] · Vyacheslav Semenov[1,2]

Published online: 14 October 2017

Abstract The main objective of this work was a comparative evaluation of hepatoprotective activity of pyrimidine derivative of the active compound of Xymedon (1-(ß-oxyethyl)-4.6-dimethyl-1.2-dihydro-2-oxopirimidon) and its derivatives with methionine and ascorbic acid. The experiment was conducted on nonlinear white rats of both sexes based on the model of toxic damage inflicted on liver by CCl_4 with prophylactic scheme. The compounds were injected at doses of 1/500 LD_{50} (13 mg/kg for Xymedon and 11 mg/kg for its derivatives with ascorbic acid and methionine). The study showed that, out of the three compounds considered, the Xymedon derivative with ascorbic acid had the most prominent hepatoprotective properties, as, given the CCl_4 poisoning, it caused the greatest decrease of liver damage area (by a factor of 3.25 over control) and change of the largest number of biochemical markers towards normalization. The Xymedon derivative with methionine had less prominent hepatoprotective properties than both the derivative with ascorbic acid and Xymedon itself.

Keywords Toxic hepatitis · Liver injure · Carbon tetrachloride · Pyrimidine derivatives · Hepatoprotective agents · Hepatoprotector

1 Introduction

As the incidence of liver diseases is increasing, researches aimed to discover effective hepatoprotective medication are getting ever more relevant. The best known drugs among other hepatoprotectors are based on plant matters, such as silymarin or essential phospholipids [1, 2]. Contemporary literature includes a wide range of researches aimed to discover new hepatoprotectors among plant extracts and natural compounds [3]. Synthetic hepatoprotective agents are less known. For instance, these include a drug called Thiotriazoline. There is not much reported information about the hepatoprotective activity of pyrimidine derivatives; however, this group of compounds commands attention due to their property to stimulate tissue regeneration. There are some works describing mild hepatoprotective properties of uracil derivatives (methyluracil, 4-methyl-5-oxymethyluracil) [4, 5], as well as hepatoprotective properties of synthetic derivatives of 2,4-dioxo-5-arylidenimino-1,3-pyrimidines [6]. We have previously obtained the results on hepatoprotective activity of a Russian pyrimidine derivative-based drug, Xymedon [7], and its derivative with ascorbic acid [8]. Moreover, we have obtained results proving the actoprotective [9, 10] and neuroprotective [11] activity of Xymedon derivatives.

The main idea of the synthesis of drug Xymedon derivatives with biogenic molecules is improvement of hepatoprotective properties. As biogenic acids, we used ascorbic acid, a

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12668-017-0461-8) contains supplementary material, which is available to authorized users.

✉ Alexandra Vyshtakalyuk
alex.vysh@mail.ru

[1] Laboratory of Biological and Chemical Researches and Laboratory of Nucleotide Base Chemistry of A.E.Arbuzov Institute of Organic and Physical Chemistry Kazan Scientific Centre Russian Academy of Sciences, 8 Arbuzova str, Kazan 420088, Republic of Tatarstan, Russia

[2] Kazan National Research Technological University, 68 Karl Marx str, Kazan 420015, Republic of Tatarstan, Russia

[3] Institute of Environmental Sciences and Institute of ManagementEconomics and Finance of Kazan Federal University, 18 Kremlevskaya str, Kazan 420008, Russia

well-known antioxidant, and methionine, known as an essential amino acid used in liver disease treatment. We hypothesized that the combination of these biomolecules with drug Xymedon in the form of conjugates will lead to better hepatoprotective properties. The aim of the study presented in the article is to test this hypothesis.

2 Material and Methods

2.1 Test Compounds

The study represents a comparative evaluation of hepatoprotective activity of three pyrimidine derivatives (Fig. 1): the active agent of Xymedon (1,2-dihydro-1-(ß-hydroxyethyl)-4,6-dimethyl-2-oxopyrimidine) (hereinafter pyrimidine (I)), its salt-like derivatives with ascorbic acid (II) and with *L*-methionine (III). All the compounds studied (the drug substance of Xymedon and its derivatives) were synthesized by us previously [12] in A.E. Arbuzov Institute of Organic and Physical Chemistry.

2.2 Experimental Design

The experiments were conducted on 16 white nonlinear rats of both sexes weighing 250–400 g. The animals were split into four equivalent groups of four rats in each. The animal care was in accordance with [13]: 12-h illumination, free access to feed and water. The animals were fed with complete feed made according to specification no. (protein 22%, fiber 4% max., fat 5% max, ash 9% max, humidity 13.5% max, caloric value 295 kcal/100 g). All animal experimentations and protocols were approved by the Local Ethics Committee of Kazan Federal University (Protocol № 4 dated 18 May 2017).

The test compounds were injected intraperitoneally according to a prophylactic scheme: 11 days of injections at doses 1/500 of LD_{50} ((I) 13 mg/kg, (II) and (III) 11 mg/kg); then, while continuing injections at the same doses, we simulated intoxication of liver by a single subcutaneous injection of 50% oil solution of CCl_4 [14], after which the drugs were injected once more the next day. The final sampling of biological material for the analysis was conducted on the 13th day of the experiment. The control group of animals was exposed to the same intoxication with CCl_4, but instead of drugs, the animals were injected with an equivalent amount of water (0.1 ml per 100 g of body weight).

2.3 Sample Preparation

In order to determine the initial blood values, we have sampled 1–1.5 ml of blood from the tip of the tail of each animal. The blood sampling after 11 days of administration of test compounds and the next day after the injection of CCl_4 was conducted in the same manner. The final blood sampling was performed on the 13th day of the experiment by sacrificing the animals by exsanguination through carotid artery under anesthesia. The sampled blood was then turned into a serum using a double centrifugation method at 3000 rpm cooled down to + 4 °C. Prior to the analysis, the samples were stored at − 25 °C. After the animals were sacrificed, the liver samples for histological examination were fixed with 4% buffered formalin.

2.4 Histological Examination

The tissues were processed by placing the samples in ethanol solutions with increasing concentration from 50 to 100%, xylene, mixture of xylene with paraffin at 37 °C and paraffin at 56 °C, then embedded in paraffin and made into blocks. The blocks were cut using microtome into sections of 5–7 μm in thickness which then were stained with hematoxylin and eosin. The tissue specimen were examined with a Nikon upright microscope with a digital camera. The morphometric analysis to evaluate the area of liver injury was conducted using the NIS B software.

The method of determining of affected liver tissue is as follows. In the NIS B software, we measured the total visible area of the histological liver slice as well as selected affected areas. The screenshot of liver photo in the NIS B software is presented in the Supplementary file Fig. 1 Sup.pptx. After the measure, all affected areas are summarized, and then, we calculated the percent of affected areas of liver tissue in formula (1):

$$\textit{Affected areas of liver}\ (\%) = \frac{\text{Sum } \textit{of affected areas}}{\textit{Total visible area}} \times 100 \quad (1)$$

2.5 Biochemical Examination

Biochemical values were determined with an automatic biochemical analyzer ARD-200 (Russia) using Chronolab reagents kits (Spain). The following indicators were examined: alanine transaminase (ALT); aspartate transaminase (AST); AST/ALT ratio (De Ritis Ratio); lactate dehydrogenase (LDH); total, direct, and indirect bilirubin; γ-glutamyltransferase (g-GT); alkaline phosphatase (ALP); creatinine; uric acid; urea; amylase; and lipase.

2.6 Statistical Analysis

The statistical analysis was performed in the IBM SPSS Statistics program. The parametric Student's *t* test and nonparametric Mann–Whitney *U* test were used to determine the statistically significant differences between the groups.

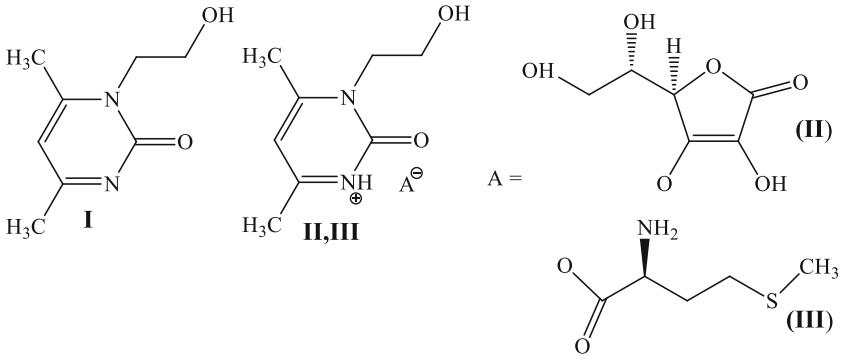

Fig. 1 Structures of studied pyrimidine derivatives

3 Results and Discussion

The study has shown that when using the compounds (I) and (II) the structural-morphological state of liver tissue is improved as shown in Fig. 2b, d. In the results of measure of injured tissue areas it was shown that the area of liver tissue affected by steatosis and necrosis significantly reduces under influence (I) and (II). In the control group, the affected area came to 40.08 ± 3.47%. In the groups that were injected with (I) and (II), this indicator came to 13.77 ± 2.48% and 12.30 ± 2.56%, respectively (these values in both groups treated with (I) and (II) are statistically significant compared to the control group at $p < 0.05$). The substance (II) showed more prominent hepatoprotective properties that the initial compound. For the group that was injected with the substance (III), the area of affected tissue (40.89 ± 3.81%) did not differ from that in the control group.

The biochemical analysis results are presented in Tables 1, 2, and 3. Alanine transaminase (ALT), aspartate transaminase (AST), AST/ALT ratio (De Ritis Ratio), and lactate dehydrogenase (LDH) were determined as the markers of hepatocyte cytolysis. Total bilirubin, γ-glutamyltransferase (g-GT) and alkaline phosphatase (ALP) were determined as the cholestasis markers. Direct bilirubin and indirect bilirubin were determined to assess the excretory functions of hepatocytes. Concomitant changes in other body systems were assessed

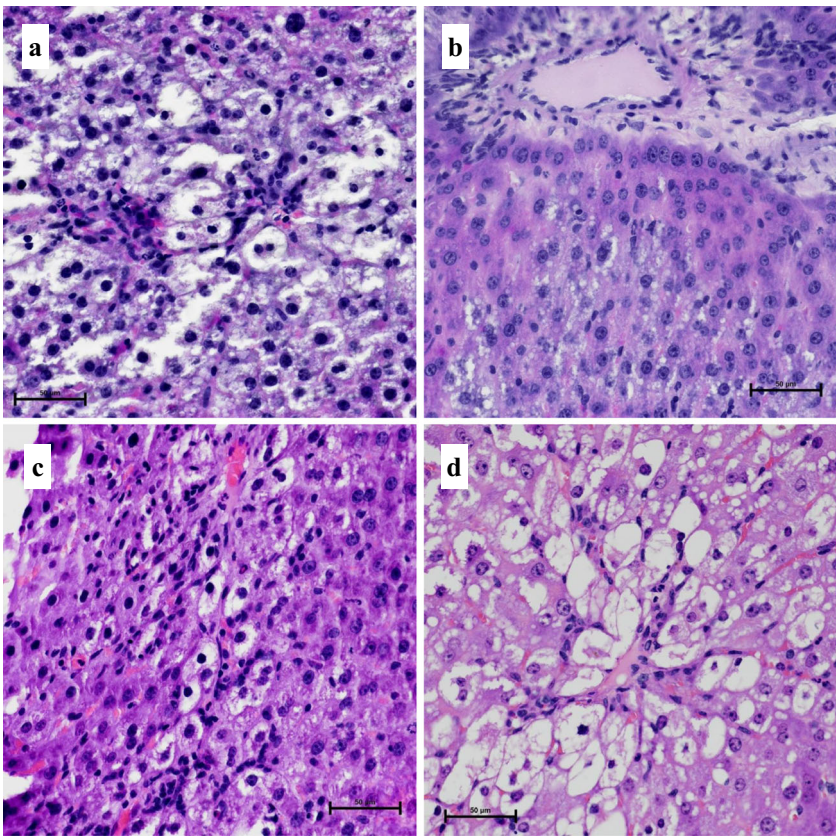

Fig. 2 Micro morphology of rats liver damaged by CCl_4. **a** Patomorphological changes of liver in control group (steatosis, necrosis, hydropic, and ballooning degeneration). **b** Improve in structural-morphological state of liver tissue in group that injected with (I) (visible areas of regeneration and areas of small-drop liposis of hepatocytes). **c** Improve in structural-morphological state of liver tissue in group that injected with (II) (a small area of hydropic and ballooning dystrophy, small-drop liposis of individual cells, and areas of uninjured cells). **d** Patomorphological changes of liver in group that injected with (III) (a large area of hydropic, fatty and ballooning dystrophy, necrosis, no difference from control group). Stained with hematoxylin and eosin. Lens 40×. Zoom 600×

Table 1 The effect of the pyrimidine derivatives on the change in liver damage markers in rats

Indices	Group	Initial	11th day	12th day	13th day
ALT (U/L)	Control	49.0 ± 0.0	96.0 ± 3.1	165.0 ± 18.4[a]	82.3 ± 11.4
	Xymedon	52.3 ± 8.5	75.0 ± 13.8	175.0 ± 25.1[a]	**69.0 ± 14.9**
	Substance (II)	67.5 ± 7.5	**64.5 ± 11.1**	**110.3 ± 11.4**	96.5 ± 33.8
	Substance (III)	51.5 ± 10.5	**69.0 ± 10.1**	394.5 ± 78.7	340.5 ± 46.6
AST (U/L)	Control	120.0 ± 5.0	183.0 ± 30.1	594.0 ± 44.0	199.3 ± 29.8
	Xymedon	106.3 ± 3.7	**131.0 ± 12.3**	717.0 ± 79.7	245.3 ± 7.5
	Substance (II)	144.5 ± 13.4	**103.5 ± 4.7**[b]	**427.5 ± 44.5**[b]	**156.0 ± 32.6**
	Substance (III)	109.3 ± 4.0	**102.0 ± 5.1**[b]	**507.0 ± 37.4**	**110.0 ± 20.8**[b]
AST/ALT (de Ritis Ratio)	Control	2.45 ± 0.10	2.13 ± 0.49	5.09 ± 2.36	2.59 ± 0.65
	Xymedon	2.12 ± 0.30	1.81 ± 0.12	**4.58 ± 1.79**	3.92 ± 0,90
	Substance (II)	2.16 ± 0.14	1.72 ± 0.23	**3.88 ± 1.16**	2.90 ± 1.28
	Substance (III)	2.40 ± 0.48	1.59 ± 0.25	**3.17 ± 0.50**[b]	2.75 ± 0.83
LDH (U/L)	Control	621 ± 117	2602 ± 564	9648 ± 745[a]	2772 ± 588
	Xymedon	783 ± 69	**1995 ± 299**	11,350 ± 796[a]	2196 ± 471
	Substance (II)	868 ± 77	**1962 ± 74**[b]	**8563 ± 307**[a]	**2003 ± 542**
	Substance (III)	925 ± 99	**1384 ± 278**[a,b]	**6147 ± 443**[a]	4486 ± 785
ALP (U/L)	Control	209.5 ± 38.5	344.0 ± 102.3	346.0 ± 48.0[a]	349.0 ± 17.7[a]
	Xymedon	174.3 ± 42.4	**223.0 ± 24.3**	**285.0 ± 37.8**	389.3 ± 23.3[a]
	Substance (II)	218.3 ± 46.5	**261.8 ± 45.5**	336.0 ± 73.9	413.0 ± 71.4[a]
	Substance (III)	226.3 ± 14.0	**263.3 ± 54.7**	**245.3 ± 99.9**	420.8 ± 30.8[a]
g-GT (U/L)	Control	5.5 ± 0.5	8.0 ± 2.7	1.0 ± 0.3[a]	9.0 ± 1.5[a]
	Xymedon	4.7 ± 0.9	7.00 ± 1.0	17.0 ± 1.4[a]	11.3 ± 2.0[a]
	Substance (II)	7.8 ± 3.6	9.0 ± 1.2	16.5 ± 9.6[a]	11.5 ± 1.5[a]
	Substance (III)	7.3 ± 3.7	9.8 ± 0.8	12.8 ± 5.9[a]	14.0 ± 4.4[a]
Total bilirubin (μmol/L)	Control	1.52 ± 0.17	1.48 ± 0.07	3.29 ± 0.22[a]	1.75 ± 0.43
	Xymedon	1.56 ± 0.21	1.71 ± 0.12	3.20 ± 0.15[a]	1.86 ± 0.13
	Substance (II)	1.21 ± 0.22	1.29 ± 0.26	**2.77 ± 0.78**	2.34 ± 0.66
	Substance (III)	1.26 ± 0.03	1.91 ± 0.25	3.38 ± 0.57[a]	4.86 ± 0.62[a]
Direct bilirubin (μmol/L)	Control	0.62 ± 0.16	0.74 ± 0.38	1.67 ± 0.18[a]	1.46 ± 0.27[a]
	Xymedon	0.77 ± 0.15	0.52 ± 0.07	1.75 ± 0.15[a]	1.49 ± 0.19[a]
	Substance (II)	0.28 ± 0.13	0.54 ± 0.12	2.03 ± 0.65[a]	2.01 ± 0.14[a]
	Substance (III)	0.49 ± 0.12	0.82 ± 0.16	2.42 ± 0.44[a]	4.03 ± 1.45[a]
Indirect bilirubin (μmol/L)	Control	0.90 ± 0.01	0.74 ± 0.45[a]	1.62 ± 0.03[a]	0.52 ± 0.10
	Xymedon	0.78 ± 0.12	1.19 ± 0.06[a]	1.45 ± 0.20[a]	0.36 ± 0.06[a]
	Substance (II)	0.94 ± 0.23	0.75 ± 0.16	**0.74 ± 0.18**	0.70 ± 0.15
	Substance (III)	0.77 ± 0.09	1.10 ± 0.11[a]	0.95 ± 0.20	0.82 ± 0.29

The positive effect of the substances studied are indicated in bold type

[a] The differences with initial indices are significant at $p < 0.05$

[b] The differences with control group are significant at $p < 0.05$

by the level of creatinine, uric acid, urea (kidney function), amylase, and lipase (pancreas function). It was shown that the initial values of different groups did not differ.

According to the monitoring dynamics, the 12th day of the experiment showed background changes of the values in the control group: the ALT level increased to 96.0 U/L, AST to 183.0 U/L, LDH to 2602 U/L, g-GT to 8 U/L, ALP to 344 U/L, creatinine to 102 μmol/L, uric acid to 95 μmol/L, urea to 12.5 mmol/L, glucose to 10.4 mmol/L, protein to 85.5 g/L, triglycerides to 1.95 mmol/L, amylase to 895 U/L, and lipase to 1518 U/L, and the level of indirect bilirubin dropped from 0.90 to 0.74 μmol/L. The changes in the levels of indirect bilirubin, creatinine, and urea were statistically valid.

After the 11 days of test compound injections, there were some differences detected for the control group, which was injected with water. ALT, LDH, and ALP increased to a lesser

Table 2 The effect of the pyrimidine derivatives on the change of metabolism indices in rats

Indices	Group	Initial	11th day	12th day	13th day
Total protein (g/L)	Control	74.5 ± 2.5	85.5 ± 5.6	72.0 ± 7.1[a]	58.7 ± 0.3[a]
	Xymedon	79.7 ± 1.7	**90.0 ± 1.7**[a]	**95.0 ± 2.5**[a,b]	53.0 ± 0.6[a]
	Substance (II)	81.0 ± 2.3	83.3 ± 3.3	**92.3 ± 4.1**[a,b]	59.3 ± 1.9[a]
	Substance (III)	76.0 ± 1.4	**90.0 ± 2.1**[a]	**85.5 ± 3.1**[a,b]	55.5 ± 0.5[a]
Glucose (mmol/L)	Control	6.2 ± 0.7	10.4 ± 1.1	10.9 ± 0.5	7.9 ± 1.9
	Xymedon	11.0 ± 0.2	12.7 ± 0.9	11.5 ± 1.5	8.7 ± 0.1
	Substance (II)	9.5 ± 0.9	9.6 ± 0.4	10.5 ± 0.6	6.9 ± 2.4
	Substance (III)	9.7 ± 1.7	14.3 ± 2.1	11.4 ± 1.3	5.0 ± 1.6
Serum cholinesterase (U/L)	Control	–	4343 ± 976	3645 ± 949	349 ± 18
	Xymedon	–	3945 ± 1478	3090 ± 615	389 ± 73
	Substance (II)	–	2313 ± 935	2330 ± 488	**421 ± 30**
	Substance (III)	–	1707 ± 440	1844 ± 273	**413 ± 72**
Triglycerides (mmol/L)	Control	1.02 ± 0.42	1.95 ± 0.25[a]	1.71 ± 0.30[a]	0.54 ± 0.07[a]
	Xymedon	1.02 ± 0.27	4.60 ± 0.54[a]	1.87 ± 0.21[a]	0.61 ± 0.13
	Substance (II)	1.06 ± 0.18	4.58 ± 0.99[a]	1.66 ± 0.07[a]	**0.77 ± 0.18**
	Substance (III)	0.93 ± 0.17	3.12 ± 0.40[a]	1.74 ± 0.14[a]	**0.80 ± 0.35**
Cholesterol (mmol/L)	Control	1.99 ± 0.45	2.12 ± 0.36	1.53 ± 0.82	1.97 ± 0.15
	Xymedon	2.04 ± 0.11	2.24 ± 0.13[a]	2.50 ± 0.59	2.66 ± 0.54
	Substance (II)	1.93 ± 0.07	2.21 ± 0.09	1.14 ± 0.38	1.64 ± 0.26
	Substance (III)	1.87 ± 0.10	1.99 ± 0.15	2.34 ± 0.18	2.17 ± 0.22

Designations are the same as in Table 1

extent; the level of AST under the effect of compounds (II) and (III) decreased and was significantly lower than in the control group (Table 1). Given the injection of compounds (I) and (III), the indirect bilirubin level increased insignificantly to 1.19 amd 1.10 μmol/L, respectively. In the groups that were injected with compounds (I) and (III) the protein level increased considerably (up to 90 g/L), as well as the level of triglycerides (up to 3.12; 4.58; and 4.60 mmol/L

Table 3 The effect of the pyrimidine derivatives on the biochemical markers of kidney and pancreas injury in rats

Indices	Group	Initial	11th day	12th day	13th day
Urea (mmol/L)	Control	7.5 ± 0.5	12.4 ± 1.4[a]	67.1 ± 14.0[a]	102.2 ± 43.0[a]
	Xymedon	6.4 ± 0.9	13.5 ± 1.1[a]	74.3 ± 9.5[a]	**71.3 ± 32.2**[a]
	Substance (II)	9.5 ± 1.3	13.7 ± 1.4[a]	**56.5 ± 11.5**[a,b]	114.0 ± 22.5[a]
	Substance (III)	5.0 ± 2.0	13.5 ± 1.8[a]	68.4 ± 17.5[a]	117.1 ± 52.7[a]
Uric acid (mmol/L)	Control	86.5 ± 0.5	97.5 ± 23.9	175.5 ± 22.5[a]	123.3 ± 41.5[a]
	Xymedon	78.7 ± 10.5	92.0 ± 6.6	193.0 ± 32.5[a]	120.2 ± 51.5
	Substance (II)	109.0 ± 7.1	99.0 ± 3.2	**102.0 ± 22.4**[b]	**118.8 ± 49.2**
	Substance (III)	73.3 ± 6.5	121.5 ± 36.5	**111.8 ± 14.0**	124.3 ± 69.7
Lipase (U/L)	Control	175 ± 31	152 ± 77	101 ± 7[a]	108 ± 10
	Xymedon	108 ± 3	161 ± 71	96 ± 3	688 ± 59
	Substance (II)	191 ± 71	**352 ± 84**[a]	100 ± 11	**145 ± 36**
	Substance (III)	151 ± 50	220 ± 48[a]	733 ± 64	110 ± 6
Amylase (U/L)	Control	521 ± 67	896 ± 222	621 ± 131	98 ± 7
	Xymedon	553 ± 56	953 ± 133[a]	942 ± 25[a]	**612 ± 38**[b]
	Substance (II)	784 ± 153	984 ± 65	991 ± 142	**904 ± 31**[b]
	Substance (III)	594 ± 49	1017 ± 69[a]	579 ± 151	515 ± 99

Designations are the same as in Table 1

under the compounds (II), (III), and (I), respectively) and cholesterol (up to 2.24 and 2.21 mmol/L with injections of compounds (I) and (II), respectively) (Table 2). The administration of pyrimidine derivatives resulted in more prominent over control increase of amylase and less prominent increase of lipase. Unlike the other groups, the group that was injected with pyrimidine derivative (II) after the 11-day course of injections showed no increase in the glucose level. The obtained results indicate that the level of cytolysis markers, increasing in the control group perhaps due to adverse background factors, decreases under the effect of pyrimidine derivatives. The pyrimidine derivatives promote metabolic processes, which lead to increase of the total blood protein and triglycerides level, and stimulate pancreatic secretion.

In 24 h after injection of CCl_4, the ALT level in the control group increased to 165 U/L, AST to 594 U/L, and LDH to 9648 U/L; the total bilirubin level increased twofold, direct bilirubin threefold, and indirect bilirubin by 1.5 times, whereas g-GT went down to 1 U/L, which might be due to decreased number of functional hepatocytes and liver suppression (Table 1). In addition, CCl_4 caused a significant increase in kidney injury markers, namely creatinine, uric acid, and urea. The level of lipase and amylase under CCl_4, on the contrary, decreased (Table 3).

Two days after the injection of CCl_4, the ALT, AST, and LDH levels in the control group decreased, which may be caused by the reduced number of functional hepatocytes due to necrosis. The level of total, conjugated and non-conjugated bilirubin, as well as the LDH level was restored. While the ALP and kidney injury markers remained elevated. In the control group, the total protein decreased to 58.67 g/L, and the triglycerides level went down to 0.54 mmol/L. The serum cholinesterase activity dropped to 349.3 U/E, amylase to 98 U/L, and lipase to 108.3 U/L.

The groups injected with test compounds and intoxicated with CCl_4 had the following peculiarities as compared to the control group. In 24 h after the intoxication with CCl_4, the group injected with the derivative (I) showed less prominent increase of transaminases ALT and AST, as well as LDH, total bilirubin and the toxic fraction of non-conjugated bilirubin, than in the control group (Table 1). In contrast to the control group, the total protein level in the groups injected with the pyrimidine derivatives reduced only 2 days after the intoxication with CCl_4, while within the first day remained at the initial level. The reduction of serum cholinesterase, amylase, and lipase as well as the increase of urea and uric acid from intoxication with CCl_4 was less prominent for the groups treated with pyrimidine derivatives than for the control group. The effect of pyrimidine derivatives on creatinine level did not determine.

The results obtained from this work are consistent with our previous studies of hepatoprotective properties of Xymedone [7] and some of the derivatives of pyrimidine with biogenic acids [12]. The model of CCl_4-induced liver damage, presented in the work [12], shows that Xymedone (I) and its derivative with ascorbic acid (II) have the most prominent effect on structural-morphological changes in the liver and outperform the hepatoprotective drug Thiotriazoline. According to the literature, Xymedon affects the key biochemical processes at the cellular and sub-cellular levels, namely it activates adenylate cyclase, which ultimately leads to cAMP's fast accumulation in a cell, to a better metabolism, first and foremost protein bio-synthesis [15].

An extended investigation of biochemical properties of blood, conducted for the first time ever in this work both on healthy animals and animals with CCl_4-induced liver damage, allowed us to obtain scientific data on the system action of pyrimidine derivatives considered, particularly on the mechanisms related to metabolic changes and on such state properties of liver as excretory, synthetic abilities of hepatocytes and cytolysis. In addition, we were able to obtain data on the system effect of pyrimidine derivatives on kidneys and pancreas. When affected by a hepatotropic poison CCl_4, the pyrimidine derivatives, (II) in particular, reduce the indicators of cytolysis, as well as bilirubin, primarily the unconjugated type, which indicates improvement in excretory function of hepatocytes. Moreover, when compared to the control group, the decline in the level of protein, triglyceride and serous cholinesterase under pyrimidine derivatives is less drastic, which means improvement in hepatic synthetic function.

One of the mechanisms of CCl_4-induced toxic liver damage is excessive formation of free radicals, intensive lipid peroxidation [14], which gives a ground to suggest that the more prominent, compared to Xymedone, hepatoprotective properties of its derivative (II) comes from antioxidative activity of the ascorbic acid fragment contained in its molecule.

4 Conclusions

Out of the three compounds considered, the Xymedon derivative with ascorbic acid had the most prominent hepatoprotective properties, as, given the CCl_4 poisoning, it caused the greatest decrease of liver damage area (by a factor of 3.25 over control) and change of the largest number of biochemical markers towards normalization. The Xymedon derivative with methionine had less prominent hepatoprotective properties than both the derivative with ascorbic acid and Xymedon itself.

Funding information The research was financed by Russian Science Foundation grant number 14-50-00014.

References

1. Morikov, D. D., Morikova, E. G., & Dvornichenko, V. V. (2015). Pharmacoeconomic analysis of the use of hepatoprotectors in management of drug-associated liver injury due to Hodgkin's lymphoma chemotherapy. *Antibiot Chemoter, 60*(7–8), 23–26.

2. Sokar, S. S., El-Sayad, M. E.-S., Ghoneim, M. E.-S., & Shebl, A. M. (2017). Combination of Sitagliptin and Silymarin ameliorates liver fibrosis induced by carbon tetrachloride in rats. *Biomedicine & Pharmacotherapy, 89*, 98–107.
3. Jimenez-Arellanes, M. A., Gutierrez-Rebolledo, G. A., Meckes-Fischer, M., & Leon-Díaz, R. (2016). Medical plant extracts and natural compounds with a hepatoprotective effect against damage caused by antitubercular drugs: a review. *Asian Pacific Journal of Tropical Medicine, 9*(12), 1141–1149.
4. Myshkin, V. A., & Enikeev, D. A. (2009). Oxymethyluracil and experimental pathology of the liver. *Bashkortostan's Medical Bulletin, 4*(2), 147–151.
5. Tauki, A. N., Fedorov, V. N., Kunitsa, Z. A., Smirnov, N. A., & Kochneva, N. V. (2010). Comparative effects of different medications in experimental toxic hepatitis. *Ivan Pavlov Russian Medical and Biological Bulletin, 1*, 66–73.
6. Krutikov VI (2004) Ashkinazi 2,4-Dioxo-5-arylidenimino-1,3-pyrimidines. Patent US N 6730787.
7. Vyshtakalyuk, A. B., Nazarov, N. G., Porfiriev, A. G., et al. (2015). The influence of the Xymedon preparation (Hydroxyethyldimethyldihydropyrimidine) on the rat liver recovery under toxic damage induced by carbon tetrachloride. *Dokl Biochem Biophy, 462*, 143–146.
8. Vyshtakalyuk, A. B., Nazarov, N. G., Zobov, V. V., et al. (2017). Evaluation of the Hepatoprotective effect of L-ascorbate 1-(2-hydroxyethyl)-4,6-Dimethyl-1,2-Dihydropyrimidine-2-one upon exposure to carbon tetrachloride. *Bulletin of Experimental Biology and Medicine, 162*(3), 340–342.
9. Nazarov, N. G., Zobov, V. V., Vyshtakalyuk, A. B., & Reznik, V. S. (2015). Research of the act-protective properties of Xymedon and its new analogs. *RJPBCS, 6*(6), 1617–1623.
10. Zobov, V. V., Nazarov, N. G., Vyshtakalyuk, A. B., Galyametdinova, I. V., Semenov, V. E., & Reznik, V. S. (2015). Efficiency of new pyrimidine derivatives influence on physical working capacity of rats in the test swimming to failure. *Human Ecology (Russian Federation)., 1*, 28–35.
11. Povysheva, T. V., Semenov, V. E., Galyametdinova, I. V., Reznik, V. S., Knni, K. S., Kolesnikov, P. E., & Chelyshev, Y. A. (2016). New Xymedon analogues for stimulation of posttraumatic regeneration of the spinal cord in rats. *Bulletin of Experimental Biology and Medicine, 162*(2), 220–224.
12. Vyshtakalyuk, A. B., Semenov, V. E., Zobov, V. V., Galyametdinova, I. V., Gumarova, L. F., Parfenev, A. A., Nazarov, N. G., Lenina, O. A., Kondrashova, S. A., Latypov, S. K., Cherepnev, G. V., Shashin, M. S., & Reznik, V. S. (2017). Synthesis and primary evaluation of the Hepatoprotective properties of novel pyrimidine derivatives. *Russian Journal of Bioorganic Chemistry, 43*(5), 604–611.
13. Washington DC (2011) Guide for the care and use of laboratory animals. Eighth Edition. Copyright by the National Academy of Sciences.
14. Mironov, A. N., Bunyatyan, N. D., Vasilyev, A. N., Verstakova, O. L., Juravleva, M. V., Lepachin, V. K., et al. (2012). *Rukovodstvo po eksperimental'nomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veshchestv [guidance on experimental preclinical trials on new pharmacological substances]. Part 1*. Moscow: Grif and K.
15. Izmaylov, S. G., & Parshikov, V. V. (2002). Xymedon: present and future. *Nizhegorodskiy meditsinskiy zhurnal [Nizhny Novgorod Medical Journal], 3*, 81–87.